Enhancing natural wastewater treatment systems: the role of particles in sunlight-mediated virus inactivation (PARVIRDIS)

Effective low-tech wastewater treatment systems are needed to combat the high level of population lacking basic sanitation. Constructed wetlands utilize natural treatment processes such as adsorption, sedimentation, and solar irradiation to attenuate the concentration of pathogens in water. These characteristics make them attractive candidates for sustainable, low-tech treatment. Two factors that limit the advancement of these systems are our inability to measure their efficiency in pathogen inactivation, and their poor removal of waterborne viruses, one of the most important classes of pathogens. Given these limitations, three major goals were planned for this project:

1. To improve molecular methods to measure virus infectivity.
2. To create an alternative wetland matrix with high viral removal capacity
3. To couple the adsorptive and photoreactive properties of the matrix to enhance virus inactivation

In two separate publications, we aimed to determine if qPCR, which measures genome integrity, could be used to estimate viral infectivity. Bacteriophage MS2 was used as the viral surrogate, and an exhaustive analysis of genome damage was done by assaying the entire coding region with qPCR. This assay was used to characterize damage after exposure to three common disinfection methods: heat, UV, and the reactive oxygen species called singlet oxygen. We found that the treatments had varying effects on genome integrity, with some treatments, such as heat, causing no damage to the genome. Using a genome-based method, like qPCR, to determine viral infectivity after treatments that do not affect the genome is therefore bound to fail. In a follow-up study, we created a framework for using quantitative PCR to estimate virus infectivity after UV inactivation. With this method we show damage to short sections of the genome (measured with qPCR) can be used to predict the total amount of genomic damage, and therefore, viral infectivity. These new methods were subsequently used to study virus adsorption and inactivation on wetland matrices.

Earlier studies had shown that metal-oxides were more effective at adsorbing viruses than the sand that is typically used as a wetland matrix. However, the fate of the viruses adsorbed onto these metal oxides was not fully understood. The new molecular methods would allow us to determine whether viruses had adsorbed or desorbed from the matrix, and whether they remained infective. In these studies, adsorption onto an iron-oxide coated sand (IOCS) caused >99.9% reduction in the concentration of viruses in solution. Furthermore, a large quantity of viruses could be loaded onto the IOCS under standard conditions (maximum adsorption density of 4e10 infective viruses/g IOCS in pH 7.5 Tris buffer with conductivity of 1250 μS/cm). However, environmentally relevant changes in the pH and the concentration of dissolved organic material prevented the adsorption of unbound viruses and led to the release of previously adsorbed viruses. Unlike earlier studies, we found that the released viruses remained in an infective state.

Given that adsorption alone was not inactivating viruses, we attempted to take advantage of the photoreactive iron-oxide surface onto which the viruses were adsorbing. Iron-oxides can react with sunlight and hydrogen peroxide via a Fenton-like reaction to produce a highly reactive oxidant, the hydroxyl radical. Given their high reactivity, hydroxyl radicals are rapidly consumed in solution; therefore, their concentrations are highest at their source, in this case, the iron-oxide surface, and decrease to negligible levels after short distances. We hypothesized that the viruses adsorbed onto the IOCS would be directly adjacent to the hydroxyl radical-producing surface, and that their proximity would increase their inactivation rate. Indeed, viruses that were adsorbed onto IOCS and exposed to simulated sunlight were inactivated more rapidly than those in suspension (99.9% inactivation vs. 90% inactivation after an 8-h sunlight exposure). The qPCR method described above was utilized to verify that the viruses were in fact inactivated by the treatment, and not merely irreversibly adsorbed onto the IOCS.

Our analysis of virus adsorption onto IOCS showed that this process is mainly governed by electrostatic interactions. These data are being used to model virus adsorption based on viral and matrix surface characteristics, as well as changes in solution conditions (pH, organic material, and ionic strength). Modeling inactivation, however, does not appear as simple because the two viral surrogates (MS2 and ΦX174) showed large differences in susceptibility to inactivation, despite having similar surface charges.

Conclusions

Creating methods to determine the viral health threat of drinking and wastewater is a top priority for environmental engineers. PCR has great potential for virus detection, but it has failed to differentiate between infective and inactivated viruses. In our two studies, we show which forms of viral disinfection are most amenable to qPCR analysis, and offer two improvements (one methodological and one analytical) to detect infective viruses. The benefits of these methods extend beyond environmental engineering into the fields of public health and medicine, where the detection of infective viruses is also of great importance.

 The iron-oxide coated sand has a high capacity for adsorbing viruses, and offers a novel method for inactivating the viruses attached to the sand grains. We propose that the sand could be used in several configurations within a wetland to take advantage of the viral adsorption and inactivation. Firstly, wetlands could be configured with removable sections of IOCS without any emphasis on maximizing sunlight exposure. In such a configuration, the IOCS would be used mainly as a substrate onto which the waterborne viruses could be concentrated. After maximizing the adsorptive capacity of the IOCS, it could be removed and exposed to the sunlight to optimize photo-inactivation. The main benefit of this set-up is that the virus is concentrated from a large volume of water onto a small amount of IOCS. Once inactivated, the IOCS could be recharged by desorbing the viruses, and reinserted back into the wetland. Alternatively, the IOCS could be used in a polishing step after the majority of the organics have been removed. In this set-up, the water could flow through shallow beds of IOCS in which both virus adsorption and photo-inactivation occur simultaneously.

Ultimately, this project offers methods to advance the pathogen removal efficiency of constructed wetlands, a low-tech wastewater treatment method. By relying on natural processes and the renewable energy of the sun, the process has the potential to be more affordable and sustainable than conventional treatments. On-going efforts to model virus adsorption would allow us to predict the viral removal efficiency of wetlands based on numerous viral, matrix, and solution parameters. Such a model would aid in the design and optimal operation of constructed wetlands.