Titre: Title:	Uncertainty Sources in the Mechanistic Modeling of Legionella within Building Water Systems
Auteurs: Authors:	Catalina Ortiz, Fatemeh Hatam, & Michèle Prévost
Date:	2024
Type:	Article de revue / Article
Référence: Citation:	Ortiz, C., Hatam, F., & Prévost, M. (2024). Uncertainty Sources in the Mechanistic Modeling of Legionella within Building Water Systems. Engineering Proceedings, 69(1), 84 (4 pages). https://doi.org/10.3390/engproc2024069084

URL de PolyPublie: PolyPublie URL:	https://publications.polymtl.ca/59393/
Version:	Version officielle de l'éditeur / Published version Révisé par les pairs / Refereed
Conditions d'utilisation: Terms of Use:	CC BY

Titre de la revue: Journal Title:	Engineering Proceedings (vol. 69, no. 1)
Maison d'édition: Publisher:	MDPI
URL officiel: Official URL:	https://doi.org/10.3390/engproc2024069084
Mention légale: Legal notice:	

engineering proceedings

Proceeding Paper

Uncertainty Sources in the Mechanistic Modeling of *Legionella* within Building Water Systems †

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† Presented at the 3rd International Joint Conference on Water Distribution Systems Analysis & Computing and Control for the Water Industry (WDSA/CCWI 2024), Ferrara, Italy, 1–4 July 2024.

Abstract: Predicting *Legionella* concentrations reaching users through building water systems requires a comprehensive water quality modeling approach. We integrate various frameworks and data to test the effect of nutrient availability, temperature, chlorine, and biofilm interactions in modeling *Legionella*. We show that neglecting biofilm detachment underestimates concentrations up to 5.5 logs, while including it increases estimates by 4.2 logs. This study identifies critical factors and uncertainty sources for characterizing the *Legionella* fate and transport phenomena within buildings.

Keywords: *Legionella*; EPANET-MSX; mechanistic modeling; building water systems

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Citation: Ortiz, C.; Hatam, F.; Prévost, M. Uncertainty Sources in the Mechanistic Modeling of *Legionella* within Building Water Systems. *Eng. Proc.* **2024**, *69*, 84. https://doi.org/10.3390/engproc2024069084

Academic Editors: Stefano Alvisi, Marco Franchini, Valentina Marsili and Filippo Mazzoni

Published: 9 September 2024

1. Introduction

Legionella propagation through aerosolized water from fixtures such as showerheads or faucets are an exposure pathway to waterborne disease in building water systems (BWS) [1]. Risk assessment models have been instrumental in estimating average infection risks under various exposure scenarios. Nevertheless, predicting spatiotemporal variations is essential for effective risk evaluation and development of mitigation strategies. To date, a sole study has used EPANET-MSX to simulate *Legionella* within a BWS [2]. Hydraulic-water quality models were fitted to 1-year data from a monitored water-efficient residence. The calibrated models show higher concentrations as consequences of increasing water stagnation, aligning to the expected trend of *Legionella* to proliferate with higher water age [1]. These results underscore the potential of EPANET-MSX to forecast exposure to *Legionella*. However, calibrated kinetic constants are only applicable to the particular system conditions. Considering nutrient sources, temperature, disinfectant, and biofilm interactions is suggested to improve the predictive capacity for *Legionella* [2]. Thus, in this study we test mechanistic modeling frameworks that incorporate these factors while pinpointing uncertainty sources and knowledge gaps.

2. Materials and Methods

2.1. Case Study

The case study residence hot and cold BWSs were built independently in EPANET 2.2 with water usage patterns in the kitchen sink and 2nd floor bathroom sink and shower [2]. EPANET-MSX files were created for every season using data from 58 discrete samplings as the boundary conditions in the heater and service line [2]. The .inp and .msx files were shared by the authors, jointly with field water quality data including temperature, dissolved oxygen (DO), free chlorine (FCL), heterotrophic plate counts (HPC), *Legionella* spp. (*L*spp) measured by qPCR in gene copies (GC)/L, and dissolved organic carbon (DOC). The focus in this investigation is summer since the highest *L*spp detection occurred during this season, totaling 12 data points from 25 June to 31 July 2018.

2.2. Modeling Frameworks

Microbial proliferation is described by four periods: initial lag, exponential growth, stationary phase, and death. The calibrated models employed a first-order growth equation constrained by the maximum observed concentrations [2]. Here, the net growth reaction of *L*spp in bulk water ($C_{LEG,b}$) follows Equation (1), where μ and k_d are the growth and mortality rates. Four mechanistic models are tested by sequentially incorporating the effect of nutrients, temperature, chlorine, and interaction with biofilm (Table 1).

$$\frac{dC_{LEG,b}}{dt} = (\mu - k_d) \cdot C_{LEG,b} \tag{1}$$

Table 1. *Legionella* spp. mechanistic modeling frameworks sequentially including the effect of nutrient limitation, temperature, chlorine, and biofilm detachment.

Model	μ	k_d	+
Nutrient-limited	$\mu_{max} \cdot \frac{O}{K_O + DO} \cdot \frac{BDOC}{K_s + BDOC}$	-	-
Temperature	$\mu_{max} \cdot \frac{BDOC}{K_s + BDOC} \cdot \frac{OD}{K_O + OD} \cdot \exp\left(-\frac{(T - T_{opt})^2}{0.2 * (T_{min} - T_{opt})^2}\right)$	$k_{d,temp}$	-
Chlorine		$k_{d,temp} + FCL/Ct$	-
Biofilm			$k_{det} \cdot C_{LEG,w} \cdot Av$

$\mu_{max} = 3.85 \times 10^{-5}$ s^{-1}: maximum growth rate from growth curves for a reference *Lp* strain grown in a minimum-nutrient medium [3]. $K_s = 0.195$ mg/L [4] and $K_O = 0.2$ mg O_2/L [5]: BDOC and DO half saturation coefficients. $T_{opt} = 37$ °C, $T_{min} = 10$ °C. $Ct = 321$ mg·s/L [6]. $k_{det} = 1.0 \times 10^{-3}$ s^{-1} or 2.17×10^{-2} s^{-1} [7] only applied to flowing pipes, $C_{LEG,w} = 39$ GC/cm^2 [7], Av = area per unit volume.

Nutrient limitation: The Monod equation is used to limit the maximum *L*spp growth according to the substrate concentration, which is considered as DO and a fraction of DOC because the biodegradable part (BDOC) was not measured. Most parameters are taken for *Legionella pneumophila* (*Lp*) because no specific parameters fit to all species. Indeed, modeling only *Lp* would be more pertinent given its association with infections [1], yet no *Lp* was found in the case study network. K_O and K_s are approximated to the substrate uptake by heterotrophs [4,5] because no *L*spp data were found. BDOC is modeled using the formulation and parameters from [4], where it reacts with FCL, facilitates bacterial growth, and increases due to cell lysis. Thus, the FCL and HPC calibrated models are utilized [2].

Temperature-dependent growth and inactivation: Temperature (T) is simulated as in [8] using measurements at the service line for the cold system, while a constant 60 °C at the heater in the hot system is used, as required by *Legionella* regulations, since no temperature data were available from the heater outlet. Temperature-dependent μ is computed using a Gaussian curve according to *Lp* growth rates at 18–47 °C [3]. For temperatures over 47 °C, first-order discrete death rates ($k_{d,temp}$) are used for the sum of culturable and viable-and-not-culturable *Lp* cells at 50, 56, 60, 65, and 70 °C [9].

Chlorination: The effect of chlorine is incorporated in k_d as the ratio between FCL and a log reduction Ct value for planktonic *Lp* [6], thus dynamically determining the disinfection rate according to the FCL levels throughout the system.

Biofilm dynamics: Attachment to biofilm is considered negligible since *Lp* growth and detachment predominates in biofilms that have reached steady state [5]. Thus, attached *L*spp concentration $C_{LEG,w}$ is assumed constant throughout the network. The *Lp* detachment rate (k_{det}) fits a two-phase first-order decay because higher release occurs during the first 5 min and is lower for the following water use time [7]. The lower rate is applied to all flowing pipes and the higher rate only to pipes right before fixtures (distal pipes) in EPANET-MSX due to the inability to differentiate them according to water use time.

3. Results and Discussion

Compared to growth without restricting μ_{max} ("Maximum growth" as referenced in Figure 1), the Monod formulation effectively limits *L*spp proliferation as a function of BDOC and DO. The nutrient-limited model represents the highest concentrations among the growth models implemented, being on average 1.2 logs higher in hot-water fixtures but 1.4 logs lower in cold-water fixtures compared to measured data. This might be due to restricted growth by low carbon levels entering from water mains, although the real DOC measurements were higher due to leaching from PEX pipes [2], which was not considered in the model. The hot system simulations align with expectations since *L*spp inhibition occurs when water temperature exceeds 50 °C during water use, while stagnated water in distal pipes reaches temperatures conductive to growth (20–45 °C) [1] within 60–70 min. Interestingly, disinfection by low chlorine residuals in hot fixtures (<0.01 mg/L) control excessive growth during prolonged stagnation/low water usage (Figure 1a). Temperature in the cold system has a negligible impact because it is stable and consistently within the growth range, leading to identical results for nutrient-limited and temperature models (Figure 1b). Average chlorine of 0.24 mg/L entering from the water mains results in high residuals at fixtures and substantial *L*spp inactivation across the cold network.

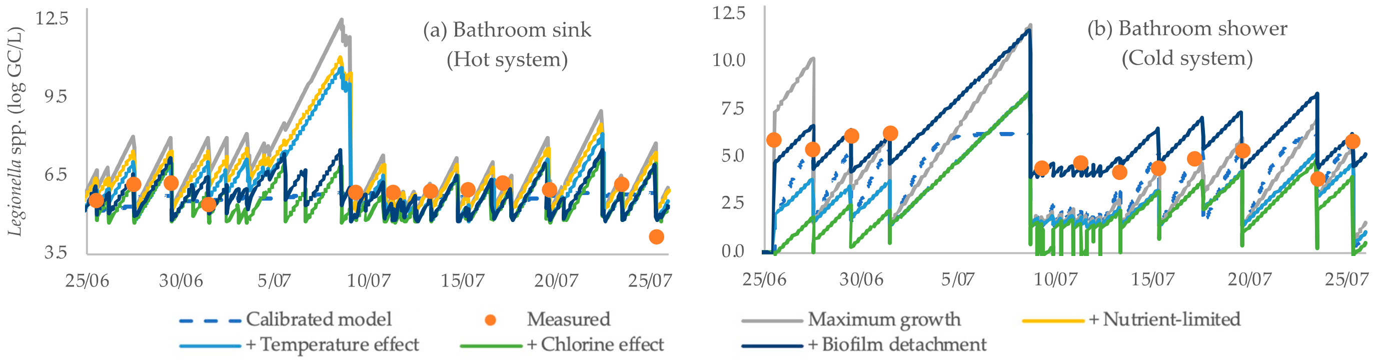

Figure 1. Measured, calibrated [2], and simulated *Legionella* spp. through sequential inclusion of nutrient limitation, temperature effect, chlorine effect, and biofilm detachment at (**a**) bathroom sink (hot system) and (**b**) bathroom shower (cold system).

Before including biofilm detachment, predictions are up to 1.1 logs lower in hot fixtures (Figure 1a) and 5.5 logs in cold fixtures (Figure 1b) than measured *L*spp. Notably, the models were calibrated assigning observed data to cold water in the shower because stagnated water reached ambient temperature quickly [2]. Modeling hot and cold water mixing would be more accurate, especially for showerheads where more aerosols are produced and there is a higher infection risk. The model including biofilm increases predictions by 4.2 logs in the cold system as a consequence of a large surface area from where biofilm detaches during water usage, approximating better elevated concentrations in first draw samples which have been attributed to detachment [10]. Yet, the model still fails to accurately predict observed *L*spp levels, implying that improved estimations require more detailed modeling frameworks and finer parameter calibration.

4. Conclusions

Employing a mechanistic framework offers a practical method to expand the application of modeling endeavors across various systems. However, mathematically describing all chemical, physical, and microbial processes influencing *Legionella* persistence within BWSs is inherently challenging. Through the comparison of mechanistic models incorporating the effect of incoming nutrients, temperature, chlorine, and biofilm detachment, we demonstrate that reasonable approximations can be achieved. Yet, incorporating additional factors is still necessary for improved simulations, such as carbon leaching from plumbing pipes, hot–cold water mixing, chlorine diffusion into biofilms, and interaction with protozoan hosts providing nutrients and protection to intracellular *Legionella*. Ultimately, as the primary goal is to quantify the overall risk of exposure, accurately predicting elevated *Legionella* concentrations as a consequence of detachment necessitates determining and calibrating biofilm model parameters, especially attached concentration and detachment rates in distal devices that offer larger surface-to-volume ratios.

Author Contributions: Conceptualization, C.O., F.H. and M.P.; methodology, C.O., F.H. and M.P.; software, C.O. and F.H.; validation, C.O., F.H. and M.P.; formal analysis, C.O., F.H. and M.P.; investigation, C.O., F.H. and M.P.; resources, F.H. and M.P.; data curation, C.O., F.H. and M.P.; writing—original draft preparation, C.O.; writing—review and editing, F.H. and M.P.; visualization, C.O., F.H. and M.P.; supervision, F.H. and M.P.; project administration, M.P.; funding acquisition, M.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Industrial Chair on Drinking Water at Polytechnique Montréal—NSERC-ALLRP 560764-20.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

Acknowledgments: We extend our gratitude to Juneseok Lee (Manhattan College, Riverdale, NY) for sharing the original data sets, reviewing, and providing feedback on the results of this study.

Conflicts of Interest: The authors declare no conflicts of interest.

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