PROCESS CONTROL FOR H_2S & THIOLS IN BEER: YEAST

Yeast Genetics

- MET2 gene disruption (+)
- MET10 gene disruption (-)
- Over expression of MET25 or NHS5 (-)
- Recombinant yeast constitutive for O-acetylhomoserine sulfhydrylase or cystothionine beta-synthase (-)
- **CB: esterase enzyme producing IAA also produces MTA...."cabbage & bananas" analogy!**
- Yeast sequential produce H_2S.....methanethiol ..... MTA in tandem.

Yeast Physiology

Hayashi of Suntory: paradigm is that CO_2 purging of H_2S is the mechanism by which H_2S is depleted late in fermentation. However by conducting a thorough daily process survey, he demonstrated the mechanism is actually via yeast assimilation from green beer, with the following observations:

1. Two peaks of H_2S at @ 200 ppb at day 1 and day 3.
2. Between these peaks H_2S drops as low a 50 ppb.
3. After the second peak, H_2S levels decline to < 10 ppb.
4. Most rapid declines in H_2S are when highest attenuation limits are reached……..in other words, when fermentable sugars are depleted
5. Purging is a very minor contributor to depletion of H_2S, with exit CO_2 reducing by @ 0.4 ppb, vs. a drop of 21.5 ppb over the same period late in green beer finishing.
6. Developed the equation: AD (Attenuation Difference) = LA (Late Attenuation) – AA (Apparent Attenuation) = residual fermentable sugar. Rapid reduction by yeast of H_2S starts at the SAME AD values in brands with different design LA's.
7. Reduction of H_2S in green beer enhanced by higher concentrations of yeast in suspension.

- Three mechanisms for H_2S formation: i) catabolic via degradation of cysteine and methionine; ii) anabolic via leaking from assimilatory sulfate reduction and iii) yeast autolysis.
- H_2S derived directly from internal pool formed as an intermediate in the biosynthesis of sulfur amino acids.
- **Minato:** "H_2S is formed as an intermediate during reductive sulfate assimilation leading to the synthesis of the sulfur-containing amino acids methionine and cysteine."
- **Thiols/thioesters mainly fermentation derived. Includes methanethiol, ethanethiol, propanethiol, methyl or ethyl thioacetate, iso-propanethiol.**
- Adding **serine or threonine** above a 200 ppm threshold significantly increases [H_2S].
- H_2S produced via cysteine breakdown (less important) or sulfite reductase (more important).

Utilizes H_2S for cysteine & methionine biosynthesis.

Yeast sulfite reductase activity (+)

H_2S Levels in Beer

Yeast Growth

- **Highest production of H_2S occurs in earlier stages of fermentation, thus being driven off in CO_2.**
- Maximum H_2S production observed when budding index nearly zero.
- Maximum H_2S production observed when wort attenuation most rapid.
- Primarily fermentation derived (not wort).
- **H_2S production burst seen at the end of growth phase.**
- Early absorption of wort methanethiol by yeast, followed by conversion to methyl thioacetate and elimination with CO_2.
- Later fermentation production of methanethiol.
- Faster fermentations > slower.
- Early literature…..claims release of H_2S timed with budding.

Yeast Strain

- H_2S production coupled with yeast growth phase....**amount** produced though influenced by temp., [CO_2], [trub], wort aeration, etc...
- **H_2S production peaks during lag & stationary phases of fermentation.....virtually nil while cell # increases.**
- Anything which suppresses growth (+) (e.g., low temp, low pitching rate, low D.O., slow fermentation, poor yeast vitality/viability).
- Methyl thioacetate increases throughout entire fermentation.
- Higher aging temps purges off H_2S more efficiently (-)
- **Suntory: production of H_2S and thiol compounds by yeast occurs mostly in the latter stages of fermentation.**
- **FAN starvation increases [H_2S], but NOT SO_2…..increasing [FAN] relieves cysteine induced H_2S production, however for SO_2 actually increases SO_2 production by yeast.**
- Less vigorous fermentation, more "unhappy" yeast, less purging of H_2S (+)

- Acetaldehyde levels (+)
- **Excessive SO_2 production elevates levels of all sulfur compounds in beer.**
- **Carlsberg yeast (+)**
- **Tuborg yeast (-)**
- **Ale yeast** produce less SO_2 and H_2S than **lager yeast**.
- **High H_2S producers**.....elevate levels in aging of mercaptans and 3-methyl-2-butene-1-thiol (+)
- Use of dried yeast (e.g. Fermentis) vs liquid yeast (e.g. Wyeast).....use of dried yeast yields beer higher in sulfur notes, especially before conditioning, although the difference diminishes with extended tank times – which depending on the beer style (eg a cream ale) can yield an even more preferred beer if pitch with a hybrid of dried/liquid cultures!!
- **Strain (+/-) ! (especially high $S0_2$ producers). Anywhere from 0-30 ppb, all else equal.**
- **High SO_2 producers** (+)

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