Uncertainty in bulk-liquid hydrodynamics and biofilm dynamics creates uncertainties in biofilm reactor design

J. P. Boltz and G. T. Daigger

ABSTRACT

While biofilm reactors may be classified as one of seven different types, the design of each is unified by fundamental biofilm principles. It follows that state-of-the art design of each biofilm reactor type is subject to the same uncertainties (although the degree of uncertainty may vary). This paper describes unifying biofilm principles and uncertainties of importance in biofilm reactor design. This approach to biofilm reactor design represents a shift from the historical approach which was based on empirical criteria and design formulations. The use of such design criteria was largely due to inherent uncertainty over reactor-scale hydrodynamics and biofilm dynamics, which correlate with biofilm thickness, structure and function. An understanding of two fundamental concepts is required to rationally design biofilm reactors: bioreactor hydrodynamics and biofilm dynamics (with particular emphasis on mass transfer resistances). Bulk-liquid hydrodynamics influences biofilm thickness control, surface area, and development. Biofilm dynamics influences biofilm thickness, structure and function. While the complex hydrodynamics of some biofilm reactors such as trickling filters and biological filters have prevented the widespread use of fundamental biofilm principles and mechanistic models in practice, reactors utilizing integrated fixed-film activated sludge or moving bed technology provide a bulk-liquid hydrodynamic environment allowing for their application. From a substrate transformation perspective, mass transfer in biofilm reactors defines the primary difference between suspended growth and biofilm systems: suspended growth systems are kinetically (i.e., biomass) limited and biofilm reactors are primarily diffusion (i.e., biofilm growth surface area) limited.

Key words | biofilm, design, diffusion, model, process, reactor, treatment, wastewater

INTRODUCTION

Biofilm reactors play an important role in environmental biotechnology, but many aspects of their design remain poorly understood. Consequently, biofilm reactors have historically been designed using empirical criteria and design formulations. Unfortunately, such an approach is not uniform and fails to provide insight to processes that are presently of concern for environmental protection (e.g., state of nitrogenous compounds: \( \text{NH}_4^+ \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^- \rightarrow \text{NO}_2 \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \)). During the past four decades, significant advances in the design, academic understanding, and mathematical modelling of biofilms has resulted in new and emerging biofilm reactors more conducive to fundamentally based design approaches and the application of fundamentally based design and operation procedures for traditional biofilm reactors. While some bioreactors and treatment applications are favourable to direct application of biofilm models for their design and operation, some are not due to their more complex bulk-liquid hydrodynamics and the associated impact on biofilm dynamics. Thus, while an understanding of kinetics, stoichiometry, and biochemical transformations are required to design any biological process and biofilm reactor, two additional
concepts are necessary to implement the evolving approach to biofilm reactor design: bioreactor hydrodynamics and biofilm dynamics (with particular emphasis on mass transfer resistances). The discussion of hydrodynamics presented in this paper is limited to the bulk of the liquid (see Wanner et al. (2006) for a mathematical description biofilm-scale hydrodynamics).

Fundamental principles describing what is known as the biofilm and mass transfer boundary layer (MTBL) compartments exist. Empirical information and design formulations are typically developed to support the design and implementation of biofilm reactors (see Boltz et al. (2009a) for additional information). Unfortunately, based on the authors’ experience there have been few efforts dedicated to linking these fundamental principles with empirical information in order to promote the use of biofilm mechanics when designing full-scale biofilm reactors. This paper will provide a basis for addressing this disconnection by (1) describing the fundamental biofilm principles that can be uniformly applied to each biofilm reactor type, and (2) identifying uncertainties that result from a fundamental-based approach to biofilm reactor design. This paper reviews biofilm theory, with a particular emphasis on the design and operational insights that it provides. Constraints on the application of this theory are then discussed in the context of biofilm reactor hydrodynamics and biofilm dynamics.

REVIEW OF BIOFILM THEORY AND APPLICATION TO PRACTICE

Biofilm and suspended growth reactors can meet similar treatment goals for carbon oxidation, nitrification, and denitrification. Identiﬁcal microorganisms are responsible for biochemical reactions, and respond in the same way to local environmental conditions (i.e., pH, temperature, electron donor (ED), electron acceptor (EA), and macronutrient availability) (Morgenroth 2008). Biofilm reactors retain biomass in a dense layer of bacterial cells and extracellular polymeric substances (EPS) that grow on a ﬁxed or movable carrier while suspended growth systems require underﬂow from a clariﬁer to accumulate mixed liquor.

In order to facilitate a mechanistic description, bioﬁlms have been compartmentalized. In a simple form the compartments include a biofilm and MTBL, which links the biofilm and bulk liquid compartments. Very little is understood about how tank and bioﬁlm carrier conﬁguration, system appurtenances (e.g., bafﬂes, mixers, aeration system), and operating mode (e.g., continuous ﬂow, sequencing batch, periodic backwashing) inﬂuences MTBL thickness. Furthermore, the impact a bioﬁlm has on bioreactor appurtenances is also poorly understood and described by existing 1-D bioﬁlm models (since a series of CFSTRs are used for the pseudo two-dimensional simulation of a bioﬁlm reactor). An example of bioﬁlm reactor design uncertainty resulting from bulk-liquid hydrodynamics and bioﬁlm dynamics is bioﬁlm accumulation and the resulting increase in hydraulic headloss in a biologically active ﬁlter. Figure 1 presents a conceptualized view of a bioﬁlm reactor and its principal components, which include the bioreactor compartment (bulk liquid), MTBL of thickness $L_L$, and bioﬁlm.

The bulk liquid consists of the inﬂuent wastewater applied to the bioreactor, the liquid as it is being treated, and the treated efﬂuent. Figure 1 illustrates the removal of reactants and product formation as the inﬂuent passes through the bioreactor. The MTBL represents the transition from the bulk liquid to the biofilm surface and captures the fact that bulk-liquid product and reactant concentrations differ from those at the biofilm surface. The bioﬁlm is where biochemical reactions occur. Mass transfer in bioﬁlm reactors is the primary mechanistic difference from suspended growth reactors. At full scale, suspended growth systems are typically kinetically (i.e., biomass) limited, while bioﬁlm reactors are typically diffusion (i.e., surface area) limited. (Note, according to Daigger et al. (2007) diffusion can be important in suspended growth bioreactors).

Bioﬁlms can be partially or completely penetrated by any soluble substrate diffusing into the gelatinous matrix of EPS, bacterial cells, organic and inorganic particulates. A partially penetrated bioﬁlm exists when the ED, EA, and/or macronutrient is depleted prior to reaching the biofilm carrier. Bioﬁlms grown in full-scale reactors might be completely penetrated immediately after a hydraulically triggered detachment event or during an organic shock load, but it is likely that most bioﬁlm reactors generally operate with partially penetrated bioﬁlms. Dynamic loadings can create unusual effects, as illustrated by a recent pilot study.
described by Stinson et al. (2009). Once a biofilm is partially penetrated, increasing the biofilm thickness \((L_F)\) has no treatment benefit and, in fact, is typically detrimental to reactor performance. Excessive biofilm thickness can have two detrimental impacts on full-scale biofilm reactors. First is the reduction in biofilm surface area, \(A\). Figure 2 illustrates (using plastic trickling filter media) that, as biofilm thickness increases the biofilm surface area decreases.

Second, biofilm zones existing near the carrier may be deprived of ED, EA, and/or macronutrients, resulting in anaerobic conditions in this ecological niche and potentially uncontrolled biofilm detachment (or sloughing) and/or odours. Excess biofilm accumulation can result in hydraulic head loss, odours, reduced carrier specific surface area, increased biofilm weight which can exceed design structural loads, and media clogging. All biofilm reactor types rely on biofilm thickness control. From a design perspective, the relevant process loading that controls biofilm reactor performance is that applied to the biofilm surface, which is generally expressed as the mass of limiting substrate applied per unit of effective biofilm carrier area (i.e., the carrier area that is expected to support the development of a biofilm) per unit time (i.e., \(\text{g m}^{-2} \text{d}^{-1}\)). Typically, the basis for defining the relevant process loading is the biofilm carrier effective specific surface area reported by a respective system manufacturer, and may be a source of
uncertainty in biofilm reactor design. While the fabricated, or manufactured, specific surface area can be easily defined, the actual biofilm area in a biofilm reactor is very likely different from the ‘manufactured’ area. Improper biofilm thickness control, inordinate biofilm accumulation, and deteriorating system performance may result from excessive substrate loading or a biofilm carrier structure that does not allow for sufficiently turbulent bulk-liquid flow in the vicinity of the biofilm surface.

Biofilm dynamics (related to formation and control) are directly influenced by bulk-liquid hydrodynamics. Biofilm thickness generally adjusts under quasi steady-state conditions so that partially penetrated biofilms are present. Conceptually, a material diffuses across the external diffusion layer and then the biofilm surface. Within the biofilm further diffuse and biochemical transformation processes will proceed until the ED, EA, or macronutrient is exhausted. State-of-the-art biofilm reactors such as the moving bed biofilm reactor (MBBR) naturally control biofilms due to the turbulent bulk liquid, and the biofilm is virtually self-regulating. Cold-weather climates retard biochemical transformation processes, and therefore the cold-weather response in a MBBR is increased $L_F$. Simply, the material diffuses deeper into the biofilm, thereby supporting a thicker biofilm (and more biomass). These effects are illustrated in Figure 3 for a tertiary nitrifying MBBR. From right to left, higher temperatures expedite biochemical processes and the ammonia-nitrogen biofilm penetration is

Figure 2 | Plastic tickling filter media plan and illustration of biofilm area ($A$) reduction with an increase in biofilm thickness ($L_F$). Electric rotary distribution systems and controlled dosing (i.e., Spülkraft) is commonly used for biofilm thickness control in trickling filters.

Figure 3 | Seasonal biomass growth in a tertiary nitrifying moving bed biofilm reactor. As the temperature increases, biofilm mass decreases (biofilm mass is normalized) (after McQuarrie 2008).
reduced. The high-turbulence bulk-liquid hydrodynamics characteristic of MBBRs promotes thin and stable biofilms (i.e., steady detachment in stead of uncontrolled sloughing).

The fate of particulate matter reflects one short-coming of biofilm theory. Biofilm models typically assume biofilms are a continuum and no advective mass transport occurs inside the biofilm. Based on these assumptions, Boltz & La Motta (2007) suggested that particles larger than those considered to be “soluble substrate” are bioflocculated at the biofilm surface and are then either (1) hydrolyzed and consumed as soluble substrate, or (2) re-enter the bulk-liquid with detached biofilm fragments. Drury et al. (1993) found that 1 µm fluorescent beads were found near the base of a biofilm only moments after they were introduced to the system, demonstrating that advection can occur inside biofilms. A majority of the COD in municipal wastewater is particulate. Thus, the fate of particulate in the biofilm is a source of uncertainty in biofilm reactor design with respect to biofilm formation, structure and function. The uncertain fate of particulates represents one reason why empirical approaches have historically been used to design and operate biofilm reactors.

Biofilm reactor classification and application of biofilm theory

Biofilm reactors can be classified as submerged or non-submerged, and into the seven types presented in Table 1 (adapted from Harremoës & Wilderer 1993). These bioreactor types vary significantly in terms of their hydrodynamic complexity and, consequently, in the ability to directly apply biofilm theory to their design and operation. By analogy to modelling suspended growth bioreactors, biofilm models represent the “completely mixed” cells used to simulate individual components of the bioreactor, but a pseudo two-dimensional approach applying cells-in-series must be constructed to simulate the complete suspended growth bioreactor. Consider a MBBR treating dissolved substrates. The overall flow pattern through the biofilm reactor and the flow past the individual biofilm carrier elements is sufficiently well understood so that a cells-in-series model can be configured and the performance of each equivalent completely mixed cell simulated using a one-dimensional biofilm model (Boltz et al. 2009b,c). In addition, the impact of biofilm development on MBBR appurtenances is expected to be negligible (e.g., no

<table>
<thead>
<tr>
<th>Phases</th>
<th>Media</th>
<th>Submerged</th>
<th>Stationary</th>
<th>Description</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air, water, biofilm</td>
<td>No</td>
<td>Yes</td>
<td>Water trickles over the biofilm surface and air moves upward or downward in the third phase.</td>
<td>Trickling filters</td>
<td></td>
</tr>
<tr>
<td>Air, water, biofilm</td>
<td>Yes</td>
<td>Yes</td>
<td>Water flows through the biofilm reactor with gas bubbles. Gravel is a fixed media while polystyrene beads are semi-fixed.</td>
<td>Aerobic biofilters</td>
<td></td>
</tr>
<tr>
<td>Air, water, biofilm</td>
<td>Yes</td>
<td>No</td>
<td>Water flows through the biofilm reactor with gas bubbles.</td>
<td>Aerobic Carbon-Oxidizing and/or Nitrifying MBBR</td>
<td></td>
</tr>
<tr>
<td>Water, biofilm</td>
<td>Yes</td>
<td>No</td>
<td>Water flows through the biofilm reactor with the electron donor and electron acceptor.</td>
<td>Denitrifying MBBR, RBC</td>
<td></td>
</tr>
<tr>
<td>Water, biofilm</td>
<td>Yes</td>
<td>Yes</td>
<td>Fixed biofilm-laden carrier material with water flowing through the biofilm reactor with the electron donor and electron acceptor.</td>
<td>Denitrifying biofilter</td>
<td></td>
</tr>
<tr>
<td>Gas, water, biofilm</td>
<td>Yes</td>
<td>Yes</td>
<td>Microporous hollow-fiber membrane with biofilm and water on one side and gas on the other.</td>
<td>Membrane biofilm reactor</td>
<td></td>
</tr>
<tr>
<td>Water, biofilm</td>
<td>Yes</td>
<td>Yes</td>
<td>Proton exchange membrane separating a compartmentalized biofilm-laden anode from a compartmentalized cathode with water on both sides, but the electron donor on one side and electron acceptor on the other.</td>
<td>Microbial Fuel Cell</td>
<td></td>
</tr>
</tbody>
</table>
biofilm induced hydraulic headloss). This contrasts with, for example, a trickling filter treating a wastewater containing particulate organic matter. Wetting of the media is often incomplete, the flow pattern over the media is indeterminate, and the fate of particulate matter uncertain. Consequently, better understanding the hydrodynamics of biofilm reactors is crucial to extending the application of biofilm theory to these types of biofilm reactors.

**BIOFILM DYNAMICS: FORMATION AND DETACHMENT**

Biofilm dynamics are of critical importance, and their thorough understanding is necessary because they govern biofilm formation and loss. This further aids biofilm reactor process designers and operators to make well informed decisions regarding optimization, design, start-up, and construction or emergency shut down.

**Biofilm formation**

A concern of process designers is bioreactor start-up and the time required to meet design objectives. Biofilm formation rate is governed by five items: (1) local environmental conditions (i.e., pH, temperature, ED, EA, and macronutrient availability); the extent of biofilm (2) external and (3) internal mass transfer resistances; (4) the kinetics and stoichiometry of principal bacterial species in a mixed-culture biofilm; and (5) detachment. Many techniques have been used to minimize biofilm reactor start-up period. The common assumption that continuously seeding biofilm reactors with suspended biomass accelerates biofilm maturation is actually incorrect. The suspended biomass competes with the bacterial cells in the developing biofilms for ED, EA, and macronutrients and retards biofilm development. If slow-growing bacteria can be retained in the suspended phase, flocs containing suspended bacterial cells generally ‘win’ the competition for substrate since mass transfer resistance is less in flocs than in biofilms. Consequently, a healthy biofilm may not develop. For integrated fixed film activated sludge (IFAS) bioreactors, for example, a common design objective is the formation of nitrifying biofilms and suspended phase heterotrophic biomass.

A low mixed liquor solids residence time (SRT) can prevent substantial suspended phase autotrophic nitrifier development, allowing them to develop in the biofilm.

**Detachment**

Biofilm detachment is a characteristic of well-operating biofilm reactors and results from proper reactor-scale biofilm thickness $L_F$ control. Bryers (1984) described four biofilm loss processes: (1) abrasion, (2) erosion, (3) sloughing, and (4) predator grazing. Abrasion (initiated by particle collision) and erosion (initiated by fluid-induced shear in the vicinity of the biofilm surface) are the removal of small groups of cells. Sloughing is the loss of large segments of biofilm approximately equal to the biofilm thickness, $L_F$ (Morgenroth 2003). Predatory higher life forms such as macro- and micro-fauna graze biofilms (Boltz et al. 2008). While abrasion and erosion can be associated with well-operating biofilm reactors, sloughing and excessive predation are detrimental to bioreactor performance.

Biofilm loss plays a role in biofilm structure. Figure 4 compares simulated bacterial mass (heterotrophs and autotrophic nitrifiers) and substrate flux (chemical oxygen demand (COD) and ammonia-nitrogen) for three detachment modes: (1) constant biofilm thickness $L_F$, (2) daily backwashing, and (3) a 7-day backwashing interval (meant to capture biofilm sloughing).

An increase in heterotrophic biomass does not produce a higher COD flux, which suggests that the system was diffusion rather than biomass limited. The ammonia-nitrogen flux and autotrophic nitrifier mass were significantly reduced after the 7-day backwashing event. The rapid loss of both fast (heterotrophs) and slow (autotrophic) growing bacterial species is advantageous to the faster growing of the two species. Morgenroth (2003) concluded that the biofilm thickness $L_F$ dynamics (i.e., formation and detachment) has a significant influence on the competition between heterotrophic and autotrophic nitrifying organisms in mixed-culture biofilms. The backwashing of biologically active filters results in very thin biofilms. Boltz et al. (2009a) stated that biofilms grown in these systems may be as thin as 20 microns. This analysis (1) describes the impact of biofilm dynamics on activity, and (2) provides some indication that even biofilm reactors capable of sustaining...
very thin biofilms may operate with partially penetrated (i.e., mass-transfer and not biomass limited) biofilms. Systems with operating cycles dedicated to biofilm thickness control increases the design uncertainty due to the impact a radically different hydrodynamic regime such a backwashing a filter has on biofilm structure and function.

Figure 5 depicts biofilms grown on granular (expanded clay) biologically active filter media. The biofilms result from shear imparted on the biofilm surface by the bulk liquid which can be differentiated by a higher Reynolds Number, \( N_{Re} \) backwashing cycle (left) and lower \( N_{Re} \) normal operating cycle (right) (photos courtesy, Sørensen 2008). The pictured biofilms are also classified according to the biofilm renderings in turbulent, high shear stress and quiescent, low shear stress hydrodynamic environments [A & B–3d simulation; C & D–2d simulation (presented as published by Xavier et al. 2005); E & F– experimental (presented as published by Kugaprasatham et al. 1992)].

Further suggests that operating cycles dedicated to biofilm thickness control increases design uncertainty by changing the microbiological composition of a biofilm reactor.

**IMPACT OF HYDRODYNAMICS ON EXTERNAL AND INTERNAL DIFFUSION**

Bulk-liquid hydrodynamics influences biofilms at all stages of their development (Lewandowski 2000) and biofilm reactor design through (1) biofilm development and control,
and (2) biofilm surface area loading, yet the hydrodynamics are an often overlooked component of biofilm reactor design. Complex biofilm reactor hydrodynamics (e.g., trickling filter) prevent widespread application of modern biofilm knowledge to process design and operation, but relatively simple bulk-liquid hydrodynamics such as the completely mixed bulk-liquid in a MBBR promote the application of mechanistic principles during biofilm reactor design. Figure 5 lists some benefits inherent to biofilm thickness control resulting from shear stress imparted by bulk-liquid turbulence.

Impact on biofilm dynamics: formation and biofilm thickness control

Reactor-scale hydrodynamics are influenced by tank and biofilm carrier configuration and type, appurtenances (e.g., baffles, mixers, aeration system), and operating mode (Grady et al. 1999) (e.g., continuous flow, sequencing batch, periodic backwashing). Bulk-liquid turbulence affects biofilm density and development (Eberl et al. 2000). Rigid segregation of bulk-liquid, MTBL, and biofilm compartments is a simplification used to model and understand biofilm reactor mechanics. Figure 5 illustrates how turbulent, high-shear stress environments result in planar and denser biofilms while quiescent, low-shear stress environments will result in rough and less dense biofilms (van Loosdrecht et al. 1995).

Resistance to mass transfer external to the biofilm

The previously presented Figure 1 illustrates the (a) biofilm, (b) MTBL, and (c) bioreactor/bulk-liquid and summarizes characteristics. The MTBL is an interfacial compartment used to model external mass transfer resistances and connects the biofilm- to the reactor compartment (Boltz et al. 2008, 2009d). The impact of external mass transfer resistances is illustrated in Figure 6 as a function of the Reynolds Number (NRe). As NRe increases (methanol) flux increases (Boltz 2005) until a plateau is reached (conceptually Lr has been minimized).

A MTBL of thickness Lr is a function of bulk-liquid hydrodynamics and substrate concentration. The mathematical biofilm model in virtually every wastewater treatment plant simulator requires a user defined MTBL thickness of Lr (Boltz et al. 2009d). At the time this paper was written the dynamical models held the MTBL thickness Lr constant when implementing a dynamic simulation. Actually the MTBL thickness varies with wastewater flow rate and pollutant loading. Increased loading conditions are typically associated with increased wastewater flow rate, organic matter and ammonium loading. Therefore, forward flow and the air flow rate in oxic zones will increase. As a result, bulk-liquid turbulence increases and it is expected
that the liquid velocity in the vicinity of the biofilm surface increases. Consequently, the MTBL thickness $L_L$ decreases. Alternatively, decreasing wastewater flow rates and pollutant loading will cause the MTBL thickness $L_L$ to increase.

Boltz et al. (2009c) applied the relationship of Frössling (1938) with coefficients presented by Rowe et al. (1965). Biofilms are nonplanar, porous, and heterogeneous biostructures, but when using 1-D biofilm models to describe biofilm reactor performance the existing science is substandard, and simplified concepts such as the empirical relationship of Frössling (1938) remain useful for capturing the basic (mass transfer) mechanisms that govern substrate transformation in the absence of improved relationships. Sufficiently turbulent bulk liquids minimize the MTBL thickness, $L_L$, such that the bulk-liquid concentration of a substrate is approximately equal to the concentration at the liquid-biofilm interface, or $S_B \approx S_{LB}$. However, virtually all full-scale biofilm reactors are subject to some degree of external mass transfer resistance. Increased external mass transfer resistance reduces substrate concentrations at the biofilm surface, $S_{LB}$, and flux across the biofilm surface. Although difficult to demonstrate in full-scale applications, some biofilm reactors may be limited by mass transfer resistances external to the biofilm (e.g., nitrifying MBBRs) (Hem et al. 1994).

CONCLUSION

Fundamental principles describing what is known as the biofilm and MTBL compartments exist. Based on the authors’ experience there have been few efforts dedicated to linking these fundamental principles with empirical information in order to promote the use of biofilm mechanics when designing full-scale biofilm reactors. This paper provides a basis for addressing this disconnection by (1) describing the fundamental biofilm principles that can be uniformly applied to each biofilm reactor type, and (2) identifying uncertainties that result from a fundamental-based approach to biofilm reactor design. Much of the uncertainty inherent in biofilm reactor design and mathematical modelling is due to reactor-scale hydrodynamics, and the strong correlation between hydrodynamics and biofilm dynamics (i.e., biofilm composition/structure, control, and function). State-of-the-art biofilm reactors such as the MBBR, improved academic understanding, and mathematical modelling has resulted in a paradigm shift from the traditional ‘black-box’ design approach to one based on sound fundamentals and a mechanistic understanding of biofilm technologies. Two fundamental concepts are required for modern biofilm reactor design: bioreactor hydrodynamics and biofilm dynamics (with particular emphasis on mass transfer resistances; in addition to the processes, kinetics, and stoichiometry inherent to activated sludge design). Significant additional research is required in these areas specifically linking the biofilm (or micro) and bioreactor (or macro) scales. Bulk-liquid hydrodynamics influence biofilm dynamics through (1) control, (2) surface area loading, and (3) development. Mass transfer in biofilm reactors presents the principle difference between full-scale activated sludge and biofilm systems: activated sludge systems are generally kinetically limited (i.e., biomass limited) and biofilm reactors are generally diffusion limited (i.e., surface area limited).

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