Antimicrobial Packaging: Inactivation Kinetics and Release Modes

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ABSTRACT: Antimicrobial packaging is a key component of food safety. An antimicrobial agent can be added to a packaging material during film formation, or applied to the food contact surface, which determines different types of antimicrobial packaging materials and antimicrobial release modes. In this work, different modes of antimicrobial agent release have been studied theoretically. A model is developed based on analyses of bacterial populations in response to the addition of an antimicrobial agent as a function of the agent release mode. This model provides a direct connection between microbial inhibition in the food system and the release rate of the antimicrobial agent, and it can be used to design more effective controlled-release packaging materials that will improve the microbiological safety and quality of food products.

INTRODUCTION

RECENT developments in active packaging (AP) have created effective method for preventing bacterial infection [1]. The AP system is designed with antimicrobial compounds to inactivate microbes and prolong the shelf-life of the packaged foods by extending the lag period of the bacterial life cycle and retarding the growth of microorganisms. Antimicrobial packaging is a key component of food safety; it constantly changes with the current needs of the consumers and food manufacturers.

AP provides unique means for allowing controlled release of an antimicrobial agent (AMA). The scope of antimicrobial packaging is very broad and has significant potential to improve the overall food safety system. To facilitate commercial applications of antimicrobial packaging materials, research needs to focus beyond basic feasibility testing and understand the release kinetics of antimicrobial agents from

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polymer films and transmittal to the food surfaces. This data need to be coupled with the information about the optimum dose of AMA and methods of specific antimicrobial agents delivery. The main technical challenge is to predict and control the optimal release rate and dose of antimicrobial agent delivered from the active package.

Although a large number of published experimental works describe the kinetics of the microbial inactivation due to the slow release of AMA, there have been no theoretical models developed that can account for the inhibition kinetics, allow predicting of the behavior of the microbial population, distinguish the most appropriate antimicrobial agents and estimate shelf-life of the food product.

THEORETICAL ANALYSIS OF BACTERIA INACTIVATION KINETICS

Controlled Release Modes of an Antimicrobial Agent

An AMA can be added to a packaging material during film formation, or applied to the food contact surface, which determines two major classes of AP materials: releasing and non-releasing films. An antimicrobial releasing film has AMA incorporated into the packaging polymer film, and this agent can migrate to the food surface and inhibit microbial growth. On the other hand, an antimicrobial non-releasing film has an AMA immobilized on the food contact surface and is the site where antimicrobial control occurs.

Generally, AMA's can be delivered to food from AP or added in the food formulation (as depicted in Figure 1). Both non-releasing film with antimicrobial coating and AMA added to the food formulation can be described as a step-like addition of antimicrobial to the bacterial culture. In this paper, these modes of AMA delivery will be referred to as "instant addition" modes. In AP materials with the continuous release of an AMA, the AMA diffuses through the package film and becomes absorbed by the food in direct contact between the product and the film surface. Depending on the properties of the packaging material, the AMA release process can either be steady state or non-steady state. Finally, it is possible for packaging to feature multimodal AMA delivery methods, i.e. the combination of instant AMA addition and its continuous release.

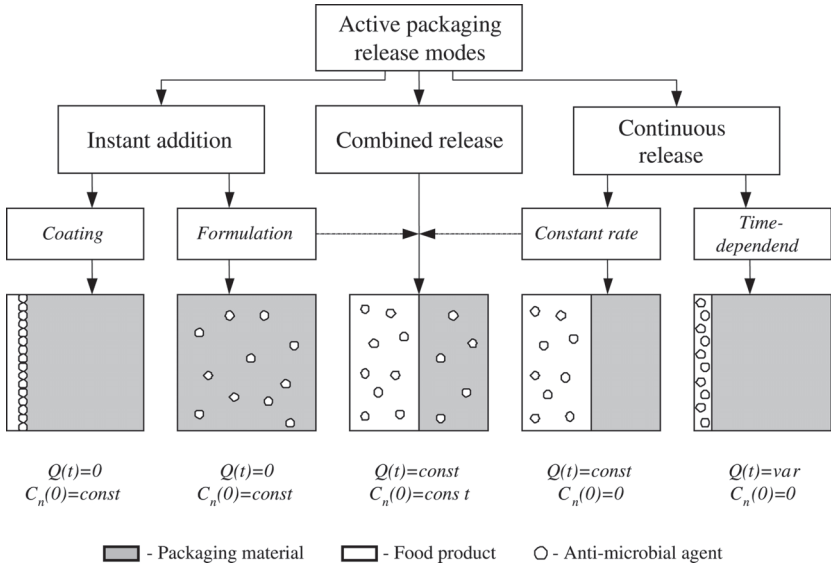

Figure 1. *Schematics of an AP system and the corresponding AMA delivery modes.*

AMA release can be characterized by the following kinetics:

$$\frac{dC_n}{dt} = Q(t) \tag{1}$$

with an initial condition $C_n\big|_{t=0} = C_{n0}$. The release rate $Q(t)$ of an AMA from the AP material is defined as:

$$Q(t) = \frac{d}{dt} M_n(t) \tag{2}$$

where $M_n(t)$ is the amount of AMA released from the packaging material.

The microbial inhibition kinetics corresponding to the AMA delivery scenarios described above can be represented by one of the following AP schemes (see Figure 1):

- AMA is instantly added to a food product (from coating or in formulation);
- antimicrobial agent is continuously added to the product over time;

- the bacterial population is exposed to a combination of the first two strategies;
- AMA is released from the packaging material with limited AMA load capacity.

Model System

The following model can be applied to a variety of packaging materials, AMA's, and target microorganisms. Specifically, we will use Nisin as the model AMA and *Listeria monocytogenes* as the representative microorganism. *L. monocytogenes,* Scott A is a widespread and virulent foodborne pathogen of great concern that can adapt to, survive in, and multiply in extreme environments. It can also survive long periods of drying and freezing followed by thawing [2]. In humans, *L. monocytogenes* causes epidemic and sporadic listeriosis. *Listeria* is one of three major pathogens (including *Salmonella* and *Toxoplasma*) responsible for 1,500 food-related deaths in the USA each year [3].

Nisin (bacteriocin produced by *Lactococcus lactis*) has been shown to be an excellent candidate for use in antimicrobial-releasing films. It has been successfully incorporated into various edible films, polyolefin-based films, and film coatings [4–9]. Nisin alone is effective against gram-positive bacteria and is able to inhibit gram-negative bacteria with the aid of a chelator such as EDTA [10].

Bacterial Kinetics

AP protects foods by inhibiting microbial growth in the food products. Initial bacterial contamination levels in food products are usually very low [11], and can be described initially by exponential growth kinetics:

$$\frac{dC_c}{dt} = \mu C_c \tag{3}$$

The microbial population dynamics in the presence of an AMA released from an AP system and/or added to a formulation is the result of two competitive processes: population growth and inactivation. One of the most frequently used models for inactivation kinetics is the well-known Chick-Watson first-order kinetic model [12]:

$$\frac{dC_c}{dt} = -K_n C_n \tag{4}$$

where: C_c—microorganism population at time t, C_{c0}—initial microorganism population at $t = 0$; C_n = antimicrobial agent residual, K_n—reaction rate.

Combining (3) and (4), one obtains an overall change of microbial concentration in time:

$$\frac{dC_c}{dt} = \mu C_c - K_n C_n \tag{5}$$

The concentration of AMA changes with time due to its release (Equation (1)) and to its reaction with the bacteria (adsorption on the cell wall and/or penetration into the cells) that can be written as:

$$\frac{dC_n}{dt} = -\frac{K_n}{\gamma} C_n + F_n(t) \tag{6}$$

where γ is the antimicrobial agent efficacy factor, i.e. the amount of AMA required to inactivate a certain number of bacteria.

The model approach described so far does not take into account the diffusion/transport of AMA from the packaging material to the food surface. Therefore, this model can be applied for packaged products where the package is in direct contact with the food surface (e.g., RTE meats), or with the thin liquid-filled headspace (hot dogs). The diffusion transport of AMA agent would not influence bacterial inactivation kinetics when diffusion time of AMA migration from the package to the food surface (t_{dif}) satisfies the following condition:

$$t_{dif} << \frac{1}{\mu} \tag{7}$$

where μ is the specific rate of bacteria growth.

Therefore, the critical distance between the package and a surface to be decontaminated can be determined as following:

$$x << \sqrt{\frac{D}{\mu}} \tag{8}$$

where D is the diffusion coefficient of AMA in the food product. Calculations performed for the *L. monocytogenes*/nisin system (bacterium doubling time is 18 min [13], molecular weight of nisin is 3.5 kDa [14])

indicate that the size of headspace should not exceed 100 μm to avoid diffusion limitations for AMA transport. Additionally, AP is deemed to be effective against bacterial contamination when the number of microorganisms during the product shelf life does not exceed its initial value:

$$C_c\big|_{t\to\infty} \le C_{c0} \tag{9}$$

Listeria Monocytogenes Inhibition Kinetics at Various Nisin Release Modes

Bacterial inactivation kinetic model describes the resulting process of exponential bacterial growth and inhibition due to AMA addition with different rates of nisin release and various initial concentrations.

Instant Addition of AMA

As mentioned earlier, AMA can be added to the food product at the time of preparation as a part of the product formulation in order to successfully inhibit bacterial contamination and to decrease the bacterial population to zero. In this case, the spoilage bacteria are exposed to the AMA and there are no other sources of AMA in the system. Therefore, the initial conditions for the system , are the following:

$$C_{n0} = \text{const}; \quad Q(t) = 0 \tag{10}$$

where C_{n0} is the initial concentration of AMA added to the food product by formulation.

Now solving the system (5), (6), one can obtain an analytical solution for the changes in the microbial population as a function of the inoculum (spoilage) size, specific growth rate (μ), inactivation rate constant, and time:

$$C_c = \left[\frac{C_{c0}(K_n\gamma + \mu) - K_n C_{n0} + K_n C_{n0} e^{-(K_n\gamma+\mu)t}}{K_n\gamma + \mu} \right] \tag{11}$$

The concentration of AMA decreases in time according to first-order kinetics:

$$C_n = C_{n0} e^{-K_n\gamma t} \tag{12}$$

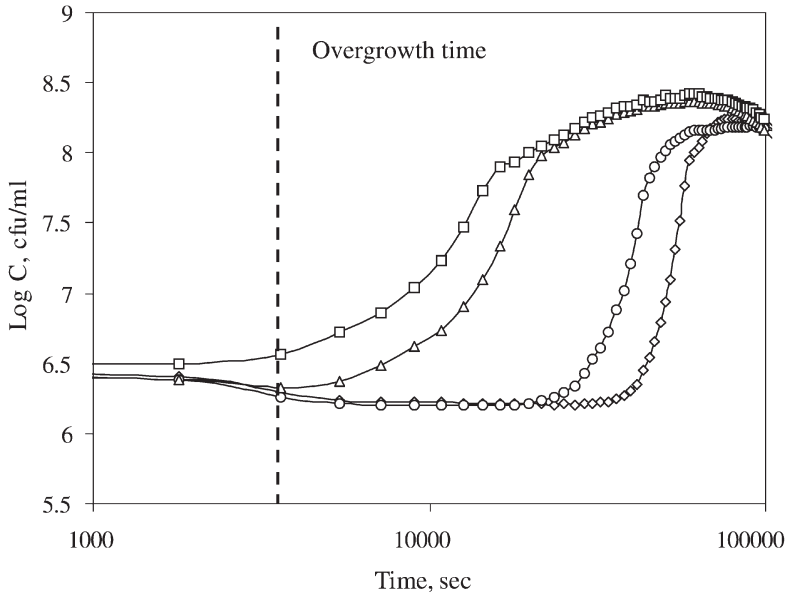

Figure 2. L. monocytogenes *growth in the presence of instantly added nisin:* C_{n0} *= 0, 750, 1000, 2000 IU/mL.*

where C_{c0} is the initial concentration of bacteria, i.e. the size of inoculum (level of bacterial contamination) from which the food product should be protected. Figure 3 depicts experimentally obtained growth kinetics of *L. monocytogenes* at various initial concentrations of nisin.

In comparison, Figure 3 represents the results of numeric calculations, based on the developed model (11). The parameters used in the model are the following: $C_{c0} = 10^5$ cfu/mL, $K_n = 20, \gamma = 0.01$. The bacterial kinetics obtained from the calculations is similar to those observed experimentally. Despite its simplicity, the model can qualitatively simulate the bacterial population dynamics.

As we can see that both experimental data (Figure 2) and theoretical model (Figure 3) show the same "overgrowth time"—the time when bacterial population will reach initial (before-treatment) level. This period of time will actually determine the shelf life of the food product.

Analysis of the solution (11) shows that it is possible to find a set of system parameters where the bacterial population would diminish. The expression in the square brackets in equation (11) consists of three terms, the last of which becomes zero at large times. The level of bacterial contamination becomes negligible only if the difference between the

first two terms approaches zero. Solving the equation, one can determine the amount of AMA (C_{n0}) that is necessary to successfully prevent bacterial spoilage with a characteristic growth rate and inactivation kinetics:

$$\frac{C_{n0}K_n}{\gamma K_n + \mu} \geq C_{c0} \tag{13}$$

The inequality in equation (13) reflects the fact that bacteria with higher growth rates require higher levels of AMA initially added to the product. This AMA delivery strategy is the traditional approach for maintaining food safety by modifying the product formulation.

Continuous Addition of AMA at a Constant Rate

The model's representation of AP system includes the continuous addition of AMA's into the product at a constant rate (Q) during its shelf life. This delivery strategy is not used in actual packaging materials, but

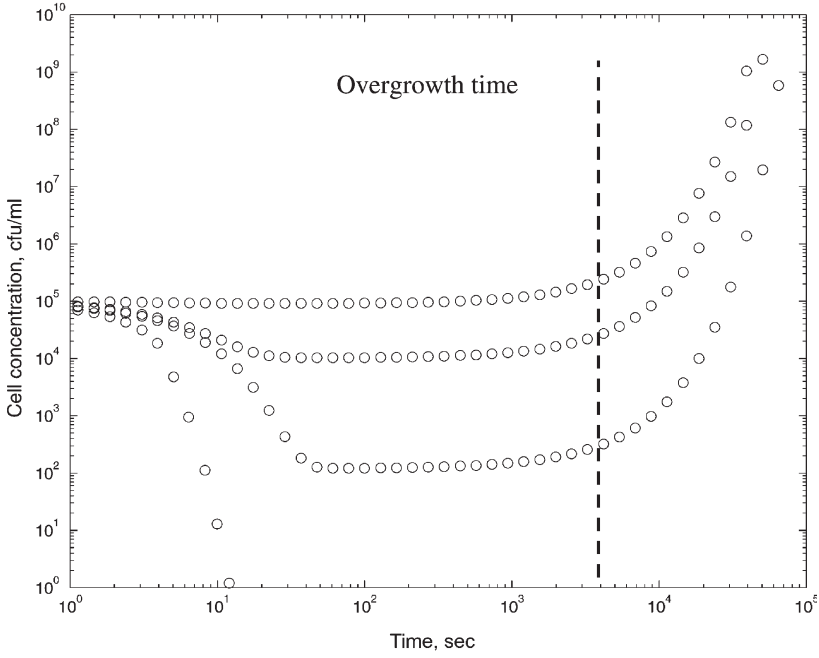

Figure 3. L. monocytogenes *growth in the presence of instantly added nisin: experimental data (a) and numerical simulation results (b).* C_{n0} *= 100, 750, 1000,1500 IU/mL.*

has been experimentally investigated in [15]. The initial conditions for this case are the following:

$$C_{n0} = 0; \quad Q(t) = Q = \text{const} \tag{14}$$

The continuous addition of AMA significantly changes the inactivation process and bacterial population dynamics, as can be seen from the solution of the system (equations (5) and (6)), with the initial conditions (14):

$$C_c = \left[\frac{Q}{\gamma\mu} e^{-\mu t} - \frac{\mu Q}{K_n\gamma + \mu} e^{-(K_n\gamma+\mu)t} + \frac{\mu C_{c0}(K_n\gamma + \mu) - K_n Q}{\mu(K_n\gamma + \mu)} \right] e^{\mu t} \tag{15}$$

The population dynamics depends on bacterial kinetics and on the antimicrobial flux Q (i.e. the material properties of the AP). The corresponding change in the AMA concentration is:

$$C_n = \frac{Q}{K_n\gamma}(1 - e^{-K_n\gamma t}) \tag{16}$$

The results of numeric simulations of *L. monocytogenes* population dynamics as a function of nisin release rate are depicted in Figure 4. It is clear that the continuous addition of AMA is very effective method to inhibit/prevent bacterial population growth. Our results are in good qualitative agreement with the published data [15]. However, this method of AMA delivery is better suited for more long-term applications, and less effective for immediate inactivation of the bacteria.

The concentration of AMA is not a simple decay, but also depends on the AMA flux. The analysis of the system parameters needed to uphold microbial safety of the food product in this case results in an inequality that shows that the continuous addition of the AMA will reduce the bacterial population to zero only for certain values of the AMA delivery rate:

$$\frac{K_n Q}{\mu(K_n\gamma + \mu)} \geq C_{c0} \tag{17}$$

Multimodal AMA Delivery

Chi-Zhang *et al* [15] have shown that microbial population dynamics depends on the mode of antimicrobial agent delivery. The initial addition

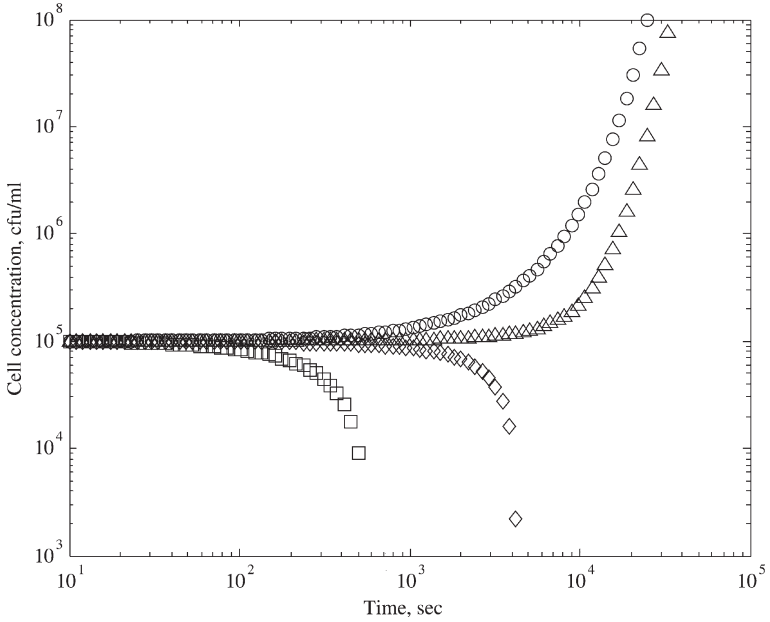

Figure 4. *Calculated* L. monocytogenes *population dynamics as a function of nisin release rate.*

of an AMA is very effective in the early stages of bacterial population development, and the continuous addition method works better for longer times, and seems more appropriate for products with an extended shelf life. In addition, the authors mentioned the synergistic effect of combining the instant (initial) addition and the continuous release modes on *L. monocytogenes* inhibition. The initial conditions in this case reflect changes in the mode of antimicrobial delivery:

$$C_{n0} = \text{const}; \quad Q(t) = (Q) = \text{const} \tag{18}$$

The solution of the system is complex and allows more flexibility in choosing strategies of bacterial population control:

$$C_c = \left[\frac{Q}{\mu\gamma} e^{-\mu t} + \frac{(C_{n0} K_n \gamma - Q)}{\gamma(K_n \gamma + \mu)} e^{-(K_n \gamma + \mu)t} + \frac{\mu C_{c0}(K_n \gamma + \mu) - K_n(Q + \mu C_{n0})}{\mu(K_n \gamma + \mu)} \right] e^{\mu t} \tag{19}$$

The corresponding changes in the AMA concentration can be expressed as follows:

$$C_n = \frac{Q + (C_{n0} K_n \gamma - Q)}{K_n \gamma} e^{-K_n \gamma t} \tag{20}$$

Bacterial kinetics obtained as a result of this model is similar to those depicted in Figure 3 and Figure 4. The combined release mode is more flexible and allows more effective inhibition of the microorganisms. The condition that allows successful suppression of growth and/or inactivation of bacteria can be expressed as:

$$\frac{K_n(\mu C_{n0} + Q)}{\mu(K_n \gamma + \mu)} \geq C_{c0} \tag{21}$$

Time-Dependent Release Rate of AMA

Understanding the release dynamics of the AMA is key in the design of antimicrobial-releasing films and packaging. Due to the finite thickness of AP layers and its limited capacity, traditional AP materials cannot release antimicrobials at a constant rate during the product shelf life period. Upon initial contact with the food product, the AP has a very low AMA release rate, because the polymer matrix is dry and the internal pores of the material are blocked. During the first minutes/hours of contact with the product, the AP material swells due to hydration, pores open, and the AMA release rate increases to its maximum value. After reaching its maximum value, the AMA release rate declines, as the AMA content of the packaging gradually decreases due to the limited amount of AMA that can be embedded into the polymer matrix.

Numerous published data on nisin-based control release films are presented in different ways and very difficult to compare. However, using equation (2) it is possible to obtain normalized data for the nisin release rate as a function of time for various materials. Data on nisin release dynamics collected from the literature are combined in Figure 5.

The bacterial population dynamics is described by the expression (5) as discussed above. As follows from the data in Figure 5, time dependence of AMA release rate can be adequately represented by an exponential function of time ($Q(t) = Qe^{-\lambda t}$) with the exponent factor ($-\lambda$) determined by transport properties of the packaging material. Now the equation (6) with new time-dependent AMA release function from the packaging material can be written as:

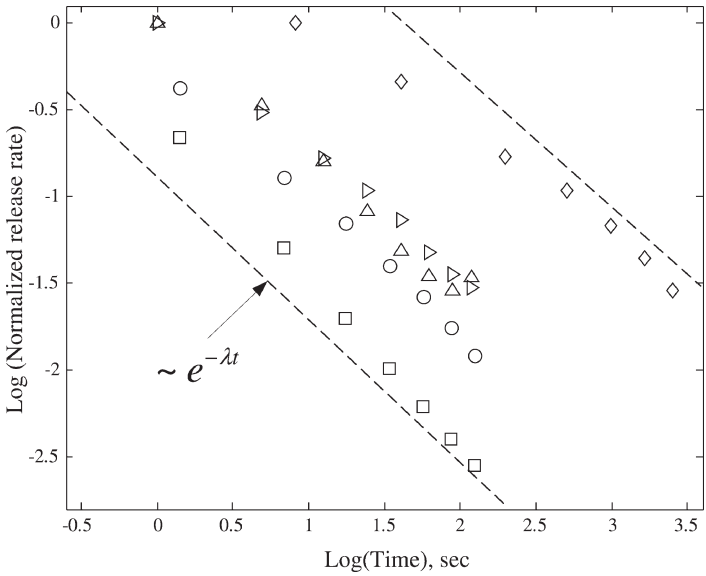

Figure 5. *Time dependence of the nisin release rate from various active packaging materials: films containing 2% (○) and 7% (□) of glyoxal PVOH [16]; (◇)—acrylic polymer film [17]; (△)—acrylic polymer film with 5% nisin [18]; (▷)—vinyl acetate-ethylene co-polymer with 5% nisin [18].*

$$\frac{dC_n}{dt} = -K_n C_n + Qe^{-\lambda t} \tag{22}$$

where $\lambda = f(M_{n0}, h, D_{ns}, D_{nl}, d)$ is the measure of AP efficacy. It is the function of diffusion coefficients of the AMA in the packaging material (D_{ns}) and food (D_{nl}), M_{n0}—the AP material storage capacity, h—mass-transfer coefficient, and d—the thickness of the packaging material.

The solution of the system (5) and (22) is the following:

a) Change of bacterial contamination with time:

$$C_c = \{K_n(K_n\gamma + \mu)Qe^{-(\mu+\lambda)t} + (\mu+\lambda)[C_{n0}(K_n\gamma - \lambda) - Q]e^{-(K_n\gamma+\mu)t}$$

$$+(K_n\gamma - \lambda)\{K_nQ + (\mu+\lambda)[C_{n0}K_n - C_{c0}(K_n\gamma + \mu)]\}\} \tag{23}$$

$$\times \frac{e^{\mu t}}{(K_n\gamma - \lambda)(\mu+\lambda)(K_n\gamma + \mu)}$$

b) Corresponding change of AMA concentration:

$$C_n = \{Q[1 - e^{-(K_n\gamma - \lambda)t}] - C_{n0}(K_n\gamma - \lambda)\}\frac{e^{-K_n\gamma t}}{K_n\gamma - \lambda} \qquad (24)$$

The microbial kinetics in this case is controlled not only by the bacterial inactivation rate constant and growth kinetics, but also by the material properties of the AP. Rearranging (23) one obtains condition for successful protection of the food product in case of time-dependent AMA release from the AP material:

$$\frac{K_n[(\mu + \lambda)C_{n0} + Q]}{(K_n\gamma + \mu)(\mu + \lambda)} \geq C_{c0} \qquad (25)$$

The results of numerical simulation of *Listeria monocytogenes* inhibition by nisin released at various rates from an AP system are depicted in Figure 6.

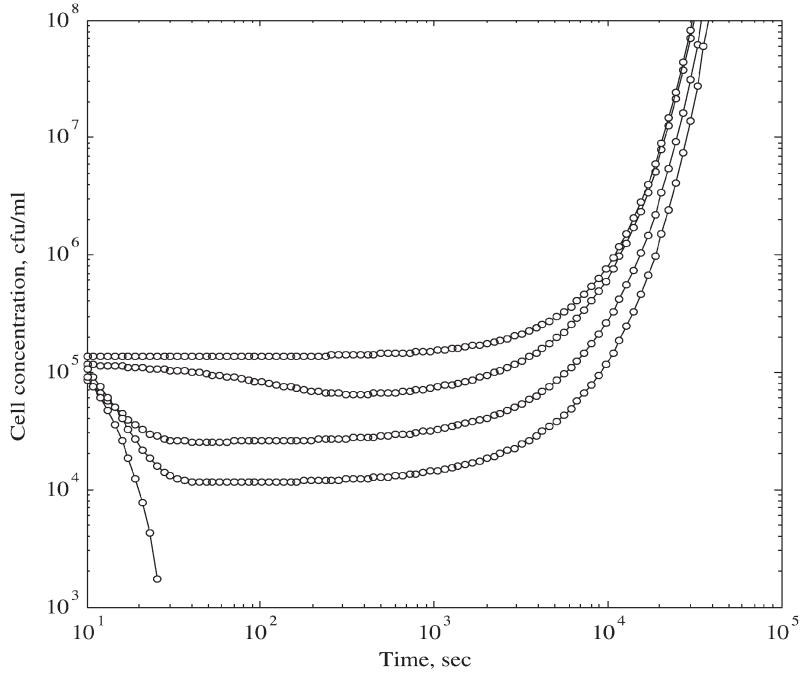

Figure 6. L. monocytogenes *population dynamics as a result of non-steady state nisin release.*

The numerical modeling results are qualitatively in good agreement with the data in reference [6], where the release of nisin from cellulose-based films has been measured and the initial inhibition of *L. monocytogenes* followed by bacteria growth was observed.

DISCUSSION

The optimal antimicrobial packaging system would have a broad antimicrobial spectrum, exert strong antimicrobial activity at low concentrations, have no adverse effects on the food product and packaging material, be cost effective, and satisfy FDA requirements [1]. The type of food, target microorganism, and desired shelf life determine the appropriate AMA and the method of delivery. The present model not only describes the inactivation kinetics of the microbial population under different scenarios, but also allows one to choose the best AMA and the most effective pathogen treatment.

Let us introduce a dimensionless process variable:

$$\alpha = \frac{K_n \gamma}{\mu} \tag{26}$$

The ratio between kinetic constants of bacterial growth and inactivation determines the overall efficacy of the treatment process. Substituting (26) into expressions (13), (17), (21), (25) and rearranging them, one obtained the new set of conditions for effective control of the bacterial population:

$$\left.\begin{array}{c} \dfrac{\alpha}{1+\alpha}\dfrac{1}{\gamma}C_{n0} \\ \dfrac{\alpha}{1+\alpha}\dfrac{1}{\gamma}\dfrac{Q}{\mu} \\ \dfrac{\alpha}{1+\alpha}\dfrac{1}{\gamma}\left(C_{n0}+\dfrac{Q}{u}\right) \\ \dfrac{\alpha}{1+\alpha}\dfrac{1}{\gamma}\left[C_{n0}+\dfrac{Q}{(\mu+\lambda)}\right] \end{array}\right\} \geq C_{c0} \tag{27}$$

Based on this system, it is possible to specify requirements for transport properties and storage capacity of active packaging material for var-

ious regimes of antimicrobial delivery. Obviously, all inequalities in (27) can be rewritten in (27) the generalized form:

$$\frac{\alpha}{\alpha+1}\frac{1}{\gamma}f(C_{n0},\ Q,\ \mu,\ \lambda)\geq C_{c0} \tag{28}$$

where $1/\gamma$ is the AMA "economy" factor specific to the bacteria/AMA pair, i.e. the amount of AMA needed to inactivate one bacterial CFU; $f(C_{n0}, Q, \mu, \lambda) \geq C_{c0}$—the function determined by specific design of the package, packaging material, and food product formulation; $\alpha/(\alpha + 1)$—the coefficient determined by the kinetics of bacteria reproduction and inactivation.

$$\frac{\alpha}{\alpha+1}\rightarrow\begin{cases}1 \text{ for } K_n>\mu\\ \dfrac{1}{2} \text{ for } K_n\sim\mu\\ 0 \text{ for } K_n<\mu\end{cases} \tag{29}$$

Hence, it is possible to choose an AMA candidate for the food product, which makes the product development and package design processes cost-effective and less time consuming.

The presented model can also be used to predict product shelf life, which can be defined as the time required for bacterial population to grow from its initial level to an unacceptable concentration. Substituting $C_c \rightarrow C_{c\,\mathrm{lim}}$, where $C_{c\,\mathrm{lim}}$ is an acceptable level of bacterial contamination, in (19) and using a Taylor series expansion, one obtains the estimated value of the product shelf life as a function of bacterial growth kinetics and AMA concentration:

$$t_{cr};\ \frac{C_{c\,\mathrm{lim}}}{1-\alpha\dfrac{C_{n0}}{C_{c0}}} \tag{30}$$

This model provides a direct connection between microbial inhibition in the food system and the release rate of the AMA, and can be used to design more effective controlled-release packaging materials that will improve microbiological safety and quality of food products.

CONCLUSION

In this work, the author attempts to develop analytical model of bacterial population response to adding of an antimicrobial agent as a function of the agent release mode. Although all calculations were performed using nisin as an AMA and *L. monocytogenes* as a model pathogenic microorganism, the model can be applied to various bacteria/AMA systems and used to predict bacterial population dynamics for active packaging applications.

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