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2D Model for Diffusion of Oxygen with Biochemical Reaction During Biofilm Formation Process in Static Aqueous Medium

[1]Y.T. Puyate and [2]A. Rim-Rukeh
[1]Department of Chemical Engineering, Rivers State University of Science and Technology,
Port Harcourt, P.M.B. 5080, Port-Harcourt, Nigeria
[2]Department of Integrated Science, College of Education, P.M.B. 2090, Agbor, Delta State, Nigeria

Abstract: A 2D model that describes diffusion of oxygen with biochemical reaction during biofilm formation process in static aqueous medium is presented. The analysis is based on X60 steel placed at the bottom of a container containing produced water inoculated with *Leptothrix discophora* (iron-oxidizing bacteria). These bacteria form biofilms on the exposed surfaces of the metal. The biofilm-microorganisms absorb oxygen from the produced water through biochemical reaction, resulting in transfer of oxygen from the bulk liquid phase to the biofilm. Predictions of the model are compared with experimental data and good agreement is obtained.

Key words: Biofilm, modeling, diffusion-reaction, oxygen, biocorrosion, microbes

INTRODUCTION

Biofilms are microbial assemblages attached to surfaces of materials such as stones in riverbeds, water and sewage pipelines, ship hulls and heat exchangers (Stickler, 1999; Stoodley *et al*., 1999; Bryers and Characklis, 1982; Costerton *et al*., 1995). Biofilms grow on surfaces in aquatic systems where nutrients are available to feed bacteria. Biofilms lead to many undesirable problems; for example, patchy biofilms could enhance corrosion by formation of differential aeration cells (Videla, 1996), destabilize corrosion inhibitor in any system (Characklis and Cooksey, 1983) and reduce the efficacy of biocides (Booth, 1971). However, application of biofilms in biotechnological processes such as wastewater treatment, formation of biobarriers in ground water treatment and bioremediation of contaminated plumes, have been reported in the literature (Chen *et al*., 1995; Chen and Kojouharov, 2000).

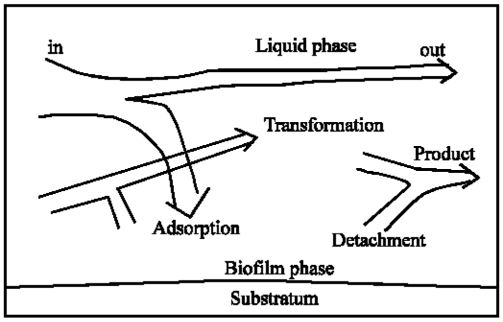

Fig. 1: A schematic representation of biofilm formation process (Characklis and Marshall, 1990)

Figure 1 shows all the processes involved during biofilm formation: (i) transport and adsorption of macromolecules at a surface to form a film called substratum; (ii) continuous transport of microbial cells to the substratum to form biofilm; (iii) growth, product and spore formation and death of cells within the biofilm and (iv) attachment/detachment of microbial cells at the biofilm-water interface. Generally, substrate and product species are transported by physical processes like molecular diffusion, convection and migration of ions. Microbial activity within biofilms can affect the kinetics of cathodic and/or anodic reactions (Jones and Amy, 2002). It is also well known that the metabolic activity of clusters of biofilm-microorganisms can change pH value to more than three units locally (Characklis and Cooksey, 1983). This means that directly at the metal-biofilm interface where the corrosion process is actually taking place, the pH value can differ significantly from that in the bulk liquid phase. Biofilm is a potential for biocorrosion and reliable information about biofilms is essential for better understanding of biocorrosion (Pritchard, 2002).

One way biofilms influence biocorrosion is through the introduction of oxygen gradient across the system and formation of patchy bacterial colonies (Videla, 1996). One direct effect of non-uniform oxygen distribution in a biofilm is the formation of differential aeration cells, where areas underneath respiring colonies are depleted of

Corresponding Author: Dr. Y.T. Puyate, Department of Chemical Engineering, Rivers State University of Science and Technology, Port Harcourt, P.M.B. 5080, Port Harcourt, Nigeria

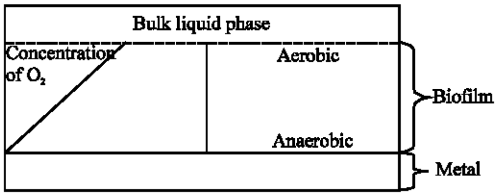

Fig. 2: Schematic representation of oxygen concentration gradient in a biofilm

oxygen relative to surrounding non-colonized areas. Thus, colony formation generates potential differences which give rise to corrosion currents. Under aerobic conditions, the areas underneath the colonies become anodic leading to metal dissolution. A schematic representation of oxygen concentration gradient in a biofilm is shown in Fig. 2 indicating that biofilm represents mainly an oxygen-consuming ecosystem.

Produced water (or formation water) is one that accompanies crude oil and gas from a producing well. It is an integral component of hydrocarbon recovery process and is usually produced during drilling and production phases of a well. Naturally, produced water contains various microorganisms which result in microbial corrosion of the inner surfaces of pipes and related systems conveying the water. Such microbial corrosion process takes place through formation of biofilms on the metal surfaces. The present analysis is based on the corrosion of X60 steel influenced by iron-oxidizing bacteria (*Leptothrix discophora*) as studied experimentally by Rim-Rukeh and Puyate (2007), where X60 steel is the material used in constructing most steel pipes carrying crude oil and produced water in the oil industry in Nigeria. Picioreanu and van Loosdrecht (2002) used Butler-Volmer kinetic equation to model the distribution of differential aeration cells during biocorrosion. While numerous mathematical models have been used as tools to simulate the structure of microbial biofilms (Costerton *et al.*, 1995; Rittman and McCarty, 1980; Wanner and Reichart, 1996), literature on the transport characteristics of oxygen during biofilm formation in biocorrosion is scarce. It is the purpose of this study to present a two-dimensional model that describes transport of oxygen with biochemical reaction during biofilm formation on the surfaces of X60 steel exposed to produced water inoculated with *Leptothrix discophora*. Such a model may be used to predict onset of biocorrosion and/or characterize oxygen transport in relation to microbial activity during biocorrosion. *Leptothrix discophora* is chosen as the microorganism that initiates the biofilm formation process because it can respire by using oxygen as its terminal electron acceptor.

UNSTEADY STATE 2D MODEL

The physical system consists of a cubical container of side 15 cm containing produced water (inoculated with *Leptothrix discophora*) to a depth of 10 cm. A metallic material (X60 steel coupon) 10 cm long, 10 cm wide and 0.5 cm thick is placed at the bottom of the container.

Figure 3 shows a schematic side view of the container with the produced water, the metal coupon at the bottom of the container and biofilm formed on the upper surface of the metal.

At the onset, planktonic cells of *Leptothrix discophora* are transported from the bulk liquid phase to the surfaces of the metal and embed themselves in slime Extracellular Polymeric Substances (EPS) to form layers called biofilms. The attached cells start to grow increasing in thickness with uneven surfaces using oxygen in the liquid phase as nutrient. The oxygen is transported from the bulk liquid phase to the biofilms in the x, y and z directions; that is, three-dimensional transport of oxygen. The biofilm formed on the upper surface of the metal is a result of transport of oxygen in the vertical y-direction, while the biofilms on the edges of the metal are due transport of oxygen in the x and z directions. The following simplifying assumptions are made in setting up the model equation that describes diffusion of oxygen with biochemical reaction during biofilm formation on the exposed surfaces of the metal in the system shown in Fig. 3: (i) there is no other supporting electrolyte in the system other than the produced water used in the study; (ii) a mono-species (*Leptothrix discophora)* and mono-substrate (dissolved oxygen) system; (iii) physical parameters (e.g., diffusion coefficients, temperature and pressure) are assumed constant; (iv) since transport of oxygen in the x and z directions are the same and the surface area of an edge of the metal is small compared to the upper surface area, the contribution of diffusion of oxygen in the x or z-direction to the entire transport process may not be significant. Hence, transport of dissolved oxygen from the bulk liquid phase to the metal surfaces is considered only in the x and y directions; diffusion of oxygen in the z-direction is neglected in the analysis.

The liquid in the container is stationary, so there is no convection of oxygen in the system. The general diffusion-reaction equation without convection that may be used to describe transport of oxygen from the bulk liquid phase to the surfaces of the metal and subsequent biochemical reaction at the surfaces is given by Welty *et al.* (1984)

$$\frac{\partial \rho_{O_2}}{\partial t} = D_{O_2}\nabla^2\rho_{O_2} + R_{O_2} \qquad (1)$$

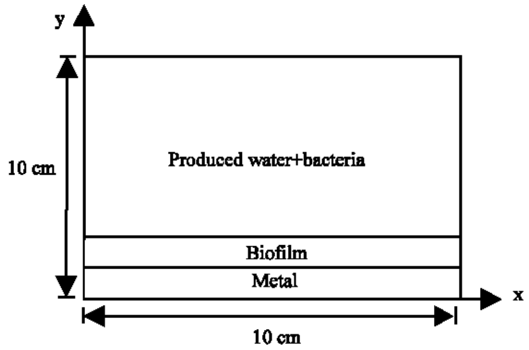

Fig. 3: Physical system showing biofilm on the upper surface of the metal (side view)

where, ρ_{O_2} is the mass concentration of oxygen, D_{O_2} is the diffusion coefficient of oxygen assumed constant and R_{O_2} is the rate of biochemical reaction between oxygen and the microorganism (*Leptothrix discophora*) at the surfaces of the metal. For a two-dimensional transport process with chemical reaction, Eq. 1 reduces to

$$\frac{\partial \rho_{O_2}}{\partial t} = D_{O_2}\left(\frac{\partial^2 \rho_{O_2}}{\partial x^2} + \frac{\partial^2 \rho_{O_2}}{\partial y^2}\right) + R_{O_2} \qquad (2)$$

where, x and y are the spatial coordinates. R_{O_2} is taken here to follow Monod equation (Characklis and Marshall, 1990; Monod, 1949) in the form

$$-R_{O_2} = q_{om}\,\rho_{LA}\,\frac{\rho_{O_2}}{K_s + \rho_{O_2}} \qquad (3)$$

where, q_{om} is the maximum specific rate of oxygen consumption in the system, K_s is the saturation constant for oxygen, ρ_{LA} is the local mass concentration of active biomass in the biofilm and the minus sign on the left-hand-side of Eq. 3 indicates that the concentration of dissolved oxygen decreases with time since it is absorbed by biofilm-microorganisms in the reaction process. Combining Eq. 2, 3 gives the unsteady-state two-dimensional model for transport of oxygen with biochemical reaction as:

$$\frac{\partial \rho_{O_2}}{\partial t} = D_{O_2}\left(\frac{\partial^2 \rho_{O_2}}{\partial x^2} + \frac{\partial^2 \rho_{O_2}}{\partial y^2}\right) - q_{om}\rho_{LA}\frac{\rho_{O_2}}{K_s + \rho_{O_2}} \qquad (4)$$

Numerical solution

Initial and boundary conditions: Initially (i.e., at $t = 0$), the concentration of dissolved oxygen in the produced water inoculated with *Leptothrix discophora* is uniform at 8.4 mg L^{-1} (or 0.0084 kg m^{-3}) as obtained experimentally by Rim-Rukeh and Puyate (2007). Biofilm consists of microorganisms which utilize the dissolved oxygen in the produced water. Since the biofilms are formed on the metal surfaces, the concentration of oxygen at the surfaces of the metal is zero at all time, while that at the liquid/air interface (y = 10) is constant at 8.4 mg L^{-1} and is the concentration of dissolved oxygen at equilibrium with the oxygen in the atmosphere. The resulting concentration gradient of dissolved oxygen in the x and y directions would lead to diffusion of oxygen from the bulk liquid phase to the surfaces of the metal in these directions. The quantity of oxygen absorbed by the biofilms on the edges of the metal would be small since the surface area of an edge of the metal is small. This implies that the concentration of dissolved oxygen in the x-direction would be approximately uniform for any value of y as assumed in this analysis. On the other hand, significant quantity of oxygen would be absorbed by the biofilm on the upper surface area of the metal so that large concentration gradient of oxygen occurs in the y-direction which is felt throughout the bulk of the liquid. In reality, the concentration gradient of dissolved oxygen in the y-direction may be nonlinear but a linear concentration gradient is assumed, with maximum oxygen concentration of 8.4 mg L^{-1} at the liquid/air interface and zero concentration of oxygen at the surface of the metal. Such approximation of nonlinear concentration gradient by a linear one is used in the two-film theory in mass transfer (Coulson and Richardson, 1977). The initial and boundary conditions of Eq. 4 may then be defined as:

$$\rho_{O_2}(x,\ y,\ 0) = 0.0084 \qquad (5a)$$

$$\rho_{O_2}(x,\ 0,\ t) = 0 \qquad (5b)$$

$$\rho_{O_2}(x,\ 10,\ t) = 0.0084 \qquad (5c)$$

$$\rho_{O_2}(0,\ y,\ t) = \frac{-0.0084(10-y)}{10} + 0.0084 \qquad (5d)$$

$$\rho_{O_2}(10,\ y,\ t) = \frac{-0.0084(10-y)}{10} + 0.0084 \qquad (5e)$$

We note that the metal is 0.5 cm thick and let y = 0 be the upper surface of the metal on which biofilm is attached. Since introduction of the metal into the liquid in the container increases the original depth of the liquid, the depth of liquid above the upper surface of the metal is assumed to be approximately 10 cm as used in the boundary condition (5b-e). The average thickness of

Table 1: Parameters used in the analysis

Parameter	Value	Reference
Diffusion coefficient of oxygen, D_{02} (m^2 sec^{-1})	5.7×10^{-6}	Kinoshita (1992)
Saturation (Monod) constant for oxygen, K_s (kg m^{-3})	0.8574	Henze *et al.* (1999)
Local mass concentration of active biomass, ρ_{LA} (kg m^{-3})	0.495	Kwok *et al.* (1998)
Initial mass concentration of oxygen in the system (kg m^{-3})	0.0084	Rim-Rukeh and Puyate (2007)
Maximum specific rate of oxygen consumption, q_{om} (kg kg^{-1} sec^{-1})	3.0×10^{-4}	Henze *et al.* (1999)

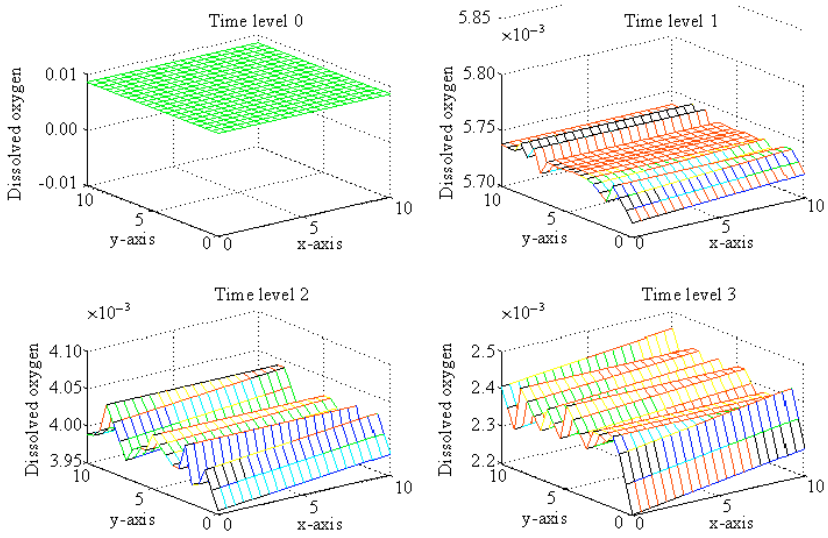

Fig. 4: Distribution of dissolved oxygen in the produced water at different time levels

biofilm is about 40×10^{-6} (Characklis and Marshall, 1990) which is very small so that the upper surface of the biofilm (where oxygen is absorbed) corresponds approximately to y = 0 at all time; this is a non-moving-boundary problem and the boundary conditions (5b-e) hold in the present analysis. If the thickness of the biofilm were to be significant (say, in the order of centimeters), then the upper surface of the biofilm will not correspond to y = 0 (in the y-direction) at all time but will be changing with time due to increase in thickness of the biofilm; such a problem is a moving-boundary problem and the proposed boundary conditions above would not hold any more. Transport parameters of oxygen and properties of *Leptothrix discophora* were not measured for the present analysis. Table 1 shows values of the various parameters used in the analysis as determined by different workers and also applied by Wijffels *et al.* (1991) in their dynamic modeling of immobilized *Nitrobacter agilis*. Equation 4 was solved numerically using conditions (5) and the parameters in Table 1 through Runge-Kutta procedure implemented in MATLAB.

RESULTS AND DISCUSSION

Figure 4 shows the distribution of dissolved oxygen in the produced water at different time levels obtained from the numerical solution of Eq. 4. The wavy structures in Fig. 4 may indicate fluctuation in the concentration of dissolved oxygen as it is transported from the bulk liquid phase to the biofilms on the metal surfaces. Thus at any time greater than zero, the concentration of dissolved oxygen is non-uniform in the y-direction and increases as the depth of liquid decreases, but uniform in the x-direction at any value of y as assumed in the analysis and reflected in the length scale of the wavy structures. Rim-Rukeh and Puyate (2007) measured experimentally the total dissolved oxygen concentration in the system shown in Fig. 3 at constant intervals of time for a period of 84 days. Since the dissolved oxygen concentration was not measured at specific values of x and y, Eq. 4 cannot be compared directly with the available experimental data.

In order to compare predictions of Eq. 4 with the experimental data, Eq. 4 was integrated numerically with respect to x and y to obtain an expression which is a function of only time. The same procedure was adopted by Puyate and Lawrence (1999, 2006) to compare experimental and predicted data on one-dimensional mass-transfer processes. Figure 5 shows the comparison between predictions of the integrated form of Eq. 4 and the experimental data (Rim-Rukeh and Puyate, 2007) presented in Table 2.

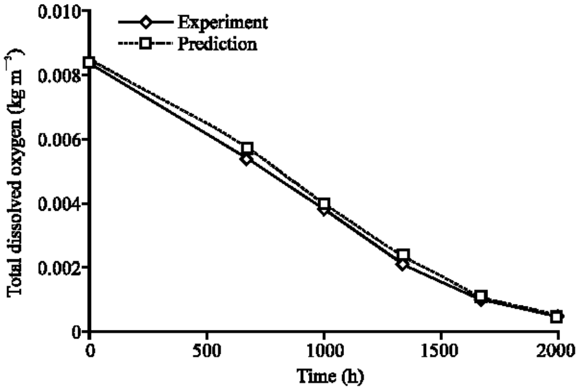

Fig. 5: Experimental (Rim-Rukeh and Puyate, 2007) and predicted concentrations of dissolved oxygen in produced water inoculated with *Leptothrix discophora* for a period of 84 days

Table 2: Measured concentrations of total dissolved oxygen (Rim-Rukeh and Puyate, 2007)

Time (h)	0	672	1008	1344	1680	2016*
Total dissolved oxygen (kg m^{-3})	0.0084	0.00542	0.0038	0.0021	0.00102	0.00048*

The values marked asterisk (*) were wrongly presented in Rim-Rukeh and Puyate (2007)

The decrease in the concentration of total dissolved oxygen in the produced water with time as obtained in Fig. 5 is consistent with the work of Picioreanu and van Loosdrecht (2002) and is attributable to the consumption of oxygen by the bacteria. The maximum deviation of the predicted oxygen profile from the experimental profile occurs at 672 h and the absolute percentage error at this point is about 6%. The error, E in a predicted value is calculated as

$$E = \frac{V_{exp.} - V_{pred.}}{V_{exp.}} \qquad (6)$$

where, $V_{exp.}$ and $V_{pred.}$ are the experimental and predicted values, respectively of total dissolved oxygen concentration. Thus, the agreement between the predicted and experimental data in Fig. 5 may be seen to be very good.

CONCLUSION

A 2-dimensional model for diffusion of oxygen with biochemical reaction during biofilm formation on X60 steel immersed in static produced water inoculated with iron-oxidizing bacteria (*Leptothrix discophora*) is presented. It is shown that predictions of the 2D model compare very well with experimental data and Monod equation may be used to describe the biochemical reaction that takes place between oxygen in the produced water and the bacteria at the metal surface. It would be worthwhile, however, to investigate the performance of a 3D model in relation to the 2D model presented though the difference between the two models is expected to be small (or insignificant) as may be inferred from Fig. 5. The closeness of the predicted and experimental data in Fig. 5 also indicates that the concentration gradient of dissolved oxygen in the y-direction may be assumed to be linear. *Nitrobacter agilis* and *Leptothrix discophora* belong to the Nitrosomonas and iron-oxidizing bacteria families, respectively. The fact that parameters of *Nitrobacter agilis* were used to model the biochemical reaction between *Leptothrix discophora* and oxygen with good results partly suggest that *Nitrobacter agilis* also consume dissolved oxygen in their environment. Dissolved oxygen plays a key role in the formation and growth of biofilm through biochemical reaction with microorganisms.

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