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Simulation of phage dynamics in multi-reactor models of complex wastewater treatment systems

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ABSTRACT

Existing population models of bacteria and phages have most commonly described single well-mixed reactors. Whilst crucial to gain an understanding of population dynamics, these models are not directly applicable to scenarios requiring multi-reactor models. Using standard approaches we have formulated these models to describe behaviour within multiple interconnected reactors. A model incorporating phage dynamics in wastewater treatment systems was then developed to investigate the potential use of phages as a tool to reduce foaming or bulking. The model couples wastewater treatment dynamics within the commercial software GPS-X (Hydromantis Inc.) with phage dynamics through a newly developed add-on called PhageDyn. Simulations predict that immediately after phage dosing there is a *lag period* during which no apparent changes are observed, which is followed by a sudden and quick increase in the phage concentration and reduction in foaming biomass. "Normalization" without foaming is achieved within 1–2 weeks after dosing. The system may subsequently relapse back to foaming, requiring additional phage dosing, or be "cured" such that the added phages keep the problematic foaming biomass indefinitely at bay. Kinetic parameters describing the behaviour of the phages, as well as reactor configuration, are key determinants of the final outcome. The model is useful for predicting potential behaviour and for determining the characteristics of phages that are likely to be successful, assisting in isolation and screening.

1. Introduction

The activated sludge process is the one of the oldest and most common approaches for treating wastewater in commercial treatment plants. This approach employs bacteria and other microorganisms to degrade contaminants so that the water is safe to discharge into the environment. The microbial flora in activated sludge exhibits great species diversity [1]. If the composition of the bacterial flora in activated sludge changes significantly, operational problems can take place that affect and reduce the performance of the wastewater treatment process.

The formation of excessive amounts of stabilized foam is a problem that affects treatment plants worldwide and has a negative impact on the treatment process. Foam is notoriously difficult to mitigate as no universal strategy is known that can address the issue without also negatively impacting on process performance [2]. It has been postulated that stable foam formation requires three factors; surfactants, air bubbles and hydrophobic particles [3]. Surfactants are present mainly due to industrial and domestic use of soaps and washing liquids, while air bubbles are generated in the aeration stages of wastewater treatment plants to enable oxidation of biodegradable waste products. As such, the first two factors cannot easily be controlled. The contribution of the third factor, hydrophobic particles, may come from certain filamentous bacteria with hydrophobic cell walls. Excessive growth of these filaments may therefore lead to excessive formation of stable foam.

Phages (or bacterial viruses) have been considered as a potential tool to treat foaming for over a decade [4–6]. Phages inject their DNA into living bacteria. Infection can follow either the lytic or the lysogenic pathway [7]. In the lytic pathway, the phage DNA diverts bacterial metabolism to the production of new phage, which results in lysis and death of the bacterial cell. In contrast, in the lysogenic pathway, the phage DNA is incorporated into the bacterial genome where it stays dormant until an environmental stimulus triggers it the activation of the lytic pathway. Preying on bacteria,

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phages help to maintain ecological balance and microbial diversity [8,9]. They are pervasive throughout natural water systems and form an integral part of natural ecological systems [10]. Changes in the abundance of different phage strains have also been shown to correlate with changes in bacterial composition in wastewater [11], lending strong support to the potential use of phages to treat foaming.

As phages have a high degree of selectivity, they can potentially be used to specifically target the foaming bacteria whilst leaving the remaining biomass untouched. In theory, this should address the cause of the foam formation whilst minimizing any negative effects on the treatment process. Nevertheless, the application of phages into large scale commercial wastewater treatment plants requires adequate planning and testing to ensure that the treatment process will not be adversely affected. Computational modelling has a role to play in predicting the behaviour of phages and likely outcome for the treatment plant. It may also assist in determining the most important factors for optimal treatment.

Computational models of wastewater treatment, as well as phage dynamics, have been studied for decades. The first widely accepted model for simulation of wastewater treatment was the *Activated Sludge Model 1* (ASM1), formulated and introduced by the International Association on Water Quality (IAWQ) in 1987 [12,13]. It is the simplest mathematical model that can realistically predict the performance of carbon oxidation, nitrification and denitrification in activated sludge systems. Subsequent more complex models have been proposed that handle additional processes such as nitrogen and phosphorus removal, processing of solubilized minerals and formation of precipitates [13,14]. Computational models of bacteria and phage populations were first reported in the early 1960s by Campbell [15] to describe population dynamics in continuous culture chemostats. Campbell's model was slightly refined 16 years later by Levin et al. [16] and this model has formed the basis for subsequent studies.

Most of the models of phage dynamics published to date have been developed to simulate experimental continuous and batch cultures [16–27], in order to increase our mechanistic understanding of the population dynamics. A number of models have been developed to simulate real-world scenarios, including phage therapy [28,29], disease outbreaks [30–32] and ocean systems [23,33]. The utility of such models in practical applications is likely to increase in the coming years, as phages are increasingly recognized as a useful tool for the control of problematic bacteria; in particular in medicine due to the increasing problem of multi-resistant pathogenic bacteria that cannot be eradicated by antibiotics [34].

To our knowledge, computational models of bacteria and phage populations geared towards simulation of complex real-world scenarios have mostly included dynamics of single well-mixed reactors. Such models provide valuable insights regarding the fundamental dynamics of phage and bacterial interactions. However, to simulate phage applications in the context of wastewater treatment plants, models involving multi-reactor systems need to be developed. Due to the delay terms present in the basic models of bacteria and phage populations, integrating multi-reactor systems presents a challenge. One way to circumvent this problem is to reformulate the *delay differential equations* (DDEs) into *ordinary differential equations* (ODEs).

No study to date has reported the integration of phage dynamics into models of activated sludge and wastewater treatment. Such integration requires proper consideration of the biological aspects of bacterial decay and recycling of material due to phage infection and lysis. In this study, we demonstrate how this can be done within ASM1 but the same principle also applies for more complex models of wastewater treatment. We achieved this integration by developing a Java-application, which based on user-specified parameter values, modifies library files of GPS-X, a commercial software for modelling wastewater treatment plants.

2. Mathematical models

2.1. Computational models of bacteria and phages

The key mechanisms in computational models of bacteria and phage populations include bacterial growth, phage infection and proliferation (see Krysiak-Baltyn et al., 2016 [35] for a recent review). Bacterial growth is typically modelled using either a Logistic or Monod growth expression [36], shown in Eqs (1) and (2), respectively:

$$\phi = X \cdot \mu_{max} \cdot \left(1 - \frac{X}{C}\right) \tag{1}$$

$$\phi = X \cdot \frac{\mu_{max} \cdot S}{k_S + S} \tag{2}$$

where

ϕ = rate of bacterial growth (h^{-1} mL^{-1})
X = concentration of bacteria (mL^{-1})
μ_{max} = maximum specific growth rate (h^{-1})
S = concentration of substrate (mg/mL)
C = carrying capacity (mL^{-1})
k_S = half velocity constant (mg/mL).

For the sake of bacterial control and reduction, only phages undergoing the lytic pathway are relevant. Phage infection according to the lytic pathway is assumed here to follow a three-step process where;

1) A phage attaches to a receptor on the bacterial cell surface.
2) The phage injects its genetic material into the cell. The metabolism of the bacteria gets hijacked and redirected to produce new phage particles inside the cells. Once a critical number of phage particles have been produced inside the bacterial cell, it undergoes lysis (bursts open) and dies.
3) Newly formed phages are released into the environment to repeat the cycle.

Three key kinetic parameters describing the process of lytic phage infection are the adsorption rate of phages to bacteria, the latency time (time between infection and lysis) and burst size (number of new phage particles produced per bacterial cell). The rate of adsorption (and infection) is assumed to be directly proportional to the concentration of phages and bacteria. The rate of production of infected cells X_I, is described by the following mathematical expression:

$$r_I = K_i \cdot X_S(t) \cdot P(t) \tag{3}$$

where

r_I = rate of formation of infected cells per time unit (h^{-1} mL^{-1})
$X_S(t)$ = the concentration of uninfected bacteria susceptible to phage infection (cells/mL)
$P(t)$ = the concentration of phages (phages/mL)
K_i = adsorption rate of phages onto bacterial cells (mL/h)

After infection, an amount of time, termed the *latency time (T),* passes before the bacterial cell lyses to release a certain number of new phage particles, termed the *burst size (b)*. Therefore, the rate at which new phages are produced due to lysis is expressed by:

$$r_P = b \cdot K_i \cdot X_S(t - T) \cdot P(t - T) \tag{4}$$

where

r_P = the rate of phage production due to bacterial lysis (h^{-1} mL^{-1})