Adopted: 5 December 2024

DOI: 10.2903/j.efsa.2025.9173

efsa JOURNAL

SCIENTIFIC OPINION

Microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVH). Part 2 – A dynamic mass balance model for handling and processing operations in ffFVH using water

EFSA Panel on Biological Hazards (BIOHAZ) | Ana Allende | Avelino Alvarez-Ordóñez | Valeria Bortolaia | Sara Bover-Cid | Alessandra De Cesare | Wietske Dohmen | Laurent Guillier | Lieve Herman | Liesbeth Jacxsens | Lapo Mughini-Gras | Maarten Nauta | Jakob Ottoson | Luisa Peixe | Fernando Perez-Rodriguez | Panagiotis Skandamis | Elisabetta Suffredini | Jen Banach | Bin Zhou | Maria Teresa da Silva Felício | Laura Martino | Winy Messens | Angela Botteon

Correspondence: biohaz@efsa.europa.eu

The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

Abstract

A dynamic mass balance model was developed to simulate contamination dynamics in the process water of fresh and frozen fruits, vegetables and herbs (ffFVH) during processing and handling operations. The mass balance relates to the flux of water and product in a wash tank and the number of microbial cells released in the water, inactivated by the water disinfectant or transferred from the water back to the product. Critical variables describing microbial dynamics in water are: (i) the chemical oxygen demand (COD), as an indicator of the concentration of organic matter; (ii) free chlorine (FC) and particularly its antimicrobial fraction, hypochlorous acid (HOCl); and (iii) the microbial population levels. Model parameters include: (i) the dilution rate of the process water, representing the speed of system saturation, equal to the water flux divided by the tank volume; (ii) the transfer rates of total bacterial counts (TBC) and COD from product to water; and (iii) the specific inactivation rate of microorganisms due to HOCl. The protective effect of COD on microbial cells against FC is encompassed in the inactivation rate. HOCl is expressed as a function of temperature, pH and total chlorine. The model can simulate '*what if scenarios*', based on user-defined process-specific and product/microorganism-specific parameters through a web R-based application. This model can help food business operators when selecting intervention strategies and conditions to maintain the microbiological quality of the process water or identify conditions that represent poor or proper water management practices. Testing alternative model structures and collecting data about operational conditions of handling and/or processing operations, microbial dynamics and the magnitude of the product-specific protective effect on microorganisms are recommended to improve the application of the model.

KEYWORDS

contamination dynamics, dynamic model, fit-for-purpose water, inactivation dynamics, industry, mathematical model, operational monitoring, water disinfection, water quality, water safety

CONTENTS

Abstract....1
Summary....3
1. Introduction....5
 1.1. Background and Terms of Reference as provided by the requestor....5
 1.2. Additional information....7
 1.3. Interpretation of the Terms of Reference....7
2. Data and Methodologies....8
 2.1. Data....8
  2.1.1. Data derived from the literature....8
  2.1.2. Data derived from outsourced activities....8
  2.1.3. Data synthesis....9
 2.2. Methodologies....9
  2.2.1. Model fitting....9
 2.3. Uncertainty analysis....9
3. Assessment....9
 3.1. Generic model structure....10
  3.1.1. The impact of COD on the antimicrobial efficacy of low FC concentrations....11
  3.1.2. Updated model structure for the needs of the mandate....12
 3.2. Fitting the water phase model to the industrial data....14
 3.3. Model structure for simulating different operational scenarios....16
 3.4. Conditions for model use and uncertainty analysis....18
4. Conclusions....18
5. Recommendations....19
Abbreviations....19
Acknowledgements....20
Requestor....20
Question number....20
Copyright for non-EFSA content....20
Panel members....20
References....20

SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Biological Hazards (BIOHAZ) to provide a scientific opinion on the microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVH), to provide guidance on the use of water in the production of ffFVH and to describe the establishment of microbiological requirements for water quality and the available prevention and control measures that can be implemented to maintain the appropriate microbiological quality of the water. In particular, the Panel was asked: (1) to describe the microbiological hazards associated with the use of water in post-harvest handling and processing operations of ffFVH and the routes and rates of contamination of the water and the ffFVH; (2) to describe specific intervention strategies (i.e. water disinfection treatments, water replenishment, good hygiene practices, etc.) needed to ensure the appropriate microbiological quality requirements of water, used for post-harvest handling and processing operations of ffFVH, taking into account their impact on the physiological state of the microbiological hazards present in the water; and (3) to describe relevant parameters to assess the appropriate microbiological quality requirements of water used for post-harvest handling and processing operations of ffFVH.

The mandate includes five outputs (scientific opinions). The Part 1 opinion (EFSA BIOHAZ Panel, 2023) contains the literature review and analysis of the outbreak data and stakeholder questionnaire. The Part 2 opinion contains a summary of the development of a dynamic mass balance model for processing operations using water in ffFVH. This Part 2 opinion describes the dynamic mass balance model with a link to data generated from the EFSA outsourced activities described in Gil et al. (2025). These data were analysed to understand the industrial practices followed by the industrial collaborators included in this tender, and the relevant outputs were used to address the TORs. Parts 3, 4 and 5 Opinions focus specifically on the fresh-whole, fresh-cut and frozen FVH sectors, respectively (EFSA BIOHAZ Panel, 2025a, 2025b, 2025c).

This Part 2 scientific opinion (SO) refers to mathematical models that can be used to simulate the contamination dynamics of process water under different scenarios, for example, (i) where water disinfection is not applied, (ii) considering the inactivation dynamics of different water disinfection treatments based on the ability of chlorine to maintain the microbiological quality of process water, as well as (iii) considering the impact of water refilling or replenishment. Based on a comprehensive review of available models in the literature for simulating the contamination dynamics of different handling and/or processing operations involving water, (EFSA outsourced activities – Gil et al., 2025) it was concluded that most models simulate batch processes in a tank (e.g. dumpling, washing, rinsing) and do not consider the influx and efflux of water and product throughout the handling or processing operation. This deviates from current practices in the industrial settings. Therefore, the current SO presents the development of a new, generic, dynamic mass balance model. This model consolidates and extends upon those available models by encompassing the necessary features for assessing the impact of different intervention strategies on the dynamics of microbial contamination in water. To do that, the parallel changes of organic load and microbial contamination reviewed in the Part 1 opinion (EFSA BIOHAZ Panel, 2023) were encompassed in the new model.

The contamination rate in the model is defined as the change (usually the increase) of the microbial load (N) in process water per unit of time (t). The mass balance between the number of microbial cells released in the water, inactivated by the water disinfectant or transferred from the water back to the product determines whether the value of the contamination rate is positive (contamination) or negative (decontamination). The translocation of microbiological cells between product and water is a continuous bi-directional process with cells present in the water becoming attached to the ffFVH, or detached from ffFVH, transferred to water, inactivated or not by water disinfection treatments and re-attached onto ffFVH from the contaminated water. It is quantified with the term '*transfer coefficient*', defined as the ratio of cells in the donor (product) over the recipient (water) when the transfer occurs from product to water or the inverse ratio when the transfer of cells takes place from water to product.

The variables identified as the most critical in describing microbial dynamics in water were: (i) the chemical oxygen demand (COD, mg/L), which is an indicator of the concentration of organic matter in the process water; (ii) free chlorine (FC, mg/L), which depends on the total chlorine addition and inactivates the microorganisms in the water and partly on the product surface but is quenched by the increasing levels of organic matter, represented by COD, forming combined chlorine; (iii) amino acids (AA) as an additional agent that determines the chlorine demand in water since their degradation kinetics are affected by chlorine concentration; (iv) the microbial population density in water (X_w in CFU/100 mL); and (v) the microbial population density on the product surface (X_p, CFU/cm^2). The COD tends to increase during processing as organic matter is shed from FVH and accumulates in water. FC refers to all active chlorine present in the water as pure chlorine (Cl_2), namely HOCl (hypochlorous acid) and OCl^- (hypochlorite ion). In an aqueous solution, hypochlorous acid partially dissociates into the anion hypochlorite (ClO^-). The antimicrobial capacity of chlorine is determined by the concentration of FC, and its efficacy is highly dependent on the pH of the medium. Total chlorine (TC) is the sum of combined chlorine and FC. Combined chlorine is the FC that has bound itself to a contaminant or organic material and does not have antimicrobial efficacy. For the needs of this mandate, FC, COD and AA concentrations in the water were considered indicators of chlorine demand under the context of organic matter. However, AA were considered to contribute to chlorine demand negligibly and were, thus, excluded from the model development.

The following evidence sources were employed to estimate model parameters and derive the final form of differential equations comprising the dynamic model: (i) data from the literature (Part 1) were used to retrieve information on the transfer coefficients, as well as for FC and COD dynamics for the most relevant microbiological hazards; (ii) data from Abnavi et al. (2021) – who modelled the dynamics of a washing process of iceberg lettuce and red lettuce – were used to

test the suitability of the model for simulating the process and to determine a reference order of magnitude for the model parameters; and (iii) data generated by EFSA outsourced activities (Gil et al., 2025) to characterise the contamination of post-harvest water used at different handling and/or processing operations of ffFVH, with the aim of evaluating the microbiological and physico-chemical quality of the processing water during the operational cycle in industry settings.

The proposed model relies on water chemistry, the average time of the ffFVH in the water tank and the ratio between water and product mass added to the tank. Water chemistry refers to the concentrations of chlorine and frequency of chlorine addition causing microbial inactivation, the changes in COD, its impact on chlorine inactivation and the consecutive outcome of FC on microbial inactivation. The initial generic model included separate differential equations for the dynamics of COD and microbial cells in product and water, as well as for the changes in FC levels in water. Focusing on the dynamics in water, which is the scope of this mandate, a simplified version of the generic model was derived with only three differential equations describing the dynamics in water of COD, FC and microbial contamination (X_w), respectively.

The parameters of this simplified generic model include (i) the dilution rate of the process water (D), (expressed in 1/min) which is equivalent to the water flux (W) divided by the tank volume and practically defines the speed of system saturation; (ii) the transfer rate of total bacterial counts (TBC) from product to water (K_x; CFU/min · kg-product); (iii) the transfer rate of COD from product to water (K_{COD}; mg_COD/min · kg_product); and (iv) the specific inactivation rate of bacteria (α), due to HOCl (1/(min · ppm-HOCl)). Evidence suggests that an increase in COD compromises the efficacy of low doses of FC and practically limits its antimicrobial efficacy, possibly by preventing the contact of FC with cells. In order to quantitatively express this observation, the rate of microbial inactivation (termed 'α') was described with a Michaelis-Mentel-based kinetic expression that includes the parameter K_m, representing the protective effect of COD on cells against FC. Two other key parameters of the model were the transfer rate of cells (K_x) and COD (K_{COD}) from product to water. In the experiments performed in industrial settings by the EFSA outsourced activities (Gil et al., 2025), FC was approximated by linear interpolation between consecutive measurements of FC. In the final 'consolidated' model (number 7 in EFSA outsourced activities – Gil et al., 2025), FC was modelled by a separate (additional) equation considering the degradation of FC by COD, represented by parameter β and the natural decay of FC, at a rate defined by the parameter γ. The impact of temperature on HOCl was accounted for by an expression describing the dependence of pKa (chlorine dissociation constant) on temperature. Levels of HOCl were estimated as a function of pKa, the pH of water and the total chlorine added in a tank through the Henderson–Hasselbalch equation.

The model was implemented in R, and for its numerical simulation, the packages *deSolve* and *pracma* were used. The uncertainty of parameter estimates was determined by fitting the generic model to data generated by the EFSA outsourced activities (Gil et al., 2025) using the cramer-rao method, which is an approximation of Monte Carlo but with a calculation that is less computationally intensive. This consolidated model enables the simulation of various '*what if scenarios*', based on user-defined process-specific and product/microorganism-specific parameters. It could help the food business operators (FBOps) in selecting the most suitable intervention strategies (e.g. the use of chlorine-based disinfectants and/or water refilling or replenishment) and the ranges of specific conditions (e.g. values for FC and rate of water refilling or replenishment) to maintain the microbiological quality of the process water. This model can help inform FBOps about conditions that could represent poor or proper water management practices that favour or prevent the accumulation of microbial contamination in water, respectively. The applicability of the model and its specificity to different handling or processing operations, products and microorganisms highly depends on the resolution of information and data available for the operational characteristics of the processing operation (frequency and amounts of FC added, rate of water refilling, rate of water replenishment, pH and temperature of the water), the product-microorganism interactions and the response of the target organisms to FC. In the absence of detailed inputs for running the model, the model provides generic quantitative information that the FBOps can use to understand the dynamics of the process and identify the main actions to be taken in their water management plan. A web application based on *Rshiny* is available to run the model at https://r4eu.efsa.europa.eu/app/WaterManage4You.

For improving the application of the model, it was recommended to collect data and information pertaining to the operational conditions of handling and/or processing operations, the dynamics of the transfer of microorganisms between products and water, the characterisation of the magnitude of the product-specific protective effect on the microorganisms against the water disinfectant and the update of model parameters, especially the microbial inactivation rate, for other bacteria than TBC and/or other water disinfectants than FC. Furthermore, it is recommended to consider alternative model structures, where different variables to COD can be used to describe the impact of organic matter on FC or other water disinfectants.

1 | INTRODUCTION

1.1 | Background and Terms of Reference as provided by the requestor

There has been an increase in the number of reported outbreaks, cases, hospitalisations and deaths associated with food of non-animal origin (FoNAO) in the EU from 2008 to 2011 (EFSA BIOHAZ Panel, 2013). A tendency has been observed for the outbreaks associated with FoNAO to involve more cases but be less severe than those associated with food of animal origin (Da Silva Felício et al., 2015). Reports by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) show an increasing trend in the implication of foodstuffs of FoNAO on the total burden of foodborne outbreaks in Europe (Machado-Moreira et al., 2019). Moreover, frozen vegetables and fruit have also been associated with major outbreaks (Murray et al., 2017; Soon et al., 2020). There has been an increase in the number of reported outbreaks associated with fresh produce in Europe and North America in recent years (Aiyedun et al., 2021), as well as in the number of fresh and frozen berry-linked viral outbreaks globally (Bozkurt et al., 2021).

Potential sources of contamination of FoNAO attributed to primary production and processing operations have been reviewed by EFSA for various commodities, including fresh and frozen fruit and vegetables (EFSA BIOHAZ Panel, 2011, 2013, 2014a, 2014b, 2014c, 2014d, 2014e, 2020). Water use during harvesting and processing has been identified as an important risk factor for contamination of fruits, vegetables and herbs (FVH). Special attention has been given to microbiological hazards associated with the use of contaminated water during harvest, post-harvest handling and processing, with a special emphasis on cross-contamination during the washing of fresh and frozen fruits, vegetables and herbs (ffFVH) (EFSA BIOHAZ Panel, 2014a). The process water used after blanching vegetables in the deep-freezing industry is also important (EFSA BIOHAZ Panel, 2020). The microbiological quality of the water that comes into contact with ffFVH is an important consideration and should be controlled by an operational prerequisite program (OPRP) activity to avoid cross-contamination (EFSA, 2020; FAO/WHO, 2019).

Large volumes of water are used during harvest and post-harvest handling and processing operations (e.g. washing, rinsing, chilling, cooling, and for general cleaning, sanitation and disinfection purposes), as well as during fresh-cut/freeze value-added operations, distribution and end-user handling of ffFVH. Therefore, most post-harvest processors are in favour of using the same water during many hours of processing operations for sustainability reasons (i.e. to save water and energy) and because, in some regions, access to potable water is limited or very expensive. According to current practices, potable water is used to fill the equipment and tanks during the first hour in the morning, and the water is not replaced for several hours or even several days in some cases, during which large volumes of ffFVH may be processed. Hence, organic matter, microorganisms, including pathogens, and chemical residues can accumulate in the water, causing cross-contamination between batches, and this is a major concern (FAO/WHO, 2019). The quality of water used in post-harvest handling practices, as well as during processing operations of ffFVH, should be monitored and controlled to avoid the accumulation of microbiological hazards.

Most current recommendations specify that post-harvest water that comes in contact with ffFVH and that is not usually subjected to an upstream microbiological inactivation or reduction treatment should be of potable quality during all post-harvest handling operations (FAO/WHO, 2019).

According to Council Directive 98/83/EC, 'water intended for human consumption'[1] shall mean, among others, 'all water used in any food-production undertaking for the manufacture, processing, preservation or marketing of products or substances intended for human consumption unless the national competent authorities (CAs) are satisfied that the quality of the water cannot affect the wholesomeness of the foodstuff in its finished form'.

Annex II – Chapter VII of Regulation (EC) No. 852/2004 on the hygiene of foodstuffs[2] states that recycled water used in processing or as an ingredient is not to present a risk of contamination. It is to be of the same standard as potable water unless the CA is satisfied that the quality of the water cannot affect the wholesomeness of the foodstuff in its finished form.

Additionally, paragraph 7.3.4.3.c in the EU Commission Notice (2017/C 163/01)[3] on guidance documents addressing microbiological risks in fresh fruits and vegetables (fFVs) at primary production through good hygiene indicates that, for primary production and associated operations at the place of such production (harvest and post-harvest), the washing water used should be at least of clean water quality for the initial washing stages. Water used for final rinses has to be of potable quality if the fFVs are often consumed as ready-to-eat (e.g. tomatoes, apples, pears, young carrots, spring onions).

According to paragraph 7.3.4.3.f in the EU Commission Notice (2017/C 163/01) as well as in relevant research papers (FAO/WHO, 2019; Gombas et al., 2017), if water is contaminated during washing and then used to process large quantities of ffFVH, it can be a vehicle for cross-contamination.

In order to avoid cross-contamination of the product due to the use of contaminated water, water disinfection treatments are needed to eliminate, or reduce to an acceptable level, microorganisms of public health concern, but these

[1] Council Directive 98/83/EEC of 3 November 1998 on the quality of water intended for human consumption (OJ L 330, 5.12.1998, pp. 32–54) has been replaced by Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption (recast) (OJ L 435, 23.12.2020, pp. 1–62).

[2] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, pp. 1–23.

[3] European Commission, 2017. Commission notice on guidance document on addressing microbiological risks in fresh fruits and vegetables at primary production through good hygiene (2017/C 163/01). OJ C 163, 23.5.2017, pp. 1–40.

treatments should not have an adverse effect on the quality and safety of the produce. Therefore, regardless of the wash method used, growers and processors should follow good practices that ensure and maintain appropriate water quality.

National rules within Member States exist and may create trade barriers since some prohibit any use of water disinfection treatments in the process water, while such practice is common in others. These risk management decisions are often based on different considerations about the reduced risk associated with microbiological contamination versus the potential added chemical risk associated with their use.

Moreover, concerns may arise regarding the maintenance of the microbiological quality of process water as well as the application of water disinfection treatments by the food business operators (FBOps). The proper operation of water disinfection treatment (e.g. application rate, in-use concentration and residual concentration on ffFVH), as well as the monitoring of the efficacy, has to be conducted properly and safely. As established by FAO/WHO (2019), water quality must be maintained throughout the processing operation, and special attention must be paid to common wash and flume systems and reused water.

Water quality and use in post-harvest handling and processing operations are an increasing concern at the global level, mostly because there is an expected reduction in the availability of water of drinking quality due to climate change (CXC 53-2003).[4] During the 43rd session of the Codex Alimentarius Commission on the Joint FAO/WHO Food Standards Programme in Autumn 2020, the future development of guidelines for the safe use and reuse of water in food production was approved. These guidelines will contain a specific Annex on the use and reuse of water in fresh produce production.

Terms of reference:

The BIOHAZ Panel is asked to issue a scientific opinion on microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVH) to provide guidance on the use of water in the production of ffFVH, the establishment of microbiological requirements for water quality and the available prevention and control measures that can be implemented to maintain the appropriate microbiological quality of the water.

More specifically, EFSA is requested to address the following terms of reference (TORs):

TOR 1 to describe the microbiological hazards associated with the use of water in post-harvest handling and processing operations of ffFVH and the routes and rates of contamination of the water and the ffFVH.

TOR 1.1: Which are the most relevant microbiological hazards associated with the use of water in different post-harvest handling and processing operations for ffFVH?

TOR 1.2: What are the routes of water contamination and the rates of contamination (increase in microbiological and pathogen load over time) for the most relevant microbiological hazards (identified in TOR 1.1.) in the water used in different post-harvest handling and processing operations for ffFVH?

TOR 1.3: Which are the contamination rates (increase in microbiological and pathogen load over time) for the most relevant microbiological hazards (identified in TOR 1.1.) between different ffFVH batches during different post-harvest handling and processing operations using the same water?

TOR 2 to describe specific intervention strategies (i.e. water disinfection treatments, water replenishment rates, good hygiene practices, etc.) needed to ensure the appropriate microbiological quality requirements of water, used for post-harvest handling and processing operations of ffFVH, taking into account their impact on the physiological state of the microbiological hazards present in the water.

TOR 2.1: Which good hygiene practices are recommended to ensure appropriate microbiological quality requirements of water used for post-harvest handling and processing operations of ffFVH?

TOR 2.2: Which are the most efficacious water disinfection treatments (dose and mode of application) to maintain the appropriate microbiological quality requirements of water used during different post-harvest handling and processing operations of ffFVH?

TOR 2.3: What is the impact of different water disinfection treatments on the induction of the viable but non-culturable (VBNC) state or injury state in bacteria in water used for different post-harvest handling and processing operations of ffFVH?

TOR 2.4: Which are the relevant parameters to establish efficacious water replenishment rates needed to maintain the appropriate microbiological quality requirements of water used for different post-harvest handling and processing operations of ffFVH?

TOR 3 to describe relevant parameters to assess the appropriate microbiological quality requirements of water used for post-harvest handling and processing operations of ffFVH.

TOR 3.1: Which relevant parameters can be used to validate and/or verify the appropriate microbiological quality requirements of the water intended to be used for different post-harvest handling and processing operations of ffFVH?

TOR 3.2: Which relevant parameters can be used to monitor the appropriate microbiological quality requirements of water that is being used during different post-harvest handling and processing operations for ffFVH?

[4] CAC (Codex Alimentarius Commission), 2003. Code of Hygienic Practice for Fresh Fruits and Vegetables CXC 53–2003, pp. 1–39.

1.2 | Additional information

The Mandate on Microbiological Risks in Water Use during Post-harvest Operations of Fresh and Frozen Fruits, Vegetables, and Herbs (ffFVH) is a self-task mandate from the BIOHAZ Panel, including multiple outputs. This mandate also integrates a work package that consists of outsourced activities, including tasks such as literature reviews, experimental data collection in industrial settings and modelling, as detailed in the external scientific report (Gil et al., 2025).

The mandate includes five outputs (scientific opinions), as illustrated in Figure 1. The already published Part 1 opinion (EFSA BIOHAZ Panel, 2023) contains the literature review and analysis of the outbreak data and stakeholder questionnaire. This Part 2 Opinion contains a summary of the development of a dynamic mass balance model for processing operations using water in ffFVH. Parts 3, 4 and 5 Opinions focus specifically on the fresh-whole, fresh-cut and frozen FVH sectors, respectively. The same approach and structure are used for each sector-specific opinion (Parts 3 to 5), aiming to produce concise opinions offering sector-specific guidance. This is achieved by extracting information from the outsourced experimental data generated through EFSA's outsourced activities coupled with modelling based on these outcomes. A user friendly tool has been also developed to allow FBOps to analyse their data and use predictive mathematical modelling to understand the impact of their intervention measures on microbial indicator levels (https://r4eu.efsa.europa.eu/app/WaterManage4You).

The sector-specific opinions only cover some of the TORs, mostly because industrial data was unavailable to provide further knowledge apart from what has already been included in the Part 1 Opinion. Thus, industrial information was available to answer the AQs included in the following TORs:TOR 1.1,TOR 2.1, TOR 2.2, TOR 3.1 and TOR 3.2. The sector-specific opinions do not address TORs 1.2. and 1.3, nor TOR 2.3. Throughout the text, all the opinions from this mandate will be referred to as 'Part 1 opinion', 'Part 2 opinion', etc.

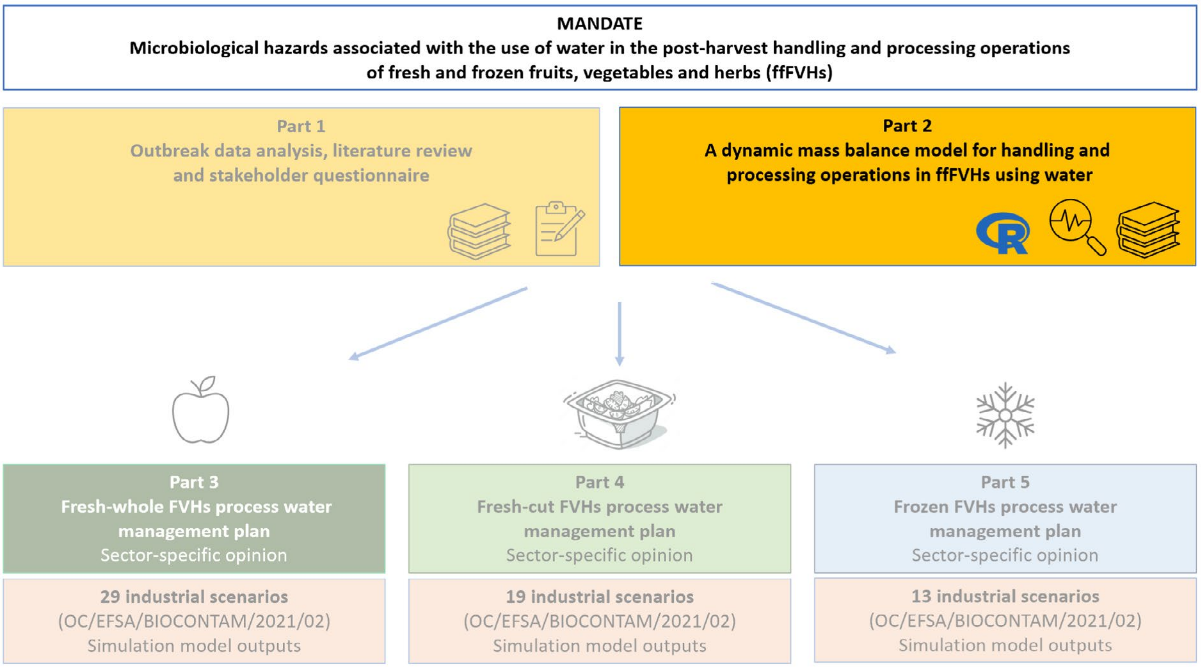

FIGURE 1 Outputs from the mandate on 'Microbiological Risks in Water Use during Post-harvest Operations of Fresh and Frozen Fruits, Vegetables, and Herbs (ffFVH)' (including EFSA outsourced activities – Gil et al., 2025).

1.3 | Interpretation of the Terms of Reference

This scientific opinion describes the mathematical models developed to simulate the contamination dynamics of process water (TOR 1.3) under different scenarios, for example, where the inactivation dynamics of different water disinfection treatments are based on the ability for chlorine to maintain the microbiological quality of the process water (TOR 2.2), as well as water replenishment (TOR 2.4). In this scientific opinion, modelling approaches were used to describe the changes over time in the microbial and organic loads (the latter being reported as the chemical oxygen demand, COD) in the process water based on the microbial transfer rates from product to water. The mathematical model applied to simulate the contamination dynamics of different handling and/or processing operations where water is applied also considers the impact of different intervention strategies on water chemistry (e.g. the concentrations and frequency of chlorine addition causing microbial inactivation), the average time of the ffFVH in the water tank, changes in COD and its impact on chlorine inactivation.

2 | DATA AND METHODOLOGIES

2.1 | Data

The information retrieved about mathematical models from the literature searches performed for the opinion 'Microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVH) Part 1', was used in this scientific opinion. Details of the methodology followed for the literature search can be found in the previously published scientific opinion (EFSA BIOHAZ Panel, 2023).

Based on the literature review for models describing the dynamics of the handling and/or processing operations of ffFVH where water is used, with or without water quality management intervention strategies, including chlorine-based disinfection and/or water replenishment, a list of variables controlling the microbial accumulation in the water was identified. These variables include:

- **Chemical Oxygen Demand** (COD, mg/L), which is an indicator of the concentration of organic matter in the process water. The COD tends to increase during processing as organic matter is shed from FVH and accumulates in water.
- **Free Chlorine** (FC, mg/L), which depends on the total chlorine addition, inactivates the microorganisms in the water and partly on the product surface but is quenched by the increasing levels of organic matter, represented by COD, forming combined chlorine (see below). Thus, FC refers to all active chlorine present in the water as pure chlorine (Cl_2), HOCl (hypochlorous acid) and OCl^- (hypochlorite ion).

$$Cl_2 + H_2O = HCl\ (\text{hydrogen chloride}) + HOCl\ (\text{hypochlorous acid}).$$

In an aqueous solution, hypochlorous acid partially dissociates into the anion hypochlorite (ClO^-):

$$HOCl \rightleftharpoons ClO - (\text{hypochlorite ion}) + H + (\text{hydrogen}).$$

Hypochlorous acid is the most active (antimicrobial) form of FC.

The most relevant parameter to determine the antimicrobial capacity of chlorine is the concentration of FC, and its efficacy is highly dependent on the pH of the medium. At a pH of 6.0, about 80% of the FC is in the form of hypochlorous acid, while at pH 8.0, only 25% of FC is in this active form.

Total Chlorine (TC) is the sum of combined chlorine and FC. Combined chlorine is the FC that has bound itself to a contaminant or organic material. For instance, when chlorine is initially added to water and contaminants such as ammonia (NH_3) are present, the formation of chloramines will be induced and the FC is converted to combined chlorine. Combined chlorine does not have antimicrobial efficacy and consequently does not contribute to the inactivation of microorganisms in process water.

- **Amino acids (AA)** are an additional (with minor contribution) agent that determines the chlorine demand in water since their degradation kinetics is affected by chlorine concentration.
- The **microbial population density in water** (X_w in CFU/100 mL).
- The **microbial population density on the product surface** (X_l, CFU/cm^2).

Data that quantitatively describe the contamination events for different washing/pre-washing operations of ffFVH were collected from the literature or newly determined through EFSA's outsourced activities (Gil et al., 2025).

2.1.1 | Data derived from the literature

The literature search performed within the framework of the scientific opinion on 'Microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVH) Part 1' was used to retrieve information on the transfer coefficients, as well as FC and COD dynamics for the most relevant microbiological hazards. Details of the methodology followed for the literature search can be found in the previously published scientific opinion (EFSA BIOHAZ Panel, 2023). Input parameters for modelling were estimated based on the extracted data related to different post-harvest handling and processing operations where water is used, such as dumping, pre-washing, washing or rinsing (e.g. removal efficiencies from rinsing experiments as input of the fraction released from product to water).

The data from Abnavi et al. (2021), who modelled the dynamics of a washing process of iceberg lettuce and red lettuce, were used to test the suitability of the model for simulating the process and to determine a reference order of magnitude for the model parameters.

2.1.2 | Data derived from outsourced activities

Due to the scarcity of available data and information on the microbiological hazards associated with the use of water in the post-harvest handling and processing operations of ffFVH, a tender was launched to generate specific data obtained via the EFSA outsourced activities (Gil et al., 2025), containing data relevant to address some of the specific assessment questions in this mandate. The tenderer was requested to characterise the contamination of post-harvest water used at different handling and/or processing operations of ffFVH with the aim of evaluating the microbiological and physico-chemical quality of the process water during the operational cycle in industry settings. Data on the dynamics of COD, FC and microbial load transfer in process water were collected from different scenarios/post-harvest handling or processing operation and included food products from the three FVH sectors: (i) fresh-whole FVH, (ii) fresh-cut FVH and (iii) frozen FVH, including at least three different food products per FVH sector.

2.1.3 | Data synthesis

The methods used for the data synthesis aimed to use the data extracted from the literature review (Part 1 Opinion – EFSA BIOHAZ Panel, 2023) as well as data obtained from EFSA's outsourced activities to feed transfer models or to directly apply available transfer models so that both data types described the contamination rates between ffFVH and water, using one or more of the following approaches: (i) direct transfer simulations based on literature models and input on post-harvest handling and/or processing practices and microbial indicators and/or pathogens of relevance to public health. Priority was given to identify and assess the performance of existing microbial transfer models based on their relevance to the process; (ii) update well-performing (i.e. describing experimental data well based on processing parameters) literature models with assumptions generated under this mandate to estimate transfer coefficients based on literature data and by re-fitting literature models to data extracted from the literature; (iii) updated (re-fitted) existing models with data obtained via EFSA's outsourced activities (Gil et al., 2025) and; (iv) evaluate the performance of the updated literature models against industrial (or pilot) data. The latter approach aimed to assess the need to update the parameter values for better agreement of the model with data representing the real industrial environment.

2.2 | Methodologies

2.2.1 | Model fitting

The new dynamic mass balance model presented in this mandate was developed within the framework of EFSA's outsourced activities (Gil et al., 2025). The structure of the dynamic mass balance model and variables used, describe the microbial population dynamics (contamination, inactivation and dilution, e.g. by water replenishment) of process water in a manner applicable to all handling and/or processing operations that may use water disinfection treatments and/or water replenishment. Certain parameter estimates are specific to product-microorganism combinations and need to be updated accordingly or grouped for certain product types and operational conditions for a given pathogen or microbial indicator group. The estimation of parameters was carried out by fitting the model to experimental data. The evaluation of the model's capacity to accurately describe the process is performed by simulating model equations based on the estimated parameters and comparing them with industrial data.

The model was implemented in R and for its numerical simulation needs the packages deSolve, pracma and readxl were used. Uncertainty of parameter estimates was calculated using the cramer-rao method, which is an approximation of Monte Carlo but with less computationally intensive calculation. The method relies on the minimisation of the log-likelihood assuming Gaussian independent noise with a 0.5 standard deviation (SD) for the concentration of total bacterial counts (TBC) in log CFU/100 mL and SD of 10 for the COD. This is equivalent to using a weighted least square method, where weights are the inverse of the assumed SDs. The problem to be solved to estimate the parameters was complex and several possible local solutions were needed. Therefore, the enhanced scatter search optimiser (Egea et al., 2007) (combining local and global estimations) was selected and ran for several hours for each of the visits and cases, repeating the procedure to ensure that the global optimum was obtained. All the computations were implemented using AMIGO2 (Balsa-Canto et al., 2016).

2.3 | Uncertainty analysis

As recommended by the EFSA guidance and related principles and methods on uncertainty analysis in scientific assessments (EFSA Scientific Committee, 2018a, 2018b), uncertainty analysis was implemented by identifying uncertainty sources influencing the model outcome as well as their relative relevance. Both uncertainty sources associated with the model structure (i.e. suitability of the model to simulate the process) as well as uncertainty sources in the data used to fit the model were considered.

3 | ASSESSMENT

The Part 1 scientific opinion detailed the biological background pertaining to the accumulation of microbial contamination in water used during handling and/or processing operations of ffFVH that use water. According to that scientific opinion, the number of microbial cells in post-harvest water at any time represents the inherent microbial load of the water, which is based on the water source, the contamination of water by the processing or handling equipment used, and the bi-directional transfer of cells from product to water. The total microbial load in the water (CFU) may be reduced either by removal of water from the tank (naturally by the outgoing product), the replenishment (or refreshment) of process water with fresh water diluting the organic matter and the microorganisms or by inactivation due to the contact of microorganisms with the disinfectant added to the water.

The translocation of microbiological cells between product and water is a continuous bi-directional process with cells present in the water becoming attached to the ffFVH, or detached from ffFVH, transferred to water and re-attached onto ffFVH from the contaminated water in use (i.e. ffFVH can become contaminated via the post-harvest water when water management practices are insufficient, thereby cross-contamination may occur). The cell transfer can be quantified with a term called '*transfer coefficient*', defined as the ratio of cells in the donor (product) over the recipient (water) when the transfer occurs from product to water or the inverse ratio when the transfer of cells takes place from water to product. For a finite time interval, the change in transfer coefficient reflects the '*transfer rate*', which is expressed as CFU/min.kg of product or mL of water.

This rate is defined as the change (usually the increase) of the microbial load (N) in process water per unit of time (t). The mass balance between the number of cells released in the water, inactivated by the disinfectant or transferred from the water back to the product determines whether the value of the contamination rate is positive (contamination) or negative (decontamination).

In parallel to microbial dynamics, the incoming product in a water tank sheds organic matter in water, which increases the COD (mg/L). The organic load may react with the disinfectant. As such, both the organic load and disinfectant are reduced (the disinfectant is degraded by the organic matter). The organic matter also interferes with the contact between disinfectant and target cells. The water replenishment and removal of product from the tank can reduce the COD and disinfectant concentrations (mg/L) similarly to what is described above for microbial populations.

The above dynamic processes can take place continuously in each handling and/or processing operation using water, resulting in momentary changes in the microbial population in the water. This change is reflected by the transfer rate of process water and the inactivation of cells by the disinfectant.

The dynamic processes can be described with a model that considers the non-autonomous behaviour of the dynamics and the reaction kinetics between COD, microbial contamination and disinfectant. The key output variable of such a model is the contamination rate over time, expressed via the following term (for momentary changes):

$$\frac{dN}{dt} = f(\text{process dynamics}) = f(\text{bi} - \text{derectional microbial trasnfer, disinfectant, COD, mass balance of product: water}, t).$$

The current mandate summarises relevant approaches existing in the literature and tailors them to the different handling and/or processing operations where water is used.

3.1 | Generic model structure

Based on the literature, the generic form of a model (named generic model in this opinion or 'general model 3' in Gil et al., 2025) that encompasses all critical variables that describe the dynamics of FC, COD, AA and their impact on microbial contamination of water and cross-contamination of FVH comprises the following first and second-order differential equations:

$$\frac{dX_\mathrm{w}}{dt} = \left(X_\mathrm{w}\_\mathrm{Inlet}\right) - \left(X_\mathrm{w}\_\mathrm{Transfer\_to}\_X_\mathrm{p}\right) - \left(X_\mathrm{w}\_\mathrm{Inactivation\_by}\_C\right),$$

$$\frac{dC}{dt} = (C\_\mathrm{Inlet}) - (C\_\mathrm{Natural\_Decay}) - (C\_\mathrm{Inactivation\_by\_COD}) - (C\_\mathrm{Degradation\_by\_AA}),$$

$$\frac{d\mathrm{COD}}{dt} = (\mathrm{COD\_Inlet}) - (\mathrm{COD\_Degradation\_by}\_C),$$

$$\frac{dX_\mathrm{p}}{dt} = \left(X_\mathrm{p}\_\mathrm{Transfer\_from}\_X_\mathrm{w}\right) - \left(X_\mathrm{p}\_\mathrm{Inactivation\_by}\_C\right) - \left(X_\mathrm{p}\_\mathrm{Dilution\_by\_Product\_Removal}\right),$$

$$\frac{d\mathrm{AA}}{dt} = \left(\mathrm{AA}_\mathrm{Inlet}\right) - \left(\mathrm{AA}_{\mathrm{Degradation}_{\mathrm{by}_C}}\right),$$

where X_w and X_p describe the microbial population density in water and the product surface, respectively, C, i.e. is the active fraction of the disinfectant concentration. For the needs of this mandate, FC, COD and AA concentrations in the water, are considered as indicators of chlorine demand, i.e. under the context of organic matter. **Table 21** of section 3.7.3 in Gil et al. (2025) provides a summary of published mathematical expressions that have been used in different studies to model the dynamics (accumulation or reduction/degradation) of microbial contamination, COD, disinfectant or reactants of COD or FC, e.g. AA in the water during washing operations.

Figure 2 depicts a tank used for different post-harvest handling and processing operations (e.g. dumping, pre-washing, washing, rinsing) of FVH (e.g. lettuce) that includes representation of the major mechanisms considered in the dynamic changes of organic matter (COD in orange), residual concentration of disinfectant (*C* in green) and pathogens (in yellow) in water (X_w blue) or in/on the product (X_p lettuce).

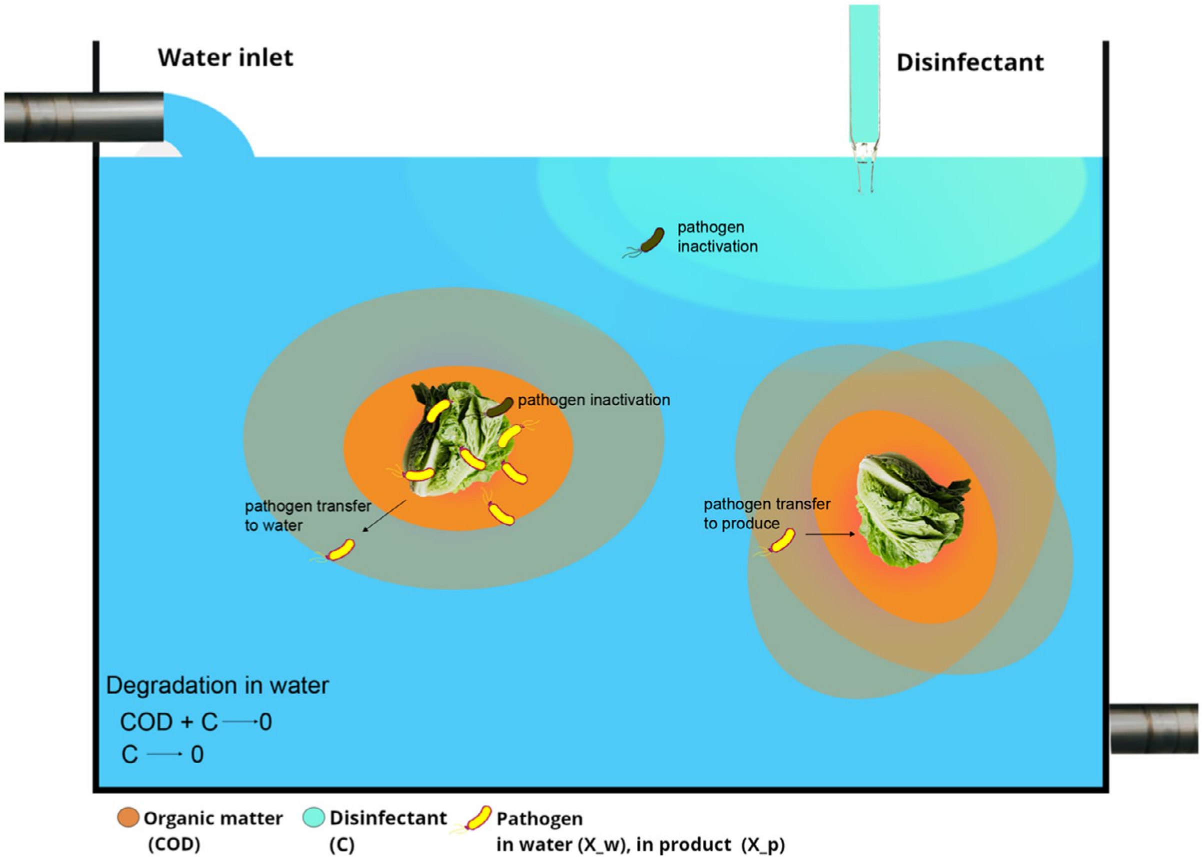

FIGURE 2 Illustration of the most common modelled mechanisms in the literature and modelled variables $(\mathrm{COD}, C, X_w, X_p)$ in a water tank used for different post-harvest handling or processing operations of FVH. Source: EFSA's outsourced activities – Gil et al. (2025).

Most literature models for such operations simulate batch processes in a tank (e.g. dumpling, washing, rinsing) and do not consider the influx and efflux of water and product throughout the handling or processing operation using water. As this is not realistic in real industrial settings, the concepts included in the generic model should be extended to incorporate these mechanisms. Such a model needs to be tailored to the different operational conditions, e.g. different FC and water addition rates (water replenishment and refilling), the flow of product in the tank, total duration of the process, temperature of the washing process, etc. and applicable for different product-pathogen/microbial indicator combinations.

3.1.1 | The impact of COD on the antimicrobial efficacy of low FC concentrations

Gómez-López et al. (2014) continuously inoculated water (in the absence of product) with ca. 5 log CFU/mL and ran two types of simulated washing (both with chlorine or chlorine dioxide) for up to 120 min. In the first experiment, COD was maintained at 0 mg/L (ppm) and in the second, the COD increased by adding concentrated process wash water (inoculated with 5 log CFU/mL of *E. coli*), up to approx. 600 mg/L COD. In both COD-based experimental conditions, FC was kept constant at 1–1.5 or 2.9–3 ppm. The results showed that 1 ppm of FC was sufficient to eliminate a constant addition of 5 log CFU/mL in the inoculum, dosed to the treatment tank (six litres) at a flow rate of 7.7 L/h. The initial COD of the treatment tank was equal to 0 mg/L, and it progressively increased during the dosing of the inoculum. The same effect was delivered by 3 ppm of FC when COD was increased during washing. However, a constant level of 1 ppm FC was gradually quenched by the increasing COD, which was incapable of eliminating (or reducing to acceptable levels) microbial populations towards the end of washing. In comparison, with 3 ppm FC, the incoming microbial load was inactivated despite the COD increase. This result undoubtedly indicates that an increase in COD compromises the efficacy of low doses of FC and practically limits its antimicrobial efficacy.

Necessary model adjustments

The proposed model describes microbial inactivation in water as a function of FC, which in turn is affected by the accumulated organic matter (reflected primarily by COD increase and AA degradation by the disinfectant) in the water tank. As such, at constant FC levels, i.e. unaffected by COD, the model predicted identical inactivation of cells both when COD was kept at 0 ppm and when COD increased up to 600 ppm (results not shown). This suggests that such concentrations of COD neutralise or 'quench' FC, rendering the same FC concentrations less effective with time. This result prompts the hypothesis that the accumulation of COD does not neutralise FC (provided that it remains constant); rather, it decreases the probability of available FC reaching the target bacteria, e.g. due to the presence of (invisible) solid particles on which FC may bind. The higher the COD, the more difficult it is for FC to contact cells, either because FC binds to organic matter (a less likely assumption, as this would also affect the measurement) or because cells are protected by the organic matter (i.e. leaving the measurement of FC undisturbed). This result would explain why this phenomenon is practically eliminated at higher FC concentrations, which are enough to counteract the protective effect of organic matter on the cells.

To simulate the above phenomenon, which is expected to be more pronounced in real practice, where COD derives from organic matter released from the product, the term ($f[COD_w, FC_w]$) reflecting the rate of microbial inactivation by FC in the model needs to be described as a function of COD. The term of Hill kinetics $\alpha\left(\frac{K_m^n}{K_m^n + COD_w^n}\right)$ is a suitable term for microbial inactivation by FC (see section 3.8.1 in Gil et al., 2025), with n offering the flexibility to describe non-linear trends. By setting n equal to 1, the term is converged to the following Michaelis-Mentel-based kinetic expression for quantifying the impact of increasing levels of COD on the efficacy of FC in reducing bacteria in water (Abnavi et al., 2021):

$$FC_{\text{microbial inactivation function}} = f\left(COD_w, FC_w, a\left(\frac{K_m}{K_m + COD_w}\right)\right),$$

where a is the inactivation rate (1/min) due to FC in the absence of COD, i.e. in pure water (e.g. tap water), COD_w is the momentary COD concentration at a given time and K_m (ppm COD) is a parameter that represents the protective effect of COD on cells against FC. The value of a represents the maximum microbial inactivation rate (also termed a_{max} in the literature, Abnavi et al., 2021) by FC, which is the microbial inactivation occurring in water due to the disinfectant without the influence of the organic matter. The overall term $FC_{\text{microbial inactivation function}}$ represents the inactivation rate due to FC, encompassing the product-dependent protective effect of COD.

3.1.2 | Updated model structure for the needs of the mandate

In addition to the mechanisms previously modelled in the literature, the updated generic model includes fluxes of water and product into and out of the tank. In the literature, this is usually modelled in a discrete form using IF-conditions, but for continuous water replenishment, the use of fluxes is a more adequate formalism. Therefore, the updated model structure incorporated the following features:

- The dynamics of water volume (F) and product mass (M) in relation to the tank volume (V). These change with time when incoming and outgoing fluxes are not equivalent, therefore being new states in the model.
- The possibility of having different and more than one product $X_{p,i}$, as shown in the work of Abnavi et al. (2021), where the subindex i takes values > 1 or is omitted when only one product is washed.
- Explicitly modelling of COD transferred from the product to the water.

To avoid a very complex model, the focus could be on processes using FC as a disinfectant and COD as an organic matter indicator, thereby disregarding the dynamics of the AA, which are assumed to have negligible impact on COD dynamics. Sub-indexes w and p, i represent concentrations in the water and product i, respectively. Therefore, the aforementioned general modelling framework is converged to the following form (Table 1).

TABLE 1 General modelling framework, based on the mass balance of water, product, chemical oxygen demand (COD), free chlorine (FC) and microbial contamination of water (X_w) and product (X_p).

$\frac{dCOD_w}{dt} = (COD_w\_Inlet) - (COD_w\_Outlet) + (COD_w\_Transfer\_from\_COD_{p,i}) - (COD_w\_Transfer\_to\_COD_{p,i}) - (COD_w\_Degradation\_by\_FC),$
$\frac{dCOD_{p,i}}{dt} = (COD_{p,i}\_Inlet) - (COD_{p,i}\_Outlet) - (COD_{p,i}\_Transfer\_to\_COD_w) + (COD_{p,i}\_Transfer\_from\_COD_w); \quad i = 1, 2, \ldots, n,$
$\frac{dFC}{dt} = (FC\_Inlet) - (FC\_Outlet) + (FC\_Injected) - (FC\_Natural\_Decay) - (FC\_Degradation\_by\_COD_w),$
$\frac{dX_w}{dt} = (X_w\_Inlet) - (X_w\_Outlet) - (X_w\_Inactivation\_by\_FC_w) + (X_w\_trans\_by\_X_{p,i}) - (X_{p,i}\_trans\_by\_X_w),$
$\frac{dX_{p,i}}{dt} = (X_{p,i}\_Inlet) - (X_{p,i}\_Outlet) - (X_{p,i}\_Transfer\_to\_X_w) + (X_{p,i}\_Transfer\_from\_X_w) - (X_{p,i}\_Inactivation\_by\_FC); \quad i = 1, 2, \ldots, n.$

This result, in turn, is expressed by the following in more detailed form, which also includes parameters that determine the process and product-microorganism-specific combinations and are to be estimated by fitting the model to experimental data for specific product-microorganism combinations. The transport terms are based on the chemical reactor theory, whereas the interactions (i.e. the reaction of FC on microbial cells or degradation of COD by FC and vice versa) are based on the law of mass action.

$$\frac{d\mathrm{COD_w}}{dt}=\frac{F_\mathrm{w}^\mathrm{in}}{V}\left[\mathrm{COD_w^{in}}-\mathrm{COD_w}\right]+\sum_{i=1}^{n}\frac{M_i}{V}\widetilde{K}_{\mathrm{COD}_{\mathrm{p},i\to\mathrm{w}}}\cdot\mathrm{COD}_{\mathrm{p},i}-\gamma\cdot\beta\cdot\mathrm{COD_w}\cdot\mathrm{FC},$$

$$\frac{d\mathrm{COD}_{\mathrm{p},i}}{dt}=\frac{F_{\mathrm{p},i}^\mathrm{in}}{M_i}\left[\mathrm{COD}_{\mathrm{p},i}^\mathrm{in}-\mathrm{COD}_{\mathrm{p},i}\right]-\widetilde{K}_{\mathrm{COD}_{\mathrm{p},i\to\mathrm{w}}}\cdot\mathrm{COD}_{\mathrm{p},i}+\frac{V}{M_{p,i}}\widetilde{K}_{\mathrm{COD}_{\mathrm{w}\to\mathrm{p},i}}\cdot\mathrm{COD_w};\qquad i=1,2,\ \dots,n,$$

$$\frac{d\mathrm{FC}}{dt}=\frac{F_\mathrm{w}^\mathrm{in}}{V}\left[\mathrm{FC^{in}}-\mathrm{FC}\right]+u(t)-\lambda\cdot\mathrm{FC}-\beta\cdot\mathrm{COD_w}\cdot\mathrm{FC},$$

$$\frac{dX_\mathrm{w}}{dt}=\frac{F_\mathrm{w}^\mathrm{in}}{V}\left[X_\mathrm{w}^\mathrm{in}-X_\mathrm{w}\right]-\alpha\left(\frac{K_\mathrm{m}^n}{K_\mathrm{m}^n+\mathrm{COD}_\mathrm{w}^n}\right)X_\mathrm{w}\cdot\mathrm{FC}+\sum_{i=1}^{n}\frac{M_i}{V}\widetilde{K}_{X_{\mathrm{p},i\to\mathrm{w}}}\cdot X_{\mathrm{p},i}-\sum_{i=1}^{n}\widetilde{K}_{X_{\mathrm{w}\to\mathrm{p},i}}\cdot X_\mathrm{w},$$

$$\frac{dX_{\mathrm{p},i}}{dt}=\frac{F_{\mathrm{p},i}^{in}}{M_i}\left[X_{\mathrm{p},i}^\mathrm{in}-X_{\mathrm{p},i}\right]-\widetilde{K}_{X_{\mathrm{p},i\to\mathrm{w}}}\cdot X_{\mathrm{p},i}+\frac{V}{M_i}\widetilde{K}_{X_{\mathrm{w}\to\mathrm{p},i}}\cdot X_\mathrm{w}-\eta\,\alpha\left(\frac{K_\mathrm{m}^n}{K_\mathrm{m}^n+\mathrm{COD}_\mathrm{w}^n}\right)\cdot\mathrm{FC}\cdot X_{\mathrm{p},i};\quad i=1,2,\ \dots,n.$$

- The sub-indexes *w* and *p* still represent concentrations in water and product, respectively. The super-index *in* represents incoming values, i.e. influx. *V* represents the volume of water, and *M* is the mass of the product in the water tank. $F_\mathrm{w}^\mathrm{in}, F_{\mathrm{p},i}^\mathrm{in}$ represent the incoming water (L/min) and product (g/min) fluxes, respectively.
- K_COD transfer rate of COD from product to water (expressed in mg/L of COD/kg-product·min).
- β the degradation rate of FC by COD, i.e. mg of FC consumed by COD (expressed in 1/(ppm-COD·min)).
- γ the yield coefficient of COD reaction with FC, i.e. mg of COD consumed *per* mg of FC (expressed in mg-COD/mg-FC)
- λ is the natural FC decay rate (expressed in 1/min).
- K_X transfer rate of microbial contamination (TBC) from product to water (expressed in CFU/kg-product·min).
- K_p transfer rate of microbial contamination (TBC) from water to product (expressed in CFU/mL·min).
- *D* the dilution rate of the process, which is equivalent to the water flux divided by the tank volume in previous models $D=F_\mathrm{w}/V$ (expressed in 1/min).
- α the inactivation rate (expressed in 1/(min·ppm-HOCl)).
- K_m the protective effect of COD (expressed in ppm-COD).

We assume that:

- $u(t)$ represents the FC added to the system (that changes with time) with units mg-FC/min·L
- 'pseudo-reactions' (are modelled using mass action law if not specifying otherwise)

$$\begin{gathered}\mathrm{FC_w}\xrightarrow{\lambda}\emptyset\\ \mathrm{FC_w}\xrightarrow{K_\mathrm{FC}\mathrm{COD_w}}\emptyset\ .\\ X_\mathrm{w}\xrightarrow{f(\mathrm{COD_w},\mathrm{FC_w})}\emptyset\end{gathered}$$

- Inactivation of microbial contamination in the product is assumed to be a fraction of the inactivation in water: $\eta\left(\frac{\alpha K_\mathrm{m}^n}{K_\mathrm{m}^n+(\mathrm{COD_w})^n}\right)\mathrm{FC}\ X_{\mathrm{p},i}$, with $\eta\in[0,1]$.

The model is flexible. It can be simplified, for example, by removing the impact of the disinfectant (FC herein), extended by incorporating the impact of other water disinfectants, or tailored to both batch and continuous processes by setting proper values (e.g. 0 or >0) for the influx and efflux terms. The above can be implemented by properly adjusting the parameter values. For instance, the impact of different products can be reflected in the model by changing the parameters associated with the COD dynamics (release from the product), the FC degradation by COD and the microbial transfer rate. Similarly, changing the microbial inactivation rate, along with the protective effect of COD on microbial inactivation, informs the model for the impact of a specific disinfectant on different microorganisms or the impact of different disinfectants on the same microorganisms. However, case-dependent adjustment of mathematical formulas may also be necessary, as detailed in Section 5 (Recommendations).

3.2 | Fitting the water phase model to the industrial data

In the following sections, three versions of the model are presented, each consisting of a set of ordinary differential equations (ODE). Two versions of the model were fitted to data from the industrial scenarios to obtain industrial-relevant parameters for the dynamics of COD and microbial contamination in the absence of disinfectant or with the addition of FC (Gil et al., 2025). The third version of the model offers the capacity to test '*what if*' operational scenarios by simulating the model with user-defined parameters for the transfer rates of cells and COD from product to water, for the microbial inactivation by FC, as well as times and concentrations of FC addition in the system throughout the process.

Based on the justification detailed in sections 3.8.1–3.8.3 of Gil et al. (2025), the following simplified and least redundant model version was defined to fit the industrial data.

$$\frac{dX_w}{dt} = -D \cdot X_w + \frac{M}{V} K_X - \alpha \left(\frac{K_m^n}{K_m^n + \mathrm{COD}_w^n} \right) X_w \cdot \mathrm{HOCl},$$

$$\frac{d\mathrm{COD}_w}{dt} = -D \cdot \mathrm{COD}_w + \frac{M}{V} K_{\mathrm{COD}},$$

$$\mathrm{HOCl} = \frac{\mathrm{FC}}{1 + \frac{10^{-\mathrm{pK_a}}}{10^{-\mathrm{pH}}}} \quad \text{with } \mathrm{pK_a} = \frac{3000}{T} + 0.0253\, T - 10.06,$$

$$M = \tau \frac{M_{\mathrm{batch}}}{t_{\mathrm{batch}}},$$

where

- X_w is the concentration of total bacterial counts (TBC in CFU/100 mL).
- COD is the chemical oxygen demand associated with the organic matter content in the process water.
- D the dilution rate of the process, which is equivalent to the water flux (F_w or W) divided by the tank volume in previous models $D = F_w / V$ (expressed in 1/min).
- HOCl is the concentration of hypochlorous acid as a function of the free chlorine (FC) concentration present in the process water, which is affected by the pH and the temperature.
- pH is the negative logarithm of the hydrogen ions and is affected by the FC levels and temperature, which controls the dissociation of chlorine added to the system.
- $\mathrm{pK_a}$ is the negative logarithm of the dissociation (acid ionisation) and depends on the temperature.
- T is the temperature, which affects the dissociation of chlorine and depends on the FC added, determines the amount of hypochlorous acid (undissociated molecule) formed in the water and is practically the most critical feature of microbial inactivation.
- Hill exponent that was assumed to be 1 ($n = 1$), as data was insufficient to identify this parameter with confidence (i.e. the confidence interval was too wide).

Since water in the handling or processing operation is initially assumed to be as pure as potable water, COD_w^{in} and X_w^{in} are zero.

The mass of the product (M expressed in kg) is calculated from information provided by the industries regarding the process washing time (τ), the duration of the operation or operational cycle (t_{batch}), and the total processed product during that time (M_{batch}).

Based on these explanations, five parameters must be estimated by fitting the above system of differential equations to experimental data:

- D is the dilution rate of the process, which is equivalent to the water flux (W) divided by the tank volume (V) = W/V (expressed in 1/min). The dilution rate practically defines the speed of system saturation, i.e. the higher the D value, the lower the saturation. Therefore, the assumption of a common value for all cases has the following trade-off: (a) if the process is operated at larger dilution rates, the transfer rates are underestimated and (b) if the process is operated at lower dilution rates, the transfer rates are overestimated.
- K_X is the transfer rate of TBC from product to water (CFU/min · kg-product).
- K_{COD} is the transfer rate of COD from product to water (mg-COD/min · kg-product).
- α is the inactivation rate of bacteria due to HOCl (1/(min · ppm-HOCl)), which is considered to be species-specific.

- K_m is the parameter reflecting the protective effect of COD and, through this, of organic matter on cells (in mg-COD/L).

Parameters α and K_m are applicable only in operations that use water disinfection with chlorine. There are mass changes with time for some of the experiments, which considerably affect the transfer of contamination from product to water. The volume of water, on the other hand, is assumed constant, although it may fluctuate minimally, due to removal when the product leaves the process and/or the tank is refilled with small volumes of water.

To simulate cases without disinfectants, FC is set to zero, and therefore, the disinfectant terms are excluded since they have no effect on the microbial dynamics in the water. The model described in this opinion at the beginning of Section 3.2 without the impact of disinfectant receives the following sub-form:

$$\frac{dX_w}{dt} = -D\,X_w + \frac{M}{V}K_X,$$

$$\frac{d\mathrm{COD}}{dt} = -D\,\mathrm{COD} + \frac{M}{V}K_{\mathrm{COD}},$$

$$M = \tau\frac{M_{\mathrm{batch}}}{t_{\mathrm{batch}}}.$$

For post-harvest handling or processing operations, where FC was used, the type of measured dynamic data was the same as in the process without disinfectant, but now with extra parameters, such as the time and levels of FC addition and the water replenishment rate that determines the dilution rate (D) of product to water. The complete set of equations listed at the beginning of Section 3.2 was used for this purpose. The drop in concentrations of added FC in the tank, as a result of natural decay and degradation by contact with COD, was not simulated at this stage. Instead, the levels of FC at any dt interval between 2 consecutive FC measurements were estimated by linear interpolation.

The impact of temperature on the outcome of the process could be double. First, it affects the dissociation of chlorine, a phenomenon that was included in the model. In addition, temperature can also affect the microbial inactivation rate caused by chlorine (Suslow, 1997). Regarding the latter, data describing the inactivation rate by FC as a function of temperature is scarce. A previous study that comparatively assessed the impact of 6, 12, 15, 21, 25 and 35°C suggests there is no significant effect of temperature on the inactivation rate by FC at temperatures above 6°C (Cheswick et al., 2020; Rasheed et al., 2016). Even though inactivation at 6°C was slower than at higher temperatures, this referred to extremely low chlorine levels, such as 0.12 ppm (Cheswick et al., 2020). Considering that FC at the concentrations used in washing operations are highly toxic to microbial cells within a very short exposure time (0.2 to 1.5 s) (Zhang et al., 2015), the temperature-dependent effect on microbial inactivation by FC was assumed to be negligible, i.e. the exposure conditions mask the temperature effect, if any. Furthermore, the uncertainty in the estimation of the microbial inactivation rate in the water (TBC) was much larger than any underlying impact of temperature on the microbial inactivation rate.

The industry did not provide dilution rates (D) for industrial cases, and the value was assumed to be less than 0.05 (1/min) but always greater than zero as the industrial data show a steady state of water volume in the tank. Such a state without disinfectant can only be reached when $D > 0$. These values are too low to have any major impact on the density of microbial contamination or COD. The contribution of D in reducing X_w and COD becomes remarkable when large volumes of water in the tank are replenished, as opposed to what most companies do. The model assumed a continuous water replenishment to meet this requirement. However, a thorough review of the available industrial data (Gil et al., 2025) suggested that no water replenishment was practically made. Instead, the volume of water added is markedly lower than the total volume of the tank, and it is primarily intended to refill the tank, compensating for the water loss with the outgoing product. Therefore, D was considered to have a negligible impact on the washing operations investigated.

Fitting estimate for transfer rates for COD from product to water was feasible for most of the industrial cases (27 out of 32) included in EFSA's outsourced activities and presented a well-defined Gaussian distribution with mean and most probable value around 2 log mg COD/min · kg_product (Gil et al., 2025).

Transfer rates for TBC were always estimated with reasonable confidence in all 32 fits (EFSA outsourced activities – Gil et al., 2025). However, their values varied from 2 to 10 log CFU/min · kg-product with a very spread distribution and a middle value of all three operation-related distributions around 7 log CFU/min · kg-product. Many factors affect these transfer rates that could not be measured and, therefore, were not included in the model. Most of these unmeasured factors are differences in product COD (representing organic matter) and TBC concentrations, for example, due to previous pre-washing treatments or changes in the type of washed product.

The parameter K_m, reflecting the protective effect of *COD* on the inactivation of TBC by HOCl, was also a highly uncertain parameter. However, for large values of K_m, the effect of COD could be disregarded, although this parameter also may vary considerably depending on the product and scenario.

3.3 | Model structure for simulating different operational scenarios

The final version of the model (model 7 in Gil et al., 2025) was developed to offer the capacity to test '*what if*' operational scenarios by simulating the model with user-defined parameters for transfer rates of cells and COD from product to water, for the microbial inactivation by FC as well as times and concentrations of FC addition in the system throughout the process. This final model considers the impact of chlorine (as in 'Model 6' described in Gil et al., 2025) but includes an additional term that enables the simulation of natural and COD-dependent degradation of FC added in the water tank:

$$\frac{dFC_w}{dt} = \left(FC_w\_influx\right) - \left(FC_w\_outflux\right) - \left(FC_w\_natural\_decay\right) - \left(FC_w\_degrad\_by\_COD_w\right).$$

a. Different types of interventions (or system controls) are enabled in the model to test different what-if scenarios. Such interventions are directly or indirectly associated with the water replenishment rate, represented in the model through the time-dependent dilution rate $D(t)$.

- Changing the water that is continuously added (F_w^{cont}).
- Adding water at certain times (F_w^{disc}).

b. The amount and frequency of FC addition in the tank, which is expressed in the model by assuming:

- Changing the FC that is continuously added (FC_{in}^{cont}).
- Addition of FC at certain times (FC_{in}^{disc}).

The detailed description of the ODE of the model is as follows:

$$\frac{dX_w}{dt} = -D(t)\cdot X_w + \frac{M}{V}K_X - \alpha\left(\frac{K_m^n}{K_m^n + COD_w^n}\right)X_w \cdot HOCl,$$

$$\frac{dCOD_w}{dt} = -D(t)\cdot COD_w + \frac{M}{V}K_{COD} - \gamma\cdot\beta\cdot COD_w\cdot FC,$$

$$\frac{dFC}{dt} = -D(t)\cdot FC + u(t) - \beta\cdot COD_w\cdot FC - \lambda\cdot FC,$$

$$HOCl = \frac{FC}{1 + \frac{10^{-pK_a}}{10^{-pH}}} \quad \text{with } pK_a = \frac{3000}{T} + 0.0253\,T - 10.06,$$

$$M = \tau\frac{M_{batch}}{t_{batch}},$$

$$D(t) = \frac{F_w^{cont} + F_w^{disc}}{V},$$

$$u(t) = \frac{FC_{in}^{cont} + FC_{in}^{disc}}{V}.$$

The parameters β and γ reflect the interactions between COD and FC, whereas the parameter λ describes the natural decay of FC in water. The protective effect of COD on the inactivation of TBC by HOCl is a highly uncertain parameter. Given the lack of sufficient experimental data to accurately determine the values of β and γ from the industrial data, they are both initially set to 0, suggesting that the total chlorine added to the system is fully converted into FC without losses due to interaction with COD. On the contrary, a value of 0.0017 1/min was used for λ, based on Abnavi et al. (2021), since this parameter is practically generic for the FC decay in process water and is not expected to vary significantly among studies. For more realistic simulations, values for all three parameters, especially β and γ, should be obtained from relevant studies in the literature or experimentally determined. In the simulations presented in the three sector-specific scientific opinions (Parts 3, 4 and 5 opinions), values greater than 0 were attributed to β and γ as best possible estimates.

The following graphs illustrate representative examples of model simulation outputs, without addition (Figure 3), with continuous (Figure 4) or with discrete (i.e. every 2 h, Figure 5) addition of disinfectant.

In the absence of FC addition (Figure 3), the COD constantly increases beyond 6000 mg/mL, and so does TBC, reaching levels of about 6 log CFU/100 mL.

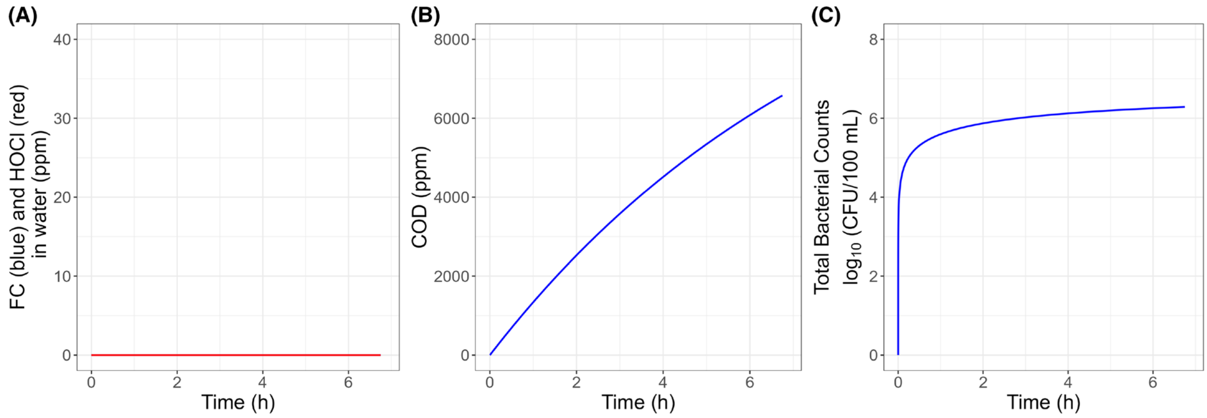

FIGURE 3 Model simulation outputs, including FC and hypochlorous acid (HOCl) (A), accumulation of the chemical oxygen demand (COD) (ppm correspond to mg/L) (B) and total bacterial counts (C), for a scenario with **no water disinfection**.

The continuous addition of FC (Figure 4) results in a continuous increase of HOCl available in the system, from 15 to 40 mg/mL, which maintains TBC at a level below 2 $\log_{10}$ CFU/100 mL even though COD increases close to 6000 mg/mL.

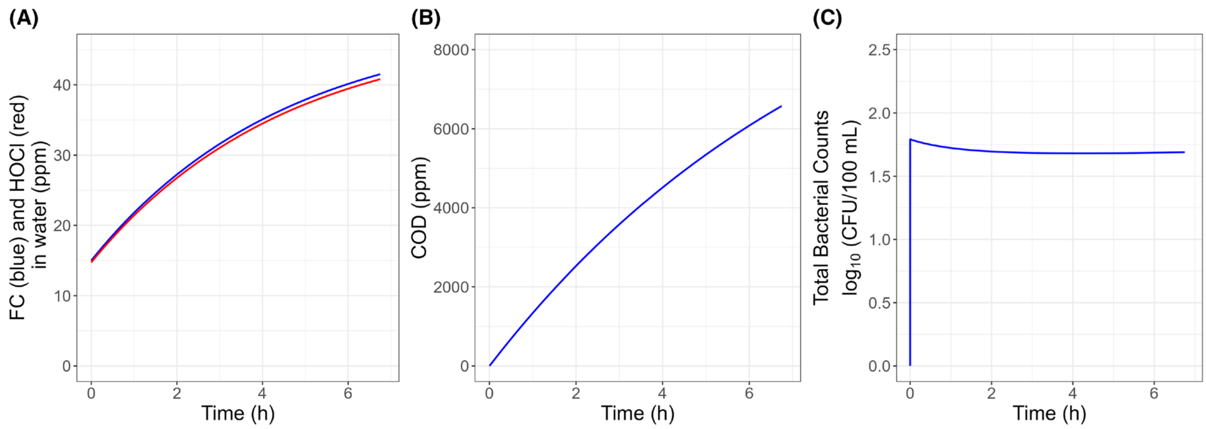

FIGURE 4 Model simulation outputs, including FC and hypochlorous acid (HOCl) (A), accumulation of the chemical oxygen demand (COD) (ppm correspond to mg/L) (B) and total bacterial counts (C), for a scenario with **continuous disinfection of the water**.

The periodic (discrete) addition of FC (Figure 5) results in limited accumulation of TBC around 2.0 $\log_{10}$ CFU/100 mL at slightly higher levels than the continuous addition of FC. COD again increases at the same concentrations as in the simulations mentioned above.

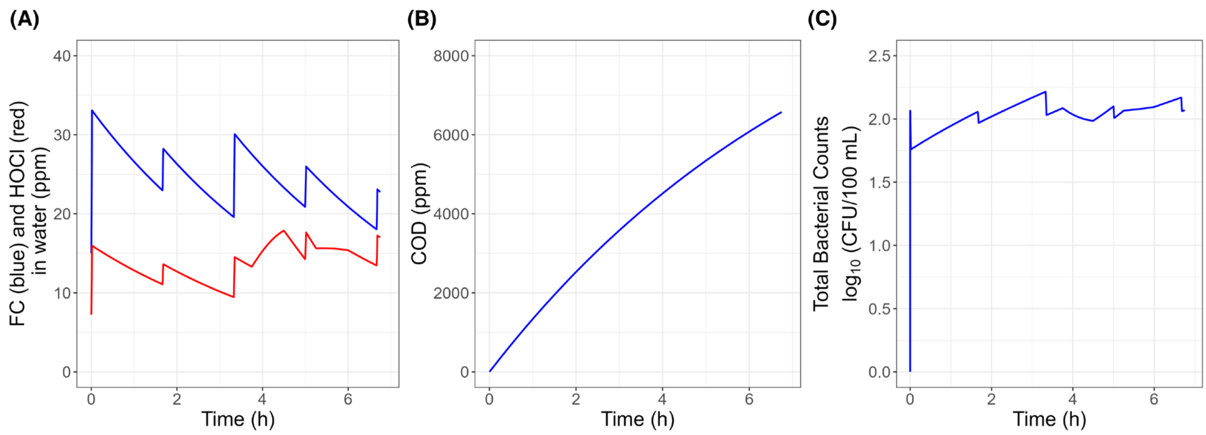

FIGURE 5 Model simulation outputs, including FC and hypochlorous acid (HOCl) (A), accumulation of the chemical oxygen demand (COD) (ppm correspond to mg/L) (B) and total bacterial counts (C), for a scenario with **discrete disinfectant addition in the water**.

3.4 | Conditions for model use and uncertainty analysis

The model (number 7 in Gil et al., 2025) is suitable for predicting the microbial contamination dynamics of process water as well as the microbial inactivation dynamics of chlorine-based water disinfection treatments in a water tank used in different handling or processing operations. Based on the industry data provided by EFSA's outsourced activities (Gil et al., 2025), a generic estimation of the parameter values was carried out by the model described in Section 3.2. The term generic refers to the estimation of (i) product-specific transfer rates for microbial cells (K_X) and COD (K_{COD}) from product to water, (ii) a common value for the protective effect of COD (K_m), independent of product and microorganism and (iii) a product-independent inactivation rate (α), for TBC.

Furthermore, the consolidated model structure described in Section 3.3 enables the simulation of various '*what if scenarios*', which could help the FBOps in selecting the most suitable intervention strategies, e.g. the use of chlorine-based disinfectants and/or water replenishment to maintain the microbiological quality of the process water at the lowest possible values for both FC and the rate of water replenishment. The model could also inform the FBOps about conditions that could represent operational practices that favour or prevent the accumulation of microbial contamination in water.

Beyond the aforementioned general scope of the model, the applicability of the model and its specificity to different handling or processing operations, products and microorganisms highly depends on the resolution of information and data available for the operational characteristics of the process, the product-microorganism interactions and the response of the target organisms to FC. In particular, when the process is thoroughly monitored (e.g. by monitoring the frequency and amounts of FC added and the rate of water refilling, the rate of water replenishment, and the pH and temperature of the water), along with experimental evidence for the sensitivity of microorganisms to FC (inactivation rate) and the product-specific protective effect of COD on inactivation of microbial cells by FC, the model can provide guidance on best practices for water disinfection (chlorine-based disinfectants) and water replenishment for specific microorganism-product combinations; these are to be experimentally validated according to the guidance provided in the 3 sector-specific opinions.

The outcome of this mandate illustrates the capacities of the dynamic (predictive) model. The major sources of uncertainty identified are associated with the final model structure and the data used to derive parameter estimates. The outcome of the sources of uncertainty associated with the model structure was compared to the uncertainty associated with the data used to fit the model. The data-related uncertainties are attributed to the incompleteness of the datasets used to fit the model, particularly regarding: (i) the time and levels of disinfectant addition, (ii) the water replenishment rate, (iii) the lack of experimental procedures enabling the estimation of microorganism and product-specific transfer coefficients of microbial cells from product to water and (iv) the lack of microbial inactivation data associated with chlorine disinfectant in pure water, i.e. in the absence of COD (e.g. tap water), for all relevant microorganisms of the current mandate.

Based on the model fitting to experimental data and the high uncertainty of parameter estimates, it seems that data-related uncertainties are more impactful than model-related uncertainties under the remit of this mandate (e.g. chlorine-based water disinfection treatments versus other water disinfection treatments). The ability of the model to describe the global trends in the dynamics of handling or processing operations was not compromised. However, there may be limitations in the accurate estimation of parameter values. The impact of such limitations pertains to the availability and suitability of data to describe the behaviour of different product-microorganism combinations, particularly when chlorine was used.

In the absence of essential (in detail) inputs discussed above for running the model, the model provides generic quantitative information that can be used by the FBOps to generally understand the dynamics of the process and identify the main actions to be taken in their water management plan. Model outputs may assess the impact of different chlorination levels and, through that, derive the minimum chlorination required or determine whether water replenishment should take place more frequently and/or with higher amounts of replenishing water added. Moreover, these outputs can be used to pre-set the operational conditions of FVH to be validated (Parts 3–5 sector-specific opinions).

4 | CONCLUSIONS

- A model was developed to simulate the microbial contamination dynamics of process water, affected by the accumulation of organic matter and the impact of chlorine-based water treatment or water replenishment on this contamination, in post-harvest handling and operations in the production of ffFVH (available also as an R Shiny app at https://r4eu.efsa.europa.eu/app/WaterManage4You).
- Based on literature data and the industry data provided by EFSA's outsourced activities, an estimation of the model parameters for certain products was achieved, considering a product-independent inactivation rate for total bacterial counts as indicator organisms.
- The model is also capable of simulating '*what if*' scenarios based on user-defined process-specific and product/microorganism-specific parameters:

  a. Process-specific parameters

    - Temperature
    - Dilution rate
    - Free chlorine concentration and the frequency of its addition

    - Ratio product/water
    - Degradation kinetics of FC by COD

  b. Product/microorganism-specific parameters

    - Transfer coefficients of microbial cells and COD from product to water
    - Inactivation rate of microorganisms by free chlorine and
    - Coefficient representing the protective effect of the COD on microbial cells.

- If the values of the above-mentioned parameters are available for a particular process and a specific product/microorganism combination, the model may be used for:

  - Identifying suitable intervention strategies and ranges of specific conditions to maintain the microbiological quality of the process water and
  - Informing FBOps about conditions that could represent poor or proper water management practices that favour or prevent accumulation of microbial contamination in water, respectively.

- In the absence of the detailed inputs discussed above for running the model, the model provides generic quantitative information that can be used by the FBOps not only to generally understand the dynamics of the process but also to identify the main actions to be taken in the water management plan.
- The more detailed the monitoring of the process and the more thoroughly characterised the behaviour of the microorganisms in the products of concern in the presence of water disinfectant, the more reliable and, therefore, suitable the model is to support operational monitoring decisions.

5 | RECOMMENDATIONS

- The following actions are recommended for improving the application of the model for the selection of the operational conditions and for facilitating monitoring of the critical parameters that contribute to the control of the microbiological quality of post-harvest water:

  - Collect data and information pertaining to the operational conditions of the handling or processing operations, i.e. detailed monitoring of the process.
  - Collect data about the dynamics of the transfer of microorganisms between products and water.
  - Collect data characterising the magnitude of the product-specific protective effect (if any) on the microorganisms against the water disinfectant (e.g. free chlorine).
  - Collect data for the parameter estimates of the model, e.g. transfer rates of cells and COD, COD dynamics, impact of the water disinfectant on microbial inactivation, of other bacteria than TBC, as well as for other water disinfectants than FC and use it to further calibrate the model.
  - Consider alternative model structures, where different variables to COD can be used to describe the impact of organic matter on FC or other water disinfectants and, thus, the microbial inactivation throughout the processing or handling operations.

ABBREVIATIONS

AQ	assessment question
BIOHAZ	Biological Hazards
CFU	colony forming units
COD	chemical oxygen demand
ECDC	European Centre for Disease Prevention and Control
FAO	Food and Agriculture Organization of the United Nations
FBOps	food business operators
FC	free chlorine
ffFVH	fresh and frozen fruit, vegetables and herbs
FVH	fruit, vegetables and herbs
HOCl	hypochlorous acid
OPRP	operational pre-requisite program
pH	potential of hydrogen
SO	Scientific opinion
T	temperature
TBC	total bacterial count
TC	total coliforms
TOR(s)	Terms of Reference

VBNC viable but non-culturable
WHO World Health Organization

ACKNOWLEDGEMENTS

The Panel on Biological Hazards wishes to acknowledge all stakeholders that provided data for this scientific output in the context of EFSA's outsourced activities (OC/EFSA/BIOCONTAM/2021/02).

REQUESTOR

EFSA

QUESTION NUMBER

EFSA-Q-2023-00076

PANEL MEMBERS

Ana Allende, Avelino Alvarez-Ordóñez, Valeria Bortolaia, Sara Bover-Cid, Alessandra De Cesare, Wietske Dohmen, Laurent Guillier, Lieve Herman, Liesbeth Jacxsens, Lapo Mughini-Gras, Maarten Nauta, Jakob Ottoson, Luisa Peixe, Fernando Perez-Rodriguez, Panagiotis Skandamis and Elisabetta Suffredini.

REFERENCES

Abnavi, M. D., Kothapalli, C. R., Munther, D., & Srinivasan, P. (2021). Chlorine inactivation of *Escherichia coli* O157:H7 in fresh produce wash process: Effectiveness and modeling. *International Journal of Food Microbiology*, *356*, 109364. https://doi.org/10.1016/j.ijfoodmicro.2021.109364

Aiyedun, S. O., Onarinde, B. A., Swainson, M., & Dixon, R. A. (2021). Foodborne outbreaks of microbial infection from fresh produce in Europe and North America: A systematic review of data from this millennium. *International Journal of Food Science & Technology*, *56*(5), 2215–2223. https://doi.org/10.1111/ijfs.14884

Balsa-Canto, E., Henriques, D., Gábor, A., & Banga, J. R. (2016). AMIGO2, a toolbox for dynamic modeling, optimization and control in systems biology. *Bioinformatics*, *32*(21), 3357–3359. https://doi.org/10.1093/bioinformatics/btw411

Bozkurt, H., Phan-Thien, K.-Y., van Ogtrop, F., Bell, T., & McConchie, R. (2021). Outbreaks, occurrence, and control of norovirus and hepatitis a virus contamination in berries: A review. *Critical Reviews in Food Science and Nutrition*, *61*(1), 116–138. https://doi.org/10.1080/10408398.2020.1719383

Cheswick, R., Moore, G., Nocker, A., Hassard, F., Jefferson, B., & Jarvis, P. (2020). Chlorine disinfection of drinking water assessed by flow cytometry: New insights. *Environmental Technology & Innovation*, *19*, 101032. https://doi.org/10.1016/j.eti.2020.101032

Da Silva Felício, M. T., Hald, T., Liebana, E., Allende, A., Hugas, M., Nguyen-The, C., Johannessen, G. S., Niskanen, T., Uyttendaele, M., & McLauchlin, J. (2015). Risk ranking of pathogens in ready-to-eat unprocessed foods of non-animal origin (FoNAO) in the EU: Initial evaluation using outbreak data (2007–2011). *International Journal of Food Microbiology*, *195*, 9–19. https://doi.org/10.1016/j.ijfoodmicro.2014.11.005

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards). (2011). Scientific Opinion on the risk posed by Shiga toxin-producing *Escherichia coli* (STEC) and other pathogenic bacteria in seeds and sprouted seeds. *EFSA Journal*, *9*(11), 2424. https://doi.org/10.2903/j.efsa.2011.2424

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards). (2013). Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 1 (outbreak data analysis and risk ranking of food/pathogen combinations). *EFSA Journal*, *11*(1), 3025. https://doi.org/10.2903/j.efsa.2013.3025

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards). (2014a). Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 2 (*Salmonella* and Norovirus in berries). *EFSA Journal*, *12*(6), 3706. https://doi.org/10.2903/j.efsa.2014.3706

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards). (2014b). Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 2 (*Salmonella* and Norovirus in leafy greens eaten raw as salads). *EFSA Journal*, *12*(3), 3600. https://doi.org/10.2903/j.efsa.2014.3600

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards). (2014c). Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 2 (*Salmonella* and Norovirus in tomatoes). *EFSA Journal*, *12*(10), 3832. https://doi.org/10.2903/j.efsa.2014.3832

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards). (2014d). Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 2 (*Salmonella* in melons). *EFSA Journal*, *12*(10), 3831. https://doi.org/10.2903/j.efsa.2014.3831

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards). (2014e). Scientific Opinion on the risk posedby pathogens in food of non-animal origin. Part 2 (*Salmonella, Yersinia*, *Shigella* and Norovirus in bulb and stem vegetables, and carrots). *EFSA Journal*, *12*(12), 3937. https://doi.org/10.2903/j.efsa.2014.3937

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis, K., Alvarez-Ordonez, A., Bolton, D., Bover-Cid, S., Chemaly, M., Davies, R., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Jordan, K., Sampers, I., … Allende, A. (2020). The public health risk posed by *Listeria monocytogenes* in frozen fruit and vegetables including herbs, blanched during processing. *EFSA Journal*, *18*(4), 6092. https://doi.org/10.2903/j.efsa.2020.6092

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis, K., Ordóñez, A. A., Bolton, D., Bover-Cid, S., Chemaly, M., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Nonno, R., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Banach, J., Ottoson, J., … Allende, A. (2023). Microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVHs). Part 1 (outbreak data analysis, literature review and stakeholder questionnaire). *EFSA Journal*, *21*(11), 8332. https://doi.org/10.2903/j.efsa.2023.8332

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Allende, A., Avelino, A.-O., Valeria, B., Sara, B.-C., Alessandra, D. C., Wietske, D., Laurent, G., Lieve, H., Liesbeth, J., Lapo, M.-G., Maarten, N., Jakob, O., Luisa, P., Fernando, P.-R., Panagiotis, S., Elisabetta, S., Jen, B., Bin, Z., … Angela, B. (2025a). Microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVHs): Part 3 (Fresh-whole FVHs process water management plan). *EFSA Journal*, *23*, 9170. https://doi.org/10.2903/j.efsa.2025.9170

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Allende, A., Avelino, A.-O., Valeria, B., Sara, B.-C., Alessandra, D. C., Wietske, D., Laurent, G., Lieve, H., Liesbeth, J., Lapo, M.-G., Maarten, N., Jakob, O., Luisa, P., Fernando, P.-R., Panagiotis, S., Elisabetta, S., Jen, B., Bin, Z., … Angela, B. (2025b). Microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVHs): Part 4 (Fresh-cut FVHs process water management plan). *EFSA Journal*, *23*, 9171. https://doi.org/10.2903/j.efsa.2025.9171

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Allende, A., Avelino, A.-O., Valeria, B., Sara, B.-C., Alessandra, D. C., Wietske, D., Laurent, G., Lieve, H., Liesbeth, J., Lapo, M.-G., Maarten, N., Jakob, O., Luisa, P., Fernando, P.-R., Panagiotis, S., Elisabetta, S., Jen, B., Bin, Z., … Angela, B. (2025c). Microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVHs): Part 5 (Frozen FVHs process water management plan). *EFSA Journal*, *23*, 9172. https://doi.org/10.2903/j.efsa.2025.9172

EFSA Scientific Committee, Benford, D., Halldorsson, T., Jeger, M. J., Knutsen, H. K., More, S., Naegeli, H., Noteborn, H., Ockleford, C., Ricci, A., Rychen, G., Schlatter, J. R., Silano, V., Solecki, R., Turck, D., Younes, M., Craig, P., Hart, A., Von Goetz, N., … Hardy, A. (2018a). Guidance on uncertainty analysis in scientific assessments. *EFSA Journal*, *16*(1), e05123. https://doi.org/10.2903/j.efsa.2018.5123

EFSA Scientific Committee, Benford, D., Halldorsson, T., Jeger, M. J., Knutsen, H. K., More, S., Naegeli, H., Noteborn, H., Ockleford, C., Ricci, A., Rychen, G., Schlatter, J. R., Silano, V., Solecki, R., Turck, D., Younes, M., Craig, P., Hart, A., Von Goetz, N., … Hardy, A. (2018b). The principles and methods behind EFSA's guidance on uncertainty analysis in scientific assessment. *EFSA Journal*, *16*(1), 5122. https://doi.org/10.2903/j.efsa.2018.5122

Egea, J. A., Rodríguez-Fernández, M., Banga, J. R., & Martí, R. (2007). Scatter search for chemical and bio-process optimization. *Journal of Global Optimization*, *37*(3), 481–503. https://doi.org/10.1007/s10898-006-9075-3

FAO and WHO (Food and Agriculture Organization of the United Nations and World Health Organization). (2019). *Safety and quality of water used in food production and processing – meeting report*. Microbiological Risk Assessment series no. 33. https://www.fao.org/documents/card/fr/c/ca6062en/

Gil, M. I., García, M. R., Abadias, M., Sánchez, G., Sampers, I., van Asselt, E., Tudela, J. A., Moreno-Razo, A. S., Vilas, C., Martínez-López, N., Vanmarcke, H., Hernandez, N., Andujar, S., Serrano, V., Sabater, D., Truchado, P., van de Kamer, D., van der Berg, J. P., Safitri, R., … Plaza, P. (2025). *Microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVHs)*. EFSA supporting publication EN-8924. 504 pp https://doi.org/10.2903/sp.efsa.2025.EN-8924

Gombas, D., Luo, Y., Brennan, J., Shergill, G., Petran, R., Walsh, R., Hau, H., Khurana, K., Zomorodi, B., Rosen, J., Varley, R., & Deng, K. (2017). Guidelines to validate control of cross-contamination during washing of fresh-cut leafy vegetables. *Journal of Food Protection*, *80*(2), 312–330. https://doi.org/10.4315/0362-028X.JFP-16-258

Gómez-López, V. M., Lannoo, A.-S., Gil, M. I., & Allende, A. (2014). Minimum free chlorine residual level required for the inactivation of *Escherichia coli* O157:H7 and trihalomethane generation during dynamic washing of fresh-cut spinach. *Food Control*, *42*, 132–138. https://doi.org/10.1016/j.foodcont.2014.01.034

Machado-Moreira, B., Richards, K., Brennan, F., Abram, F., & Burgess, C. M. (2019). Microbial contamination of fresh produce: What, where, and how? *Comprehensive Reviews in Food Science and Food Safety*, *18*(6), 1727–1750. https://doi.org/10.1111/1541-4337.12487

Murray, K., Wu, F., Shi, J., Jun Xue, S., & Warriner, K. (2017). Challenges in the microbiological food safety of fresh produce: Limitations of post-harvest washing and the need for alternative interventions. *Food Quality and Safety*, *1*(4), 289–301. https://doi.org/10.1093/fqsafe/fyx027

Rasheed, S., Hashmi, I., & Campos, L. (2016). Inactivation of *Escherichia coli* and *salmonella* with chlorine in drinking waters at various pH and temperature levels. *Proceedings of the Pakistan Academy of Sciences*, *53*, 83–92.

Soon, J. M., Brazier, A. K. M., & Wallace, C. A. (2020). Determining common contributory factors in food safety incidents – A review of global outbreaks and recalls 2008–2018. *Trends in Food Science & Technology*, *97*, 76–87. https://doi.org/10.1016/j.tifs.2019.12.030

Suslow, T. V. (1997). *Postharvest chlorination: Basic properties and key points for effective distribution*. https://doi.org/10.3733/ucanr.8003

Zhang, B., Luo, Y., Zhou, B., Wang, Q., & Millner, P. D. (2015). A novel microfluidic mixer-based approach for determining inactivation kinetics of *Escherichia coli* O157:H7 in chlorine solutions. *Food Microbiology*, *49*, 152–160. https://doi.org/10.1016/j.fm.2015.01.013

How to cite this article: EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Allende, A., Alvarez-Ordóñez, A., Bortolaia, V., Bover-Cid, S., De Cesare, A., Dohmen, W., Guillier, L., Herman, L., Jacxsens, L., Mughini-Gras, L., Nauta, M., Ottoson, J., Peixe, L., Perez-Rodriguez, F., Skandamis, P., Suffredini, E., Banach, J., Zhou, B., … Botteon, A. (2025). Microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVH). Part 2 – A dynamic mass balance model for handling and processing operations in ffFVH using water. *EFSA Journal*, *23*(1), e9173. https://doi.org/10.2903/j.efsa.2025.9173