Journal of Theoretical Biology 259 (2009) 670–683

Contents lists available at ScienceDirect

Journal of Theoretical Biology

journal homepage: www.elsevier.com/locate/yjtbi

# Modeling how soluble microbial products (SMP) support heterotrophic bacteria in autotroph-based biofilms

Brian V. Merkey [a],*, Bruce E. Rittmann [b], David L. Chopp [c],**

[a] Department of Environmental Engineering, The Technical University of Denmark, 2800 Kgs. Lyngby, Denmark
[b] Center for Environmental Biotechnology, The Biodesign Institute, Arizona State University, Tempe, AZ 85287-5701, USA
[c] Department of Engineering Sciences and Applied Mathematics, Northwestern University, Evanston, IL 60208, USA

ARTICLE INFO

*Article history:*
Received 9 July 2008
Received in revised form
4 April 2009
Accepted 18 May 2009
Available online 27 May 2009

*Keywords:*
Biofilm model
Multi-species
Multi-component
*r*-strategist
*K*-strategist

ABSTRACT

Multi-species biofilm modeling has been used for many years to understand the interactions between species in different biofilm systems, but the complex symbiotic relationship between species is sometimes overlooked, because models do not always include all relevant species and components. In this paper, we develop and use a mathematical model to describe a model biofilm system that includes autotrophic and heterotrophic bacteria and the key products produced by the bacteria. The model combines the methods of earlier multi-species models with a multi-component biofilm model in order to explore the interaction between species via exchange of soluble microbial products (SMP). We show that multiple parameter sets are able to describe the findings of experimental studies, and that heterotrophs growing on autotrophically produced SMP may pursue either *r*- or *K*-strategies to sustain themselves when SMP is their only substrate. We also show that heterotrophs can colonize some distance from the autotrophs and still be sustained by autotrophically produced SMP. This work defines the feasible range of parameters for utilization of SMP by heterotrophs and the nature of the interactions between autotrophs and heterotrophs in multi-species, multi-component biofilms.

## 1. Introduction

The surface-attached microbial communities known as biofilms are the dominant form of microbial life, with more than 90% of bacteria living in biofilm communities (Characklis and Marshall, 1990). Biofilms have a huge impact on many natural and engineered systems that support human life, in particular for applications of wastewater treatment (Rittmann, 2004; Rittmann and McCarty, 2001). For purposes of water reclamation, an important type of biofilm is the combination of autotrophic and heterotrophic bacteria (Eberl et al., 2006). Autotrophic bacteria species use an inorganic electron donor, such as $NH_4^+$ or $H_2$, and inorganic carbon ($CO_2$ or $HCO_3^-$) as their carbon source. Due to their need to reduce inorganic carbon for synthesis, autotrophs have low true yields and maximum specific growth rates (1 per day) (Rittmann and McCarty, 2001). Conversely, heterotrophs use organic compounds as their electron donor and carbon source, and when respiring oxygen or nitrate, have high true yields and fast maximum specific growth rates (10–20 per day). Despite their differences, heterotrophs and autotrophs coexist in natural biofilms, as well as in biofilms in wastewater-treatment processes used to remove organic and ammonia pollution at the same time.

Whether or not a biofilm is multi-species, all biofilms are also inherently multi-component because they include electron donors and acceptors, active biomass, extracellular polymeric substances (EPS), residual inert material, and soluble microbial products (SMP) produced during normal metabolic activity (Laspidou and Rittmann, 2002a, b; Barker and Stuckey, 1999). The schematic in Fig. 1 illustrates the metabolic connections between these different components of a biofilm, whose properties include the following:

- The electron donor and acceptor substrates and the carbon sources are essential components that the bacteria consume to gain energy and grow.
- Continuous decay of active bacteria produces residual, inert biomass that accumulates as a solid.
- Normal metabolic activity releases extracellular polymeric substances, a solid that acts as a “glue” to hold the biofilm together and to the surface.
- Normal metabolic activity and hydrolysis of EPS release organic soluble microbial products that are available to heterotrophs as an electron donor and carbon source; when

---

* Corresponding author.
** Also corresponding author.
*E-mail addresses:* brim@env.dtu.dk (B.V. Merkey), chopp@northwestern.edu (D.L. Chopp).

0022-5193/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jtbi.2009.05.010

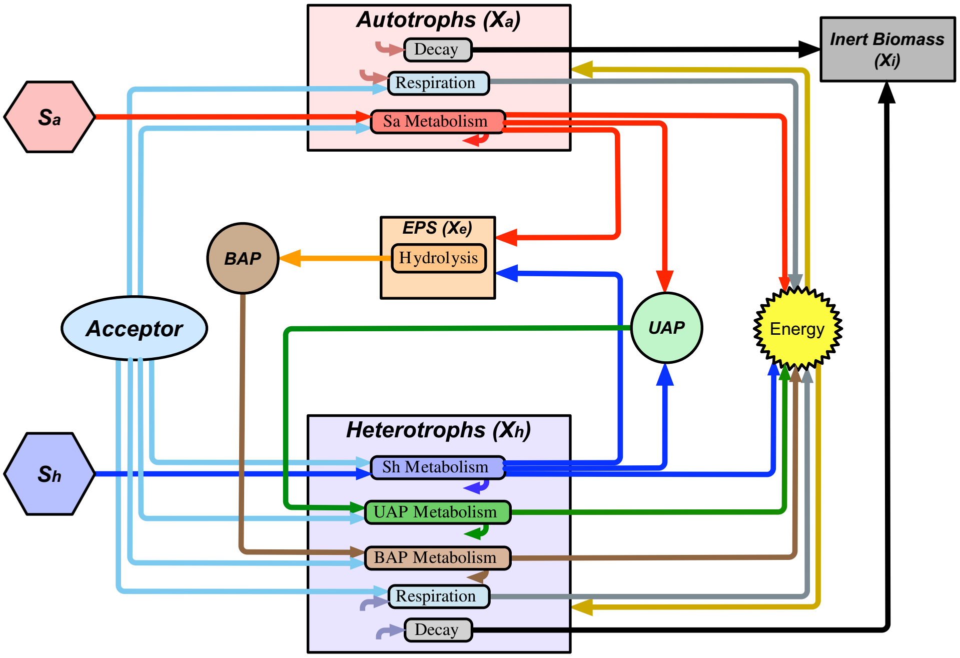

**Fig. 1.** Model schematic. This schematic follows Laspidou and Rittmann (2002a) and describes the interaction of the dissolved components (hexagons, ovals, circles) and solid components (rectangles) in a biofilm. Within the solid components are shown the reactions either carried out by or occurring to the component, with reactants entering and products leaving. Electron donors $S_a$ and $S_h$ and the electron acceptor $A$ are supplied externally, while an initial bacterial population will yield the remaining components. Energy is not tracked explicitly, but rather is included in the diagram to show that the conversion processes are not 100% efficient.

the SMP comes from autotrophs, it augments the organic material available to the heterotrophs.

These components all play a role in the growth and evolution of a biofilm community. EPS help maintain the integrity of the biofilm structure, but EPS production diverts electrons from biomass growth which may affect long-term species success (Kreft and Wimpenny, 2001; Xavier and Foster, 2007). SMP capture the natural products of bacterial growth (the utilization-associated products, UAP) and hydrolysis (the biomass-associated products, BAP) and allows for an interaction between bacterial species (Barker and Stuckey, 1999). The possibility for interactions between heterotrophs and autotrophs expand when the multiple components are included, and in an autotrophically based system, the existence of the heterotrophs depends on autotrophic production of EPS and SMP (Furumai and Rittmann, 1992, 1994; Zhang et al., 1994; Bishop et al., 1995; Okabe et al., 1996; Rittmann et al., 2002; Kindaichi et al., 2004). Zhang et al. (1994) in particular expressed the need for a multi-species biofilm model that tracks these by-products in addition to the bulk substrates, and it is this need that we address here.

The ability to closely model the growth of a multi-species, multi-component biofilm is hampered, though, because the parameters that describe SMP and EPS production and SMP consumption are not well-established (Barker and Stuckey, 1999). Reasonable parameter values for SMP utilization have been given by Laspidou and Rittmann (2002a) and Noguera et al. (1994), but these parameters were calculated for a single-species *Pseudomonas atlantica* system, and, as such, are not necessarily applicable to all bacterial species or biofilm systems. In particular, it is not known which type of growth strategy heterotrophs growing on the limited resource of autotrophically produced SMP tend to pursue: an *r*- or *K*-strategy (Andrews and Harris, 1986; Velicer et al., 1999; Rittmann and McCarty, 2001) or even a yield-strategy (Kreft, 2004; Kreft and Bonhoeffer, 2005). A heterotroph species that is an *r*-strategist has a high maximum specific growth rate and would be capable of fast growth on a relatively high concentration of SMP, but this growth could be impaired under low SMP concentrations. On the other hand, a heterotroph species that is a *K*-strategist would be able to scavenge low SMP concentrations, but its lower maximum specific growth rate could cause it to be out-competed by the autotrophs for space or oxygen. Alternatively, a yield-strategy approach would mean efficient conversion of SMP to biomass by the heterotrophs, but again a low specific growth rate could potentially lead to the heterotrophs being out-competed. It is likely that different heterotrophic species pursue different strategies, but it is not immediately clear which strategy provides the best fit with experimental findings, or whether growth on SMP is only possible with one of these strategies.

The objective of the work presented here is to use a multi-species, multi-component biofilm model to quantify and evaluate the importance of including SMP when modeling biofilm systems and, in particular, to explore the effects of SMP-parameter values on the ability of heterotrophs to subsist solely on SMP. Multi-species biofilm models have previously been applied to study many different systems (Kissel et al., 1984; Wanner and Gujer, 1986; Rittmann and Manem, 1992; Wanner and Reichert, 1995; Rauch et al., 1999; Stoodley et al., 2002b; Alpkvist and Klapper, 2007b), and previous work in multi-component biofilm models includes Laspidou and Rittmann (2004a, b), Furumai and Rittmann (1992, 1994), Rittmann et al. (2002), and Wood and Whitaker (2000). Combining the multi-species and multi-component approaches allows us to investigate systems not addressable by previous models: for example, neither the early multi-species model of Wanner and Gujer (1986) nor the Unified Model of Laspidou and Rittmann (2004a, b) is useful for modeling a system where the heterotrophs subsist entirely on organic carbon in the form of SMP produced by the autotrophs; the combined model we present here overcomes this limitation. In order to explore these complex biofilms, we began with a multi-dimensional biofilm model similar to that of Alpkvist and Klapper (2007b), who described the benefits of a continuum model for biofilm growth over cellular automata or individual-based models. Expanding