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Towards Efficient Disinfection in Heterogeneous Bacterial Populations: Model-Based Multi-Objective Optimisation of Dosing Strategies

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Resumen

La desinfección periódica es una práctica fundamental en higiene clínica, industrial y ambiental para limitar la transmisión de patógenos. Estos protocolos crean presiones selectivas que promueven, potencialmente, resistencia y tolerancia. La persistencia de compuestos como el cloruro de benzalconio en los entornos tratados pueden favorecer poblaciones adaptadas con el consiguiente coste ambiental y económico. Esto evidencia la necesidad de estrategias de dosificación más eficientes. Este trabajo propone un enfoque de optimización multiobjetivo para el diseño de protocolos de desinfección que minimicen la población de bacterias y, a su vez, el uso máximo y total de desinfectante. El enfoque considera dinámicas de competencia entre una cepa salvaje y una mutante con mayor supervivencia a la desinfección y crecimiento más lento. A través de la identificación de compromisos bacterianos de crecimiento y supervivencia, junto con las condiciones para su selección o eliminación, nuestro método establece una base sistemática para una desinfección eficiente que equilibra eficacia e impacto ambiental y económico.

Palabras clave: Modelado matemático, Tolerancia y resistencia antimicrobiana, Desinfección periódica, Compromiso entre crecimiento y supervivencia bacteriana, Optimización multi-objetivo

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Abstract

Periodic disinfection is a fundamental practice in clinical, industrial, and environmental hygiene to limit pathogen transmission. These protocols create dynamic selective pressures by alternating phases of disinfectant exposure and bacterial regrowth, potentially promoting resistance and tolerance. The persistence of compounds such as benzalkonium chloride in treated environments, along with suboptimal concentrations, may favour adaptive populations while increasing environmental and economic costs, underscoring the need for more efficient dosing strategies. This work presents a model-based multi-objective optimisation approach to design disinfectant protocols that minimise bacterial populations while reducing both maximum and total disinfectant use. The approach accounts for competitive dynamics between a wild-type bacterial strain and a mutant with increased disinfection survival at the expense of reduced growth, enabling comparison of optimal dosing strategies depending on the duration of growth periods. By identifying trade-offs and conditions driving bacterial selection or eradication, our approach provides a systematic basis for efficient disinfection strategies that balance efficacy with lower environmental and economic impact.

Keywords: Mathematical modelling, Antimicrobial resistance and tolerance, Periodic disinfection, Bacterial growth-survival trade-offs, Multi-objective optimisation

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1. Introduction

Periodic disinfection is widely used across hospital Warren et al. (2022), industrial Knight and Craven (2010) and water treatment Hwang et al. (2012) settings to prevent the spread of pathogens. These interventions are generally intended to reduce microbial contamination before stable surface colonisation and biofilm establishment occur. Disinfection protocols, as with antibiotic therapies in clinical practices, typically occur as cyclic, highly dynamic processes. These often combine periods of disinfectant addition, nutrient exposure of the treated environment—e.g., food-contact surfaces Knight and Craven (2010)—and subsequent bacterial regrowth, generating dynamic selective pressures that may promote adaptation (Nordholt et al., 2021).

The increased disinfection survival of resistant or tolerant strains often entails a fitness cost in bacterial growth (Melnyk et al., 2015), creating a selective disadvantage relative to faster-growing, more susceptible competitors during growth in the absence of disinfectant (Martínez-López et al., 2026b). This disadvantage can be exploited to optimise periodic disinfection protocols. Such an optimisation approach is inherently multi-objective, since the aim is not only to minimise bacterial populations but also to achieve the lowest effective treatment in terms of disinfectant use. This is particularly relevant for quaternary ammonium compounds such as benzalkonium chloride, relatively stable chemicals widely used as disinfectants. Suboptimal concentrations of such compounds can arise in areas with incomplete coverage or after dilution, potentially favouring the selection of adaptive subpopulations (Holah et al., 2002; García and Cabo, 2018). After treatment, antimicrobial residues may persist or be discharged into the environment, thereby imposing selective pressures that potentially promote tolerance, resistance or cross-resistance to other compounds, including clinically relevant antibiotics. Therefore, reducing disinfectant use during treatment can significantly help lower environmental impact, limit tolerance and resistance selection, and reduce economic costs.

Although research on mathematical modelling of resistance and tolerance dynamics during antimicrobial protocols (using not only disinfectants, but also antibiotics, preservatives, and other chemicals) has increased substantially over the years, relatively few studies use model-based optimisation to design optimal protocols. Most of them focus on clinical applications, aiming to design best dosing strategies to reduce an indicator of bacterial viability at the end of treatment—bacterial load (Paterson et al., 2016) or, in stochastic models, treatment failure rate (Ochoa et al., 2020) or extinction probability (Molina-Hernández et al., 2026)—while minimising cumulative antibiotic use, and considering constraints in antibiotic peaks reflecting toxicity limits for the host. Other works also optimised non-antimicrobial methods, namely bacteriophages (viruses infecting bacteria), to treat patients affected by susceptible and resistant strains, where the total amount of applied bacteriophages was also considered an objective to minimise risks to the patient (Dere et al., 2025).

However, mathematical models explaining within-host bacterial dynamics differ substantially from those used in disinfection. The unique approach to optimal disinfection in this case focuses on persister dynamics, characterised by a small, non-growing subpopulation that serves as a protection mechanism against disinfection (Cogan et al., 2012). Bacterial competition for growth-limiting resources was thus not considered, as persisters are not allowed to grow, nor the pharmacodynamics describing the dependence of bacterial killing on disinfectant dose. Moreover, the influence of growth-survival trade-offs arising from competitive interactions has not been explored elsewhere in relation to optimise disinfection protocols. These bacterial trade-offs are strongly dependent on the duration of the regrowth periods, seriously affecting the protocol outcome (Martínez-López et al., 2026b).

Note that, most studies minimise both the bacterial population at the end of treatment and total antimicrobial use by transforming the multi-objective problem into a weighted, single-objective optimisation. This approach, though simple, obscures the interpretation of optimal strategies, as these depend strongly on the relative importance assigned to the different objectives. To the authors' knowledge, a model-based optimisation approach based on Pareto optimality has been developed only for periodic antibiotic dosing to treat infected patients (Ochoa et al., 2020) and for finding optimal antibiotic cycling to exploit bacterial collateral sensitivity (Molina-Hernández et al., 2026). However, the use of Pareto optimality to address multi-objective optimisation of periodic disinfection has not been analysed previously.

This work proposes a model-based multi-objective optimisation approach using Pareto optimality to identify best dosing strategies for periodic disinfection. The objectives correspond to minimise a heterogeneous population—formed by a wild-type strain and a mutant with increased survival but decreased growth—at the end of the protocol, while minimising both the maximum per-cycle and total disinfectant use. The maximum per-cycle dose is minimised to account for, e.g., toxicity limits (Choi et al., 2018; Rogov et al., 2020). The mathematical model considers growth-survival trade-offs for the strains arising from competitive interactions and dynamic selective pressures generated by alternating growth and disinfection. The influence of varying the time between disinfectant applications on the optimal dosing strategies is also analysed.

2. The mathematical model

We consider a bacterial population formed by two strains, wild-type (W) and mutant (M), subject to a periodic disinfection protocol; this consists of successive cycles alternating growth periods in the absence of disinfectant—during which the strains compete for growth-limiting resources—and disinfection periods. The duration of the growth ($t_g > 0$) and disinfection ($t_k > 0$) periods remains fixed, whereas the applied disinfectant concentration may vary between cycles. Dilution into fresh medium by a factor $0 < D < 1$ follows each disinfection period to start the next treatment cycle.

We use the following model to describe population dynamics under the periodic disinfection protocol (see details and extension for an arbitrary number of strains in Martínez-López et al. (2026a)). The cell concentrations of W and M are obtained as the solution of the following system of Ordinary Differential Equations (ODEs):

$$\frac{dX_s}{dt} = \left(\mu_s I_g(t) - k_s(C_c) I_k(t)\right) I_e(X^0_{s,c}) X_s, \quad t \in \left(t^0_c, t^f_c\right), \qquad (1)$$

where t denotes time, $X_s = X_s(t)$ are the cell concentrations of strains $s = W, M$, and $t_c^0 = (c-1)(t_g + t_k)$ and $t_c^f = c(t_g + t_k)$ are the initial and final times of the cycle $c \geq 1$.

Each cycle starts with a growth period, during which strains $s = W, M$ grow exponentially at rates $\mu_s > 0$ until the growth period ends or the population reaches the medium's carrying capacity ($K > 0$). In this case, the strains reach the stationary phase due to resource exhaustion and can no longer reproduce for the rest of the period. The time needed for the population to reach the stationary phase at the c-cycle (saturation time t_c^{sat}) is obtained as the solution of the following implicit equation:

$$K = X_{W,c}^0 \exp\left(\mu_W t_c^{sat}\right) + X_{M,c}^0 \exp\left(\mu_M t_c^{sat}\right), \tag{2}$$

which is well-defined ($t_c^{sat} \geq 0$) as long as $X_{W,c}^0 + X_{M,c}^0 \leq K$. The indicator function for exponential growth (I_g) in ODEs (1) is then defined as:

$$I_g(t) = \begin{cases} 1, & t \in \left(t_c^0, t_c^0 + \min\{t_g, t_c^{sat}\}\right), \\ 0, & \text{otherwise.} \end{cases} \tag{3}$$

Growth periods are followed by disinfection, during which the strains $s = W, M$ decay exponentially at rates $k_s(C_c)$ depending on the disinfectant concentration applied at each cycle (C_c). This is modelled through the Hill dependence:

$$k_s(C_c) = k_{max} \frac{C_c^{H_s}}{C_c^{H_s} + EC_{50,s}^{H_s}}, \tag{4}$$

where $k_{max}, EC_{50,s}, H_s > 0$ are the typical Hill parameters—maximum kill rate, half-maximal inhibitory concentrations, and Hill coefficients, respectively. The indicator function for the disinfection periods (I_k) in ODEs. (1) is defined as:

$$I_k(t) = \begin{cases} 1, & t \in \left(t_c^0 + t_g, t_c^f\right), \\ 0, & \text{otherwise.} \end{cases} \tag{5}$$

The last indicator (I_e) accounts for the biological constraint that strains $s = W, M$ cannot grow or die along the c-cycle if they are extinct. This is modelled in ODEs (1) through an extinction limit ($X_e > 0$) for the cell concentrations at the start of each cycle—denoted as $X_{s,c}^0$—using the expression:

$$I_e(X_{s,c}^0) = \begin{cases} 1, & X_{s,c}^0 \geq X_e, \\ 0, & \text{otherwise.} \end{cases} \tag{6}$$

The initial conditions for ODEs (1) at the c-cycle are (except when $c = 1$) the concentrations of strains $s = W, M$ at the end of the disinfection period of cycle $c-1$, following the dilution step. Dilution is assumed to simultaneously remove the disinfectant from the population and replenish growth-limiting resources (Martínez-López et al., 2026b). For $c = 1$, the initial conditions of ODEs (1) are the strains' inocula, X_s^0 ($X_e < X_s^0 < K$). Then, the initial conditions at the c-cycle are:

$$X_s(t_c^0) = X_{s,c}^0 = \begin{cases} X_s^0, & c = 1, \\ DX_s\left(t_{c-1}^{f-}\right), & c > 1. \end{cases} \tag{7}$$

Finally, the mutant strain M shows increased disinfection survival compared to the wild-type W, at the expense of a fitness cost during growth in the absence of disinfectant. The survival advantage conferred by the mutation on strain M is modelled as a decreased kill rate—$k_M(C) < k_W(C)$, for any disinfectant concentration $C > 0$—whereas the fitness cost is expressed in terms of decreased growth rate, $\mu_M < \mu_W$. The mutant M is thus favoured by disinfection periods over the wild-type W, whilst W has the selective advantage during competition for growth-limiting resources, as it grows faster.

3. Multi-objective optimisation problem

To explore efficient strategies for periodic disinfection on heterogeneous bacterial populations, we propose a model-based Multi-Objective Optimisation (MOO) approach to minimise the population at the end of treatment, while reducing the maximum per-cycle and total disinfectant use. Minimising the maximum disinfectant dose applied in each cycle can account for toxicity limits (Choi et al., 2018; Rogov et al., 2020). In contrast, the total applied disinfectant is minimised to avoid misuse or overuse that ultimately results in environmental impact, while saving unnecessary economic costs. The decision variables for the MOO problem are then the disinfectant doses $\{C_c, 1 \leq c \leq n_c\}$ to apply in $n_c > 1$ cycles (duration of the periodic protocol), where the objectives are:

- **Objective 1.** Minimise the maximum per-cycle applied disinfectant dose, $C_{max} = \max\{C_c, 1 \leq c \leq n_c\}$.
- **Objective 2.** Minimise the total disinfectant concentration applied, $C_T = \sum_{c=1}^{n_c} C_c$.
- **Objective 3.** Minimise the total bacterial concentration at the end of the protocol, $X_W\left(t_{n_c}^f\right) + X_M\left(t_{n_c}^f\right)$.

Mathematically, this MOO problem can be expressed as:

$$\min_{\{C_c, 1 \leq c \leq n_c\}} J := \left[X_W\left(t_{n_c}^f\right) + X_M\left(t_{n_c}^f\right),\ C_{max},\ C_T\right] \in \mathbb{R}^3, \tag{8}$$

subject to:

$$\begin{cases} \text{The mathematical model in Eqs. (1)-(7),} \\ C_c \geq 0,\ 1 \leq c \leq n_c. \end{cases}$$

The ϵ-constraint method (Ehrgott and Ruzika, 2008) is selected to solve the MOO problem in Eq. (8), so that two of the objectives are transformed into inequality constraints with upper bounds ϵ_1 and ϵ_2. This procedure transforms the MOO problem into a Single-Objective Optimisation (SOO) problem that must be solved for different values of the pair (ϵ_1, ϵ_2). Then, considering **Objectives 1** and **2** as inequality constraints, the MOO problem in Eq. (8) results in the following SOO problem:

$$\min_{\{C_c, 1 \leq c \leq n_c\}} J := X_W\left(t_{n_c}^f\right) + X_M\left(t_{n_c}^f\right) \in \mathbb{R}, \tag{9}$$

subject to:

$$\begin{cases} \text{The mathematical model in Eqs. (1)-(7),} \\ C_{max} \leq \epsilon_1, \\ C_T \leq \epsilon_2, \\ C_c \geq 0,\ 1 \leq c \leq n_c. \end{cases}$$

A hybrid—combination of local and global—optimisation algorithm is used to solve the SSO problem in Eq. (9) and

avoid convergence to local, sub-optimal solutions. In this regard, the global meta-heuristic population-based algorithm Enhanced Scatter Search (ESS) (Egea et al., 2007) is applied in combination with MATLAB's Nelder-Mead simplex method *fminsearch*. Convergence of ESS to optimal solutions is fast because the mathematical model underlying the MOO approach (Eqs. (1)–(7)) can be solved analytically, even though the saturation times over the cycles lack closed-form expression (Martínez-López et al., 2026a).

4. Results and discussion

The proposed model-based MMO approach is illustrated through the experiment performed in Martínez-López et al. (2026b), consisting of periodic disinfection with benzalkonium chloride (BAC) on a heterogeneous population formed by two *Escherichia coli* strains: a wild-type (W) and a tolerant mutant (M). The mutant strain M showed an increase in survival fraction of $8 - 725$ times (depending on applied BAC concentration) relative to W after disinfection, and an approximately 20% decrease in growth rate due to fitness costs. The parameters for the mathematical model in Eqs. (1)–(7) were extracted from the source publication—except the Hill parameters in Eq. (4) describing disinfectant killing on strains W and M—and are listed in Table 1. We consider $n_c = 5$ cycles for the periodic disinfection protocol, though the approach remains valid for any other $n_c \geq 1$.

Table 1: Parameters and units of the mathematical model in Eqs. (1)–(7).

Parameter	Meaning	Value	Units
t_g	Duration of growth periods	$4, 8, 24$	h
t_k	Duration of disinfection periods	10	min
K	Carrying capacity	5.00×10^8	CFU/mL
X_W^0, X_M^0	Strains' inocula	5.00×10^5	CFU/mL
X_e	Extinction limit	1.67	CFU/mL
D	Dilution factor	1:100	–
μ_W	Growth rate of W	$2.00{\times}10^{-2}$	1/min
μ_M	Growth rate of M	$1.61{\times}10^{-2}$	1/min
k_{max}	Maximum kill rate	7.04	1/min
$EC_{50,W}$	Half-maximal inhibitory conc. of W	6.71×10^1	µg/mL
$EC_{50,M}$	Half-maximal inhibitory conc. of M	7.53×10^1	µg/mL
H_W	Hill coefficient of W	4.00	–
H_M	Hill coefficient of M	4.47	–

Note: CFU = colony-forming units.

The optimal strategies for periodic BAC disinfection on the heterogeneous *E. coli* population are obtained by solving the MOO problem in Eq. (8) with the ϵ-constraint method, as described in Section 3. **Objectives 1** and **2**—minimising the maximum per-cycle and total applied BAC concentrations, respectively—are treated as constraints for the decision variables—BAC doses applied in $n_c = 5$ cycles—of the transformed SOO problem given in Eq. (9). The values considered for the inequality constraint corresponding to **Objective 1** are selected in the range $\epsilon_1 \in [5, 55]$ µg/mL. For each value of ϵ_1, the inequality constraint associated with **Objective 2** is selected within the range $\epsilon_2 \in [\epsilon_1, n_c\epsilon_1]$, with a BAC concentration step of 5 µg/mL. The SOO problem in Eq. (9) is then solved for each pair (ϵ_1, ϵ_2) defining the values for the two constraints. This procedure is performed for different values of the growth time (t_g), given in Table 1, to analyse the influence of this parameter on the optimal BAC profiles.

The optimisation results for the different growth times are summarised in Figure 1. Figure 1A) shows the Pareto surfaces formed by points coloured according to the value of **Objective 3**, which minimises the total bacterial concentration after five cycles of periodic disinfection—the optimal dosing strategies achieving population eradication at the end of the protocol are indicated by pink dots. We can observe that the maximum per-cycle BAC dose required to eliminate the bacterial population increases with the duration of the growth periods, from 34 to 51 µg/mL, whereas the total BAC concentration to be applied throughout the protocols remains approximately constant (between $49 - 51$ µg/mL). This is the range of BAC concentrations needed to eliminate a homogeneous, wild-type population in a single cycle. The Pareto surface narrows as the growth time increases because bacteria can better compensate for the effects of disinfection, until it collapses to a line for $t_g = 24$ h. In this case, the population has time to regrow to cell concentrations close to the carrying capacity (K) if it is not effectively eliminated in a single cycle, so that the optimal dosing strategies that minimise the maximum per-cycle and total BAC application become the same.

The best BAC dosing strategies that eliminate the heterogeneous bacterial population within five cycles using the lowest per-cycle dose are shown in Figure 1B) (top plots). As the growth period lengthens, the optimal BAC profile shifts from sustained exposure across all cycles to a single high dose applied in the final cycle. For short growth periods ($t_g = 4$h), the mutant M cannot grow fast enough to offset population losses due to disinfection and dilution carried out at the end of each cycle, even in the absence of competition with strain W (see Figure 1B), bottom plots). Under these conditions, strain W remains dominant, and the optimal strategy is to prevent its expansion by applying the minimum per-cycle BAC dose required to achieve complete elimination in five cycles. In contrast, as the growth period increases ($t_g = 8$h and 24 h), higher BAC doses are needed in each cycle to eradicate the population, and the optimal dosing strategy shifts BAC application toward later cycles; this allows strains W and M to compete during the early stages of the protocol, reducing the bacterial population through competition and thereby lowering the per-cycle required BAC dose.

Conversely, Figure 1C) (top plots) shows the optimal disinfection profiles that achieve population eradication while minimising total BAC use throughout the protocol. In this case, the best dosing strategy is to apply a single high-BAC dose, regardless of the duration of the growth periods. Nevertheless, the timing of this unique dose depends on the growth time: it is applied in the first cycle when growth periods are short ($t_g = 4$ h), but shifts to the final cycle when longer regrowth periods are allowed between disinfection steps ($t_g = 8$ h and 24 h). Again, the optimal dosing strategy seeks to prevent early propagation of the wild-type W when $t_g = 4$ h, while the mutant strain M is effectively removed through successive disinfection and dilution steps. When strains are allowed to regrow for longer periods ($t_g = 8, 24$ h), the optimal profile delays BAC application to the final cycle, allowing bacterial competition to reduce the abundance of strain M before complete population eradication is achieved with the min-

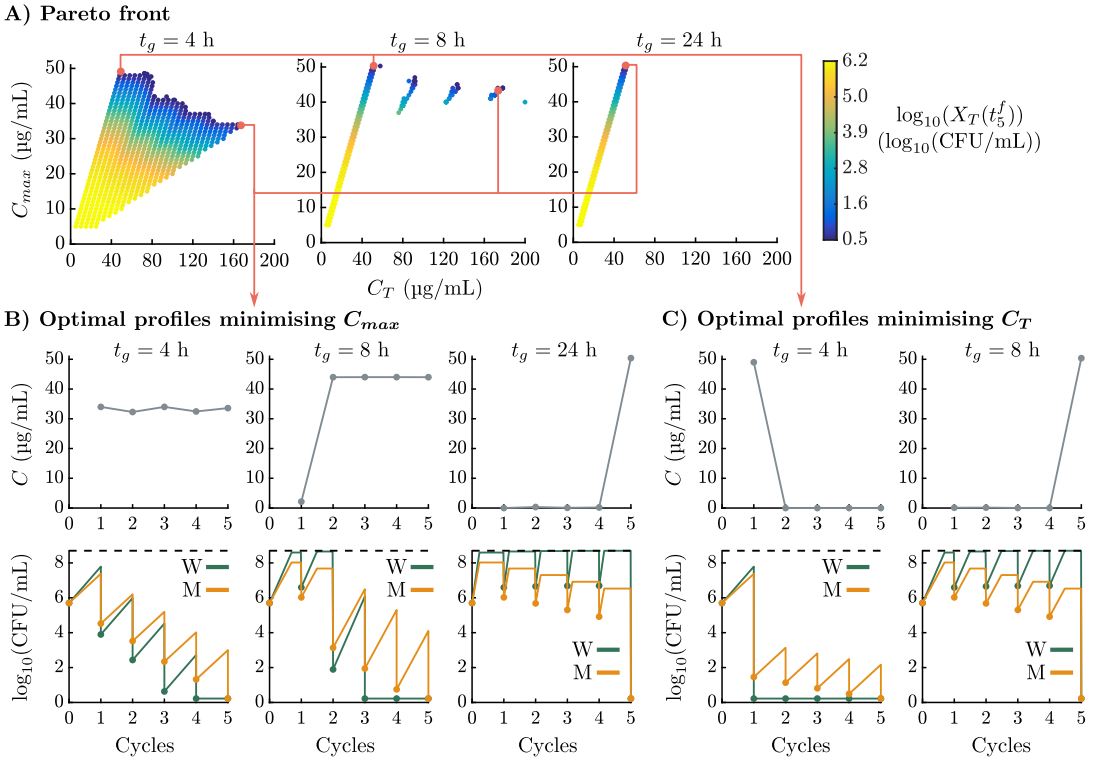

Figure 1: Results of optimising periodic BAC disinfection using the proposed model-based MOO approach, comparing three different values for the growth time: t_g = 4h, 8h, and 24h. A) Pareto fronts, where colour represents **Objective 3**, i.e., minimising the bacterial population. The optimal dosing strategies achieving population eradication at the end of the protocol are indicated by pink dots; B) Optimal BAC profiles that eradicate the population while minimising C_{max}, and resulting population dynamics; C) Optimal BAC profiles that eradicate the population while minimising C_T, and resulting population dynamics.

imum effective BAC concentration (Figure 1C), bottom plots).

The best strategy for periodic disinfection that eliminates the bacterial population and minimises total BAC use is not shown in Figure 1C) for t_g = 24 h, as it coincides with the profile minimising per-cycle BAC application in Figure 1B). As discussed previously, the bacterial population always reaches the carrying capacity (K) at the end of growth periods under these conditions, except when the strains are below the extinction limit (X_e) at the start of the period. Then, disinfection efficacy depends on the population composition at the end of the preceding growth period, specifically on which strain dominates the population near K. Since complete eradication means reducing the strains' concentration below X_e, the required per-cycle BAC doses are high, and the mutant strain M needs substantially higher doses to be eliminated than the wild-type W. Then, the optimal strategy exploits bacterial competition to successively reduce the concentration of strain M in the stationary phase at the end of growth periods; a high BAC dose applied in the last cycle can then eradicate the remaining population using a concentration close to that required for a stationary wild-type W population alone. This point is further illustrated in Figure 2, which shows the result of periodic disinfection when a BAC dose of 51 µg/mL—the same concentration that achieves population eradication in Figure 1B) for a growth time of t_g = 24h—is applied across all cycles.

Such concentration is sufficient to efficiently reduce the wild-type population W below the extinction limit after a single BAC application; however, suppressing the competitive pressure exerted by strain W leads to the survival of the mutant strain M. Because the growth time is large and in the absence of competition with strain W, the mutant M subsequently reaches the carrying capacity during regrowth periods, exceeding the concentration attained during the first cycle. Therefore, the same disinfectant concentration used by the optimal BAC strategy to eradicate the total population selects for the mutant strain under this scenario.

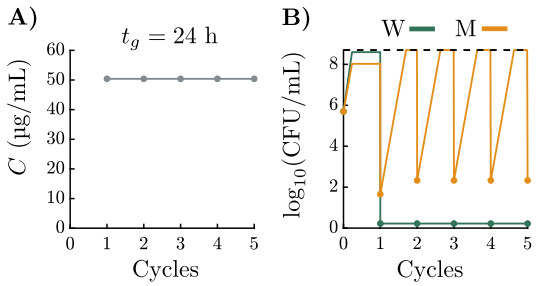

Figure 2: A) Periodic disinfection profile consisting of applying 51 µg/mL BAC in all cycles; B) Dynamics of the wild-type W and mutant M subpopulations under the BAC profile shown in A).

Future research will integrate phenotypic heterogeneity leading to multimodal time-kill kinetics—e.g., heteroresistance (Martínez-López et al., 2024a)— that are not captured by the current mathematical model. To this end, the proposed model-based MOO approach will be extended with a stochastic birth-death modelling framework using extinction probability as an indicator of protocol success, thereby enabling consideration of the inherent stochasticity of such bacterial populations in optimal design. This is particularly challenging when considering bacterial competition, since the stochas-

tic dynamics for the different subpopulations become highly complex and interconnected (Martínez-López et al., 2024b).

5. Conclusions

This work developed a model-based approach to optimise disinfection protocols consisting of successive cycles alternating growth and disinfectant-exposure periods. The mathematical model considers a heterogeneous bacterial population formed by a wild-type strain and a (resistant or tolerant) mutant with increased survival but a fitness cost during growth in the absence of disinfectant, which generates growth-survival trade-offs determining bacterial selection or eradication. By exploiting dynamic changes in these trade-offs induced by periodic disinfection, the proposed model-based multi-objective optimisation approach determines the disinfectant dose to apply in each cycle for minimising the heterogeneous population at the end of treatment while reducing maximum and total disinfectant use, reflecting different constraints on environmental impact or economic costs.

The proposed approach was applied to optimise periodic disinfection with benzalkonium chloride (BAC) across different scenarios, varying the time between BAC applications (growth time). The results show that growth time plays a critical role in shaping optimal disinfection strategies through bacterial growth-survival trade-offs. The consequences of fitness costs on the mutant strain during growth periods are exacerbated by short growth times, as the wild-type rapidly consumes the available resources, strongly limiting mutant reproduction. This selective pressure, combined with the disinfection and dilution steps, ultimately drives mutant extinction. Strategies that minimise per-cycle BAC dose favour sustained application to efficiently remove the wild-type, whereas those that minimise cumulative BAC use prevent propagation from early cycles. In contrast, optimal dosing strategies for longer growth periods shift toward eliminating the mutant strain, allowing bacterial competition and concentrating disinfectant use in the last cycle. This strategy exploits the mutant's competitive disadvantage, thereby reducing the overall BAC requirement for successful eradication.

The present study provides valuable insights into the design of efficient and adaptive disinfection protocols that balance competing biological and operational objectives, enabling successful bacterial eradication while minimising economic costs and environmental impact, including the release of suboptimal concentrations promoting resistance, tolerance, or cross-resistance. Furthermore, the proposed approach can be readily extended to optimise periodic protocols involving other bactericidal compounds and an arbitrary number of competing strains, allowing the straightforward incorporation of pharmacodynamic models describing bacterial killing as a function of bactericide concentration.

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