



Review

# Micro-organism inactivation during drying of small droplets or thin-layer slabs – A critical review of existing kinetics models and an appraisal of the drying rate dependent model

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## Abstract

In this article, we have reviewed the commonly used inactivation kinetics models in order to explore a fundamental basis for modelling the inactivation mechanism during drying of small droplets or thin-layer slabs of bioactive liquid-based materials. In the literature, there are only a few studies dealing systematically with the theoretical and/or experimental aspects of the inactivation kinetics during drying of small objects. In most studies, the inactivation kinetics model for enzymes and micro-organisms was formulated by incorporating the average temperature and water content of the material. The experimental validation of these models has been limited. In general, the inactivation kinetics models published in the literature were validated using ‘heating-only’ experiments (no evaporation involved) and large deviations were reported between the predicted and measured survival rates. It is uncertain if drying parameters such as the average moisture content and average temperature are most appropriate for correlating the inactivation kinetics. Recently, the drying-rate and temperature-rate dependent models were proposed and tested against the micro-organisms survival during drying experiments in order to develop a more accurate approach. The rate-dependent models seem to be more useful compared to the traditional models with the average temperature and water content as two prime parameters. In this paper, the authors have presented an assessment considering the spatial distribution of the inactivation kinetics emphasizing the difference between the surface moisture content and the centre moisture content of the material. This appraisal provides a fundamental qualification of the rate-dependent models, which have been obtained empirically.

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*Keywords:* Inactivation kinetics; Drying kinetics; Drying; Micro-organisms; Enzymes

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## Nomenclature

$a$	fitting parameter	$X_0$	water content at the centre of the droplet (kg water/kg dry solids)
$\bar{a}$	fitting parameter	<i>Greek symbols</i>	
$b$	fitting parameter	$\alpha$	fitting parameter
$E_d$	apparent activation energy ( $\text{J mol}^{-1}$ )	$\beta$	fitting parameter
$k_0$	pre-exponential factor ( $\text{s}^{-1}$ )	$\gamma$	parameter used in <a href="#">Appendix B</a>
$\bar{k}_0$	fitting parameter	$\theta$	dimensionless temperature
$k_d$	inactivation rate constant ( $\text{s}^{-1}$ )	$\Phi$	unknown parameter in Eqs. (13) and (16)
$k_{\text{drying}}$	first-order rate constant ( $\text{s}^{-1}$ )	<i>Subscripts</i>	
$N$	number of the live micro-organism	s	surface conditions
$N_0$	initial number of the live micro-organism	$\infty$	bulk gas phase conditions
$r$	radial distance within a droplet (m)	ref	reference conditions
$R$	droplet radius (m)		
$R_g$	universal gas constant ( $\text{J mol}^{-1} \text{K}^{-1}$ )		
$t$	time (s)		
$T$	temperature (K)		
$X$	water content on a dry basis (kg water/kg dry solids)		

## 1. Introduction

Drying is a widely employed unit operation in processing of bioactive materials such as foodstuff, dairy powders, drugs, nutraceuticals, biochemicals and various biological suspensions containing enzymes, proteins, antibodies and vitamins. Drying can be considered as a preservation technique, by means of which the activity of biological components can be preserved for a longer period of time. Materials can be dried either in a small liquid droplet form (e.g. in spray drying) or a thin-layer liquid film or slab form (e.g. in tunnel drying). Drying technologies such as spray drying require high air temperatures to facilitate water evaporation and hence formation of a solid structure. Low-temperature drying processes such as freeze-drying are costly and time consuming for the bulk bioactive powder production compared with high-temperature drying processes. It has been estimated that the cost per kilogram of water removed for spray drying is six times lower than the cost for freeze-drying (Knorr, 1998).

Water is known to play a major role to the stability of biological molecules. The removal of water from the material during drying may cause irreversible changes in the structural and functional integrity of the bacterial membranes and configurations of the protein contents. Furthermore, the exposure of materials to high temperatures may cause damage to the protein and enzyme structures and also reduces the viability and hence the activity of micro-organisms in the dried product. When drying conditions are rigorous, heating and subsequent dehydration causes severe stresses that could exert a substantial negative impact on the bioactivity of the labile biological components. The loss of bioactivity is termed as inactivation or bio-degradation of the product which is undesirable when useful bacteria (e.g. probiotic bacteria) need to be main-

tained. Preservation of the functionality of the proteins and enzymes and hence the activity of useful micro-organisms during bioactive product drying is essential because it directly affects the final quality and market-value of the product.

Predicting the survival/activity of micro-organisms and bioactive constituents during drying is necessary for ensuring the high retention of bioactivity and also for optimization and scale-up or scale-down purposes. This requires a good understanding about the inactivation mechanism and parameters that may affect the inactivation rate during processing. For a dryer-wide simulation, it is essential to couple the inactivation kinetics model with the heat, mass and momentum conservation equations under drying conditions in order to predict the survival of micro-organisms accurately. Many studies have been published in the literature to correlate the inactivation rate with temperature and/or moisture content in a mathematical form by simply incorporating the average temperature and/or moisture content terms to the inactivation kinetics (Goula, Adamopoulos, Chatzidakis, & Nikas, 2006; Lievense, Verbeek, Taekema, Meerdink, & Van't Riet, 1992). For instance, recently Goula et al. (2006) proposed a mathematical model to describe a rate of lycopene (a carotenoid polyene) loss during drying of a tomato pulp. They evaluated the inactivation rate constant as a function of average moisture content and average temperature of the droplet being dried and validated a proposed model using experimental outcomes. However, this type of model may not demonstrate the effects of water content and water content distribution on the inactivation rate of bioactive substances. In the literature, these kinds of inactivation kinetics models have been proposed and perhaps been used for some years to predict the degradation rate of various bioactive components.

In the next section, several well-known inactivation kinetics models originally proposed in context to fast drying processes for deactivation of various enzymes and micro-organisms have been discussed. Inactivation kinetics models, which were worked out recently considering the average droplet drying rate and the average heating rate are also reviewed in the following section. A theoretical appraisal of the drying and heating rates dependent model has been also presented. A relationship between the static water content- and temperature-dependent functions and the rate dependent parameters has been suggested in this paper. This paper is mainly focused at inactivation kinetics of bioactive components during fast drying processes, such as spray drying or tunnel drying that involves high drying medium temperatures and short exposure times of the material being dried.

## 2. A critical review

A review of the literature has revealed a number of attempts that has been made to demonstrate the inactivation kinetics for selected enzymes and micro-organisms during the drying processes. Majority of the work was carried out using various enzymes by Wijlhuizen, Kerkhof, and Bruin (1979), Liou, Luyben, and Bruin (1985), Yamamoto and Sano (1982), Meerdink and Van't Riet (1995) and Etzel, Suen, Halverson, and Budijono (1996). Several publications were also reported for inactivation of micro-organisms (Elizondo & Labuza, 1974; Fu & Etzel, 1995; Johnson & Etzel, 1993; Lievense et al., 1992) in the context of drying processes. In most studies, the measurement of inactivation parameters as a function of time were performed using isolated heating-only experiments with no 'drying' involved. The proposed kinetics models are still lacking in desirable accuracy. Nevertheless, these models have been used for some years to describe inactivation of various enzymes, proteins and micro-organisms during convective drying of the materials.

In general, inactivation kinetics of micro-organisms and enzymes has been conventionally expressed using a first-order reaction kinetic in most studies published in context to inactivation during drying processes (Elizondo & Labuza, 1974; Goula et al., 2006; Johnson & Etzel, 1993; Lievense et al., 1992; Liou et al., 1985; Meerdink & Van't Riet, 1995; Wijlhuizen et al., 1979; Yamamoto & Sano, 1982). In this case, Eq. (1) can be used to describe the inactivation rate:

$$\frac{d(N/N_0)}{dt} = -k_d(N/N_0) \quad (1)$$

where  $N$  is the number of the live micro-organisms in suspension ( $\text{cell} \cdot \text{m}^{-3}$ ) or the live enzyme concentration ( $\text{units} \cdot \text{m}^{-3}$ ),  $k_d$  is the inactivation rate constant ( $\text{s}^{-1}$ ) and  $N_0$  is the initial cell concentration ( $\text{cell} \cdot \text{m}^{-3}$ ).

Eq. (1) is argued to be not the most appropriate inactivation kinetics for describing the microbial inactivation because it is claimed to be structurally not correct (Geer-

aerd, Herremans, & Van Impe, 2000). The same authors proposed a structured model, which deal with a non-loglinear behaviour of micro-organisms during the mild heat treatment of various food products. Geeraerd et al. (2000) took into account the shoulder and tail behaviour of the non-loglinear survivor curve, which is significant during the mild heat treatment that does not involve high evaporation rates. Industrial spray drying processes are associated with high evaporation rates and significantly short processing times. As a result, a tailing behaviour of the non-loglinear survivor curve may be negligible and not very important. Therefore, it should be reasonable to consider the first-order inactivation kinetics as far as spray drying or other fast drying processes are concerned.

For micro-organisms suspended in a solution without being dried (i.e. in a saturated liquid medium),  $k_d$  is usually considered to be a function of temperature only and is described using the Arrhenius equation (Johnson & Etzel, 1993; Meerdink & Van't Riet, 1995; Yamamoto & Sano, 1982):

$$k_d = k_0 \exp\left(-\frac{E_d}{R_g T}\right) \quad (2)$$

Fast drying such as spray drying is considered to be a 'safe' processing route to convert liquid bioactive feedstock into a powder form because the mean drying time of the droplet is small, usually 10–30 s (Masters, 1991; Písecký, 1997). Removal of moisture takes place quickly in spray drying process and hence the temperature change, moisture concentration change, solid formation and inactivation processes are also fast (Chen, 2004). In general, industrial drying operations involve reduction in the moisture content of the material during processing, as well as an increase in the temperature of the material. It should be noted that the inactivation rate is usually elevated with rising temperature and reduced with falling moisture content. Hence, the overall inactivation is a 'competitive' process between the two different inactivation mechanisms. However, the contribution of each inactivation mechanism during processing is not very well understood. It has now become an interesting task to discover the controlling inactivation parameter during different phases of a drying process. This information could be very helpful for process design and optimization to achieve the highest bioactivity of different biological compounds.

As far as drying is concerned, two obvious controlling parameters for inactivation are temperature and water content. In literature, the temperature dependence of the inactivation rate constant  $k_d$  during drying is described using Eq. (2). The temperature distribution within a small droplet or a thin slab can be considered to be negligible. This is a reasonable consideration if the Biot number is smaller than its critical value, which is 0.1 based on the classical Biot number theory (Incropera & DeWitt, 2002). However, when a significant evaporation takes place, an evaporative droplet can have negligible temperature gradients even for larger Biot numbers, i.e. larger than 0.1 (Chen & Peng,

2005; Patel, Chen, & Kar, 2005). Moisture content, on the other hand, has a significant non-uniformity within the droplet/particle being dried due to the nature of the drying processes, especially during the falling rate drying period (Chen, 2006). If the temperature is considered to be non-uniform, the simulation becomes more complicated due to the requirement of solving an additional partial differential equation governing a spatial distribution of temperature.

During drying, with the appearance of an unsaturated porous material, the water-content-dependent  $k_d$  has to be considered since it is expected that the water content plays a significant role during the inactivation process. Pre-equilibrated materials imbedded with micro-organisms show a strong water content (or water activity) dependence, which is a complex phenomenon. There is a local maximum of the heat resistance of the bacteria in water activity range of 0.2–0.5 (Laroche, Fine, & Gervais, 2005; Xu & Wang, 2005). Drying of high water content food materials such as milk would exhibit droplet surface from water activity lower than 1 (Chen, in press).

In the literature, a few attempts have been observed to describe the inactivation kinetics using the uniform temperature (i.e. the average temperature,  $\bar{T}$ ) and a radial moisture concentration within a droplet/particle being dried (Lievens et al., 1992; Meerdink & Van't Riet, 1995; Wijnhuizen et al., 1979; Yamamoto & Sano, 1982). For example, Wijnhuizen et al. (1979) presented the early theoretical analysis to illustrate thermal degradation during drying of a single skim milk droplet containing the alkaline phosphatase enzyme. Their model has eight coefficients, which needed to be derived experimentally. The inactivation rate constant  $k_d$  was described as a function of average temperature ( $\bar{T}$ ) and average moisture content ( $\bar{X}$ ). The average moisture content was calculated by solving the unsteady-state diffusion equation and integrating the local moisture content. The spatial distribution of water content was resolved with a partial differential equation for the Fickian diffusion process. The effective diffusivity was empirically expressed against water content and temperature. Effects of various drying parameters on the activity of enzymes were estimated in the study; however, no experimental proof was presented.

Lievens et al. (1992) proposed an inactivation kinetics model for degradation of *Lactobacillus plantarum* during drying by considering thermal and dehydration inactivation as two separate influences but operating simultaneously. The model had 10 parameters to be obtained from the experimental work. Measurements of the drying parameters were, however, obtained from the fluidized-bed drying with drying temperatures lower than 50 °C. Again, the effective diffusivity concept was used to take into account the spatial moisture distribution. The inactivation parameters were measured from 'non-drying', heating experiments in which approximately 1 mm thick *L. plantarum*-starch granulate was placed in a Petri dish and stored

at  $5.0 \pm 0.5$  °C in a vacuum desiccator for 48 h. After 48 h, the glucose fermenting activity and moisture concentrations of the sample were measured. They illustrated in their work that thermal inactivation is insignificant at drying temperatures lower than 50 °C. Furthermore, they stated that the dehydration inactivation depends on the reached moisture content of the material only and is independent of the drying rate. However, Lievens et al. (1992) did not show if this observation could be true for high-temperature drying processes, where drying rates are much higher.

A similar trend was followed by Yamamoto and Sano (1982), who proposed a five-parameter model for enzyme inactivation during drying using a single suspended droplet drying experiment. A sucrose solution of fixed water content containing different enzymes such as  $\beta$ -galactosidase, glucose oxidase and alkaline phosphatase was incubated at a constant temperature. The thickness of the material used and air temperatures were not reported in the study. The deactivation energy  $E_d$  was described as a function of average water content. Again, a binary (water and dissolved solids) diffusion coefficient was introduced to the drying analysis for taking care of the water distribution inside a droplet. They concluded that air temperatures and droplet size significantly affect the inactivation rate. The effect of initial water content is shown to be insignificant. However, the enzyme activity was experimentally measured using constant-temperature and constant moisture content heating experiments, where no evaporation was involved. Again, this work may be classified into the 'pre-equilibration' experiments.

Meerdink and Van't Riet (1995) studied the inactivation of the enzyme  $\alpha$ -amylase during drying, describing deactivation energy  $E_d$  to be water content dependent. Drying parameters such as temperature and moisture content were experimentally measured as a function of time during drying of equal-sized free-falling droplets under spray-drying conditions in order to couple with inactivation kinetics. However, thermal inactivation kinetics of the enzyme was determined again using the constant temperature (75–100 °C) and constant water concentrations (0.09–0.82 kg water/kg dry solids) conditions from separate non-drying, heating-only, pre-equilibration experiments. The moisture concentration profiles inside a droplet being dried were calculated using an unsteady-state diffusion equation. The approach considered by Meerdink and Van't Riet (1995) is attractive, as it requires only four parameters ( $a$ ,  $b$ ,  $k_0$  and  $E_d$ ) to be acquired experimentally. The inactivation rate constant ( $k_d$ ) was expressed using the following formulae:

$$k_d = k_0 \exp \left( aX - \frac{E_d + bX}{R_g T} \right), \quad (3)$$

where  $X$  is the water content on a dry basis. Meerdink and Van't Riet (1995) concluded that the inactivation rate is more sensitive to droplet temperature changes than the drying rate. However, their comparison of model simulations with experimental data showed that the inactivation

rate was underestimated. The average discrepancy between experimental inactivation rates and model prediction was above 34%. In other words, the water content and temperature dependent function may not work well for drying simulations. They argued that the prevalent spray-air mixing pattern in a spray dryer could be a reason for this large discrepancy.

Recently, Li, Lin, Chen, Chen, and Pearce (2006) investigated the inactivation kinetics of two probiotic strains, *Bifidobacterium infantis* and *Streptococcus thermophilus*, during the milk droplet drying process. Droplet temperature, moisture content, drying rate and change in droplet diameter were measured as a function of time during drying of a single suspended milk droplet. The weight loss and temperature–time profiles were measured using the glass-filament method described by Sano and Kee (1982) and Lin and Chen (2002). As an innovative approach, the survival of probiotic strains was also measured by obtaining the droplets at different time intervals from the droplet drying experiments. The work by Li et al. (2006) was based on the earlier experimental approach reported by Woo, Stevenson, Chen, Pearce, and Harnett (2000). In their work, a probiotic strain was uniformly mixed with a milk solution. Single milk droplets, which contain probiotic bacteria, were suspended and dried using hot air. The dried droplets were captured at different time intervals, then dissolved and diluted in a broth for cell death counting. A MatLab optimization procedure was adopted with the known temperature–time and water content–time profiles to ‘back’ calculate the inactivation kinetics constants for a proposed kinetics formula.

In the study by Li et al. (2006), a number of inactivation kinetics correlations were tested for their appropriateness during modelling the inactivation kinetics of the probiotic bacteria, suspended in milk and dried in a droplet form of smaller than 2 mm in diameter. They incorporated the average droplet drying rate and/or the average heating rate into the inactivation kinetics models to predict the survival rate of probiotic bacteria used in the study. In their study, predictions were found to be more accurate when the inactivation rate was described as a function of the average drying rate and/or the rate of change of temperature. Traditional inactivation kinetics models, which take into account the average temperature and moisture content only, were found not to be appropriate when correlating the experimental data, yielding very large errors in the predictions. In particular, traditional models did not follow the effect of the particle temperature as expected since the predicted inactivation trends at different air temperatures tended to stay together rather than being separated.

It is shown that the inactivation occurs mainly in the early part of the drying process, where the water is rapidly removed while temperature stays very close to the wet-bulb temperature of the drying air. Therefore, relating the kinetics to the droplet temperature in the early stage of drying, where the change occurs most dramatically, did not seem to be sensible when using traditional (non-rate dependent)

inactivation kinetics models. As such, the drying rate and the rate of temperature rise were considered as a part of the inactivation kinetics in the work by Li et al. (2006). The better-fit rate-dependent correlations were

$$k_d = k_0 \left( 1 + b \cdot \left| \frac{dX}{dt} \right| \right) \exp \left( -\frac{E_d}{R_g T} \right), \quad (4)$$

$$k_d = k_0 \left( 1 + a \cdot \left| \frac{dT}{dt} \right| \right) \cdot \left( 1 + b \cdot \left| \frac{dX}{dt} \right| \right) \exp \left( -\frac{E_d}{R_g T} \right), \quad (5)$$

$$k_d = k_0 \left( 1 + a \cdot \left| \frac{dX}{dt} \right| + b \cdot \left| \frac{dX}{dt} \right|^2 \right) \exp \left( -\frac{E_d}{R_g T} \right). \quad (6)$$

These three correlations were found to be useful in correlating the inactivation rates with the drying kinetics. The overall best fit correlation, for probiotic strains used by Li et al. (2006), was found to be Eq. (6). Eq. (5) gave better predictions than Eqs. (2)–(4) except Eq. (6).

The rate-dependent inactivation rate expression was thought to be caused by the spatial distribution of the water content in a droplet or particle being dried. The results of Li et al. (2006) illustrated that the rate of temperature change had little effect on the survival of microorganisms compared to the drying rate. They concluded that the rate-dependence of microbial inactivation is greater when the drying condition is more severe, especially at higher drying rate conditions and also during the early stage of drying. This finding is in agreement with Kuts and Tutova (1983), who mentioned that a high drying rate negatively affects the inactivation rate, and stated that the drying rate must be considered as one of the most important parameters that influence the residual activity during drying. From the work by Li et al. (2006), Eqs. (2) and (3) were found to be inappropriate for describing the inactivation rate of both probiotic strains during drying. They speculated that the inactivation kinetics could perhaps be modelled appropriately with a ‘non-rate dependent’ inactivation rate expression such as Eqs. (2) and (3), when gentle drying conditions are imposed or when the drying rates and heating rates are rather small.

In the next section, a fundamental appraisal for the rate expressions such as Eqs. (5) and (6), is described based on a simple integration of Eq. (3) over the droplet radius and some extended derivations. This appraisal, which is by no means full proof, is expected to help in understanding the role played by water distribution within a material during modelling the inactivation kinetics.

### 3. A theoretical appraisal of the rate dependent model by Li et al. (2006)

Taking Eq. (3) as a valid inactivation kinetics model for describing the local phenomena, one can integrate Eq. (1) over the sample dimension. The droplet radius, as an example in the current analysis, is considered as the sample dimension. Similar analysis can be carried out by considering the material’s thickness for a thin slab. From Eqs. (1) and (3), one may write the following:

$$\frac{d\bar{N}}{dt} \approx - \left[ \frac{k_0}{R} \int_{r=0}^{r=R} \exp \left( aX - \frac{E_d + bX}{R_g T} \right) dr \right] \bar{N}, \quad (7)$$

where  $\bar{N}$  is the mean survival number of the live microorganisms within the material being dried. Firstly, Eq. (7) has to be further simplified so that the effect of water content and the effect of temperature could be considered in ‘isolation’. This is done by rewriting Eq. (7) as

$$\frac{1}{\bar{N}} \frac{d\bar{N}}{dt} = - \frac{k_0}{R} \int_{r=0}^{r=R} \exp \left( \left( a - \frac{b}{R_g T} \right) X - \frac{E_d}{R_g T} \right) dr, \quad (8)$$

which may be appropriate over a small temperature range. Another simplification comes through the well-known Frank–Kamenestii approach to simplify the Arrhenius temperature dependency as follows (Bowes, 1984; Chen, 2007):

$$\exp \left( - \frac{E_d}{R_g T} \right) \approx e^{\frac{E_d}{R_g T_{\text{ref}}}} \cdot e^{-\frac{E_d}{R_g T_{\text{ref}}^2} (T - T_{\text{ref}})} = e^{\frac{E_d}{R_g T_{\text{ref}}}} \cdot e^{\theta}, \quad (9)$$

where  $\theta = - \frac{E_d}{R_g T_{\text{ref}}^2} (T - T_{\text{ref}})$ .

Here,  $T_{\text{ref}}$  is a reference temperature and  $\theta$  is a dimensionless temperature. Again this simplification is more appropriate for a moderate range of temperature different from the reference temperature. From the above analysis, one can re-write Eq. (8) as

$$\frac{1}{\bar{N}} \frac{d\bar{N}}{dt} = - \frac{\bar{k}_0}{R} \int_{r=0}^{r=R} \exp(\bar{a}X + \theta) dr, \quad (10)$$

where  $\bar{k}_0 = k_0 e^{E_d/R_g T_{\text{ref}}}$  and  $\bar{a} = \left( a - \frac{b}{R_g T} \right)$ .

By rearranging Eq. (10), one can obtain the following:

$$\begin{aligned} \frac{1}{\bar{N}} \frac{d\bar{N}}{dt} &= - \frac{\bar{k}_0}{R} \left[ \int_{r=0}^{r=R} d(e^{\bar{a}X + \theta} r) - \int_{r=0}^{r=R} r d(e^{\bar{a}X + \theta}) \right] \\ &= - \frac{\bar{k}_0}{R} \left[ e^{\bar{a}X_s + \theta_s} R - \int_{r=0}^{r=R} r d(e^{\bar{a}X + \theta}) \right]. \end{aligned} \quad (11)$$

The last integral term on RHS of the above equation can be viewed as an ‘‘area’’ under the curve and can be further approximated by introducing a characteristic radius ( $r_c < R$ ) in such a way that Eq. (11) could be presented for a small droplet where the surface temperature would be close to the centre temperature (i.e.  $\theta_s \approx \theta_0$ ) as following:

$$\frac{1}{\bar{N}} \frac{d\bar{N}}{dt} \approx - \frac{\bar{k}_0}{R} [e^{\bar{a}X_s} (R - r_c) + r_c e^{\bar{a}X_0}] \cdot e^{\theta_0} \quad (12)$$

Detailed mathematical derivation to get Eq. (12) from Eq. (11) is illustrated in Appendix A. Above expression illustrates the dynamics of the inactivation kinetics being dependent on the surface water content ( $X_s$ ) and the centre water content ( $X_0$ ) as well as the progression of the characteristic radius into the droplet/slab. When the drying kinetics is modelled using a common lumped-parameter model where the inactivation rate is expressed as a function of the average water content, the different but perhaps rather significant roles played by the surface and centre water contents can not be addressed. This is because the accurate

inactivation modelling such as that using Eq. (3) would require information about the water content distribution within a material being dried.

The characteristic radius  $r_c$  in Eq. (12) is a dynamic parameter and is expected to be a function of the water content. The characteristic radius  $r_c$  should be reduced as the water content declines due to evaporation. The main purpose of incorporating the characteristic radius and simplifying the integral expression in terms of two extreme water concentration dependent functions is to show qualitatively but definitely the different roles performed by the surface water content and the centre water content on the inactivation rate.

Another possible way to look at the water content dependence of the inactivation rate is to replace  $X$  in Eq. (10) by the average water content ( $\bar{X}$ ), assuming the average water content being a linear function of the surface water content ( $X_s$ ), the centre water content ( $X_0$ ) and the radius of the droplet or the thickness of the slab ( $R$ ) being dried. This assumption will lead to a simple and short approach to demonstrate the effects of the surface and centre water contents on the inactivation rate, as illustrated in Appendix B. However, an assumption of a linear water content distribution may not be appropriate for a particle or a thin-layer slab under ‘high’ drying rate conditions (Chen, 2006).

In order to see if there was any relationship between Eq. (12) and the empirical models which were found to be useful by Li et al. (2006), i.e. Eqs. (5) and (6), the authors assume  $r_c$  is equal to  $\Phi \cdot R$ , that is just averaging of a characteristic length over the radius of the droplet. The unknown parameter  $\Phi$  should be constant, and for simple averaging it may be assumed as 0.5. Then, Eq. (12) can be re-written by incorporating  $r_c = \Phi \cdot R$  and by extending a surface water content term in a series form as:

$$\frac{1}{\bar{N}} \frac{d\bar{N}}{dt} \approx - \phi \bar{k}_0 \left[ 1 + \bar{a}X_s + \frac{1}{2} \bar{a}^2 X_s^2 + \dots + e^{\bar{a}X_0} \right] e^{\theta_0}. \quad (13)$$

Under a constant drying condition, the surface water content  $X_s$  should decrease in such a way that approximately follows the first-order kinetics (Akpınar, 2006; Kadja & Bergeles, 2003):

$$\frac{dX_s}{dt} \approx -k_{\text{drying}} (X_s - X_\infty), \quad (14)$$

where  $k_{\text{drying}}$  is a first-order rate constant. This expression is consistent with the findings for thin sample materials where no constant-rate period is involved. In general, the experimental runs conducted by Li et al. (2006) had a low ambient humidity ( $\sim 0.0001$  kg water/kg dry air). For this case, it is reasonable to describe the surface water content for the first third of the drying period using the following expression:

$$X_s \approx - \frac{1}{k_{\text{drying}}} \frac{dX_s}{dt}. \quad (15)$$

Another reasonable assumption for the first third of the drying period is that the centre water content  $X_o$  remains constant. Sano and Kee (1982) simulated the skim milk droplet drying under similar conditions considered in Li et al. (2006), and they demonstrated that during the initial one third of the drying period, there was an insignificant change in the centre water content. The majority of the initial water loss occurs within a region half of the radius or thickness from the surface of the droplet or a thin-layer slab, respectively. Substituting Eq. (15) into Eq. (13) and also considering  $X_o$  being unchanged, one can have the following:

$$\begin{aligned} \frac{1}{\bar{N}} \frac{d\bar{N}}{dt} &\approx -\phi \bar{k}_0 \left[ 1 + \frac{\bar{a}}{k_{\text{drying}}} \left| \frac{dX_s}{dt} \right| + \frac{1}{2} \left( \frac{\bar{a}}{k_{\text{drying}}} \right)^2 \left| \frac{dX_s}{dt} \right|^2 + \dots + e^{\bar{a}X_o} \right] e^{\theta_0} \\ &= -\phi \bar{k}_0 \left[ 1 + \alpha \left| \frac{dX_s}{dt} \right| + \beta \left| \frac{dX_s}{dt} \right|^2 + \dots + e^{\bar{a}X_o} \right] e^{\theta_0}, \end{aligned} \quad (16)$$

where  $\alpha = (\bar{a}/k_{\text{drying}})$  and  $\beta = \frac{1}{2} \left( \frac{\bar{a}}{k_{\text{drying}}} \right)^2 = 0.5\alpha^2$ .

It is noted that the parameter  $\bar{a}$  can be negative or positive depending upon the net effect of  $\{a - (b/R_g T)\}$ . In the study by Li et al. (2006), where Eq. (6) was found to be the most favourable one for data correlation, the fitting parameter  $\bar{a}$  was negative and in the order of several hundreds. If this is the case, the exponential term  $e^{\bar{a}X_o}$  was small compared with other terms in Eq. (16). Then, Eq. (16) becomes similar as the original inactivation kinetics model, i.e. Eq. (6), being fitting parameters  $\alpha$  and  $\beta$  equivalent to coefficients  $a$  and  $b$ .

Above simplified analysis and also the mathematical analysis suggested in Appendix B illustrate that the fitting parameter  $\beta$  would be roughly in the order of  $0.5\alpha^2$  or a ratio of  $\beta/0.5\alpha^2$  should be close to the value of 1. In the paper by Li et al. (2006), the value of  $\beta$  for *B. infantis* and *S. thermophilus* were 9012.8 and 13647, respectively and the corresponding values of  $\alpha$  were  $-154.24$  and  $-203.03$ , respectively. It is very interesting to see that the corresponding ratios of  $\beta/0.5\alpha^2$  for *B. infantis* and *S. thermophilus* were 0.76 and 0.66 respectively. These values are close to the value of 1, which perhaps illustrates a favourable result that supports the analysis given above. The occurrence of a unit ratio of  $\beta/0.5\alpha^2$  could be due to the assumptions such as  $r_c = \Phi R$  and the inactivation rate being proportional to the  $r_c(e^{\bar{a}X_s + \theta_s} - e^{\bar{a}X_o + \theta_0})$ . The order of magnitude predicted would be of a good interest in any case. The emphasis in this study is regarding the effect of a spatial water distribution. The effect of spatial water distribution in a droplet or a thin slab seems to be captured when using the drying-rate dependent approach. It should be noted that this appraisal is a semi-qualitative illustration for using average water content as a parameter in order to correlate with inactivation kinetics. We do not suggest that the inactivation kinetics model presented by Eq. (3) is the most accurate description of the inactivation mechanism. This remark is supported by results of Li et al. (2006).

#### 4. Further remarks

The current analysis has provided an indicative confirmation about the initial thought reported by Li et al. (2006) that the spatial distribution of the water content played a key role in determining the apparent ‘drying rate-dependent’ inactivation rate found in their study. To be able to comprehensively predict the micro-organism survival in the droplet/particle domain, their distribution effect would have to be taken into account. The spatially distributed model such as the liquid diffusion model where Fick’s law is employed may be more useful when one uses the inactivation model such as Eq. (3). However, there is no proof yet that the liquid diffusion model, without the water content dependent diffusivity, predicts the actual local distribution of water content for small objects such as droplet/particle well enough to suit the purpose of predicting bacteria inactivation. This is because of the assumptions made on the liquid diffusion and the liquid/gas interface condition (Chen, 2006). This remains to be a topic of interest. On the other hand, the lumped parameter model such as Eqs. (4)–(6) can be more readily solved when incorporated in a Computational Fluid Dynamics package or other simulation tools (Huang, Kumar, & Mujumdar, 2003).

There are several areas, which require immediate attention in order to improve the inactivation kinetics model. The first topic is the arrangement or distribution of living micro-organisms in the domain material at the beginning of drying as well as during processing. Unfortunately, this topic has basically remained untouched to date. Knowledge regarding the living cell distribution together with moisture and temperature distributions within a droplet or a slab is crucial for studying and correlating the inactivation kinetics. Because the surface properties of the droplet could be different from the centre core regions, the survival of living cells would be different at the surface and the centre. It is likely that the survival number would be very small if the living cells are concentrated in the surface region and high if more cells are located in the centre. However, cells could be randomly grouped or distributed into some zones of the matrix material depending on pre-mixing conditions, for instance homogenization during milk processing. Living cells may be located more at one region in the dried particle, which may not be symmetrical. For example, Gardiner et al. (2000) studied the survival rates of human-derived probiotic strains during spray drying of skim milk. They found during optical imaging of a single broken spray-dried particle that bacteria were not uniformly distributed in a skim milk particle. Nevertheless, information regarding the cell arrangement would be very helpful when following a single droplet drying process for inactivation modelling purposes. To date, we have assumed a uniform distribution of the living cells within a material to visualize the inactivation process in mathematical terms.

Another interesting research area may be to see if the addition of other materials (e.g. sugars) to the biological solution improves the survival of micro-organisms during processing. In the literature, some studies have been published to show the protection of micro-organisms against drying using various carbohydrates. Researchers have proposed that carbohydrates stabilize the membrane and protein chains in the dry state by bonding to the macromolecular assemblages and offer extra protection against the cell rupture (Leslie, Israeli, Lighthart, Crowe, & Crowe, 1995; Oldenhof, Wolkers, Fonseca, Passot, & Marin, 2005). Several studies demonstrated a significant increase in the survival rate of micro-organisms when protective agents such as lactose, sucrose, maltodextrin and trehalose were added to the feed prior to freeze drying (Berny & Hennebert, 1991; Zhao & Zhang, 2005). However, survival rates of yeast during fluidised-bed drying in the work by Bayrock and Ingledew (1997a, 1997b) show only a little improvement in the final survival number of yeast upon adding trehalose to the feed. Later studies show that protective agents do not work efficiently at high temperatures or in other words high drying rates conditions, which are common in the industrial spray drying operations. This may become an interesting research task now to discover the role of protective agents during low temperature operations such as freeze-drying and vacuum desiccation and high temperature operations such as spray drying, convective drying and fluidized-bed drying. Also, literature shows that the protective effects of a particular agent may vary for different micro-organisms. This is an additional and perhaps even harder task to find out suitable protective agents for each group of micro-organisms.

Another important research aspect is to evaluate whether the inactivation mechanism of enzymes and micro-organisms is similar or different during different drying operations. The fundamental structure of micro-organisms and enzymes are somewhat different and hence they may have different responses and stability against drying-induced stresses during processing. In the literature, inactivation for enzymes and micro-organisms has been mathematically worked out using the same approach considering the first-order reaction kinetics (Etzel et al., 1996; Li et al., 2006; Meerdink & Van't Riet, 1995). In fact, inactivation kinetics models have been interchangeably used for enzymes and micro-organisms and also for protein denaturation. However, inactivation of micro-organisms would largely depend on damage to the protective membranes around the cell and is perhaps a different mechanism compared with inactivation of enzymes and denaturation of proteins. The later mechanism involved uncoiling, segmentation and even aggregation in molecular structures. Future work could be focused on the sub-cell level inactivation mechanisms so that the overall inactivation of micro-organisms during drying of food and bioactive products could be modelled more mechanistically.

## 5. Concluding remarks

A critical review of 'non-rate dependent' and 'rate-dependent' inactivation kinetics models has been presented. A semi-qualitative appraisal has been worked out to take into account the effect of spatial water distribution during modelling the inactivation kinetics. The current analysis has shown that there is a relationship between the 'drying-rate dependent' inactivation kinetics model and the 'non-rate dependent' inactivation kinetics model. The drying-rate dependent inactivation kinetics is an empirical model, which seems to be able to capture the effect of water content distribution within a material being dried in a hot air environment. There is a scope for more research into this matter so that a balance between the fundamental and practicality of the model approaches can be addressed. Furthermore, it is suggested that the sub-cell level changes in micro-organisms, when subject to heating and drying, should somehow be explored, so that a more accurate approach of the inactivation kinetics can be evaluated.

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## Appendix A. Intermediate steps of author's mathematical analysis

Eq. (11) can be integrated as following by incorporating  $r_c$  to get Eq. (12):

$$\begin{aligned} \frac{1}{\bar{N}} \frac{d\bar{N}}{dt} &\approx -\frac{\bar{k}_0}{R} \left[ e^{\bar{a}X_s + \theta_s} R - r_c \int_{r=0}^{r=R} d(e^{\bar{a}X + \theta}) \right] \\ &= -\frac{\bar{k}_0}{R} [e^{\bar{a}X_s + \theta_s} R - r_c (e^{\bar{a}X_s + \theta_s} - e^{\bar{a}X_0 + \theta_0})] \\ &= -\frac{\bar{k}_0}{R} [e^{\bar{a}X_s + \theta_s} (R - r_c) + r_c e^{\bar{a}X_0 + \theta_0}]. \end{aligned} \quad (i)$$

For the case of a small droplet, where the surface and the centre temperature are similar (i.e. negligible temperature distribution within a material, so  $\theta_s \approx \theta_0$ ), Eq. (i) can be re-written as shown in Eq. (12). If the characteristic radius  $r_c$  may be replaced by  $\Phi R$ , Eq. (12) would result in the following expression:

$$\frac{1}{\bar{N}} \frac{d\bar{N}}{dt} \approx -\phi \bar{k}_0 [e^{\bar{a}X_s} + e^{\bar{a}X_0}] e^{\theta_0}. \quad (ii)$$

The above equation can be expressed in a series form, as shown in Eq. (13).

## Appendix B. Referee's suggested mathematical analysis

A simple and short way to demonstrate the water dependence of the inactivation rate is to replace  $(X)$  in Eq. (10) by the average water content  $(\bar{X})$  and to assume the average water content being a linear function of the surface water content  $(X_s)$ , the centre water content  $(X_0)$  and the radius of the droplet or the thickness of the slab  $(R)$  as  $\bar{X} = (X_0 + X_s)/2\gamma R$ . Although a shape of this relationship depends on the unknown parameter  $\gamma$ , which can be taken as 1 for a linear relationship. Based on the average water content function, Eq. (10) can be re-written as

$$\frac{1}{\bar{N}} \frac{d\bar{N}}{dt} = -\frac{\bar{k}_0}{R} e^{\frac{\bar{a}X_0}{2\gamma R}} e^{\frac{\bar{a}X_s}{2\gamma R}} e^{\theta}. \quad (\text{iii})$$

This expression may be used to show the dynamics of the inactivation kinetics being dependent on the surface and centre water contents.

Then, Eq. (iii) may be expressed in a series form as following:

$$\frac{1}{\bar{N}} \frac{d\bar{N}}{dt} \approx -\bar{k}_0 e^{\frac{\bar{a}X_0}{2\gamma R}} e^{\theta} \left[ 1 + \frac{\bar{a}}{2\gamma R} X_s + \frac{1}{2} \left( \frac{\bar{a}}{2\gamma R} \right)^2 X_s^2 + \dots \right]. \quad (\text{iv})$$

After introducing  $k_{\text{drying}}$  from Eq. (15), Eq. (iv) will become Eq. (v) which is similar to Eq. (16):

$$\begin{aligned} \frac{1}{\bar{N}} \frac{d\bar{N}}{dt} &\approx -\bar{k}_0 e^{\frac{\bar{a}X_0}{2\gamma R}} e^{\theta} \left[ 1 + \frac{\bar{a}}{2k_{\text{drying}}\gamma R} \frac{dX_s}{dt} \right. \\ &\quad \left. + \frac{1}{2} \left( \frac{\bar{a}}{2k_{\text{drying}}\gamma R} \right)^2 \left( \frac{dX_s}{dt} \right)^2 + \dots \right] \\ &= -\bar{k}_0 e^{\frac{\bar{a}X_0}{2\gamma R}} e^{\theta} \left[ 1 + \alpha \left| \frac{dX_s}{dt} \right| + \beta \left| \frac{dX_s}{dt} \right|^2 + \dots \right], \quad (\text{v}) \end{aligned}$$

where  $\alpha = \left( \frac{\bar{a}}{2k_{\text{drying}}\gamma R} \right)$  and  $\beta = \frac{1}{2} \left( \frac{\bar{a}}{2k_{\text{drying}}\gamma R} \right)^2 = 0.5\alpha^2$ .

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