Microbiological Indicators: Low Acid Aseptic Production

Key to Systematic Troubleshooting

Systematic Troubleshooting: Data Collection

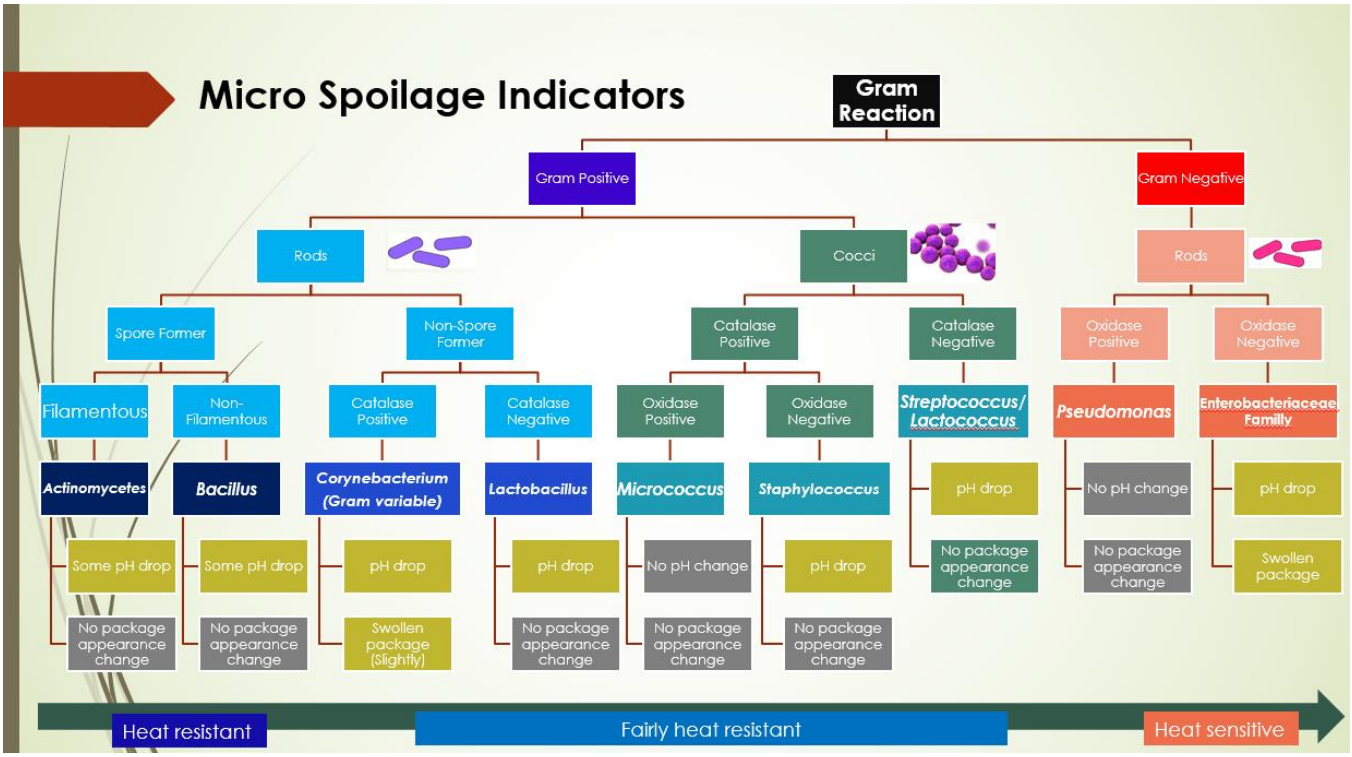
- % Defective in production run and spoilage pattern (Timeline Information)
- History of unsterility (Previous runs ok? Certain times of the year problematic?)
- Ingredient information (as applicable)
- Package Integrity/ Seal Integrity Test Results (last good check, packaging material information, etc…)
- Operational events, data and records (Operator log, other manual or electronic records, downtime, observations in the line, new equipment installation or design change, etc.)
- Operations, Maintenance and PM process and history
- CIP/SIP, Sanitation Logs (parameters check, water quality, etc…)
- Plant layout analysis (What processor feeds what tank/s and filler/s, etc.)
- Change Control implementation deviation; or lack thereof
    - May have been influenced by other functions (Procurement, Sales and Marketing, etc…)

Microbiological Indicators

- **pH of the affected product/s**
- **Sensory of affected product: gas formation, coagulation, smell, curdling, etc…**
- **Rough/ full Micro identification of the organisms responsible for the spoilage**

Rough Micro Identification

- Groups of bacteria or specific Genus are indicative of certain contamination source/s
- Very useful in the process of elimination – “The bugs don’t lie!”
- First step: description of spoilage indications
  - pH of affected product
  - Gas formation, coagulation, acid formation or flavor change
  - Smell
  - Separation, etc
- Second step: Spoilage organism isolation and other tests
  - Plating in appropriate agar (BHIA, PCA, TSA, etc.)
  - Mixed or pure culture?
  - Gram stain, Catalase and Oxidase test (as applicable)
  - Spore identification (if applicable)
  - Heat shock at 80C or 100C (as applicable)



Bacillus sp. (Heat resistant spore-formers)

Production source

- Process survivors
- CIP (sub-optimal) / Biofilms
- Pre-processing (inadequate soaking)
- Recontamination from area of high temperature

Resistance

- **Very resistant** against heat, chemicals
- and dry conditions

Bacillus subtilis - Mesophilic sporeformer - Flat sour reaction
Cells are rod shaped and straight, and fairly large, roughly 1-2 x 2-4 μm, and often arranged in pairs or chains. Spores are most often oval. Irregular, convex and wrinkled colonies, which easily are spread out on the agar surface. Thick and opaque and cream-colored brown colonies are usual.

Bacillus licheniformis - Mesophilic sporeformer - Flat sour reaction
Cells are rod shaped and straight, and fairly large, and often arranged in pairs or chains. Spores are most often oval. Colonies are Irregular, convex and **wrinkled colonies usually strongly attached to the agar and often slime is accumulated on the colony and looks like waterfilled bubbles**. Very common in raw paper. K-creases, creating contact between product and raw paper often result in *B. licheniformis* unsterilities.

Bacillus cereus - Psycrophilic sporeformer – Coagulation, no gas, no ph change
Cells are rod shaped and straight, and fairly large, and most often arranged in chains. Spores are most often oval. Irregular, convex and wrinkled colonies, which easily is **spread out on the agar surface. Thick and opaque and cream-colored to very white colonies** are usual. Compared to most strains of *B. subtilis and B. licheniformis, B.cereus* has a quite low heat resistance. Left in a system after a weak CIP, *B.cereus* has extremly adhesion properties and can grow to large populations, that are very heat resistant.

Bacillus stearothermophilus - Thermophilic sporeformer - Flat sour reaction
Cells are rod shaped and slender, and much longer than their width and often in long chains. Spores are most often oval. Irregular, convex and somewhat wrinkled colonies. Opaque and cream-colored brown colonies are usual. Unsterilities caused by thermophilic sporeformers are a good indicator of survivors from the UHT-treatment.

Streptococcus/ Lactococcus (G+ve cocci – lactic, oval, pairs & short chains),

Lactobacillus (G+ rods, long chains)

Production source

- Reinfection - Streptococcus
- CIP – Lactobacillus

Resistance

- Withstand dry conditions; some fairly heat resistant
- (thermoduric)

***Streptococcus**:* Gram-positive and catalase negative cocci. Cells spherical or ovoid, 0,5-2,0 μm, occurring in pairs or chains. Poor growth on ordinary laboratory media. Certain strains have pinpoint white and raised colonies.

Unsterilities with *Streptococcus* point in direction of **reinfection in the regenerative heating section**. Contamination with raw milk is due to cracks/pinholes in the heat exchanger in combination with pressure fluctuations in the system/malfunctioning back pressure valves.

***Lactobacillus**:* Gram-positive and catalase negative rod. Cells usually long rods, 0,5-1,2 μm x 1,0-10 μm, occuring commonly in short chains. Poor growth on ordinary laboratory media. Certain strains have pinpoint translucent colonies.

Unsterilities with *Lactobacillus* **point in direction of weak CIP**.

Staphylococcus/Micrococcus (G+ve cocci)

Production source

- Staph: Splices – handling
- Micrococcus: Airborne, Package Integrity (PI), also handling

Resistance

- Withstand dry conditions
- Some fairly heat resistant (thermoduric)

Staphylococcus: Gram-positive, catalase and oxidase negative cocci –Spherical cells, 0,5-1,5 μm, occuring singly, in pairs and in irregular clusters (grapes). Colonies are white/cream/yellow/orange.

Unsterilities caused by *Staphylococcus* is most often due to non-hygienic handling of packaging material. Packaging material sterilisation can not kill *Staphylococcus*, when occuring in greasy fingerprints. Deep bath machines are not as sensitive for bad packaging material handling as the non-deep bath machines, but splices unsterilities occur also in the deep bath machines.

Micrococcus: Gram-positive, catalase positive and oxidase positive cocci –Spherical cells, 0,5-2,0 μm, occuring in pairs, tetrads and in irregular clusters (grapes). Colonies are usually pigmented in shades of yellow or red.

Micrococcus species point generally in the direction of air contamination.

Some *Micrococcus* species with cell arrangement in tetrads are also borne on human skin and may occur as unsterilities due to non-hygienic handling of the packaging material.

Corynebacterium

(G+ve/variable staining and cell shape, characteristic "chinese letter" cell grouping)

Production source

- Reinfection
- Air (Mixed), Manometers

Resistance:

- Withstand dry conditions and
- fairly heat resistant (thermoduric)

Gram positive or variable in gram reaction. Non sporeformer.

Cell morhology: Straight and slightly curved, slender rods with clubbed ends in young cultures. Cell arrangement of rods: often cells in V, L or K figurations.These rods can be very long, up to 18 μ m. Later, in about a day, the rods are loosen up and cocci are liberated i.e. in an old culture there are only cocci. Colonies are often pigmented in shades of yellow.

Corynebacterium is together with *Micrococcus* a **good indicator of air contamination**. They do not occur simultaneously, either is it *Corynebacterium* or *Micrococcus*.

Common production sources for unsterilities are:

- Compressed air
- CPM (constant pressure modulating) valve
- Manometers

Pseudomonas (G –ve rods)

Production source

- Reinfection
- Package integrity
- Leakage/final cooling

Resistance

- Very heat sensitive; cannot survive dry conditions.

Gram negative, oxidase positive rod. Catalase positive and psycrotrophic. Small rod-shaped cells, slightly curved. Relatively large colonies, flat and elevated. Pigmentation of agar usually in light brown or green colour.

Unsterilities with Pseudomonas are a good indicator of **water leakage. *Pseudomonas* is always present in water**. Besides fault in packaging integrity, the most common source to unsterilities, is leakage in final cooling of heat exchanger is a common reason.

Lipolytic and proteolytic enzymes cause aggregation of the milk fat globules. Sweet coagulation can also occur as a result of solely enzymatic activity. If Pseudomonas got the possibility to grow to high numbers in the raw milk, which Pseudomonas can do at refrigeration temperatures as it is psycrotrophic, then proteolytic enzymes are produced in the raw milk. Pseudomonas is very heat sensitive and is killed by pasteurisation, but the enzymes are very heat stable and survive UHT-treatment. Sweet coagulation due to remaining enzymatic activity is a very slow reaction, which takes months.

Enterobacteriaceae Bacteria group

(G –ve rods: *Salmonella, E. coli, Klebsiella, Shigella, Yersinia*)

Production source

- Reinfection
- Package integrity
- Bad gaskets/tightness
- Non hygienic design

Resistance

- Very heat sensitive; can not survive dry conditions

Gram negative, oxidase negative straight rods. Usually catalase positive. Mesophilic. Many genera are pathogenic as *Salmonella* and *Shigella.*Small straight rod-shaped cells. Relatively small colonies, most often in shadows of beige.
They are facultative anaerobic (have aerobic and fermentative metabolism). Some of them can ferment lactose and are collectively named coliforms.

Unsterilities of *ENTEROBACTERIACEAE* is a **good indicator of bad hygiene, due to handling as well as of design**.

Examples of leakage creating unsterilities of *ENTEROBACTERIACEAE* are

- Package integrity (far most common)
- System tightness (gaskets etc.)

Certain groups of microorganisms will give an indication of possible root cause of unsterility in the system. This is key to the *systematic troubleshooting process*.

This microbiological indicator information must be reviewed with all the other investigativedata. It is then that pieces of the puzzle will start to show the spoilage picture.

- Using this fact based and data driven approach, the team can:
  - Identify the true root cause
  - Implement appropriate corrective and preventative actions
  - **Prevent future spoilages**