Water Science & Technology

IWA PUBLISHING

Water Science & Technology Vol 86 No 11, 2820 doi: 10.2166/wst.2022.391

UV Disinfection sensitivity index of spores or protozoa: A model to predict the required fluence of spores or protozoa

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ABSTRACT

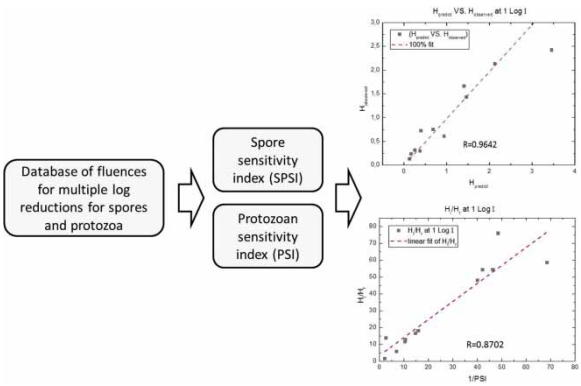
During UV disinfection, the required UV dose in terms of fluence depends upon the species of bacteria spore and protozoa. To rank their UV disinfection sensitivity, spore sensitivity index (SPSI) and protozoan sensitivity index (PSI) are defined. For spores, shoulder effect exists, therefore, SPSI is defined as the ratio between the k_i of any spores for the linear portion of the dose response curve to the k_{ir} of *Bacillus subtilis* as the reference spore. After statistical analysis, the fluence of any spore can be predicted by SPSI through equation, $H = (0.8358 \pm 0.126)*LogI*SPSI + H_0$. PSI is defined as the ratio between the inactivation rate constants of a protozoa in reference to that of *Cryptosporidium parvum*. The equation predicting the fluence of any protozoa in reference to *Cryptosporidium parvum* is: $H = 107.45*(3.86 \pm 2.68)*LogI/PSI$. Two regression equations suggest that protozoa require significantly higher UV dose than bacteria spores.

Key words: *Bacillus subtilis* and *Cryptosporidium parvum* as reference, protozoan, sensitivity index, spore, UV disinfection

HIGHLIGHTS

- UV sensitivity index of bacteria spore and protozoa were defined.
- The UV fluence could be predicted by the UV sensitivity indexes.
- Protozoa required significantly higher UV dose than that required by spores.

GRAPHICAL ABSTRACT



1. INTRODUCTION

UV disinfection technology becomes more and more important in water and wastewater industries, because UV radiation is an effective inactivation process against pathogenic micro-organisms such as *Cryptosporidium* and *Giardia* which poses a major threat to the safety of drinking water (Lonnen *et al.* 2005). To determine the inactivation equivalent fluence in UV disinfection system is more complex than medium pressure mercury vapor polychromatic. Because the spectral sensitivity of the microorganisms should be known toward the various wavelengths emitted by the medium pressure lamp as well as the spectral transmittance of the water (Mamane-Gravetz *et al.* 2005). Different spores and protozoa require different UV irradiation doses, depending upon the cultivation method used. The difference in UV susceptibility may also be related to the individual spectral UV sensitivity of the spores and protozoa (Cabaj *et al.* 2001, 2002).

Currently, the relationship between the fluence required for different spores and protozoan at a specific Log I have been reported in various publications. Some papers reported the UV dose and response data. The UV disinfection of different spores and protozoa at different degrees of Log I was published by Malayeri *et al.* (2016). This current research aims to develop a simple and universal model to systematically predict the fluence required to achieve specific reduction log I by using the spores sensitive index (SPSI) and protozoan sensitive index (PSI) during UV disinfection. Two independent universal equations were developed for fluence required to achieve a specific inactivation level Log I for different spores and protozoan in wastewater by using *Bacillus subtilis* and *Cryptosporidium parvum* as reference spores and protozoan, respectively.

2. MATERIAL AND METHODS

2.1. Databases

The database developed by Malayeri *et al.* (2016) was used to obtain a uniform set of first-order inactivation rate constants of spores and protozoa during UV disinfection. The inactivation rate constants of other spores and protozoa were divided by the mean k_r as a reference spores and protozoa to derive their corresponding SPSI. The SPSI developed was then used to derive the statistical equation between H_i/H_r and SPSI and Log I.

2.1.1. Spores mathematic model

Shoulder effect during UV disinfection refers to the initial delay of inactivation of bacteria spores (spores for simplicity) to achieve observable inactivation rate of Log I (Severin *et al.* 1983, 1984). Shoulder effect can be mathematically described as follows:

$$\frac{N_d}{N_0} = 1 - (1 - 10^{-k_H H})^{10^d} \tag{1}$$

where N_0 is the initial spore concentration before UV disinfection, N_d is the spore concentration after it received UV fluence of H. When fluence equals zero, the shoulder effect d is the log (N_d/N_0), which will be referred to as Log I for simplicity. k_H is the first order initial rate constant (cm^2/mJ) of the linear portion of log (N_d/N_0) vs. H.

To simplify the mathematical model of Equation (1), a spore survival curve during UV disinfection can be considered as two portions (Chick 1908; Watson 1908). First, shoulder portion of the curve can be approximated as shoulder broadness (SB), H_0, which is the minimal fluence required to have observable Log I. Mathematically, H_0 is the intercept of H when log (N_d/N_0) equal to zero. The actual SB would be either less or greater than the H_0 which is obtained from the intercept from the linear portion of the survival curve. However, the predicted H could be either less or greater than actually observed fluence H. Second, after the initial shoulder, the linear portion follows the first order kinetics which is characterized by the inactivation rate constant, k_H. As a result, a simple kinetic model is expressed as follows:

$$LogI = k_H * H + b \tag{2}$$

where log I is log (N_d/N_0). k_H is the first order initial constant (cm^2/mJ) of the linear portion of log(N_d/N_0) vs. H. b is the intercept on the y-axis of Log I of the spore survival dose cure.

Equation (2) can be solved to get H:

$$H = k_i * LogI + H_0 \tag{3}$$

where H_i is the fluence required at a given Log I. k_i equals $1/k_H$. H_0 is the shoulder broadness and equals b/k_H.

Similarly, Equation (3) can be also applied to a reference spore such as *Bacillus subtilis*, which is recommended as a reference spore by the US EPA, as follows:

$$H_r = k_{ir} * LogI + H_{0r} \tag{4}$$

To obtain a simplified predictive model, Equations (3) and (4) can be re-arranged as follows:

$$H - H_0 = k_i * LogI \tag{5}$$

$$H_r - H_{0r} = k_{ir} * LogI \tag{6}$$

When Equation (5) is divided by Equation (6) at the both sides at the same inactivation Log I, a simple linear equation is obtained:

$$\frac{H - H_0}{H_r - H_{0r}} = \frac{\Delta H_i}{\Delta H_r} = \frac{k_i}{k_{ir}} \tag{7}$$

Equation (7) suggests that the ratio between the fluence differences required for any spore is proportional to the ratio of their inactivation rate constants at the linear portion, if the same level inactivation rate of Log I is to be achieved for the specific spore.

In this study, a new concept of SPSI similar to bacteria sensitivity index Tang & Sillanpää (2015) is defined as the ratio between the k_i of any spores for the linear portion of the dose response curve to the k_{ir} of the reference spores as follows:

$$SPSI = \frac{k_i}{k_{ir}} \tag{8}$$

With this definition, the ratio between the fluence differences required to achieve a specific inactivation Log I, $\Delta H/\Delta H_r$ can be theoretically related to SPSI as follows:

$$\frac{\Delta H_i}{\Delta H_r} = SPSI \tag{9}$$

This equation suggests several important points between $\Delta H/\Delta H_r$ and SPSI at a specific Log I: first, $\Delta H/\Delta H_r$ should be linearly proportion to SPSI; second, the slope of the equation should be one in theory. In reality, however, the uncertainty during the measurement of H and the determination of the corresponding inactivation rate constants ki, which depends upon the accuracy of fluence measured will have slope different from one; third, when the approximated shoulder H_0 from the intercept of the linear portion at the Log $I = 0$ is used to replace the actual SB, the uncertainty of the coefficient, α, also increase. All of these uncertainty factors will affect the regression coefficient, α, which will deviate from one. To determine the coefficient α, regression analysis is carried out according to the following linear model:

$$\frac{\Delta H_i}{\Delta H_r} = \alpha * SPSI * (H_r - H_{0r}) \tag{10}$$

After regression analysis, the equation can be re-written as follows:

$$H - H_0 = \alpha * SPSI * (H_r - H_{0r}) \tag{11}$$

Substituting Equation (4) into the above equation:

$$H = \alpha * SPSI * (k_{ir} * LogI + H_{0r} - H_{0r}) + H_0 \quad (12)$$

Finally, a predictive equation is obtained as follows:

$$H = \alpha * (k_{ir} * LogI) * SPSI + H_0 \quad (13)$$

Since the shoulder broadness, H_{or}, was cancelled to each other, Equation (13) indicates that the predictive model is independent of H_{or}.

2.1.2. Protozoa mathematic model

During UV disinfection of protozoa, no shoulder effect was reported. Therefore, the disinfection kinetic model of protozoa is described by the first-order kinetics as proposed by Chick (1908) and Watson (1908) as follows:

$$N = N_0 e^{-k_t C} \quad (14)$$

where N_0 is the initial concentration of protozoa to applying UV, N is the number of protozoa after exposure time t to UV, k_t is the disinfection rate constant of a protozoa and C is the concentration of a disinfectant. For UV disinfection, the concentration is replaced by UV irradiance intensity (mW/cm^2). The product of UV intensity and the exposure time is defined as fluence (H), which has the unit of mJ/cm^2. *Cryptosporidium parvum* is used as the reference protozoa because the US EPA had specific concern and regulation of the protozoa, therefore:

$$Log\left(\frac{N_0}{N}\right)_i = LogI_i = k_i tC = k_i * H_i \quad (15)$$

$$Log\left(\frac{N_0}{N}\right)_r = LogI_r = k_r tC = k_r * H_r \quad (16)$$

Dividing Equation (3) by Equation (4) at the both sides, the following equation resulted:

$$\frac{LogI_i}{LogI_r} = \frac{k_i * H_i}{k_r * H_r} \quad (17)$$

To achieve the same level of Log I for both a specific protozoa and *Cryptosporidium parvum*, the left side of the equation becomes unity 1. Inspecting Equation (17), a new concept of PSI is defined as the ratio between the ki of any protozoa to the kr of a reference protozoa, *Cryptosporidium parvum*, as follows:

$$PSI = \frac{k_i}{k_r} \quad (18)$$

Therefore, the relative fluence required to achieve a given order of inactivation Log I, H_i/H_r can be theoretically related to PSI according to the theoretical Equation (17) as follows:

$$\frac{H_i}{H_r} = \frac{1}{PSI} \quad (19)$$

Similarly, this equation suggests two important points about the relationship between H_i/H_r and 1/PSI at the same order of Log I: first, the slope of the equation should be 1 and linearly proportional to 1/PSI; second, for the reference protozoa *Cryptosporidium parvum*, both sides become one. In reality, the uncertainty in the measurement of H and the uncertainty in the quantification of inactivation rate constants k_i will result in a slope different from one. To determine the coefficient β,

regression analysis is carried out according to the following linear model:

$$H_i = \beta * LogI * \frac{1}{PSI} \quad (20)$$

2.2. Statistic analysis

By using the database which compiled by Malayeri *et al.* (2016), the inactivation UV dose at different Log I was modelled through a linear correlation analyses using SPSS of the IBM. The inactivation rate constant of each spore and protozoan were divided by the corresponding inactivation rate constants of the reference spores such as *Bacillus subtilis*, or the reference protozoa such as *Cryptosporidium parvum*, respectively. The regression analysis was conducted between H_i/H_r and SPSI using linear to determine which model fits best to the data sets. Once the model was chosen, it was used throughout the rest of the statistical analysis. The same statistical analysis procedure was applied for regression analysis between the required fluence and the 1/PSI.

3. RESULTS AND DISCUSSIONS

3.1. Spores sensitivity index

The calculated values of k_H and b are listed under their corresponding spores in the second and third column, respectively. To facilitate the linear regression, the coefficients of $k_i = 1/k_H (mJ/cm^2)$ and the shoulder broadness, $H_0 = b/k_H$ (mJ/cm^2), of Equation (3) are presented in the Table 1 so that SPSI can be defined for each spores.

Depending on the coefficient of reference data and Table 1, SPSI can be defined in Table 2.

Histogram with probability density function (PDF) and cumulative distribution function (CDF) of SPSI using the fluence required for *Bacillus subtilis* as the reference in Figure 1 shows that up to five spores have the same inactivation rate constants as the linear portion of the survival curve as that of the reference spores *Bacillus subtilis*.

3.1.1. Transformation of H into $\Delta H/\Delta H_r$

To assess which set of SPSI is the best, following the Equation (3), Table 3 is presented. Through Table 4, H can be transformed to $\Delta H/\Delta H_r$ as shown in Table 4.

Table 1 | Transformation of k_H into k_i during UV disinfection of spores

	$-Log\ I = -k_H + b$		$H = k_i Log\ I + H_0$		
Inactivation kinetics	**$k_H (cm^2/mJ)$**	**B**	**$k_i = 1/k_H (mJ/cm^2)$**	**$H_0 = b/k_H (mJ/cm^2)$**	**Reference**
Bacillus subtillis as reference	11.235	8.372	0.089	0.745	
Spores for model development					
Clostridium pasteurianum	1.65	1.83	0.606	1.109	Clauß (2006)
Streptomyces griseus ATCC10137	3.25	5.67	0.308	1.745	Clauß (2006)
Bacillus strophaeus ATCC9372	8	1.33	0.125	0.166	Sholtes *et al.* (2016)
Sterne	12	15	0.083	1.25	Nicholson & Galeano (2003)
Bacillus astrophaeus ATCC9372	16.5	5.33	0.061	0.323	Zhang *et al.* (2014)
34F2(sterne) method: Schaeffer's sporulation medium	28.5	10.67	0.035	0.374	Rose & O'Connell (2009)
Thermoactionmyces	30	26.67	0.033	0.889	Clauß (2006)
Bacillus cereus ATCC11778	44	7	0.023	0.159	Clauß (2006)
Bacillus pumilus ATCC27142	68	0.67	0.015	0.010	Boczek *et al.* (2016)
Aspergillus brasiliensis ATCC16404	85.5	42.67	0.011	0.499	Taylor-Edmonds *et al.* (2015)

Table 2 | Definition of SPSI

SPSI = k_i/k_{ir}

Reference spores: Bacillus subtilis	SPSI	Reference
Clostridium pasteurianum ATCC6013	6.809	Clauß (2006)
Streptomyces griseus ATCC10137	3.457	Clauß (2006)
Bacillus astrophaeus ATCC9372	1.404	Sholtes *et al.* (2016)
Sterne	0.936	Nicholson & Galeano (2003)
Bacillus astrophaeus ATCC9372	0.680	Zhang *et al.* (2014)
34F2 (sterne) method: Schaeffer's sporulation medium	0.394	Rose & O'Connell (2009)
Thermoactinomyces vulgaris ATCC43649	0.374	Clauß (2006)
Bacillus cereus ATCC11778	0.255	Clauß (2006)
Bacillus pumilus ATCC27142	0.165	Boczek *et al.* (2016)
Aspergillus brasiliensis ATCC16404	0.131	Taylor-Edmonds *et al.* (2015)

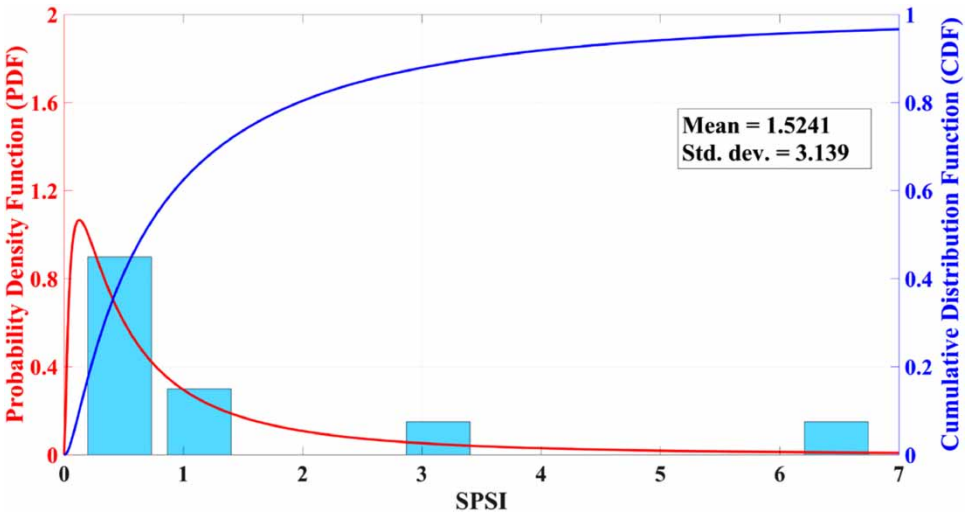


Figure 1 | Histogram with probability density function (PDF) and cumulative distribution function (CDF) of SPSI using the fluence required for *Bacillus subtilis*.

Table 3 | SPSI in reference to *Bacillus subtilis*

Fluence difference	$\Delta H = H\text{-}H_0$				
Log I	**0**	**1**	**2**	**3**	**Reference**
34F2(sterne) method: Schaeffer's sporulation medium	0.374	22.625	35.625	79.626	Rose & O'Connell (2009)
Aspergillus brasiliensis ATCC16404	0.499	121.501	225.501	292.501	Taylor-Edmonds *et al.* (2015)
Bacillus astrophaeus ATCC9372	0.323	21.676	37.677	54.677	Zhang *et al.* (2014)
Bacillus cereus ATCC11778	0.159	51.841	92.841	139.841	Clauß (2006)
Bacillus pumilus ATCC27142	0.010	67.834	137.990	203.990	Boczek *et al.* (2016)
Bacillus strophaeus ATCC9372	0.166	9.833	15.833	25.83375	Sholtes *et al.* (2016)
Bacillus subtilis	0.745	17.1	28.09	38.753	
Clostridium pasteurianum	1.109	2.291	4.191	5.591	Clauß (2006)
Sterne	1.25	26.75	35.75	50.75	Nicholson & Galeano (2003)
Streptomyces griseus ATCC10137	0	6.755	11.255	13.255	Clauß (2006)
Thermoactionmyces	1.745	54.111	89.111	114.111	Clauß (2006)

Table 4 | $\Delta h/\Delta H_r$ at specific inactivation log I of *Bacillus subtilis* as reference

Fluence difference	$\Delta H = H\text{-}H_0$				
Log I	**0**	**1**	**2**	**3**	**Reference**
ΔHr, *Bacillus subtilis* as reference					
Bacillus subtilis	0.745	16.354	37.703	52.009	Zhang *et al.* (2014)
Thermoactionmyces	1.745	0.302	0.423	0.455	Clauß (2006)
Aspergillus brasiliensis ATCC16404	0.499	0.135	0.167	0.177	Taylor-Edmonds *et al.* (2015)
34F2(sterne) method: Schaeffer's sporulation medium	0.374	0.722	1.058	0.653	Rose & O'Connell (2009)
Bacillus astrophaeus ATCC9372	0.323	0.754	1	0.951	Zhang *et al.* (2014)
Bacillus strophaeus ATCC9372	0.166	1.663	2.381	2.013	Sholtes *et al.* (2016)
Bacillus cereus ATCC11778	0.159	0.315	0.406	0.371	Clauß (2006)
Clostridium pasteurianum	1.109	7.139	8.996	9.302	Clauß (2006)
Sterne	1.25	0.611	1.954	1.024	Nicholson & Galeano (2003)
Bacillus pumilus ATCC27142	0.010	0.241	0.273	0.254	Boczek *et al.* (2016)
Streptomyces griseus ATCC10137	0	2.421	3.349	3.923	Clauß (2006)

3.1.2. Correlation analysis between $\Delta H/\Delta H_r$ and SPSI

According to Equation (10), $\Delta H/\Delta H_r$ should linearly correlate with SPSI. Figures 2–4 are plots a typical predicted output $\Delta H/\Delta H_r$ against the observed $\Delta H/\Delta H_r$ at 1 Log I, 2 Log I and 3 Log I, respectively. It shows that the correlation is very robust with $R = 0.9642$, $R = 0.9713$ and $R = 0.9917$ between $\Delta H/\Delta H_r$ which suggests that the theoretical equation is valid. On the other hand, it means the H_0 and H_r are not the major errors to effect the UV disinfection efficiency. Such robust linear relationship suggests that Equation (10) should have robust predictive power of the fluence requirement for specific inactivation rate of Log I as long as SPSI is defined. According to the results, the equation can be obtained to predict the required fluence of any spores as follows:

$$H = (0.8358 \pm 0.126) * \text{LogI} * \text{SPSI} + H_0 \tag{21}$$

3.2. Protozoa sensitivity index

The protozoa sensitivity index can be defined by Equation (3). In Table 5 are listed the k_i and b, and after linear regression, k_i and H_0 can be calculated.

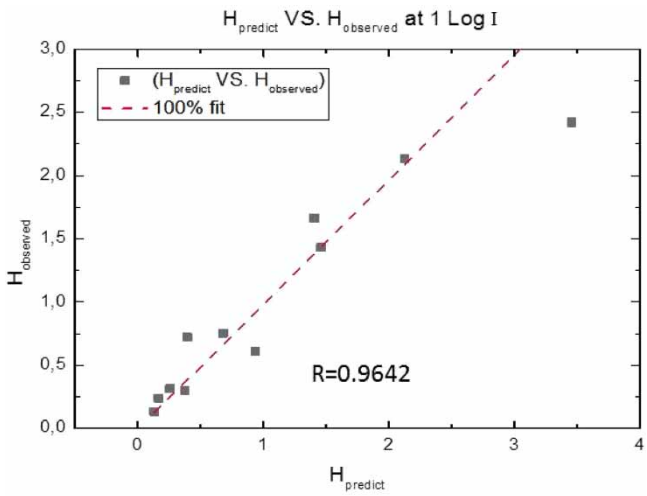


Figure 2 | $H_{predicted}$ vs. $H_{observed}$ at 1 Log I.

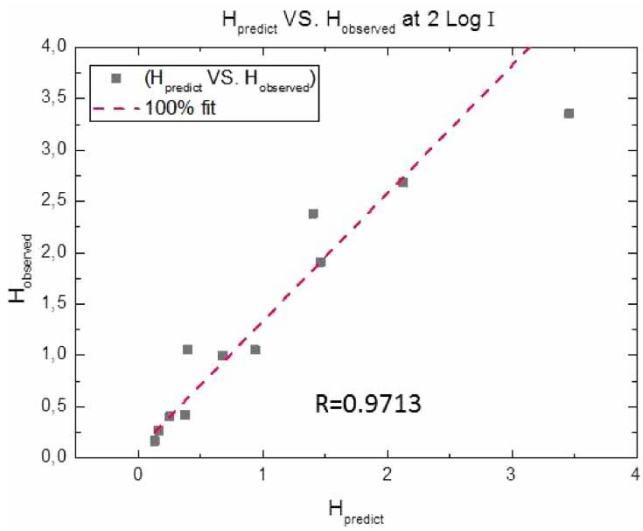


Figure 3 | $H_{predicted}$ vs. $H_{observed}$ at 2 Log I.

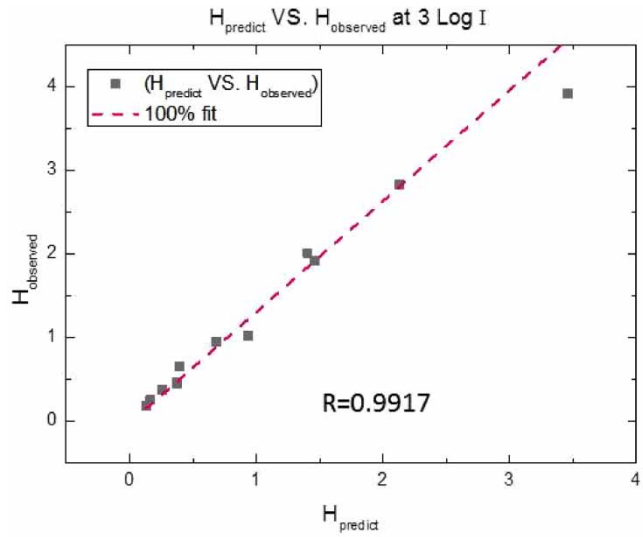


Figure 4 | $H_{predicted}$ vs. $H_{observed}$ at 3 Log I.

Using reference data and Table 5, PSI can be defined in Table 6.

Histogram with probability density function (PDF) and cumulative distribution function (CDF) of PSI using the fluence required for *Cryptosporidium parvum* as the reference in Figure 5 shows two protozoas have the same inactivation rate constants of the linear portion of the survival curve as that of the reference protozoa *Cryptosporidium parvum*.

3.2.1. Transformation of H into H/H_r

To assess which set of PSI is the best, following the Equation (3), Table 7 is presented. Through Table 7, H can be transformed to H/H_r as shown in Table 8.

3.2.2. Correlation analysis between H/Hr and 1/PSI

According to Equation (10), H/H_r should linearly correlate with 1/PSI regardless which set of reference fluence is used; Figures 6–8 are plots of a typical predicted output H/H_r against the observed H/H_r at 1 Log I, 2 Log I and 3 Log I, respectively.

Table 5 | Transformation of k_H into k_i during UV disinfection of protozoa

	$-\text{Log } I = -k_H + b$		$H = k_i \text{Log } I + H_0$		
Inactivation kinetics	**k_H(cm²/mJ)**	**b**	**$k_i = 1/k_H$(mJ/cm²)**	**$H_0 = b/k_H$(mJ/cm²)**	**Reference**
Cryptosporidium parvum as reference	0.475	0.869	2.105	1.829	
Protozoa for model development					
Acanthamoeba castellanii CCAP15342 (life stage: cysts)	23	24.33	0.043	1.058	Cervero-Aragó *et al.* (2014)
Acanthamoeba spp. 155 (life stage: trophozoites)	19	3.67	0.053	0.193	Cervero-Aragó *et al.* (2014)
Acanthamoeba spp. 155 (life stage: cysts)	32.5	1.67	0.031	0.051	Cervero-Aragó *et al.* (2014)
Giardia lamblia	1.25	0	0.8	0	Cervero-Aragó *et al.* (2014)
Giardia lamblia	1	0.53	1	0.53	Campbell & Wallis (2002)
Naegleria fowleri	5	3	0.2	0.6	Mofidi *et al.* (2002)
Toxoplasma gondii oocysts	4.9	2.6	0.204	0.530	Mofidi *et al.* (2002)
Toxoplasma gondii	3.3	0.13	0.303	0.039	Amoah *et al.* (2005)
Vermamoeba vermiformis CCAP 15434	7.5	3.67	0.133	0.489	Ware *et al.* (2010)
Vermamoeba vermiformis 195 (life stage: trophozoites)	7	3	0.143	0.429	Cervero-Aragó *et al.* (2014)
Vermamoeba vermiformis 195 (life stage: cysts)	22	12	0.045	0.545	Cervero-Aragó *et al.* (2014)

Table 6 | PSI of reference dose required data

$PSI = k_i/k_{ir}$

Reference protozoa: cryptosporidium parvum	**PSI**	**1/PSI**	**Reference**
Giardia lamblia	0.475	2.105	Mofidi *et al.* (2002)
Toxoplasma gondii	0.144	6.947	Ware *et al.* (2010)
Toxoplasma gondii oocysts	0.097	10.316	Amoah *et al.* (2005)
Naegleria fowleri	0.095	10.526	Mofidi *et al.* (2002)
Vermamoeba vermiformis 195 (life stage: trophozoites)	0.068	14.737	Cervero-Aragó *et al.* (2014)
Vermamoeba vermiformis CCAP 15434	0.063	15.789	Cervero-Aragó *et al.* (2014)
Vermamoeba vermiformis 195 (life stage: cysts)	0.055	46.316	Cervero-Aragó *et al.* (2014)
Giardia lamblia	0.38	2.632	Campbell & Wallis (2002)
Acanthamoeba spp. 155 (life stage: trophozoites)	0.025	40.000	Cervero-Aragó *et al.* (2014)
Acanthamoeba castellanii CCAP15342 (life stage: trophozoites)	0.023	42.105	Cervero-Aragó *et al.* (2014)
Acanthamoeba castellanii CCAP15342 (life stage: cysts)	0.021	48.421	Cervero-Aragó *et al.* (2014)
Acanthamoeba spp. 155 (life stage: cysts)	0.015	68.421	Cervero-Aragó *et al.* (2014)

It shows that the correlation is not more robust than SPSI. $R = 0.8702$, $R = 0.8925$ and $R = 0.9646$ between H/H_r which suggests that the theoretical equation is valid. According to the results as before mentioned, the equations can be obtained to predict the required fluence of any protozoa by using three different sets of fluence requirements of *Cryptosporidium parvum* as follows:

$$H = 107.45*(3.86 \pm 2.68)*\text{LogI}*\frac{1}{\text{PSI}} \quad (22)$$

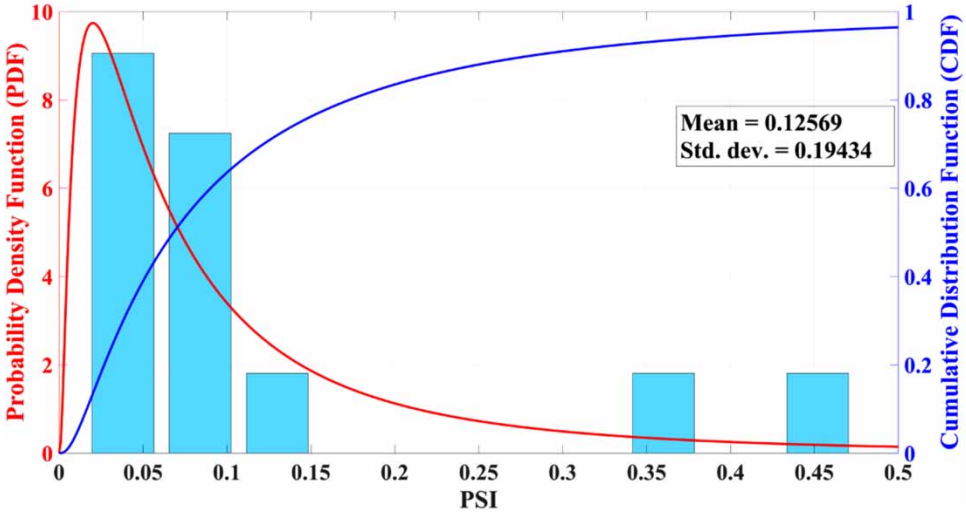


Figure 5 | Histogram with probability density function (PDF) and cumulative distribution function (CDF) of PSI using the fluence required for *Cryptosporidium parvum*.

Table 7 | Fluence required at different Log I of protozoa

Fluence difference				
Log I	**1**	**2**	**3**	**Reference**
Hr, *Cryptosporidium parvum* as reference				
Cryptosporidium parvum				
Acanthamoeba castellanii CCAP15342 (life stage: trophozoites)	31.4	51.4	71.4	Cervero-Aragó *et al.* (2014)
Acanthamoeba castellanii CCAP15342 (life stage: cysts)	43.942	73.942	89.942	Cervero-Aragó *et al.* (2014)
Acanthamoeba spp. 155 (life stage: trophozoites)	27.807	30.807	65.807	Cervero-Aragó *et al.* (2014)
Acanthamoeba spp. 155 (life stage: cysts)	33.948	66.949	98.948	Cervero-Aragó *et al.* (2014)
Giardia lamblia	8	10	20	Cervero-Aragó *et al.* (2014)
Giardia lamblia	0.97	1.47	3.47	Mofidi *et al.* (2002)
Naegleria fowleri	7.4	12.4	17.4	Mofidi *et al.* (2002)
Toxoplasma gondii oocysts	6.67	12,469	16.469	Amoah *et al.* (2005)
Toxoplasma gondii	3.361	6.761	9.961	Ware *et al.* (2010)
Vermamoeba vermiformis CCAP 15434	10.511	18.511	25.510	Cervero-Aragó *et al.* (2014)
Vermamoeba vermiformis 195 (life stage: trophozoites)	9.571	16.571	23.571	Cervero-Aragó *et al.* (2014)
Vermamoeba vermiformis 195 (life stage: cysts)	31.455	59.454	75.455	Cervero-Aragó *et al.* (2014)

3.3. Comparison with different bateria and virus

3.3.1. SPSI compare with bacteria sensitivity index (BSI)

For the previous study, the BSI used *E. coli* as the reference bacteria, the fluence recommended by the US EPA is used in the correlation analysis to obtain the following equation:

$$H = (0.914 \pm 0.044) * LogI * (BSI)_E + H_0 \quad (23)$$

Compared with SPSI, the UV fluence is lower than BSI. As a result, it would significantly reduce the trial and error experiment in deciding which fluence should be used to achieve a specific inactivation rate Log I for a specific spores providing the corresponding SPSI is known.

Table 8 | H/H_r data specific inactivation log I of *Cryptosporidium parvum* as reference

Fluence				
Log I	**1**	**2**	**3**	**Reference**
ΔHr, *Cryptosporidium parvum* as reference				
Acanthamoeba castellanii CCAP15342 (life stage: trophozoites)	54.286	299.570	192.153	Cervero-Aragó *et al.* (2014)
Acanthamoeba castellanii CCAP15342 (life stage: cysts)	75.970	430.951	242.054	Cervero-Aragó *et al.* (2014)
Acanthamoeba spp. 155 (life stage: trophozoites)	48.074	179.549	177.100	Cervero-Aragó *et al.* (2014)
Acanthamoeba spp. 155 (life stage: cysts)	58.692	390.191	266.292	Cervero-Aragó *et al.* (2014)
Giardia lamblia	13.831	58.282	53.824	Cervero-Aragó *et al.* (2014)
Giardia lamblia	1.677	8.567	9.338	Mofidi *et al.* (2002)
Naegleria fowleri	12.793	72.270	46.827	Mofidi *et al.* (2002)
Toxoplasma gondii oocysts	11.530	72.674	44.322	Amoah *et al.* (2005)
Toxoplasma gondii	5.810	39.402	26.806	Ware *et al.* (2010)
Vermamoeba vermiformis CCAP 15434	18.171	107.884	68.654	Cervero-Aragó *et al.* (2014)
Vermamoeba vermiformis 195 (life stage: trophozoites)	16.547	96.582	63.436	Cervero-Aragó *et al.* (2014)
Vermamoeba vermiformis 195 (life stage: cysts)	54.380	346.514	203.065	Cervero-Aragó *et al.* (2014)

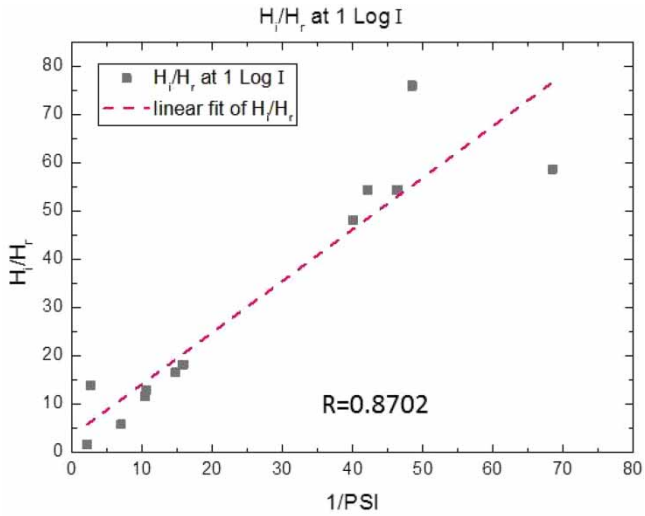


Figure 6 | Linear correlation between H/H_r and 1/PSI at 1 Log I.

3.3.2. PSI compare with virus sentivity index (VSI)

Without shoulder broadness, VSI was used to predict the required UV fluence for virus to be inactivated at Log I. Log I is log inactivation and VSI is the VSI in reference to MS2-phage as follows:

$$H = 21.469*(1.027 \pm 0.0166)*LogI*\frac{1}{VSI} \quad (24)$$

Obviously, to disinfect protozoa, more UV fluence is needed than bacteria spores. The major advantage of the method developed in this study is rooted in its dimensionless parameters such as H_i/H_r versus PSI.

4. CONCLUSIONS

Scientific analysis unveils a linear relationship between fluence required for inactivation of any spores and that required by reference spores such as *Bacillus subtillis* is proportional to the ratio of their corresponding inactivation rate constant k_i/k_{ir}.

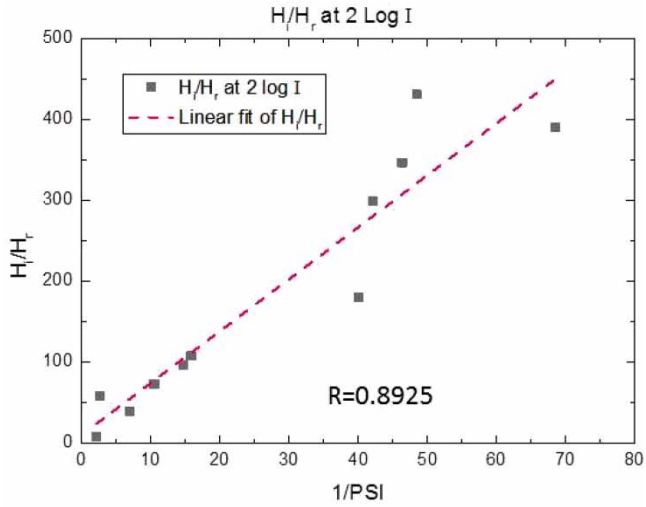


Figure 7 | Linear correlation between H/H_r and 1/PSI at 2 Log I.

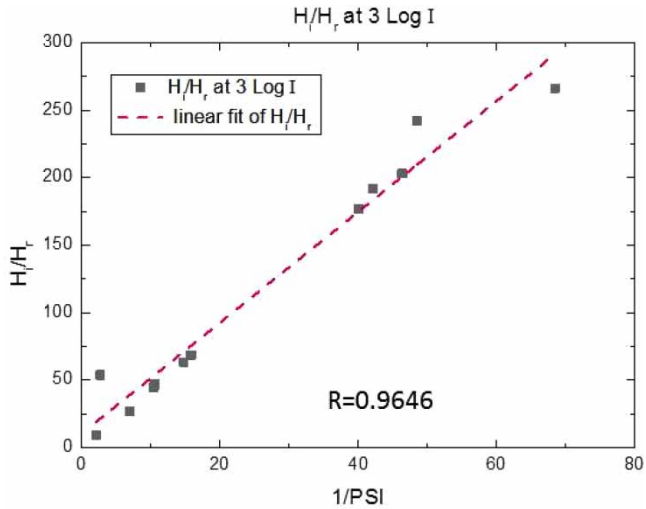


Figure 8 | Linear correlation between H/H_r and 1/PSI at 3 Log I.

The SPSI has been successfully used to predict the fluence. The developed model tends to overpredict the fluence required at low Log I while it would underpredict the fluence required as Log I level increased. Up to 3 Log I, the model underpredicts all the fluence with the maximal errors less than 15%. The PSI was defined as the ratio between the inactivation rate constants of a protozoa in reference to that of *Cryptosporidium parvum*. PSI can be used to rank the relative UV disinfection sensitivity. For example, most protozoa have a PSI greater than that of *Cryptosporidium parvum*, and if there are no protozoa, which has very low PSI, *Cryptosporidium parvum* should be used as an adequate indicator in the validation of a UV disinfection system. Using statistical equations developed in this paper, PSI can be used to accurately predict the fluence required, Hi, for any given protozoa at a specific Log I by using Equation (22).

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Zhao Wang: statistical analysis and writing. **Walter Z. Tang**: conceptualization, methodology, statistical analysis, investigation, writing, review and editing, and supervision. **Mika Sillanpää**: review and editing, **Jinze Li**: review and editing.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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First received 15 July 2022; accepted in revised form 11 November 2022. Available online 26 November 2022