A Formal Model of Early Biofilm Formation

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Abstract. Biofilms are structured communities of bacterial cells adherent to a surface. This bacterial state is called *sessile*.
This presentation focuses on the modelling of the transition between planktonic and sessile state using Real-time Maude as the modelling language. With more and more bacteria joining the sessile community the likelihood of producing a biofilm increases. Once the percentage of bacterial cells that adheres to the surface reaches a threshold, which is specific for the considered bacterium species, a permanent biofilm is created. An important challenge is to predict the time needed for the formation of a biofilm on a specific surface, in order to plan when the material infrastructure that comprises such a surface needs to be replaced. We exploit the model-checking features of Real-time Maude to formally prove that a regular cleaning or replacement of the infrastructure prevents the biofilm formation.

Keywords: Biofilms · Formal Methods · Rewriting logic · Real-Time Maude.

1 Introduction

Biofilms are microstructured bacterial communities that live at interfaces. They are the most common mode of life for microorganisms in both terrestrial and marine environments. Usually biofilms thrive at liquid/solid interfaces like the inner surface of water pipes [9] and catheters [8], or in soil and sediments [2]; biofilms play a beneficial role in wastewater treatment, where they increase organic carbon degradation and contaminant removal. However, biofilms harbor pathogens and protect them from antimicrobial agents, thus posing serious health threat in health settings. Biofilms consist of bacterial cells encased in self-produced extracellular polymeric substances, collectively termed biofilm matrix. The biofilm matrix is composed of carbohydrates, proteins and extracellular DNA. The relative proportion of these components is species-dependent and varies with biofilm age and nutrient concentration [4]. In the biofilm life cycle, the most important step is the initial attachment, in which the cells transition from free-swimming state (so-called *planktonic*) to *sessil* state, in which they attach to solid surface, lose their motility and start producing the extracellular matrix, in addition to the normal growth process [1]. Modelling of initial biofilm formation is important to predict the extent of biofilm growth on removable biomedical devices (e.g., catheters) and drinking water pipes, thus allowing planned replacement or cleaning of biofilm-contaminated parts and minimising the risk of infections [3]. The effectiveness of cleaning treatment depends also on biofilm concentration, thus the formal modelling of initial biofilm formation will allow optimising the frequency and the duration of antimicrobial application in biomedical devices and water systems.

Our presentation illustrates a formal model of the transition from planktonic to sessile state. We use Real-Time Maude [5, 7], a formal modeling language and high-performance simulation and model checking tool for distributed real-time systems, to model this transition. It is based on Full Maude, the object-oriented extension of Core Maude, which is the basic version of Maude [6].

In Section 2 we present the model of the bacterial population and the transition of bacteria from planktonic to sessile, which leads to the biofilm formation, as well as the model of the planned intervention. In Section 3 we briefly illustrate how the model-checking features of Real-time Maude can be used to anayse the effect of an intervention and formally prove that a regular cleaning or replacement of a material infrastructure prevents the biofilm formation.

2 Biofilm Formation Model

We model a bacterial population in real-time Maude as a multiset of object of a class `Bacterium` with two attributes: `state`, which can be either `planktonik` or `sessile`, and `toDuplication`, which is the average lifetime of a cell before duplication. We define a `transition` constructor to record the number of transitions from planktonic state to sessile state that occurred during the last minute. Thus `transitions: M out of TOTAL during the last minute` records that `M` bacteria out of `TOTAL` changed their state during the last minute.

The population evolution is controlled using three states, `reproduce`, `aged` and `done`, as follows:

- State `aged` denotes that the population is initially set at time 0 or has been aged by 1 time unit (1 minute); this population state enables the rewrite rule that models the transition from planktonic to sessile without changing the population state and the rewrite rule that models the population state change to `reproduce` when the population has completed the transitions to sessile for the current minute.
- State `reproduce` denotes that old cells are ready to reproduce and enables the rewrite rule that models reproduction for the cells that have reached the required age.
- State `done` denotes that the population has completed reproduction for the current minute; this population state enables the *tick* rewrite rule, which ages the population by 1 minute and changes the population state back to `aged`.

Since we are interested in considering an intervention to prevent the biofilm formation, we actually need thrtee `tick` rewrite rules for the three cases of absence of intervention, planned cleaning and planned replacement.

For example, the rewrite rule with replacement intervention is as follows:

```
rl [tick-replacement] :
  {transitions: M out of TOTAL during the last minute
   population(done, N)
   (intervention replacement in T1 time units)
   REST}
=>
  {transitions: 0 out of TOTAL during the last minute
   population(aged, N)
   (intervention replacement in (T1 monus 1) time units)
   idle(REST,1)}
in time 1 .
```

The rewrite rule determines the population aging by 1 minute by using the operation `idle`, which is recursively defined as follows:

```
var B : Oid .
var M : Msg .
var REST : Configuration .
vars T T1 : Time .

eq idle(none, T1) = none .
eq idle(< B : Bacterium | toDuplication : T > REST, T1) =
        < B : Bacterium | toDuplication : T monus T1 >
        idle(REST, T1) .
eq idle(M REST, T1) = M idle(REST, T1) .
```

where `monus` is a subtraction that returns 0 when the standard subtraction gives a negative number. Thus all bacteria are recursively aged by 1 minute.

The `tick-intervention` rewrite rule also decreases the time to the next replacement intervention by 1 minute. Then, when the time to the next replacement intervention becomes 0, the following rewrite rule becomes enabled:

```
rl [replacement] :
  {(intervention replacement in 0 time units)
   REST}
=>
  init (intervention replacement
            in INTERVENTION-FREQUENCY time units) .
```

where `init` is the population with all bacteria in planktonic state.

3 Formal Analysis of the Intervention

Our model uses a number of parameters to characterise the bacterial population: initial population size, average number of bacterial state transitions per minute, average cell age at the time of reproduction and a sessile population size threshold that characterises the formation of a biofilm. The values of these parameters are determined through experiments.

Additionally, the `INTERVENTION-FREQUENCY` parameter models the time between two consecutive interventions. We use the Real-time Maude model-checking features to determine whether a specific value of the `INTERVENTION-FREQUENCY` parameter may prevent the biofilm formation. For example, in an initial setting that includes a planned intervention, we can use the Maude `search` command to look for a system state in which the biofilm threshold is reached. If the search does not returns any solution then the planned intervention is effective.

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