IWA 17th SWWS AND 9th ROS
10th–14th November, 2024
Curitiba-Brazil

Aerobic Spore-Forming Bacteria as indicators of microbiological water safety in Point-Of-Use systems

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Highlights:

- Aerobic Spore-Forming Bacteria (ASFB) can be determined using a simplified and cost-effective methodology, contrasting with the complexity of protozoan quantification.
- ASFB exhibit resistance and removal behavior similar to protozoa.
- ASFB can be used as an alternative indicator for protozoan removal in Point-Of-Use systems.

Keywords: Protozoa; Household Slow Sand Filtration; Chlorination

INTRODUCTION

The recent "Guidelines for drinking-water quality: small water supplies" published by the World Health Organization establishes that the parameters used for water monitoring in small systems should not only protect the health of users but also reflect the technical, financial, and human limitations of each locality (WHO, 2024). Furthermore, the guide suggests that regulations for these systems should prioritize ensuring microbiological water safety (WHO, 2024).

Microbiological risks associated with drinking water are mostly related to the presence of bacteria, viruses, helminths, and protozoa with the potential for transmitting infectious diseases (WHO, 2024). Regarding protozoa, monitoring in Point-Of-Use systems is challenging since the analysis is costly and methodologically complex, resulting in the need for alternative indicators (Pereira et al., 2023).

Aerobic Spore-Forming Bacteria (ASFB) share similarities with protozoa (life cycle, resistance to chemical and physical stress, morphology, transportation, and retention due to size approximation), making them efficient indicators and easily monitorable in real systems compared to resistant pathogens (Headd; Bradford, 2016). Therefore, this study aimed to analyze the viability of aerobic spore-forming bacteria as an indicator of microbiological water safety assurance in a three-barrier Point-Of-Use system.

METHODOLOGY

The Point-Of-Use system consisted of (i) pre-treatment by sedimentation and filtration through a non-woven synthetic fabric, (ii) household slow sand filtration, and (iii) disinfection via chlorination with calcium hypochlorite. The system was supplied daily with surface water sourced from the Monjolinho

River, located in São Carlos, São Paulo, Brazil. The ASFB was analyzed according to procedure 9218 (APHA, AWWA, WEF, 2017). Aliquots of 100 mL were stored in borosilicate bottles and subjected to thermal shock to inactivate vegetative cells. This process involved heating in a water bath with constant agitation at 80 °C for 10 minutes, followed by cooling in an ice bath. Finally, the aliquots were filtered through 0.45 µm membranes and inoculated onto nutrient agar containing trypan blue at 35±0.5°C for 24±2 hours. The results were expressed as the number of spores.100 mL^{-1}.

The estimated inactivation of *Giardia* cysts by the adopted disinfection process was estimated using the model proposed by the United States Environmental Protection Agency (USEPA, 1991), which relates the concentration of Free Residual Chlorine - C ($mg.L^{-1}$), contact time – t (minutes), pH, and water temperature - T (°C). Equation 1 describes the model.

$$Giardia\ cysts\ inactivation\ (log) = \frac{C*t}{0{,}2828 * pH^{2{,}69} * c^{0{,}15} * 0{,}933^{T-5}} \quad (1)$$

Normality distribution was evaluated by Shapiro-Wilk test, while hypothesis tests by Student's t-test or Mann-Whitney test. All statistical analyses were performed on PAST 4.03 software (PAlaeontological Statistics) considering a significance level of 5% ($p < 0.05$).

RESULTS AND CONCLUSIONS

Over 140 days of Point-Of-Use system operation, it was possible to observe the removal of ASFB across the multiple barriers proposed in the treatment system (Figure 1a). Spores were removed in the employed pre-treatment, although without statistical significance ($p > 0.05$), which aligns with the findings of Cai et al. (2022) regarding the effectiveness of ASFB removal through clarification processes involving sedimentation. On the other hand, through the filtration and chlorination disinfection steps, spores were significantly removed and inactivated ($p < 0.01$).

Using the proposed chlorination process, with a fixed contact time (C) of 30 minutes and free residual chlorine of 0.91±0.55 $mg.L^{-1}$, the product C*t (free chlorine versus contact time) was 27.3±16.5 $mg.min.L^{-1}$. Thus, the average inactivation of *Giardia* cysts was estimated to be 1.39±0.90 log (Figure 1b). Comparatively, ASFB removal through the chlorination process resulted in removals of 0.90±0.69 log, a value lower than the estimated inactivation for *Giardia*.

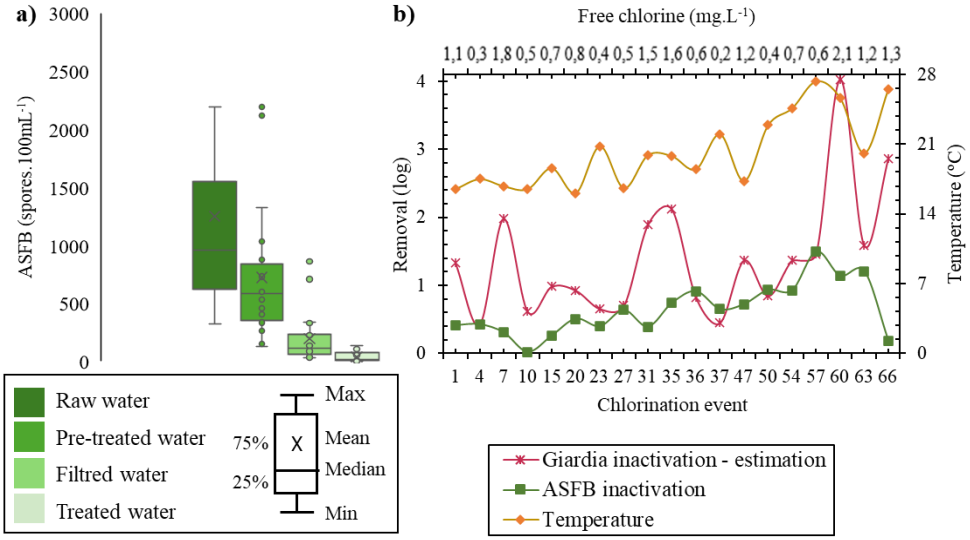

Figure 1: (a) Reduction of ASFB by the treatment system and (b) estimation of *Giardia* cysts inactivation through chlorination.

According to Oliveira et al. (2018), an alternative parameter must possess specific characteristics to serve as an indicator of water treatment efficiency, including: (i) containing a concentration higher than that of pathogenic organisms in raw water; (ii) being quantifiable using simplified and low-cost techniques; (iii) exhibiting removal mechanisms similar to pathogenic organisms; and (iv) being removed at a similar or lower rate than pathogens. Through the protozoan inactivation estimated, it is evident that ASFB were reduced at a lower rate compared to *Giardia* cysts and via a simplified quantification technique, indicating that these organisms can be alternative indicators of chlorination efficiency for inactivate resistant organisms.

Based on the experience of using ASFB as an alternative indicator of protozoan removal in a Point-Of-Use system, it can be inferred that these organisms have the potential to be viable indicators for assessing the microbiological safety of treated water in Point-Of-Use systems due to their ease of determination and behavior similar to protozoa. This finding can facilitate the monitoring, enhancing the efficacy of implementing water treatment technologies, particularly in remote, rural, and isolated communities.

ACKNOWLEDGMENTS

This work was supported by The Royal Society (ICA\R1\201373 - International Collaboration Awards 2020), the Brazilian National Council for Scientific and Technological Development (proc. 442074/2023-9, 308070/2021-6), and the Coordination for the Improvement of Higher Education Personnel (88887.683608/2022-00, CAPES-PROEX - Brazil).

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