

**MBAA District New England
Spring Meeting, March 8 2002
Hosted by The Shipyard Brewery, Portland, ME
John Seabrooks, MBCo., Presenter**

District New England was fortunate to have John Seabrooks of Miller Brewing Co. speak at our spring meeting this year on the topic of "Microbiological Q.C. For Small Brewers." John was at one point MBCo.'s microbrewery liaison, coordinating activities between the large parent company and some of its smaller subsidiary breweries, and he is currently director of R&D at Miller. His insights on the topic of running a small brewery were invaluable, and the following are highlights of some of the questions he fielded from our membership.

Q. What medium would you suggest for general purpose plating in a very small brewery (a pub) that may only be able to use one? Why?

- There is no ideal medium. The choice of media depends on what you want to detect.
- Universal Beer Agar (UBA) is a good general medium that will support the growth of a wide spectrum of microorganisms. This would be the best choice if only one medium could be selected.
 - It will support the growth of yeasts (wild and brewing yeasts) and most bacteria encountered in a brewery. Growth of *lactobacilli* and *pediococci* is promoted by anaerobic incubation conditions.
 - Addition of cycloheximide (Actidione) to about 2 to 3 ppm will inhibit the growth of yeast while allowing the growth of bacteria.
- If you were most interested in detecting lactic acid bacteria, Lee's Multi-differential Agar (LMDA, also known as SDA and sold through Siebel) would be a very good choice. This medium is as good or better than UBA+ for detecting brewing bacteria.
 - LMDA contains cyclohexamide to inhibit yeast growth.
 - Lactic acid bacteria grow well under incubation anaerobic conditions.
 - Acetic acid bacteria can be detected under aerobic incubation conditions.

Q. It has been noted that bright beer tank counts drop slightly overnight on those occasions when sampling can't be done until the day after filtration. Why?

- These counts may be due to the presence of non-beer spoiling bacteria that were introduced through filter aids – DE. Bacteria from DE are typically aerobic non-beer spoilers and do not survive in the beer matrix. This would account for the decreasing counts.
- Another possibility, although less likely, is that the bacteria are settling below the sample point, due to the cold temperatures at which bright beer is typically stored, resulting in an apparent drop in counts.
- One last explanation is that the apparent drop in counts could simply be due to the precision of the plating test – normal method error.

Q. When should bright beer tanks be sampled from?

- When – immediately after filtration. It would also be important to test just before running to packaging.
 - The reason for sampling early is to get the incubation started so results will be available ASAP – it takes 5 to 7 days for lactic acid bacteria to grow.
 - The reason for sampling later is to determine if there was an increase in counts, which would indicate a process problem that requires attention.

- Information from the above two bullets should be used to drive changes in cleaning schedule or procedures. The goal is to eliminate sources of contamination. The problem here is that the plating information won't be available until after the beer has already been packaged.

Q. Would the same rule of thumb (regarding sampling times) apply to bottled and draft packages?

- Yes. The same testing program should be applied to bottled and draft packages (as to bright beer tanks). Also, the fact that there are counts in the bright beer indicates a fundamental process problem that should be addressed through fact-based cleaning and root-cause analysis. The presence of any bacteria in the product puts beer quality at risk.

Q. What is the best time to draw a sample from the fermenter to assay for microbial activity other than yeast?

- At fermenter-full.
- Fermenter-full sampling is important to:
 - Detect wort spoilers, typically aerobes that will not survive the low pH and anaerobic conditions of brewery fermentations, but may contribute to flavor defects.
 - To determine the presence of lactic acid bacteria. Since lactic acid bacteria grow slowly, plating at fermenter-full ensures plating results will be ready by EOF so data-based decisions can be made in regards to product disposition, yeast acid washing, and fact-based cleaning.

Q. Recommended sample volumes for some typical control points? (i.e. yeast slurry, wort, fermenting beer, packaged product, rinse water, etc).

I assume the question refers to volume for microbiological testing.

- Yeast slurry – 1.0 ml for plating
- Wort – 1 ml for plating
- Fermenting beer – 100 mls - filtered
- Packaged product – 350 mls - filtered
- Rinse water – 500 to 1000 ml - filtered

Q. Please describe the dangers of cyclohexamide, it's potential replacements, and it's availability issues.

- Cyclohexamide, also known by the product trade name Actidione, is an antibiotic produced by the bacterium *Streptomyces griseus*.
 - It acts by inhibiting protein synthesis of eukaryotic organisms, including yeasts and other higher organisms. Bacteria are not affected.
 - It is a teratogen – it causes birth defects. For this reason pregnant women should not be exposed to cyclohexamide.
 - It is also a skin irritant and potential mutagen.
 - Target organs - nerves, kidneys, skin
- Possible replacements - Nystatin. Currently being evaluated by the ASBC.
 - Nystatin is more expensive than cyclohexamide and it is not soluble in water.
 - Nystatin acts by interfering with membrane permeability by binding to sterols.
- Availability of cyclohexamide is not currently an issue.

Q. Please describe some of the tricks and inherent dangers of multiple yeast strains in the brewery.

There are three obvious issues with using multiple yeast strains:

- (A) Keeping the strains separate;
- (B) Being able to distinguish between the different yeasts; and
- (C) maintaining each of the yeasts in a healthy state.

A. Keeping the strains separate

- Special attention must be paid to isolating yeast movement and storage to avoid accidental mixing or cross contamination.
 - Hoses, transfer lines, and storage tanks must be kept clean and free of other yeasts.
 - Fermenters and storage tanks should be appropriately labeled to avoid inadvertent mixing of yeasts and to ensure the correct yeast is used in the appropriate fermentation.

B. Distinguishing between different yeasts.

- Brewing yeast identification can be difficult, especially when trying to distinguish yeasts of the same type, i.e., different ale yeasts and different lager yeasts.
- Lager yeasts are very closely related and look very similar under the microscope and on the petri dish. Accurate identification requires chromosome fingerprinting (electrophoretic karyotyping) or PCR fingerprinting. Fermentation characteristics, such as cell size distribution, flocculation characteristics, and volatiles profiles, can also be used, but are less reliable.
- Ale yeasts are a more diverse group of brewing yeasts. Different strains often have unique characteristics that can be used to distinguish them, e.g., chain formation or lack thereof, and colony morphology.
- Lager and ale yeasts can be easily distinguished by their ability to grow at 37°C or by colony color on x-alpha-galactoside plates.
 - Lager yeasts won't grow at 37°C while ale yeast will. Therefore, you can detect ale yeast contamination of lager yeast using this method.
 - Lager yeasts form blue colonies on x-alpha-galactoside medium, while ale yeasts form white colonies. This medium can be used to detect lager yeast contamination of ale yeasts.
 - Weiss yeasts are similar to ale yeasts - top cropping, prefer warmer fermentation temperatures, grow at 37C, and form white colonies on x-alpha-galactose. They can be distinguished from ale yeasts from their characteristic phenolic or clove aroma, the result of the production of 4-vinyl-guaiacol.

C. Maintaining yeasts in a healthy state.

- This is not an issue if the different yeasts are promptly used following cropping.
- However, if yeast must be stored for more than two weeks between fermentations, it would be wise to revitalize the yeast before reuse. Revitalization involves repropagation using the following steps:
 - Wasting 95% of the stored slurry
 - Topping the storage tank off with fresh wort containing 2X zinc (0.30 ppm a good target) and saturated levels of dissolved oxygen.
 - If possible, supplement with additional aeration periodically during the first 8 to 12 hours after top off.
 - Allowing the yeast to end ferment.

Q. What are some common product defects caused by RD's in the brewery? How can a small brewer monitor for them? How common a problem is this for the small brewer?

- RD = respiratory deficient. Results from loss of yeast cellular mitochondria.
- Symptoms are slow, lagging, stuck fermentations and high VDK.
- RD's can be monitored using Wallerstein Laboratories Nutrient (WLN) medium. RD's form small, dark green colonies.
- Occurrence of RD's is not a wide spread or common problem, but it is the most commonly encountered genetic-based problem with yeast. It is thought to be somewhat strain dependent.

Q. What is the most common micro issue/problem you have observed with regards to very small breweries? How is this issue commonly addressed?

- Contamination of product with lactic acid bacteria and wild yeast, resulting in off-flavors and product inconsistencies.
- Cleaning.
- Effective quality control – knowledge of critical control points.

Q. During filtration of a very light colored and bodied product the following phenomena were observed:

- Pre-coat of the DE filter was as normal.
- Filtration of the product yielded bright beer until approximately 25% of the way thru.
- At 1/4 of the way through, the product at the outlet turned hazy.
- No pressure increase was associated with this (pressure never exceeded 3 bar psig on a filter rated up to 8 bar).
- Subsequent re-filtration of the hazy batch yielded bright beer.
- The product was bottled and showed no unusual effects throughout its shelf life.
- The QC/QA lab carefully screened the product and no unusual microbial activity was found. Also, the fact that the second filtration (same method as the first) cleaned up the haze rules out both bacteria and other physical haze issues, in the brewer's opinion.

This happened twice, about 5 months apart, last year. Same product recipe both times. The brewer never found an acceptable explanation.

Any ideas, detective Holmes?

- This problem may likely be due to a cracked filter cake perhaps due to improper precoating or variable pump speed / pressure spikes.
- A less likely explanation is bleed through of DE fines.

Q. What are your thoughts on the indispensable core elements of microbiological QC at any brewery, no matter how small?

- Monitoring of the critical control points in the process – HACCP, including the following points:
 - Brink yeast
 - Fermentation
 - Lagering
 - Bright beer
 - Draft
- Couple this with data-based cleaning

Q. Can you discuss the yeast acid rinsing controversy?

- I assume the controversy is whether acid washing deteriorates yeast quality.
- Yeast washing does not impact yeast quality if done properly.
- Temperature – 35 to 40 F

- pH – 2.2 to 2.5
- Time – 1 hour
- Hop acids are critical for effectiveness of bacterial kill.

Q. What preparations should a brewer make (in advance) to respond to a QC crisis that is microbiological in nature?

- HACCP – make sure collecting the right data from the right sample points.
- Accessible data system
- Proper collection and formatting of process microbiological data.

Q. Should small brewers propagate/store their own yeast? Is it either financially or technically possible to do as good a job as necessary?

- This depends on the size, the brewing/fermentation frequency, and microbiological cleanliness of the brewery.
- Many breweries have operated for years by simply serially repitching their yeast and never restarting from a master culture. The important point here is to be able to maintain clean yeast.
- The ability to propagate your own yeast adds flexibility to your operation and gives you greater control over the quality of the yeast you use.
- It is relatively simple to maintain and propagate yeast. Yeast can be maintained on agar slants for 3 to 6 months without problems (viability and genetic drift) or indefinitely without genetic change by storage at –80C. Propagation from slant or cryogenic stock to a small fermenter for step up takes 2 to 3 weeks.
- You will be able to maintain consistent yeast quality by storing the yeast cryogenically and periodically restarting the working culture.
- It is also helpful if the yeast curator has microbiological expertise.

Q. What criteria should we employ to help make the decision whether to run a batch to drain or not? I mean objective, not emotional/subjective criteria.

- Beer flavor and aroma – is the beer consistent organoleptically with customer and brewmasters' expectations?
- Never, never sell the customer a bad beer.
- It is also important to have specifications for each beer produced to be able to measure quality of outgoing products.