

Kinetics And Monte Carlo Simulation of UV Disinfection *B. Subtilis* Spores And SARS-CoV-2 In Dried Saliva Droplets

John Gibson (✉ jh.gibson@utoronto.ca)

University of Toronto <https://orcid.org/0000-0003-0915-8130>

Ramin Farnood

University of Toronto

Benoit Barbeau

Polytechnique Montréal: Polytechnique Montreal

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Abstract

Surfaces can be contaminated by droplets produced coughing or sneezing. In this short, exploratory work, UV disinfection data from *B. subtilis* spores in dried saliva droplets were fitted to a first-order model. The model has a disinfection rate constant for single organisms, and a smaller one for aggregates ($R^2 \geq 0.97$). Changes in the fraction of organisms in aggregates (β) alone could account for the effects of different sized droplets in the experimental work. Since a wide spectrum of droplet sizes can be produced and some of the rate constants were uncertain, Monte Carlo simulation was used estimate the UV inactivation performance in dried saliva droplets in a range of conditions. Using conservative lognormal distribution for β , the model was applied to the UV disinfection of SARS-CoV-2 in dried droplets. It was shown that one-log reduction of SARS-CoV-2 was very likely ($p > 99.9\%$) and two-log reduction was probable ($p = 75\%$) at a dose of 60 mJ/cm^2 . Aggregates tend to be variable and limit the log reductions that can be achieved at high UV doses.

Introduction

We are in what is now, hopefully, the final stages of a global pandemic of the novel human coronavirus, known as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). This is the latest in a series of coronavirus outbreaks that also includes Severe Acute Respiratory Syndrome (SARS CoV-1) and Middle East Respiratory Syndrome (MERS) (Noorimotlagh, 2020). It is reasonable to expect more outbreaks in the future, and methods to control the spread of these viruses are important areas for research.

UV radiation, particularly at a wavelength of 254 nm, has long been understood to be absorbed by the pyrimidine bases, thymine in DNA and uracil in RNA, causing dimers that slow or halt reproduction (Jagger, 1967; Masschelein, 2002). When it comes to viruses, Pfaender et al. (2015) showed direct damage of the hepatitis C virus genome, slowing replication after UV treatment. Beck et al. (2016) showed RNA damage of the bacteriophage MS2 after UV treatment at wavelengths of 210 and 290 nm.

Many viruses, including SARS-CoV-2, have been shown to persist on surfaces for several days (Noorimotlagh, 2020). One source of surface contamination is droplets produced by speaking, sneezing, or coughing (Dbouk and Drikakis, 2020; Johnson et al., 2011). The ability of UV to disinfect viral pathogens in dried droplets remains largely absent from the current literature. Ma et al. (2021), after exploring the UV disinfection of the MHV, Phi6, and coronavirus HCoV 299E in liquid suspensions, suggest a need for better understanding UV disinfection on surfaces. A recent meta-analysis by Chiappa et al. (2021) report that just 11% (2 of 18) of the selected studies examined the effects of UV on viruses in dried droplets, with the majority (14 of 18) using liquid suspensions. The two dried droplets studies focused on demonstrating new technologies: a rotating full-room disinfectant (Bedell et al., 2016) and a pulsed xenon emitter (Simmons et al., 2021). Though both studies were able to show reductions in coronaviruses, their unique emitters make it difficult to apply these results elsewhere.

Experimental work on dried droplets is challenging due to the small size of the droplets and the large number of variables. Johnson et al. (2011) showed that the largest droplets produced by speaking had a mode diameter of just 145 μm , corresponding to a volume of just 0.0016 μL . Lab work with such small volumes is challenging. The ASTM test E3135–18 *Standard Practice for Determining Antimicrobial Efficacy of Ultraviolet Germicidal Irradiation Against Microorganisms on Carriers with Simulated Soil* suggests using droplets of 1 μL instead. ASTM test E2721–16 *Standard Practice for Evaluation of Effectiveness of Decontamination Procedures for Surfaces When Challenged with Droplets Containing Human Pathogenic Viruses* aims to produce droplets 15 μm in diameter. However, this is done by atomizing the virus, which is potentially hazardous if they were to escape before, during, or after the experiment. Significant losses are also observed during the aerosolization procedure. In addition, Barancheshme et al. (2021) showed there is wide variability in the UV transmission of human saliva, another source of variability in experiments.

Computer modeling can inform us about UV disinfection in droplets without these restrictions. Monte Carlo Simulation (MCS), with an appropriate mathematical model, creates the opportunity to examine tens of thousands of different scenarios in a few minutes. As such, this work aims to develop a kinetic model of UV disinfection in dried droplets and use this model to inform us about the disinfection of viruses in dried droplets using MCS.

Model Development

Disinfection is typically modelled as first order process where the rate of change of the number of surviving organisms is proportional to the dose received. The ratio of surviving organism (N/N_o) is given by:

$$\frac{N}{N_o} = e^{-kD} \quad (1)$$

In the case of UV, dose (D) is the product of the light intensity (mW/cm^2) the organism receives and the duration of exposure (s). The parameter k is a disinfection rate constant (cm^2/mJ).

Tseng and Li (2007) showed good agreement with the first order model when disinfecting-viruses with UV on surfaces, with R^2 values of 0.94, 0.96, 0.96, and 0.98 for MS2, Φ X174, Φ 6, and T7 viruses, respectively. The lower UV sensitivity of viruses on surfaces when compared to aerosols observed by Tseng and Li (2007) was postulated to involve the presence of aggregates on the surface, which is addressed below.

Hard-to-disinfect aggregates of organisms, often associated with small particles, is a historical problem in UV disinfection (Azimi et al., 2013, 2012; Emerick et al., 2000; Loge, 1996). Mathematical models developed to describe this behavior include that of Emerick et al. (2000), which introduced a parameter for the number of particle associated organisms (N_p). Since they are harder to disinfect, the rate of

inactivation of N_p was assumed to be linear with time, rather than exponential. Other authors have used double-exponential models where the process remains first order, with different exponential rate constants for single organisms (k_S) and a smaller one for particle-associated aggregates (k_A) (Azimi et al., 2012; Barbeau et al., 2005). The model used in this work is of the double-exponential type, and is adapted from Barbeau et al. (2005) shown below:

$$\frac{N}{N_0} = (1 - \beta)e^{-k_S D} + \beta e^{-k_A D} \quad (2)$$

In this model, k_S is the rate constant for single organisms; k_A is the rate constant for aggregates; D is the UV dose; and β is the fraction of the population in aggregates. The rate constant for aggregates is smaller than that of individual organisms, consistent with the fact that they are harder to disinfect. In reality, there is most likely a distribution of rate constants (i.e. more than two) but the current scientific literature precludes us from defining rate constants based on aggregate characteristics. One advantage of this relatively simple model is the small number of parameters that must be estimated, which is important for modelling. This model can also help to identify what are the aggregate characteristics that control the value of k_A .

Monte Carlo Simulation

Monte Carlo Simulation can be used to account for variability and uncertainty. Variables can be assigned statistical distributions that reflect the level of uncertainty in their estimation. In this case, the model is executed 10,000 times, each time taking a random sample of the uncertain variables. Increasing the number of model executions to 15,000 produced results that agreed with 10,000 executions within 1%, suggesting a sufficient number had been used. The results are tabulated to see what fraction of a given log reduction in N/N_0 is exceeded. This allows an estimate of performance, even if some of the parameters are uncertain or variable.

The most uncertain parameter in this work is the fraction of the viral population in aggregates (β). Johnson et al. (2011) showed that the diameter of saliva droplets produced by speaking followed a lognormal distribution, so β is also assumed to be lognormal. Since there is a high degree of uncertainty in estimating β , a relatively large standard deviation is assumed for this lognormal distribution: 80% of the mean. Figure 1 show the distribution of β used in this work. Note that the mean value is about 2%, but values as low as 0.4% and as high as 7.6% are possible.

There is less uncertainty in the single organism disinfection rate constant (k_S), since published values are often available. However, the aggregate rate constant (k_A), as discussed previously, is more uncertain, with few values found in the literature. To account for this uncertainty, k_A is assumed to be normally distributed with a standard deviation of 50% of the mean.

SARS-CoV-2 Modelling

One of the advantages of the relatively simple model used here is that only three parameters are needed: k_S , k_A , and β . Of these, k_S is the easiest to estimate. Based on the recent review of Hessling et al. (2020), the median observed value for k_S for several different coronaviruses is approximately 0.22 cm²/mJ. This is consistent with observed cluster of values for SARS-CoV-1, MS2, and Φ 6 viruses published by Iii et al. (2020). The published value for SARS-CoV-1 in this cluster has a k_S value of approximately 0.18 cm²/mJ. Taking the average, a value of 0.20 cm²/mJ is used for the k_S of SARS-CoV-2 in this work.

The fraction of viruses in aggregates (β) is more uncertain. From the model validation work, described below, the values of β were observed to be 2% and 13% for 2 μ L and 10 μ L droplets, respectively. This suggests β decreases as droplets get smaller. The actual droplets we are interested in are much smaller than 2 μ L, on the order of 0.002 μ L. Nonetheless, we will conservatively assume a mean value for β of 2% for disinfection of SARS-CoV-2. This ensures there is some safety factor in any disinfection predictions.

Estimating k_A for SARS-CoV-2 is also difficult, but there is some previous work on particle-associated bacteria on which we can draw. Azimi et al. (2012) observed a ratio of k_A/k_S of 6%. Kollu and Örmeci (2012) observed an approximate ratio of k_A/k_S of 15%. The ratio of k_A/k_S in the model validation in this work was 12%. Therefore, we assign a median value of k_A/k_S of 10% in this work based on other observations. This gives a value of k_A of 0.020 cm²/mJ for SARS-CoV-2 (10% of k_S). Table 1 shows an overview of the model inputs used for *B. subtilis* spores and SARS-CoV-2.

Table 1 Monte Carlo Simulation parameters*

Test Organism	Model Parameter	Distribution type	Mean Value	Standard deviation (% of mean)
<i>B. subtilis</i> spores	Single organism rate constant (k_S)	n/a	0.10 cm ² /mJ	n/a
	Aggregate disinfection rate constant (k_A)	Normal	0.012 cm ² /mJ	0.006 (50%)
	Fraction of particle associated organisms (β)	Lognormal	2%	1.8% (80%)
SARS-CoV-2	Single organism rate constant (k_S)	n/a	0.20 cm ² /mJ	n/a
	Aggregate disinfection rate constant (k_A)	Normal	0.020 cm ² /mJ	0.010 (50%)
	Fraction of particle associated organisms (β)	Lognormal	2%	1.8% (80%)

* See Model Development section for details of parameter estimation

Results

Model Validation

Laboratory disinfection work commonly uses less hazardous surrogates in place of more dangerous ones. The work of Barancheshme et al. (2021) used relatively difficult to disinfect, *B. subtilis* spores in dried human saliva droplets in a UV disinfection study.

Figure 2 shows the results of Barancheshme et al. (2021) on *B. subtilis* spores fitted to a double-exponential model for 2 μL and 10 μL droplets. Note that both rate constants (k_S and k_A) have the same values independent of droplet size. The only difference between the figures is the fraction of organisms found in aggregates (β), which decreases with droplet size from 13–2%. The two-population, double-exponential model effectively described the UV disinfection of *B. subtilis* spores in dried human saliva resulting in R^2 values of 0.98 and 0.97 for 2 μL and 10 μL droplets, respectively. These results suggest that the rate constants are indeed constant, and the effects of droplet size can be approximated by changing the β alone.

Monte Carlo Simulation of *B. subtilis* Spore UV inactivation

In reality, the number of organisms associated with aggregates is both variable and uncertain, since not two coughs are exactly the same. Based on the previous model validation, this variability is approximated by changing the parameter β . The precise value of k_A is also not known, only approximated, so a normal distribution is used to account for this. Allowing for this uncertainty, Table 2 shows the expected log reduction in *B. subtilis* spores in dried saliva droplets.

Table 2 Probability of achieving different log reductions in *B. subtilis* spores as a function of UV dose (Monte Carlo simulation with 10,000 model executions)

	Dose (mJ/cm ²)					
	20	40	60	80	100	120
>1 log	<0.1%	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%
>2 log	<0.1%	<0.1%	33%	64%	75%	81%
>3 log	<0.1%	<0.1%	<0.1%	<0.1%	1%	4%

The results of the MCS show that there are diminishing returns as UV dose is increased. One-log reduction can be achieved at a dose of 40 mJ/cm². However, doubling this dose to 80 mJ/cm² shows that a two-log reduction will be achieved only 64% of the time. Tripling the dose to 120 mJ/cm² only increases the chances of two-log (or more) reduction to just 81%. The rate constant for aggregates,

important at higher log reductions, is an order of magnitude smaller than that of single organisms. Also, aggregates are variable in this model, and it gets more unlikely to achieve complete inactivation at the higher doses where they dominate. For example, some of the time, aggregates will contain such a large fraction of the total population a high log reduction is difficult to achieve.

Monte Carlo Simulation of SARS-CoV-2 Inactivation

The single organism rate constant (k_S) for SARS-CoV-2 used in this work is twice that of *B. subtilis* spores (0.20 vs. 0.10 cm²/mJ). In addition, the aggregate rate constant (k_A) is assumed to be proportional to the single organism disinfection sensitivity (k_S), making both rate constants larger. These higher rate constants make the log reductions that can be achieved with SARS-CoV-2 greater than that of *B. subtilis* spores (Table 3).

For example, a one-log reduction in SARS-CoV-2 is very likely ($p > 99.9\%$) at a dose of just 20 mJ/cm², but very unlikely for *B. subtilis* spores ($p < 0.1\%$). At 40 mJ/cm², the probability of exceeding a two-log reduction in SARS-CoV-2 is 55%, where it is $< 0.1\%$ for *B. subtilis* spores. Overall, this makes *B. subtilis* spores a conservative surrogate for SARS-CoV-2.

Table 3 Probability of achieving different log reductions in SARS-CoV-2 as a function of UV dose (Monte Carlo simulation with 10,000 model executions)

	Dose (mJ/cm ²)					
	20	40	60	80	100	120
>1 log	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%
>2 log	<0.1%	55%	75%	84%	89%	91%
>3 log	<0.1%	<0.1%	1%	7%	19%	33%

Once again, even for the more UV sensitive SARS-CoV-2, aggregates limit the ability to achieve high log reductions, in this model. Doubling the dose from 60 to 120 mJ/cm² increases the probability of two-log reduction from 75–91%, an increase of just 17%. The probability of achieving greater than a three-log reduction never exceeds 33%. This suggests the three-log reductions are likely difficult to achieve in this conservative model. In practical terms, a two-log reduction of SARS-CoV-2 in dried saliva droplets is likely a reasonable target.

According to the model, a one-log reduction is very likely ($> 99.9\%$) and a two-log reduction is probable ($p = 75\%$) for SARS-CoV-2 at a dose of 60 mJ/cm². This is likely a practical dose to aim for. Figure 3 shows the distribution of log reductions at this dose. Note that both low (< 1.3) and high (> 3.2) log reductions are observed, illustrating the variable nature of dried droplets in this model.

Model Limitations

The assumptions of the model are described in the Model Development section. Briefly, we assume that UV disinfection of dried saliva is similar to UV disinfection in other liquids containing aggregates and/or particles. The Model Validation section shows that the observed disinfection of *B. subtilis* spores in dried saliva could be described in this way with $R^2 \geq 0.97$. The fraction of organisms in aggregates is estimated using a single parameter β , where in reality the situation is likely much more complex. However, even if β could be determined experimentally, there is no guarantee that a different cough or sneeze would produce the same result. Nonetheless, a better understanding how viruses are distributed in dried droplets could improve this model. The single organism rate constant for SARS-CoV-2, on which so much depends, is approximate and based on the literature and not actual experiments. This also could be improved.

Lastly, the validation work was done using bacterial spores, and some of this information was extrapolated to viruses. This size discrepancy between viruses and bacteria likely contributes more uncertainty in β and k_A for SARS-CoV-2. However, some uncertainty in these parameters was accounted for in the MCS. In addition, an appropriate single organism rate constants (k_S) was used for SARS-CoV-2, and k_S describes most of the behavior at low log reductions. High log reductions are uncertain, and this is reflected in the model.

Overall, double-exponential, three-parameter model that can describe UV disinfection in dried saliva droplets is proposed. Applying this simple model to SARS-CoV-2 disinfection suggests the one-log reductions are very likely ($p > 99.9\%$) and two-log reductions are probable ($p = 75\%$) at a dose of 60 mJ/cm².

Conclusions

In this work, we demonstrated the UV disinfection of *B. subtilis* spores in dried human saliva could be described by a two-population, double-exponential model ($R^2 \geq 0.97$) where β is the fraction of organisms in aggregates. The value of β was 2% for dried 2 μ L droplets and decreased with droplet size. Since no two coughs are exactly the same, we assume that β is variable and it was assigned a lognormal distribution. Monte Carlo simulation (MCS) of *B. subtilis* spores and SARS-CoV-2 showed increases in the resistance to UV disinfection and increases in the variability in the results at higher log reductions. This was attributed to the lower disinfection rate constant for aggregates (k_A) and the variable nature of droplets. It was shown, using a conservative estimation of β for very small droplets, that a two-log reduction of SARS-CoV-2 was probable ($p = 75\%$) at a dose of 60 mJ/cm². Higher log reductions are more uncertain and difficult to achieve. Future work should investigate the impact of dried droplets characteristics on the kinetic parameters of the double-exponential model.

Declarations

Ethics approval and consent to participate:

This study did not involve human participants, human data or human tissue.

Consent for publication:

Consent was provided by one the co-authors of this study (Barbeau) to use experimental results from and his earlier study (Barancheshme et al., 2021).

Availability of data and materials:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests:

The authors declare that they have no competing interests.

Funding:

No outside funding was provided for this low-cost, modelling study.

Author Contribution:

JG conceptualized work, did the numerical analysis, and wrote first draft of report

RF provided financial support in the form of a research associate position for JG. Also provided editorial comments on draft report.

BB provided experimental data used in validating model and editorial comments and suggestions for draft report.

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Figures

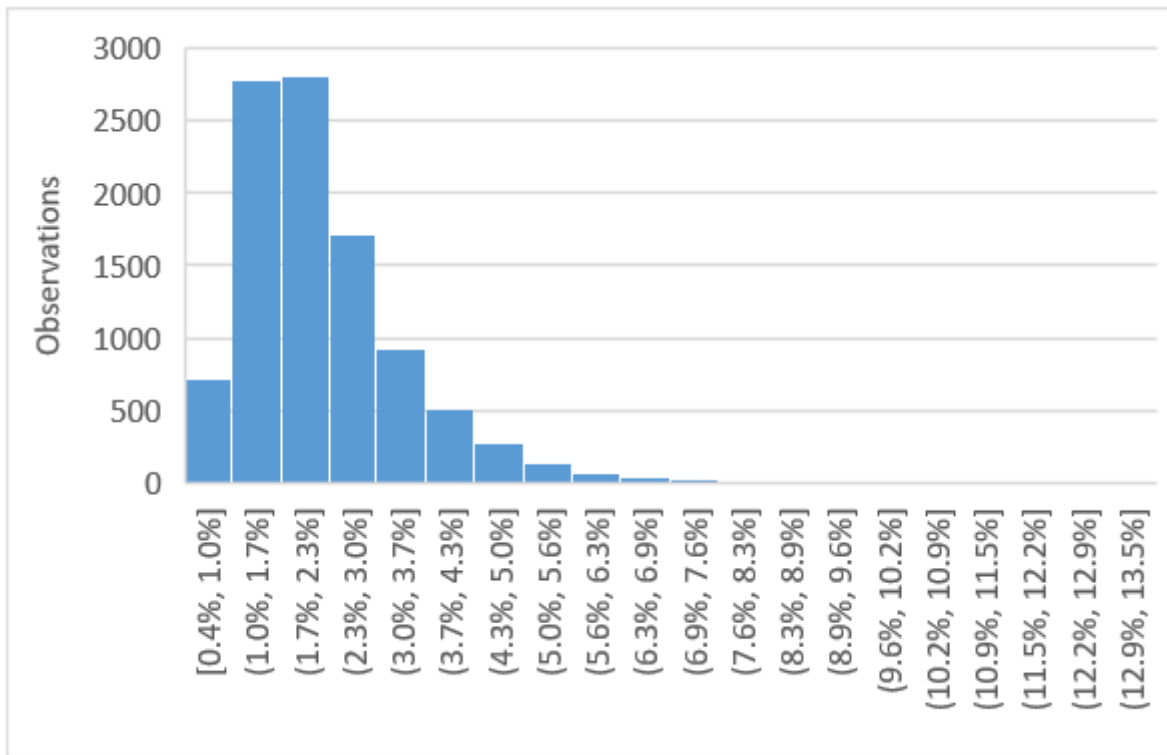


Figure 1

Distribution of the fraction of the population found in aggregates (β) used in this work (10,000 simulations).

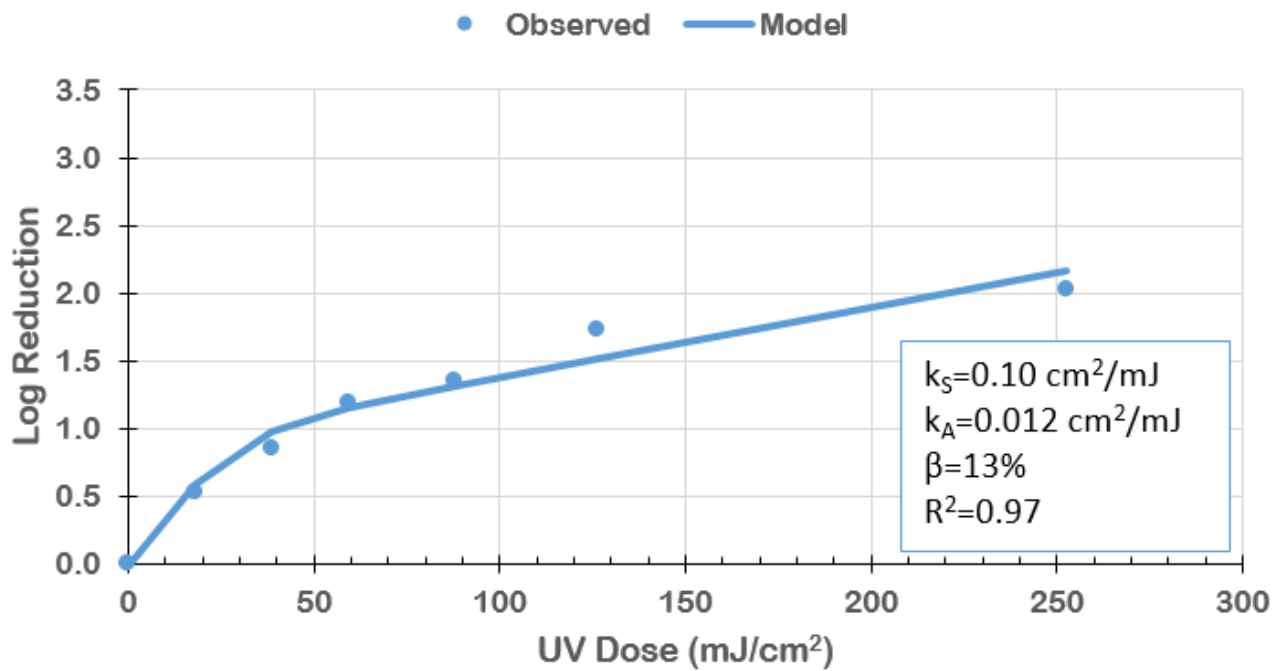
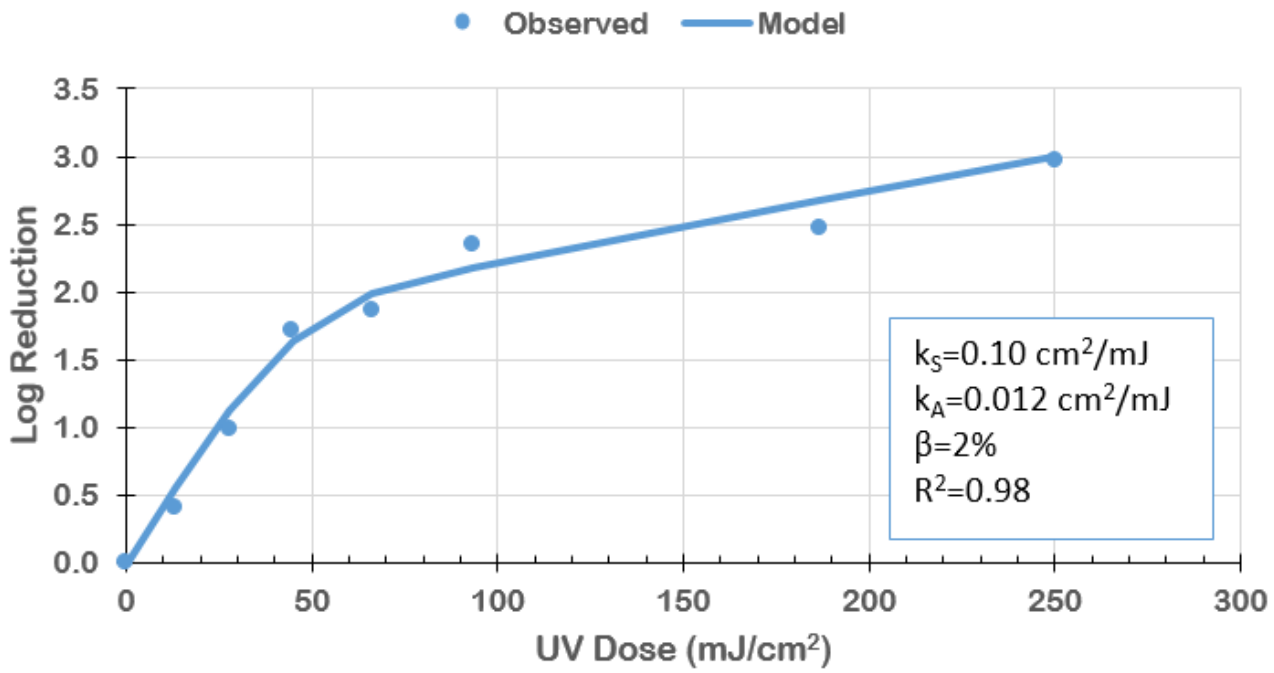


Figure 2

UV disinfection of *B. subtilis* spores in human saliva in dried 2 µL (A) and 10 µL (B) droplets fitted to a double-exponential model. (k_S = rate constant for single organisms; k_A = rate constant for aggregates; β = fraction of organisms in aggregates)

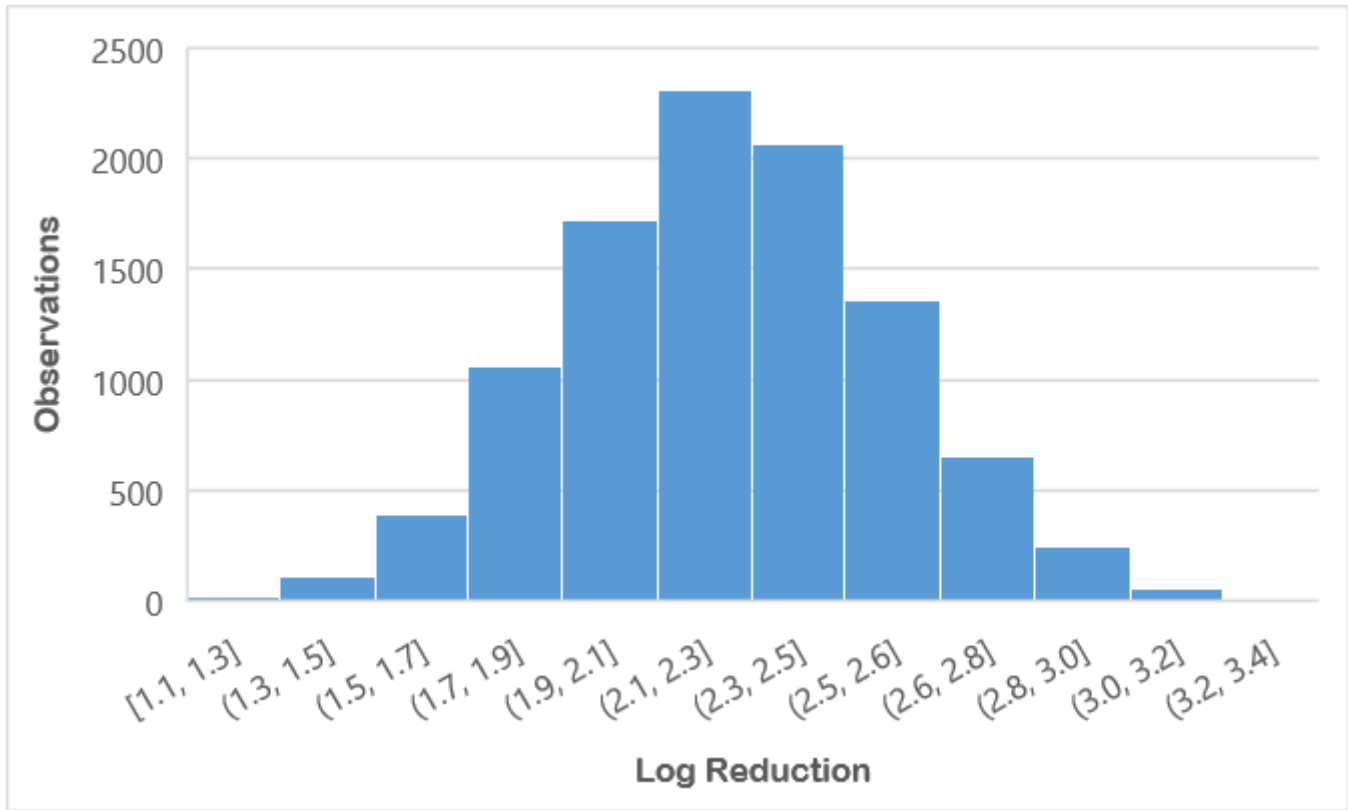


Figure 3

Log reductions of SARS-CoV-2 in dried saliva droplets at a UV dose of 60 mJ/cm² (10,000 model executions)