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# Bio-safe drinking water with or without chlorine: a review

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## ABSTRACT

Drinking water biosafety has become an increasing concern for public health. Chlorination is widely used as the main disinfection strategy worldwide but has clear and well-known byproduct issues. The Netherlands has successfully demonstrated unchlorinated approach for almost 20 years but has not been widely adopted by other countries. To chlorine or not chlorine is becoming a critical question in front of all the water utilities. This review aims to provide a good overview of current biosafety management strategies, their disadvantages, as well as the latest developments and future trends. Firstly, the advantages and deficiencies of conventional disinfection and non-disinfection were discussed. Secondly, the commonly used and promising methods for biostability assessment are described. Finally, critical views on the strategy selection for ensuring drinking water biosafety were discussed. It is recommended to achieve both biological and chemical balance by removing pathogens while minimizing the organic matter and dosing a minimum level of disinfectants, which would represent the compromise choice between the current chlorine-based disinfection and chlorine-free strategy. It's worth noting that the complexity of ensuring biosafety lies in the variations among different regions, the selection of suitable methods should be tailored to specific situations on a case-by-case basis.

**Key words:** biostability, chlorination, drinking water biosafety, unchlorinated

## HIGHLIGHTS

- Recent development in drinking water biosafety is reviewed.
- Disadvantages of chlorination are discussed.
- Successful unchlorination demostration is given regarding key points and limitations.
- Monitoring technologies are presented and reviewed.
- Chlorination and unchloriantion are compared, with a focus on disucssing future trends and directions.

## 1. INTRODUCTION

Ensuring the biosafety of drinking water is essential for promoting public health, preventing outbreaks of waterborne diseases, protecting vulnerable populations, supporting economic development, and preserving the environment. This is especially true for drinking water distribution systems, where the growth of microbes may result in water quality deterioration, such as undesirable aesthetic (e.g., tastes, odors, and visual turbidity) water quality (Van Der Kooij 2000). It may also cause infrastructure failures, such as the blockage of filters at the point of use, biofouling on distribution pipes, and corrosion caused by biological factors (Lee *et al.* 1980). In an extreme situation, the consumption of water harboring pathogens poses a hygiene threat to consumers and affects public health (Vital *et al.* 2007).

Particularly, waterborne microbial infections are recognized as a significant public health issue worldwide (Azimirad *et al.* 2018; Bailey *et al.* 2021). Approximately 4.0% of global deaths and 5.7% of the overall disease burden may be attributed to waterborne pathogens (Prüss *et al.* 2002). In 2015, over 1.3 million global deaths resulted from diarrhea, making it the fourth most prevalent cause of death in children under the age of five

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(Wang *et al.* 2016). This poses a significant health threat on a global scale, affecting not only low-income regions with limited access to safe water, sanitation, and prompt medical assistance, but also high-income regions where acute infectious diarrhea often leads to hospital visits (Wazny *et al.* 2013).

To ensure the biosafety of drinking water, water supply systems typically employ a series of water treatment processes designed to efficiently eliminate or deactivate microorganisms associated with hygiene concerns (Favere *et al.* 2021a). These approaches can generally be categorized into two groups based on the presence or absence of chlorination. Chlorine disinfection can diffuse and enter the cell to inactivate microbes primarily by reacting with cell components (Young & Setlow 2003), while the unchlorinated route is producing and distributing biologically stable water based on nutrient limitation during water treatment. Besides, in terms of monitoring and testing, microbiological indicators of water quality are utilized globally to ensure the biosafety of drinking water. Traditional detection methods like heterotrophic plate count (HPC) are commonly used to monitor microorganisms in drinking water (Allen *et al.* 2004). For instance, European Union directives specify that no total coliforms, *Escherichia coli*, or Enterococci should be detected in every 100 mL of drinking water (Sandra & Rachel 2013) In China, the total plate count in chlorinated drinking water must be below 100 CFU/mL (Colony Forming Units), and no total coliforms, Clostridium perfringens, or fecal coliforms like *E. coli* and Enterococci should be detected in 100 mL of water.

The development of science and technology in the field of drinking water keeps updating the knowledge and our understanding on biosafety. Particularly, the fast detection and advance characterization techniques on microbes and organic matter providing new insights into this specific field of research. In this review, we provide a comprehensive assessment on the limitations and advantages of widely used disinfection methods and non-disinfection methods, the biostability assays for monitoring and mitigating the risk of microorganism contamination, and the recommendations on future research and applications, with a special emphasize on the development of new methodologies and technologies.

## 2. DRINKING WATER DISINFECTION

### 2.1. Disinfection strategies

Disinfection typically serves as the final step in water treatment, aimed at eliminating pathogenic microorganisms (Sharma *et al.* 2016). The widely applicable disinfection methods include chlorine-containing disinfectants (primarily free chlorine, chloramine, and chlorine dioxide), ozone disinfection, and ultraviolet (UV) disinfection (Sedlak & von Gunten 2011), among which chlorine is extensively used as a drinking water disinfectant owing to its stable disinfection effectiveness and affordability. It penetrates cells and primarily inactivates microbes by reacting with cell components (Young & Setlow 2003). The concerns about the formation potential toxic byproducts have led water utilities to switch residual disinfection from chlorine to chloramine, which is believed to maintain a more stable residual than free chlorine, providing prolonged protection against regrowth, and it is thought to penetrate biofilms more effectively (Lee *et al.* 2011).

Ozonation disinfection works by destroying bacterial cell walls, releasing cellular components, damaging nucleic acids, and breaking down carbon-nitrogen bonds in proteins, ultimately leading to depolymerization (Hollender *et al.* 2009). Its effectiveness depends on factors such as the susceptibility of the organism, reaction time, and radical concentrations (Alexander *et al.* 2016). UV irradiation is known for its strong disinfection capabilities, disrupting the DNA structure of organisms by creating pyrimidine dimers and destroying cell membranes through reactive oxygen species (ROS) during photolysis (Gong *et al.* 2012; Liu *et al.* 2019). Although UV is efficient in eliminating chlorine-resistant protozoa such as *Cryptosporidium parvum* (Linden *et al.* 2002), it cannot maintain prolonged inactivation ability. In cases of emergencies, such as drinking water contamination due to pipeline leakage during distribution, disinfection can promptly inactivate microorganisms (Tong *et al.* 2015). Overall, disinfection serves as a relatively quick and efficient treatment approach to ensure the biosafety of drinking water.

### 2.2. Limitation of disinfection (chlorination)

As chlorine-based disinfection is the widely used strategy worldwide for drinking water production and disinfection, this section will focus on the limitations of chlorination, such as chlorine gas, sodium hypochlorite, or chloramines. The main limitations include the presence of chlorine-resistant bacteria, formation of disinfection byproducts (DBPs), generation of growth-promoting carbon sources, and promotion of antimicrobial resistance (AMR) in water (Figure 1).

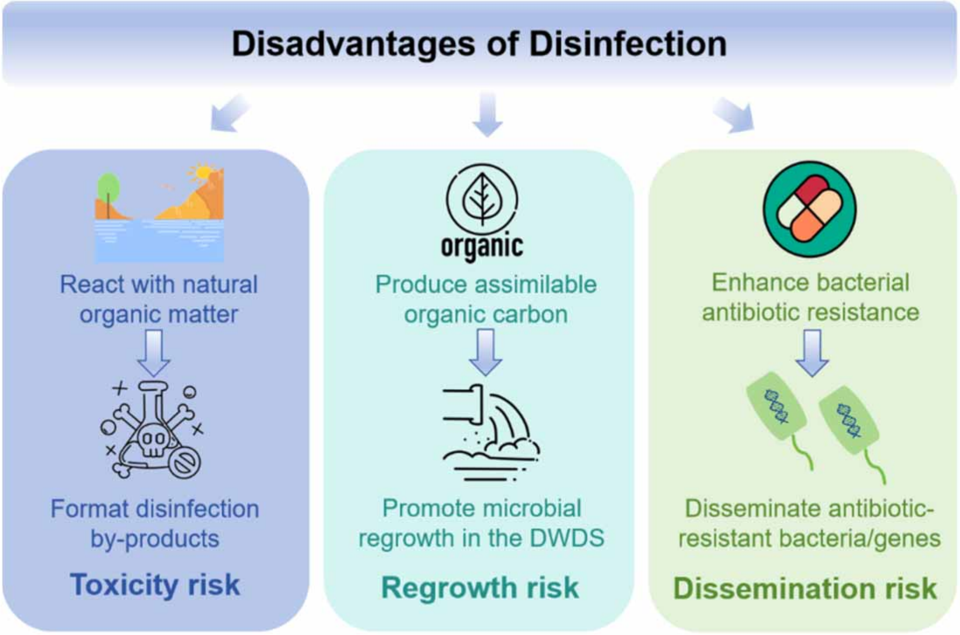

**Figure 1** | Main disadvantages of (chlorine-based) disinfection.

### 2.2.1. Disinfection-residual bacteria

Almost none of the disinfection technologies are capable of completely eliminating all microorganisms in water on a large scale (Proctor & Hammes 2015). The residual microorganisms that have the ability to grow or maintain virulence after disinfection are referred to as disinfection-residual bacteria (DRB) (Wang *et al.* 2021). Disinfection exerts a notable selection pressure on bacterial types in drinking water (Luo *et al.* 2021), meaning that not all bacterial groups behave in the same way when exposed to disinfection, e.g., chlorination (Di Cesare *et al.* 2020). The efficacy of disinfection against microorganisms is significantly influenced by intrinsic properties of microbial cells, such as a robust cell envelope observed in Mycobacteria or the ability to form endospores in certain microorganisms, enabling these microbial communities to withstand disinfection stress (Ivone *et al.* 2013). Another important mechanism is the bacteria's ability to recover from a damaged state, notably seen in certain groups like Beta- and Gammaproteobacteria (Becerra-Castro *et al.* 2016). Undesirable DRBs may pose various risks related to changes in regrowth potential, biofouling potential, antibiotic resistance levels, etc. (Garner *et al.* 2018; Yu *et al.* 2018). In the study by Wang *et al.* (2021), at the genus level, *Pseudomonas*, *Mycobacterium*, *Legionella*, *Sphingomonas*, and *Bacillus* showed the highest increase in relative abundance after chlorine disinfection.

### 2.2.2. Disinfection byproducts

The disinfection process can lead to the formation of haloacetic acids (HAAs) and trihalomethanes (THM4), such as chloroform, dibromochloromethane, dibromo-dichloromethane, and bromoform, which are identified as byproducts resulting from the reaction of disinfectants with precursors present in raw water. The concentrations of DBPs increased with increased chlorine dosages and high levels of precursors (Niu *et al.* 2017). These precursors may originate from natural organic compounds (NOM) (Ruan *et al.* 2021; Maqbool *et al.* 2022), as well as anthropogenic contaminants such as endocrine-disrupting chemicals (EDCs), pharmaceutical and personal care products (PPCPs), pesticides and herbicides, cyanotoxins, textile dyes, surfactants (Richardson & Postigo 2015; Postigo *et al.* 2017). These DBPs are considered toxicological contaminants with mutagenic, teratogenic, and carcinogenic properties. For instance, epidemiological research has indicated links between the consumption of chlorinated tap water with increased THM4 levels and negative health effects, such as bladder cancer (Costet *et al.* 2011), miscarriages (Waller *et al.* 1998), and birth defects (Grellier *et al.* 2010; Wright *et al.* 2017), among others. It is important to note that measuring THM4 concentrations to assess exposure to DBPs is not because they have been demonstrated to be the main contributors to cancer risk; rather, they are carcinogens, and their concentrations are assumed to be associated with those of other DBPs (Li & Mitch 2018). Furthermore, these links do not necessarily represent a cause-and-effect relationship; for example, epidemiology studies addressing different types of birth defects do not universally support a causal connection between exposure to chlorination DBPs and any birth defects (Hrudey 2009).

However, the dangers of DBPs cannot be overlooked. Firstly, humans are exposed to DBPs through various pathways, including direct consumption of water and dermal absorption during activities such as showering, bathing, and swimming in treated pools (Li *et al.* 2017b; Qiu *et al.* 2023). Secondly, the analysis and regulation of DBPs present challenges due to the diversity of precursors, resulting in the formation of a highly complex mixture of DBPs likely numbering over 1,000 (Li & Mitch 2018). Some of these DBPs, including both regulated and unregulated compounds, have demonstrated higher genotoxicity and cytotoxicity compared to certain regulated compounds (Richardson & Postigo 2015; Plewa *et al.* 2017), with insufficient understanding of their toxicological risks. Thirdly, although the causal linkage between diseases and DBP exposure is unclear, a positive correlation has been found between bladder cancer and THM4 and HAAs (Grellier *et al.* 2015).

Utilities have made efforts to optimize the use of disinfectants to achieve both pathogen reduction and regulatory limits on DBPs. For instance, utilities are transitioning from relying solely on chlorine disinfection to combining primary disinfectants (e.g., ozone, UV) with chloramines as secondary disinfectants (Seidel *et al.* 2005; Dotson *et al.* 2012). However, each disinfectant promotes the formation of distinct classes of DBPs. Chloramination promotes the production of nitrosamines (Liu *et al.* 2020)and iodinated DBPs (Hu *et al.* 2018), ozone leads to the formation of haloacetaldehydes and halonitromethanes (McCurry *et al.* 2016), while medium pressure UV irradiation has the potential to generate dichloroacetonitrile (Ye *et al.* 2018).

#### 2.2.3. Disinfection-generated organic matter (DgOM)

Another concern arises from the possibility that disinfected natural organic matter present in surface water may change the molecular composition of dissolved organic matter (DOM) and transform into assimilable organic carbon (AOC) (Ramseier *et al.* 2011; Ye *et al.* 2018), introducing uncertainty to the biological stability of the finished drinking water (Liu *et al.* 2015a). This type of disinfection by-product, related to biodegradable organic matter, could form following various disinfection approaches, such as ozone, ferrate, permanganate, chlorine dioxide, chlorine, and chloramine (Ramseier *et al.* 2011; Liu *et al.* 2015b; Li *et al.* 2018). For instance, it is acknowledged that several disinfection methods, including ozonation (Park *et al.* 2016) and chlorination (Liu *et al.* 2002), could increase the AOC concentration during the drinking water treatment processes. The heightened availability of nutrients promotes microbial regrowth, which is contrary to the original objectives of water disinfection, namely ensuring biological safety. Previous studies have indicated a strong correlation between bacterial growth and AOC concentrations. In a study by Li *et al.* (2018), an AOC concentration of less than 135 μg/L in the distribution water distribution system (DWDS) limited intact cell concentrations.

#### 2.2.4. Antimicrobial resistance

Disinfection impacts the antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in drinking water, which has garnered considerable research attention (Karkman *et al.* 2018). Current disinfection methods not only fail to completely eradicate ARB and ARGs before entering the drinking water distribution system (DWDS), but they may also actively contribute to their development and spread (Sanganyado & Gwenzi 2019). Disinfection methods known to enhance AMR include chlorination, chloramine, and UV irradiation. Less commonly used disinfection technologies, such as ozonation and other advanced oxidation processes, are not considered selective factors (Guo *et al.* 2017; Zhang *et al.* 2020). For example, UV and chlorine treatments effectively inactivated bacterial cells but incompletely degraded ARGs (Stange *et al.* 2019). Whereas, low-dose oxidants may stimulate the production of ROS (Jin *et al.* 2020), increasing the permeability of bacterial cell membranes and facilitating the transfer of ARGs among bacteria (Zhang *et al.* 2017). Chlorine exposure has been found to induce the overexpression of efflux pumps (Hou *et al.* 2019), enhancing bacterial antibiotic resistance (Blair *et al.* 2015). The remaining ARGs could be assimilated into pathogenic microorganisms through transformation and transduction in water settings, posing a health risk to humans (Zhang *et al.* 2020).

## 3. THE BIOSAFETY AND BIOSTABILITY OF UNCHLORINATED DRINKING WATER

### 3.1. Chlorine-free drinking water supply in The Netherlands

In practice, the whole country of the Netherlands, and some regions of Switzerland, Austria, and Germany realized producing and distributing chlorine-free drinking water (van der Kooij *et al.* 2002; Hammes *et al.* 2010a;

Rosario-Ortiz *et al.* 2016). The well-known example is the Netherlands, for which the chlorination was halted in 2005 (Hijnen *et al.* 2018a). Instead, the approach shifted toward suppressing growth in the distribution network by inducing nutrients limited biostability, rather than relying on chemical disinfectant residuals. This was accomplished through systematic optimization of drinking water system by combining efforts from water source, water treatment, and water distribution.

Firstly, prioritize the use of the best available sources, starting with microbiologically safe groundwater, followed by surface water with soil passage like managed artificial recharge (MAR) or riverbank filtration (RBF), and the direct treatment of surface water (e.g., abstracted from dunes, and the rivers Meuse and Rhine) employing multiple barrier treatments (Figure 2).

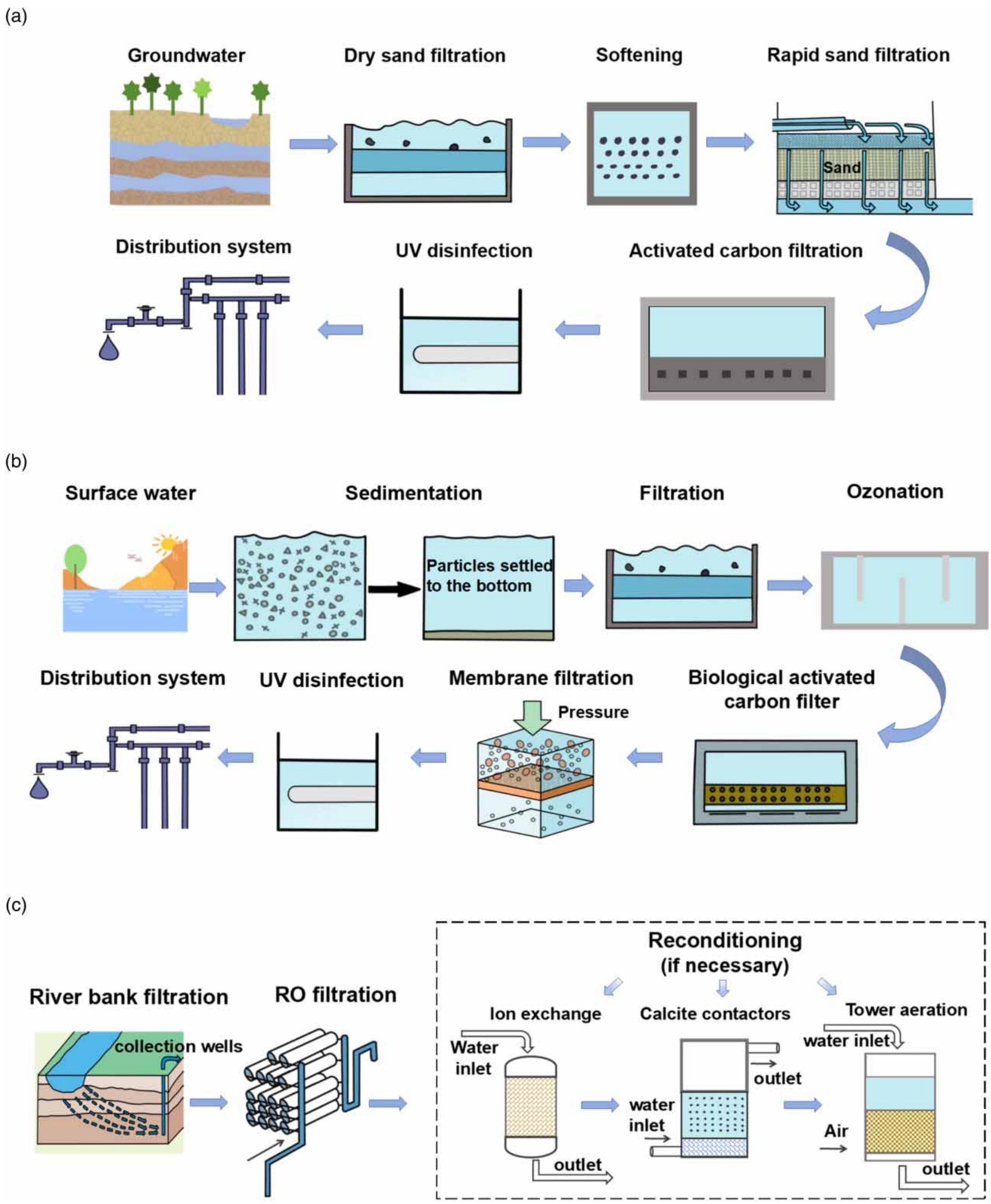

**Figure 2** | (a) Full-scale conventional water treatment processes at the drinking water treatment plant, adapted from Sousi *et al.* (2020b); (b) The direct treatment of surface water employing a series of measures, adapted from Smeets *et al.* (2009b); (c) One-step reverse osmosis system based on RBF, adapted from Zhai *et al.* (2021).

Secondly, the direct treatment of surface water requires a series of measures, opting for preferred physical process treatments including sedimentation, filtration (e.g., ultrafiltration, nanofiltration, and reverse osmosis (RO) (Kamp *et al.* 2000), and UV disinfection, if indispensably necessary, consider oxidation using ozone (often combined with biological treatment such as sand filters or biologically active carbon filtration), peroxide or $H_2O_2$-UV irradiation (Kruithof *et al.* 2007), but chlorine usage is avoided. Pre-treatment through soil infiltration or applying post-treatment via slow sand filtration proved effective in achieving low levels of high molecular weight organic carbon and microbial growth potential in the production of drinking water (van der Kooij *et al.* 2017). Besides, employing ultrafiltration post-treatment in traditional surface water treatment facilities demonstrated a promising method to improve the biological stability of drinking water (Schurer *et al.* 2019).

Thirdly, avoid growth and contamination during distribution, and set strict hygiene procedures for the construction, maintenance, and repair of DWDS. To prevent the growth of microorganisms, it also requires using biostable pipe material (Park *et al.* 2021; Duong *et al.* 2023). Keeping the monitoring of water quality and system performance on a regular frequency is also important for preventing potential adverse health impacts, for example monitoring program for tap water quality and the malfunction of the system (e.g., avoidance of stagnant areas and prevention of sediment accumulation (Liu *et al.* 2014; Ling *et al.* 2018)). The remarkably low number of outbreaks and complaints in the Netherlands suggests the effectiveness and safety of the Dutch approach (de Moel *et al.* 2006; Smeets *et al.* 2009a). Moreover, certain circumstances in the Netherlands, encompassing population density, geography, and economy, were conducive to these procedures (Smeets *et al.* 2008).

Beyond the established achievements, the Dutch scientist proposed and realized the one-step reverse osmosis (OSRO) concept recently (Zhai *et al.* 2021), which combines RBF and RO for drinking water treatment. In brief, river water flows through the soil passages to eliminate particles, organic compounds, and microorganisms. Subsequently, the well-pretreated water by nature is abstracted and pumped through RO membrane to provide high-quality drinking water. Alternatively, RBF can be utilized not only as its natural form but also integrated with artificial recharging, constructed wetlands, and various other methods of natural water purification (D'Alessio *et al.* 2018). The OSRO treatment is recognized for its capability to efficiently remove particles, pathogens, and nutrients (Albergamo *et al.* 2020). Moreover, it has been proved that even artificially adding pathogens into OSRO-treated water, the pathogens could not grow in the water and simulated plumbing system, confirming it is a green and safe technology for producing high-quality drinking water (Learbuch *et al.* 2019).

The implementation of water safety plans regarding emergencies is rapidly increasing in the Netherlands. Some general corrective actions are presented here. For example, emergency response measures adopted include shutting down the intake at moments of poor raw water quality and discharging the pollutants from the intake reservoir back into the river. Moreover, emergency power supplies and dividing the system into water-tight compartments are employed to address risks such as power loss or flooding. In case of contamination occurs during the distribution, the affected area is isolated by selectively closing valves while keeping pressure in the system. Meanwhile, to avoid pressure losses, the water supply security plans enable alternative systems to partially take over the water supply in the affected region. Additionally, flushing the system where feasible and chlorination may be employed to deactivate pathogens that could persist in the distribution system after flushing. Customers will receive notifications through door-to-door boiling notices, the internet, and radio broadcasts. Regional crisis centers are activated in cases of considerable-scale events. The boiling notice will be lifted upon microbial sampling confirms water safety (van Lieverloo *et al.* 2006; Smeets *et al.* 2009b).

## 3.2. Biostability assays

When producing and distributing biostable drinking water (i.e., without the use of additional residual disinfectants), precisely monitoring the biological stability of drinking water is of high priority (Favere *et al.* 2021b).

### 3.2.1. Chemical assays: biodegradable organic matter

Several methods such as biodegradable dissolved organic carbon (BDOC) and AOC are employed to evaluate the biodegradable organic carbon in drinking water, which are recognized as the primary nutrients that bacteria preferentially consume because they are prone to bacterial degradation. Generally, a low AOC level (below 50 μg/L) can be recognized as biostability in chlorinated drinking water (Kooij 1992), which should be below 10 μg C/L in non-chlorinated systems (van der Kooij 1992).

Although valuable insights have been gained through these indicators, BDOC and AOC as representations to assess biodegraded organic carbon are limited to scenarios where growth relies solely on biodegradable carbon and overlook the quantity of other organic matter available for bacterial utilization in drinking water (van der Kooij 2000). AOC was initially assessed using strains *Pseudomonas fluorescens* strain P17 and *Spirillum* strain NOX, but not all biodegradable organic carbon present in drinking water could be assimilated by these strains (Wu *et al.* 2022). Moreover, AOC tests by definition do not evaluate autotrophic growth and exhibit limitations in assessing nutrients beyond organic carbon (Prest *et al.* 2016). An improved method has been developed to measure AOC in drinking water, which is based on batch growth of a natural microbial community until the stationary phase is reached and all AOC is consumed ($1\ \mu g\ \mathrm{AOC} = 1 \times 10^7$ cells) (Hammes & Egli 2005). For instance, slowly biodegradable compounds have been demonstrated to influence bacterial regrowth in DWDS significantly (Hijnen *et al.* 2018b). Furthermore, other growth-limiting nutrients, e.g., phosphate (Miettinen *et al.* 1997), are also underestimated, which may be more crucial in certain situations for comprehending microbial growth in full-scale drinking water systems. In addition, considering the complex nature of AOC regarding a mixture of chemicals (Terry & Summers 2018) and the conventional assays for DOM only from a specific aspect (Hem & Efraimsen 2001; Kim *et al.* 2017; Wang *et al.* 2022). For instance, the absorbance at 254 nm in the UV–visible absorbance spectroscopy is indicative of aromatic compounds (Yan *et al.* 2017). Excitation-emission matrix employs parallel factor analysis to differentiate various fluorescent components (Lin & Guo 2020), but it cannot identify nonfluorescent DOM components. High-performance size exclusion chromatography can determine the relative molecular weight component of DOM (McAdams *et al.* 2018). However, these methods yield relatively abstract conclusions and cannot offer crucial information about the source and specific compositions of organic matter.

Therefore, it is desirable to detect accurately and understand the transformation of organic matter at the molecular level as the type and composition of nutrients are essential for both bacterial growth and shaping the bacterial community (Elhadidy *et al.* 2016; Nescerecka *et al.* 2018).

Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) is a promising method to provide crucial information in terms of element combination, compound compositions, and the fate of DOM in drinking water (Lavonen *et al.* 2015). In the study by Huang *et al.* (2019), Pseudomonas aeruginosa was selected to evaluate the extent of AOC formation from NOM upon chlorination, UVC irradiation, and photocatalysis ($TiO_2$-UVA). and FT-ICR-MS was utilized to establish connections between transformations of NOM to the corresponding AOC formation. In another study by Hou *et al.* (2022), a pilot-scale system was implemented to enhance real drinking water treatment procedures employing the coagulation coagulation/ ozonation/catalytic ceramic membrane filtration process in a continuous mode and FT-ICR MS was utilized to offer insights into DOM transformation at the molecular level and the correlation between AOC and various classes of compounds. However, the application of this technology has its limitation. Firstly, the FT-ICR-MS data employed for analysis typically possess a relatively high mass cutoff (namely, mass-to-charge, m/z), indicating the exclusion of molecules with lower molecular weight, while these fractions are demonstrated to contribute to biodegradable substances. Secondly, electrospray ionization prefers easily ionized compounds, thus the detection of compounds with low ionization efficiency would be limited (Huang *et al.* 2020). To date, the utilization of the FT-ICR MS technology in analyzing the DOM transformation of drinking water at the molecular level is promising.

### 3.2.2. Biological assays: microbiological parameters

Another aspect of biological stability concerns microbiological parameters, with the widely used parameter being traditional HPCs for monitoring microbial drinking water quality (Srinivasan & Harrington 2007; Favere *et al.* 2021b). However, this assay is time- and labor-consuming, and the results only represent a limited and specific fraction of culturable microbial communities in water samples. For instance, regulated bacteria like *E. coli* are more susceptible to disinfectants than most pathogens (van Lieverloo *et al.* 2007), while certain protozoa like *Cryptosporidium* can be exposed to similar dosages of chlorine disinfectant with little or no effect. This could lead to prolonged undetected serious pathogenic contamination in actual operations, thus highlighting the limitations of traditional detection methods such as HPC.

In view of this, it is necessary to use culture-independent methods to detect the activity and number of microorganisms. Bacterial cell viability can be evaluated by labeling cells using fluorescent dyes that target specific bacterial physiological features, which include cell membrane integrity, membrane potential, respiratory activity, that can be detected with epifluorescence microscopy or flow cytometry (FCM) (Prévost *et al.* 1998; Hoefel *et al.* 2005; Berney *et al.* 2008; Hammes *et al.* 2011). For example, the flow-cytometric total cell concentration proved to be a credible and reasonable parameter for illustrating bacterial growth throughout the 18-month sampling campaign in both drinking water treatment and the distribution system (Hammes *et al.* 2010b).

Moreover, adenosine triphosphate (ATP) can serve as a valuable assay of biological activity (Pan *et al.* 2021). This method is capable of specifically detecting intracellular and extracellular ATP and estimating the average bacterial ATP content per cell by integrating it with FCM (Hammes & Egli 2010; Hammes *et al.* 2010c). FCM and ATP measurements have been demonstrated to be effective for precise detection and rapid analysis, offering more descriptive value for assessing the treatment effectiveness of drinking water than conventional HPC measurements (Siebel *et al.* 2008; van der Wielen & van der Kooij 2010; Vital *et al.* 2012).

Bacterial community profiling has expanded the assessment scope of biological stability by examining community structures. A shift in the microbial community composition may indicate instability, emphasizing the need to understand bacterial dynamics for evaluating microbial risk and ensuring the delivery of safe drinking water (Pinto *et al.* 2014). Molecular methods for this purpose are typically categorized into fingerprinting methods and high-throughput sequencing techniques (HTS). While fingerprinting methods like denatured gradient gel electrophoresis (DGGE)and terminal-restriction fragment length polymorphism (T-RFLP)track changes in microbial communities, they primarily identify predominant species (Douterelo *et al.* 2014). In contrast, HTS analyze microbial diversity and structure across different water settings, offering deeper insights. For instance, in a study by Pinar-Méndez *et al.* (2022), 16S rRNA metabarcoding and microbial water quality indicators were used to study bacterial community dynamics in a full-scale DWTP.

## 4. CONCLUSION AND OUTLOOK: TO CHLORINATE OR NOT TO CHLORINATE?

To summarize (Figure 3), this review provides a comprehensive overview of drinking water biosafety by chlorination and unchlorinated but by nutrients limitation approach. The main disadvantage of chlorination includes formation of DBPs and AOC, promotion of chlorine-resistant microbes and AMR. By the successful demonstration of the Netherlands, we propose the combination of efforts in source, treatment, and distribution to achieve unchlorinated water supply. At the end, chemical and microbiological based monitoring methods for biosafety and biostability were reviewed.

### 4.1. Balancing the immediate microbial risks and chronic chemical risks

Optimizing the combinations of disinfectants has been preferred as a useful approach to balance the immediate threat from pathogens in contrast to the chronic risk linked with DBPs. However, this is a highly complex balance

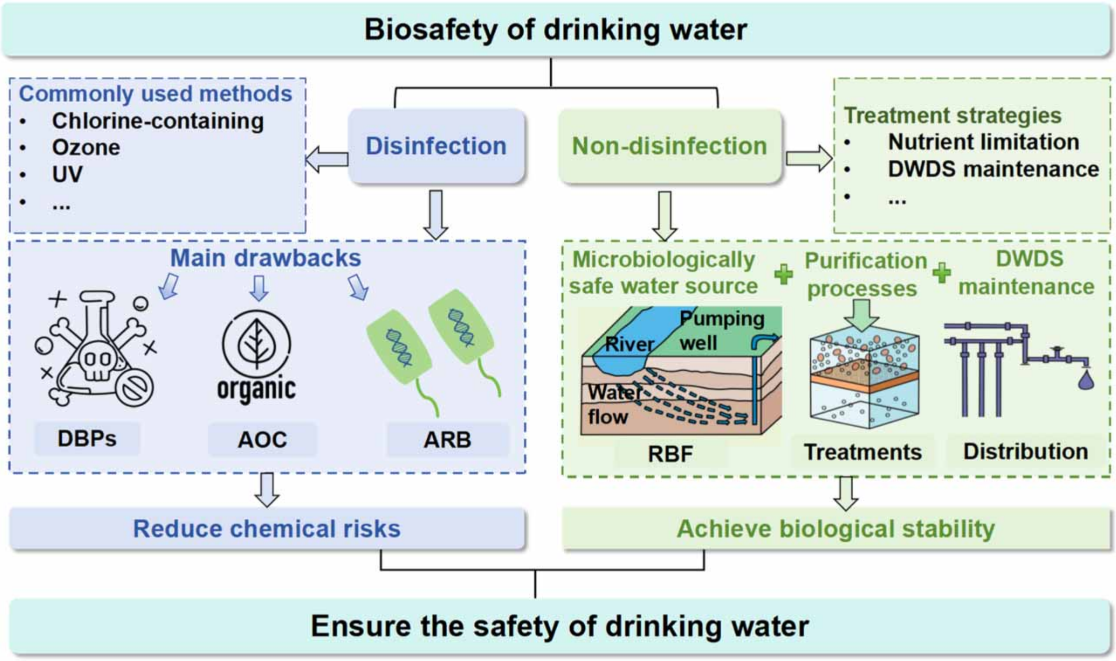

**Figure 3** | A schematic illustration of insights into the use of disinfection and non-disinfection strategies to ensure drinking water biosafety.

(Li & Mitch 2018). In addition, employing chlorination to more than one step by adjusting the time interval and dosage ratio could improve the disinfection efficiency like a higher overall Ct value and a shorter recovery time, and this strategy could reduce chlorine consumption costs. However, the formation of DBPs has not been clarified and needs further research to suit the production conditions (Li *et al.* 2017a). Additionally, sensor-based monitoring and evaluation systems can be utilized to collect and analyze data to obtain precise measurement results and these data gained at the passive chlorinator and throughout the distribution network can help to develop a predictive model for determining an optimal initial chlorine dose (Wilson *et al.* 2017; Lindmark *et al.* 2022). The WHO guidelines for chlorination suggest maintaining chlorine levels equal to or exceeding 0.5 mg/L across the distribution system and a minimum level of 0.2 mg/L chlorine at the point of delivery in piped infrastructure (Lindmark *et al.* 2022). Real-time monitoring and timely detection of system malfunctions could trigger alerts for the maintenance of passive chlorinators to improve long-term service delivery (Andres *et al.* 2018). These technologies enable real-time monitoring and control of water quality parameters, guiding prompt adjustments in disinfectant dosages and treatment processes to lie on water quality standards while maintaining optimal disinfection levels and minimizing the formation of DBPs. However, there are some limitations of passive in-line chlorination such as frequent manual testing (labor and time-consuming) to detect technical failure and long-term sustainability not well-studied (Lindmark *et al.* 2022). In this review, we only discussed the commonly used disinfection methods. Other physical and chemical approaches, such as dry and moist heat, ethylene oxide, hydrogen peroxide, and alcohol were not mentioned due to their limited application in full-scale systems (Bharti *et al.* 2022). The direction of less-toxic disinfectant alternatives would mean the possibility of having greener disinfection approaches.

In the case of the Netherlands, drinking water is produced without chlorine, which completely eliminates the chronic risk associated with lifelong exposure to potentially carcinogenic DBPs. Achieving this requires multi-barrier water treatments and a well-maintained distribution system, which may not be applicable for other cases. For example, utilizing soil infiltration as a pre-treatment or slow sand filtration as a post-treatment requires substantial area of spaces, the adequate land area for abstracting groundwater is in total about 1,500 square kilometers, 4.4% of the ground in the Netherlands (Smeets *et al.* 2009b).In addition, unique geographical conditions such as sandy aquifers covered by impermeable clay layers act as a protective barrier, shielding the groundwater from potential surface contamination (Schijven & Hassanizadeh 2002). Moreover, optimized membrane technologies (e.g., nanofiltration, RO) can remove all microbes from the water when the integrity of the membrane and connections is guaranteed (Kamp *et al.* 2000), but with high costs and energy consumption (Vingerhoeds *et al.* 2016; Sousi *et al.* 2020a). Furthermore, it is also associated with advanced and expensive monitoring and maintenance of the distribution system. As discussed, organic matter enables the consumption of disinfectants and conversion into DBP (including AOC). Therefore, another point of interest for achieving such biological and chemical balance would be removing pathogens while minimizing the organic matter and maintaining a minimum level of disinfectants. This would be the compromise choice between the current chlorine-based disinfection and the successful but challenging chlorine-free in the Netherlands.

### 4.2. Advancing high-resolution and high frequency monitoring methods

It is more than clear that HPC and *E. coli* are not good indicators for monitoring and managing drinking water biosafety and bio-quality. Methodology that could provide fast, accurate, and preferably molecular and fundamental insights on microbiological processes to bring forward the microbiological water quality measurements to actionable management. Examining native bacterial community compositions and community dynamics could be linked to the proposed actions, providing an evaluative framework for the microbial management of drinking water. Precise and sensitive microbial monitoring is crucial for evaluating microbial processes and further ensuring the biosafety of drinking water for the end consumer. Recognizing the need for these aspects, methods including ATP, FCM, and HTS technologies, especially the combination of multi-omics (e.g., metagenomics, metatranscriptomics, metaproteomics, and metabolomics) could expand our understanding on the activity and diversity drinking water microbes. Moreover, Parameters aimed at monitoring more specific microbial groups, such as heterotrophic or Aeromonas plate counts, Mycobacterium spp., and fungi, are recognized as reliable indicators for detecting regrowth in DWDS (van der Wielen *et al.* 2016). In return, this would offer valuable insights on how disinfectants impact microbial ecology and guide the practical work on biological water quality monitoring. Additionally, some of these technologies (e.g., ATP and FCM measurement) could be (or already) implemented in real-time monitoring in water supply utilities, combing with machine learning models, it will bring revolutionary changes in drinking water supply paradigm, particularly regarding the dosage of disinfectants and the response from distribution system (Czyczula Rudjord *et al.* 2022; Kang *et al.* 2023).

It should be mentioned that the complexity of ensuring biosafety lies in the variations among different countries, which exhibit various source water characteristics, employ distinct conditions of treatment and maintenance, and implement different analytical methods for monitoring (van der Kooij 2000; Laurent *et al.* 2005). The selection of suitable methods should be on a case-by-case basis regarding specific situations.

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## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

## CONFLICT OF INTEREST

The authors declare there is no conflict.

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