The Hunt for the Ideal Indicator Organism

by Melanie Mann

Routine monitoring of indicator organisms is an important tool for reducing the risk of disease from waterborne pathogens, whether disease occurs from consumption of drinking water or from incidental ingestion of recreational waters. Recreational waters of concern include ambient waters at beaches, lakes, and rivers where ingestion of water may occur during swimming or water contact may occur during other activities such as fishing. Recreational waters also include venues that use treated water, such as public swimming pools and water parks. Indicator organisms are also used to monitor water quality and microbial risks in other arenas, including shellfish harvesting, food production, biosolids treatment, and non-potable water reuse applications such as irrigation and aquaculture.

Indicator organisms are used because it is not practical to test waters for each and every potentially waterborne human pathogen that may be present in a given water sample. Pathogenic microorganisms include bacteria, viruses, and protozoan parasites.

This article reviews current and proposed groups of fecal indicator organisms used to monitor water resource recovery facility (WRRF) effluents and ambient recreational waters in the U.S., as well as the characteristics of ideal indicator organisms as compared to indicator organisms currently in use.

Characteristics of an Ideal Indicator Organism

The perfect fecal contamination indicator organism has not been found, but it is useful to consider what properties an ideal indicator organism would have. The ideal indicator organism should:

1. Be present when fecal contamination is present (in raw and treated waters)
2. Occur at concentrations higher than pathogens of interest
3. Persist in the environment similarly to pathogens of interest
4. Should not reproduce outside the host organism
5. Be relatively safe compared to pathogens to reduce the exposure risk to laboratory staff
6. Be detectable by a method that is simple, fast and inexpensive, as compared to specific pathogen detection methods
7. Occur in fecal waste but not occur from other sources. (list adapted from Pepper (2009) and Edzwald (2011)).

Although none of the current fecal indicator organisms meet all the criteria of an ideal indicator as listed above, the U.S. Environmental Protection Agency (USEPA) has periodically refined its list of recommended indicator organisms to better meet many of these criteria. In the U.S., the indicator organisms that have been used to establish regulatory limits for WRRF effluents and recreational waters have been specific groups of bacteria. Viruses and protozoa have traditionally not been used as indicator organisms for these waters due to several limitations.

Individual pathogens do not make good fecal contamination indicator organisms. While human fecal waste always includes non-pathogenic bacteria, it will only include pathogens when individuals are infected. Moreover, concentrations of pathogens tend to be lower than concentrations of traditional indicator bacteria in fecal waste, which can make the pathogen quantification more difficult.

Viruses meet the ideal criterion of not reproducing outside the host organism, since they require host cells for reproduction. By contrast, typical indicator bacteria, which are usually present when fecal contamination is present, can reproduce outside their host organism in the environment, posing some challenges when assessing the presence of fecal contamination. However, detecting and enumerating viruses is more complex, more time-consuming, and more expensive than detecting and quantifying typical indicator bacteria.

Although bacteria are overall a better match with the ideal criteria than pathogens or viruses, all bacterial indicators used in the U.S. have some potential nonfecal sources. Since the ideal indicator organism has yet to be found, several bacteria species which best meet the criteria are currently used as regulatory indicator organisms.

Current Indicator Organisms in WRRF Effluents and Recreational Waters

Several bacteria species, or groups of bacteria species, have been used in the U.S. as fecal indicator organisms in WRRF effluents and in recreational waters. Regulated bacterial indicators have included total coliforms, fecal coliforms, Escherichia coli (E. coli), and Enterococcus. These indicators have been incorporated into many states’ water quality criteria and into National Pollutant Discharge Elimination System (NPDES) permits for WRRF effluents. The concentration of indicator organisms in effluent is an indication of the effectiveness of the disinfection process at the facility; however, treatment processes upstream of disinfection also contribute to reducing the concentrations of pathogens and indicator organisms in the WRRF effluent.

Total coliforms are a large group of many bacterial species in the family Enterobacteriaceae. Total coliforms are found in higher concentrations in fecal waste than any other fecal indicator, and for this reason they are a good indicator of potential fecal contamination. Some members of the total coliform group can also grow in the environment, so while the presence of total coliform indicates potential fecal contamination, total coliforms alone do not prove fecal contamination. For example, the Revised Total Coliform Rule for drinking water (USEPA, 2013) requires testing for total coliform to indicate potential fecal contamination, with a positive result requiring additional tests for fecal coliform or E. coli for added specificity. Total coliforms are also sometimes used as indicator organisms in WRRF NPDES permits; however, most states now use one of the other more specific bacterial indicators for this purpose.

Fecal coliforms are a subset of total coliforms, including Escherichia coli, Enterobacter, and Klebsiella. Fecal coliforms have fewer nonfecal sources than total coliforms and as such they are a more specific indicator of potential fecal contamination. However, some members of the fecal coliform group can also occur in nonfecal wastes. Fecal coliforms are still the primary regulated indicator organism for many WRRF effluents in the U.S. E. coli is a member species of the fecal coliform group and is even less likely than fecal coliforms as a group to come from nonfecal sources. E. coli is thus a more specific indicator of fecal contamination than total coliforms and fecal coliforms. The USEPA also found E. coli was better correlated than fecal coliforms to rates of gastroenteritis in swimmers in fresh water, based on studies conducted in the 1970s and 1980s (USEPA, 1986). Therefore both
While USEPA’s studies in the 1970s and 1980s showed that Enterococcus and E. coli were both correlated to rates of gastroenteritis in swimmers in fresh water, the correlation in marine waters was much better for Enterococcus than for E. coli. Accordingly, the USEPA’s 1986 and 2012 RWQC recommended Enterococcus as the indicator of choice for marine recreational waters. Many states that used fecal coliforms as indicator organisms for WRRF effluents discharging to marine waters have changed, or are in the process of changing, to NPDES permit limits based on Enterococcus in marine waters.

Other organisms have been used for various purposes and for water quality and epidemiology research efforts; however, in the U.S. fecal coliforms, E. coli and Enterococcus are the most widely used indicator organisms for WRRF effluents and recreational waters.

Potential Future Indicator Organisms

Viruses that infect the current indicator organisms may themselves be used in the future as indicator organisms. Bacteriophages are viruses that infect bacteria, and coliphages are a group of viruses that infect E. coli and other coliform bacteria. Coliphages are classified as either somatic coliphages or male-specific coliphages, depending on the way they access and infect E. coli. Coliphages are of interest to the microbial monitoring community because of the potential that a viral indicator will more accurately indicate the presence of viral pathogens as compared to traditional bacterial indicators. Coliphages are of fecal origin, and are always present in raw wastewater, although they are not present in fecal waste of all humans at all times. Coliphages are similar in size to many pathogenic viruses, and they may have similar response to wastewater treatment and disinfection processes as human enteric viruses.

The U.S. Ground Water Rule (USEPA, 2006) already allows the use of coliphage to indicate potential fecal contamination of groundwater. Some water reuse regulations, such as those used in North Carolina (NCAC, 2011), also allow the use of coliphage for monitoring and disinfection of reclaimed water.

Challenges of Using Indicator Organisms to Monitor Wastewater Disinfection

Water resource recovery facilities in the U.S. use a variety of disinfectants, and each disinfectant has different rates of inactivation for the various fecal indicator bacteria (E. coli and Enterococcus), for the two types of coliphages (somatic and male-specific), and for the large range of waterborne human pathogens (bacteria, viruses, and protozoa). In addition, the relative density of the different bacterial and viral indicator organisms can vary with each facility’s effluent, and may even change with season. Therefore, effluents that have the same concentration of E. coli or Enterococcus may have different concentrations of coliphages or pathogens.

Wastewater NPDES permits are administered by each state, and WRRF disinfection process design and permit requirements vary among the states. As one example of differing permit requirements, some states require a chlorine contact time of 30 minutes at average flow rate, but require no minimum chlorine residual after the contact time as long as the effluent E. coli concentration is less than 126 colony forming units (cfu) per 100 mL, or the Enterococcus concentration is less than 35 cfu/100 mL, as a monthly geometric mean. Other states require the same 30 minutes of contact time with chlorination, and also require a minimum total chlorine residual such as 1.0 mg/L after the contact time, in addition to requiring the same E. coli or Enterococcus standard. The facility that is required to maintain a minimum total chlorine residual after the contact time is likely to use a higher chlorine dose and have lower effluent E. coli concentration and lower effluent pathogen concentrations than the facility that is required to meet the E. coli standard but has no minimum chlorine residual requirement.

A further difference among water resource recovery plants in the U.S. is that most of their effluents contain at least some ammonia. Therefore, upon addition of chlorine, the free chlorine species combine with ammonia to form chloramines, which are a slower-acting disinfectant than free chlorine. In contrast, facilities with very low effluent ammonia can have faster-acting free chlorine species in the disinfection process. The facility with a free chlorine residual is likely to inactivate pathogens more effectively - even at a lower applied chlorine dose or residual - than the facility with a combined chlorine residual. Both facilities may have the same indicator bacteria concentration based on E. coli in fresh waters.
ria permit limit, but they are likely to have different concentrations of pathogens in the effluent.

More than twenty percent of larger U.S. WRRFs disinfect with ultraviolet (UV) radiation instead of chlorine. UV disinfection differs from chemical oxidative disinfection in that UV radiation does not kill microorganisms. In sufficient doses, UV radiation inactivates microbes by damaging their genetic material, leaving them unable to reproduce and therefore non-infective. Molecular methods for pathogen detection such as polymerase chain reaction (PCR) can detect the presence of pathogen DNA or RNA, and quantitative polymerase chain reaction (qPCR) methods have been developed to estimate pathogen concentration. However, neither PCR nor qPCR can distinguish live pathogens from dead pathogens. Among the living pathogens, PCR and qPCR do not distinguish those that are capable of reproducing and causing infection from those that are inactivated and non-infectious. Therefore, the use of PCR and qPCR techniques in recreational waters under the impact of UV-treated wastewaters is likely to overestimate the number of infectious pathogens, as many will have been rendered non-infectious via UV disinfection.

Summary

The USEPA 2012 RWQC recommends *E. coli* (in fresh water) and *Enterococcus* (in fresh and marine waters) as fecal indicator organisms. The USEPA no longer recommends total coliforms or fecal coliforms for this purpose. However, the USEPA is currently developing recommendations for the use of coliphages as viral indicator organisms in recreational waters. Assuming this effort results in the incorporation of coliphages into USEPA RWQC, states may eventually adopt coliphage limits into their NPDES permits for WRRFs. Research is currently underway by the USEPA and other researchers to evaluate whether coliphages will be closer to the ideal indicator organism than *E. coli* or *Enterococcus*. 

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References


