Impact of the physiological state of mold spores on their resistance to chlorinated disinfectant

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Introduction

Fungi are a major source of contamination of agri-food environments because of their ability to survive and grow on foods, particularly through the production of spores that also facilitate their dissemination. They can directly or indirectly cause the contamination of food products. To reduce the risks of contamination, manufacturers use a wide variety of chemical disinfectants. Their fungicidal efficacy is evaluated according to the protocols described in many standards, including the standard NF EN 17272 (2020) for the specific evaluation of process-product couples for airborne room disinfections. However, these standards may have some limitations. Indeed, the chosen species, the physiological conditions of fungi during sporulation and the presence of a soiling substance can have an impact on the survival rate of conidia in response to a treatment with a disinfectant. The impact of these parameters was then investigated in this study.

Material and methods

Two strains of *Penicillium commune* isolated from spoiled cheeses were used. Spores were produced on reduced aw (aw 0.95) or non-reduced aw (aw 0.99) media. Then, spores were collected without being rehydrated or being rehydrated in different interfering substances (whole or skim milk, BSA 0.3 g/l or 1/20th skim milk) and subjected to a 0.1% chlorine treatment for 12.5 min after adhesion on stainless steel surfaces.

Results and conclusion

Where spores were in contact with skim or whole milk, the activity of the chlorine solution is nil or greatly reduced. In contrary, when spores were suspended in 0.3 g/L BSA or 20-fold diluted skim milk (standard conditions), no surviving spores were counted in almost all cases with a log reduction greater than or equal to 3-3.5 log. Dry spores produced on a medium with reduced aw displayed intermediate phenotypes.

This study then showed that the use of spores rehydrated in 1/20th skim milk or in BSA at 0.3 g/l could cause an overestimation of the efficiency of chlorine towards dry spores produced under suboptimal growth conditions and could call into question the normative conditions used to validate the fungicidal effect of biocides. Besides, these results demonstrate once again the importance of cleaning prior to disinfection.

Impact of environmental and application conditions on the inactivation of dry fungal spores by disinfectants

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Fungi are a major source of contamination of agri-food environments because of their ability to survive and grow on foodstuff, but also because of their sporulation ability, which eases their dissemination in production environments. Biocidal products are key tools for reducing contamination risks, and their efficacy can be evaluated through normative assays (ex. NF EN 17272 (2020) standard). Though the fungicidal effectiveness of biocide products can be assessed with several standards, few studies were carried out to assess the effectiveness of disinfection procedures under near-reality conditions. This work aims to investigate the impact on dry fungal spores of disinfection procedures used in the field.

The dry harvest of fungal spores after cultivation under a moderate water stress increases the intrinsic resistance to biocides and is assumed to better mimic the actual physiological state of spores in food production environments. Dry spores of *A. flavus, C. cladosporioides, M. circinelloides and P. commune* were then adhered to stainless steel surfaces and submitted in pilot-plant conditions to 4 commercial disinfectants containing chlorine, hydrogen peroxide, triamine or glycolic acid. Different application modes were used, *ie* fumigation, nebulization and foam gun. Inactivation of spores was assessed according to the temperature, the humidity and the combination of biocide concentration and contact time for instance, in order to identify the most impacting factor and leading to the best results.

According to the disinfection process, results were highly variable, with a mean inactivation ranged from 1,6 to >7 log reduction with the highest inactivation using fumigation. The target fungal species, the contact time, the biocide concentration but also the ambient temperature and foam density can affect the disinfection effectiveness depending on the tested procedure. The results will help foodborne operators adapt and optimize their C&D procedures in order to reduce the occurrence of food spoilage by molds.