

PROCESS CONTROL FOR MICRO STABILITY OF BEER

Beer Attributes

Micro

IAAs inhibit at 10 ppm.

Descending order of microbiological inhibition: alpha-acids.....hexa IAAs...tetra IAAs.....rho.....IAAs.

Beer is a nasty environment for most bugs due to: a) high ethanol levels; b) acidic pH's – in some cases below 4.0; c) only polymeric forms of carbohydrate & protein left once yeast have finished with the wort; d) in heavily hopped brands, antibacterial properties of hops acids; e) if you need oxygen to grow you are out of luck in the anaerobic, carbonated environment of beer – it's a tough place to make a living!

Increasing micro stability by adding hops at the end of boiling or in the whirlpool as keeps [AAs] relatively high.

Hop alpha-acids are 3 to 4 times more active against microbes than IAAs.

Fosters: MF beer sample....24 hr enrichment....remove non-microbial ATP with ATPaseextract microbial ATP by adding 500 uL of NRM.....pipette 200 uL sample to cuvette...add 100 uL LUMIT-QM.....read as light RLUs.....any sample 3x RLUs of sterile control sample scored as "positive". Found :

- i) 10.7% of production samples that were positive by RLUs were negative by traditional plating media.
- ii) Determined most false positives were associated with poorly cleaned fillers, with most of these occurring at start-ups due to the presence of non-beer spoilers.
- iii) In bottled sample, 1.2% of samples were positive for both RLUs/traditional media.....95% negative for both.....3.8% positive by RLUs and negative for media culturing.
- iv) When in-line sampling for ATP by RLUs, debris build-up can block assay step to extract microbial ATP.....

Effect on Beer Microbiological Stability

Spoilage potential of lactobacilli increases if it contains a plasmid with the HorA gene (hop resistance gene). If also contains HorC, almost 100% chance will be a spoiler.

Wild yeast can produce **nitrosamines** through nitrate reduction to nitrite which then reacts with secondary amines during fermentation to produce nitrosamines. Examples include species of *Brettanomyces*, *Hansenula* and *Candida*.

German survey of spoilage organisms.....@ 49% are *Lactobacillus brevis*, 12% *Pediococcus damnosus*, 15% *Lactobacillus lindneri*, 13% "other" lactobacilli and 11% "others" (non-lactobacilli).

VNC strains"Viable but non-culturable" strains that can grow well in beer but not in synthetic media.....high adaptability to beer makes them less able to grow in MRSincreased numbers of transfer in beer retards growth of *L. lindneri* and *L. paracolindes* in MRS.

Plasmid borne PMF (Proton Motive Force)-dependent transporter protein suspected to enable spoilage.

Brauwelt: spoilage frequency is @ 45%, 4%, 5% and 46% for *L. brevis*, *P. damnosus*, *L. lindneri* and others, respectively.

Subculturing at 30°C induces *L. lindneri* to lose its plasmids.

CLEN supports growth of @ 88% of wild yeast species, including all strains that could be detected on LYSINE agar, as well as LYSINE – strains which in theory should have been lysine +. Of the 44 strains tested, # positive for detection was 32, 30, 24, 21, 14 and 12 for Growth at 37°C, Copper Sulfate, CLEN, Lysine, XMAC & CYC media, respectively. XMAC slowest of all taking 14 days to allow inducing of enzymes for the strange CHOs used. Most WY grew on only one, maybe two of the media, few on 3-6, none on all seven.

L. paracollinoides is a beer spoilage species.

L. lindneri is "an innate beer spoiler".

"Beer Spoiler": those exhibiting positive growth in 20 BU/pH 4.2 lager.

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- v) 100% correlation between samples by ATP vs. cultural when > 5 cells per sample. # cells/membrane vs. RLUs 1, 2, 11 and 49 yielded 198, 80, 127 and 1,000 RLUs.
- vi) Frequently observed *Pseudomonas diminuta* due to poor CIP..... detected by ATP, but not cultural methods.
- vii) Rinsing membranes with antifoam reduces interference even further as foam inhibits extraction of microbial ATP.
- viii) Require higher [ATPase] in samples with higher foam levels (e.g. dark ales and highly carbonated lagers).

L. casei 316 bp PCR primer RNA data base *L. brevis* a 739 bp.... *L. plantarum* a 590 bp primer..... Able to detect 10 bacteria/bottle of beerneed to rinse membranes with water, otherwise beer completely inhibits PCR technology. Procedure: MF (polycarbonate) beer sample...wash with water....extract/ppt DNA...amplify DNAgel electrophoresis....score as positive or negative.

Microbiological Issues

Micro

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