Mathematical modeling of diffusion regulated *Quorum* sensing in Artificially Structured Microbial Consortia (ASMC).

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Abstract A mathematical model, involving diffusion of extracellular compounds and cell interactions, has been adapted, in order to assess the *quorum* sensing process occurring in biofilms. *Quorum* sensing is a diffusion dependent phenomenon; therefore, accumulation levels of extra-cellular substances can directly affect cellular development. In order to modeling *quorum* sensing from artificially structured microbial consortia (ASMC), accumulation of N-acyl-L-homoserine lactone (AHL) was studied within a biofilm formed with three bacterial species, *Agrobacterium tumefaciens* KYC6, *Escherichia coli* JM109 and green fluorescent protein (GFP), producer and reporter, *Escherichia coli* JM105. Different artificial biofilm structures or ASMC and conditions were considered when modeling cell behavior under particular experimental situations, such as: accumulation from external addition or internal production of N-acyl-L-homoserine lactone (AHL) in mixed mono- and multi-layered biofilms. Mathematical modeling results include: internal concentration profiles of extra-cellular compounds, accumulated within the biofilm; time related patterns of cell distribution in ASMC constructed with different strains and dynamic estimations of biofilm thickness.
Keywords: *Quorum* sensing; mathematical modeling; diffusion; ASMC and N-acyl-L-homoserine lactone (AHL).

INTRODUCTION

Mathematical modeling of metabolic interactions in biofilms has been of large interest, mainly when explaining cell interactions that might lead to defined internal architectures microbial consortia. The mathematical model was based on a more general deterministic model, which was previously developed by Wanner and Gujer (1986). The model was capable of describing the cell distribution and concentration profiles of extra-cellular substances (ES) in the biofilm and was able to predict the preferred architecture in a mixed biofilm. However, metabolites exchange is merely an example of systems in which biofilm architecture is of importance. Other phenomena in which biofilm architecture is important, include intercellular communication through messenger molecules, also referred to as *quorum* sensing (Kjelleberg and Molin, 2002; Webb *et al*., 2003 and Parsek and Greenberg, 2005), and biofilm resistance against environmental challenges (Stewart and Costerton, 2001; Hentzer *et al*., 2002; Davies, 2003; Russell, 2003 and Anguige *et al*., 2004). Both phenomena are diffusion dependent and, therefore, accumulation levels of extra-cellular substances can directly affect cells' development. For that reason, it would be useful to simulate internal mass transport and metabolic processes, in order to accurately evaluate biofilm cell growth and distribution.

***Quorum* sensing.**

This is the process through which bacteria, specifically Gram-negative bacteria (Whitehead *et al*., 2001), can self-monitor their population density by producing and sensing extra-cellular substances. A typical example of such substances is the acetyl homoserine lactones (AHL) such as *N*-3-oxohexanoyl-L-homoserine lactone (OHHL), which modulate transcriptional responses in the cell. Cell density regulation is one of the most important responses to *quorum* sensing. However, other responses such as the production of luminescence (James *et al*., 2000), the release of cyto-toxic substances (Shirtliff *et al*., 2002), swarming and migration (Daniels *et al*., 2004) and the induction of toxicity resistance have also been observed (Anguige *et al*., 2004). After reaching a threshold level of extra-cellular AHL, in the microbial environment, AHLs trigger complex genetic responses inducing internal and external mechanisms for AHL production/accumulation leading to the *quorum* sensing response, which has been described and mathematically modeled (Chopp *et al*., 2003; James *et al*., 2000). In these models *quorum* sensing is considered to be an AHL diffusion-controlled process, with AHL diffusing either through the cell membrane (for internal production and accumulation) or across the external medium. *Quorum* sensing is probably one of the main metabolisms for cell communication within the biofilm. Therefore mathematical modeling of the production, diffusion and accumulation of AHL substances in biofilms provides useful information about this process.

Production of *N*-3-oxohexanoyl-L-homoserine lactone (OHHL) in *Vibrio fischeri*.

Maximum OHHL production and phenotype expression, by *Vibrio fischeri* cells, normally occurs in the late stationary phase. Initial OHHL production and accumulation stages are intra-cellular, and then its concentration levels increase causing diffusion through the cell membrane with further extra-cellular accumulation. OHHL production is genetically auto-induced by the so called *lux* genes. In case of the *Vibrio fischeri* system, as described by James *et al*. (2000), the presence of extra-cellular OHHL initiates an auto-induction process. Autoinducer substance (OHHL) binds to the LuxR protein, generated from *lux* genes, to form a complex that triggers binding between OHHL/LuxR complex and *lux* operons to activate gene induction, along with more production of LuxR and OHHL. A high population density of cells increases concentration levels of intra-cellular and extra-cellular OHHL, initiating an auto-induction process among the cells. This process generates a phenotypic response from the cells, which produce bioluminescence.

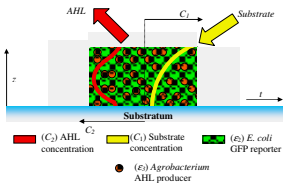
OBJECTIVE

To mathematically model the diffusion process, related to the *quorum* sensing phenomenon on artificially structured microbial consortia (ASMC).

METHODS

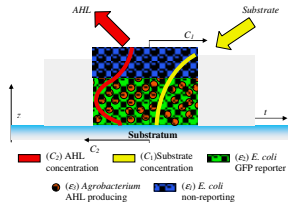
Mason *et al.* (2005) performed experimental studies on OHHL (*N*-3-oxohexanoyl-L-homoserine lactone or 3O-C6-HSL) and OOHL (*N*-3-oxooctanoyl-L-homoserine lactone or 3O-C8-HSL) accumulation in biofilms with artificially defined internal architectures. The systems were used for studying AHL accumulation in single and double layered biofilms. The *E. coli* JM105 strain, which has a GFP reporter gene that produces green fluorescence in presence of AHL substances, was used to make any AHL interactions visible and measurable. The experiments could broadly be divided into two different types: a) OHHL accumulation in mono layered biofilms and b) double layered biofilms. The biofilm structures and situations under study were:

a)



A single-layer mixed biofilm constructed with *E. coli* with a GFP reporter gene for AHL and *Agrobacterium tumefaciens* producing OOHL (*N*-3-oxooctanoyl homoserine lactone or 3O-C8-HSL) within the biofilm.

b)

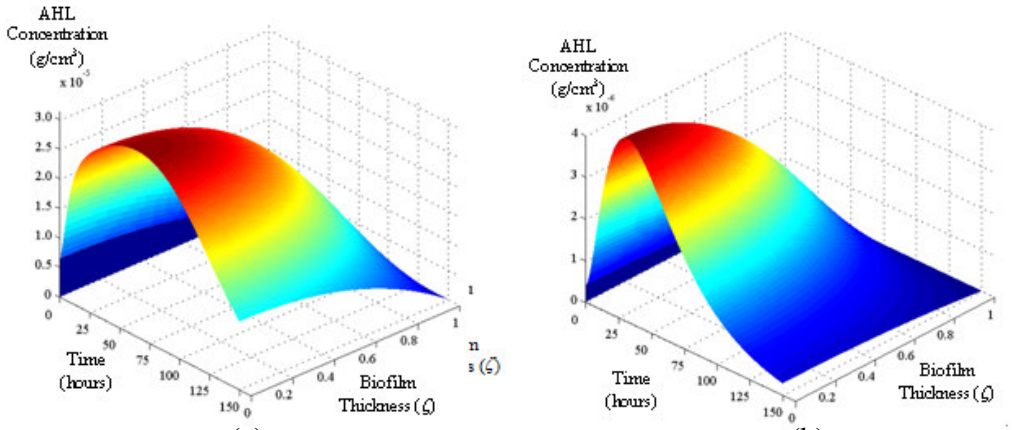


A double-layer mixed biofilm constructed with a mixed layer of *E. coli* with a GFP reporter gene for OOHL and *Agrobacterium tumefaciens* producing OOHL, at the bottom, and *E. coli* without any reporter for OOHL (*N*-3-oxooctanoyl homoserine lactone or 3O-C8-HSL) at the top.

These systems were studied using a multi-species biofilm model to predict cell distribution, concentration profiles of extra-cellular substances and biofilm thickness over time. Such calculations were useful for studying the effect of the biofilm structure on diffusion and accumulation of AHL, as well as the effect of AHL concentration gradients on cell growth.

RESULTS

Figure 1 shows the calculated AHL concentration profiles for each one of the four experimental ASMC architectures aforementioned.



(a) (b)

Figure 1. AHL concentration profiles for: (a) mono-layered; (b) double layered ASMC with internal production.

These results show that internal production of AHL takes longer and follow a different diffusion process than external addition. Therefore Figure 1(c) and (d) represent internal production and accumulation according to a more natural *quorum* sensing process, in which internal diffusion plays an important role in cell-cell communication. A double layered ASMC with internal production of AHL exhibits a faster and larger internal accumulation of AHL, which might lead to a diffusion regulated gene transcriptional processes from within multispecies biofilms.

CONCLUSIONS

A deterministic mathematical model, based on diffusion mass transport, has been applied for modeling artificially structured microbial consortia (ASMC), in order to

study diffusion regulated *quorum* sensing phenomenon, which affects cellular behavior of environmental biofilms. Finally, diffusion barriers within biofilms play an important role in cell-cell communication and regulates gene response of cells, therefore, extracellular mass transport by diffusion and internal biofilm structure are related to metabolic interactions of multispecies cell consortia

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