



HAL
open science

Applications of predictive modeling techniques to fungal growth in foods

Philippe Dantigny

► **To cite this version:**

Philippe Dantigny. Applications of predictive modeling techniques to fungal growth in foods. Current Opinion in Food Science, 2021, 38, pp.86 - 90. 10.1016/j.cofs.2020.10.028 . hal-03493157

HAL Id: hal-03493157

<https://hal.science/hal-03493157v1>

Submitted on 15 Dec 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Applications of predictive modeling techniques to fungal growth in foods

Philippe Dantigny

Univ Brest, Laboratoire Universitaire de Biodiversité et Ecologie Microbienne, F-29280 Plouzané,
France

Corresponding author: Dantigny, Philippe (philippe.dantigny@univ-brest.fr)

1. Introduction

The objective of this mini-review is to guide microbiologists through the selection and implementation of suitable predictive models for specific mold growth applications. Most food microbiologists including mycologists are not familiar with modelling techniques while people involved in modelling are unaware of mold specificities. As compared to bacteria, the development of mold is characterized by distinct features, such as the ability to form mycelium and to produce asexual conidia and sexual spores for some species [1**]. Several fundamental laws that have been developed in the process control field [2], can be extended to process modelling as, i/ the simplest model that described the data satisfactorily is the best, ii/ you must understand the process before you can model it. A model cannot describe all complexities of a system. Therefore, it is necessary to determine the relevant biological responses and select the most significant factors that impact these responses, in addition to attaining proven predictive capacity.

2. Biological responses

In a recent paper [3], the major fungal responses were listed, inactivation, growth, germination, and production of metabolites, mycotoxins mainly. Modeling the effect of environmental factors on mycotoxins concentration was addressed recently [4**,5**]. The biological responses and the model parameters that should be determined with respect to the studied phenomenon are reported Table 1. Primary models are kinetic and describe how biological responses vary with time, secondary models describe how primary model parameters depend on environmental and biological factors.

3. Model characteristics and requirements

3.1. Primary models

Simplicity is the first quality. A simple linear model with breakthrough is widely used by mycologists to determine the fungal growth rate, μ (mm/h).

$$r = r_0 + \mu (t - \lambda) \quad \text{for } t > \lambda \quad \text{Eq (1)}$$

where r (mm) the radius of the colony, r_0 (mm) the radius of the inoculation suspension droplet or agar plug, λ (h) the lag time for growth and t (h) the time. In more than 9 cases out of 10, the linear

model described the kinetics satisfactorily with r^2 greater than 0.995. Bacteriologists are using the Baranyi's model, which has been developed to describe the decrease in the growth rate in liquid medium due to substrate limitation. In one case out of 10, a decrease in the fungal growth rate is noticed, especially for long incubation duration. In such a case, the Baranyi's model can be used.

$$r = r_0 + \mu A - \text{Ln} \left\{ 1 + \frac{[\exp(\mu A) - 1]}{\exp(r_{max} - r_0)} \right\} \text{ with} \quad \text{Eq (2)}$$

$$A = t + \left(\frac{1}{\mu} \right) \text{Ln} [\exp(-\mu t) + \exp(-\mu \lambda) - \exp(-\mu t - \mu \lambda)] \quad \text{Eq (3)}$$

It appears that this model is rather complex, the significance of the parameters can be found elsewhere [6]. By omitting the logarithmic terms in Eq (2-3), the Baranyi's model leads to the linear one [7]. Applying the first law reported in the introduction, the linear model is simple and should be preferred to the Baranyi's one.

Model accuracy is the second quality. Accuracy is evaluated by the goodness of fit and the quality of the parameter estimates by means of the (root) mean square error, (R)MSE, and the 95% confidence intervals, respectively. Usually accuracy is the only criterion to choose a model. This is not a good approach because the "best" model can depend on the studied organism.

Versatility is the third quality of a model. Although the shape of the curves obtained by plotting biological responses *versus* time are different, the same model should be used to compare the same parameters. Depending of the experimental conditions such as a water stress, germination curves can be either asymmetric or symmetrical. For this reason, a versatile model capable to fit both shape of curves was proposed recently [8].

$$P = P_{max} \left[1 - \frac{1}{1 + \left(\frac{t}{\tau} \right)^d} \right] \quad \text{Eq (4)}$$

where P(%) the percentage of germinated spores, P_{max} (%) the maximum percentage of viable spores, τ (h) the germination time, d(-) a shape parameter.

The model described Eq (4) is asymmetric but tends to be symmetrical as the shape parameter d increases.

The Weibull model, Eq (5) was used for describing the effect of ethanol vapour on the inactivation of three *Penicillium* species [9], Figure 1:

$$\log \left[\frac{N}{N_0} \right] = - \frac{1}{2.303} \left(\frac{t}{\alpha} \right)^\beta \quad \text{Eq (5)}$$

Depending of the value of the shape parameter β (-), the inactivation curve can be upward concave ($\beta > 1$) or downward concave ($\beta < 1$). Therefore, the Weibull model allows the comparison between different kinetics by means of the same parameters.

Fitness is the fourth model quality. The model should fit the experimental data with a correct shape. The effect of pH on the growth rate of chalk yeasts [10], illustrates this concept. pH has almost no effect on the growth rate in a wide range, pH 3-7, Figure 2. The cardinal model with inflection, CMI, is based on the cardinal values, pH_{min} , pH_{max} , minimum and maximum pH at which no growth occurred, respectively, and pH_{opt} , pH at which growth is the greatest.

$$\frac{\mu}{\mu_{opt}} = \frac{(pH - pH_{min})(pH - pH_{max})^2}{(pH_{opt} - pH_{max})[(pH_{opt} - pH_{max})(pH - pH_{opt}) - (pH_{opt} - pH_{min})(pH_{opt} + pH_{max} - 2 pH)]} \quad \text{Eq (6)}$$

A plateau can be observed, therefore there is no precise pH_{opt} value. For this reason, a specific model to describe plateau based on $pH_{-1/2}$ and $pH_{+1/2}$, acid and alkaline pH at which $\mu = \mu_{opt}/2$, was developed.

$$\frac{\mu}{\mu_{opt}} = \left[1 - \frac{1}{1 + \left(\frac{pH}{pH_{-1/2}} \right)^d} \right] \cdot \left[1 - \frac{1}{1 + \left(\frac{14 - pH}{14 - pH_{+1/2}} \right)^{d/2}} \right] \quad \text{Eq (7)}$$

where d (-) a design parameter.

The lack of fitness of the CMI leads to an overestimation of the optimal growth rate, Fig. 2. The estimated μ_{opt} value was 1.25 mm/day by the CMI, whereas the "true" value was around 1.00.

3.2. Secondary models

Previous sections of this paper were concerned with primary models. All parameters listed in Table 1 depend on many factors such as environmental conditions, i.e., pH, water activity, gas composition, inhibitors concentration according to secondary models. The great majority of these models aimed at modelling the effect of these factors on the radial growth rate.

3.2.1. Environmental factors

Polynomial models were widely used to describe the effect of environmental factors on the radial growth rate [11*]. These polynomial models are very versatile but their parameters are not comparable in biological or physical terms. Therefore, their changes are not intuitive for mycologists. In contrast to polynomial models, secondary models, based on cardinal limits for growth or minimal inhibitory concentrations, MIC, were developed for fungi.

A cardinal model with inflection [12] was proposed to describe the effect of temperature on bacterial growth rate based on three cardinal parameters (T_{\min} , T_{opt} and T_{\max}). At T_{opt} the growth rate (μ_{opt}) is optimum. The min and max sub-indices correspond to values below and above no growth occurs. This model extended its use to describe the effect of water activity on the fungal growth rate [13]. Eventually, it was suggested to set the maximum water activity to 1 (pure water) [14*]. The final model consisted of only three parameters, i.e., $a_{w\min}$, $a_{w\text{opt}}$ and μ_{opt} .

$$\frac{\mu}{\mu_{\text{opt}}} = \frac{(a_w - 1)(a_w - a_{w\min})^2}{(a_{w\text{opt}} - a_{w\min})[(a_{w\text{opt}} - a_{w\min})(a_w - a_{w\text{opt}}) - (a_{w\text{opt}} - 1)(a_{w\text{opt}} - a_{w\min} - 2a_w)]} \quad \text{Eq (8)}$$

In its normalized form, the ratio μ/μ_{opt} varies between zero to one, and the right hand-side of Equation (8) is named $\gamma(a_w)$.

3.2.2. Inhibitory molecules

Ethanol is used in the bakery industry at low concentrations to inhibit mold growth in food products. The inhibitory effect of ethanol [15] was described by a model, Eq (8) capable to fit either upward or downward concave shape curves, by means of a design parameter, K (%). The right hand side of Eq (9), $\gamma(E)$, is a gamma function for ethanol.

$$\frac{\mu}{\mu_{\text{opt}}} = \frac{K(E_{\max} - E)}{KE_{\max} - 2KE + EE_{\max}} \quad \text{Eq (9)}$$

$E(\%)$ was the ethanol concentration, and E_{\max} (%) represented the MIC value for ethanol. The studied species exhibited MIC values in the range 2.14-6.43%.

Fungicides prepared from copper sulfate are used in vineyards against downy mildew (*Plasmora viticola*) and *Botrytis cinerea*. In contrast to ethanol, the inhibitory effect of copper sulfate concentration on the radial growth rate of *B. cinerea* and *Penicillium expansum* [16] exhibited a S-shaped curve, Fig. 3, and accordingly was described by another model, Eq (10).

$$\frac{\mu}{\mu_{\text{opt}}} = \frac{1}{1 + \left(\frac{Cu}{Cu_{50}}\right)^d} \quad \text{Eq (10)}$$

Cu (mM) was the fungicide concentration, Cu_{50} (mM) the concentration at which $\mu = \mu_{\text{opt}}/2$, and d (-) a design parameter. The right hand side of Eq (10), $\gamma(Cu)$, is a gamma function for copper sulfate concentration. These models are potentially useful to combine the effect of inhibitors and environmental factors because they are compatible with the gamma concept.

3.3. Gamma concept

The gamma concept [17^{*}] was introduced to describe the combined effect of various environmental factors on the relative growth rate, μ/μ_{opt} . For example, the combined effect of water activity and temperature could be expressed as:

$$\gamma = \frac{\mu}{\mu_{opt}} = \gamma(a_w)\gamma(T) \quad \text{Eq (11)}$$

By using the gamma concept, the effect of each factor could be assessed independently from the others. Usually the gamma functions are based on parameters that are independent from the medium such as cardinal values. In a first step, these parameters can be estimated on synthetic or semi-synthetic medium. Then in a second step, the only parameter, μ_{opt} , that depends on the medium or the food product can be calculated. This approach demonstrated its suitability for predicting the growth of *B. cinerea* and *P. expansum* on grape berries as a function of T and a_w [18^{*}], and the growth of chalk yeasts on bread as a function of T, a_w and pH [19].

4. Mould-free shelf-life of food products

Fungal growth rate is not the major biological response to be considered [3] for determining the mould free shelf-life of food products. In this case, the time for visible growth, t_v (d), is more relevant because a food product is considered to be spoiled when the mould colony is visible, say 2-3 mm. Food products are usually contaminated by a low number of conidia after processing. The mould-free shelf life of food products is usually assessed by adding a known number of spores at the same spot. In contrast to the fungal growth rate, the time to visible growth depends greatly on the inoculum size [20]. The model parameters can be estimated for inoculum size in the range 10^2 - 10^5 spores/spot, then t_v can be determined with the model for 1 spore/spot by extrapolation [21]. However, it does not make sense to determine t_v for a single spore. Due to biological variability within a population of spores, different statistical distributions (Exponential, Normal, Lognormal, Weibull, Logistic or Pareto) of t_v can be applied. It was reported that at 53° Brix, 0.883 a_w , the 95th percentile t_v was four fold the 5th percentile for *Byssosclamyces fulva* [22^{**}].

5. Conclusion

Last, but not least, the physiological state is the neglected factor. In the laboratory, fungal spores are produced under optimal conditions, then partially re-hydrated for obtaining standardized spore suspensions [23^{*}]. In contrast to lab spores, spores present in the air that are a major source of

contamination of dairy [24] and bakery [25] products are un-hydrated. It was shown that hydration modify greatly and rapidly the physiological state of fungal spores [26], thus affecting the distribution of the germination time among a population of spores. Because germination time can represent up to one fourth of the time to visible growth [21], the time to visible growth should be estimated by inoculating airborne conidia at the surface of dairy and bakery products [27]. It can be concluded that many of existing data reported in the literature may be biased because no sufficient attention has been paid to the impact of the physiological state on biological responses.

References and recommended reading

1. Dantigny P, Bensoussan M: **Introduction to predictive mycology**. In *Predictive Mycology*. Edited by Dantigny P, Panagou EZ. Nova Science Publishers; 2013:1-26.
- (●●) A basis of predictive mycology.
2. Luyben WL: *Process Modeling, Simulation and Control for Chemical Engineers*. 2nd Edition. Mc Graw-Hill International Editions, 1990, 725p.
 3. Dantigny P: **Relevant issues in predictive mycology**. *Curr Opin Food Sci* 2016, **11**:29-33.
- (●●) The major factors, that affect fungal responses in food and in the environment, are listed.
4. Aldars-García L, Ramos AJ, Sanchis V, Marín S: **Modeling postharvest mycotoxins in foods: recent research**. *Curr Opin Food Sci* 2016, **11**:46-50.
- (●●) Media, intraspecific variability, and microbial interactions should be integrated in model design for predicting mycotoxins production.
5. Coton M, Dantigny P: **Mycotoxin migration in moldy foods**. *Curr Opin Food Sci* 2019, **29**:88-93.
- (●●) The list of the major factors that affect mycotoxin migration in moldy foods.
6. Baranyi J, Roberts TA: **A dynamic approach to predicting bacterial growth**. *Int J Food Microbiol* 1994, **23**:277-294.
 7. Gibson AM, Baranyi J, Pitt JI, Eyles MJ, Roberts TA: **Predicting fungal growth: the effect of water activity on *Aspergillus flavus* and related species**. *Int J Food Microbiol* 1994, **23**:419-431.
 8. Dantigny P, Nanguy SP-M, Judet-Correia D, Bensoussan M: **A new model for germination of fungi**. *Int J Food Microbiol* 2011, **146**:176-181.
- (●) A new versatile model specifically developed to fit either, symmetric and asymmetric germination curves.
9. Dao T, Dejardin J, Bensoussan M, Dantigny P: **Use of the Weibull model to describe inactivation of dry-harvested conidia of different *Penicillium* species by ethanol vapours**. *J Appl Microbiol* 2010, **109**:408-414.
 10. Dantigny P, Burgain A, Deniel F, Bensoussan M: **A model for the effect of pH on the growth of chalk yeasts**. *Int J Food Microbiol* 2014, **186**:49-54.
 11. Deschuyffeleer N, Samapundo S, Devlieghere F: **Secondary models for fungi**. In *Predictive Mycology*. Edited by Dantigny P, Panagou EZ. Nova Science Publishers; 2013:153-194.
- (●) An extensive review of the existing secondary models for fungi.
12. Rosso L, Lobry JR, Flandrois JP: **An unexpected correlation between cardinal temperatures of microbial-growth highlighted by a new model**. *J Theor Biol* 1993, **162**:447-463.
 13. Rosso L, Robinson TP: **A cardinal model to describe the effect of water activity on the growth of moulds**. *Int J Food Microbiol* 2001, **63**:265-273.

14. Sautour M, Dantigny P, Divies C, Bensoussan M: **A temperature-type model for describing the relationship between fungal growth and water activity.** *Int J Food Microbiol* 2001, **67**:63-69.
- (●) An accurate model for estimating the cardinal water activities for fungal growth.
15. Dantigny P, Guilmart A, Radoi F, Bensoussan M, Zwietering M: **Modelling the effect of ethanol on growth rate of food spoilage.** *Int J Food Microbiol* 2005, **98**:261-269.
16. Judet-Correia D, Charpentier C, Bensoussan M, Dantigny P: **Modelling the inhibitory effect of copper sulfate on the growth of *Penicillium expansum* and *Botrytis cinerea*.** *Lett Appl Microbiol* 2011, **53**:558-564.
17. Zwietering MH, Witjes T, de Wit JC, van't Riet K: **A decision support system for prediction of microbial spoilage in foods.** *J Food Prot* 1992, **55**:973-979.
- (●) The paper that described the gamma concept.
18. Judet-Correia D, Charpentier C, Bensoussan M, Dantigny P: **Modelling the inhibitory effect of copper sulfate on the growth of *Penicillium expansum* and *Botrytis cinerea*.** *Lett Appl Microbiol* 2011, **53**:558-564.
- (●) This paper demonstrated how easy was the validation of predictive models on food by using the gamma concept.
19. Burgain A, Bensoussan M, Dantigny P: **Validation of a predictive model for the growth of chalk yeasts on bread.** *Int J Food Microbiol* 2015, **204**:47-54.
20. Gougouli M, Kalantzi K, Beletsiotis E, Koutsoumanis K: **Development and application of predictive models for fungal growth as tools to improve quality control in yogurt production.** *Food Microbiol* 2011, **28**:1453-1462.
21. Burgain A, Bensoussan M, Dantigny P: **Effect of inoculum size and water activity on the time to visible growth of *Penicillium chrysogenum* colony.** *Int J Food Microbiol* 2013, **163**:180-183.
22. Santos JL, Samapundo S, van Impe J, Sant'Ana SA, Devlieghere F: **Effect of sugar concentration (°Brix) and storage temperature on the time to visible growth of individual ascospores of six heat-resistant moulds isolated from fruit products.** *Food Control* 2020, **108**:106880.
- (●●) In the short list for the best predictive mycology paper award 2020.
23. Dantigny P, Nanguy SP-M: **Significance of the physiological state of fungal spores.** *Int J Food Microbiol* 2009, **134**:16-20.
- (●) A short review on the effect of physiological state of fungal spores on various biological responses such as germination time, viability and inactivation.
24. Salustiano VC, Andrade NJ, Cardoso Brandão SC, Cordeiro Azerado RM, Kitakawa Lima SA: **Microbiological air quality of processing areas in a dairy plant as evaluated by the sedimentation technique and a one stage sampler.** *Braz J Microbiol* **34**:255-259.
25. Legan JD: **Mould spoilage of bread: the problem and some solutions.** *Biodeterior Biodegrad* 1993, **32**:33-53.
26. Nanguy SP-M, Perrier-Cornet J-M, Bensoussan M, Dantigny P: **Impact of water activity of diverse media on spore germination of *Aspergillus* and *Penicillium* species.** *Int J Food Microbiol* **142**:273-276.

27. Burgain A, Dantigny P: **Inoculation of airborne conidia of *Penicillium chrysogenum* on the surface of a solid medium.** *Food Microbiol* 2016, **54**:162-166.

(●●) Towards a standardized method to inoculate airborne conidia on food.

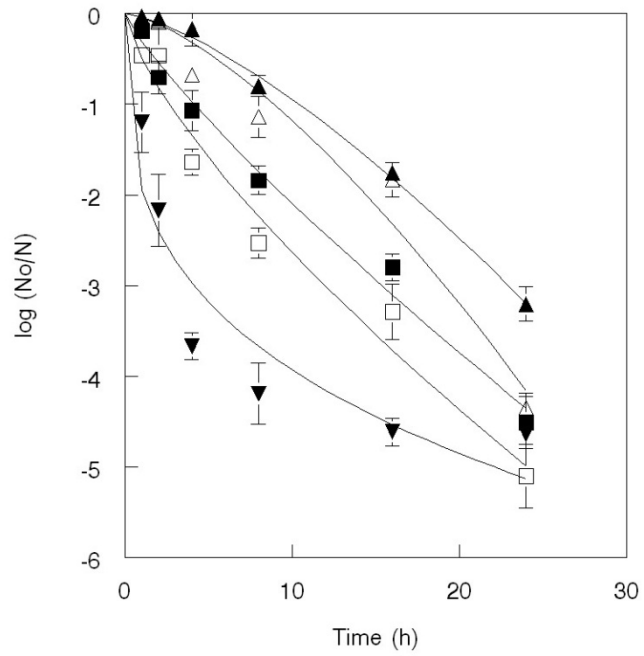


Fig. 1: Survival curves of dry-harvested conidia of *Penicillium chrysogenum* exposed to ethanol vapour, (▲) 0.7 kPa, (△) 1.5 kPa, (■) 2.8 kPa, (□) 4.6 kPa, (▼) 7.5 kPa. (Dao et al., J. Appl. Microbiol, 109, 408-414, 2010)

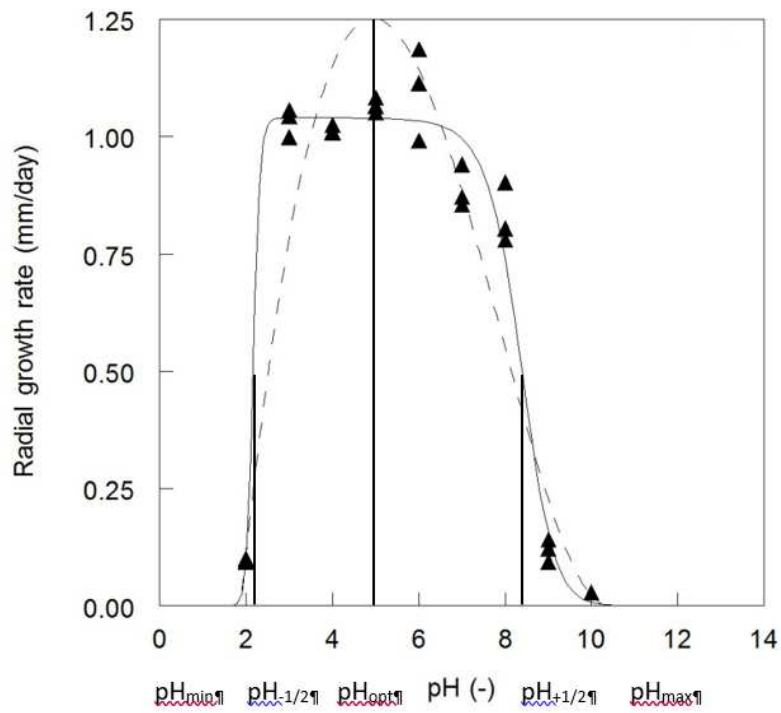


Fig 2 : Influence of pH on the growth of *Hyphopichia burtonii*. Broken line: cardinal model with inflection. Solid line: plateau model. Adapted from Dantigny et al., 2014 [19].

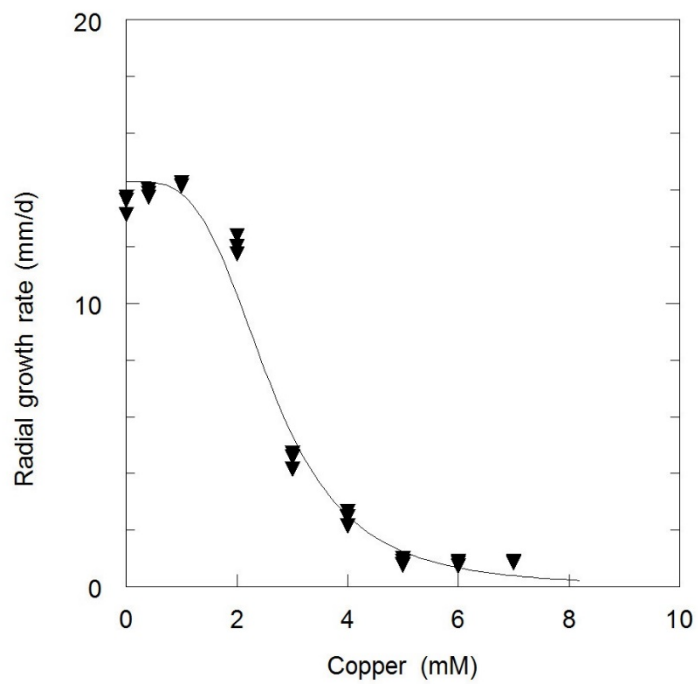


Fig. 3: Effect of copper concentration on the radial growth rate of *Botrytis cinerea* (Adapted from Judet-Correia et al., Lett. Appl. Microbiol, 53, 558-564, 2011)

Table 1: Structure of **primary** models used in predictive mycology.

Phenomenon	Biological response	Model structure	Biological parameters
Inactivation	Number of survivors : N (spores/ml)	$\text{Log } (N) = f(t)$	Inactivation rate: $k \text{ (min}^{-1}\text{)}$
Growth	Radius of the colony : r (mm)	$r = f(t)$	Radial growth rate: $\mu \text{ (mm/d)}$
Germination	Percentage of germinated spores: P (%)	$P = f(t)$	Germination time: $\tau \text{ (h)}$ Viability: $P_{\text{max}} \text{ (%)}$

