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Modeling phage induced bacterial disinfection rates and the resulting design implications

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ABSTRACT

The phage induced disinfection rates of *Escherichia coli* K-12 MG1655 in the presence of coliphage Ec2 were determined under a wide range of phage and bacterial concentrations. These rates were elucidated to determine if phages could be used in water and wastewater treatment systems as a biological based disinfectant. Disinfection rates ranging from 0.13 ± 0.1 to $2.03 \pm 0.1\ h^{-1}$ were observed for *E. coli* K12. A multiple linear regression model was used to explain the variance in the disinfection rates, and this model demonstrated an interaction effect between the initial phage and bacterial concentrations. Furthermore, the results were modeled with particle aggregation theory, which over predicted the disinfection rates at higher phage and bacterial concentrations, suggesting additional interactions. Finally, the observed and predicted disinfection rates were used to determine additional design parameters. The results suggested that a phage based disinfection process may be suitable for the inactivation of specific pathogens in plug flow reactors, such as the pathogens in hospital wastewater effluents and the bacteria responsible for foaming and sludge bulking in activated sludge processes.

1. Introduction

In spite of the advantages of our current disinfection processes, there are situations where it would be advantageous to specifically inactivate a desired organism. Specifically, for wastewater treatment, the filamentous bacteria responsible for foaming and sludge bulking are problematic, because they are difficult to control without interfering with the activated sludge process (Martins et al., 2004; Soddell and Seviour, 1990). Therefore, a disinfectant that could target the specific filamentous bacteria and not interfere with the activated sludge process would be ideal.

The use of phages as a disinfection agent is one possible option for controlling specific bacterial targets. Phages are obligate viruses that are specific to bacteria, and globally phages outnumber bacteria 10 to 1. Due to their abundance, phages are considered the most diverse type of life on the planet (Hendrix, 2002). Phages are promising disinfection candidates because of the following: they are easy to isolate from a variety of environmental sources; they are specific to an organism, which causes them to avoid unintended targets; and finally, phages have autocatalytic properties, in which the phage population increases over time (Abedon, 2009; Bergh et al., 1989). Furthermore, phages have been found effective in controlling bacterial growth in a wide variety of settings ranging from human health, agriculture, animal husbandry, biofilms, and engineered systems (Choi et al., 2011; Goodridge, 2004; Kay et al., 2011; Kudva et al., 1999; Merril et al., 1996; Sharma et al., 2005;

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Thomas et al., 2002; Worley-Morse et al., 2013; Zhang and Hu, 2013).

Regarding the use of phages in engineered applications, phages may be an advantageous option for inactivating the filamentous bacteria responsible for foaming and sludge bulking, because they can specifically target the desired organisms (Choi et al., 2011; Petrovski et al., 2012; Thomas et al., 2002). Since phages are autocatalytic, and the phage concentration increases after cellular lysis, the return sludge may be used to produce a continual supply of phages, which may control the long-term overgrowth of filamentous bacteria. Furthermore, due to the low cost of phages, it might be advantageous to use phages to inactivate pathogens in hospital wastewater effluents on-site when the construction of a traditional on-site treatment facility is cost prohibitive (Pauwels and Verstraete, 2006). Unfortunately, the application of phages in industrial and commercial settings has remained limited due to challenges with phage resistance, challenges in biofilm penetration, and difficulties in consistently modeling and predicting their treatment efficacy.

Various methods have been employed to understand and predict the final bacterial and phage concentrations after an interaction event. Generally, a multiplicity of infection (MOI) ratio, which is the ratio of phages in plaque forming units (PFU) to bacteria in colony forming units (CFU), is used to characterize and describe phage and bacterial interactions. A MOI of 10 with sufficient time for the autocatalytic phage growth is considered sufficient for treatment (Abedon, 2009). However, there have been concerns that different ratios of phages to cells with the same MOI have different treatment outcomes. For example, an MOI of 10 with a ratio of $10^6/10^5$ PFU/CFU might have a different outcome compared to a ratio of $10^8/10^7$ PFU/CFU, which also has an MOI ratio of 10, because phage and bacterial collisions are much more likely with the higher initial phage and bacterial concentrations.

In addition to using MOI as a tool to understand the treatment outcome, various mathematical models have been proposed (Abedon, 2009; Cairns et al., 2009; Kasman et al., 2002a, 2002b; Payne and Jansen, 2000). These models highlighted the complexities in dealing with the autocatalytic systems of phages and bacteria, and the importance of the initial phage and bacterial populations in determining the treatment outcome. For example, Payne and Jansen (2000) demonstrated that the autocatalytic effect of phages, which results in more bacterial infections due to the resulting cellular lysis and release of additional phages, is greatly diminished or absent at lower bacterial concentrations. Experimentally, this has been verified by Wiggins and Alexander (1985), who demonstrated that the phage 80α population did not increase in *Staphylococcus aureus* cultures of less than approximately 10^4 CFU/mL. Additionally, even the timing of the phage treatment drastically affects the outcome as Payne and Jansen (2001) demonstrated with kinetic models. While these models were useful in highlighting the complexities of the dynamics between phage and bacterial interactions, further work is needed to predict the treatment disinfection rates as function of the initial phage and bacterial concentrations, so that the potential uses of phages as disinfection agents in engineered systems can be evaluated.

Thus, in order to determine the potential role of phages as a disinfectant in engineered systems, it is pivotal to perform the following: determine the disinfection rates of bacteria in the presence of phages; accurately model and predict these disinfection rates under a wide range of phage and bacterial concentrations; determine the appropriateness of using MOI for characterizing phage and bacterial experiments; and understand the design constraints based on these parameters. To this end, in the present study, we set out to measure disinfection rates for *Escherichia coli* K-12 MG1655 and coliphage Ec2 under a wide variety of phage and bacterial concentrations, ranging from 10^6 to 10^8 CFU/mL and 10^6 to 10^8 PFU/mL at room temperature (20–22 °C) in a substrate limiting growth medium. To predict the disinfection rates in terms of the initial phage and bacterial concentrations, two different models were developed and evaluated: first, a numerical method involving a particle aggregation model was used; and second, a statistical method involving a multiple linear regression model was used. Finally, the observed and modeled disinfection rates were used to determine the implications for treatment design in a plug flow reactor (PFR) and a continuous stirred-tank reactor (CSTR).

2. Materials and methods

2.1. Strains and growth conditions

E. coli K-12 MG1655 (American Type Culture Collection® 700926) and the coliphage Ec2 were used in all experiments. *E. coli* K-12 MG1655 was propagated in Luria Bertani (LB) growth medium and fresh cultures were obtained weekly by plate streaking from frozen stocks stored at −80 °C. Coliphage Ec2 was isolated from the North Durham water reclamation facility (Worley-Morse et al., 2013). Phage stocks of Ec2 were created by a modified phage stock plate lysis and elution method of Sambrook and Russell (2001). Briefly, 200 mL of late-log *E. coli* K-12 MG1655 was mixed with 100 μL of a phage stock dilution that gave semi-confluent lysis. To each plate with semi-confluent lysis, 5 mL of SM buffer (100 mM NaCl, 10 mM $MgSO_4$, 50 mM Tris-CL [pH 7.5], and 0.01% gelatin) was added and the plates were placed on an orbital shaker for 4 h at 100 revolutions per minute (RPM). The plate was briefly washed with an additional 1 mL of SM buffer and the resulting liquid was added to the previous mixture. The phage stocks were centrifuged at 6500 relative centrifugal force (RCF) for 5 min. The supernatant was filtered with a 2 μm polypropylene syringe filter to remove any smaller bacterial debris. Stocks were quantified, diluted, and re-quantified to verify the concentration and stored at 4 °C. Coliphage Ec2 stocks were stable at 4 °C, and after one month no observable phage titer loss was detected.

2.2. Experimental design

E. coli K-12 MG1655 was exposed to a range of concentrations of coliphage Ec2, and the bacterial populations were quantified hourly over 4 h using a viable colony plate count method, which is explained in further detail below. In addition to the bacterial counts, phage counts were enumerated for the