LAB #5
MICROBIAL REMOVAL AND INACTIVATION VIA FILTRATION

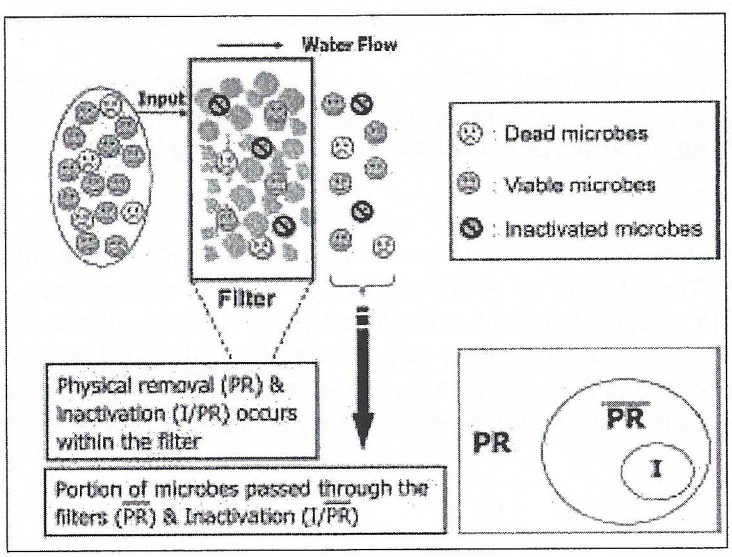
Introduction

First implemented circa 2000 B.C., filtration is one of the oldest water treatment technologies known to mankind. In the 1880s, filtration was implemented in the United States in an attempt to improve water quality by removing turbidity. In 1989, the Surface Water Treatment Rule, which requires widespread filtration of drinking water, was passed by the U.S. Environmental Protection Agency. Today, rapid filtration through granular media is the most common filtration technology. It relies on the use of coagulants to destabilize particulate matter prior to filtration. Conventional filtration following coagulation, flocculation, and sedimentation processes can be used with most surface waters, even those with turbidity in excess of 1,000 NTU (Crittenden *et al.*, 2005).

Particles are removed during filtration by several mechanisms. Straining is the dominant mechanism in precoat and slow sand filtration. It occurs when the particles are larger than the void spaces in the filter. When the particles are smaller than the void spaces, they are removed by attachment resulting from close-range molecular forces, such as van der Waals. Rapid sand filtration, which is far more common than slow sand filtration, minimizes straining while encouraging adsorption. Filter performance is affected by the properties of the media, including grain size and size distribution, density, hardness, bed porosity, and specific surface area. The most common granular materials used in filters are sand, anthracite, coal, garnet, and ilmenite (Crittenden *et al.*, 2005).

Strategy to Calculate and Differentiate Between Removal and Inactivation in Combined Processes (Abbaszadegan *et al.*, 2006)

In many circumstances, microbes can be inactivated during filtration, and still go through the filter media. This exaggerates physical microbial removal since the dead microbes are not measured, but are instead assumed to be physically removed. To accurately assess microbial removal during filtration processes, the physical removal of the organisms as well as their inactivation should be measured. The diagram below shows mechanisms of microbial removal and inactivation as a result of filtration



Schematic mechanisms and Venn diagram of filtration including physical removal (PR) and inactivation (I) processes.

Culturable assays may be used to calculate total removal of microorganisms, including physical removal and inactivation. Total removal (TR) is computed using Eq. (1), and log removal of microorganisms (L) is defined by Eq. 2;

$$\text{Total Removal: } (TR) = (C_I - C_O) / C_I \qquad (1)$$

$$\text{Log Removal } (L) = -\text{Log}_{10}(1 - TR) \text{ for } 0 \leq TR < 1 \qquad (2)$$

where C_I is the number of culturable organisms in the influent, C_o is the number of culturable organisms in the effluent, and TR is total removal reported as a fraction of 1. Note that $TR = 0$ for no removal and $TR = 1$ for total removal. If there is total removal (TR = 1), the computed log removal would be the same as the original number of microorganisms spiked.

The total number of microorganisms (including both viable and inactivated) in the influent and effluent can be represented by T_I and T_o, respectively. Equations 3 and 4 could be used to differentiate between physical removal and inactivation.

$$\text{Physical Removal: } (PR) = (T_I - T_O) / T_I, \; PR_{bar} = 1 - PR \qquad (3)$$

$$\text{Inactivation: } (I/PR_{bar}) = [(C_I/T_I) - (C_O/T_O)] / (C_I/T_I) \qquad (4)$$

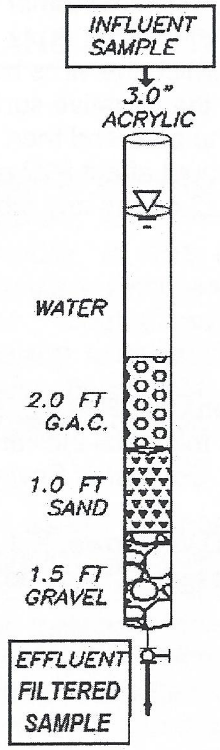
where PR and (I/PR_{bar}) are both fractions of one, PR_{bar} is the complement of PR (and is equal to the portion of microorganisms that passed through filters, whereas PR is the portion that did not pass through), and (I/PR_{bar}) is a portion of the microorganisms passing through the filter which are inactivated. Total log removal is the summation of physical log removal and log inactivation.

Procedure

During this lab, we will use a pilot-scale filtration apparatus to evaluate the physical removal and inactivation of indicator microorganisms. The following parameters will be used for the filtration experiments:

Flow Rate: 0.2 gallons/minute
Hydraulic Retention Time: 3 minutes
Filter Liquid Volume: 3.5 Liters

A schematic of the filtration apparatus is shown in the following figure.



A water sample containing the bacteria *E.coli* and the bacteriophage P22 will be filtered through the filtration apparatus. Samples will be collected from the influent and the effluent, and will be diluted and assayed for culturable microorganisms using the double agar layer technique (as described in Lab #3) for the bacteriophage and the spread plate technique (as described in Lab #1) for the bacteria. The appropriate plate counts for the microorganisms represent the number of culturable organisms, C_I and C_o.

In order to enumerate the total number of organisms (T_I and T_o), the number of microbes (both infectious and noninfectious) must be determined. This could be done by manually counting the number of organisms using a microscope (particularly effective for *Cryptosporidium*, but not for viruses) or automatically using real time quantitative PCR (qPCR).

Using the values of the four parameters (C_I, C_o, T_I, and T_o), the total removal, log removal, physical removal, and inactivation can be calculated using Eqs. 1 – 4, respectively.

Questions

1. What is the difference between microbial removal vs. inactivation? 1 pt
2. How does the removal/inactivation of P22 compare for the different treatment processes that we have studied (coagulation/flocculation/sedimentation [Lab 4], filtration [Lab 5], and UV disinfection [Lab 7])? Is this what you would expect? 6 pts
3. List three factors that may affect the removal of pathogens in filtration systems. Briefly describe how you would qualitatively expect these factors to impact removal (more or less removal). Why? 6 pts

4. Bacteria and viruses are generally smaller than the pore size of a filter, which limits physical removal by straining. Describe another mechanism that may play a role in microbial removal/inactivation by filtration. 3 pts
5. The isoelectric point (the pH at which the virus has no charge) of P22 is between 3 and 4. This means that at pH 3 – 4, the negative surface charge characteristic of viruses in natural waters (pH 7 – 8) drops to zero and then becomes positive. How do you think this change in surface charge would affect P22 removal during filtration? Would it affect the removal of bacteriophage P22 during any other treatment processes? Why or why not? 4 pts

References

Abbaszadegan, M., Monteiro, P., Ouwens, R., Ryu, H., & Alum, A. 2006. Removal and inactivation of *Cryptosporidium* and microbial indicators by a quaternary ammonium chloride (QAC)-treated zeolite in pilot filters. *Journal of Environmental Science and Health, Part A.* 41:1201-1210.

Crittenden, J.C., Trussell, R.R., Hand, D.W., Howe, K.J., & Tchobanoglous, G., Eds. 2005. *Water Treatment: Principals and Design, 2nd Ed.* John Wiley & Sons: Hoboken, NJ.