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

Fouling and Cleaning in Food Processing 2022
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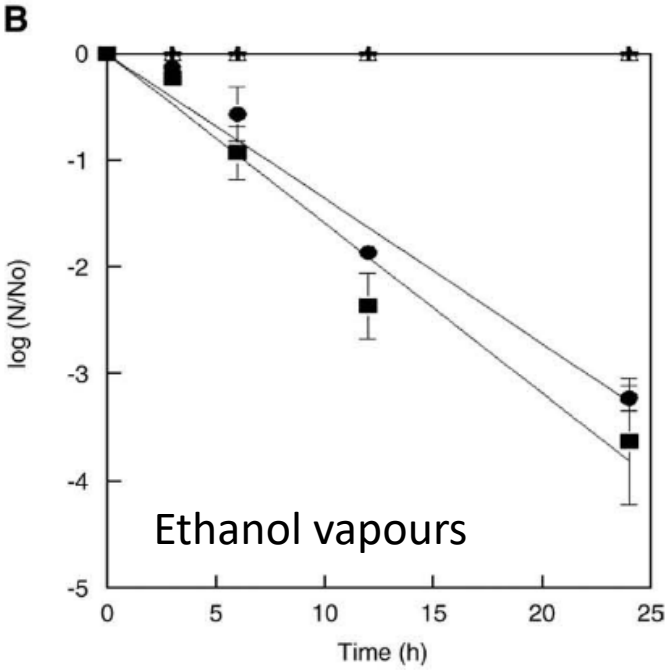
Dry fungal spores

Dry harvested-spores were previously shown to be more resistant than hydrated spores to ethanol and chlorine (cf. poster).



(Dao & Dantigny, 2009)

Dry-harvested spores

Hydrated spores

European standards to assess the effectiveness of disinfectants use hydrated spores and may overestimate the actual effectiveness of disinfection procedures.

Objectives & methodology

Objectives:

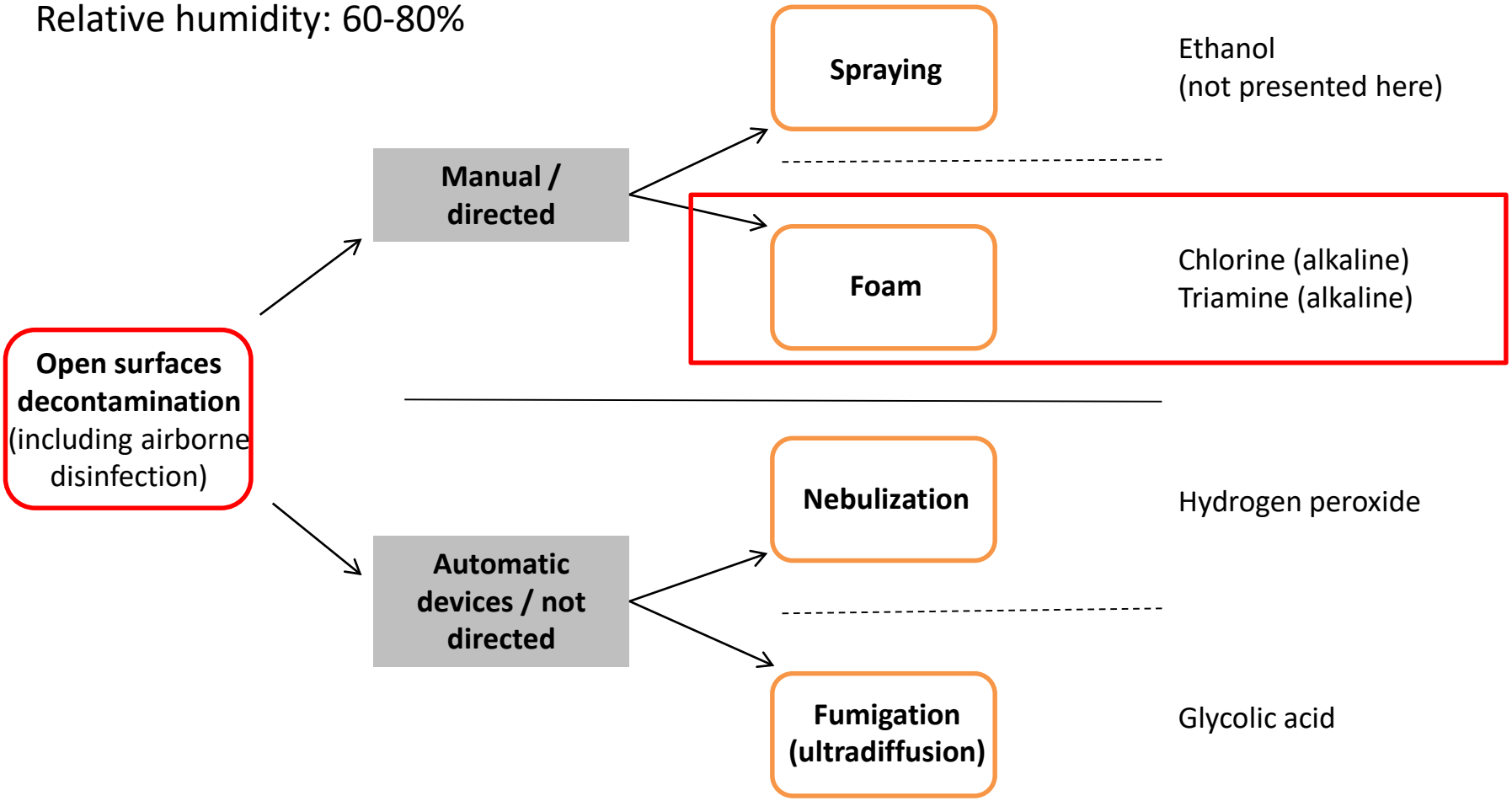
- Assess the actual effectiveness of disinfection protocols towards dry-harvested spores in real application conditions
- Identify levers to improve dry spores inactivation

Methodology:

- Survey the current practices in dairy industries for the decontamination of open surfaces
- Prepare « dry » spores and adhesion through electrostatic forces to stainless steel coupons
- Implement disinfection protocols in ACTALIA's BSL3 pilot plant
- Assess procedures effectiveness a a function of some relevant environmental and application-related factors

Disinfection practices in dairy industries (open surfaces)

Temperature: 15-20°C
Relative humidity: 60-80%



Foam gun

Foam gun

Implementation of a factorial design to assess the effectiveness of both biocides according to 5 factors

Disinfectant	Temperature	Time	Concentration	Foam
chlorine	20 °C	30 min	4 %	Wet
	15 °C	30 min	4 %	Dry
	20 °C	5 min	1 %	Dry
	15 °C	5 min	1 %	Wet
triamine	20 °C	30 min	2 %	Dry
	15 °C	5 min	5 %	Dry
	15 °C	30 min	2 %	Wet
	20 °C	5 min	5 %	Wet

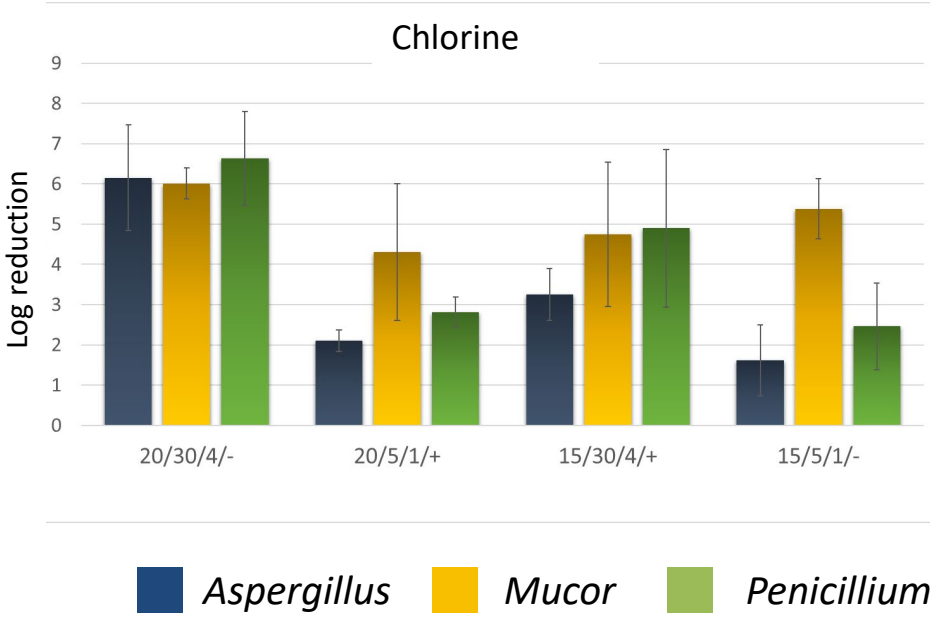
« Wet » foam

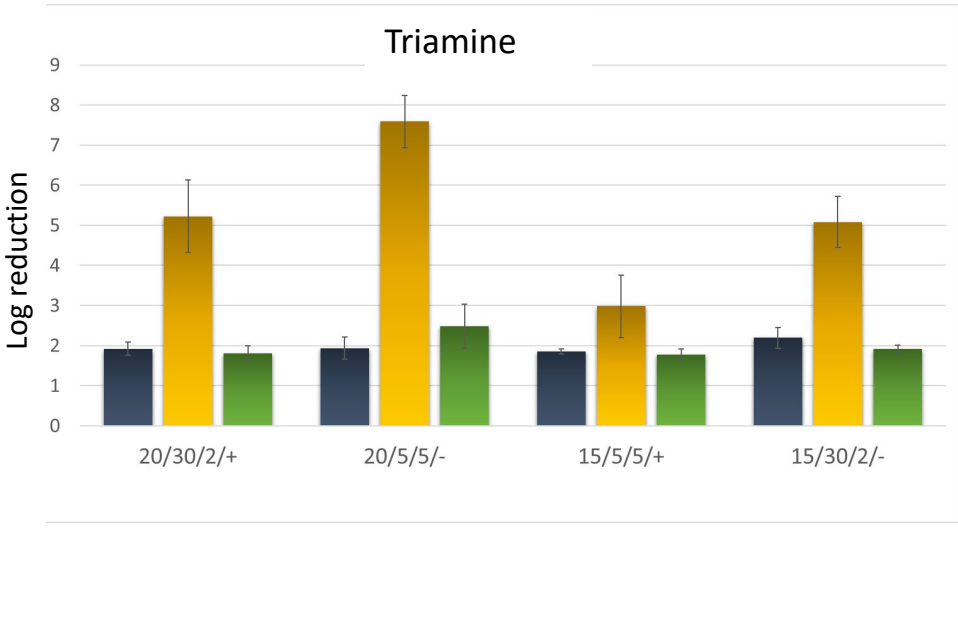
« Dry » foam

<u>3 target fungal species (dry-harvested spores):</u>

- *Penicillium commune*
- *Mucor circinelloides*
- *Aspergillus flavus*

Foam gun





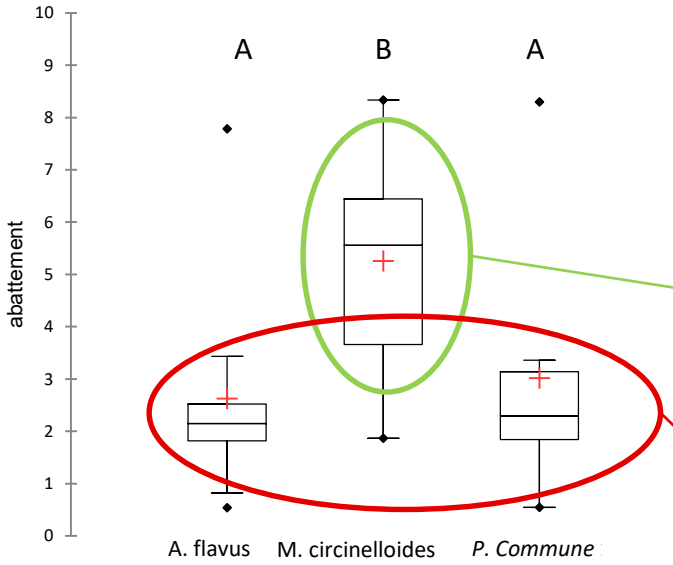
Aspergillus *Mucor* *Penicillium*

Log reductions:

- *Aspergillus*: 1.6-6.2 log with chlorine / 1.8-2.2 log with triamine
- *Mucor*: 4.3-6 log with chlorine / 3->6.9 log with triamine
- *Penicillium:* 2.5-6.6 log with chlorine / 1.8-2.5 log with triamine

Foam gun

Fungal species have different susceptibilities to treatments with foams:
Mucor is more sensitive.



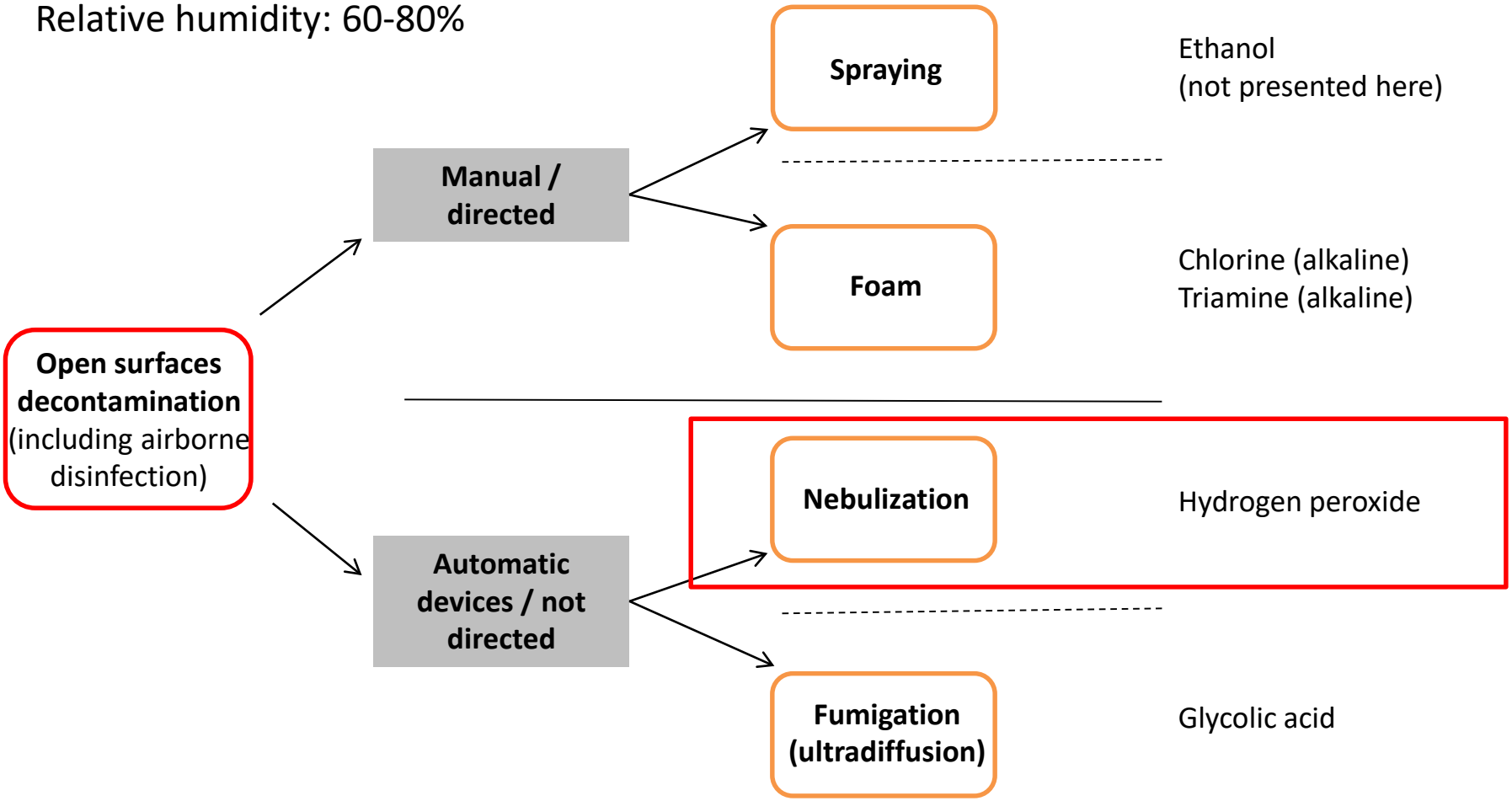
And factors having an impact also differ between species:

Temperature, foam dryness ($p<0.05$)
+0.9 log at 20°C +3 log with wet foam

+1-1.5 log +1.5 log +0.9-1.9 log with chlorine
Concentration, contact time and biocide ($p<0,01$)
Foam dryness ($p<0.05$)
+0.8-1 log with wet foam

Disinfection practices in dairy industries (open surfaces)

Temperature: 15-20°C
Relative humidity: 60-80%



Nebulization

Implementation of a factorial design to assess the effectiveness of H_2O_2 nebulization according to 3 factors

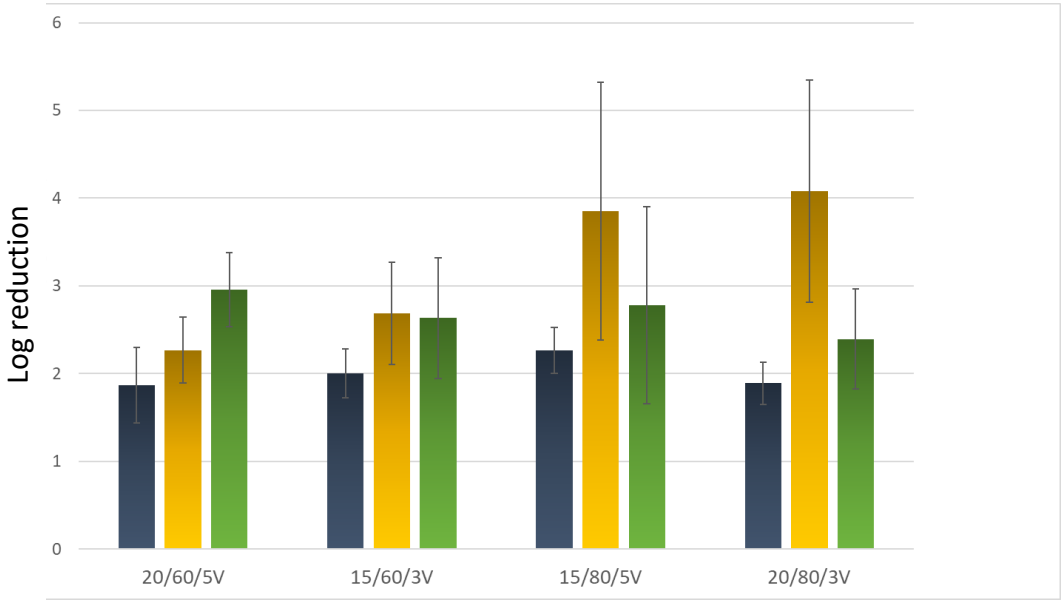
Temperature	Relative humidity	Concentration
20°C	80%	3ml/m³
15°C	80%	5ml/m³
20°C	60%	3ml/m³
15°C	60%	5ml/m³

One contact time: 2 hours

<u>3 target fungal species (dry-harvested spores):</u>

- *Penicillium commune*
- *Mucor circinelloides*
- *Aspergillus flavus*

Nebulization

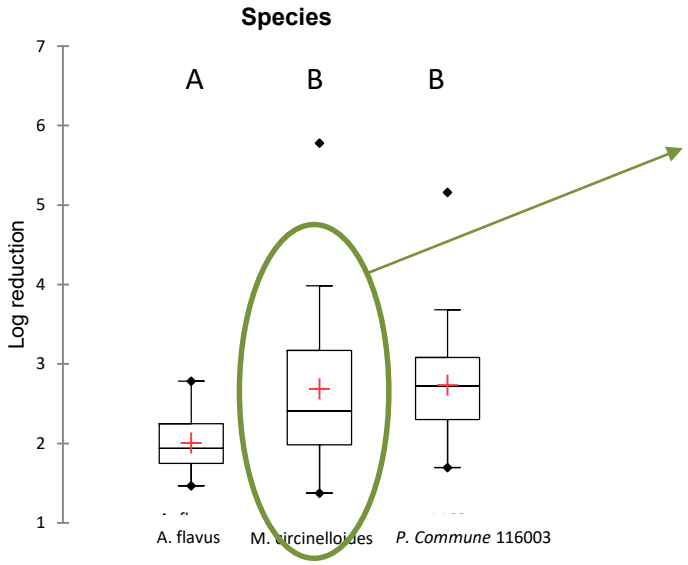


Log reductions:

- *Aspergillus*: 1.9-2.3 log
- *Mucor*: 2.3-4.1 log
- *Penicillium:* 2.4-3 log

Nebulization

Fungal species have different susceptibilities to treatments with H_2O_2:
A. flavus is more resistant.

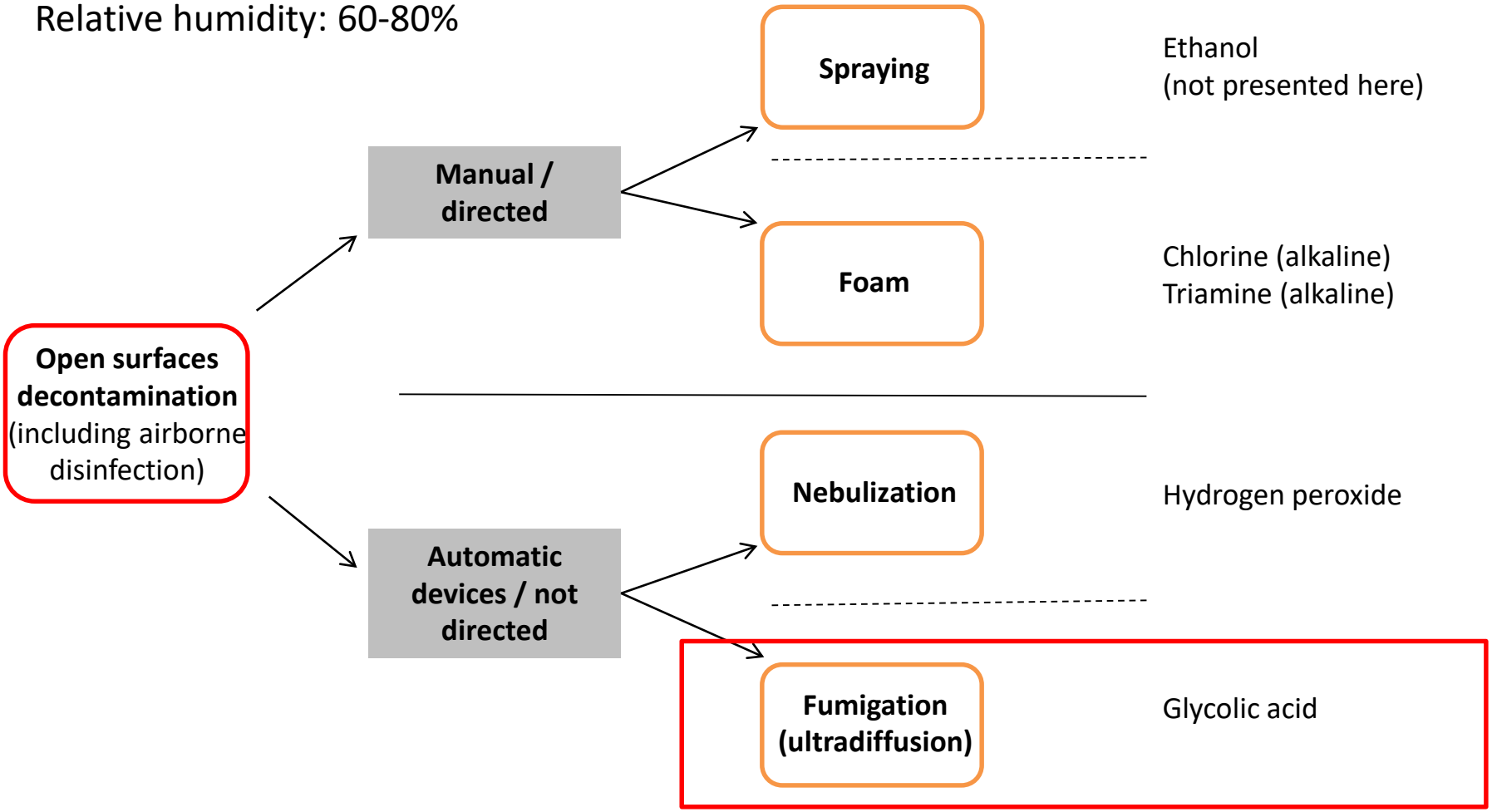


Only two factors had a statistically ($p<0.05$) and biologically significant influence on spores inactivation:

- at 15°C, the inactivation of *Mucor* is 0.9 higher than at 20°C;
- At 5ml/m^3, the inactivation of *Mucor* is 0.6 higher than at 3ml/m^3.

Disinfection practices in dairy industries (open surfaces)

Temperature: 15-20°C
Relative humidity: 60-80%



Fumigation

Implementation of a factorial design to assess the effectiveness of fumigation of glycolic acid according to 3 factors

Temperature	Relative humidity	Protocol
15°C	80%	4h, 0,8g/m^3
20°C	80%	16h, 1,2g/m^3
15°C	60%	16h, 1,2g/m^3
20°C	60%	4h, 0,8g/m^3

<u>3 target fungal species (dry-harvested spores):</u>

- *Penicillium commune*
- *Mucor circinelloides*
- *Aspergillus flavus*

Fumigation

Fumigation with glycolic acid was highly effective with many conditions resulting in the absence of surviving spores.

Species	Temperature (°C)	Relative humidity (%)	Concentration and contact time	Log inactivation (N/N0)	% of replicates with counting < limit of detection
A. flavus	20	80	0,8 g/m^3 for 4h	2,4 ± 0,3	0%
	20	60	1,2 g/m^3 for 16h	> 8,8	67%
	15	80	1,2 g/m^3 for 16h	> 5,7	33%
	15	60	0,8 g/m^3 for 4h	3,2 ± 0,5	0%
P. commune	20	80	0,8 g/m^3 for 4h	6,5 ± 0,1	0%
	20	60	1,2 g/m^3 for 16h	> 8,8	100%
	15	80	1,2 g/m^3 for 16h	> 9,2	100%
	15	60	0,8 g/m^3 for 4h	> 7,6	33%
M. circinelloides	20	80	0,8 g/m^3 for 4h	3,7 ± 0,7	0%
	20	60	1,2 g/m^3 for 16h	> 8,5	100%
	15	80	1,2 g/m^3 for 16h	> 8,2	100%
	15	60	0,8 g/m^3 for 4h	4,1 ± 0,6	0%

Fumigation

Statistical analysis difficult due to many censored data but some tendency were highlighted:

- Log reductions higher at 20°C compared to 15°C for *Aspergillus* (+1.8 log)
- Log reductions higher at 60% RH compared to 80% for *Aspergillus* (+2.7 log)
- Higher inactivation rates obtained for the 3 species with the reinforced protocol (+1.9 to 4.8 log)

Conclusion and perspectives

Some answers but more questions raised!

- Effectiveness of disinfection procedures highly dependent on the target fungal species (differential susceptibility between Mucorales and Ascomycota);
  → Further research needed to better understand where this difference comes from.

- In our test conditions, the effectiveness of the different procedures towards dry-harvested spores is greater with fumigation followed by foaming with chlorine;
  → Is it specific to dry spores? Would a preliminary detergency make dry spores more sensitive?
  → What is the threshold to consider a procedure as effective in real test conditions?

- Optimization levers depend on the target species and the disinfection method → no universal recomendations
  → Further assays needed with surface responses experimental designs to be able to predict the effectiveness of disinfection procedures, as a function of most relevant factors.

- Results obtained in pilot conditions differ from that obtained in laboratory conditions, especially for airborne decontamination with nebulization
  → Study the interactions between fungal spores and disinfectant droplets

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Thank you for your attention

Any question?