

GLOBAL WATER PATHOGEN PROJECT

PART FOUR. MANAGEMENT OF RISK FROM EXCRETA AND WASTEWATER

UNDERSTANDING PATHOGEN REDUCTION IN SANITATION SYSTEMS: UNITS OF MEASUREMENT, EXPRESSING CHANGES IN CONCENTRATIONS, AND KINETICS

Marcos von Sperling

Federal University of Minas Gerais

Belo Horizonte, Brazil

Matthew Verbyla

San Diego State University

San Diego, United States

James Mihelcic

University of South Florida

Tampa, United States

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Summary

The purpose of this chapter is to provide readers of the individual chapters on onsite and sewer Sanitation System Technologies with background information so they may better understand: a) how pathogens are measured, b) how to express changes in pathogen concentrations, and c) how pathogen reduction (as covered here in the Sanitation Technologies chapters) relates to pathogen decay rates (as covered in the GWPP Persistence chapters) for sanitation technologies that are configured as batch or continuous flow systems with different degrees of mixing.

1.0 Enumerating Pathogens and Expressing Changes in Their Concentrations

1.1 Enumerating Pathogens

Pathogens in excreta and water are a diverse group of microorganisms that include from smallest size to largest, viruses, bacteria, protozoa, fungi, and the eggs of helminth worms. They are too small to see with the naked eye and are often present in low concentrations in water and biosolids. As such, there are several different ways pathogens are measured and enumerated in the laboratory. Each method has its own strengths and weaknesses. In fact, two different methods used to measure the same pathogen in the same sample may yield vastly different results! It is important for readers of these GWPP chapters to take this into consideration when interpreting data from the scientific literature.

The most common methods used to enumerate pathogens can be divided into three different classes: 1) culture-based methods, which measure the ability for pathogens to replicate and/or express certain enzymes *in vitro*, under specified laboratory conditions; 2) molecular and immunological methods, which involve the detection of specific antigens or segments of the pathogen's genome; and 3) microscopy-based methods, which require the laboratory technician to identify and count pathogen cells under a microscope (based on their physical characteristics). Sometimes, a combination of methods is used, such as immunofluorescence microscopy (combination of immunological and microscopy-based methods) or integrated cell culture and polymerase chain reaction (culture- and molecular-based method) for enumerating enteric viruses.

No single method is perfect. For molecular and immunological methods, the presence of a specific genome or antigen may indicate the presence of the pathogen, but does not necessarily confirm if the pathogen is viable or infectious. Also, with culture-based methods, some viable and infectious pathogens may not replicate or express enzymes in the laboratory, where conditions are not exactly the same as they are in the pathogen's natural habitat (i.e., inside the host). Microscopy-based methods are time-consuming and require a trained microbiologist to identify pathogen species (which is not always possible as some pathogens are morphologically indistinguishable from other microorganisms). Tables 1a and 1b provide a summary of some common types of culture-based, molecular, immunological, and microscopy-based methods used to enumerate pathogens, and show the units of expression that are associated with each method.

Table 1a. Common culture-based methods used to enumerate pathogens and determine their concentrations at different points in sanitation systems

Type of Method	Description of Method	Units of Expression
Endpoint Dilution	<p>MPN refers to “most probable number” which is a statistical approach used to estimate the number of pathogens in a sample based on dividing the sample into smaller sub-volumes (or by doing dilutions) and determining if the pathogen is present or not in replicates of each sub-volume (or dilution). One of the more familiar applications of this method is the IDEXX Colilert and Enterolert kits that are used to measure <i>E. coli</i> and Enterococci (which are not actually pathogens but faecal indicator bacteria; see GWPP Chapter entitled “General and host-associated bacterial indicators of faecal pollution”). This method is most commonly used for bacteria, but can also be applied to viruses in cell culture.</p>	MPN
	<p>TCID₅₀ stands for the “50% tissue culture infective dose” and is a statistical approach very similar to the most probable number method, but more frequently used for enteric viruses in cell culture. There are some fundamental differences between the statistical models used to calculate MPN and TCID₅₀, but the methods used to collect the raw data in the laboratory (endpoint dilution) are essentially the same.</p>	TCID ₅₀
Direct count (e.g., membrane filtration)	<p>CFU stands for “colony-forming units” and is based on the in vitro growth of bacterial pathogens in a selective and/or differential media. Samples are poured directly onto a Petri dish with growth media, or passed through a membrane that is transferred to a petri dish with growth media. After an incubation period, discrete colonies with the desired characteristics are counted. The assumption is that each bacterial colony (visible to the naked eye after incubation) originated from a single bacterium. This method is often stated to be more accurate and precise than the MPN method, though there are some exceptions, especially when the number of pathogens is low (Sutton, 2010).</p>	CFU
Plaque assay	<p>PFU stands for “plaque-forming units” and is used to enumerate enteric virus pathogens. The sample is added to a plate with a confluent layer of cells from a susceptible continuous cell line (commonly monkey kidney cells or human adenocarcinoma cells). Viable enteric viruses may infect one cell, and then spread to neighboring cells, causing cytopathic effects that create a plaque (which is visible to the naked eye after incubation and cell staining). The assumption is that each plaque originated from a single virus.</p>	PFU

Table 1b. Common molecular- and microscopy-based methods used to enumerate pathogens and determine their concentrations at different points in sanitation systems

Type of Method	Description of Method
Quantitative polymerase chain reaction (qPCR) and Quantitative polymerase chain reaction with reverse transcription (RT-qPCR)	Gene copies are used to describe the concentration of pathogens that were enumerated using the molecular method known as quantitative polymerase chain reaction (qPCR) or qPCR with reverse transcription (RT-qPCR). Samples are lysed to release genetic material, and biochemical reactions take place with specific primers that target a segment of the genome that is unique to the pathogen in question. Concentrations are determined by comparing unknown samples with a standard curve constructed from standards containing known quantities of pathogens or their genome segments. RT-qPCR is used to enumerate pathogenic viruses that contain an RNA genome instead of a DNA genome, it is different from qPCR because the RNA first needs to be reverse transcribed to produce cDNA, which can then be used in a polymerase chain reaction.
Modified Bailenger Method	Helminth eggs are often quantified in wastewater and sludge by concentrating a large volume liquid sample or extracting the eggs from the solids in a sludge sample, and then identifying and counting them under a microscope. Sedimentation, centrifugation and flotation techniques are most commonly used to separate the eggs from liquid and other constituents based on their specific gravity. The identification of eggs from helminth species that infect humans is done by experienced lab technicians, although image processing tools with pattern recognition algorithms have also been used (Jimenez et al., 2016). The units of measurement are reported as eggs. The following are challenges associated with the interpretation of data in the literature generated using these methods: The lab technician may only observe a fraction of the concentrated sample (some haemocytometers can only accommodate small volumes), which affects the limit of detection; the concentration process results in the loss of some portion of helminth eggs, and potentially favors some species over others (Ayres and Mara, 1996), however the percent recovery is seldom reported; assessing the viability of eggs requires a month-long incubation, so viability is often not assessed; the units of measurement (typically eggs/L) alone do not reflect whether or not the eggs are viable; the limit of detection is seldom reported in the literature.
Immunomagnetic separation and immunofluorescence microscopy	The cysts and oocysts of protozoan parasites such as <i>Giardia</i> and <i>Cryptosporidium</i> are typically concentrated from large volume water using either membrane filtration or high-speed centrifugation (Efstratiou et al., 2017). Immunomagnetic separation is then used to purify the cysts and oocysts from the concentrated sample. Specific antibodies conjugated to magnetic beads are added to the sample and magnets are used to separate the beads (to which the cysts and oocysts are attached). Fluorescent stains are applied to the sample to make the (oo)cysts easier to see under the microscope.

Units of concentration are simply reported as the unit of expression divided by some unit volume. By convention, this unit volume is most commonly 100 mL for bacteria, 1 L for viruses, and 1 L for protozoan parasites and helminth eggs.

1.2 Expressing Changes in Pathogen Concentrations

There are several important terms that are used to describe changes in the measured concentrations of pathogens along the sanitation service chain. Removal refers to the physical elimination of pathogens from wastewater. Often, pathogens removed from wastewater are simply transferred to sludge, where they may remain viable. Inactivation refers to the physical destruction of pathogens resulting in a loss of viability—this can happen to pathogens in wastewater or in sludge. Growth refers to the replication of pathogens in a sanitation system. Some opportunistic, zoonotic, and bacterial pathogens are capable of regrowth within sanitation systems (Jjemba et al. 2010), but parasites and enteric viruses require a human host to replicate, and cannot regrow within sanitation systems. Pathogens may become volumetrically

concentrated in smaller volumes of water or sludge due to evaporation or moisture removal (e.g. thickening, dewatering), which may cause the illusion of regrowth. To avoid this, pathogen concentrations in sludge can be expressed in terms of their number per dry mass instead of number per unit volume. In summary, pathogen concentrations in wastewater should be expressed as number per unit volume (typically per 100 mL for bacteria, per L for parasites and viruses, and sometimes per mL for viruses such as the coliphage). Pathogen concentrations in sludge or biomass should be expressed as number per unit dry mass (typically per g or kg for all pathogen types). Dry mass is sometimes reported by g TS, meaning grams of Total Solids, in the sense that the total solids analysis would give an indication of the dry matter present in the sludge or biomass.

The term reduction will be used throughout the sanitation technologies chapters to refer to the combined removal and inactivation of pathogens in wastewater systems. Unless stated otherwise, it is assumed that pathogen regrowth in sanitation systems is negligible. The efficiency of pathogen reduction in a particular sanitation system can be expressed as the percent (%) reduction

efficiency. The percent reduction efficiency, $E(\%)$, is quantified as follows, where N_0 is the influent pathogen concentration, and N is the effluent pathogen concentration (the units of concentration must be the same for N and N_0):

$$E(\%) = \frac{N_0 - N}{N_0} \times 100 \quad (1)$$

Pathogen concentrations are often discussed with respect to their order of magnitude, which is a way of comparing their relative size; in this context, base 10 comparisons are implied. For example, if one number is 10 ($=10^1$) times greater than another number, it is one order of magnitude greater. If a number is 1,000 ($=10^3$) times greater than another number, it is said to be 3 orders of magnitude greater.

In the case of pathogens and indicators of fecal contamination, the concentrations can be very high, and thus more attention is given to the order of magnitude of the concentrations instead of the absolute values themselves. For instance, a concentration of 183,098,765 MPN/100 mL is usually expressed as 1.83×10^8 MPN/100 mL, giving more emphasis on the order of magnitude of 108 and recognizing that there is not much accuracy on the digits that come after 183.

Given these high numbers, another way of expressing this concentration is by taking the \log_{10} of the original value (this is known as the **log₁₀-transformed concentration**). For instance, a concentration of 1.00×10^8 MPN/100 mL has a log-transformed value of 8.00 (i.e. $\log_{10}(1.00 \times 10^8) = 8.00$). Likewise, 1.46×10^8 MPN/100 mL has a log-transformed value of 8.16 (i.e. $\log_{10}(1.46 \times 10^8) = 8.16$).

An alternative to expressing pathogen reduction as a

percentage is to use the \log_{10} reduction value (LRV), which is defined as the difference between the log-transformed pathogen concentrations of the influent and effluent across a particular sanitation technology or across the whole system:

$$LRV = \log_{10}N_0 - \log_{10}N = \log_{10} \left(\frac{N_0}{N} \right) \quad (2)$$

The LRV is related to percent reduction, and one can be calculated from the other as shown in the following two equations:

$$LRV = -\log_{10} \left(1 - \frac{E(\%)}{100} \right) = \log_{10} \left(\frac{100}{100 - E(\%)} \right) \quad (3)$$

$$E(\%) = 100 \times (1 - 10^{-LRV}) \quad (4)$$

For instance, if the influent concentration is 1.00×10^8 MPN/100 mL and the effluent concentration is 1.00×10^5 MPN/100 mL, from Equation 1 it is seen that the reduction efficiency E is $(1.00 \times 10^8 - 1.00 \times 10^5) / (1.00 \times 10^8) = 0.999$, which, expressed as percent reduction efficiency $E(\%)$ is $0.999 \times 100 = 99.9\%$. In order to express in terms of LRV, using Equation 2, one has: $LRV = \log_{10}(1.00 \times 10^8) - \log_{10}(1.00 \times 10^5) = 8 - 5 = 3$. Alternatively, the calculation can be done using the second part of Equation 2: $LRV = \log_{10}[(1.00 \times 10^8) / (1.00 \times 10^5)] = \log_{10}(1.00 \times 10^3) = 3$.

Equations 3 and 4 can be used to convert $E(\%)$ into LRV and vice-versa. Using Equation 3 or 4, it is seen that an efficiency of 90% corresponds to an LRV of 1 \log_{10} unit; 99% @ 2 \log_{10} units; 99.9% @ 3 \log_{10} units; 99.99% @ 4 \log_{10} units; 99.999% @ 5 \log_{10} units and so on. This relationship between percent reduction efficiency (E) and \log_{10} reduction value (LRV) is shown in Table 2.

Table 2. Relationship between equivalent percent reduction efficiency and \log_{10} reduction values

Percent Reduction Efficiency (%)	Equivalent Log ₁₀ Reduction Value (LRV)
10%	0.05
25%	0.1
50%	0.3
75%	0.6
90%	1.0
95%	1.3
99%	2
99.9%	3
99.99%	4
99.999%	5
99.9999%	6
99.99999%	7
99.999999%	8
99.9999999%	9
99.99999999%	10
99.999999999%	11
99.9999999999%	12 ^a

^aNote: In California, USA, a 12 \log_{10} reduction of viruses is required for potable wastewater reuse

In the Sanitation Technologies chapters and other locations of the Global Water Pathogen Project, pathogen reduction efficiencies are generally expressed as LRVs, using \log_{10} units. This is because in some cases, pathogens in wastewater must be reduced by six or more orders of magnitude for the treated effluent or sludge to be safely reused, for example in agriculture (WHO, 2006). A reason for this is because it is cumbersome to refer to reduction as 99.9999%; it is much easier to say “6 log” reduction. Note that the term “log” here implies a base 10 logarithm (\log_{10}), even if the subscript 10 does not necessarily appear after “log”. Pathogen reduction in sanitation technologies is almost never described in terms of natural logarithms, but if the natural logarithm is used in this context, it is denoted by the notation “LN”.

It is possible to have a LRV that is greater than the order of magnitude of the pathogen concentration in the influent. For instance, a pathogen that has an influent concentration of 1.00×10^5 CFU/100 mL can be subjected to a treatment with a LRV of, say, 7. Rearranging Equation 2, it is seen that this will lead to $\log_{10}(10^5) - 7 = 5 - 7 = -2$. The effluent concentration will then be $10^{(-2)} = 0.01$ CFU/100 mL. Of course, in this case, one must check whether this value is below the detection limits of the lab method used to enumerate the pathogen in question (considering the dilutions made to the sample prior to analysis and whether or not the sample was concentrated from a larger volume). If the value is above the detection limit, it can be reported as such. If it is below the detection limit, this could be mentioned after the calculation.

For sanitation treatment units placed in series, the overall efficiency of the combined treatment units is given by the multiplication of the remaining fractions of the constituent in each unit, which can be difficult to conceptualize. For instance, in a complete treatment system there may be three unit processes (i.e. sanitation technologies) placed in series, with the following reduction efficiencies in Unit A = 90%, Unit B = 99.9%, and Unit C = 99%. In this situation, the overall reduction efficiency will be:

$$E_{\text{overall}}(\%) = 100 \times \left(1 - \left[\left(1 - \frac{90}{100} \right) \times \left(1 - \frac{99.9}{100} \right) \times \left(1 - \frac{99}{100} \right) \right] \right) = 99.9999\% \text{ (or } 6 \log_{10} \text{ unit reduction)}$$

However, if the pathogen reduction efficiencies in each unit are expressed as LRVs (Unit A = 1 \log_{10} , Unit B = 3 \log_{10} , Unit C = 2 \log_{10}), then the relationship between the units in

series is additive in terms of their individual LRV values, and much easier to calculate:

$$LRV_{\text{overall}} = 1 + 3 + 2 = 6 \log_{10} \text{ unit reduction (which is equivalent to } 99.9999\%)$$

2.0 Pathogen Reduction Kinetics and Process Models

Individuals who estimate (model) how pathogens are reduced across a particular sanitation treatment technology are interested in whether pathogen concentrations are reduced over time and how to mathematically describe the rate of pathogen reduction. Kinetics deals with the rate of a reaction of a constituent in a reactor, that is, its transformation. Reactors can be operated in a batch or continuous flow regimens. For continuous flow regimens, when you have both the kinetics of pathogen inactivation and the transport of pathogens through the reactor, the model is called a process model. The following are two idealized types of reactors used in process models that have different hydraulic behavior and mixing conditions: completely-mixed flow reactors (CMFRs) and plug-flow reactors (PFRs). It is important to note that these two idealized reactors never occur in real life, where hydraulic behavior and mixing conditions lie somewhere between these two idealized extremes. Other models that represent more realistic hydraulics and mixing have been developed, and the use of these process models together with reaction kinetics is covered in many textbooks on chemical engineering and wastewater treatment, such as Levenspiel (1999), Arceivala (1981), von Sperling and Chernicharo (2005), von Sperling (2007), Kadlec and Wallace (2009), Metcalf and Eddy (2014), Mihelcic and Zimmerman (2014).

2.1 Batch Systems

The reduction of pathogenic organisms frequently involves several mechanisms working simultaneously, and therefore it is usually difficult to model them individually. In almost all cases, pathogen reduction rates in sanitation technologies are sometimes expressed as pseudo first-order reactions. Note there may be some pathogen inactivation mechanisms that follow second-order kinetics, such as indirect sunlight-mediated mechanisms for the inactivation of viruses (e.g. Kohn et al., 2016). However, for the purpose of this book, we will assume that pathogen reduction in sanitation system technologies generally follows pseudo first-order kinetics. In this case, the rate of pathogen reduction is assumed to be proportional to the concentration at any given time, and is expressed as follows, where N is the pathogen concentration (i.e. MPN/100mL) at time t (i.e. minutes, hours, days) and k is

the reduction or decay rate coefficient (inverse units of time; i.e. minutes⁻¹, hours⁻¹, days⁻¹, etc.):

$$\frac{dN}{dt} = -kN \quad (5)$$

The integration of Equation (5) yields the following, where N_0 is the concentration at time $t = 0$:

$$N = N_0 e^{-kt} \quad (6)$$

Note that the effluent concentration (N), and thus the reduction efficiency, depends on the unitless pair $k \times t$. Therefore, to estimate the reduction efficiency of a sanitation treatment unit process that operates in batch mode, the reduction coefficient k and the retention time t are necessary inputs. For more information about typical pathogen reduction rates and coefficients, refer to the GWPP chapters on Persistence and Transport.

In batch experiments, the value of k may be estimated by regression analysis, having different pairs of values of t and N , that is, based on several samples taken at different times t and having different concentrations N_t (concentrations at time t). The value of k (and sometimes N_0) is the unknown in the regression analysis, that is, the parameter to be estimated. Simple linear regression, using the Method of Minimum Squares, can be done after linearization of Equation (6) by applying log-transformation on both sides of the equation, leading to $\ln(N_t) = \ln(N_0) - k.t$, with k being the slope of the line of best fit to the several experimental pairs of values (t , N_t). Alternatively, one can use Equation (6) directly, without linearization, and use non-linear regression, obtaining the value of k based on an algorithm of minimization of the sum of the squared errors.

The more data points one has, the more reliable is the estimate of k . The least reliable estimate of k would be based on only two data points: one at the beginning of the experiment ($t=0$; N equal to the initial concentration N_0 or N_{initial}) and one at the end of the experiment, conducted for a duration over the total time t ($t=t_{\text{total}}$; N equal to the final concentration N_{final}). By inserting these values into Equation (6), one could calculate k by rearrangement of Equation (6), leading to $k = \ln(N_{\text{initial}}/N_{\text{final}})/t_{\text{total}}$. This approach is not recommended because it does not allow for the confirmation that the decay rate is indeed indicative of first-order (i.e., log-linear). Also, microbial decay rate curves frequently display shoulders and tails (Haas and Joffe, 1994), which would not be visible in a decay experiment with only two time points.

The first method, using a series of values collected over time and applying a regression analysis (linear or non-linear), is more frequently applied by researchers when doing kinetic studies aiming at deriving the value of the reduction coefficient k .

The relative resistance of different pathogens to a particular set of conditions can be compared using the t_{90} time, which is the time required for 90% ($1 \log_{10}$) reduction, or the t_{99} time, which is the time required for 99% ($2 \log_{10}$)

reduction. For first-order and pseudo-first order reactions, the k value can be estimated from the t_{90} or t_{99} time, for a batch reactor as shown below:

$$k = \frac{\ln(100/10)}{t_{90}} = \frac{2.3}{t_{90}} \quad \text{or} \quad t_{90} = \frac{2.3}{k} \quad (7)$$

$$k = \frac{\ln(100/1)}{t_{99}} = \frac{4.6}{t_{99}} \quad \text{or} \quad t_{99} = \frac{4.6}{k} \quad (8)$$

It is very important to emphasize that Equations (7) and (8) are only associated with batch reactors. Most estimates of the decay rate coefficient k for different pathogens reported in the literature come from bench or lab-scale studies that use batch system experiments to investigate the kinetics of pathogen inactivation, using the approach described above. However, it should be understood that a reliable determination of the “true” intrinsic decay rate coefficient k is not simple. As a result, reported values often vary considerably from study to study, even when highly-controlled batch systems are used. To further complicate the usage of such kinetic coefficients, it should be remembered that full-scale sanitation technologies seldom operate in batch mode. Batch reactors are distinguished by the fact that, during the reaction time, they have no inlet or outlet flows. For instance, two examples of sanitation systems that can approach the behavior of batch reactors are a pit from a latrine that has been taken offline and is no longer in use or a sequencing batch reactor treating wastewater using the activated sludge process. Most sanitation technologies operate as continuous flow reactors, with liquid always entering and leaving the reactor. Furthermore, flow rates often have considerable variation both diurnally and seasonally. Therefore, in full-scale sanitation systems, unless the hydraulic behavior and mixing conditions are very well-characterized, decay rate coefficients obtained from bench or lab-scale studies should not be used, as they could produce very misleading results. This is an important warning regarding the widespread utilization of the expected “true intrinsic kinetic” k values derived from batch experiments: even if they may represent relatively well the decay rate of the constituent under study, the estimation of the effluent concentrations needs to take into account the hydraulic behavior and mixing conditions of the reactor, especially taking into account continuous flow reactors, as described in sections 2.2 and 2.3.

2.2 Continuous Flow Systems

Most sanitation technologies utilize continuous flow reactors. Due to mixing in these reactors, not all pathogens spend the same amount of time in the reactor. Some flow through quickly, while others take longer to exit the reactor. The simplest estimate of the average time a pathogen or a water molecule spends in the reactor is calculated as the theoretical mean hydraulic retention time (HRT), using Equation (9), where V is the reactor volume (units of m^3 or L, for example) and Q is the average flow entering and leaving the reactor (with units of volume/time, e.g., m^3/d or L/d):

$$HRT = \frac{V}{Q} \quad (9)$$

Equation (9) shows that the dimension of HRT is time (typically days or hours). In order to estimate the effluent concentration of a continuous flow tank, it is necessary to know not only the decay rate coefficient k , but also the hydraulic behavior of the reactor. It should be remembered that the reactors can have different shapes (rectangular, square, irregular), different depths, the presence or absence of packing material, the presence or absence of

plants and roots, different arrangements of inlet and outlet structures, different influences from wind, thermal stratification and circulation, dead zones, hydraulic short circuiting and several other factors that may substantially contribute to its departure from any idealized behavior.

Idealized flow regimens (Table 3) are often assumed when designing or modeling the behavior of reactors, however these represent idealized and extreme flow conditions. Actual flow conditions will be somewhere between these idealized and extreme conditions.

Table 3. Characteristics of the most frequently used (idealized) reactors in the design of sanitation technology systems

Hydraulic model	Schematics	Characteristics
Plug flow		<p>The fluid particles enter the tank continuously in one extremity, pass through the reactor, and are then discharged at the other end, in the same sequence in which they entered the reactor. The fluid particles move as a piston, without any longitudinal mixing. The particles maintain their identity and stay in the tank for a period equal to the theoretical hydraulic detention time. This type of flow is approached in long tanks with a large length-to-width ratio, in which longitudinal dispersion is minimal. These reactors are also called tubular reactors. Plug-flow reactors are idealised reactors, since complete absence of longitudinal dispersion is difficult to obtain in practice.</p>

Hydraulic model	Schematics	Characteristics
Completely mixed		<p>The particles that enter the tank are immediately dispersed in all the reactor body. The input and output flows are continuous. The fluid particles leave the tank in proportion to their statistical population. Complete mixing can be approached in circular or square tanks in which the tank's contents are continuously and uniformly distributed. Completely-mixed reactors are often labelled completely-mixed flow reactors (CMFR) or completely stirred tank reactors (CSTR). Completely-mixed reactors are idealised reactors, since total and identical dispersion is difficult to obtain in practice.</p>

Equations 10 and 11 respectively present formulations for estimating the **effluent concentration N** in an idealized **plug-flow reactor** and in an idealized **completely-mixed reactor**, assuming steady-state conditions and first-order kinetics. Both use the concept of the dimensionless pair $k \times t$, or, in this case, $k \times \text{HRT}$. This dimensionless pair imbeds not only the reaction kinetics, but also the hydraulic behavior of the reactor.

$$\text{Idealized plug-flow reactor: } N = N_0 e^{-k \cdot \text{HRT}} \quad (10)$$

$$\text{Idealized completely-mixed reactor: } N = \frac{N_0}{1 + k \cdot \text{HRT}} \quad (11)$$

Note that the idealized plug-flow reactor (Equation 10) behaves, theoretically, exactly as the idealized completely-mixed batch reactor (Equation 6). Examining both of these equations shows that for a certain value of the product $k \times \text{HRT}$, the ideal plug-flow reactor leads to lower values of the effluent concentration N , compared with the

completely-mixed reactor.

There are other models that can be used in order to try to approach the actual hydraulic behavior of a tank, such as the dispersed-flow model and the tanks-in-series model. Both give better predictions of the actual behavior in a real reactor, but their application is beyond the scope of this chapter. Nonetheless, it is important to understand that when using the dispersed-flow model, a reactor with zero dispersion yields the same results as the idealized plug-flow reactor (Equation 10), and a reactor with infinite dispersion reproduces the idealized completely-mixed model (Equation 11). Similarly, the tanks-in-series model equals the completely-mixed model when the number of reactors in series is one, and reproduces the plug-flow model when the number of reactors in series is infinite. Of course, in existing reactors, the behavior is never idealized, and a certain degree of dispersion is likely to occur. In terms of a simplified hydraulic representation, each reactor may be represented by its dispersion number, which is a

dimensionless variable that represents the relative importance of dispersion versus advection (which by definition is greater than zero and can theoretically approach infinity) for the dispersed-flow model or by the equivalent number of tanks in series (usually 1 or greater) for the tanks-in-series model.

2.3 Estimation of the reduction coefficient k in actual continuous-flow reactors

For design purposes, one needs to select the flow conditions (batch or continuous flow reactor) and the hydraulic model for continuous flow reactors (plug flow, completely-mixed or intermediate variants), and adopt values for the hydraulic retention time HRT and the reduction coefficient k. If it is considered that the hydraulic model represents well the fluid behavior and mixing conditions in the reactor, then the k value may be taken from literature, usually based on batch studies, as discussed in Section 2.1.

But in many cases, researchers opt to derive reduction coefficient ("k" values) based on real, existing, reactors, either in pilot or full-scale units. These "k" values that are calculated from pilot or full-scale continuous flow reactors should be labeled k', to distinguish them from the true intrinsic coefficients, which we will label k. Considerable care needs to be exercised in the interpretation and use of such k' values, here understood, not as true intrinsic coefficients, but rather as process coefficients, because they may incorporate in themselves, not only the influence of reaction, but also the unwanted influence of hydraulics and mixing.

In an existing batch reactor, the reduction coefficient k can be estimated based on measurements of the concentrations N at different time intervals t, as explained in Section 2.1.

In continuous-flow reactors, the mean value of the reduction coefficient k is usually calculated based on:

measurements of the influent concentration (No) and effluent concentration (N). Usually average values of No and N are used
 assumption that the reaction follows a first-order reaction
 assumption of steady-state conditions
 adoption of the theoretical hydraulic retention time (HRT) as the actual time. Usually the mean value of HRT is used
 adoption of one of the idealized flow regimens (plug flow or completely-mixed) (dispersed-flow models or tanks-in-series models can also be used if the flow dispersion is characterized).

For the two idealized flow regimens, rearrangement of Equations 10 and 11 leads to the following two equations for estimating the reduction coefficient (denoted k' here to distinguish it from the true intrinsic decay rate coefficient as measured in batch system experiments):

$$\text{Idealized plug-flow reactor: } k' = \frac{\ln(N_0/N)}{HRT} \quad (12)$$

$$\text{Idealized completely-mixed reactor: } k' = \frac{(N_0/N) - 1}{HRT} \quad (13)$$

It is very important to understand again that the k reduction coefficient calculated this way for a full-scale sanitation system reactor is fundamentally different from the true kinetic coefficient (as determined in batch experiments in the laboratory) because it reflects not only the kinetics but also the particular hydraulic behavior of the reactor under study, and assigns it to a specified idealized regime (plug flow or completely-mixed). These idealized conditions are never achieved in practice. Additionally, the actual HRT is likely to be different from the theoretical mean HRT (i.e. the volume divided by the flow rate; Equation 9), because of dead zones, short circuits, stratification, wind and other interferences (Verbyla et al., 2013).

For a given pathogen reduction efficiency, the estimation of k based on the hydraulic retention time (HRT) and on the influent concentration (No) and effluent concentration (N) on an existing continuous-flow reactor leads to the two following divergent situations:

- adoption of the idealized completely-mixed model leads to k values which are greater than those representing the true kinetics (as determined in batch experiments)
- adoption of the idealized plug-flow model leads to k values which are lower than those representing the true kinetics (as determined in batch experiments)

Example

The following example should help to clarify the point. An existing reactor has the following average values of performance indicators: (a) influent E. coli concentration: N = 1.00 x 10⁸ MPN/100 mL; (b) effluent E. coli concentration: N = 1.00 x 10⁶ MPN/100 mL; (c) theoretical hydraulic retention time (V/Q): HRT = 30 days.

From the data, it can be seen that the reduction efficiency is (N-N)/N = (1.00 x 10⁸ - 1.00 x 10⁶) / (1.00 x 10⁸) = 0.99 = 99%. Expressing in log₁₀ units removed gives log₁₀[(1.00 x 10⁸) / (1.00 x 10⁶)] = log₁₀(100) = 2.00, or **2.00 log units reduced**.

Use of Equations 12 and 13 will lead to the following estimated k reduction coefficients: **plug flow: k = 0.15 d⁻¹** (actually 0.1535 d⁻¹ to allow accurate calculations); **completely-mixed: k = 3.30 d⁻¹**. Therefore, for the same reactor, different k values are estimated in practice, depending on the hydraulic regime assumed (i.e., plug flow or completely-mixed). The true kinetic coefficient (as obtained by batch experiments in the laboratory) will lie between the two values obtained for idealized plug flow and completely-mixed models applied to actual continuous-flow reactors.

In principle, there should be only one coefficient,

representing the decay rate of the microorganism, according to its kinetics. However, the inadequacy of idealized models in representing the real hydraulic pattern in the reactor leads to the deviations that occur in practice when obtaining k values based only on influent and effluent concentrations in an existing reactor. The reason for the differences observed in the example above is that, since completely-mixed reactors are the least efficient for first-order reduction kinetics, the lower efficiency is compensated by a higher k value. Conversely, since plug-flow reactors are the most efficient reactors, the k value is reduced to produce the same effluent quality. Depending on the dispersion characteristics of the reactor, the deviation can be very large, inducing considerable errors in the estimation.

An improvement in the estimation of k values from existing reactors is when the values are derived, not only from influent and effluent concentrations, but rather on a profile of concentrations measured along the reactor (for reactors that operate closer to plug flow conditions than completely-mixed). For instance, sampling at different distances from the inlet (hence different travelling times) may give indications on the mixing conditions within the reactor and allow a better adoption of the selected hydraulic model. Furthermore, regression analysis (linear or non-linear), using different pairs of travelling time t and concentration N can be used to derive k (as explained in Section 2.1, applied to batch systems), for plug-flow or dispersed-flow models.

When reporting k values, it is essential that the researcher or practitioner specifies the conditions employed in the calculations:

- batch experiments with estimation of the intrinsic k value from time and concentration data, thus representing a more accurate estimate of the true kinetic coefficient; or
- continuous flow reactor (i.e., chemostat, bench-scale system, pilot-scale system, or full-scale system) with calculations based on measurements of influent and effluent concentrations (or at intermediate points), an assumed hydraulic retention time (usually the theoretical one, corresponding to volume divided by flow), and an assumed hydraulic model (idealized plug flow, idealized completely-mixed, dispersed flow, tanks in series, or other).

2.4 Configuration and volume of individual units in the treatment chain

As shown previously in Section 1, the overall log reduction in the treatment chain can be obtained by simply summing together the individual LRVs for each unit in series. This is why it is emphasized that, for this book, reporting the performance of each individual unit helps to clarify the actual behavior of each unit in the reduction of pathogenic organisms. However, for the same influent flow and concentration, increasing the volume of a single reactor (and hence its hydraulic retention time), adding extra treatment units in series, splitting a single reactor into several smaller ones, and improving flow conditions (reduction of dead zones and short circuits) can increase the efficiency of pathogen reduction in the overall system. However, the extent to which the efficiency improves depends on the flow regimen as shown in Figure 1.

Assuming that the flow rate Q does not change and the pathogen reduction coefficient k stays the same for each reactor throughout the treatment chain, for idealized plug-flow reactors, doubling the total volume of the reactor will also double the expected pathogen reduction. However, under the same assumptions, increasing the number of units in series assuming idealized plug-flow reactors without changing the overall volume will not affect the calculated pathogen reduction. For completely-mixed reactors, this is not the case. Splitting a completely-mixed reactor into smaller reactors in series with the same total volume (Figure 1b) substantially increases the calculated pathogen reduction, but not proportionally to the increase in volume, as is the case for plug-flow reactors. Table 3 shows this effect in greater detail.

Adding a second completely-mixed reactor after the first one (to double the overall volume) will double the pathogen LRV (again, assuming that k value remains the same in both reactors). The relative volume, expressed as the product of $k \times \text{HRT}$ (from Equations 10 and 11), is a way to demonstrate how much larger a treatment reactor needs to be in order to lead to a calculated higher percentage reduction efficiency or log reduction value. Using this approach, the relative volume required to achieve different reduction efficiencies in the idealized hydraulic regimens of completely-mixed and plug flow can be calculated. For any given reduction efficiency, the idealized plug-flow reactor requires a smaller volume than the idealized completely-mixed reactor, as shown in Table

Table 4. Relative volumes (expressed as the product $k \times \text{HRT}$) required to achieve different reduction efficiencies in the idealized hydraulic regimens of completely-mixed and plug flow reactors

Percent Reduction Efficiency (E) (%)	50	80	90	95	99	99.9	99.99	99.999
Log_{10} Reduction Value (LRV)	0.3	0.7	1.0	1.3	2.0	3.0	4.0	5.0
$k^a \times \text{HRT}^b$ value for ideal completely- mixed flow reactor (CMFR)	1	4	9	19	99	999	9999	99999
$k \times \text{HRT}$ value for ideal plug flow reactor (PFR)	0.7	1.6	2.3	3.0	4.6	6.9	9.2	11.5
Ratio of $V_{\text{CMFR}}^c : V_{\text{PFR}}$	1.4	2.5	3.9	6.3	21.5	145	1087	8696

^a kinetic coefficient; ^b hydraulic retention time; ^c volume

For example, if the relative $k \times \text{HRT}$ value for 50% reduction efficiency is 1 for a completely-mixed reactor (Table 4), it can be shown (using Equations 10 and 11) that to achieve the same percent or log_{10} reduction, the volume of a plug flow reactor would be 70% as big to achieve the same percent (or log_{10}) reduction. In other words, the idealized completely-mixed reactor would need to be 1:0.7 = 1.4 times as large as the idealized plug-flow reactor to achieve 50% reduction. As the percent or log_{10} reduction goals get larger, this discrepancy between the idealized flow regimens increases. The volume of a completely-mixed reactor would need to be 1087 times larger than a plug-flow reactor to achieve a calculated 4- log_{10} reduction. Therefore, as the log_{10} pathogen reduction requirements increase for wastewater treatment processes, having a good understanding about the extent of flow mixing becomes very important when trying to estimate pathogen decay rates. For this reason, estimates of pseudo-first order decay rates for pathogens from studies of full-scale systems can be quite confounded by the lack of a detailed understanding about hydraulic efficiency of the systems. For this reason, in the subsequent chapters, we focus on reporting the log_{10} pathogen reductions (LRV) in different sanitation technologies rather than attempting to report apparent decay rate coefficients k (based on assumed flow regimens). For a detailed review of pathogen decay rates, refer to the chapters on Persistence (e.g. Pathogen Specific Persistence Modeling Data).

3.0 Concluding Summary

While reading the subsequent chapters related to the

management of microbial risk from excreta and wastewater using specific sanitation technologies, here are a few important concepts to keep in mind:

- Excreta and wastewater have solid and liquid components
 - Pathogens in the liquid fraction can be removed or inactivated
 - Pathogens in the solid fraction must be inactivated
- Pathogen Reduction = Removal + Inactivation - Growth
 - Removal is the transfer of pathogens from the water to the sludge
 - Inactivation is the destruction of pathogens, resulting in a loss of viability
 - Growth refers to the replication of (only bacterial) pathogens
- Pathogen reduction can be expressed as a percent reduction or a log_{10} reduction value (LRV)
- The rate of pathogen reduction (decay rate) in sanitation systems is typically assumed to be pseudo first-order, meaning that it depends only on the concentration of pathogens in the system and the average amount of time spent in the system
- Any discussion about the rate of pathogen reduction in sanitation systems is not complete without an assumption about the flow conditions (batch vs. continuous flow, idealized vs actual flow regimens)
- Because of the difficulty in expressing reduction coefficients and flow conditions, the chapters on sanitation technologies in this book report pathogen removals based on log_{10} reduction values (LRV).

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