Biofilm in an aseptic production facility

A case study of spoilage diagnosis & eradication

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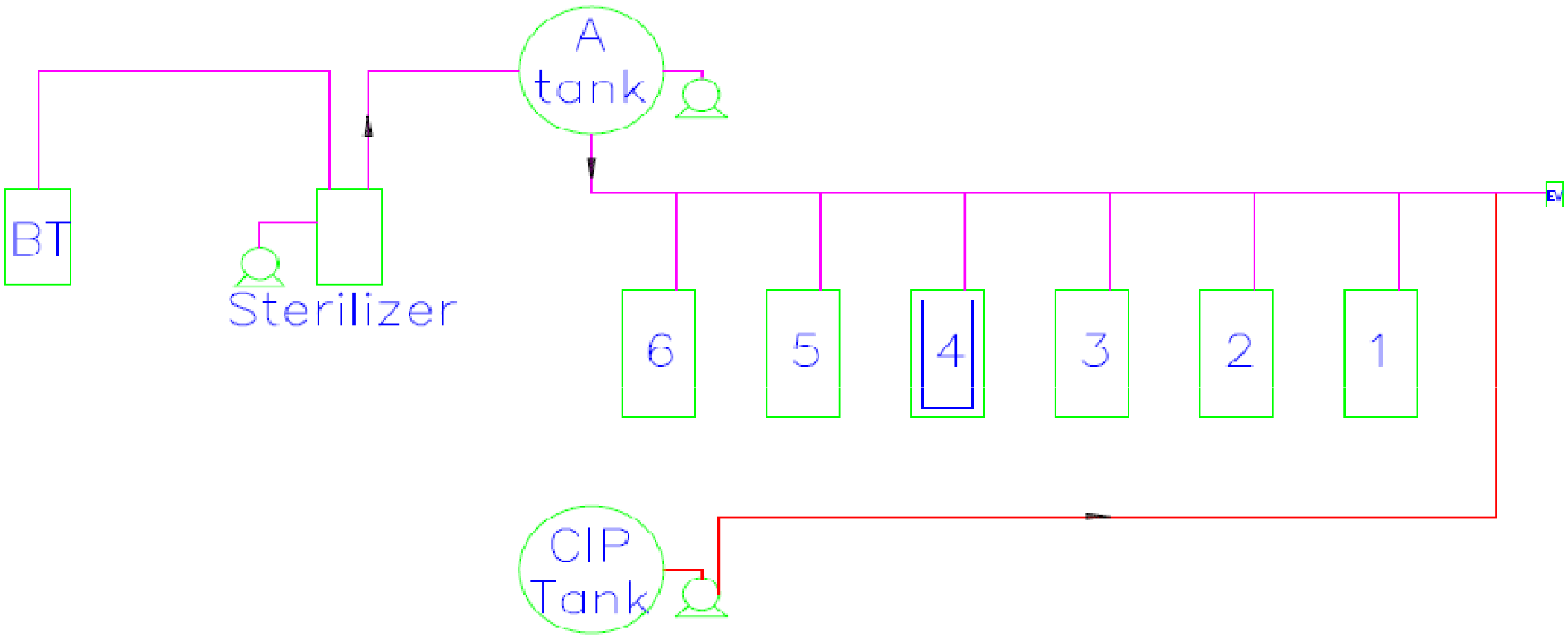
Equipment Layout



Background information

- Increasing frequency of spoilage incidents
  - 5 incidents Jan-Dec 2008
  - 14 incidents Feb-Aug 2009
- Single common spoilage family – affecting different lines, different products
  - Heat resistant spore forming *Bacillus* species, predominantly *Bacillus licheniformis*
- Common source of organism
  - Confirmed by 16 s rRNA sequencing and FTIR (Fourier Transform Infrared spectroscopy) cluster analysis
- Spoilage organism isolated from product lines and B valve of filling machine <u>after</u> CIP cycle
- Onset of spoilage detected 4-6 hours after production start, occasionally directly after production start

Spoilage Summary

Production Date	Product	Line No.	Machines in Production	Machine affected	Defect Type	Contaminating microbe***
Jan. 24, 2008	Créma	2	3, 4	4	Flat sour	*Bacillus spp.*
Feb. 12, 2008	Créma	2	3, 4	4	Flat sour	*Bacillus spp.*
Feb. 20, 2008	Créma	2	3, 4	4	Flat sour	*Bacillus spp.*
July 3, 2008	Créma	1	3, 4	4	Flat sour	*Bacillus spp.*
Dec. 5, 2008	Créma	2	3, 4	4	Flat sour	*B. licheniformis*
Feb. 18, 2009	Créma	2	3, 4	4	Flat sour	*Brevibacillus Agri*
March 18, 2009	Créma	2	3, 4	4	Flat sour	*B. licheniformis*
April 17, 2009	Créma	2	4	4	Flat sour	*B. licheniformis*
May 7, 2009	Yamoo Choco	2	3, 5	5	Flat sour / blown	*Bacillus spp.*
May 15, 2009	Sweet Milk	2	5, 6**	5	blown	*Bacillus spp.*
June 8, 2009	Créma	2	3 only	3	Flat sour	*B. licheniformis*
June 24, 2009	Sweet Milk	2	3, 5, 6	5	Flat sour / blown	*B. licheniformis*
August 1, 2009	Yamoo Choco	2	3, 5	5	Flat sour / blown	*B. licheniformis*
August 7, 2009	Choco	1	3, 5	5	blown	*Bacillus spp.*
August 13, 2009	Choco	1	3, 5	5	Flat sour / blown	*B. cereus (flat sour)*
Aug. 26, 2009	Choco	1	1, 2, 6	6	blown	-
Aug. 29, 2009	Choco	1	3, 6	6	blown	-
Sept. 2, 2009	Créma	2	3, 4	4	Flat sour	*B. licheniformis / B. cereus*
Sept. 4, 2009	Choco	1	3, 6	6	Flat sour / blown	*B. licheniformis / B. cereus / B. flexus / Paenibacillus sp.*

Spoilage on Filler 4 – ***Filler is <u>not</u> the source of the spoilage***

- *Bacillus licheniformis* isolated from spoiled product produced on "old" filler 4 on 3 occasions
- "New" filler 4 is similarly affected by unsterility on Sept 2 with the same micro-organism indicating the same source of contamination

Diagnosis based on findings - presence of *biofilm* in the system

A biofilm is an aggregate of <u>microorganisms</u> in which cells are stuck to each other and/or to a surface. These cells are embedded within a self-produced matrix layer of DNA, proteins and polysaccharides.

Biofilms can develop on wet food processing surfaces that are not completely cleaned or not cleaned often enough. The presence of biofilm reduces the effect of conventional sanitizers. Sanitizers have a limited ability to penetrate a protective layer consisting of microbial polymers.

The biofilm phenotype

Biofilms in aseptic systems

Biofilms in the pipelines of UHT facilities are regularly subjected to CIP and pre-sterilization. The high temperatures involved in these processes will kill all micro-organisms except for heat resistant survival forms (spores) contained in the biofilm.

When conditions become favorable for growth, the spores germinate and multiply rapidly, hence the lag phase of 4-8h before spoilage is detected.

If cleaning is adequate in all aspects (time, temperature, concentration, flow rate), biofilms cannot develop in the system.

Removal of the biofilm - challenges

Specific conditions that have resulted in biofilm formation should be rectified. Some examples

- System design & installation eg weld quality
- Equipment layout
- Operational procedures eg frequency of cleaning
- Nature of products being processed
- Processing time &/or temperature
- Mix/blend procedures
- Entrained air in product
- CIP critical points

Biofilms and their resident bacteria must be removed completely – "shock treatment" eg deep cleaning often essential in tenacious cases

Sanitation program essential after biofilm removal

Similarity to case in USA

Investigation extended over 4 months

Characterized by

- Flavored milk
- Intermittent spoilage incidents – 6 in 10 weeks
- Flat sour spoilage up to 100%
- Clean for 6-8 hours then rises up to 100%
- Spoilage organism pure culture of heat resistant spore-forming *Bacillus* species

Similarity to case in USA

Significant findings

- Spoilage organisms isolated from CIP rinse water **after CIP cycle**
- Spoilage organisms isolated from caustic storage tank – scale build up and liquid
- Caustic frequently re-used – spores can survive in caustic
- Cleaning water supplied by on-site deep well – Bacillus species originate from soil and water in nature
- Spoilage reduced to **zero incidents in 8 weeks** during Sept/Oct after introduction of program designed to address biofilm

Similarity to case in USA

Program details

- Initial aggressive deep cleaning CIP program of sterilizer, product supply lines and filler
- Aggressive manual clean of caustic storage tank
- Using fresh caustic for every CIP cycle (discontinue re-use)
- Increased chlorination of plant cleaning water
- Sanitizing filler and product line if idle for more than 2 hours (sanitizing solution in rinse water recommended and supplied by major supplier. PAA most effective according to literature)
- Full CIP cycle with acid <u>every 24 hours</u>
- Periodic system deep cleans (weekly at beginning – reduce with time)

Indicator - Monitoring of CIP final rinse water for spores

On-site investigation

Nov/Dec 2009

Initial Findings (1)

- Presence of heat resistant organisms confirmed in CIP rinse water and Crema pre-mix prior to UHT processing
- Sub-optimal heat processing conditions confirmed on UHT 2 (140C/2.8 sec)
- Crema mix/blend process constitutes a risk that may encourage spore outgrowth (extended time at elevated temperature)
- Locust bean gum stabilizer has a high dissolving point (70-80C) above the temperature used in the blending process (55-70C)
- System pre-sterilization conditions out of specification
- CIP system –system requires reliance on operator interface which poses a risk to sustainable delivery of optimal cleaning process

Note : The ***combined impact*** of these findings constituted a current and ongoing risk to the production of quality product

Main Findings (2)

Too rapid cooling of aseptic tanks poses risk to integrity of sterile filters

Hygiene issues regarding excessive condensate in filler aseptic chambers and bad welds on product pipelines to and from aseptic filling machines

Strategy proposed

- Eliminate all sources which provide a risk of heat resistant spores going into the process

  - Manual mix/blend operation
  - High dissolving point ingredients
  - Heat processing
  - Cleanability of circuit

- Rid existing system of all biofilm that could harbor heat resistant spores

  - CIP (check program, equipment, cleaning chemicals, verify cleaning result)

- Secure optimum parameters for CIP program and ensure repeatability of delivery

Proposed action plan – main points

- Adopt recommended cleaning program with Ecolab chemicals (supported by successful US experience with identical spoilage case)
- Gasket change program and manual cleaning & sanitation of gasket seats (places where spores can collect and shelter from CIP and pre-sterilization)
- Inspect installation of product line for cleanability, low spots, weld quality etc (some welds of suspect quality from external observation only)
- Review processing time/temp for cream and chocolate milk (supported by experience in Brazil with chocolate milk and TP Pilot plant experience)
- Review critical points of CIP, especially flow rate
- Review product mix/blend protocol & ingredients (supported by record of periodic high count in thermophilic cells from mix tank)

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CIP Rinse water quality

Micro results 22 October 2009 from rinse water tank

Mesophilic spore count	80cfu/ml
Thermophilic count (3 samples)	**860/880/990 cfu/ml**

Results confirm a source of heat resistant spore-forming organisms.

Recommendation: Spores need to be eliminated in CIP rinse water.

Mix/blend process (1)

All Purpose Cream (APC) micro results – thermophilic cells

Dec 5/08	>65 000cfu/ml
Nov 4/08	>65 000cfu/ml
Nov 18/08	20 240cfu/ml
Nov 19/08	>65 000cfu/ml
Nov 24/08	>65 000cfu/ml
Nov 25/08	>65 000cfu/ml

These results confirm the presence of heat resistant cells capable of forming spores in the APC raw materials.

Recommendation: All possible precautions should be taken with this product to ensure thorough wetting of powders to ensure heat penetration in the UHT process

Mix/blend process (2)

Overall process poses a risk for spore contamination due to extended time at elevated temperature after initial heat shock in mix/blend operation. The risk is confirmed by periodic high counts of thermophilic cells in the APC mix.

Recommendation for risk reduction/improved heat penetration and overall improvement in product consistency – fat particles not properly emulsified.

Institute homogenization step upstream of UHT process. This can be located immediately after the mix/blend procedure.

Processing of créma APC: Formulation and mixing

CIP process mapping(1)

Filler	No of filler runs	Unsterilities	CIP flow direction
1	22	0	
2	5	0	
3	153	1	
4	67	9	
5/6	76	6	
6		3	

No correlation between no of filler runs and unsterility, but seems to be a pattern associated with CIP flow direction and unsterility – increases towards the end of the line – nothing in the first 2 fillers in line.

CIP process (2)

CIP audit 5 Nov together with chemical supplier

- Manual mixing process involving use of caustic flakes
- Rinse between caustic and acid on Alcip 10 is 150 sec. Confirmed by titration that it will take approx 300 sec water rinse to remove all traces of caustic.

  Risk: Residual caustic will partially neutralize acid thus reducing effectiveness of acid wash.
- After only 220 sec water rinse, the conductivity meter reads 0.7 (as for water!)

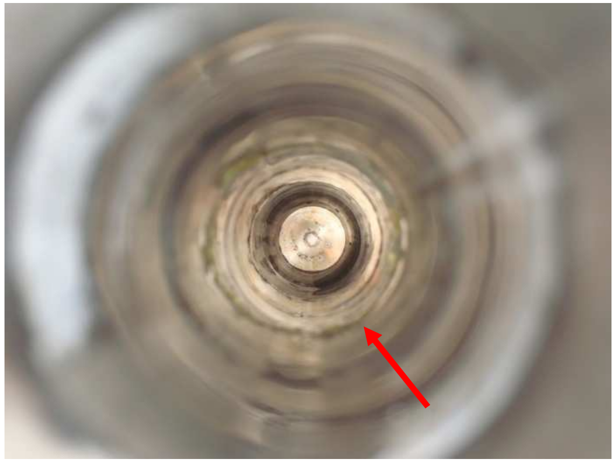
Conductivity meter needs calibrating and/or probe needs replacing

Recommend interlock between concentration and timer

CIP (3)

Aseptic damper on aseptic homogenizer before CIP.

Aseptic damper after CIP – residues still visible.



Cleaning inadequate. Recommend extra manual cleaning of dampers.

UHT process for APC

Proc system	Volume (litres) 08/09	% vol split	Proc parameters	Unsterility cases
Flex 1	1 522 875	40	140C/6 sec	1
Flex 2	2 316 300	60	140C/2.8 sec	9

Data supports the high probability of underprocessing on Flex 2

Processing of APC: UHT processing in TA Flex 1 and 2

TA Flex 1 holding time and temperature:

One holding tube only, designed for 4 seconds at 4000 l/h

Gives ~6 seconds holding time for APC at ~2600 l/h

UHT temperature 140-141C

TA Flex 2 holding time and temperature:

Special holding tube for APC designed for 4 sec at 2000 l/h

Gives ~2,8 seconds holding time for APC at ~2600 l/h

UHT temperature 140-141C

Processing of APC:
UHT heat treatment in TA Flex 2

Improvements to be done:

Holding tube for APC will be increased to ~6 seconds to compensate for occasional laminar product flow and give sufficient time for heat treatment of spores and any particles.

Processing of Chocolate Milk: Formulation and mixing

No homogeniser in the preparation process =>

Particles not split will not be properly soaked

Particles not split before UHT heat treatment

Processing of Chocolate Milk: UHT heat treatment in TA Flex 1 and 2

UHT temperature 140 C and 4 seconds =>

Not a safe combination for commercial sterility on this product

Reasons:

-Raw material with high and varying number of heat resistant spores

-No homogenisation before heat treatment

UHT processing on TA Flex 2

Present available product/holding tube combinations:

APC	2,8 sec at 2600 LPH	
SW MILK	4 sec at 4000 LPH	Same holding tube as for CHOCO
CHOCO	4 sec at 4000 LPH	

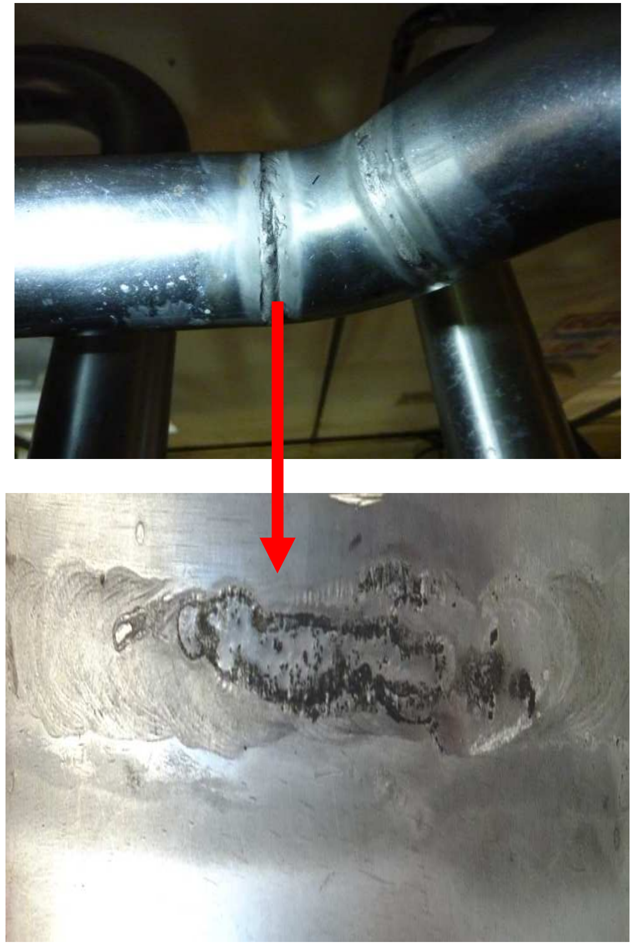
Available combinations after rebuilding:

APC	6 sec at 2600 LPH	
SW MILK	4 sec at 4000 LPH	Same holding tube as for APC
CHOCO	6 sec at 4000 LPH	

System cleanability

Welds on product lines to fillers are not good when viewed from the outside.

When these pipes were cut open, these welds were extremely rough on the product contact surface. They pose a challenge to achieve thorough cleaning and therefore will support the development of biofilms in these areas (weld located on product line immediately before product inlet valve on filler 4 on right.)



Overview of findings

- Spores and heat resistant vegetative cells in CIP rinse water
- High numbers of heat resistant cells in APC before UHT treatment
- Incorrect setting of pre-sterilization temperature guards on Flex 2
- Sub-optimal heat treatment of APC and Choco products
- CIP audit – see recommendations
- Cleanability – welds on product lines to fillers poor quality. Questionable cleaning result
- Hygiene – condensate on aseptic chamber doors of idle fillers

Summary of recommendations (1 – CIP improvements)

- Water treatment of CIP rinse water

✓ CIP program – continue for 6-8 weeks with Ecolab chemicals and protocol to ensure removal of biofilm. Check micro on CIP rinse water to verify results (ongoing)

  - After 8 weeks of daily cleaning with Ecolab chemicals, continue deep cleaning program with Ecolab chemicals once per week for a total of 3 months
  - Avoid using solid form of alkali in the future (pearls/flakes)

- CIP equipment, program and process refinements

  - Secure concentration of alkali and acid by means of automation and re-programming
  - Interlock time/concentration and time/temperature in program so that set points are achieved before timer begins
  - Secure repeatability of flow rate on all cleaning combinations
  - Prevent manual intervention in program

Summary of recommendations (2 – Process Improvements)

- ✓ Raise temperature of the mix water to 80C to ensure thorough dissolving of Locust Bean gum
- ✓ Revised UHT heat processing parameters
  - 6 sec holding for APC and Choco on Flex 2
  - run Choco heat process at 144C
- ✓ Restore standard setting on pre-sterilization temperature guards
- ✓ Orifice plates for cooling water to Alsafe should be secured in place
- Install homogenization step upstream of UHT process
- Consistently follow the mix/blend protocols – computerize instructions to ensure consistency

Summary of recommendations (3 – GMP Improvements)

- Re-direct airflow in Filling Room to avoid excessive condensate build up on idle fillers

✓ Check all welds on product lines to all fillers – replace pipes if necessary (line 4 completed)

Quality Update – Nov 13th 2009

- Crema has been produced on 6 days per week and either Choco or Sweet Milk on 1 day per week since the production re-start on October 22nd
  - 30 Crema productions
  - 5 Choco productions
  - 5 Sweet Milk productions
- It has been 5 weeks and > 40 production runs since the investigation began on Nov 2nd and no case of unsterility reported

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Topline Summary

- Based on facts/findings, root cause of unsterility is biofilm
- Biofilm develops in the system due to sub-optimal CIP & poor quality welds in product line
- Source of heat resistant spores is combination of ingredients and sub-optimal mix/blend procedure
- Root cause eradication package:
  - CIP improvements
  - Process improvements (UHT + mix/blend)
  - GMP improvements

QUESTIONS?