CBC Online Seminar Q&A

Yeast Storage Solutions: Theory and Practice

Q: Is agitation during storage recommended?

A: Yes, highly. The main issue here is ensuring that temperature is evenly maintained throughout the slurry. Yeast slurry can heavily insulate itself and temperatures internal may be much higher than temperatures external. It is crucial to make sure the slurry is homogenous in consistency and in temperature. Agitation makes this possible.

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Q: Any recommendations on yeast brink tank geometry?

A: I would keep considerations made when deciding tank geometry the same when determining yeast brink geometry. The factors are much the same and the big stress of yeast can be hydrostatic pressure. Taller, thinner vessels create more pressure on yeast slurries that like to settle to the bottom due to their higher density.

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Q: How do you recommend storing yeast in a small yeast library, if you don't have access to a -80 degree C freezer? Maintaining an in-house library?

A: There is much literature out there on how to produce yeast slants that have a very long lifespan. There are specific procedures to prepare yeast for long term storage, and these can be stored at refrigeration temperatures for a long period of time. Liquid nitrogen tanks are also plausible but the procedures to prepare yeast for deep freeze must be followed absolutely.

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Q: Do you have recommended dosing of oil for, say, a 1/6- or 1/2-barrel brink if we wanted to conduct our own continuing tests?

A: I would most definitely start very low, i.e. 0.06 mL per gallon. You can step up slowly but I would not recommend anything beyond a total contribution of 60 mL per 10 barrels of total batch contribution.

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Q: Could I make a yeast transfer tank from 50-liter keg?

A: Yes, with a good sanitary welder you could redesign the keg to hold yeast. Having adequate pressure relief and sufficient sanitization is key however, and modifying these to work as yeast

brinks has been done and are available to purchase. You will need a way to measure the overall weight of the slurry, and this should be considered in the design.

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Q: What would be a proper oil and KH2PO4 concentration for yeast storage? And how do you dose and mix it with the slurry?

A: Proper is a matter of opinion but it is key to note that the solution of KH2PO4 we used was 2% as directed by research articles. This was diluted in a sterile distilled water base. Oil should be started very low, and we used a micro-pipettor to measure out the oil. Keep all things as sanitary as possible. Autoclaving is the best way to sanitize. Then dose to your slurry and make sure to adequately mix it.

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Q: When serial pitching cone to cone, have you ever taken samples throughout the pitch to see if density and viability is homogeneous or not throughout the slurry?

A: We have not but it is important to note that this density can change quickly, especially as you run out of yeast slurry and get thinner closer to the top of yeast pellicle. You also can pull to fast from the slurry and create a gap that pulls the thinner volume through before you get all of the slurry. You should be able to see this visually but sampling more often is a great way to ensure you know what you have.

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Q: Do you suggest a recirculation loop or an agitator?

A: I tend to prefer agitation. If run properly, it is a much gentler way to mix yeast. Avoid centrifugal pumps where possible, and lobe pumps are better suited to moving yeast gently. I want to emphasize gently as the possibility exists to shear yeast and kill it from handling it too roughly.

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Q: How do you add the sunflower oil in a sanitary manner to a yeast brink, tank, etc. and do you recommend dosing sunflower oil to your yeast before potential yeast propagation?

A: We have transitioned to adding the sunflower oil in the boil to help as an anti-foam. But that being said, the process would be the same as adding fermentation additives like ALDC or Clarex where you must sanitize all equipment where possible and ensure it is clean. Brewers have done this through top ports or manways, but keep cleanliness and sanitation paramount at all times. Adding to yeast before propagation would likely have similar results to adding pre-fermentation as that is a similar environment, and what you are preparing the yeast for.

However, you should oxygenate during propagation regardless as yeast will not replicate adequately unless in an aerobic environment.

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Q: So if yeast prefers to live and be stored in a pH of 5.2, and by nature a lager takes longer to produce, D-rest, etc., at the time the pH is probably around 4.3-4.5. So is it to be assumed that storing and keeping lager yeast is always going to be harder to do? Or did I simply misunderstand your point?

A: In this case, the experiments displayed other results than lager yeast being more sensitive to long term storage. In fact, lager yeast demonstrated the most stability in long term cold storage. This was a surprise, but this is not fully understood yet. It might have something to do with the integrity of the cell walls of specific strains. What is key to note is that cell wall strength and durability have large impacts on the ability for yeast to maintain its intracellular pH.

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Q: Any thoughts on testing KH2PO4 AND sunflower oil in a storage brink?

A: Go for it, We did play around with this, and the results were not necessarily definitively showing benefit but we are continuing to work on this theory. It is likely that these two compounds act independent of each other on different aspects of cell health so it would make sense for them to work cooperatively on improving yeast storage health.

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Q: How much is “a little positive pressure?” Do you recommend a specific PSI?

A: Keep it as low as possible with still having some positive pressure. 1 to 3 PSI is adequate to ensure there are no points of ingress or vacuums being pulled.

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Q: Do you have recommendations for agitation rates with an automated low shear agitator? For example, "run for 2 minutes every 30 at 5 rpm" or some such thing.

A: This will be dependent on a few factors but the overall goal is to ensure good mixing over time. Whatever agitation rate with your agitator design ensures good homogenous mixing is recommended.

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Q: How often should one mix the contents of a keg with yeast in storage? Would too much agitation add unwanted oxygen which you want to avoid during storage (due to glycogen depletion)?

A: The overall goal is to prevent too much stress on the stored yeast and having aerobic conditions will cause the yeast to replicate potentially too much. Yeast will release CO2 in storage and one can assume if this is demonstrated that the natural CO2 will blanket the environment. As long as you do not have leaks or are pulling a vacuum, your yeast should be in an adequately purged environment. Mixing then should be done regularly (every couple of hours) to ensure it is homogenous and evenly cooled.

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Q: How many times were these experiments carried out?

A: We ran the first set of experiments about 3 times or so and the second set 5 times. We had a few more going that we had to stop when the shelter in place order took effect. Results over all showed immensely similar results.

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Q: Can I be confident that the density I calculate from a sample pulled from a fermenter cone is representative of the bulk of what's in the cone? I thought that part of the advantage of an agitated brink would be the ability to get a homogeneous slurry (so a sample is representative of the whole).

A: In all honesty, no. But there are a lot of factors to consider here. The stored fermentation vessel cone is not agitated, nor is it homogenized. It is a settled mass, with temperature stratification and density highly likely. A brink does in fact provided the benefit of a homogenous mixture if the agitation regime is designed to adequately mix the slurry.

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Q: How do you decant from a stainless brink (e.g. how do you know when you have all the top liquid/draw from the top)?

A: Great question and I don’t have a good answer for this. Potentially a racking arm above the settled slurry would be imaginable, but I have never seen such a thing. In that case, you could “drop” the decanted beer from the slurry. However, we adjusted our experiments to fit the reality that this does not exist to our knowledge and stopped decanting to see if the results would be maintained. Initial results showed promise.

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Q: Have you noticed any increase in oxidative flavors in the finished product or premature staling from the addition of fatty acids?

A: In the case of research and what we have seen, no. In fact, in specific experiments it was demonstrated that shelf life was improved when oil replaced oxygen in storage.

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Q: Is there enough protein from the sunflower oil that we would need to worry about nut allergies or is it such small quantities that it wouldn't matter?

A: My limited research and understanding into this is that the process by which the oil is extracted prevents MOST allergens from being carried over into the oil. However, I highly recommend you do your research before trying this.

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Q: Are you using sunflower oil in place of a silicone-based or other defoamer? How well does it work in that role?

A: Yes, we are. Birko has a good article on their website about a plant based anti-foam. Our results showed some promise in that we did not have boil over issues, but getting the dosage rate dialed in is key to its success. We started low and have gradually increased over time.

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Q: Knowing that serial re-pitch is a good way to ensure consistent fermentation profile, would you recommend "dead yeast" discharge from tank's cone during fermentation (before yeast cropping day) as a way to prevent "bad/dead cells" perpetuation into following batch(es)?

A: I think I misunderstood this question in the presentation and I apologize. If I get your meaning, you mean dropping dead yeast to the drain before harvesting. And the answer is absolutely yes. You will be able to visually see a change between dead yeast/trub and live yeast slurry. A discharge drop should always take place before harvesting yeast so that these dead cells do not transfer over to the next fermentation.

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Q: Any idea what the alcohol content of the samples were pre-oil dosing? Wondering if residual ethanol is a necessary solvent for oil-based acid uptake into cells.

A: The American ale strain experiments were carried out with a 7% ABV IPA fermentation and the W34/70 experiments were carried out with yeast harvested from a 4.8% ABV fermentation. So one was about middle of the road as to what we produce and one was the low-average end. It’s possible that ethanol may assist in dissolving oil but you must consider the stress that higher alcohol beer will put on yeast slurry. So diluting to a common ethanol rate would be recommended.

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Q: How would you recommend to monitor the temperature of the yeast in the yeast tank to minimize the risk of contamination?

A: In this, to monitor the temperature, you should always strive to measure from the center, i.e. “core”, of the yeast slurry. A deep thermowell will tell you the temp internal but may disrupt agitation equipment. It is key though to know your internal yeast slurry temperature.

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Q: Have you considered measuring glycogen content through an iodine test (Lugol), rather than "dead or alive" cells with mthylene blue? What would you expect the results to be?

A: Moonjai et al actually did something to this effect and demonstrated an increase in glycogen with oil dosing. We did not do this as of yet, but want to focus most on vitality going forward as that is the big indicator of future performance.

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Q: Would you recommend using a buffer such as KH2PO4 for yeast propagation as well?

A: Likely not, as the big key for yeast propagation is to supply the yeast with key nutrients such as FAN and trace metals, as well as carbohydrates and oxygen. KH2PO4 only supplies specific nutrients (K and PO4) that are inadequate to sufficiently prepare yeast for further fermentation.

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Q: Do storage solutions vary greatly when propagating and/or maintaining spontaneous fermentation microbes? I.e. if propagated from Lambic or Sour Ales.

A: This I cannot speak to as we did not focus on this. It would be interesting to see what if any effect the storage solutions would have on storing bacteria and wild yeast, but they tend to be very resilient and perhaps more so than Saccharomyces strains.

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Q: Was rapeseed (canola) oil investigated as one of the vegetable oils with higher linoleic acid content in yeast propagation or early yeast generation fermentation?

A: No, but it is high on my list going forward. It does have the benefit of higher linoleic content but we wanted to target a nice balance between oleic and linoleic which sunflower oil suited well. Hopefully we will have future results to demonstrate how varying oils impact yeast health.

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Q: If you're harvesting from a centrifuge, would you expect to have a bit of decreased vitality from sheer force damage?

A: Potentially, yes. But there are many other reasons that centrifuges have been considered less ideal for yeast collection. Often it is the difficult sanitary collection methods. Yeast

separating centrifuges are generally designed with light treatment of yeast in mind so it is likely that they would take the health of the yeast into consideration.

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Q: Have you quantified the amount of oil added into the kettle that survived trub precipitation and made it into the fermenter?

A: We have not, nor developed the method to identify and differentiate these from standard fatty acids derived from malt. It would be difficult to separate these from each other but likely is possible.