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# Hypochlorite inactivation kinetics of lactococcal bacteriophages

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**Abstract**

Ten heat-resistant lactococcal phages in M17 broth were inactivated by exposure to a range of different hypochlorite concentrations (2000–5000 mg $l^{-1}$). Deviations from first-order kinetics as sigmoidal shapes were observed in the survival curves of all bacteriophages. Therefore, an empirical model with four parameters was used to describe hypochlorite inactivation. The model provided good fit for all phages; however, it was observed that there was a high correlation between the parameters of this model. That is why, the number of parameters was reduced from four to two with a slight loss of goodness-of-fit and this reduced model produced fits comparable to the original model. Alternative *D* and *z* values were also proposed for the model. By comparing the times necessary to reduce the number of phages six- or seven-logs, the most hypochlorite-resistant phages were found as: $\phi$pld67 37, $\phi$pll47 21 and $\phi$pld66 36. Demonstration of this model may also be used for other bacteriophages inactivated by other biocides.


*Keywords:* Lactococcal bacteriophages; Hypochlorite; Sigmoidal survival curves; First-order kinetics; Predictive microbiology

## 1. Introduction

Lactococcal bacteriophages are the most serious threat to dairy fermentations (Daly, Fitzgerald, & Davis, 1996). Technological and biochemical functions of the starter cultures are severely affected when the fermentation environment is contaminated by phages brought into the dairy via raw milk or air. Once a starter culture is infected, phages multiply rapidly, attacking more and more bacterial cells. By the inhibition of the starter culture activity, poor product quality or even complete fermentation failure occurs (Müller-Merbach, Raucher, & Hinrichs, 2005). Therefore, bacteriophages must be removed from the processing environment or inactivated.

Exposure to a lethal chemical agent is an effective means to reduce the microbial population in foods, packages and on equipment. It is therefore a widely used approach of disinfection and preservation in food and other industries (Peleg, 2002). Chlorine is widely used as a disinfectant in the food industry and for the treatment of drinking water in community distribution systems (Erkmen, 2004). Its efficacy against a broad spectrum of microorganisms is well documented (Nguyen & Carlin, 1994; Paz, Duaigues, Hanashiro, D'Aquino, & Santini, 1993; Sapers, Miller, & Mattrazzo, 1999).

Traditionally, microbial mortality has been treated as a process that follows first-order kinetics; however, there is now enough evidence that first-order model is an exception rather than the rule for both bacterial cells (van Boekel, 2002) and bacterial spores (Periago et al., 2004). Although there are some studies related with bacteriophage inactivation and nonlinear models (Avsaroglu, Buzrul, Alpas, Akcelik, & Bozoglu, 2006; Chen, Joerger, Kingsley, & Hoover, 2004; Müller-Merbach et al., 2005), to the best of our knowledge, there is no study related to bacteriophage inactivation by a lethal chemical agent (hypochlorite) and the proposed model describing the sigmoidal shapes of bacteriophages. The lactococcal phages used in this study are all heat-resistant and are all common in Turkish dairy plants. Therefore, more studies should be carried out to have further knowledge about their inactivation kinetics, i.e., different mathematical models should be used in order to describe the inactivation accurately. The objectives of

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this study were to define the hypochlorite inactivation of several lactococcal bacteriophages by use of a suitable nonlinear model and further to determine the effect of hypochlorite concentration on the parameters of the proposed model.

## 2. Materials and methods

### 2.1. Bacteria, bacteriophages and culture conditions

All bacteria and bacteriophages were obtained from Ankara University, Faculty of Science, Department of Biology Culture Collection (Ankara, Turkey). The complete list of bacteriophages and their respective host strains are given in Table 1. Bacteria were grown routinely in M17 medium (Merck KgaA, Darmstadt, Germany) at 30 °C overnight and were maintained in M17 medium supplemented with 20% (ml/100 ml) sterile glycerol (Sigma-Aldrich, USA) at −20 °C. Phage propagation and counts were also performed in M17 medium at 30 °C and overnight incubation. Phage stocks were prepared in M17 medium with the addition of 40% (ml/100 ml) sterile glycerol and stored at –20 °C. Phages were enumerated by using double-layer agar method and expressed as plaque-forming unit per mililiter (PFU $ml^{-1}$) (Terzaghi & Sandine, 1975).

### 2.2. Chemical treatment

Sodium hypochlorite, which was diluted in M17 medium (2000, 3000, 4000, 5000 mg $l^{-1}$ with 14% active chlorine), was used for chemical inactivation of phages. Phage suspensions (approx. $10^7$ PFU $ml^{-1}$) in M17 medium were mixed with sodium hypochlorite in eppendorf tubes with respect to final concentration of the biocide. Sodium hypochlorite and phages were mixed in 1:1 range where 2 × concentrations of hypochlorite were used, respectively. For each experiment, nonbiocide-treated phages were enumerated as controls (Quiberoni, Guglielmotti, & Reinheimer, 2003). All experiments were repeated three times at 25 °C and the average determined.

### 2.3. The model, data analysis and model evaluation

Sigmoidal survival curves can be described by a variety of mathematical models having 3–4 adjustable parameters; therefore, the following empirical equation suggested by Peleg (2003) was used:

$$\log_{10} S(t) = -\frac{[1 + bt^n]t}{k_1 + k_2 t}, \quad (1)$$

where $S(t)$ is the survival ratio, i.e., $S(t) = N(t)/N_0$, $N(t)$ and $N_0$ are the number of survivors after an exposure time $t$ and initial number of microorganisms (microbial cells and spores; CFU $ml^{-1}$or in case of viruses; PFU $ml^{-1}$). The parameters $b$, $n$, $k_1$ and $k_2$ are concentration-dependent coefficients.

SigmaPlot 2000 Version 6.00 (Chicago, IL, USA) was used for nonlinear regression analysis and to determine the parameters of the suggested model. The goodness-of-fit of the model was assessed using adjusted regression coefficient ($R^2_{adj}$) and mean square error (MSE) values.

## 3. Results and discussion

Preliminary experiments showed that application of hypochlorite solutions between 100 and 300 mg $l^{-1}$, up to 60 min, had no effect on significant reduction ($P < 0.05$) of the bacteriophages studied. Therefore, higher concentrations were tried (2000–5000 mg $l^{-1}$). Fig. 1 shows the hypochlorite inactivation of the phage *ϕpll*36 14 at 2000 and 3000 mg $l^{-1}$; the higher the concentration of hypochlorite, the faster was the phage inactivated. About 8-log reduction for the phage *ϕpll*36 14 was observed at 30 and 20 min treatments for the concentrations 2000 and 3000 mg $l^{-1}$, respectively. Fig. 1 also indicates the existence of sigmoidal survival curves for the phage *ϕpll*36 14, i.e., deviation from first-order kinetics. This is also true for all other phages studied (results not shown). The visual inspection of Fig. 1 indicates a good fit obtained by the model and support for this statement comes from the corresponding $R^2_{adj}$ and MSE values which were 0.99 and 0.05 at 2000 mg $l^{-1}$ and 0.99 and 0.003 at 3000 mg $l^{-1}$, respectively. It should also be noted that adequate description of the data by Eq. (1) does not exclude other nonlinear modeling approaches. In fact, any other model with at least

Table 1
Bacteriophages, host starins, isolation material and places

| Bacteriophages | Host strain | Isolation material | Isolation site |
|---|---|---|---|
| *ϕ*pld6434 | *L. lactis* subsp. *lactis* biovar. *diacetylactis* PLD64 | Raw milk | İzmir (Ödemiş) |
| *ϕ*pll105 | *L. lactis* subsp. *lactis* PLL10 | Raw milk | Eskişehir |
| *ϕ*pll4721 | *L. lactis* subsp. *lactis* PLL47 | Whey | Kilis |
| *ϕ*pld6737 | *L. lactis* subsp. *lactis* biovar. *diacetylactis* PLD67 | Raw milk | Erzincan |
| *ϕ*pld6636 | *L. lactis* subsp. *lactis* biovar. *diacetylactis* PLD66 | Raw milk | Niğde (Kemerhisar) |
| *ϕ*plc6158 | *L. lactis* subsp. *cremoris* PLC61 | Raw milk | Adıyaman (Tut) |
| *ϕ*plc6154 | *L. lactis* subsp. *cremoris* PLC61 | Raw milk | Adana |
| *ϕ*pll62 | *L. lactis* subsp. *lactis* PLL6 | Raw milk | Burdur (Varollar) |
| *ϕ*pll356 | *L. lactis* subsp. *lactis* PLL35 | Raw milk | Antalya |
| *ϕ*pll3614 | *L. lactis* subsp. *lactis* PLL36 | Raw milk | Eskişehir |