Brewing Microbial Monitoring and ATP Testing

Gusmer Enterprises

Jill Gallegos

Outline

- Intro: Why should I monitor for microbes?
- Micro Monitoring in the Lab
  - Monitoring the product: Beer
  - Tools to monitor the beer
- Micro Monitoring in the Brewery
  - Monitoring the Brewery: Process Sanitation
  - Best practices
  - Common contamination points and troubleshooting
- ATP Testing

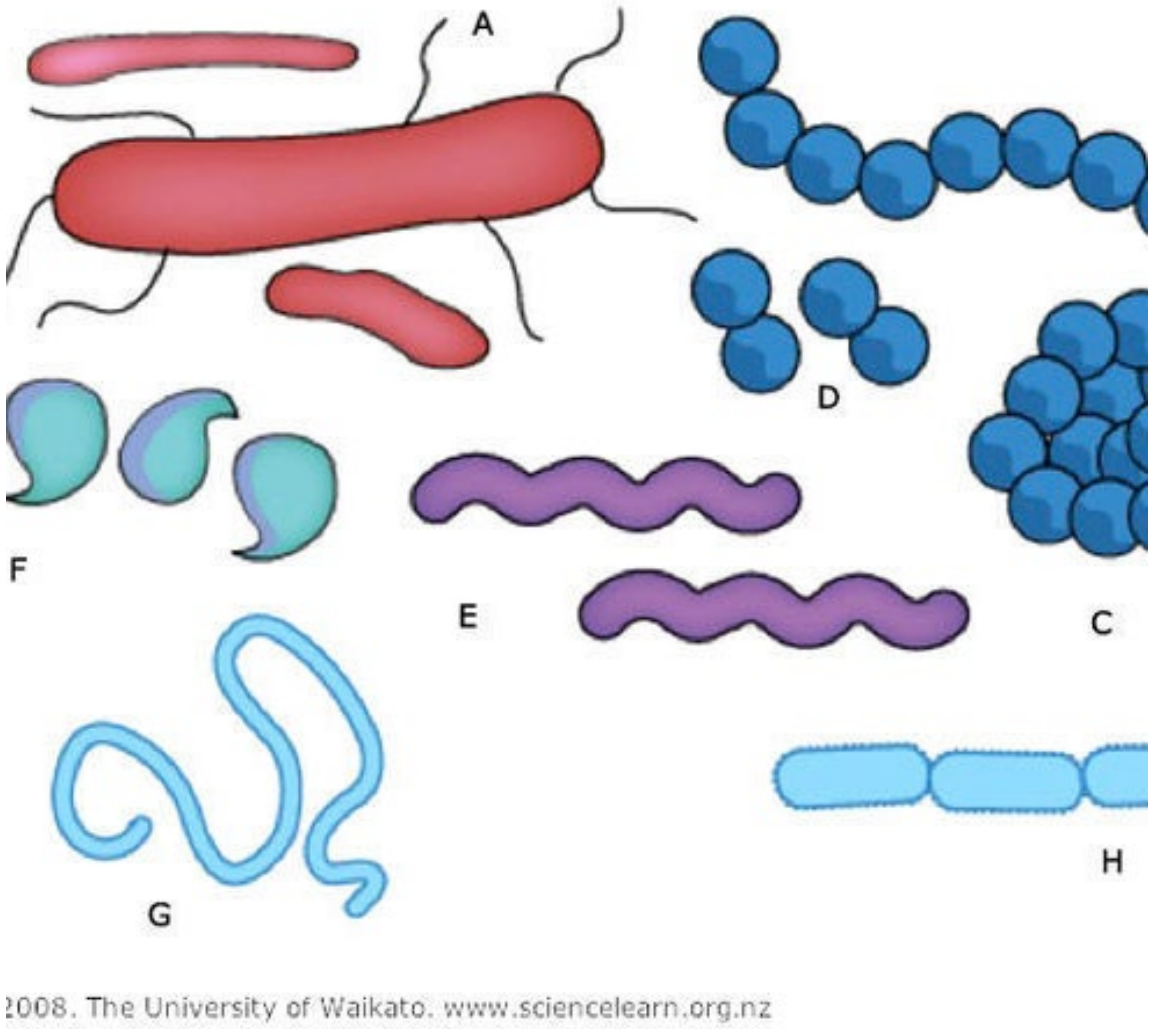
Why Should I Monitor for Microbes?

- Ensures no contamination and spoilage of finished product
  - If contamination, determine what
- Find contamination points
- Identify equipment failures or inadequate sanitation procedures
- Identify contaminated product from good product

Why Should I Monitor for Microbes?

Common Spoilage Organisms

- **Wort Spoilers (aerobic environment)**
  - Enterobacteriaceae
- **Lactic Acid Bacteria (facultative anaerobe)**
  - Lactobacillus, Pediococcus
- **Anaerobic Beer Spoilers**
  - Pectinatus, Megespheara
- **Beer Spoiler in the presence of Oxygen**
  - Acetobacter, Gluconobacter



Laboratory Equipment

Membrane Filtration

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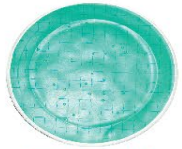
Yeast and Mold

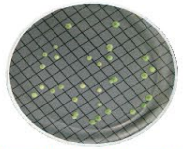


Orange Serum Media

Catalogue Number	MHA000P2O (50/Pk) NP0SA0150 (150/Pk)
Application	Used for the isolation and enumeration of acidophilic and aciduric microorganisms in water, beverages and foods.
Incubation Time & Temperature	48 hrs – 5 days at 24 – 32°C
Typical Colony Appearance	Yeast appear white, creamy and large. Bacteria are smaller, white or transparent.
pH at 25 °C	MHA000P2O: 5.6 ±0.2 NP0SA0150: 4.5 ± 0.2 (with time, a slight reduction of pH may be noticed, which will not affect the recovery performance of the product)
Packaging Type	MHA000P2O: Non-luer tip ampoule NP0SA0150: Dehydrated nutrient pad in 47mm dish

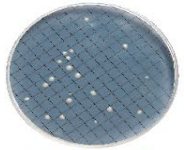
Wallerstein Nutrient Broth (WLN)

Catalogue Number	MHA000P2N (50/Pk) MX0OWN220 (20/Pk)
Application	Used to detect yeast and mold in worts, beers and other fermentation products. Used in cojunction with WLD.
Incubation Time & Temperature	48 hrs – 5 days for yeast at 20°C 35°C for bacteria
Typical Colony Appearance	Mold can appear non-pigmented to white, with various texture. Yeast appear as creamy, white larger colonies. Bacteria appear blue-green.
pH at 25 °C	5.5 ±0.2
Packaging Type	MHA000P2N: (Non-luer tip ampoule) MX0OWN220: (Luer tip ampoule)



m-Green/Schauffus Pottinger Media

Catalogue Number	MHA000P2M (50/Pk) NPSPY0150 (150/Pk)
Application	A low pH culture medium used to detect yeast and mold and other aciduric organisms
Incubation Time & Temperature	48 – 72 hrs at 28 – 32°C
Typical Colony Appearance	Yeast are large green opaque colonies. Mold appears green and filamentous. Bacteria able to grow at this pH form smaller clear to white colonies.
pH at 25 °C	MHA000P2M: 4.6 ±0.2 NPSPY0150: 4.5 ±0.2 (with time, a slight reduction in pH may be noticed, which will not affect the recovery performance of the product)
Packaging Type	MHA000P2M: (Non-luer tip ampoule) NPSPY0150: (Dehydrated nutrient pad in 47mm dish)

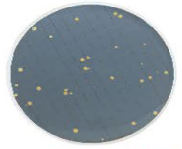


Yeast & Mold Broth

Catalogue Number	MX00YM220 (20/Pk)
Application	Used for the isolation and cultivation of yeast, mold and other aciduric organisms.
Incubation Time & Temperature	48 – 72 hrs at 20 – 30°C
Typical Colony Appearance	Yeast produce white colonies with a creamy texture. Mold is rough textured and/or filamentous. Bacteria are smaller and clear to white.
pH at 25 °C	4.6 ±0.3
Packaging Type	Luer tip ampoule

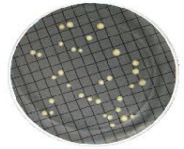
Brettanomyces Broth

Catalogue Number	MHA00BSM2 (50/Pk)
Application	Detection of *Brettanomyces* in wine and beer. Bacteria and other yeasts are inhibited.
Incubation Time & Temperature	5 – 7 days at 25°C
Typical Colony Appearance	Colonies appear small, white and creamy.
pH at 25 °C	3.5 ±0.2
Packaging Type	Non-luer tip ampoule



PRY (Preservative Resistant Yeast) Broth

Catalogue Number	MHA00PRY2 (50/Pk)
Application	A low pH selective culture medium for the detection of spoilage microorganisms resistant to acetic acid.
Incubation Time & Temperature	48 – 72 hrs at 30°C
Typical Colony Appearance	Yellow
pH at 25 °C	3.6 ±0.2
Packaging Type	Non-luer tip ampoule



Wort Media

Catalogue Number	NPWYM0150 (150/Pk)
Application	Used for the isolation and enumeration of yeast in beverages, beer and wine.
Incubation Time & Temperature	48 hrs - 5 days at 22.5°C
Typical Colony Appearance	Yeasts develop smooth white or colored colonies.
pH at 25 °C	4.5 ± 0.2 (with time, a slight reduction in pH may be noticed, which will not affect the recovery performance of the product)
Packaging Type	Dehydrated nutrient pad in 47mm dish

Morris Orange Serum

Catalogue Number	MHA00MSO2
Application	Used to detect yeast and mold resistant to sodium benzoate (like *Zygosaccharomyces bailii*) in beverage.
Incubation Time & Temperature	2 -7 days at 25-30°C
Typical Colony Appearance	Yeast colonies appear as white, creamy and large colonies.
pH at 25 °C	3.5 ± 0.2
Packaging Type	Non-luer tip ampoule

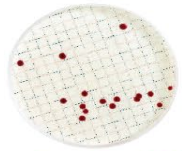
Yeast and Mold Selective Broth

Catalogue Number	MHA00P2SM (with Chloramphenicol) (50/Pk)
Application	Used to detect yeast and mold. Chloramphenicol is used to suppress background bacterial growth.
Incubation Time & Temperature	48 – 72 hrs at 28 – 35 °C
Typical Colony Appearance	Yeast appear as large green and opaque colonies. Mold is green and filamentous.
pH at 25 °C	4.4 ±0.2
Packaging Type	Non-luer tip ampoule

Total Viable Organism/Total Viable Count

Heterotrophic Plate Count (HPC) Broth

Catalogue Number	MHA000P2S (50/Pk)
Application	Recovery of heterotrophic bacteria found in various types of water, aspecially high-purity or potable. Prior to use, warm the media at 30-50 °C until liquefied.
Incubation Time & Temperature	48 – 72 hrs at 25 – 35°C
Typical Colony Appearance	Clear to white colonies; some may produce pigment.
pH at 25 °C	7.1 ±0.2
Packaging Type	Non-luer tip ampoule



Tryptone Glucose Extract (TGE) Broth

Catalogue Number	MHA000P2T (50/Pk) With TTC Indicator: MHA00P2TT (50/Pk)
Application	A non-selective medium to detect total heterotrophic microorganisms in watar and other liquids.
Incubation Time & Temperature	48 - 72hrs at 25-35°C
Typical Colony Appearance	Colonies appear clear to creamy white; some may produce pigment. Tryptone Glucose Extract Broth with Indicator (TTC) produce red colonies.
pH at 25 °C	7.0 ± 0.2
Packaging Type	Non-luer tip ampoule

Tryptic Soybean Broth (TSB)

Catalogue Number	MX00TT220 (20/Pk)
Application	Used to detect total heterotrophic microorganisms in water and other liquids.
Incubation Time & Temperature	18 – 72 hrs at 30 – 35°C
Typical Colony Appearance	Clear to white colonies; some may produce pigment.
pH at 25 °C	7.3 ±0.2
Packaging Type	Luer tip ampoule

Standard Count Media

Catalogue Number	NPSTC0150 (150/Pk) With TTC Indicator: NPTTC0150 (150/Pk)
Application	Used for the cultivation of fastidious and other microorganisms found in water, wastewater, raw materials, beverages, beer, food, etc.
Incubation Time & Temperature	24 – 48 hrs at 35°C 48 – 96 hrs at 25°C
Typical Colony Appearance	Morphology and color vary depending on the microorganisms caught on the membrane. The majority of microorganisms develop pink to red colonies (formation from TTC indicator).
pH at 25 °C	7.1 ± 0.2 (with time, a slight reduction in pH may be noticed, which will not affect the recovery performance of the product)
Packaging Type	Dehydrated nutrient pad in 47mm dish

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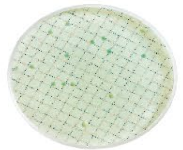
MILLIPORE™

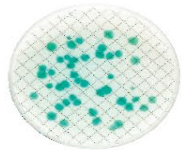
Microbial Growth Media
Selection Guide

Bacterial Selective



Wallerstein Differential Broth (WLD)

Catalogue Number	MHA000P2D (50/Pk) MX0OWD220 (20/Pk)
Application	Used in breweries to detact and enumerate bacteria present in small numbers in a mixed flora sample. Used in conjunction with WLN broth. Cycloheximide inhibits the growth of most yeast and mold, allowing bacteria to grow.
Incubation Time & Temperature	48 – 72 hrs at 30 – 35°C
Typical Colony Appearance	Bacteria appear small with green-blue color. If cycloheximide rasistant yeast grow, thay are creamy, green white.
pH at 25 °C	5.5 ±0.2
Packaging Type	MHA000P2D: Non-luer tip ampoule MX0OWD220: Luer tip ampoule

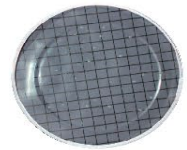


Pseudomonas Selective Broth

Catalogue Number	MHA000P2P (50/Pk)
Application	Used for the detection of *Pseudomonas* species.
Incubation Time & Temperature	24 – 72 hrs at 25 – 35°C
Typical Colony Appearance	All growth on this medium indicate the presence of *Pseudomonas* species. Colonies that are blue-green, brown or show fluorescance are presumptive *P. aeruginosa*.
pH at 25 °C	7.1 +/-0.2
Packaging Type	Non-luer tip ampoule

Lactose TTC Tergitol Media

Catalogue Number	NPECC0150 (150/Pk) EZPDLT150 (150/Pk + 150 EZ-Pak®membranes
Application	Used for the detection and enumeration of coliform organisms, thermotolerant coliform organisms and presumptive *Escherichia coli* in water, food and other samples.
Incubation Time & Temperature	24 – 48 hrs at 35°C. For specific *E. coli* detection, 24 hrs at 44 ± 0.5°C.
Typical Colony Appearance	*Escherichia coli* form small yellowish colonies (after 12–16 hours) which later change to orange and develop a yellow halo. *Enterobacter* and *Klebsiella* form yellow-green colonies. *Salmonella, Proteus and Pseudomonas* form red colonies with a bluish halo.
pH at 25 °C	8.2 ± 0.2 (with time, a slight reduction in pH may be noticed, which will not affect the recovery performance of the product)
Packaging Type	Dehydrated nutrient pad in 47mm dish

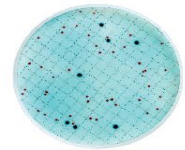


MRS Media

Catalogue Number	NPMRS0150 (150/Pk) MHA00MRS2 (50/Pk)
Application	Used for the isolation and enumeration of lactic acid bacteria species in food and other samples.
Incubation Time & Temperature	3 – 5 days at 32.5°C in a 5% CO_2 or in anaerobic atmosphere.
Typical Colony Appearance	Colonies formed are small and white.
pH at 25 °C	6.3 ± 0.2 NPMRS0150: (with time, a slight reduction in pH may be noticed, which will not affect the recovery performance of the product)
Packaging Type	NPMRS0150: Dehydrated nutrient pad in 47mm dish MHA00MRS2: Non-luer tip ampoule

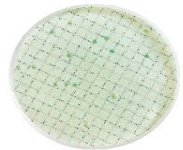
m-Endo Total Coliform Broth

Catalogue Number	MHA000P2E (50/Pk)
Application	Used for the recovery of *E. coli* and coliform organisms in potable waters.
Incubation Time & Temperature	24 hrs at 35°C
Typical Colony Appearance	Red colonies with green metallic sheen.
pH at 25 °C	7.2 ±0.2
Packaging Type	Non-luer tip ampoule



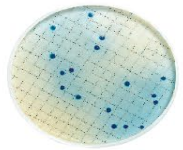
m-ColiBlue24® Broth

Catalogue Number	MOOPMCB24 (50/Pk)
Application	Used to detect both total coliforms and *E. coli* in water and beverages. This broth contains special inhibitors that prevent the growth of non-coliform bacteria but does not inhibit the growth of stressed organisms.
Incubation Time & Temperature	24 hrs at 35°C
Typical Colony Appearance	*E. coli* form blue colonies and other coliforms form red colonies
pH at 25 °C	7.0 ±0.2
Packaging Type	Non-luer tip ampoule



Pseudomonas CN Media

Catalogue Number	NPPCN0150 (150/Pk)
Application	Used for the detection and enumeration of *Pseudomonas aeruginosa* in water.
Incubation Time & Temperature	24 – 72 hrs at 35°C
Typical Colony Appearance	Colonies formed are green-blue with blue halos and fluorescence under short wavelength (254 nm) ultraviolet light.
pH at 25 °C	7.1 ±0.2 (with time, a slight reduction in may be noticed, which will not affect the recovery performance of the product)
Packaging Type	Dehydrated nutrient pad in 47mm dish



m-FC Broth

Catalogue Number	MHA000P2F (with Rosolic Acid) MHA00FCR2 (without Rosolic Acid)
Application	Enumeration of fecal coliforms by membrane filtration technique at an elevated temperature for waste or effluent water.
Incubation Time & Temperature	24 hrs at 44.5°C
Typical Colony Appearance	Fecal coliforms appear blue, other colonies appear gray to cream. In some rare cases, a membrane may have confirmed fecal coliforms that are pink in color.
pH at 25 °C	7.4 ±0.2
Packaging Type	Non-luer tip ampoule

Monitoring the Brewery: Process Sanitation

Process sanitation reduces unwanted microbes to at or below an acceptable level

- **Cleaning** – Removing some contaminants
- **Sanitation** – greatly reduces micro load
- **Sterilization** – Completely eliminates microbes from process

Three steps to brewery sanitation

- **Clean…Rinse…Sanitize**

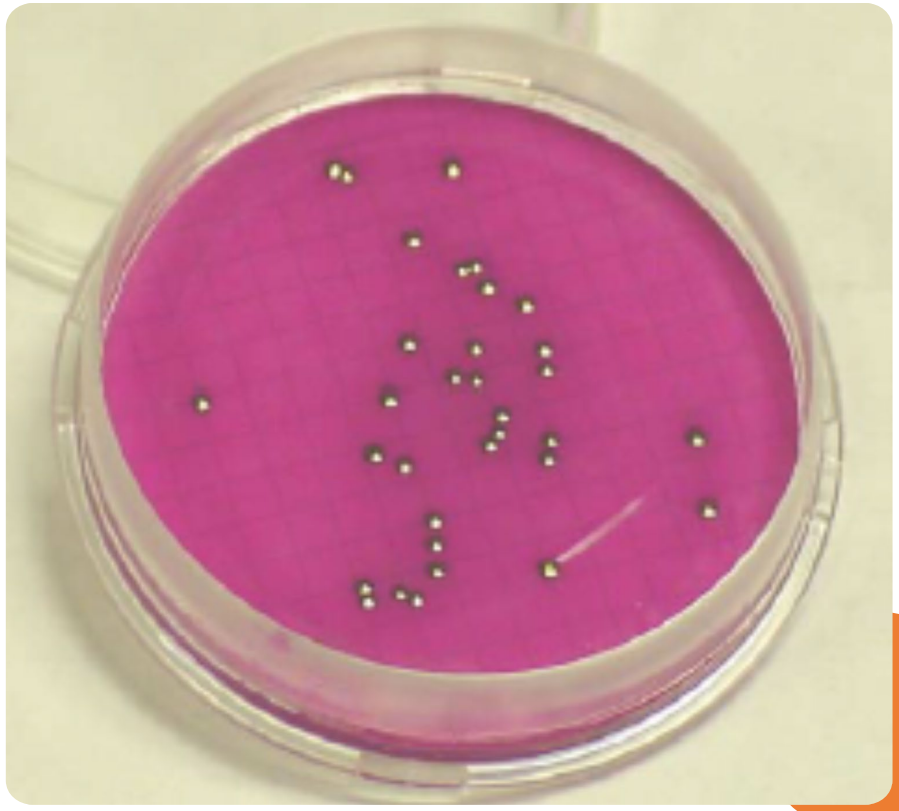
Process Sanitation

All contact surfaces must be sanitized at start-up

- Hot **180+ F° water**
- Ensure each surface reaches temperature

If line remains down for extended period it should be re-sanitized

- Typically 1-2 hours specification



Sanitation Audits

- **Regular audits** should be performed to ensure sanitation cycles are effective and being carried out correctly
  - **ATP** and **Swab/Samplers** to check piping and equipment
  - **Temperature checks** on suspect areas
  - **Visual inspections** of hard to clean areas
  - **Procedure checks** and operator training

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Hot Spot “Mapping”

- Tracking contamination points or failed swab tests can create a map of where contamination is occurring
- Focus improvements
- Create check lists or check points during or after sanitation

Sanitation Watch Outs

- Not opening all valves
- Not closing valves ***while still under flow***
  - This, often combined with drain placement, is a leading cause of periodic micro hits
- Not sanitizing the top of containers (surge tanks, filler bowls)
- Failure to reach temperature due to dead legs
- Outside of filling spouts (sanitation cups)
- Not using adequate water flow
  - Typically the same flow rate the process is run at should be the CIP/sanitation flow rate
  - There should be pressure on the line, occasionally throttle back valves to create a little back pressure for better cleaning/sanitation

Common Contamination Concerns

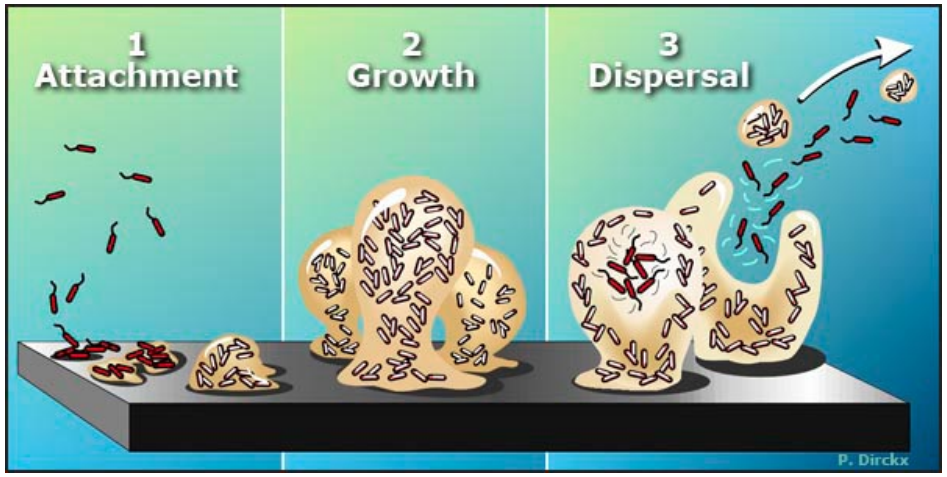
- **Floor Drains**
  - Many drains have positive pressure (watch for steam) which push microbes up and out into the bottling area
  - Unfortunately, many lines are designed so that sampling and drain valves are located directly above floor drains
  - Opening without flow or not closing under pressure during CIP will cause contamination

Common Contamination Concerns

- **Dead legs**
  - Long T's for drain/sample valves
  - Old piping, reclaim or recycle lines
- **Filling spouts**
  - Sanitation cups allow the outer rim and portion of the filling spout to be cleaned
- Drip and drain lines, gas lines, addition lines

Biofilms

- Poorly performed or too infrequent of sanitations can lead to the build up of biofilms
  - Biofilms harbor and protect microbes from sanitation
  - Typically in hard to clean areas
  - Difficult to remove
  - Prevention is key



Sanitary Sampling

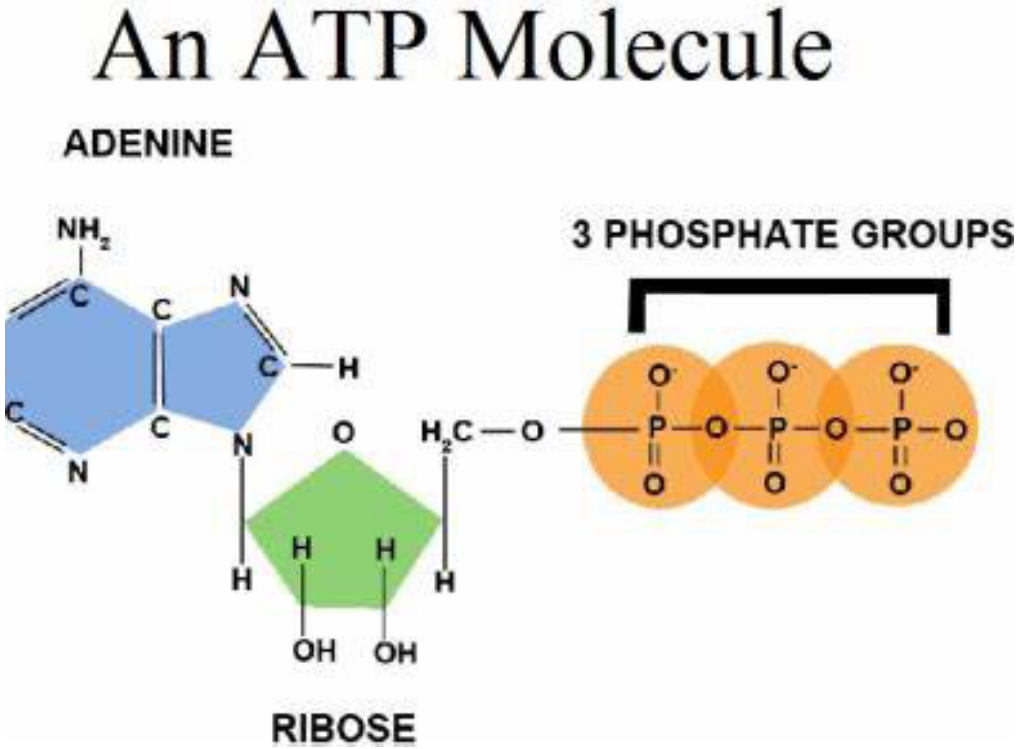
- Only open and close valves while under pressure
- Use smallest valve possible
- **Spray valve outlet with alcohol** and allow a second or two of flow before collecting sample
- Install specially designed **sanitary sampling valves**
- Use **sterile containers** for sample collection
- **Avoid dead legs** between product flow and valve outlet (long T's)

Sanitation Verification with ATP Testing

- ATP Technology
  - Firefly Luciferase is a light emitting enzyme responsible for the bioluminescence of fireflies
  - When ATP is combined with luciferin light is emitted.
- luciferin + ATP → luciferyl adenylate + PP
- luciferyl adenylate + O_2 → oxyluciferin + AMP + light

Sanitation Verification with ATP Testing

- ATP or Adenine Triphosphate is the primary energy carrier in all organisms
  - This molecule when found on surfaces will combine with Luciferase and produce light
  - ATP instrumentation that is used in food and beverage manufacturing will quantify the amount of light and yield a reading in RLU units



Sanitation Verification with ATP Testing

- What can produce a positive test with an ATP instrument in my brewery?
  - Bacteria
  - Yeast
  - Malt
  - Hops
  - Any material that is or was living

Sanitation Verification with ATP Testing

- Why would I be concerned about material that is not bacteria or yeast remaining on my brewing equipment after sanitation?
  - Remaining biological material after sanitation provides the perfect environment for microorganisms to multiply
  - When there is a food and water source available it is inevitable that microorganisms will thrive

Sanitation Verification with ATP Testing

- What are some examples of ATP technology testing sites in my brewery?
  - Product contact surfaces
    - Filler heads
    - Fermentation and Bright Tanks
    - Tank valves
    - Transfer Hoses

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Sanitation Verification with ATP Testing

- Where should I sample with ATP technology in my brewery?
  - Non Product Contact Surfaces
    - Drains (source of microbial growth and spread)
    - Sanitation equipment (mops, squeegees)

Sanitation Verification with ATP Testing

- MVP ICON is an ATP monitoring instrument that was created by BioControl Systems based in Seattle WA
  - Gusmer distributes the ICON system in the US under the Millipore brand

Sanitation Verification with ATP Testing

- How does the MVP ICON differ from its competition?
- The software that comes with the ICON allows users to create sampling plans and perform testing without writing down results
    - The software then stores all results which will allow for tracking of failing results and therefore makes troubleshooting easy

Sanitation Verification with ATP Testing

- How does the MVP ICON differ from its competition?
  - BioControl used thousands of data point on varying surfaces including stainless steel to identify Pass, Warn and Fail results making the process simplified for users

Sanitation Verification with ATP Testing

- What are some important facts to remember when using ATP technology for sanitation verification?
  - A failed reading does not necessarily mean that microbes are present, but it does means that the surface is not clean and therefore can promote growth
  - It is important to test after sanitation and use a failed result as a indication that the equipment needs to be cleaned again production begins
  - It is important to track results over time and use this information to identify trends
  - This technology is the quickest and easiest way to identify issues that can lead to spoiled beer before big problems arise

Questions?

Thank you!

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