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Effect of Chemicals on the Microbial Evolution in Foods

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ABSTRACT

In contrast with most chemical hazardous compounds, the concentration of food pathogens changes during processing, storage, and meal preparation, making it difficult to estimate the number of microorganisms or the concentration of their toxins at the moment of ingestion by the consumer. These changes are attributed to microbial proliferation, survival, and/or inactivation and must be considered when exposure to a microbial hazard is assessed. The number of microorganisms can also change as a result of physical removal, mixing of food ingredients, partitioning of a food product, or cross-contamination (M. J. Nauta. 2002. *Int. J. Food Microbiol.* 73:297–304). Predictive microbiology, i.e., relating these microbial evolutionary patterns to environmental conditions, can therefore be considered a useful tool for microbial risk assessment, especially in the exposure assessment step. During the early development of the field (late 1980s and early 1990s), almost all research was focused on the modeling of microbial growth over time and the influence of temperature on this growth. Later, modeling of the influence of other intrinsic and extrinsic parameters garnered attention. Recently, more attention has been given to modeling of the effects of chemicals on microbial inactivation and survival. This article is an overview of different applied strategies for modeling the effect of chemical compounds on microbial populations. Various approaches for modeling chemical growth inhibition, the growth–no growth interface, and microbial inactivation by chemicals are reviewed.

Different types of antimicrobial compounds can be used to inactivate or inhibit growth of microorganisms in food products, but chemical compounds can never replace sterilization treatment. To ensure sound usage of such compounds in industrial practice, a quantitative understanding of their antimicrobial activity is necessary. To quantify the effect of an antimicrobial compound on a specific microorganism, the concept of the MIC, i.e., the lowest concentration which results in maintenance or reduction of inoculum viability, is often applied *(20).* The procedure for determination of MIC values has been described *(60),* but MIC values do not give any information about the extent of inhibition or inactivation at a specific concentration of the antimicrobial compound. An excellent way of quantifying the effect of a compound on microbial behavior is by development of predictive models. When effects on microorganisms are modeled, a clear differentiation is made between inactivating (i.e., microbiocidic) and growth inhibiting (i.e., microbiostatic) compounds. The microbiostatic compounds are classified by their mechanism of action. Most of the microbiocidic agents, which are used for disinfection, are classified on the basis of the oxidizing properties of the agent.

The most commonly applied microbiostatic agents in foods are probably those lowering the water activity (a_w). Typical examples of these agents are NaCl and sucrose. The effect of those compounds can be attributed to a difference between the intracellular and extracellular osmotic pressures created by the solute present in the water phase of the food. Other compounds must pass the negatively charged surface of the microbial cell to exert their action *(69).* The antimicrobial activity of, for example, organic acids resides therefore only in the undissociated molecule dissolved in the water phase of the food product. Typical examples of preservatives sensu stricto, i.e., those compounds that are effective at the molecular level at concentrations $<0.5\%$, are benzoic acid, sorbic acid, propionic acid, parabens, nitrite, and sulfites. The antimicrobial action of these preservatives is solely due to the metabolic activity of the molecule.

The concentration of the undissociated form of an acid (RCOOH) is a function of pH and depends on the pK value of the acid in question. The relationship is given by the following equation:

$$\mathrm{pH} = \mathrm{pK} + \log\frac{[\mathrm{RCOO^-}]}{[\mathrm{RCOOH}]} \qquad (1)$$

Other organic acids, such as lactic acid and acetic acid, are used at higher concentrations, and their effect is a combination of depression of the pH, reduction in the water activity, and exertion of metabolic inhibition by the undissociated acid molecules. Some general information about the action systems of chemical preservatives has been reported by Davidson *(28),* Gould *(48),* and Kabara and Eklund *(56).*

The use of this type of organic acid as a preservative is gaining interest because preservatives sensu stricto have a negative consumer image and by legislation are very limited in their use. However, preservatives are still commonly

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used in a wide range of foods such as cooked meat products *(106)* and mayonnaise-based salads *(30)* and are used as decontamination agents for fresh meat and poultry *(110)*.

Several gases have a microbiostatic effect on microorganisms. Carbon dioxide is the most widely used gas, especially as part of the modified atmosphere packaging technology, which is widely applied by the food industry for use with chilled food products. Despite numerous reports on the effect of CO_2 on microbial growth and metabolism, the mechanism of CO_2 inhibition remains unclear *(29, 35)*. The different proposed mechanisms of action are (i) reduction in the pH of the food, (ii) cellular penetration followed by a decrease in the intracellular pH, (iii) specific actions on cytoplasmic enzymes, and (iv) specific actions on biological membranes. When CO_2 is introduced into the package, it is partly dissolved in the water phase and the fat phase of the food. Devlieghere et al. *(32)* clearly demonstrated that only the CO_2 dissolved in the water phase of the food will exert an antimicrobial action.

Other natural antimicrobial compounds, such as bacteriocins, spices, and essential oils, have only recently been incorporated into practical applications in the food industry. Therefore, few models are available to model their antimicrobial effect of these compounds.

Commonly, a traditional two-step procedure is employed in predictive microbiology. In a first step, microbial evolution curves are generated. The experimentally measured profiles of cell concentration versus time are fitted with a primary growth model (e.g., the model of Baranyi and Roberts *(5)*), and growth curve descriptors or parameters are obtained, e.g., the lag phase duration λ (hours), the maximum specific growth rate μ_{max} (1/hours), and the maximum population density N_{max} (CFU/ml) *(80)*. In a second step, λ, μ_{max}, and N_{max} are related to environmental conditions such as temperature, pH, and concentration of an antimicrobial compound by a secondary model *(74)*.

MODELING OF MICROBIAL GROWTH INHIBITION BY CHEMICALS

Primary modeling. Inhibition of microbial proliferation is one of the key objectives of food preservation. The most important factor in retarding microbial growth in minimally processed food products is storage under chilled conditions. However, in many cases low temperature is not enough to ensure substantial shelf life extension and to guarantee safety and quality. Thus, microbial cells should be additionally stressed by other factors such as the addition of NaCl, preservatives, or organic salts or a CO_2-enriched atmosphere. Application of these stressors will result in reduced growth, which will be expressed as longer lag time, decreased maximum specific growth rate, and in some cases lower maximal cell density. These phenomena can easily be modeled using a primary growth curve. Classically, a sigmoidal growth curve is used to model cell growth as a function of time, although a three-phase linear model is sometimes used as a simplification *(19)*. During the development of the field of predictive microbiology, the Gompertz model and the logistic growth model *(46)* were mostly applied. In the late 1990s, the dynamic growth model of Baranyi and Roberts *(5)* became more popular, e.g., see *(84)* (for an overview of the different primary growth models, see *(74)*).

Secondary modeling. When the effect of a growth inhibiting compound is expressed, related secondary model types are used, with the following types of equations:

$$\begin{aligned} \mu_{max} &= f([\text{antimicrobial compound}]) \\ \lambda &= g([\text{antimicrobial compound}]) \\ N_{max} &= h([\text{antimicrobial compound}]) \end{aligned} \quad (2)$$

Secondary modeling: the water phase. Microorganisms are metabolically active in the water phase of a food product. Therefore, the active part of a microbiostatic compound is the concentration of this compound in the water phase of the food product. The concentration in the water phase should be taken into account in the different secondary models developed to express the effect of microbiostatic compounds on microbial proliferation. In the case of hydrophilic compounds, the concentration in the water phase can be easily calculated when the water content of the food is known, as is typically the case for NaCl, nitrite, and organic acids such as lactic acid or acetic acid and their salts. For hydrophobic compounds, such as benzoic acid, sorbic acid, and parabens, the distribution of the compound in question between the water phase and the fat phase of the food product (i.e., the partition coefficient) should be taken into consideration. The part of the preservative that ends up in the fat phase must be considered a loss of antimicrobial activity.

For antimicrobial gases such as CO_2, only the part dissolved in the water phase of the food product will exert its antimicrobial action *(32)*. The amount of CO_2 dissolved in the water phase will depend on the initial concentration of CO_2 in the headspace and on the gas/product ratio, temperature, pH, the amount of fat, and the saturation degree of the fat.

When microbial behavior in food products is modeled, the structure of the food must be considered. The concentration of an active compound in the water phase of an emulsion will be determined by the structure of this emulsion, which must be factored into predictive models when food products are considered *(124)*. The distribution of hydrophilic compounds between the intracellular and extracellular water phases of a food such as meat or vegetables could also play a role in the effectiveness of these compounds. The active and passive transport possibilities of the active compounds across the cell membrane will probably be the determining factor in this distribution. However, very little information is available concerning this distribution, and future research should focus on this phenomenon to increase the transferability of results in broth to results in real food systems.

Secondary modeling: the effect of a_w and pH-independent antimicrobials. The following secondary model types have been described: Arrhenius type models, Bělehrádek type models, polynomial models, neural network

TABLE 1. *Overview of secondary models for the maximum specific growth rate as a function of lactic acid*[a]

Model equation	Reference
Dependence on $[La^-]$, [LaH], and $[H^+]$	
$\mu_{opt}\left(1 - \frac{[La^-]}{[La^-]_{max}}\right)\left(1 - \frac{[LaH]}{[LaH]_{max}}\right)\left(1 - \frac{[H^+]}{[H^+]_{max}}\right)$	*98*
Dependence on [LaH] and $[H^+]$	
$\mu_{opt}\left(1 - \frac{[LaH]}{[LaH]_{max}}\right)^{\alpha}\left(1 - \frac{[H^+]}{[H^+]_{max}}\right)^{\beta}$	*94*
$\mu_{opt}\left(1 - \sqrt{\frac{[LaH]}{[LaH]_{max}}}\right)\left[\frac{(pH - pH_{min})(pH - pH_{max})}{(pH - pH_{min})(pH - pH_{max}) - (pH - pH_{opt})^2}\right]$	*64*
Dependence on NaLa (sodium lactate)	
$\mu_{opt}\left[\frac{\gamma(NaLa_{max} - NaLa)}{NaLa_{max}(\gamma - NaLa)}\right]$	*51*
$\mu_{opt}\left(1 - \frac{NaLa_{max}}{NaLa}\right)$	*31*

[a] μ_{opt} is the specific growth rate in the absence of lactic acid; max (or min) indicates a concentration at which growth is no longer possible; α, β, and γ are parameters with no clear biological interpretation.

models, and cardinal values models *(119).* When the effect of a pH-independent antimicrobial compound is transferred to a secondary model, the Bělehrádek type *(79)* (also called Ratkowsky model or square root model) and the polynomial models are most often applied.

An example of a polynomial model for the effect of two microbial compounds comp1 and comp2 on the maximum specific growth rate μ_{max} is given here:

$$\mu_{max} = a_1[comp1] + a_2[comp2] + a_3[comp3]^2 + a_4[comp2]^2 + a_5[comp1][comp2] \quad (3)$$

A typical Bělehrádek model has the following structure *(79)*:

$$\sqrt{\mu_{max}} = a_1(T - T_{min})\sqrt{(a_w - a_{w_{min}})} \quad (4)$$

in which the index min (minimum) indicates a (theoretical) value at which growth ceases. It has been extended for specific antimicrobial compounds:

$$\sqrt{\mu_{max}} = a_1(T - T_{min}) \times \{(a_w - a_{w_{min}})([comp1_{max}] - [comp1]) \times ([comp2_{max}] - [comp2])\}^{1/2} \quad (5)$$

Examples can be found for CO_2 *(26, 31),* lactate anion *(33, 51),* and nitrite *(81).* Similar equations can be derived for the lag phase λ, where μ_{max} is replaced by $1/\lambda$.

Secondary modeling: the effect of pH-dependent antimicrobial compounds. Most of antimicrobial compounds in which the antimicrobial effect is pH dependent are organic acids. Various mathematical models can be used to predict the microbiostatic effect of organic acids.

Appropriate modeling methodology depends largely on how the organic acids are introduced in food products. An organic acid can be present as a natural or purposefully added ingredient, or it can be produced in situ by microbial organisms, such as lactic or propionic acid bacteria *(52).* Although the inhibitory mechanisms remain the same, each addition mechanism calls for a specific modeling approach; when the inhibitory agent is added the concentration of the agent must be considered an invariant environmental factor, whereas when the agent is produced in situ, it must be considered a time-dependent variable.

Modeling the inhibitory effect of an added organic acid on microbial growth when the initial acid concentration is not (or only negligibly) altered during storage is the simplest approach. The traditional two-step procedure in predictive microbiology can be applied, resulting in a primary model type and a secondary model type relating λ, μ_{max}, and N_{max} to the organic acid concentration. In the literature, most secondary models available are for μ_{max}. An overview of these models for one of the most studied organic acids, lactic acid, is given in Table 1. The model of Presser et al. *(98)* accounts for all three antimicrobial components, whereas those of Passos et al. *(93)* and Le Marc et al. *(64)* consider only the two most inhibitory agents, [LaH] and $[H^+]$. A number of models for the maximum specific growth rate as a function of the sodium salt have been described. Because this easily dissolved salt has only a minor effect on pH, addition of sodium lactate to nonacidic foods principally comes down to addition of $[La^-]$, and the models of Houtsma et al. *(51)* and Devlieghere et al. *(33)* thus merely describe the specific effect of lactate.

With respect to the functional form, the models in Table 1 that include more than one variable all use a multiplicative approach. Le Marc et al. *(64)* suggested expanding this approach by the inclusion of a novel factor to describe the interactive effects of the variables (see ''Modeling of Growth–No Growth Boundaries''). This factor allows for a more accurate description of the experimental data near the growth limits.

When an organic acid is produced in situ by microbial metabolism, e.g., during a fermentation process, its concen-

tration increases with time. Microbial growth constitutes the driving force of acid production, but the acid itself exerts a negative feedback influence on the growth process of the producing organism and of other organisms present. Over the years, a number of researchers have presented models for the combined processes of growth and acid production, expressing the mutual influence between them. Considering again lactic acid as a type case, several models have been described *(12, 70, 71, 88, 92, 97, 121).* All of these models more or less fit in the same general frame, in which three so-called model building blocks can be distinguished.

A first block considers the growth characteristics of the microorganisms.

$$\frac{dN_i}{dt} = \mu_i([\text{LaH}], [\text{H}^+], \cdot)N_i \quad (6)$$

where N (CFU/ml) symbolizes the cell concentration, t (hours) is the time, and μ (1/hours) is the specific growth rate. The index i indicates the organisms, which are typically a lactic acid bacterium (the antagonist, $i = A$) and a contaminant (the target, $i = T$). Usually, the antimicrobial action of [LaH] and [H^+] is accounted for, and the influence of lactate is assumed to be negligible. Although both organisms experience negative effects, the lactic acid bacterium is usually less sensitive than the target. Functional forms for μ are often inspired by those listed in Table 1 *(12, 92).* However, [LaH] and [H^+] are now time dependent and do not refer to an invariant (initial) concentration.

A second block describes the production of lactic acid ($i = A, T$):

$$\frac{d\text{La}H_{\text{tot}}}{dt} = \sum \mu_i([\text{LaH}], [\text{H}^+], \cdot)N_i \quad (7)$$

where LaH_{tot} (M) is the total lactic acid concentration (i.e., the sum of [LaH] and [La^-]) and π is the specific production rate. According to equation 7, both antagonist and target contribute to the total lactic acid concentration. However, lactic acid bacteria are generally the most productive. Analogous to μ, π depends on [LaH] and [H^+]. Often, the specific production rate is related to the specific growth rate through the so-called linear law, i.e., $\pi = Y\mu + m$, where Y (mmol/CFU) is a yield coefficient and m (mmol/[CFU·h]) is a maintenance coefficient *(70, 71, 92, 97).* In this way, total growth (both associated and not associated with lactic acid production) is incorporated.

The last block concerns the dissociation kinetics of lactic acid in the medium, which can be included in equation 6:

$$[\text{H}^+] = f(\text{LaH}_{\text{tot}}, \text{medium})$$

$$[\text{LaH}] = g(\text{LaH}_{\text{tot}}, [\text{H}^+], \text{medium}) \quad (8)$$

Naturally, the protons and undissociated lactic acid molecules are dependent on the medium characteristics or more precisely the buffering capacity. A major difficulty is that for a lot of complex media and food products, the buffering components are not known in detail. Classical chemical laws (e.g., chemical equilibria, mass, and charge balances) therefore cannot be applied directly, and most of the models make use of purely empirical expressions for this last block. Two semimechanistic approaches, which are maximally inspired by chemical laws, are those of Wilson et al. *(125)* and Vereecken and Van Impe *(121).* An application of equations 6 and 8 to the growth inhibition of *Yersinia enterocolitica* in mono- and coculture with *Lactobacillus sakei* was reported by Vereecken et al. *(120).* An overview of some current models in the area of modeling microbial growth inhibition by antimicrobial compounds is given in Table 2.

Tertiary modeling. Within the field of modeling microbial growth inhibition as influenced by chemicals, some tertiary models can be mentioned. The Pathogen Modeling Program (PMP 7.0, 2004) is a software package developed by the U.S. Department of Agriculture Agricultural Research Service (Eastern Regional Research Center, Wyndmoor, Pa.) that can be downloaded freely from the internet (http://www.arserrc.gov/mfs/pathogen.htm). In addition to the classic growth factors such as temperature and the physiochemical factors of pH and a_w, the effect of some chemicals such as sodium nitrite or sodium pyrophosphate on the aerobic or anaerobic growth of some selected pathogens can be estimated. PMP also includes studies on heat inactivation, survival (see ''Modeling of Microbial Inactivation by Chemicals, Tertiary Modeling''), cooling, irradiation, time to turbidity, and time to toxin (fish). Unfortunately, the model is unable to make estimations when organic acids are applied, because only the pH is incorporated for the growth models. A second example is the Seafood Spoilage Predictor *(25).* This tertiary model can be used to estimate the time to spoilage for some fish products for a user-specified input temperature profile. The concentration of CO_2 in the headspace is incorporated in the model. This freeware program was developed at the Danish Institute for Fisheries Research (http://www.dfu.min.dk/micro/ssp/).

In general, few tertiary models are available for chemicals as inhibitory agents for microbial growth. More efforts should be made to integrate this phenomenon in ready-to-use tertiary models to enable an understanding of the actions of these agent to be disseminated to the food industry.

MODELING OF GROWTH–NO GROWTH BOUNDARIES

Many large food producers choose no growth-supporting conditions to guarantee the microbial safety of their food products, i.e., conditions that do not allow growth of any food pathogen in the product. For these producers it is essential to know the interface between conditions supporting growth and conditions where growth is not possible because treatment conditions that are too severe on the nongrowth side can deterioriate food quality and are more expensive. The growth–no growth interface can be defined by a single factor such as temperature (cardinal value) while other factors remain constant, but more often a combination of factors such as temperature, pH, a_w, and concentrations of chemical compounds is considered. Several models have been developed to describe these multiple factor interfaces.

TABLE 2. *Overview of some current models of microbial growth inhibition by antimicrobial compounds*

Microorganism	Growth medium[a]	Antimicrobial component	Modeling approach	Reference(s)
Acinetobacter calcoaceticus	Ready-to-drink beverages	Titrable acidity, Na benzoate, K sorbate	Polynomial with backward regression	*8*
Aeromonas hydrophila	TSB, pork meat	Lactic acid, HCl	Square root model	*47*
Brochotrix thermosphacta	Fresh red mullet (*Mullus barbatus*)	CO_2	Polynomial, Bělehrádek, Arrhenius	*59*
Clostridium botulinum	Cheese products	NaCl, citrate phosphate	Polynomial, probability model	*113, 114*
	PYGS	CO_2	Time-to-growth polynomial model	*40*
Escherichia coli O157:H7	Apple cider	K sorbate, Na benzoate	Probability model, polynomial	*117*
Gluconobacter oxydans	Ready-to-drink beverages	Titrable acidity, Na benzoate, K sorbate	Polynomial with backward regression	*8*
Lactic acid bacteria	Endive	CO_2	Arrhenius, Ratkowsky	*118*
	Fresh red mullet	CO_2	Polynomial, Bělehrádek, Arrhenius	*59*
	Lager beer	SO_2, beer components	Polynomial	*39*
	Modified-atmosphere-packed cooked meat products	CO_2, Na lactate	Square root model, polynomial	*31, 33*
	Vacuum-packaged meat	Lactic acid	Model of Yeh et al. (1991)	*88*
Listeria spp.	BHI	NaCl, methyl paraben, Na propionate, Na benzoate, K sorbate	Probability model	*101*
Listeria innocua	BHI	Lactic, acetic, and propionic acids	Extended cardinal model	*64*
Listeria monocytogenes	BHI	CO_2	Polynomial model	*38*
	Cooked ham simulation medium	CO_2, Na lactate	Ratkowsky, response surface	*34*
	LEB	NaCl, phenolic compounds	Combined exponential-polynomial approach	*85*
	Pork meat	Lactic acid, HCl	Square root model	*47*
	Shrikhand (a fermented milk product) standardized broth	Pediocin K7	Polynomial	*53*
		NaCl, Na lactate, Na acetate	Polynomial	*87*
	TPB	$NaNO_2$	Polynomial	*18*
	TSB	Lactic acid, HCl	Square root model	*47*
		NaCl, $NaNO_2$	Polynomial	*73*
		CO_2	*Z*-value concept	*96*
	TSB-YE	Nisin, NaCl	Polynomial	*11*
		Curvaticin 13, NaCl	Polynomial	*10*
		Nisin, leucocin F10, NaCl, EDTA	Probability model	*89*
	TSYGB	Lactic and acetic acids	Polynomial	*45*
		CO_2, NaCl	Polynomial	*41*
Mesophillic bacteria	Endive	CO_2	Arrhenius, Ratkowsky	*118*
Penicillium brevicompactum	MY50	Sorbic acid, Na sorbate, propionic acid	Cardinal model	*82*
Pseudomonas spp.	Fresh red mullet	CO_2	Polynomial, Bělehrádek, Arrhenius	*59*
Psychrotrophic, gram-negative bacteria	Endive	CO_2	Arrhenius, Ratkowsky	*118*
Saccharomyces cerevisiae	Laboratory medium	K sorbate	Probability model	*67*
	YEPD broth	Sulphite, nitrite, sorbic acid, benzoic acid	Mechanistic	*61*
Saccharomyces rosei	Cucumber juice medium	Lactic acid, acetic acid, hydrochloric acid, NaCl	Semimechanistic	*91*
Shewanella putrefaciens	Fresh red mullet	CO_2	Polynomial, Bělehrádek, Arrhenius	*59*
Staphylococcus aureus	BHI	Acetic, lactic, and hydrochloric acids	Modified Gompertz plus polynomial	*36*

[a] TSB, tryptic soy broth; PYGS, peptone yeast extract glucose starch medium; BHI, brain heart infusion; LEB, *Listeria* enrichment broth; TPB, tryptose phosphate broth; TSB-YE, TSB with yeast extract; TSYGB, tryptone soya broth with yeast extract and glucose; MY50, malt extract, yeast extract, peptone, 50% suger agar; YEPD, yeast extract–peptone–dextrose.

TABLE 3. *Overview of some current models of the growth–no growth interface for some antimicrobial compounds*

Microorganism	Strain	Growth medium[a]	Antimicrobial component	Modeling approach	Reference
Aspergillus niger		Ready-to-drink beverages	Na benzoate, K sorbate, titrable acidity	Logit based, polynomial	*6*
Bacillus cereus		BHI	Ethanol	Logit based, square root model based	*62*
Candida lipolytica		Ready-to-drink beverages	Na benzoate, K sorbate, titrable acidity	Logit based, polynomial	*7*
Escherichia coli	M23	Nutrient broth	Lactic acid	Logit based, nonlinear square root model	*99*
	O157:H7	TSB	Acetic acid, NaCl, sucrose	Logit based, polynomial	*77*
		Mayonnaise model system	Acetic acid, NaCl, sucrose	Logit based, polynomial	*78*
Listeria monocytogenes		TSB-YE	Lactic acid	Logit based, nonlinear square root model	*116*
			Nisin, leucocin F10, NaCl, EDTA	Logistic regression (=logit based after recalculation), polynomial with backward regression	*89*
		Mexican-style cheese	Lactic acid, NaCl	Logit based, linear model	*9*
Listeria innocua		BHI	Acetic, lactic, propionic acids	Extended cardinal model	*64*
Penicillium brevicompactum		MY50 solid medium	Sorbic acid, propionic acid, Na benzoate	Cardinal model	*82*
Penicillium spinulosum		Ready-to-drink beverages	Na benzoate, K sorbate, titrable acidity	Logit based, polynomial	*6*
Saccharomyces cerevisiae		Ready-to-drink beverages	Na benzoate, K sorbate, titrable acidity	Logit based, polynomial	*7*
Salmonella Entiritidis		BHI	Ethanol	Logit based, square root model	*62*
Staphylococcus aureus		BHI	Ethanol	Logit based, square root model	*62*
			K sorbate	Polynomial	*112*
			K sorbate, Ca propionate	Polynomial	*111*
Shigella flexneri		Broth	Na nitrite	Logit based, square root model	*100*
Zygosaccharomyces bailii		Mango puree	K sorbate, Na benzoate	Logit based, polynomial	*68*
		Ready-to-drink beverages	Na benzoate, K sorbate, titrable acidity	Logit based, polynomial	*7*
		YNB	Acetic acid, NaCl, fructose	TTG based, polynomial[b]	*54*

[a] BHI, brain heart infusion; TSB, tryptic soy broth; TSB-YE, TSB with yeast extract; MY50, malt extract, yeast extract, peptone, 50% sugar agar; YNB, yeast nitrogen broth.
[b] TTG, time to growth.

When modeling the growth–no growth interface, many data points near the growth boundaries are needed because the transition between growth and no growth is often quite abrupt. The primary data are binary: growth is observed or not observed (with some exceptions). A classical primary model will not be used in this specific area of predictive modeling. The data (growth or not) will be modeled immediately using secondary models, describing the possibility of growth as a function of environmental conditions. Tienungoon et al. *(116)* develped a model where a pH decrease of 0.1 to 0.2 units can cause growth of *Listeria monocytogenes* to cease, and Salter et al. *(105)* showed that an a_w decrease of 0.001 to 0.004 forms the growth–no growth boundary for *Escherichia coli.*

Because the use of growth–no growth modeling is recent, there are no tertiary models currently available, and more attention should be given to this research area.

Secondary models. A first attempt to model the growth–no growth interface was made by Ratkowsky and Ross *(100).* They transformed the square-root model (a kinetic model) from McMeekin et al. *(81)* and adjusted it with a nitrite factor (equation 9) to a logit-based model (a probability model):

$$\sqrt{\mu_{max}} = a_1(T - T_{min}) \times \sqrt{(pH - pH_{min})(a_w - a_{w_{min}})(NO_{2max} - NO_2)} \quad (9)$$

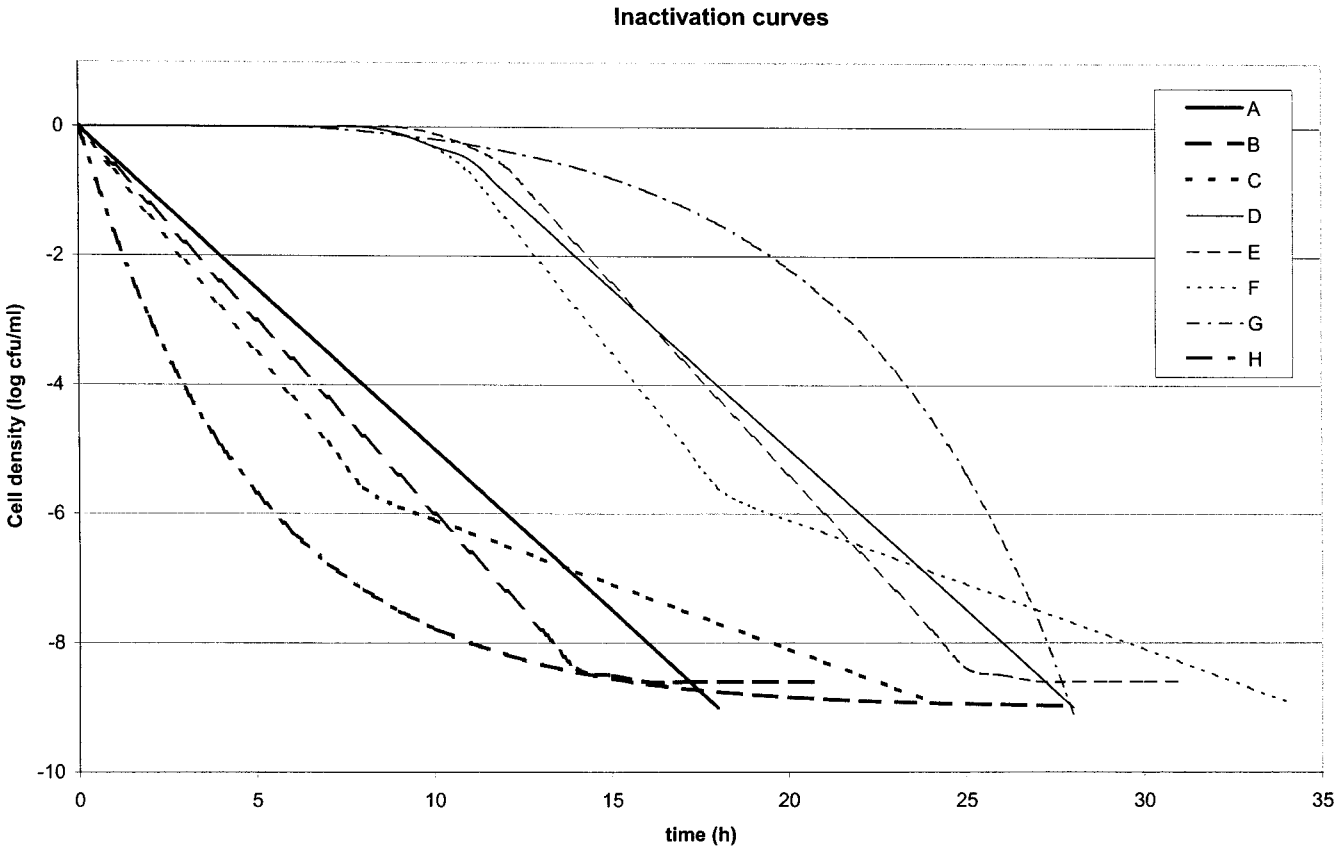

FIGURE 1. *Different types of inactivation codes. Figure adapted from Xiong et al. (126): log-linear curves (A), log-linear curves with a tailing (B), biphasic curves (C), log-linear curves with a shoulder (D), sigmoidal curves (E), and biphasic curves with a shoulder (F). Two other shapes of chemical inactivation curves were reported by Peleg and Penchina (95): concave downward (G) and concave upward (H).*

From both sides of the equation, the natural logarithm was taken, and the left side was replaced by the Logit $(p) = \ln[p/(1 - p)]$, where p is the probability that growth occurs. The data set is considered to be binary: $p = 1$ if growth can be observed for that defined combination of environmental conditions, and $p = 0$ if no growth is observed. The new equation (10) is

$$\text{logit}(p) = b_0 + b_1\ln(T - T_{\min}) + b_2\ln(\text{pH} - \text{pH}_{\min}) + b_3\ln(a_w - a_{w_{\min}}) + b_4\ln(\text{NO}_{2\max} - \text{NO}_2) \quad (10)$$

The coefficients b_0, b_1, b_2, b_3, and b_4 must be estimated by fitting the model to experimental data. The other parameters ($T_{\min}$, $\text{pH}_{\min}$, $a_{w_{\min}}$, and $\text{NO}_{2\max}$) should be estimated independently or fixed to constant values so the coefficients can be estimated using linear logistic regression. Once the *b*-values are identified, the position of the interface can be estimated by choosing a fixed *p*. Often $p = 0.5$ is preferred, corresponding to a 50:50 likelihood that the organism will grow under those fixed environmental conditions. Then, $\text{logit}(0.5) = \ln(0.5/0.5) = \ln(1) = 0$.

Another example was given by Presser et al. *(99)*, who transformed a Bělehrádek type of model to a logit-based model (equation 11) to predict the chance for *E. coli* M23 to grow in response to a_w, temperature, pH, and lactic acid concentration:

$$\text{logit}(p) = b_0 + b_1\ln(a_w - a_{w_{\min}}) + b_2\ln(T - T_{\min}) + b_3\ln[1 - 10^{(\text{pH}_{\min} - \text{pH})}] + b_4\ln\left[1 - \frac{\text{LAC}}{U_{\min}(1 + 10^{(\text{pH} - \text{pK}_a)}}\right] + b_5\ln\left[1 - \frac{\text{LAC}}{D_{\min}(1 + 10^{(\text{pK}_a - \text{pH})}}\right] \quad (11)$$

An extended model was developed by Tienungoon et al. *(116)* (model not shown). Similar models were developed by Lanciotti et al. *(62)* presenting the effect of pH, a_w, temperature, and ethanol concentration on the growth–no growth interface of *Bacillus cereus*, *Staphylococcus aureus*, and *Salmonella* Enteritidis.

In these models, linear regression was used to estimate the parameters. Salter et al. *(105)* made a model estimating the growth boundaries for *E. coli* R31 using a nonlinear regression logistic model:

$$\text{logit}(p) = b_0 + b_1\ln(a_w - a_{w_{\min}}) + b_2\ln(T - T_{\min}) + b_3\ln\{1 - \exp[b_4(T - T_{\max})]\} \quad (12)$$

A similar logit-based modeling approach was used by McKellar and Lu *(77)*, but they preferred a quadratic polynomial to the adapted square root model. They focused on the growth–no growth interface of the pathogenic *E. coli*

TABLE 4. *Static models for survival curves, adapted from (67) and (23)*

Model	Mathematical formula	Reference
First order kinetics	$N(t) = N_0 e^{-kt}$ or $\log\frac{N(t)}{N_0} = -\frac{t}{D}$	*22*
Cerf	$\frac{N(t)}{N_0} = fe^{-k_1 t} + (1 - f)e^{-k_2 t}$	*21*
Kamau	For linear survival curves: $\frac{N(t)}{N_0} = \frac{2}{1 + e^{bt}}$	*57*
	For survival curves with a lag phase: $\log\frac{N(t)}{N_0} = \log(1 + e^{-b_1 t_{1/2}}) - \log(1 + e^{b_2 t_{1/2}})$	
	For biphasic survival curves: $\log\frac{N(t)}{N_0} = \log\left[\frac{2f}{1 + e^{b_1 t}} + \frac{2(1 - f)}{1 + e^{b_2 t}}\right]$	
Whiting-Buchanan	$\log\frac{N(t)}{N_0} = \log\left[\frac{f(1 + e^{-b_1 t_{lag}})}{1 + e^{b_1(t - t_{lag})}} + \frac{(1 - f)(1 + e^{-b_2 t_{lag}})}{1 + e^{b_2(t - t_{lag})}}\right]$	*122*
Gompertz	$\log\frac{N(t)}{N_0} = Ce^{-e^{BM}} - Ce^{-e^{-B(t-M)}}$	*65*
Daugthry	$\log\frac{N(t)}{N_0} = -k_d t e^{-\lambda, t}$	*27*
Cole	$\log N(t) = \alpha + \frac{\varpi - \alpha}{1 + e^{4\sigma(\tau - \log t)/(\varpi - \sigma)}}$	*24*
Buchanan	$\text{Log } N(t) = \begin{cases} \log N_0 & (t \le t_{lag}) \\ \log N_0 - \frac{t - t_{lag}}{D} & (t > t_{lag}) \end{cases}$	*17*
Xiong	For $t \le t_{lag}$: $\log\frac{N(t)}{N_0} = 0$	*126*
	For $t > t_{lag}$: $\log\frac{N(t)}{N_0} = \log[\, fe^{-k_1(t - t_{lag})} + (1 - f)e^{-k_2(t - t_{lag})}]$	
Geeraerd	$N(t) = (N_0 - N_{res})e^{-k_{max} t}\left(\frac{1 + C_{c0}}{1 + C_{c0}e^{-k_{max} t}}\right) + N_{res}$	*44*
Membre	$\log N(t) = (1 + \log N_0) - e^{kt}$	*88*
Chiruta	$\ln\left[1 - \log\frac{N(t)}{N_0}\right] = \varepsilon + \varpi \ln(t) + \Omega[\ln(t)]^2$	*23*

O157:H7 as a function of temperature, pH, acetic acid concentration, salt concentration, and glucose concentration.

A second type of model was developed by Masana and Baranyi *(72).* They investigated the growth–no growth interface of *Brochotrix thermosphacta* as a function of pH and water activity. The a_w values were recalculated as $b_w = \sqrt{(1 - a_w)}$. The model could be divided into two parts: a parabolic part (equation 13) and a linear part at a constant NaCl level:

$$pH_{boundary} = a_0 + a_1 b_w + a_2 (b_w)^2 \qquad (13)$$

A third type of modeling was proposed by Le Marc et al. *(64).* Their model, which describes the *L. monocytogenes* growth–no growth interface, is based on a four-factor kinetic model estimating the value of μ_{max} (equation 14). Functions are based on the cardinal values of temperature *(104)* and were adjusted for *L. monocytogenes* by Bajard et al. *(2)* and for pH by Rosso et al. *(103)*:

$$\mu_{max} = \mu_{opt}\rho(T)\gamma(pH)\tau([RCOOH])\xi(T, pH, [RCOOH]) \qquad (14)$$

TABLE 5. *Model features with respect to the eight possible inactivation curve shapes, partly originating from (126)* and *(44)*

Model	Log-linear	Log-linear + shoulder	Log-linear + tail	Log-linear + shoulder + tail	Biphasic	Biphasic + shoulder	Multiphasic: Concave up	Multiphasic: Concave down
Suitable for use in both constant and changing environmental conditions								
First order	+	−	−	−	−	−	−	−
Cerf	+	−	+	−	+	−	−	−
Geeraerd	+	+	+	+	−	−	−	−
To be used only under constant environmental conditions (inherently static, inconsistent at time zero, or inconsistent at changing conditions)								
Gompertz	+[a]	+[a]	+[a]	+[a]	−	−	−	−
Kamau	+[b]	+	+	−	+	−	−	−
Whiting/Buchanan	+[b]	+	+	+	+	+	−	−
Buchanan	+	+	−	−	−	−	−	−
Cole	+	+	+	+	−	−	−	−
Membré	−	−	−	−	−	−	−	+
Daugthry	+	−	−	−	+	−	+	−
Chiruta	+	−	−	−	+	−	+	−
Xiong	+	+	+	+	+	+	−	−

[a] + indicates that the slope of the log-linear part cannot be directly related to one (or a combination of) parameter value(s), see, e.g., Membré et al. *(42)*.

[b] + indicates that the model only approximates log-linear behavior.

The growth–no growth interface was obtained by reducing the μ_{max} to zero, which can be done by making one of the environmental factors equal to zero or by making the interaction term $\xi(T, \text{pH}, [\text{RCOOH}])$ equal to zero.

An overview of some current models in the area of modeling growth–no growth in response to various antimicrobial compounds is given in Table 3.

MODELING OF MICROBIAL INACTIVATION BY CHEMICALS

Beyond the growth–no growth interface, microbial death induced by high temperatures and radiation has been well described. Less information is available for nonthermal and nonradiation methods of inactivation. Most of the modeling work regarding survival or inactivation of chemically stressed microorganisms has focused on development of primary models to describe the evolution of the cell population as a function of time.

Primary models. The concept of *D*-values known from thermal heat treatments, assuming a semilogarithmic survival curve being linear, has been extended to microbial inactivation by chemicals. Obviously, the concept of a *D*-value becomes problematic when experimentally determined semilogarithmic survival curves are clearly nonlinear *(95)*, which is often the case for inactivation by chemicals. Six commonly observed types of survival curves have been distinguished by Xiong et al. *(126)*: log-linear curves, log-linear curves with a shoulder, log-linear curves with a tailing, sigmoidal curves, biphasic curves, and biphasic curves with a shoulder. Figure 1 represents graphically these six different shapes of survival curves. Two other shapes of inactivation curves have been reported *(95)*: concave downward and concave upward. Microbial survival data for *Salmonella* Typhimurium exposed to several chlorine concentrations clearly showed concave upward characteristics *(63)*, whereas exposure of *L. monocytogenes* to potassium sorbate resulted in concave downward curves *(37)*. Peleg and Penchina *(95)* noted that the nonlinearity of inactivation curves has been resolved in four different ways: (i) ignore the curvature and force a straight line through the data points, (ii) divide the survival curves to several segments, each small enough to be approximated by a straight line, (ii) use population balance and kinetic models that are consistent with the actual shapes of the survival curves, and (iv) do not treat the survival curve in kinetic terms but as a cumulative form of a temporal distribution of lethal events. An overview of the most commonly applied primary models for static survival curves is given in Table 4. In Table 5, an overview of the same primary models is given with respect to their ability to describe one or more of these (in total) eight different survival shapes. A subdivision has been made with respect to the static or dynamic character of the different model types. For more details on the motivation of this subdivision, see the article by Geeraerd et al. *(44)*.

When a chemical agent is used as a disinfectant against microbial contamination of food, its concentration tends to decrease with time as the result of various processes, including reaction with other compounds, evaporation, and degradation. Recently, primary models were developed to take into account this change in concentration *(94)*, indicating the necessity for suitable dynamic model types.

Secondary models. Most of the model development has focused on primary models for microbial inactivation by chemicals at the expense of secondary model development. In most cases, the influence of environmental factors

TABLE 6. *Overview of some current models of microbial inactivation by antimicrobial compounds*

Microorganism	Strain	Growth medium[a]	Antimicrobial component	Modeling approach	Reference(s)
Escherichia coli	NRRL B-3704	Simulated milk ultrafiltrate	Nisin	Polynomial	*115*
	O157:H7	Eggplant salad	Oregano essential oil	Polynomial	*108*
				Vitalistic modeling approach of Kilsby et al. (2000)	*107*
		Green peppers	Chlorine dioxide gas	Polynomial	*49*
			Ozone gas	Polynomial	*50*
Listeria monocytogenes		BHI	Citric acid	Exponential equation	*13*
			Lactic acid, NaCl, Na nitrite	Polynomial	*14, 15*
				Logistic-based equation	*17*
		LEB	NaCl, phenol compounds	Combined exponential-polynomial approach	*85*
Salmonella Enteritidis		Taramasalad	Oregano essential oil	Response surface	*58*
Salmonella Typhimurium		Reduced-calorie mayonnaise	Citric acid, glucose	Second order polynomial	*83*
Yersinia enterocolitica		Laboratory media	NaCl, lactic acid	Semimechanistic own model	*55*

[a] BHI, brain heart infusion; LEB, *Listeria* enrichment broth.

in the microbial cell on the primary model parameters are described by black box polynomial equations. In many cases, the time for a 4D reduction (t_{4D}) is described by a polynomial equation as a function of factors including temperature, pH, and concentration of the applied chemical(s), as was done for an expanded model of nonthermal inactivation of *L. monocytogenes (15)* and the survival of *S. aureus (123).* Growth, survival, and death of *Y. enterocolitica* was also modeled with a new primary model with four parameters, A, B, C, and D, which were in turn described with a polynomial model having terms for temperature, pH, and the concentrations of NaCl and undissociated lactic acid *(55).*

Another applied approach is the use of probabilistic models to predict the probability of a specific reduction of a food pathogen or the probability of survival starting from a specific inoculum level. This approach was used to model the effects of nisin, leucocin F10, pH, NaCl, and EDTA on the survival of *L. monocytogenes* in broth *(89)* and to model the effects of pH, storage temperature, concentration of sorbate or benzoate, and freeze-thaw treatment combinations on the reduction of *E. coli* O157:H7 in apple cider *(117).* An overview of some current models in the area of modeling microbial inactivation by antimicrobial compounds is given in Table 6.

Tertiary models. Within the PMP, inactivation can be modeled for stressful environmental conditions, although these model results are referred to as survival curves. In addition to the effects of sodium nitrite and sodium pyrophosphate, lactic acid also was incorporated as an environmental factor.

Incorporation into tertiary models of chemical agents used to control microbial proliferation is rather limited. More effort is needed in this field to transform this knowledge into helpful tools for the food industry.

TOWARD AN INDIVIDUAL APPROACH

Most actual predictive models are developed using high inoculum levels (for growth, 10^3 to 10^4 CFU/ml; for inactivation, 10^6 to 10^8 CFU/ml), but in reality, foods often initially contain only low levels of pathogens. When low inoculation levels were applied, an increase in variability of growth characteristics of the cell population was clearly demonstrated by Robinson et al. *(102).* They proved that the variability in detection time, using optical density measurements, increased when the inoculum level was lowered, and the effect became more pronounced when the environmental conditions became more stressful. Similar research was done by Augustin et al. *(1),* Llaudes et al. *(66),* and Pascual et al. *(90).* This variation should be taken into account when exposure assessments are performed in the framework of microbial risk assessment. Therefore, new modeling approaches should be developed, using individual-based modeling techniques. A first attempt expressing the need of individual modeling was made by Buchanan et al. *(19)* and Baranyi *(3).* Later models were developed by McKellar and Knight *(76),* McKellar *(75),* Smelt et al. *(109),* and Baranyi *(4).* A method for isolating single cells in the cups of a microtiter plate, combining high yield with a high likelihood of having a single cell, was developed by Francois et al. *(42).* This method was applied to determine the individual cell lag phase of *L. monocytogenes* in broth as a function of temperature and pH *(43).*

The field of predictive microbiology is still in its embryonic state, but the knowledge that can be gained will be essential for increasing the credibility of this approach as a powerful tool in microbial risk assessment.

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