Technical Brief

Microfiltration Devices to Control Populations of Thermo-tolerant Acid-resistant Bacteria *Alicyclobacillus* (ACB)

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Abstract
This paper addresses the capability of microporous filter devices to retain *Alicyclobacillus* and *Bacillus* spores. Included are design features, operation, and deployment in filtering production-scale beverages and beverage ingredients.

Data are presented to show that both microporous depth filter media and microporous membrane (surface) media deliver complete retention according to the pores size rating selected and the retention rating respectively.

Introduction
Alicyclobacillus species and in particular *Alicyclobacillus acidoterrestris* are nonpathogenic food spoilage bacteria which are problematic for the beverage industry due to their inherent ability to resist thermal stress (via sporulation) and grow in low pH environments. Typically, the food industry has relied upon acidic environments and pasteurization conditions to served as antimicrobial measures, yet A. acidoterrestris has shown itself able to grow in elevated temperatures (19.5 - 58 ºC), consistent with cooling post-pasteurization and at a low pH 2.5 - 6.0 which is consistent with acidic fruit juice containing beverages (Jensen and Whitfield, 2003). *A. acidoterrestris* is soil-borne with global distribution and can enter the beverage manufacturing process on the outer surface of raw fruit, from ingredients and water sources. Pasteurization conditions which inhibit growth of microorganisms, actually stimulates spore formation in *A. acidoterrestris*, thus selecting for the organism in the final bottled product. In the bottled beverage *A. acidoterrestris* spores grow into vegetative cells and metabolically convert vanillin to guaiacol, a potent taint that results in a foul odor and smoky, phenolic like taste (Pettipher et at. 1997). The spoilage process can occur quickly, less than 48 hours post bottling and only minimal bacterial loads of 1 CFU/10 mL are required to produce detectable taints (reviewed by Walls and Chuyate, 1999). Previous research has primarily focused on using physical and chemical processes to prevent the growth of *A. acidoterrestris* spores in the final product (reviewed by Jensen, 1999). Unfortunately, the processes described typically lead to negative organoleptic changes that compromise the finished beverage quality. Filtration has demonstrated itself in the bottled water, beer and wine industries as an effective microbial control measure just prior to final packaging. Here we investigate filtration as a means to remove a model spore forming bacteria in aqueous environments as well as *A. acidoterrestris* in apple juice and 75% fructose solutions.

Materials and Methods
Filter Media - Depth Filters: 0.5, 0.8, 1 and 2 µm nominally rated cellulosic filter media.

Depth filters are rated "nominally" which refers to the filter media's ability to protect a membrane filter of a similar reported pore size. Cellulosic depth media is typically 3-5 mm thick and captures contaminants via several mechanisms including, surface retention, depth straining, electrostatic attraction and impaction (see figure 1). The depth filters tested here are composed of a blend of refined cellulose fiber, filter aids such as diatomite or perlite and a food-grade binding resin. The binding resin provides both; mechanical strength to the structure, as well as, imparting a net positive electrostatic charge to the filter surfaces. This "charge" aids in the adsorption of particles in the fluid.

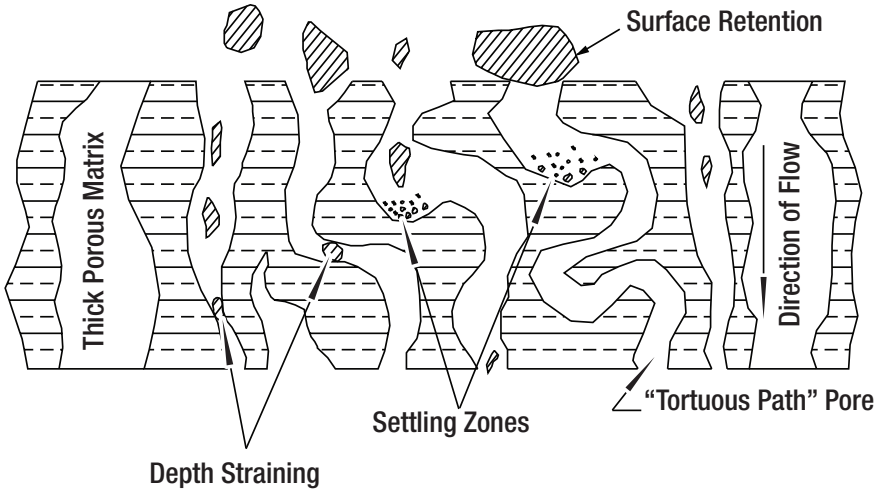

Figure 1. Mechanisms involved in depth filtration.

Membrane Filters: 0.2, 0.45, 0.65 and 0.8 µm absolute rated nylon 6,6 or polyethersulfone microporous membrane.

Membrane filters are rated upon their ability to remove organisms of a size similar to the pore size of the membrane. The absolute rating indicates that 100% of organisms equal to or larger than the rated pore size are retained. Membrane filters remove contaminants primarily via the size exclusion mechanism.

Challenge Spores

- *Bacillus subtilis* (Institute for Fermentation, Osaka Japan - IFO 3134)
- *Alicyclobacillius* acidocaldarius (IFO 15652)
- *Alicyclobacillius acidoterrestris* (isolated from apple juice by 3M Sumitomo laboratory)

Challenge Procedure

To limit microbial contamination all filter challenge testing was conducted in a laboratory laminar flow hood (for challenge test stand see figure 2). Filter media was secured into test housings (either 47 or 90 mm diameter) so that no bypass would occur. Membrane media was wetted with sterile water prior to installation into the filter housing.

Depth media was installed into filter housings without pre-wetting or flushing. The filter housing was fitted with a pressure gauge so that upstream versus downstream differential pressure could be monitored. To pass the culture solution through the filter media, peristaltic pump tubing was plumbed from the filter housing to the culture vessel. A calibrated peristaltic pump was added midstream to control flow rate. Depth media was flushed with a volume equivalent to 54 liters of water per square meter of effective filter area. Following depth media flush, excess water trapped in the media was purged by allowing the pump to run until no further water exited the housing.

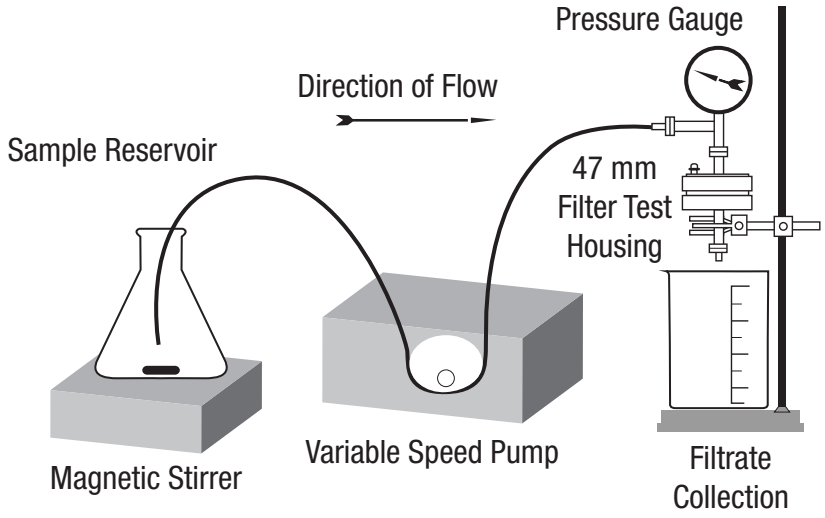

Figure 2. Constant flow test assembly, used in challenge of filter media

Up to 1000 mLs of spore solution was passed through the filter media at 5.2, 9.5, 23.7 or 28.6 liters per minute per square meter of filter area (LPM/m^2) at a constant rate. Flux for each trial is noted. Differential pressure was monitored as a function of time and testing was halted when the filter differential pressure reached 25 psid or the culture solution was exhausted. The filter effluent was then passed through a recovery membrane. The recovery membrane was then incubated on media as noted. The Bacterial challenge cultures were grown to a cell count of 106 - 108 colony forming units (CFU) per mL. Cultures were heated to 80 ºC for 10 minutes to insure that challenge solutions contained only spores. CFUs were determined by serial dilution and the pour culture plate method.

Culture media used in filtration trials

Media 1: standard agar and glucose broth

Media 2: A+B mixed.

A. (pH:3.0 to 4.0); (1 g) yeast extract, 0.2 g $(NH_4)_2SO_4$, (0.5 g) $MgSO_4$ $7H_2O$, (0.25 g) $CaCl_2$ $2H_2O$, KH_2PO_4, purified water (500 mL)

B. (1 g) glucose, (20 g) agar, purified water (500 mL).

Media 3: 2 g yeast extract, (2 g) soluble starch, (1 g) glucose, (15 g) bacto agar, purified water 1000 mL.

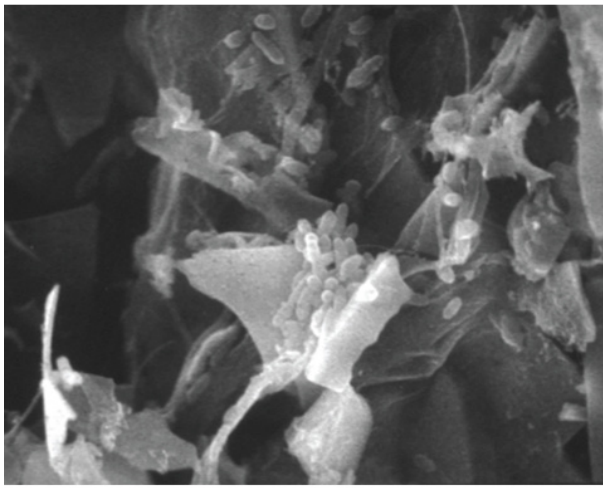

Figure 3. Scanning electron micrograph of bacteria retention in cellulosic depth filter media (Zeta Plus™ Series filter).

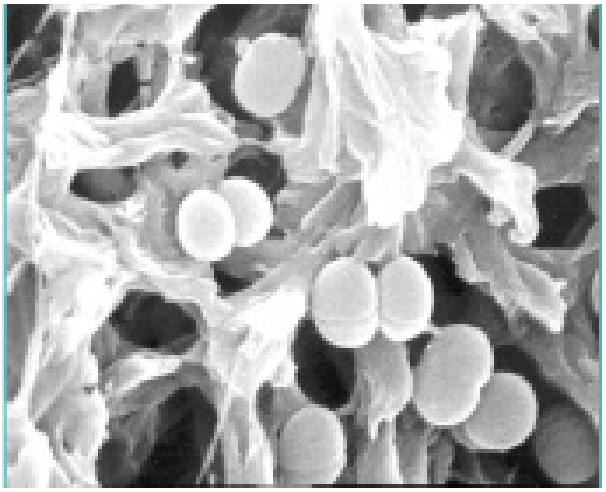

Figure 4. Scanning electron micrograph of bacterial retention by microporous nylon membrane (LifeASSURE™ BA Series filter)

Results

A culture of ~10^6 CFUs *Bacillius subtilis* spores were dispersed in water and used to challenge depth and nylon membrane filter media. The challenge solution was passed through the filter media at a constant flux rate of 9.5 LPM/m^2 reaching a total throughput of 190 L/m^2. Challenge conditions and filter effluent quality is outlined in Tables 1 and 2. Filter effluent was collected and then passed through a recovery membrane. Colonies were counted after the recovery membrane was incubated on Media 1 at 35 ºC for 2 days.

B. subtilis spore retention by four grades of depth media ranging from 0.5 - 2 µm is described in Table 1. Depth media nominally rated at less than or equal to 1 µm retained 100% of the *B. subtilis* spores. Even though the 2 µm depth media did not retain 100% of the spores a log reduction value (LRV) of > 4.5 was observed.

Log reduction values were calculated using the following formula:

$$LRV = \log_{10}\left(\frac{\text{total number of organisms entering the filter}}{\text{total number of organisms exiting the filter}}\right)$$

Total number of organisms entering the filter = based on CFU of the challenge solution.
Total number of organisms exiting the filter = based on CFU from capture membrane culture.

Table 1. Cellulose depth filter media (Zeta Plus™ Series filter) challenged with *Bacillus subtilis* spores dispersed in water. (n = 2)

Depth Filter Grade	Nominal Pore Size (µm)	Bacterial Challenge (x 10^6) CFU/mL	Filter Effluent Colony Counts	LRV
Zeta Plus™ 10H	2	2.9 - 3.9	110	> 4.5
Zeta Plus™ 30H	1	(6.0 x 10^5) - 2.6	0	> 5.8
Zeta Plus™ 50H	0.8	2.8 - 3.1	0	> 6.4
Zeta Plus™ 60H	0.5	3.8 - 5.3	0	> 6.6

Table 2 describes challenge conditions for nylon microporous membrane ranging in pores size from 0.20 - 0.80 µm (absolute pore size rating). Nylon membranes from 0.2 to 0.65 µm retained 100% of Bacillus spores. The 0.80 µm membrane retained all but one CFU and achieved a LRV greater than 6.8.

Table 2. Nylon membrane filter media (LifeASSURE™ BLA Series filter) challenged with *Bacillus subtillis* spores dispersed in water. (n = 2)

Surface Filter Grade	Pore Size (µm)	Bacterial Challenge (x 10^6) CFU/mL	Filter Effluent Colony Counts	LRV
LifeASSURE™ BLA080	2	7.4 - 8.0	1	> 6.8
LifeASSURE™ BLA065	1	5.3 - 5.8	0	> 6.8
LifeASSURE™ BLA045	0.8	6.7 - 7.8	0	> 6.7
LifeASSURE™ BLA020	0.5	6.1 - 6.5	0	> 6.9

Data in Table 3 summarizes a challenge study in which *Alicyclobacillus acidocaldarius* spores were mixed in 20% apple juice (Brix ~ 2.2 º) and used to challenge depth filters nominally rated at 0.5 and 1 µm pore size. Challenge solution was passed through the depth media at a constant flux rate of 23.7 LPM/m^2 to a total throughput volume of 190 L/m^2. Filter effluent was collected and then passed through a recovery membrane. Colonies were counted after the recovery membrane was incubated on a Media 2 at 60 ºC for 3 - 5 days. The data set shows that the 1 µm media resulted in an incomplete retention of *A. acidocaldarius* spores, yet a LRV of 4.6 was achieved. In comparison, the 0.5 µm media retained 100% of the challenge spores and yielded a LRV of greater than 6.2.

Table 3. Cellulose depth filter media (Zeta Plus™ Series filter) challenged with *Alicyclobacillus acidocaldarius* spores dispersed in 20% apple juice. (n = 2)

Depth Filter Grade	Nominal Pore Size (µm)	Bacterial Challenge (x 10^6) CFU/mL	Filter Effluent Colony Counts	LRV
Zeta Plus™ 30H	1	2.7 - 3.0	62 - 68	4.6
Zeta Plus™ 50H	0.5	1.5 - 1.7	0	> 6.2

Two grades of depth filters were challenged with 10^6 CFU/mL of *Alicyclobacillius acidoterrestris* spores in a milieu of apple juice (Brix ~ 11º). The depth filters were challenged at a flux rate of 28.6 LPM/m^2 and to a throughput capacity of at least 425 L/m^2. Data in table 4 indicates that the 1 µm media retained most but not all of the spores. In comparison, the 0.5 µm media retained 100% of the challenge spores with a net LRV exceeding 6.5.

Table 4. Cellulose depth filter media (Zeta Plus™ Series filter) challenged with *Alicyclobacillius acidoterrestris* spores dispersed in apple juice. (n = 1)

Depth Filter Grade	Nominal Pore Size (µm)	Bacterial Challenge (x 10^6) CFU/mL	Filter Effluent Colony Counts	LRV
Zeta Plus™ 30H	1	5.4	1.8×10^3	3.5
Zeta Plus™ 50H	0.5	3.3	0	> 6.5

Data shown in table 5 summarizes the results of a challenge study in which *A. acidoterrestris* spores dispersed in 75% fructose (Brix 75º) were used to challenge depth media nominally rated at 0.8 µm and two 0.45 µm (nylon and polyethersulfone) microporous membranes. The challenge solution was passed through the depth media at a constant flux rate of 5.2 LPM/m^2 to a total throughput volumeof ≥ 53 L/m^2. Filter effluent was collected and then passed through a recovery membrane. Colonies were counted after the recovery membrane was incubated on Media 3 at 45 ºC for 4 days. The data set shows that all media tested retained 100% of the challenge spores.

Table 5. Cellulose depth (Zeta Plus™ Series filter) and membrane filter media (LifeASSURE™ BA and BNA Series filters) challenged with *Alicyclobacillius acidoterrestris* spores dispersed in 75% fructose. (n = 2)

Surface Filter Grade	Media Pore Size (µm)	Bacterial Challenge (x 10^8) CFU/mL	Filter Effluent Colony Counts	LRV
Zeta Plus™ 60H	0.80 (nom)	1.1 - 1.3	0	> 8.0
LifeASSURE™ BA045	0.45 (abs N)	1.4	0	> 8.1
LifeASSURE™ BNA045	0.45 (abs P)	1.1 - 1.3	0	> 8.0

Nom = nominally rated cellulose depth media, Abs N = absolute rated nylon membrane, Abs P = absolute rated polyethersulfone membrane

Discussion

For the filter trials conducted in this report, the spore challenge load was much higher than typically observed under normal beverage manufacturing conditions. The high challenge level was done purposely to exceed the worst case process scenario and present a difficult challenge to the filter media.

In the first series of experiments (see Tables 1 and 2) a surrogate spore forming bacteria was used to challenge depth and membrane filter media. Surrogate spores (*Bacillus subtilis*) were dispersed in water and used to challenge depth (0.5 - 2.0 µm) and membrane (0.2 - 0.8 µm) filter media. In both trials, the open porosity media resulted in substantial log reduction of spores, yet did permit passage of a limited number of spores into the filter effluent. The media grades on the tighter end of the porosity range showed complete retention of spores. These results show that depth and membrane filter media can be used to completely remove bacterial spores in an aqueous environment. These findings support the idea of filtering ingredient and utility water used in the manufacturing facility as a bio-security measure.

In the next experiment (see Table 3) depth filter media (0.5 and 1.0 µm) was challenged with *Alicyclobacillus acidocaldarius* spores in a dilute apple juice solution to model filtration of a juice containing beverage. The 0.5 µm media completely retained the *A. acidocaldarius* spores. For the 1.0 µm media, the differential in spore retention, as compared to *B. subtilis* data, may be attributed to the decreased pH, higher flux rate or species spore filtration behavior. The low pH in this trial would have the tendency to ameliorate the clarification character imparted by the net positive charge on the depth filtration media. The surface of *Alicyclobacillus* species tend to have a neutral outer membrane charge and may escape the trapping function of the depth filters net positive charge. In a related experiment depth media (0.5 and 1.0 µm) media was challenged with *A. acidoterrestris* spores dispersed in apple juice. The results in Tables 3 and 4 are similar and again, *A. acidoterrestris* spores were 100% retained by 0.5 µm depth media.

In the final experiment (see Table 5) depth (0.8 µm) and two membrane (0.45 µm) chemistries were challenged with *A. acidoterrestris* spores in a 75% fructose solution. This trial was designed to model filtration of a soft drink ingredient. In this experiment all media type showed 100% retention of the *A. acidoterrestris* spores. As compared to other experiments presented the flux rate and throughputs were relatively low. However, the filter media was not run until exhaustion. In this experiment, one replicate was run at a constant flow rate for the duration for the experiment. For the other replicate, the pump was stopped and started intermittently to model an extreme process condition. The results from each replicate did not differ, thus these results indicate that intermittent flow through the filter media does not negatively impact its spore retaining ability.

In the bench scale experiments presented the variables include flux and carbohydrate level (Brix). The differential is the result of experiments run in different laboratories at different times. Even though the challenges occurred under a differential of conditions the filter media behaved similarly across experiments, lending support to the robustness of the filter media. The data shows the filter media threshold for spore retention appears to be at 0.8 µm.

Rising international specifications, pressures of HACCP and constantly improving detection methodologies coupled with the relatively low numbers of organisms that can force a product recall, the beverage industry continues to seek technologies that may prevent passage of food spoilage microbes into the final product. In the laboratory models presented we show that microfiltration has the ability to prevent passage of *B. subtilis*, *A. acidocaldarius* and *A. acidoterrestris* spores into the filter effluent. Thus in appropriate processes microfiltration may play a role singly or as part of a multifaceted approach to prevent food spoilage microbes from entering the final product.

Practical Application of Microfiltration Technology

Due to the structure and contaminant holding capacity of the filtration media discussed here, microfiltration is appropriate for relatively clean fluid streams, that is, those that have a relatively low particulate load; and microfiltration is not recommended for solutions that are negatively impacted by changes in fluid turbidity due to haze and insoluble material removal. The data presented indicates that in low turbidity streams depth and membrane microfiltration media can be used to protect the beverage product just prior to bottling. In sanitization and upstream preparative processes microfiltration is also applicable to prevent microbial migration downstream (i.e utility and sanitization water).

Media Deployment

Cellulosic depth filters are typically deployed in cartridges that are made up of stacks of lenticular cells (see Figure 6). These cartridges are then fitted into housings (see Figure 7) and it is important to note that each filter system is uniquely sized to match the beverage producer needs and process requirements.

Membrane filters almost universally are of pleated construction and fabricated into a cylindrical cartridge, lengths range from 2.5" to 40" (see figure 5). Membrane filters can be used singly or in housings that contain multiple cartridges flowing in parallel and systems are sized for the process needs.

Follow-up Experiments

Based on the data generated here the next set of experiments should try to answer the following questions.

- Over long term exposure will *Alicyclobacillius* species growth through depth and/or membrane filter media and if so under what conditions?
- What is the throughput capacity of depth and membrane filters for low population *Alicyclobacillius* species in juices, ingredients and make up water?

References

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Walls, I. and Chuyate, R. (1999) Spoilage of fruit juices from *Alicyclobacillus acidoterrestris*. *Workshop proceedings from the 10th World congress of Food Science & Technology*. 286-288.

Jensen, N. (1999) Spoilage of fruit juices from *Alicyclobacillus acidoterrestris*. *Workshop proceedings from the 10th World congress of Food Science & Technology*. 286-288.

Acknowledgements

Portions of the data presented here were generated by Dennis G. Battersby and John J. Churey, 3M Purification Inc. Internal Testing Report (1998); and Ichiro Nagaski, 3M Sumitomo, Internal Testing Report (2006).

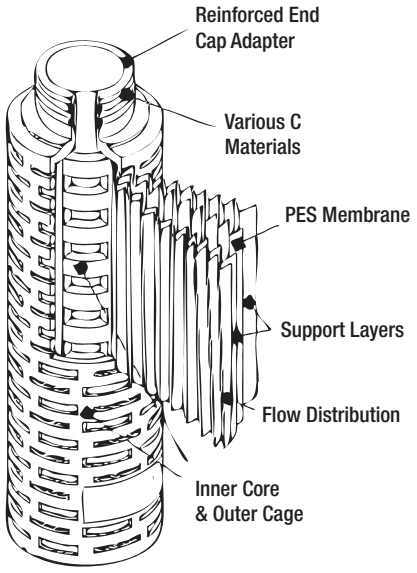

Figure 5. Anatomy of a typical membrane filter cartridge

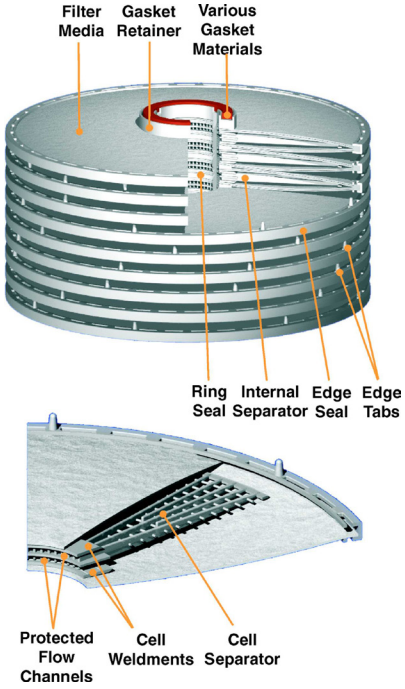

Figure 6. Anatomy of a typical lenticular depth filter cartridge

Figure 7. Depth filters fitted into sanitary design, stainless steel housings.

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