Distilling Best Practices: The Thermophile Files

A Test for Thelma

Thelma Castillo was smiling as she cheerfully opened the incubator to assess the results of her routine microbial analysis of the distillation vats. It was her fourth month on the job as a microbiologist for the Research and Development section of Negrosanon Distillery and she felt like she was living a dream. When the distillery hired her fresh out of college, she could not believe that they picked her over eight other applicants. She loved her job. Nevertheless, on this day she raised her eyebrows as she looked at the first plate.

"Is this contaminated? How careless of me!" Small specks of bacterial colonies were present in the culture. She proceeded to the next plate, and the next ...

She was no longer smiling. All the plates were the same; they were filled with thermophilic bacterial growth. She reached for the intercom and called the distillery manager.

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Thermophilic Bacteria: Characteristics and Physiological Features

Thermophiles grow optimally in temperatures of 50ºC-120ºC. These microorganisms are categorized on the basis of their temperature tolerance: facultative thermophiles can grow in temperatures of 50ºC-65ºC, but also grow at 37ºC; obligate thermophiles have maximum growth temperatures of 65ºC-70ºC, but will not grow below 40ºC; extreme thermophiles can grow between 40ºC-70ºC with an optimal growth temperature of 65 ºC; and hyperthermophiles can grow at over 90ºC with optimal temperatures of 80ºC-115ºC.[1]

The ability of these bacteria to survive and produce enzymes at high temperatures can be attributed to their cell membranes which contain saturated fatty acids that provide a hydrophobic environment for the cell and allow it to maintain rigidity at elevated temperatures.[2] The presence of certain metals, inorganic salts, and substrate molecules also aid in thermostability.[3]

Sources of Thermophilic Bacteria

Thermophiles prefer living in temperatures not commonly found in nature, but in areas such as hot springs and water heaters. One would assume that thermophiles require hot environments in order to survive. It is quite startling to find that they can be found everywhere including soil, manure, and compost piles.[4] Therefore any material which comes into contact with these substrata can become contaminated.

Common Problems with Fermentation

Problem fermentations can be divided into two broad categories: issues with fermentation rate progression and off-character formation; which are sporadic and chronic; and display a dependence upon substrate (juice) composition and strain variability.[5] In distilleries, the fermentation process is the most important aspect for production of alcohol. Several rate and progression issues can arise during this process. Among these are: long lags before onset of fermentation, a too slow or too rapid rate of fermentation, a sluggish maximal rate of fermentation, and actual cessation of sugar consumption.[6] These can be caused by numerous factors and careful analysis of fermentation conditions and of the fermentation profile can provide the accurate reason for poor fermentation performance.

The Quest for a Solution

Thelma looked up from her laptop and massaged the crick in her neck. She had been pouring over articles to find the protocols for eliminating bacterial contamination. It seemed as though they were doing everything right. They had been following the Hazard Analysis Critical Control Point (HACCP) system to the letter. These were thermophiles! No ordinary bacteria could survive the heat undergone during the pasteurization process.

"Time to take a field trip to the plant," Thelma thought to herself.

Environmental Manipulation

Bacteria, like most living organisms, require a certain environment to survive and persist. Generally, six basic environmental conditions are considered for bacterial growth; and these are the factors which are focused on in bacterial elimination and control. One way to easily remember these conditions is use of the acronym, FAT TOM: Food, Acidity, Time, Temperature, Oxygen, and Moisture[7].

Control of bacterial growth may be achieved through manipulation of their environment, based on the aforementioned conditions. The following are ways to control bacteria through environmental manipulation, as recommended by USDA in the guidebook, "Introduction to Microbiology of Food Processing:

- Control by pH

  Bacterial growth can be controlled by reducing pH below the minimum level for growth of the organism. For example, acidification can be used to prevent the growth of *Clostridium botulinum*. However, because some bacteria, such as E. coli O157:H7, have low infectious doses, preventing growth alone will not provide a safe product. Reduced pH can be combined with other factors to control the pathogens of concern. For example, a reduced pH, combined with a mild heat treatment, is used to achieve commercial sterility in canned, acidified meat and poultry products.

  - Acidification procedures

    To produce products with a pH of 4.6 or less, acidification must be properly done. Two procedures are recommended; direct batch acidification wherein ingredients are mixed in a kettle with an acid or more commonly, an acid food is added directly to the batch; or addition of acidified brine, such as pickled pig's feet.

  - Determination of pH

    The most important factor in the production of acidified foods is the timely attainment and maintenance of a pH level that will inhibit the growth of *C. botulinum* spores. To achieve this goal, it is necessary to measure pH. Although pH can be measured using dye solutions and pH paper, the recommended method for determining pH is with a pH meter. Table 1 shows the pH requirements for some indicator microorganisms found in food.

- Control by temperature

  Bacterial growth can be controlled by keeping food at temperatures above or below the temperatures at which bacteria grow. Thus, refrigeration, freezing, and hot holding can be used to control growth. However, the most effective way to control microorganisms is to kill them with heat. The amount of heat needed depends on a number of factors, including:
  - The specific microorganism (the species and whether or not it is in the spore form);
  - The number of microorganisms to be inactivated;
  - The food product; and
  - Factors of the food, such as its pH, a_w, the presence of preservatives (such as nitrate), and the amount of fat.

Table 1

Approximate pH Requirements for Microorganism Growth

Microorganism	Minimum	Optimum	Maximum
Clostridium perfringens	5.5–5.8	7.2	8.0–9.0
Campylobacter spp.	4.9	6.5–7.5	9.0
Clostridium botulinum toxin	4.6		8.5
Clostridium botulinum growth	4.6		8.5
Staphylococcus aureus growth	4.0	6.0–7.0	10.0
Staphylococcus aureus toxin	4.5	7.0–8.0	9.6
Enterohemorrhagic *Escherichia coli*	4.4	6.0–7.0	9.0
Listeria monocytogenes	4.39	7.0	9.4
Salmonella spp.	4.2	7.0–7.5	9.5
pH minimum as low as 3.8 has been reported for *Salmonella* spp.			

Source: Introduction to the Microbiology of Food Processing (USDA, 2012)

The "danger zone" is a term used to describe the temperature range that provides bacteria the optimal growth conditions. This range is 40°F to 140°F (4°C to 60°C). The following are therefore considered:

- Design food processes to ensure that food products spend as little time possible in the danger zone.
- Control the entire danger zone temperature. At the high end, bacteria will grow slowly, and at the low end, many bacteria will survive and some may grow.
- Temperatures above the danger zone begin to destroy most microbes, although the time needed for cell destruction is longer at lower temperatures. At refrigeration temperatures, some spoilage bacteria and a few pathogens can grow very slowly; however, for most bacteria, refrigeration temperatures are too cold for optimal growth. No significant bacterial growth occurs below freezing.

Table 2 shows the approximate temperature requirements for some indicator microorganisms.

Table 2

Approximate Temperature Requirements for Microorganism Growth in °F (°C)

Microorganism	Minimum	Optimum	Maximum
Campylobacter spp.	90 (32)	108–113 (42–45)	113 (45)
Clostridium botulinum types A & B	50–54 (10–12)	86–104 (30–40)	122 (50)
Clostridium botulinum type E	37–38 (3–3.3)	77–99 (25–37)	113 (45)
Clostridium perfringens	54 (12)	109–117 (43–47)	122 (50)
Enterohemorrhagic Escherichia coli	45 (7)	95–104 (35–40)	115 (46)
Listeria monocytogenes	32 (0)	86–99 (30 –37)	113 (45)
Salmonella spp.	41 (5)	95–99 (35–37)	113–117 (45–47)
Staphylococcus aureus growth	45 (7)	95–104 (35–40)	118 (48)
Staphylococcus aureus toxin	50 (10)	104–113 (40–45)	115 (46)

Source: Introduction to the Microbiology of Food Processing (USDA, 2012)

- Control by a_w

  When substances are dissolved, there is a substantial reaction between the substance and the water. The water-binding capacity of a particular dissolved ingredient influences the amount of water left for the growth of bacteria.
  - A number of the molecules of the water are bound by the molecules of the substances dissolved.
  - All of the substances dissolved in the water reduce the number of unattached water molecules and in this way, reduce the amount of water available for bacterial growth.
  - The extent to which the water activity is lowered depends primarily on the total concentration of all dissolved substances. The dissolved substances compete with the bacteria for available water.

  For most bacteria, yeasts, and molds will grow above a water activity of 0.95, and most foods have a water activity above 0.95. Spores of *C. botulinum* are generally inhibited at an a_w of about 0.93 or less. Thus, one food preservation method is to reduce the amount of water available to spores to a point where they are inhibited and apply a mild heat treatment to destroy the vegetative cells. Examples of meat products preserved with mild heat and reduced a_w are jerky and some dry sausages.

  - One commonly used method is an electric hygrometer with a sensor to measure equilibrium relative humidity (ERH). The sensors are the same as those used to measure relative humidity in air.
  - A dew point instrument is also commonly used to measure a_w

Table 3 shows some minimum a_w requirements for microorganism growth.

Table 3

Some Minimum Requirements for Microorganism Growth

Microorganism	Minimum a_w for Growth
Most molds (e.g., *Aspergillus*)	0.75
Most yeasts	0.88
C. botulinum	0.93
S. aureus	0.85
Salmonella	0.94
L. monocytogenes	0.92

Source: Introduction to the Microbiology of Food Processing (USDA, 2012)

- Control by Chemicals

  Chemicals may be added to foods to inhibit bacterial growth or to kill microorganisms. However, at normal levels of use (which must be approved by regulatory agencies such as USDA's Food Safety and Inspection Service and the Food and Drug Administration), most chemicals cause inhibition rather than inactivation. Acids and their salts (e.g. lactic acid and sodium lactate), nitrites, some phosphates, and sodium chloride (salt) are common chemicals used in the industry. Chemicals are often used in combination with other factors, such as heat or reduced a_w.

One can control bacteria by utilizing the interaction of various factors that were just reviewed. Combinations of inhibitory factors that alone may be insufficient to control microorganisms can often be effective when used together. This is referred to as the "hurdles" or "multi-hurdle" concept – if enough hurdles or barriers are included, bacteria will not be able to overcome the hurdles and grow. For example, when the water activity is lower, the pH range at which an organism can grow is more limited.

Hazard Analysis and Critical Control Point (HACCP): Principles and Guidelines

HACCP (Hazard Analysis Critical Control Point System) is a management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement and handling, to manufacturing, distribution and consumption of the finished product.

This system is designed for use in all segments of the food industry from growing, harvesting, processing, manufacturing, distributing, and merchandising to preparing food for consumption.

Creation of a successful HACCP system employs proper organization, logical and scientific planning based on food safety principles and intensive investigation and research, and strict implementation of the planned-out approach. According to the USDA Food Safety and Inspection Program (2016), design of this system is based on the seven principles of HACCP, which encompasses a systematic approach to the identification, prevention, and control of food safety hazards, which are as follows:

1. Conduct a Hazard Analysis
2. Determine the Critical Control Points
3. Establish Critical Limits
4. Establish Monitoring Procedures
5. Establish Corrective Actions
6. Establish Record Keeping and Documentation Procedures
7. Establish Verification Procedures

Little Bright Ideas, Big Solution

Twenty-four hours later, Thelma woke up with a start from the notes and the books she has been pouring over in her office. She started to furiously scribble more notes on her project paper, but this time with more confidence and assuredness. Her ideas were all based from the basic knowledge she has learned in her microbiology subjects; concepts which have long been learned, but once again remembered, when the need arose. Her plans were simple, but she knew for sure that they would work. She now knew what to do.

End Notes

[1] Kikani, B.A., R.J. Shukla, and S.P. Singh. “Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology..” Formatex Research Center , Vol.2. pp. 1000-1007. Jan. 2010. Accessed 27 August, 2016 <https://www.researchgate.net/publication/236179064Biocatalytic_potential_of_thermophilic_bacteria_and_actinomycetes>.
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[4] United States Department of Agriculture. “Introduction to the Microbiology of Food Processing.” Aug. 2012. Accessed 29 Aug. 2016. <www.fsis.usda.gov/shared/PDF/SPN_Guidebook_Microbiology.pdf>.
[5] Viticulture and Enology: Problem Fermentations. Accessed 1 Oct. 2016 http://wineserver.ucdavis.edu/industry/enology/fermentationmanagement/wine/problemfermentations.html
[6] Viticulture and Enology
[7] Introduction to the Microbiology of Food Processing.