Characterization of microbial activity within biofilms grown on trickling filters by microsensors measurements

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ABSTRACT
In this study, respirometry of heterogeneous media is advised as a valuable technique for characterizing mass transport and biological activity of H_2S-oxidizing biofilms growing attached to a trickling bed. Controlled flows of liquid and H_2S-containing air were recirculated through a closed heterogeneous respirometer while oxygen concentration through gas, liquid and biofilm phase was simultaneously recorded. Respirometer monitoring results were used to calibrate a model developed to describe the HR operation. Results highlighted that using DO concentration within biofilm in model calibration improve microbial activity characterization, obtaining a more accurate parameters estimation.

INTRODUCTION
Biodegradation of pollutants contained in waste gases can be performed in biotrickling filters (BTF) by specific bacteria attached to the packing material that grow as a biofilm. Although many parameters can be monitored and controlled during gas biofiltration in these systems, the activity in the biofilm is usually unknown. An improved activity monitoring technique, named heterogeneous respirometry (HR) (Bonilla-Blancas et al. 2015), has been developed and applied to characterize sulfur oxidizing biofilms immobilized on trickling beds. In HR, the aerobic oxidation of sulfide can be studied directly with the colonized trickling bed and also mathematically described in order to validate this improved technique. Nevertheless, the interpretation and modeling of oxygen profiles in the liquid and gas bulk phases of a HR is complex since mass transport phenomena between gas, liquid and biofilm phases must be considered. Moreover, reliable kinetic models describing trickling beds must consider the biodegradation processes and activity occurring along the biofilm thickness.
Dissolved oxygen microsensors (DO-MEA) have been already developed to monitor oxygen consumption in aerobic heterotrophic biofilms cultivated in a flat plate bioreactor, which allowed estimating the effective diffusivity within the biofilm, besides the biokinetics of the microbial culture (Guimerà et al. 2015). The improvement of the HR technique used in previous works (Bonilla-Blancas et al. 2015) has been performed herein by using a DO-MEA sensor during respirometric tests to obtain 8 DO profiles in 1 mm of biofilm and in a single measurement. This enhanced configuration of the HR allows describing the processes occurring during substrate oxidation in the liquid, gas and biofilm phases from the oxygen profiles obtained during experimental tests.

EXPERIMENTAL METHODS
Microsensors measurements
DO monitoring within biofilm was performed using a DO microsensor (DO-MEA sensor) based on microfabrication technology as described elsewhere (Moya et al. 2014). This microsensor, specially designed for biofilm profiling, consisted of an array of eleven gold-disk working electrodes and a rectangular gold counter electrode, mounted on a minimally invasive microfabricated needle. Additional information of microsensor characteristics can be found in Moya et al. 2014. The array of microelectrodes was designed to be inserted vertically inside a biofilm, allowing the simultaneous monitoring of DO concentration at different biofilm depths. The DO was monitored using an external

reference electrode (REF321, Radiometer analytical, France). Microelectrodes were polarized at an oxygen reduction potential of -850 mV using an 8-channel potentiostat (1010C, CH-Instruments, USA). Generated reduction currents were used to quantify the DO concentration. The electrodes were simultaneously calibrated before and after biofilms monitoring in order to quantify sensitivity drift during monitoring.

DO microsensor was set up in the reactor through the sampling port (shown in Figure 1c). The sensor was manually inserted in the biofilm, before sealing the sampling port, positioning eight of the electrodes from the biofilm surface inward. The microsensor was kept in the same position during the whole procedure.

Heterogeneous respirometer setup

The experimental system consisted of a lab-scale BTF, manufactured in PVC, with a bed diameter and height of 0.06 m and 0.23 m, respectively. Respirometric setup is presented in Figure 1a-b. The reactor was designed with an easy-to-open system in order to fill the bed volume (0.63 L) with the biofilm-covered packing material, from the system under study. During respirometric assays the liquid phase was continuously recirculated at a flowrate of 10.8 m h^{-1}, using a peristaltic pump (77200-12, Cole Parmer, USA), while the gas phase was counter-currently recirculated with a gas compressor (Model 3112, Boxer, UK) at 43.4 m h^{-1}.

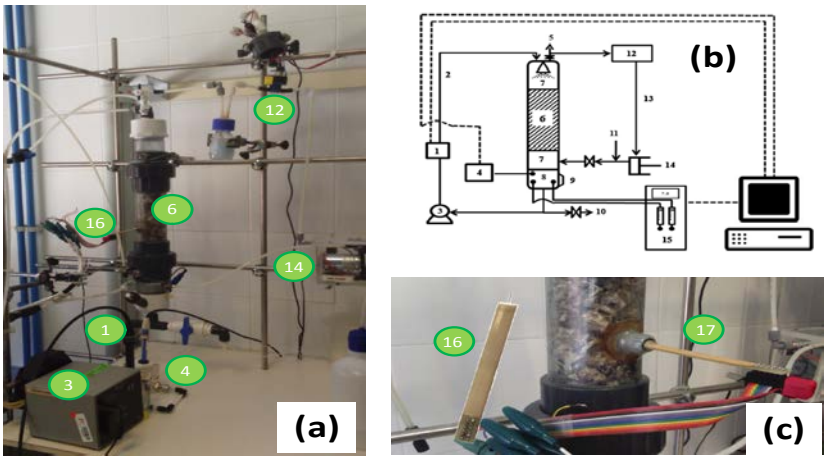

Figure 1. Schematic of the heterogeneous respirometer. (1) Dissolved oxygen sensor, (2) liquid recirculation, (3) liquid recirculation pump, (4) pH sensor, (5) as out, (6) packed bed, (7) gas free (gas volume out of the packed bed), (8) liquid reservoir, (9) pulse port, (10) liquid purge, (11) gas in, (12) O2/CO2 sensor, (13) gas recirculation, (14) gas recirculation compressor, (15) micro-burette for pH control, (16) DO microsensor and (17) microsensor port.

The HR operation was exhaustively controlled from the monitoring of oxygen concentration within gas phase, the liquid phase and biofilm. For this purpose the HR was provided with an oxygen gas analyzer (SIDOR module OXOR-P, SICK, Germany), a galvanic DO sensor (CellOx 325, WTW, Germany) placed on liquid phase recirculation, and a sampling port for microsensors monitoring. The pH was also monitored (SenTix 20, WTW, Germany) throughout the experimental tests, and was accurately controlled at pH 7.0±0.1 by a high-precission two-channel micro-burette (Multi-burette 2S, Crison, USA) adding1 M HCl and 1 M NaOH solutions.

Experimental determinations in heterogeneous respirometer

Abiotic and biotic experiments were performed to characterize the mass transfer phenomena and the H_2S-oxidizing activity within the biofilm. First, abiotic assays were carried out to estimate the overall volumetric mass transfer coefficient ($K_L{\cdot}a_{G\text{-}L}$) corresponding to oxygen. The procedure to obtain

experimental data and to estimate $K_L \cdot a_{G\text{-}L}$ was applied as described in Bonilla-Blancas et al. 2015.

For biotic assays, the HR was filled with PP pall rings (15.9 mm diameter) from a pilot BTF for biological (aerobic) removal of H_2S (López et al. 2016). Biotic experiments started with the addition of 126 mL of mineral medium into the HR, which was continuously recirculated and aerated for 24 hours in order to achieve endogenous conditions. Afterwards, the HR was closed, while both phases were recirculated, and pulses of a certain volume of H_2S were injected. Gas and liquid phase were recirculated at lineal velocities of 101.2 and 10.8 $m \cdot h^{-1}$ respectively, until total degradation of injected H_2S. During the overall operation oxygen concentration was simultaneously monitored within the three phases. Oxygen evolution through the HR was used to calibrate the biokinetic model included in mathematical model developed to describe HR operation.

MATHEMATICAL MODEL DEVELOPMENT

The HR model include the mathematical expressions for the description of mass transport by advective flow in gas and liquid phase, mass transfer through the gas-liquid interface, mass transfer at the liquid-biofilm interface, internal mass transport in biofilms and biological kinetics within biofilms. The model equations were based on the above mechanism and assumptions typically assumed in BTF models (López et al. 2016). The following subsections describe mass balances in the gas, liquid and biofilm phases and their initial conditiond in the HR.

Mass balances for the gas phase

Mass balance for gas phase in the HR trickling bed (1) and the HR reservoir (2),

$$\frac{dC_{G,i}}{dt} = \frac{Q_G}{V_{Bed} \cdot \varepsilon_G^{Bed}} \cdot \left(C_{G,i}^{Free} - C_{G,i}\right) - \frac{K_L \cdot a_{G-L}}{\varepsilon_G^{Bed}} \cdot \left(\frac{C_{G,i}}{H_{e,i}} - C_{L,i}\right) \quad (1)$$

$$\frac{dC_{G,i}^{Free}}{dt} = \frac{Q_G}{V_G^{Free}} \cdot \left(C_{G,i} - C_{G.i}^{Free}\right) \quad (2)$$

Where $C_{G,i}$ is the concentrations of the specie *i* in the HR gas phase, Q_G is the gas flow rate, V_{Bed} is the bed volume, ε_G^{Bed} is the volumetric fraction of gas phase within the trickling bed, $C_{G,i}^{Free}$ is the concentration in the HR gas reservoir, $H_{e,i}$ is the gas/liquid partitions coefficient, $C_{L,i}$ is the concentration in the HR liquid phase and V_G^{Free} is the volume of the HR reservoir.

The initial conditions were: $t=0$, $C_{G,i}=0$, $C_{G,i}^{Free}$=*pulse concentration*.

Mass balances for the liquid phase

Mass balance for liquid phase in the HR trickling bed (3) and the HR reservoir (4),

$$\frac{dC_{L,i}}{dt} = \frac{Q_L}{V_{Bed} \cdot \varepsilon_L^{Bed}} \cdot \left(C_{L,i}^{Res} - C_{L,i}\right) + \frac{K_L \cdot a_{G-L}}{\varepsilon_L^{Bed}} \cdot \left(\frac{C_{G,i}}{H_{e,i}} - C_{L,i}\right) - \frac{K_B \cdot a_{L-B}}{\varepsilon_L^{Bed}} \cdot \left(C_{L,i} - C_{B,i}\right) \quad (3)$$

$$\frac{dC_{L,i}^{Res}}{dt} = \frac{Q_L}{V_L^{Res}} \cdot \left(C_{L,i} - C_{L,i}^{Res}\right) \quad (4)$$

Where Q_L is the liquid flow rate, ε_L^{Bed} is the volumetric fraction of liquid phase within the trickling bed, $C_{L,i}^{Res}$ is the concentration in the HR liquid reservoir, $K_B \cdot a_{L\text{-}B}$ is the overall mass transfer coefficient at liquid-biofilm interphase, $C_{B,i}$ is the concentration within biofilm and V_L^{Res} is the volume of the HR reservoir.

The initial conditions were: $t=0$, $C_{L,i}=0$, $C_{L,i}^{Res}=0$.

Mass balances for the biofilm

Mass balances for the biofilm (5),

$$\frac{dC_{B,i}}{dt} = \frac{K_B \cdot a_{L-B}}{\varepsilon_L^{Bed}} - D_{B,i} \cdot \frac{\partial^2 C_{B,i}}{\partial \delta^2} + \sum \upsilon_{ij} \cdot r_{B,j} \quad (5)$$

Where $D_{B,i}$ is the diffusion coefficient within biofilm, δ is the biofilm thickness, ν_{ij} is the stoichiometric coefficient for the reaction j and $r_{B,j}$ is the rate of the reaction j. The H_2S consumption within biofilm is described using the kinetic model presented in Mora et al. 2016. Kinetic expressions are not included in herein.

The initial conditions were: $t=0$, $C_{B,i}=0$.

Model solution

Since mass transport of compounds in the axial direction is modelled as plug flow, the model equations were solved discretizing the HR in *nvs* vertical layers, simulating a sequence of continuous stirred tank reactors. In the same way, biofilm layers were also divided in *nb* subdivisions for model solution.

RESULTS AND DISCUSSION

Microsensors monitoring

Microsensros performance for HR monitoring was analyzed assessing the variations of the device response during HR operation. Microsensor characterization before and after HR monitoring showed a slightly decrease on sensitivity for DO detection due to biofilm monitoring, as stated in Moya et al. 2014. Even so, sensitivity decrease was lower than 10% after 4 h of biofilm monitoring, highlighting the suitability of DO microsensor for HR monitoring.

HR characterization parameters

Abiotic tests and trickling bed determinations allowed model parameters estimation. Results of most relevant parameters are shown in Table 1.

Table 1. Parameters estimated from the system.

Parameter	Value	Parameter	Value
Liquid volume	0.125 L	Dynamic hold-up	23.5e-03 L
Gas volume	1.49 L	Static hold-up	17.7e-03 L
Biomass	1.78 g X	OUR_{end}	25.0 mg O_2 g^{-1} X h^{-1}
Biofilm	3.23 g TS	$K_L a_{G-L}$	54.1 h^{-1}

Microbial assays

Experimental tests performed in the heterogeneous respirometer to study S-oxidation consisted of spiking the respirometer gas phase with a certain volume of H_2S (200µL, 1mL, 5mL and 10mL), which corresponded to initial gas phase concentrations ranging from 135ppm_v to 6720ppm_v. In Fig. 2 the complete respirometric profile and respirometric profiles from tests performed with 5mL and 10mL of H_2S are presented. Endogenous activity and k_La were calculated from the endogenous phase and the re-aireation phase. k_La obtained was much higher compared to those reported in previous works (Bonilla-Blancas 2015) for polyurethane foam indicating that with pall rings mass transfer resistance was further reduced.

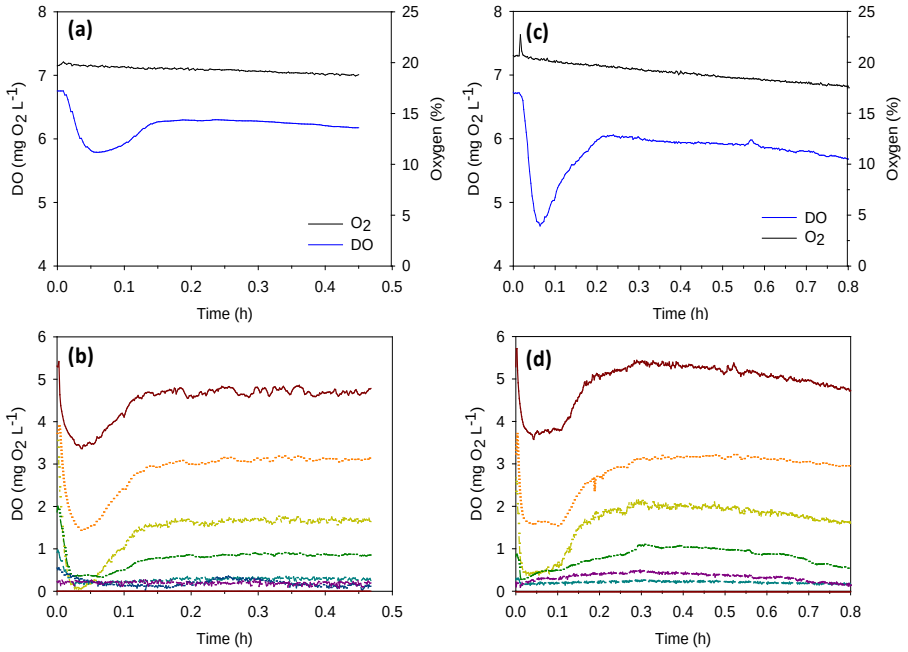

Figure 2. Respirometric profiles obtained from oxygen monitoring in gas and liquid phases and in the biofilm. (a,b) Pulse of 5mL H_2S (b,c) Pulse of 10mL H_2S.

The most relevant parameters of the biokinetic model selected for microbial activity description were estimated using the monitoring results (Figure 2) to calibrate the HR model (equations 1-5).

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