CFD modeling of a fixed-bed biofilm reactor coupling hydrodynamics and biokinetics

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Abstract
Rigorous modeling of transport phenomena is essential to reproduce accurately biofiltration systems performance. In this sense, the aim of this study was to investigate the effect of integrating fluid flow dynamics in the development of these bioreactor models, mimicking their hydrodynamics and behavior in a fixed biofilm reactor. 2D bioreactor models were developed using three different well-established tools for modeling bioreactors (AQUASIM, MATLAB, and Computational Fluid Dynamics - CFD), considering from ideal flow patterns to more complex fluid dynamics. A detailed comparison was performed among the results, taking into account the simulation of dissolved oxygen profiles in the liquid phase, inside the biofilm and in the boundary layer along a bioreactor. These models were validated by comparing the simulations with direct measurements obtained by means of dissolved oxygen microsensors of high spatial resolution. In all cases, deviations were below 6%, nevertheless CFD predictions obtained the lowest deviations below 3.5%. Thus, these results underline that CFD techniques are appropriate to model more accurately the performance of fixed-bed biofilm reactors, allowing the study in detail of all the hydrodynamics variables involved in the process. In addition, a 3D CFD model, combining hydrodynamics and biological reactions, was developed and solved to simulate local transient flow and dynamic behaviors of oxygen consumption in the bioreactor. The results of CFD simulations were evaluated in order to know the effect of mass transport phenomena (advection and diffusion) by characterizing hydrodynamics and, finally, to predict the oxygen degradation along the bioreactor.

Keywords:
CFD modeling; Fixed biofilm reactor; Biological reactions; Hydrodynamics; Dissolved oxygen

Nomenclature

List of symbols:
A_l is the available surface area between biofilm and liquid phase (m^2)
BT is the biofilm thickness (m)
BLT is the boundary layer thickness (m)
C(t) is the concentration-time function of the tracer
C_BDO is the concentration of oxygen in the biofilm phase (kg DO m^{-3})
C_Bn is the concentration of n-compound in the biofilm phase (kg m^{-3})
$C_Bn(i,1)$ is the concentration of n-compound in the first layer of biofilm (kg m^{-3})
C_Ln is the concentration of n compound in the liquid phase (kg m^{-3})
C_Ln0 is the concentration of n compound in the inlet liquid phase (kg m^{-3})
C_n is the concentration of the n variable (kg m^{-3})
D is the kinematic diffusivity for the n variable (m^2 s^{-1})
D_Bn is the diffusion coefficient of n-compound in biofilm phase (m^2 s^{-1})
D_Ln is the diffusion coefficient of n-compound in liquid phase (m^2 s^{-1})
D_r is the relative diffusivity
E(t) is the residence time distribution function of the tracer
k_d is the maintenance factor for oxygen (kg DO kg VSS^{-1} s^{-1})
$K_{S,DO}$ is the Monod half-saturation coefficient for oxygen (kg DO m^{-3})
M is the tracer mass (kg)
n_B is the number of discretizations in the biofilm phase
n_L is the number of discretizations in the liquid phase
q is the volumetric flow rate of the tracer (m^3 s^{-1})
Q is the volumetric flow rate in the inlet bioreactor (m^3 s^{-1})
$q_{max,DO}$ is the maximum specific growth rate (kg DO kg VSS^{-1} s^{-1})

R_Bn is the biological reaction rate of n-compound (kg m^{-3} s^{-1})
Re is Reynolds number
S_φ is a volumetric source term (kg m^{-3} s^{-1})
t is the time (s)
t_m is the mean residence time (s)
U is the fluid velocity (m s^{-1})
V_L is the liquid phase volume (m^3)
X is biomass density (kg m^{-3})
$Y_{DO/S}$ is the oxygen-glucose yield (kg DO kg S^{-1})

Greek letters:
ρ is the mixture density (kg m^{-3})
Φ is the conserved quantity of n variable per unit mass of fluid

1. INTRODUCTION

Over the past years biofilters and biotrickling filters have emerged as an efficient and reliable biological process to treat pollutants from air emissions. These techniques employ the metabolic diversity of microorganisms to degrade, transform or accumulate a wide range of compounds. Then, microorganisms are an essential element in these bioreactors operation, where they grow as a fixed film, while interacting with the environment through the gas/liquid phase that flows over them. This fluid flow influences biofilm development and activity by the transport of dissolved solutes into and out of the biofilm, and the application of shear forces to the biofilm [1]. An adequate characterization of this fluid flow is required in order to represent accurately the biological behavior.

Several mathematical models have been used to describe hydrodynamics when modeling liquid-phase bioreactors performance, from plug flow or complete mixed ideal models to computational fluid dynamic (CFD) models [2]. These authors compared performance-prediction models of the most common aerobic bioreactors, considering ideal and non-ideal flows, and concluded that CFD models are the most complete because they allow us to describe space-time evolution of physical and biological phenomena. Therefore, CFD techniques have been employed as a useful tool for understanding hydrodynamics and biochemical reactions in wastewater treatment field [2–6], where the bioreaction behavior is associated to the liquid phase dynamics. Biofilms mathematical modeling has been well-established by IWA Task Group on Biofilm Modeling [7], describing the basic features to model biofilms, developing numerous mathematical models in different dimensions and defining a set of benchmark problems to evaluate model responsiveness under established scenarios [8]. Specifically, the Benchmark problem 2 (BM2) is focused on estimating hydrodynamics influence over mass transfer in heterogeneous biofilms, considering diffusion and advection mass transport in the fluid phase and concluding that 2D and 3D models should be applied when a detailed resolution inside the biofilm is required. Moreover, other works [9,10] have demonstrated the suitability of 3D CFD models to study biofouling phenomenon coupled with hydrodynamics variables in membrane reactors.

Regarding to gas-phase biofiltration, most of published models consider that the liquid movement through a porous bed occurs by an ideal plug flow pattern [11–15], since axial dispersion effect is generally neglected for the conditions on which such bioreactors are operated [16]. Nonetheless, residence time distribution techniques (RTD) have been used for characterizing the hydrodynamics in

biotrickling filters [17,18], measuring the liquid dispersion of circulating fluids in packed beds. These experimental analysis allow detecting axial dispersion, due to the formation of some preferential flow channels or stagnant regions as a result of packing structure and biofilm growth. Hence, mathematical models based on generally accepted assumptions seem not suitable for developing rigorous dynamic models, since hydrodynamics might critically influence the biofilm dynamics of the biofiltration system.

Procedures adopted for validating these bioreactor models are commonly based on experimental data measured in the liquid phase, despite the biological activity is mainly located at the biofilm. Over the last few decades, microelectrodes have demonstrated a notable potential in characterizing biofilms, allowing for spatially measurement of species, such as dissolved oxygen (DO) at the liquid phase and at the bulk liquid/biofilm interface, as well as in the biofilm [19]. Determination and modeling of oxygen profiles in biofilm reactors at laboratory scale have been previously addressed [20–22], showing that the concentration profiles in the biofilm are considerably influenced by boundary layers and flow characteristics of the liquid phase. Accurate experimental measurements and hydrodynamics description coupled with the biological reactions should be considered to develop rigorous models [23]. In contrast, models present in the literature neither take into account the simulation of the oxygen gradients at the liquid phase nor describes in detail boundary layer profiles.

According to the stated above, the aim of this work was to contribute to the existing knowledge of how considering fluid flow dynamics can aid to develop more rigorous models for biofiltration processes. In particular, in this research, a bioreactor model is developed employing CFD techniques and their performance is validated with experimental measurements inside the biofilm recorded using DO microsensors. A fixed-bed biofilm reactor is used to characterize the biodegradation phenomena inside an aerobic heterotrophic biofilm, reproducing at laboratory-scale the operating conditions of an industrial biotrickling filter, and studying in detail the relevant phenomena in both liquid and biofilm phases. Additionally, to confer a coherent identity model, the CFD simulation results are compared qualitative and critically with another results obtained from a well-established modeling tool, such as AQUASIM, and the results from using traditional assumptions to model biological systems. The developed 3D CFD model is used to study the influence of hydrodynamics over mass transport and to predict species presence anywhere in the bioreactor.

2. EXPERIMENTAL METHODS

2.1. Experimental setup

Experimental measurements were conducted through an aerobic heterotrophic biofilm grown on a flat plate bioreactor (FPB), manufactured in accordance with [24]. The bioreactor was made in methacrylate (PMMA). The flat plate, the surface for the biofilm development, was 18 cm long, 3.5 cm wide and 0.3 cm tall. The reactor included two reservoirs, located at the inlet and the outlet of the flat plate, which were 2 cm long, 3.5 cm wide and 2.8 cm tall. The FPB was inoculated and operated as described in [25]. During DO profiles recording, operating conditions were set up reproducing those typical of a conventional biotrickling filter (liquid phase velocity and residence time were adjusted to approximately 1 m h^{-1} and 12 hours respectively by using a peristaltic pump to recirculate the phase through the FPB).

In the FPB, DO profiles were recorded at different regions along bioreactor. Specifically, P1 and P2 profiles were measured in zones close to the inlet (at 6 and 9 cm from the beginning of the flat plate respectively), while P3 and P4 profiles were recorded in regions near the outlet (at 15 and 18 cm from the beginning of the flat plate respectively). Experimental data were recorded in less than one hour, in order to avoid the biofilm dynamic effect on the profile. These profiles were recorded using a commercial Clark-type microsensor (OX-25, Unisense, Denmark). The electrodes were connected to a 4-channel amplifier (Microsensor Multimeter, Unisense, Denmark) and polarized at -0.80V (vs Ag/AgCl). Data acquisition was performed using specific data acquisition software (Sensor Trace Basic, Unisense, Denmark). Linear two-point calibrations were performed in the measurement medium solution. Oxygen saturation conditions, taking into account salinity and temperature, were achieved aerating solutions with standard air. Glucose, fed in excess to avoid biomass growth limitation, was used as the sole carbon source. In addition, anaerobic conditions were obtained adding Na_2SO_3 to the solution. The sensor positioning within the biofilm was possible through the use of a three-dimensional micromanipulator (MM33-2, Unisense, Denmark), with a precision in z-axis of 10 µm, and in x-/y-axis of 100 µm. Biofilm density was monitored along the flat plate using protein analysis [26]. These measurements were related to the volatile suspended solid (VSS) concentration according to the correlation described in [27].

2.2. Biofilm characterization

Recorded DO profiles, obtained at different positions along the flat plate and recorded during both endogenous and substrate consumption conditions, were used to estimate the microbial consortium kinetic parameters. In this way, the evaluation of the recorded microsensor profiles was performed using the methodology proposed by Wäsche et al. [28], obtaining the boundary layer thickness and the dissolved oxygen profiles inside the biofilm, thus locating liquid and biofilm interfaces.

Moreover, the effective diffusivity was estimated using the correlation described in [27], in which the diffusion coefficient of n-compound in biofilm phase (D_Bn) is quantified as function of biomass density and Reynolds number (Expression 1).

$$D_r = D_B n \cdot D_L n^{-1} = 0.93 - 0.023 \cdot X + 1.17 \cdot 10^{-2} \cdot \mathrm{Re}^2 + 1.1 \cdot 10^{-4} \cdot X^2 \quad (1)$$

where D_r is the relative diffusivity, D_Ln is the diffusion coefficient of n-compound in liquid phase in (m^2 s^{-1}), X is biomass density (g L^{-1}) and Re is Reynolds number (-).

Finally, the parameters of the biological kinetics expressions were fitted from recorded DO oxygen profiles following the procedure defined by [29], where a dynamic model is developed to estimate oxygen evolution using one-dimensional diffusion-reaction equation.

2.3. RTD experimental procedure

The residence time distribution (RTD) was obtained in order to validate models hydraulic behavior, performing the experimental tracer technique in the FPB. The measuring control point was located at the outlet of the bioreactor, taking samples at a 60 seconds frequency. Methylene blue was used as a dye tracer following a pulse as the injection method. After a sampling period of 3000 seconds, tracer

sorption studies were carried out processing the samples analytically with an ultraviolet/visible spectrometer (UV/Vis Lambda 25, Perkin-Elmer, United States).

To perform the RTD analysis, the concentration-time (C(t)) and the residence time distribution (E(t)) curves (Expressions 2 and 3 respectively) were obtained, linking mass and volumetric flow rate of the tracer along the experiment. Finally, the mean residence time (t_m) was calculated according to the Expression 4 and non-ideal hydrodynamic model was proposed.

$$\frac{M}{q} = \int_0^\infty C(t)\ dt \qquad (2) \qquad E(t) = \frac{C(t)}{\int_0^\infty C(t)\ dt} \qquad (3) \qquad t_m = \int_0^\infty E(t) \cdot t\ dt \qquad (4)$$

where M is the tracer mass (kg), q is the volumetric flow rate of the tracer ($m^3\ s^{-1}$), C(t) is the concentration-time function of the tracer, t_m is the mean residence time (s), E(t) is the residence time distribution function of the tracer and t is the time (s).

3. MODELS DEVELOPMENT

3.1 General assumptions

The developed models were built coupling the description of the physical transport phenomena and the biological processes occurring along this type of biofilm reactors, considering the most relevant phenomena such as advection, diffusion and biodegradation [30]. Two phases were considered: bulk solution and biofilm. The liquid phase (composed of water, mineral salts, glucose and dissolved oxygen) was circulated from the inlet to the outlet of bioreactor, passing over the biofilm, which remains immobile inside de bioreactor. Oxygen and glucose were transferred from the liquid phase to the biofilm phase, where the aerobic glucose biodegradation takes place.

The general assumptions of the model, based on consolidated models reported in literature [8,12,15], are: (1) The biofilm is considered as a flat surface with a constant thickness (BT), according to bioreactor design. (2) The biofilm is covered by a liquid film of constant thickness. (3) Biomass is considered heterogeneous, since it has been observed in previous studies for this type of bioreactors [25]. Therefore, a biomass density gradient has been defined along the biofilm. (4) Degradation within the biofilm is described by the Monod equation (for more details see section 3.2). (5) The biofilm system is at pseudo-steady state. (6) Liquid phase circulation regime in the flat plate is modeled as plug flow pattern. (7) No reaction is considered in the liquid phase (i.e. there is a negligible amount of biomass in the liquid phase). (8) The concentration of dissolved oxygen in the liquid is kept constant at saturation concentration and glucose is supplied in excess. (9) Mass transport from the liquid phase to the biofilm and throughout the biofilm occurs by diffusion, following Fick's law. The diffusion coefficients for the substrate and dissolved oxygen in the biofilm were calculated by Expression 1. In the liquid phase, the diffusion coefficients are equal to those in pure water (Table 1). (10) Mass-transfer resistance at the interface between the biofilm and the bulk fluid is considered.

3.2 Biological kinetics expressions

Aerobic heterotrophic bacteria use dissolved oxygen (DO) and glucose (S) as electron acceptor and carbon source respectively. Oxygen and glucose consumption are related to microbial growth

limited by oxygen, also considering an endogenous term, and glucose in excess (Expressions 5 and 6). This last assumption was verified experimentally, finding glucose concentration through the bioreactor far superior to half-saturation coefficient for glucose ($K_{S,G}$).

$$R_B DO(i,j)\Big|_{\substack{i=1\\j=1}}^{\substack{i=n_L\\j=n_B}} = q_{\max,DO} \cdot \frac{C_B DO(i,j)}{K_{S,DO} + C_B DO(i,j)} \cdot X(i,j) + k_d \cdot X(i,j) \quad (5)$$

$$R_B S(i,j)\Big|_{\substack{i=1\\j=1}}^{\substack{i=n_L\\j=n_B}} = \frac{1}{Y_{DO/S}} \cdot q_{\max,DO} \cdot \frac{C_B DO(i,j)}{K_{S,DO} + C_B DO(i,j)} \cdot X(i,j) \quad (6)$$

where X is the biomass concentration in kg m^{-3}; $q_{max,DO}$ is the maximum specific growth rate in kg DO kg VSS^{-1} s^{-1}; C_BDO is the concentration of oxygen in the biofilm phase in kg DO m^{-3}; $K_{S,DO}$ is the Monod half-saturation coefficient for oxygen in kg DO m^{-3}; k_d is the maintenance factor for oxygen in kg DO kg VSS^{-1} s^{-1}; and $Y_{DO/S}$ is the oxygen-glucose yield in kg DO kg S^{-1}.

Values of kinetic and stoichiometric parameters are summarized in the subsection 4.1.

3.3 Description of the models

Three different models were developed and tested in order to evaluate the effect of considering hydrodynamics when the bioreactor model is defined. The models were used to evaluate pseudo steady-state glucose degradation and oxygen consumption within the biofilm at different positions in the flat plate bioreactor. All modeling approaches consider several mechanisms occurring in the bioreactor, schematized in Figure 1, such as mass transport by advective flow in the liquid phase, mass transfer by diffusion at the liquid-biofilm interface, internal diffusion in the biofilm phase and biological reaction in the biofilm.

Regarding to the computing solution methodologies, two of the models were solved as nodal models defining mass balances equations in the phases, and the last one was worked out using CFD techniques. The first nodal model is defined as a traditional diffusion-reaction model, discretizing the set of partial differential equations in space along the bioreactor height and biofilm thickness and solving them using MATLAB. The second nodal model was implemented using a well-established tool as AQUASIM simulation package. In the last model, which is developed using a CFD software, the biological reactions are introduced into the CFD code, coupling them with system hydrodynamics. The main parameters characterizing bioreactor performance are shown in Table 1. A mesh sensitivity analysis was performed to find an optimal discretization of the bioreactor models, using dissolved oxygen variable as optimization criterion. As a result, a minimum of eight mesh points were used for discretizing the reactor length, while thirty nodes more to discretize the biofilm thickness (for more details see subsection 4.2).

Table 1. Main parameters for the bioreactor models formulation.

Parameter	Symbol	Value	Units	Determination method /Reference
Volumetric flow rate in the inlet bioreactor	Q	$4.37 \cdot 10^{-8}$	$m^3\ s^{-1}$	Operating conditions
Liquid phase volume in the flat plate	V_L	$4.46 \cdot 10^{-6}$	m^3	Bioreactor design
Interfacial area liquid-biofilm	A_I	$5.94 \cdot 10^{-3}$	m^2	Bioreactor design
Boundary layer thickness	BLT	0.0002	m	Indirectly determined from

				experimental data [28]
Biofilm thickness	BT	0.003	m	Bioreactor design
Substrate influent concentration	C_LG0	13	kg m^{-3}	Operating conditions
Oxygen influent concentration	C_LO0	0.00685	kg m^{-3}	Operating conditions
Number of discretizations in liquid phase	n_L	8	-	Determined by sensitivity study to n_L
Number of discretizations in biofilm phase	n_B	30	-	Determined by sensitivity study to n_B
Glucose diffusivity in liquid phase	D_{LG}	$6.73 \cdot 10^{-10}$	m^2 s^{-1}	[31]
O2 diffusivity in liquid phase	D_{LO}	$1.88 \cdot 10^{-9}$	m^2 s^{-1}	[32]
Glucose diffusivity in biofilm phase	D_{BG}	Expression 1	m^2 s^{-1}	[27]
O2 diffusivity in biofilm phase	D_{BO}	Expression 1	m^2 s^{-1}	[27]

3.3.1. Conventional Diffusion-Reaction modeling approach

The theoretical model describing the aerobic glucose biodegradation in the bioreactor was based on mass balances in the liquid and within biofilm phases. Model equations were established according to the mechanisms and assumptions mentioned above. Mass transport throughout the liquid phase was modeled as a sequence of single continuous stirred tank reactors (CSTR's) in order to simulate a plug flow. As can be seen in Figure 1, horizontal layers (n_L) were defined from bioreactor inlet to the outlet. Similarly, the biofilm layers were also divided in several layers (n_B) starting from the biofilm surface to the biofilm layer in contact with the substratum. Then, the set of partial differential equations was discretized in space along the bioreactor length and biofilm thickness. The resulting set of simultaneous ordinary differential equations was solved in MATLAB® Release 2015a, using a variable order method based on the numerical differentiation formulas (NDFs) to solve stiff differential equations.

Mass balances in liquid and biofilm phases are described in the following subsections.

3.3.1.1. Mass balance for the liquid phase.

Components considered in the liquid phase were oxygen and glucose. In this phase, the dynamic mass balance for a compound *n*, C_Ln, in a segment *i* was expressed by two expressions: Expression 7 is for the first segment (i=1), which bear boundary constraints, and Expression 8 is from the second segment (i=2) to the last bioreactor segment (i= n_L).

$$\left.\frac{dC_Ln(i)}{dt}\right|_{i=1}^{i=1} = \frac{Q}{\left(V_L / n_L\right)} \cdot \left(C_Ln0 - C_Ln(i)\right) - \frac{D_{Ln} \cdot A_I}{BTL \cdot V_L} \cdot \left(C_Ln(i) - C_Bn(i,1)\right) \quad (7)$$

$$\left.\frac{dC_Ln(i)}{dt}\right|_{i=2}^{i=n_L} = \frac{Q}{\left(V_L / n_L\right)} \cdot \left(C_Ln(i-1) - C_Ln(i)\right) - \frac{D_{Ln} \cdot A_I}{BTL \cdot V_L} \cdot \left(C_Ln(i) - C_Bn(i,1)\right) \quad (8)$$

where V_L is the liquid phase volume in m^3; n_L is the number of discretizations in the liquid phase; C_Ln is the concentration of compound n in the liquid phase in kg m^{-3}; Q is the volumetric flow rate in the inlet bioreactor in m^3 s^{-1}; C_Ln0 is the concentration of compound n in the inlet liquid phase in kg m^{-3}; D_{Ln} is the diffusion coefficient of n-compound in the liquid phase in m^2 s^{-1}; A_I is the available surface

area between the entire biofilm and the liquid phase in m^2; BLT is the boundary layer thickness in m; $C_Bn(i,1)$ is the concentration of n-compound in the first layer of biofilm in kg m^{-3}.

3.3.1.2. Mass balance for the biofilm.

In the biofilm phase, the same compounds as in the liquid phase were considered (oxygen and glucose). The dynamic mass balance for a compound *n*, C_Bn, in a segment *i* of the liquid phase and in the *j* layer of the biofilm depth is expressed by Expression 9, except for the first layer near the interface (j=1) and the last before the substratum (j=n_B), which bear boundary constraints (Expressions 10 and 11 respectively).

$$\left.\frac{dC_Bn(i,j)}{dt}\right|_{\substack{i=1\\ j=2}}^{\substack{i=n_L\\ j=n_B-1}} = \frac{D_{Bn}}{\left(BT/n_B\right)^2}\cdot\left(C_Bn(i,j-1)-2\cdot C_Bn(i,j)+C_Bn(i,j+1)\right)-R_Bn(i,j) \quad (9)$$

$$\left.\frac{dC_Bn(i,j)}{dt}\right|_{\substack{i=1\\ j=1}}^{\substack{i=n_L\\ j=1}} = \frac{D_{Ln}}{BTL\cdot\left(BT/n_B\right)}\cdot\left(C_Ln(i)-C_Bn(i,j)\right)-\frac{D_{Bn}}{\left(BT/n_B\right)^2}\cdot\left(C_Bn(i,j)-C_Bn(i,j+1)\right)-R_Bn(i,j) \quad (10)$$

$$\left.\frac{dC_Bn(i,j)}{dt}\right|_{\substack{i=1\\ j=n_B}}^{\substack{i=n_L\\ j=n_B}} = \frac{D_{Bn}}{\left(BT/n_B\right)^2}\cdot\left(C_Bn(i,j-1)-C_Bn(i,j)\right)-R_Bn(i,j) \quad (11)$$

where C_Bn is the concentration of n-compound in the biofilm phase in kg m^{-3}; D_Ln is the diffusion coefficient of n compound in liquid phase in m^2 s^{-1}; BTL is the boundary layer thickness in m; C_Ln is the concentration of n compound in the liquid phase in kg m^{-3}; D_{Bn} is the diffusion coefficient of n-compound in biofilm phase in m^2 s^{-1}; BT is the biofilm thickness in m; n_B is the number of discretizations in the biofilm phase; R_Bn is the biological reaction rate of n-compound in kg m^{-3} s^{-1} (see Expressions 5 and 6 for more details).

3.3.2. AQUASIM modeling

The heterotrophic biofilm model was simulated using AQUASIM 2.1 [33]. Pseudo 2D and 3D bioreactors modeling can be solved using this package. The mathematical model was established in accordance with the assumption stated above, and considering that oxygen and glucose diffuse from the liquid phase to the biofilm phase, where the biological reaction occurs. The model was developed using a set of biofilm reactor compartments (n_L), where in each of them mass transport in the biofilm occurs in the direction perpendicular to the substratum and the biofilm evolution is calculated independently in each compartment. These compartments are combined through advective links between the bulk fluid zones, so that mass transfer occurs in the flow direction [8].

Biofilm reactor compartments were configured following the guidelines suggested in [34]. The reactor type was chosen as unconfined to model free-surface flow over the biofilm and the pore volume contains only liquid phase and dissolved substances. Oxygen and glucose were introduced as dissolved variables. The biofilm matrix was assumed to be rigid and the particulate variable of the system, biomass (X), was specified. Moreover, the bioreactor parameters were introduced in variables section and Expressions 5 and 6 were implemented as processes. In addition, the number of grid points was defined as n_B + 2, since AQUASIM keeps 2 boundary points [35]. The properties of

particulate and dissolved variables were defined and initial conditions and influent value were also provided.

3.3.3. CFD modeling

The commercial CFD software ANSYS® Academic Research, Release 16.2, was used to solve the equations of continuity and momentum. This is a finite-volume solver, using body-fitted grids. ANSYS CFX uses a co-located (non-staggered) grid layout and all variables are evaluated at the cell centers. An improved version of the Rhie–Chow algorithm is used to calculate the velocity at the cell faces. The pressure–velocity coupling is obtained using a version of the SIMPLEC algorithm. The code uses a high order advection scheme, with a numerical advection correction term. ANSYS-CFX uses a Multigrid (MG) accelerated Incomplete Lower Upper (ILU) factorization technique for solving the discrete system of linearized equations.

A single-phase model is used to characterize the bioreactor performance, following the general assumption previously discussed. The bioreactor is defined by a single domain, and the region of the biological system is introduced as a subdomain in the lower part of the flat plate. The boundary conditions of inlet, outlet and free slip are defined in the respective areas of the bioreactor (figure 1A), and the remaining surfaces are bounded as walls. A hydraulic pressure loss model is introduced in the momentum equation in order to model physical characteristics of biomass.

The implementation of biological reactions in the CFD software is performed using the methodology described by [6], derived from bioreactors modeling in wastewater treatment field [5,36]. Variables on kinetic expressions were defined as additional variables (oxygen, glucose and biomass) in the computational space, including an extra transport equation for each additional variable:

$$\frac{\partial(\rho\varphi)}{\partial t} + \nabla \cdot \left(\rho\, U\, \varphi\right) = \nabla \cdot \left(\rho\, D\, \nabla\varphi\right) + S_{\varphi} \qquad (12)$$

where U is the fluid velocity in m s^{-1}, ρ is the mixture density in kg m^{-3}, C_n is the concentration of the n variable in kg m^{-3} (C_Ln in liquid phase and C_Bn in biofilm phase), $\varphi = C_n/\rho$ is the conserved quantity of n variable per unit mass of fluid, S_φ is a volumetric source term in kg m^{-3} s^{-1} and D is the kinematic diffusivity for the n variable in m^2 s^{-1} (D_Ln in liquid phase and D_Bn in biofilm phase). The left-hand side terms include the temporal and the convective, and in the right-hand side appears the diffusion and the term for the sources. All terms in Expression 12 are considered for oxygen and glucose, whereas the diffusive term was neglected for the biomass additional variable. In addition, the expressions for the biodegradation kinetics (Expressions 5 and 6) were included as source terms.

During the meshing process, the guidelines detailed in *CFX Best Practices Guide for Numerical Accuracy* [37] were took into account, testing mesh dependence and discretization schemes. Then, a sensitivity analysis of the mesh confirmed the null impact of the mesh size element on the simulation results (for more details see subsection 4.2).

Finally, simulations were calculated either in transient and steady state. In both states, laminar flow regime was defined since operating conditions were characterized by low Reynolds numbers, and a time step of 0.1 seconds was defined, fulfilling the condition of the Courant number. Moreover, in order

to reduce computing time, the simulation results were obtained after following two steps [6]. First, a converged steady state solution for the fluid was calculated. Secondly, the resulting hydrodynamic variables were kept constant for the transient simulation, and only the transport equations for the additional variables were solved, since biochemical reactions do not affect on the hydrodynamics calculation. Convergence was assumed when the maximum normalized root mean square in each cell for all the equations (momentum, mass and additional variables) reached a value less than $1 \cdot 10^{-4}$ and additional variables kept in a constant value.

4. RESULTS AND DISCUSSION

4.1. Microprofiles experimental measurements and biokinetics estimation

DO microsensors measurements were performed in order to characterize the biological activity within the biofilm under both endogenous and substrate consumption conditions and to study biofilm heterogeneity, as it has been reported in previous works [25]. Twenty-four oxygen concentration profiles (twelve during endogenous conditions and twelve substrate consumption conditions) were measured at six different regions along the flat plate bioreactor (FPB). Four representative profiles during substrate operating conditions (P1, P2, P3 and P4) are depicted in Figure 2.

The characterization of these profiles, using the methodology proposed by [28], allowed to distinguish three phases (see Figure 1): the liquid phase, in which DO concentration can be assumed to be constant; the boundary layer (liquid-biofilm interface), in which DO mass transfer from liquid to biofilm occurs; and the biofilm, in which DO is diffused and consumed by the microbial activity.

Since the interface between liquid boundary layer and biofilm is located at the inflection point in which the profile changes the curvature from convex to concave, the profiles are presented positioning the inflection point as the zero of axis coordinate. Then, positive values in Figure 2 are measurements in the bulk and the concentration boundary layer, while the negative ones correspond to measurements inside the biofilm. As can be seen, the profiles showed the characteristic shape for substrate profiles diffusing into a matrix where it is consumed [24]. In general, the experimental data registered along the bioreactor (from the inlet P1 to the outlet P4) exhibit a tendency to increase the oxygen profile slope, indicating higher oxygen consumption rate, since biomass concentration increases along the reactor (Table 2). In all cases, oxygen was completely exhausted before reaching the substratum, being oxygen penetration depths in the biofilm between 500–1500 µm. Besides, boundary layer thickness ranged from approximately 100–200 µm, which is close to the values measured in other biofilm systems [28].

In addition, to determine biokinetic parameters of Expressions 5 and 6, biofilm density was measured using protein analysis at bioreactor regions where profiles were recorded, obtaining a biomass density gradient as is shown in Table 2. Oxygen-glucose yield was calculated from the oxidation reaction of glucose, obtaining a value of 1.07 kg DO kg S^{-1}. Moreover, the effective diffusivities within the biofilm were estimated by Expression 1. Consequently, using one-dimensional diffusion-reaction equations [29], different sets of the biological kinetics parameters (the maximum specific oxygen uptake rate (q_{max}), the Monod half saturation coefficient of oxygen ($K_{S,O}$), and the

maintenance-decay coefficient for oxygen (k_d)) were fitted from the DO profiles, reflecting the heterogeneity of the community along bioreactor. Values of these parameters are summarized in Table 2.

Table 2. Kinetic and stoichiometric parameters for bioreactor model.

Location	X (kg VSS m^{-3})	k_d (kg DO kg VSS^{-1} s^{-1})	$K_{S,O}$ (kg DO m^{-3})	q_{max} (kg DO kg VSS^{-1} s^{-1})
P1	9,30	$1.60 \cdot 10^{-7}$	$2.46 \cdot 10^{-4}$	$1.27 \cdot 10^{-6}$
P2	13,25	$1.72 \cdot 10^{-7}$	$2.32 \cdot 10^{-4}$	$4.47 \cdot 10^{-7}$
P3	18,20	$5.66 \cdot 10^{-7}$	$1.42 \cdot 10^{-4}$	$7.01 \cdot 10^{-7}$
P4	25,60	$3.02 \cdot 10^{-7}$	$3.02 \cdot 10^{-4}$	$4.82 \cdot 10^{-6}$

The maintenance coefficient for oxygen and growth parameters varied in a considerable range, which is consequence of the differences observed in profiles of Figure 2. Despite these variations, fitted parameters were in the ranges that other authors have reported for biokinetic parameters of immobilized biofilm [25,29].

4.2. Mesh sensitivity analysis

Since the procedures to solve the developed models are either finite difference or finite volume methods, it is necessary to select the number of grid points used for discretizing the bioreactor length (n_L) and the biofilm thickness (n_B). The number of discretizations is an essential parameter which influences in the resolution of the numerical calculation and, additionally, directly affects on the accurate reproduction of the experimentally observed gradients within and outside the biofilm.

A wide range of values for mesh grid points can be found in the literature of biofiltration modeling, from lower values below 10 [38–40] to larger ones over 50 [14,41,42], without indicating a standard criterion to select this number of divisions. In addition, a systematic evaluation of biofilm reactor models [43] showed that the number of discretizations in the boundary layer and the biofilm are highly sensitive parameters. These authors examined the impact of the discretization points on predicted values and recommended to choose a number of layers appropriate to represent the substrate and biomass gradients.

Therefore, a mesh sensitivity analysis was performed to check the accuracy of the computational mesh size used in the current simulations. As shown in Figure 2, the biological activity varies with biomass density along the bioreactor, being essential to examine the suitability of the mesh size in order to determine whether it can be used for capturing in detail dissolved oxygen transport and consumption inside and outside the biofilm.

Predicted values from three different scenarios are compared in Figure 3. Then, three different values of mesh element size are considered. The fine mesh (element size of 0.05 mm, n_B=60), the medium mesh (element size of 0.1 mm, n_B=30) and the coarse mesh (element size of 0.2 mm, n_B=15). All the three meshes presented a well behaved convergence. The sensitivity of n_B with respect dissolved oxygen variable is depicted in Figure 3A for two profiles with different depths. As can be seen, the oxygen profiles reproduce adequately the expected behavior and follow the same trend for

fine and medium meshes, but they have significantly change for the coarse mesh. In order to compare the fine mesh with the medium and coarse meshes, the normalized root mean square errors (NRMSE) [44] was calculated, obtaining deviations for P1 of 0.17% and 1.45% respectively; and for P3 are 0.29% and 1.21%. Therefore, the improvements between fine and medium meshes do not justify the increase in computational time. Hence, the medium mesh with n_B=30 discretizations points (for CFD mesh an element size of 0.1 mm) was used as the base case for the simulations presented herein.

A sensitivity analysis of n_L was performed to know the effect of the biomass density gradient considered along the bioreactor. The results from the above simulations allowed to study the variation of oxygen concentration along the reactor at the interface level, as depicted in Figure 3B. These results showed a minimum of 8 discretizations needed in order to reproduce correctly the DO gradients along the bioreactor. In addition, mesh quality parameters (orthogonal quality and skewness) had been checked in the defined mesh for CFD simulations with an element size of 0.1 mm. Orthogonal quality average of 0.99023 and skewness average of 0.020903 were obtained in the active section of the bioreactor, which were in the range of recommended values by ANSYS CFX (0.95-1 and 0-0.25 respectively).

4.3. Comparison and validation of the models performance

Three different modeling tools (AQUASIM, MATLAB and CFD software (ANSYS CFX)) were used for modeling the bioreactor performance and compare the results. A first set of simulations was carried out using 2D steady-state models, which were implemented as indicated in subsection 3.3. The results of the simulations performed according to the operating conditions specified in Table 1 are presented in Figure 4, in order to compare models performance and to validate the implementation of biological expressions into the CFD code. In this sense, results of simulating the bioreactor performance in AQUASIM, MATLAB and ANSYS CFX were compared with the corresponding experimental DO profiles (obtained with microelectrodes), along the bioreactor for the different biofilm densities (A) 9.3 g VSS $L^{-1;}$ B) 13.5 g VSS L^{-1}; C) 18.2 g VSS L^{-1}; and D) 25.6 g VSS L^{-1}).

Qualitatively, AQUASIM, MATLAB and CFD simulated profiles present the same behavior and the same trend throughout the biofilm, reaching the anaerobic limit at similar depths for all the studied biofilm densities. Therefore, fitting the kinetic constants and their implementation into CFD software can be stated as successful. However, the characterization of the liquid phase using these three techniques shows marked differences. MATLAB and AQUASIM models do not explicitly simulate the external concentration gradients to the biofilm surface, modeling the reduction in concentration as a mass-transfer resistance in the boundary layer. Consequently, the concentration value remains constant in the liquid phase. In the CFD model, the transport of oxygen in the liquid phase is defined with numerical hydrodynamic models so, more accurate results are obtained with this technique.

For a more quantitative comparison with the experimental results, the normalized root mean square errors (NRMSE) [44] between the experimental DO profiles and the DO simulated profiles for the different biofilm densities were determined (Table 3). The deviations obtained using any of the three techniques for modeling the bioreactor are below 6%, reproducing with great accuracy the biological behavior. Although the deviations obtained with nodal models are the largest, AQUASIM program

described with exactitude the mean value of the oxygen concentration in the liquid phase. MATLAB model also simulated biofilm profiles with lower deviations, but the profile along the liquid phase is steeper, reducing excessively the concentration in the zones near the bioreactor outlet. CFD results obtained the lowest deviations, reproducing the DO values at the liquid-biofilm interface with high accuracy, despite they require more computing time. Hence, comparing AQUASIM and MATLAB simulated profiles with the CFD ones, in which a detailed simulation of mass transport phenomena in the liquid has been considered, the fact of including hydrodynamics models improves the simulations results, reducing the NRMSE around 2% for the studied conditions. It should be noted that P1 profile has the minor deviations in all simulated results, since the convective term affects in a lesser extent in that region. Therefore, CFD model describes more rigorously the convective term than nodal models.

Table 3. Normalized root mean square errors (NRMSE) between the experimental DO profiles and the DO simulated profiles.

	P1 (9.3 g VSS L^{-1})	P2 (13.5 g VSS L^{-1})	P3 (18.2 g VSS L^{-1})	P4 (25.6 g VSS L^{-1})
AQUASIM	3.04%	5.36%	4.96%	4.48%
MATLAB	3.47%	4.35%	4.48%	5.13%
CFD	2.35%	3.47%	2.65%	3.34%

Depending on the purpose for which the bioreactor model is developed, the results obtained with the three techniques will have advantages and disadvantages. In brief, when a bioreactor model is developed, it should be taken into account that AQUASIM is easy and quick to configure, MATLAB can have a tedious implementation (all equations model need to be programmed), but it allows to define 'tailored' used-defined models, and CFD allows to couple the biological equations with hydrodynamic models easily, but it introduces computational intensity and increases simulation time. Even so, it should be pointed out that the use of CFD techniques allows a much more realistic description of hydrodynamic effects over the transport of substrates in the bulk phases, which directly affects the model accuracy on the mass transfer at the boundary layer [45].

As shown in Figure 4, oxygen and concentration gradients external to the biofilm surface can be simulated using the developed CFD model, also reproducing the behavior in the boundary layer. Therefore, the developed CFD model can be used to determine the effective thickness of biofilm and boundary layer and to differentiate between aerobic and anaerobic zones in the bioreactor. Nevertheless, resulted prediction should be improved at the boundary layer in the cases near the outlet (Figures 4C and 4D). The reason for having less accurately fitting in these areas is because the thicker and denser biofilm sections were found at the bioreactor outlet, as it was observed experimentally in these cases and in a previous work [20]. These phenomena cause a reduction of the liquid layer thickness in these zones, thus raising the liquid velocity with the consequent increase in the slope of the measured oxygen profile. Therefore, these phenomena should be considered to improve the present model. In spite of that, the developed CFD model allows to adequately simulate the DO concentration trend throughout the boundary layer, being a novel tool to study in detail mass transfer phenomena in this type of bioreactors. Most notably, the use of CFD techniques to model biofiltration systems provides a scenario where hydrodynamics and biological reactions are coupled

and, thus, the effect of velocity profiles over mass transport and biological activity can be rigorously described.

4.4. CFD simulation results in transient conditions

A set of CFD simulations was carried out, describing the bioreactor performance using 3D model in transient conditions. From the results, the fluid flow characteristics, mass transport mechanism and biodegradation phenomena along the bioreactor in unsteady state were analyzed.

The hydrodynamics characterization of the bioreactor was described from streamlines, velocity profiles and RTD analysis. Streamlines and velocity profiles along the bioreactor are shown in Figure 5. The streamlines (Figure 5A) show that the liquid phase was correctly distributed over the bioreactor, contacting the overall of the biomass (specified in grey color). Moreover, the velocity profiles (Figure 5B) identify bioreactor zones which support the highest and lowest velocities. The bioreactor zones which supported lower velocities were mainly in the biofilm section, thus the diffusive phenomena were predominant over the mass transport in these zones, as it was indicated by [24]. Finally, the velocity profiles in the liquid phase (represented by L1 line in the cross section of the bioreactor in Figure 5B) match satisfactory the velocity distribution of a laminar flow regime (Figure 5D).

In the RTD analysis, the concentration-time (C(t)) and the residence time distribution (E(t)) are obtained according to Expressions 2 and 3 respectively. Both experimental and simulated E(t) curves are represented in Figure 6. Then, a mean residence time (Expression 4) of 812 seconds is obtained for the complete bioreactor, being this value close to the theoretical with a deviation of 2.7%.

CFD hydrodynamic model was validated using RTD test. As can be seen in Figure 6, the designed CFD model reproduced correctly the experimental data obtained in the control point. RTD shows an exponential curve displaced in time. The initial time (240 seconds) was related to plug-flow behavior in the flat plate region, while the two reservoirs (located at the inlet and the outlet of the flat plate) corresponded to the CSTR in series, contributing to obtain mixed flux. Maximum concentration was obtained at 420 seconds and its value was used to calculate the number of tank-in-series [45]. From this, it was possible to develop a hydrodynamics study on the bioreactor by means of a non-ideal hydraulic model, concluding that the bioreactor configuration is equivalent to a plug-flow reactor with a CSTR in series model.

An accurate global behavior of the bioreactor was reproduced, since the hydrodynamics in the reactor were precisely reproduced and validated. So, other variables arduous to measure in the experimental set-up, which are involved in bioreactor performance, can be analyzed from the results of this simulation. This opens the door to future optimal reactor design in terms of hydrodynamic behavior.

In figures 5B and 5C oxygen and velocity contours are both depicted in a transverse plane to the direction of the liquid phase. Also, figure 5D shows axial profiles of these variables in the line L1 of the transverse plane. In these figures, DO concentration profiles and velocity distributions describe the behavior of the studied biofilm with a uniform structure. The flow velocity in the liquid phase forms a profile, decreasing from the maximum value when is in contact with the gas phase (free-slip condition)

to zero at the surface biofilm (non-slip condition). It is noted that, as other studies have described [24], this velocity profile has a direct consequence in the formation of the boundary layer in the oxygen profile above the biofilm. At the interface, where velocity distribution reaches zero, the oxygen profile changes the curvature from convex to concave due to effect of combining microbial activity and diffusion within the biofilm.

In addition, in relation with mass transport mechanism inside and outside the biofilm, the velocity profiles and the oxygen gradients in direction X and Y are studied in detail (see Supplementary material, section SM-1 for further detail). Along the bioreactor it is observed that advection phenomena in the liquid phase are governing in the direction of the fluid flow (X direction) over the direction perpendicular to the flow (Y direction), being negligible the diffusivity effect. In contrast, as can be seen in Figure 7A and section SM-1 of Supplementary material, in the boundary layer diffusion is greatly notable in the Y direction and convection decreases its effect, but still it remains higher than the diffusion. Finally, the mass transport is purely diffusive within the biofilm as demonstrated in Figure 7A. These phenomena are also observed analyzing in detail the convection and diffusion along the P1, P2, P3 and P4 simulated profiles. The velocity profiles in X direction (see Supplementary material, section SM-2 for further detail) have the same behavior that the profile above presented in Figure 5D, being larger in the liquid phase, reducing their velocities through de boundary layer and reaching zero at biofilm interface. Moreover, the diffusive effect begins in the boundary layer as depicted in Figure 7B, where the oxygen gradient in Y direction is represented, increasing up to the maximum value within the biofilm, and decreasing through the active region of biofilm. Therefore, it can be assumed that a duality of transport mechanism (convection plus diffusion) is present in the liquid phase and the diffusive effect governs within the biofilm, as it is discussed in [24,27].

Finally, the CFD model allows us to characterize the behavior of the dissolved oxygen variable along the bioreactor length. As can be seen in a top view of the bioreactor (Figure 8A), CFD model reproduces consistently oxygen degradation at interface level, decreasing its concentration along the flat plate and showing the zone with lower concentration at the end of the flat plate. Moreover, in a frontal view of the bioreactor (Figure 8B), CFD model reproduces expected oxygen profile along the biofilm, showing deeper oxygen profiles near the bioreactor inlet, as it was found experimentally, and the anaerobic volume (specified in blue color) inside the biofilm region, which was also measured in the experimental set-up.

Therefore, this simulation technique allows us to determine nutrients and substrate limitations anywhere in the bioreactor geometry. This characteristic could be very useful to simulate complex geometries of reactors, e.g. packed beds, where the mass transfer can be affected by the packing material geometry and porosity, creating preferential channels or changing the liquid phase velocity. In order to model rigorously gas-liquid mass transfer, it should be considered a wide range of factors such as the pollutant physical–chemical properties, the medium properties, the internal reactor characteristics, as well as the operating conditions. All these factors can be coupled using CFD codes, defining the different flow regimes, the properties of fluid phases and the reactors geometry, although introducing computational expense. Hence, the introduction of a powerful tool, such as CFD, to model biofiltration systems offers a great potential for the detailed analysis of local gas-liquid mass transfer

behavior in bioreactors, besides allowing the optimization of advanced reactor operations. In addition, the developed 3D CFD model could be useful to predict species behavior and biofilm growth under several operating conditions at different phases and bioreactor zones.

5. CONCLUSIONS

To the best of our knowledge, this is the first study where a flat plate bioreactor with an immobilized biofilm has been completely modeled coupling the hydrodynamic and the biological phenomena. It is also the first time that the use of CFD techniques was satisfactorily validated comparing simulated DO profiles with DO profiles experimentally measured within the biofilm by means of microsensors. The use of different techniques for modeling bioreactors was evaluated and used to determine in which scenarios would be suitable the use of advanced modeling. Results indicated that the hydrodynamic behavior should be considered in the liquid phase for modeling accurately the performance of biofilm reactors, since this phase acts as a major medium to transfer oxygen and nutrients to the biofilm. Another important finding is that CFD techniques are more appropriate to satisfactorily model the performance of biological systems in a wide range of conditions, reproducing accurately measured experimental data along the bioreactor. These findings suggest that the application of CFD techniques for modeling bioreactors can help to develop more rigorous models, especially when mass transfer in the biofilm is limiting, allowing to underline the role of the hydrodynamic in the process. Present research provides a novel approach regarding the application of CFD tool to the simulation of biological reactions in fixed-bed biofilm reactors, obtaining successful results. With this novel tool, the spatial and temporal behavior of biological systems coupled with hydrodynamics effects could be analyzed in detail, being the key to optimize the performance of this type of bioreactors under a wide range of conditions.

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