Inactivation of dairy phages applying photocatalytic paint under diverse environmental conditions: a kinetic study

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A first-order kinetic model regarding the Plaque Forming Units per mL (PFU/mL) of dairy phages and correlating the inactivation constant with operating conditions of radiation flux and relative humidity was developed. Results showed that as the radiation flux and relative humidity increased, phage inactivation turned higher for both uncoated and coated plates with photocatalytic paint (1.43 log orders/h for phage J-1 and 0.92 log orders/h for M13-G1b for 80% of relative humidity and 100% of radiation flux). The novel model showed a strong agreement with experimental data (RMSE of 4.43% for J-1 and 2.98% for M13-G1b) and it would be useful to predict phage inactivation under diverse combinations of operating conditions levels, different from those tested in this work.

Introduction

Bacteriophages are ubiquitous in dairy plants and represent an important risk, since when a phage infection is produced on a fermentative process, it might cause the decline on product quality or even the complete block of the process [1]. Our work group had demonstrated the feasibility of a photocatalytic paint (carbon doped TiO_2) in the inactivation of 16 dairy phages working with visible light [2]. The photocatalytic efficiency is strongly influenced by the interaction between the catalyst and biological entity to be degraded, which also depends on the amount of water molecules present, the photocatalyst hydrophilicity, incident radiation flux, among others [3]. The aims of this work were to study the effect of the relative humidity and of the irradiation level on inactivation of two phages using a photocatalytic paint under typical indoor radiation (λ = 360 - 720 nm) and to obtain a kinetic model that fits the experimental results.

Material and Methods

Suspensions of phages J-1 and M13-G1b (10^6-10^7 PFU/mL) were deposited (20 µL) on the borosilicate plates (coated and uncoated) and dried in a dark and sterile environment. Plates were put into the reactor and exposed to radiation for a maximum time of 20 h. At scheduled times, samples were removed from the reactor for phage quantification through the double layer plaque titration method [2]. Three levels of irradiation, regulated by using optical filters with different transmittances (100%, 56% and 18%) were tested, keeping the value of relative humidity constant (RH, 80%). The maximum incident net radiation flux employed was 3.03×10^{-2} W/cm^2 (100%). Three different conditions of relative humidity were tested: 30%, 50% and 80% by placing different oversaturated salt solutions inside the reactor and keeping the irradiation flux level constant in 100% (λ = 360 - 720 nm).

Experimental data were fitted with Eq.1, minimizing the Root Mean Square Error (RMSE). This equation was obtained by solving the mass balance of PFU applying a first order kinetic and considering that the inactivation constant depends on RH (as a potential function) and on radiation flux (as a logistic function).

$$P = P_0 \times exp\left(\frac{-k_1 \times \langle q_{in}\rangle_{A_{irr}}^{k_2} \times t}{1 + exp(-k_4 \times (C_w - k_3))}\right) \qquad (1)$$

Results and Discussion

At 100% radiation (80% RH), inactivation of both phages reached its highest value (1.43 log orders/h for phage J-1 and 0.92 log orders/h for phage M13-G1b). On the contrary, phages reached their lowest inactivation rate (0.80 log orders/h for phage J-1 and 0.55 log orders/h for phage M13-G1b) working with a level of irradiation of 18%. Similar performance was observed when phages were tested on uncoated plates. In these assays, the loss of infectivity can be attributed exclusively to a photochemical process caused by an interaction between the phages and radiation [4].

The greatest phage reduction was obtained working with 80% of RH (100% radiation). The lowest titer reduction was obtained at 30% RH (0.14 log orders/h for both phages), being the reduction in the inactivation rate less significant than when the relative humidity is reduced from 80% to 50%. Similar performance was observed when phages were tested with uncoated plates: as the relative humidity decreased, phage inactivation became lower. No loss of infectivity on coated plates in the dark (0% radiation, 80% RH) was observed.

Experimental data were fitted with the proposed kinetic model (Eq. 1), in which the effect of relative humidity and incident radiation flux on phage inactivation were considered and kinetic parameters were estimated (Table 1). Working with coated plates, the reaction order regarding the radiation flux

for both phages was similar (according to kinetic parameter k_2) and significantly lower than 1: as the radiation flux increased, a saturation of photons occurred on the catalytic surface, which causes the inactivation rate not improve in the same proportion as the increase in flux. Phage J-1 showed a similar value of k_2 for both coated (photocatalytic) and uncoated (photochemical) plates. This may be attributed to a great radiation absorption by the phage, in contrast to phage M13-G1b, which is likely to absorb less photons, as evidenced by the higher value of k_2 estimated for uncoated plates (close to 1). This different behavior may be related to differences in the genomic sequences and expressed proteins of each phage, which may influence phage inactivation. Regarding the logistic function to take into account the relative humidity influence, this model can explain that the phages inactivation increases slightly between RH of 30 and 50%, but when RH is increased to 80 % the inactivation rate is accelerated significantly. k_3 represents the midpoint of the logistic function and k_4 represents the steepness of the curve of the logistic function. For all situations, the order of magnitude of these parameters is similar. However, for both phages the steepness of the inactivation rate regarding the relative humidity is increased when the photocatalytic process comes into action (k_4 is higher for coated plates). On the other hand, the predicted water concentration at which the inactivation curve reaches the inflexion point is reduced for the photocatalytic assays. This would indicate that the maximum inactivation rate could be reached with a lower water vapor concentration when combined photocatalytic and photochemical processes take part.

A good agreement between experimental data and model predictions is observed for both phages and for uncoated and coated plates, reaching an average RMSE for all conditions tested of 4.43% for phage J-1 and 2.98% for phage M13-G1b. Figure 1 shows phage M13-G1b inactivation as a function of time, fitted with the model.

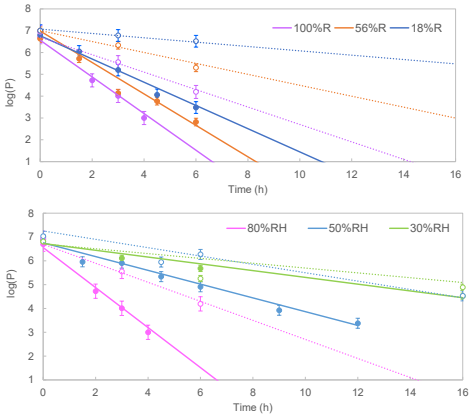

Figure 1. Phage M13-G1b concentration as a function of time (log(P) vs time, with P in [PFU/mL]), fitted with the proposed kinetic model, for assays with coated (experiments: fill dotes, model: fill line) and uncoated (experiments: empty dotes, model: dotted line) plates; varying the incident radiation flux (80% RH, top chart) and relative humidity (100% R, bottom chart).

Table 1. Estimated parameters obtained from the fitted kinetic model and the minimizing the RMSE for both tested phages and assays with uncoated and coated plates with photocatalytic paint.

	J-1		M13-G1b	
	Uncoated	**Coated**	**Uncoated**	**Coated**
k_1 [$cm^{2\,k2}\ s^{k2}\ Einstein^{-k2}\ h^{-1}$]	3.52×10^{4}	2.71×10^{4}	2.33×10^{7}	3.74×10^{17}
k_2 [-]	0.36	0.33	0.82	0.27
k_3 [$mol\ cm^{-3}$]	2.77×10^{-3}	2.00×10^{-3}	2.77×10^{-3}	1.31×10^{-2}
k_4 [$cm^3 mol^{-1}$]	2.47×10^{3}	3.95×10^{3}	2.30×10^{3}	2.94×10^{3}
RMSE [%]	1.90	6.12	2.59	3.16
Total RMSE [%]	4.43		2.98	

Conclusions

The results showed that as the radiation flux increased, the photocatalytic inactivation turned higher, reaching the highest log orders/h reduction working with 100% irradiation. Regarding to the relative humidity, as it increases, phage reduction was greater, reaching the maximum log orders/h reduction working with 80% relative humidity. A good agreement between the model predictions and experimental data was observed. The proposed model would be useful to predict phage inactivation under others combinations of levels of the studied operating conditions, different from those tested in this work.

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