

Brettanomyces:

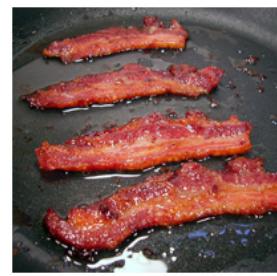
Flavors and performance of single and multiple strain fermentations with respect to time.

Greg Doss
Wyeast Laboratories
AHA NHC June 20, 2008



“Brett” Descriptors

- Horse Blanket
- Barnyard
- Pie Cherry
- Sweetarts/ Smarties Candy
- Smokey
- Band-aids
- Bacon
- Spice
- Cloves
- Cheesy
- Rancid
- Mousy/ Mouse Urine
- Bready
- Dirty Socks
- Clean????



Outline of Talk

- General Brettanomyces Information
- Wyeast Experiment
- Tasting/ Discussion

General

- Isolated by Claussen 1904 as cause of 2° fermentation of English Stock Beer
- Brettano-myces = British Brewing-Fungus
- Dekkera
 - Interchangeable name for Brettanomyces
 - Sporogenous form or sexual anamorph
 - Spore form is rare in nature <1.0%

General

- Major spoilage organism in wine.
 - Responsible for \$ millions annual losses in the wine industry.
 - Most research performed on Brettanomyces in wine.
- Very little research in beer.
- Common character of Lambics and Orval
- Homebrewers Gaining Popularity
 - (Burgundian Babble Belt, Mad Fermentationist, etc.)
- US Commercial Brewers
 - Russian River, Pizza Port, Jolly Pumpkin, The Shed etc.

General

Brettanomyces/ Dekkera Species Taxonomy

- *B. bruxellensis*
 - *B. lambicus*, *B. intermedius*
- *B. anomala*
- *B. custersianus*
- *B. naardenensis*
- *B. nanus*

Each species very diverse with many strains

General

- Slow growing
 - Grow to large populations from very few cells
- Fastidious
- Very adaptable
- Resilient to harsh conditions
 - Low pH
 - High alcohol
 - Low nutrient
- Resistant to Cycloheximide

Morphology

- Variable Morphologies
 - “Boat” shaped (Ogival)
 - Spherical
 - Elongated (Pseudohyphae)



Metabolism

- Can use a large number of substrates for carbon source
 - Simple Sugars
 - Glucose, Fructose, Sucrose, Trehelose, Maltose, Maltotriose
 - Dextrins
 - Maltotetraose completely
 - Maltopentose & Larger dextrans partially
 - Alcohol
 - Ethanol, glycerol
 - Cellibiose (Wood)
 - Most of the problems in wine industry

“Brett” Flavor Contribution



- Volatile Phenols
 - Medicinal (Band-Aid, plastic)
 - Farmyard (Horse blanket, Wet animal, Wet leather)
 - Spicy Clove
- Acetic Acid (Vinegar)
 - O₂ required
 - More found in barrel/ carboy aging
 - Less if added at bottling
- Isovaleric Acid (Rancid, Cheesy)
 - Leucine conversion
- Tetrahydropyridines (Mouse urine [High] , Bready [Low])
 - Ethanol + Lysine (O₂)
- Ethyl Lactate
 - Fruit, Coconut

Volatile Phenols



- Why do they do that? .
 - Survival mechanism.
 - Way for cell to replenish coenzyme NADH pool
- Reaction is dependant on enzyme activity and substrate levels
 - Not necessarily dependant on growth
 - Population
 - Enzyme activity is dependant on fermentation conditions.
 - ★ • Manipulated by O₂, pH, Density, Temperature
 - Level of substrates - Hydrocinnamic Acids (Penolic Acids)
 - Abundant in plants including cereal grains.
 - Anti-oxidants
 - Natural antimicrobials
 - ★ • Manipulated by Grist/ Mash profile

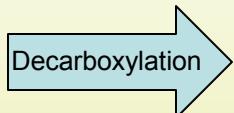
Volatile Phenols

Phenolic Acid

P-Coumaric Acid

Ferulic Acid

Caffeic Acid

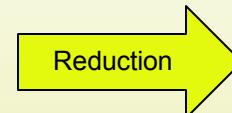


Vinyl Phenol

4-vinyl phenol

4-vinyl guaiacol

Vinyl calechol



Ethyl Phenol

4-ethyl phenol
(Barnyard)

4-ethyl guaiacol
(Smokey)

Ethyl calechol

Common

Saccharomyces

Most Wild yeast

Lactic Acid Bacteria

Unique

Brettanomyces

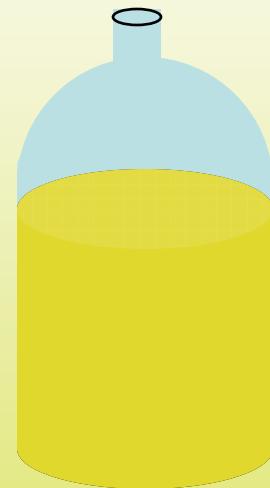
Pichia spp.

Candida spp.

Few Lactic Acid Bacteria

Sanitation

- Why are we scared?
 - Don't know a lot about it.
 - Will survive in low pH, high alcohol, low nutrient environments.
 - Very recognizable flavor profile.
 - Bio-film
- Don't be scared!
 - Brettanomyces dies like everything else.
 - Use good sanitation techniques – no problems
 - Susceptible to heat and chemicals.
 - 155F
 - Avoid plastic fermenters
 - No cracks in gaskets
 - Take all parts of corni apart
 - Chemicals
 - Cleaner
 - » Caustic
 - » Glo-san/ PBW
 - » Can add chlorine if concerned with biofilm.
 - Sanitizer
 - » Iodophor
 - » Acid Sanitizer
 - » Peracetic acid



How do we use Brett in the Brewhouse?

- Commercial Examples
 - Orval @ Bottling 5000 cells/ ml.
 - No priming sugar added
 - Flavor production around 3 months.
 - Lambic Breweries
 - Natural inoculation
 - Flavor production 6 months
 - Russian River
 - Number of different beers.
 - Most 2° Brett in barrel
 - Sanctification all Brett
 - Bottles at 1.010, Ends up at 1.004
 - Jolly Pumpkin
 - 1° stainless, 2° Natural Brett in barrel
 - Not a lot of density drop from Brett
- Homebrew
 - Bergundian Babble Belt Brett Exchange
 - <http://madfermentationist.blogspot.com/>
- Not a lot of difference between US Comercial and Homebrewers
 - Pioneers. A lot to experiment with.

How do we use Brett in the Brewhouse?

- Problems
 - Not a lot of research on beer.
 - Very complex flavor production.
 - Lengthy fermentations/conditioning
 - Many variables involved.
 - Profile changes with CO₂

Brewing Variables

Brettanomyces Strain

Saccharomyces Strain

Timing of Incoculation

Pitch Rate

Temperature

Grist

Mash

Density

pH

O₂

Wyeast Experiment

- Goal
 - Determine the affect the timing of inoculation.
 - Determine the affect of other yeast strains on Brettanomyces flavor production
- Protocol
 - Brew standard/simple wort every month for 3 months
 - Inoculate 4.5 gal. fermentations
 - Test pH, density, and sensory each week

Recipe

General

Vol. 44 gal
IBU 10

Malt

25# Briess Pilsen DME (63%)
10# Briess Wheat DME (25%)
5# Maltose (12%)

Boil

30 min 8oz. Williamette (5.5%AA)
12 min 7 g. Tan B
10 min 20 g. Whirlflock
5 min 20 g. Wyeast Nutrient

Fermentation

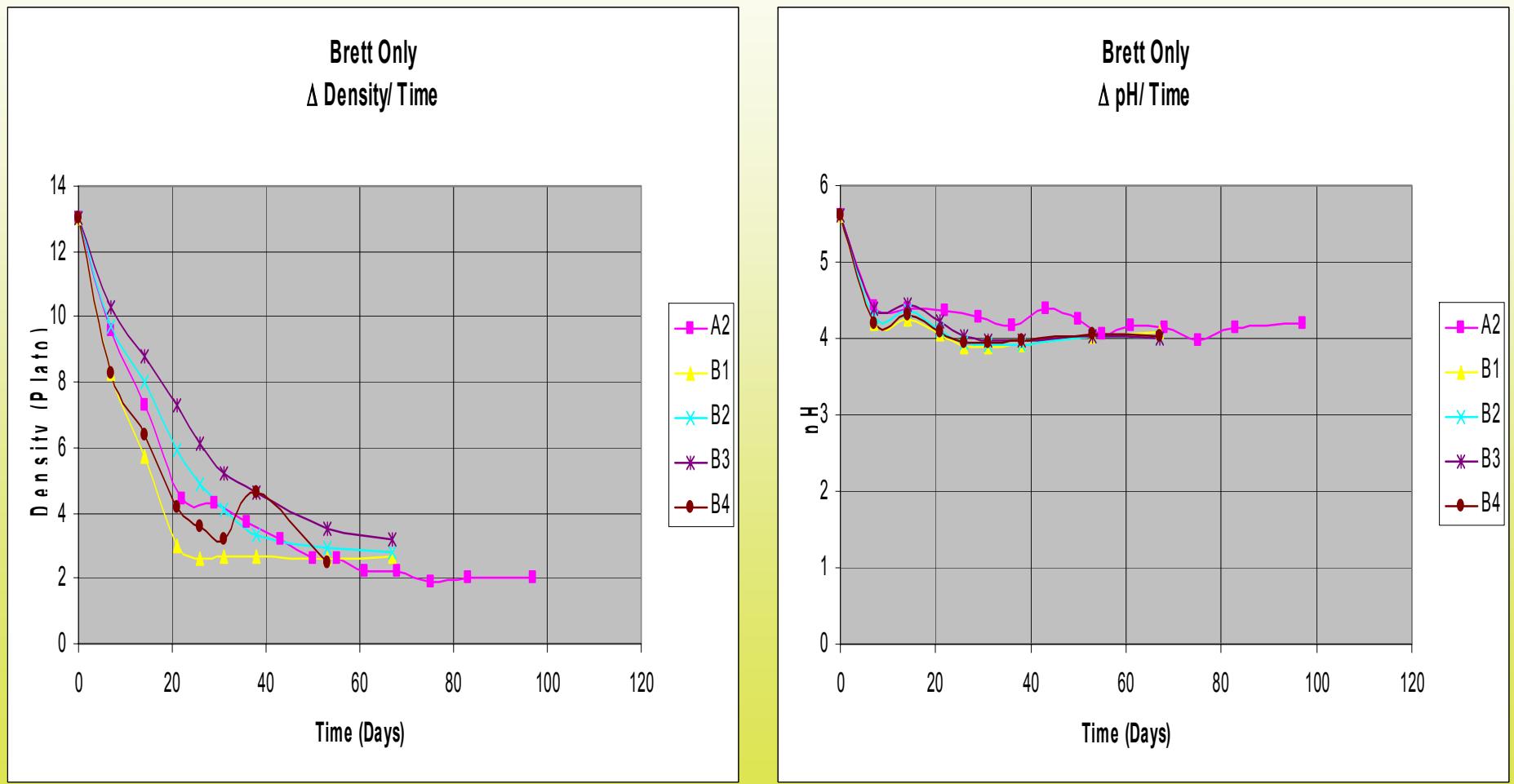
OG 13.0°P
O₂ 13 ppm.
Fermentation Temperature 70F
Saccaromyces Pitch Rate 6 million cells/ ml.
Brettanomyces Pitch Rate 3 million cells/ ml.

Inoculations

- #1
 - 1° Brettanomyces (5526)
- #2
 - 1° Saccharomyces (1007, 1056, 3787)
 - 2° Brettanomyces (5526)
- #3
 - Co-inoculation Saccharomyces-Brettanomyces
 - (1007-5526, 1056-5526, 3787-5526)

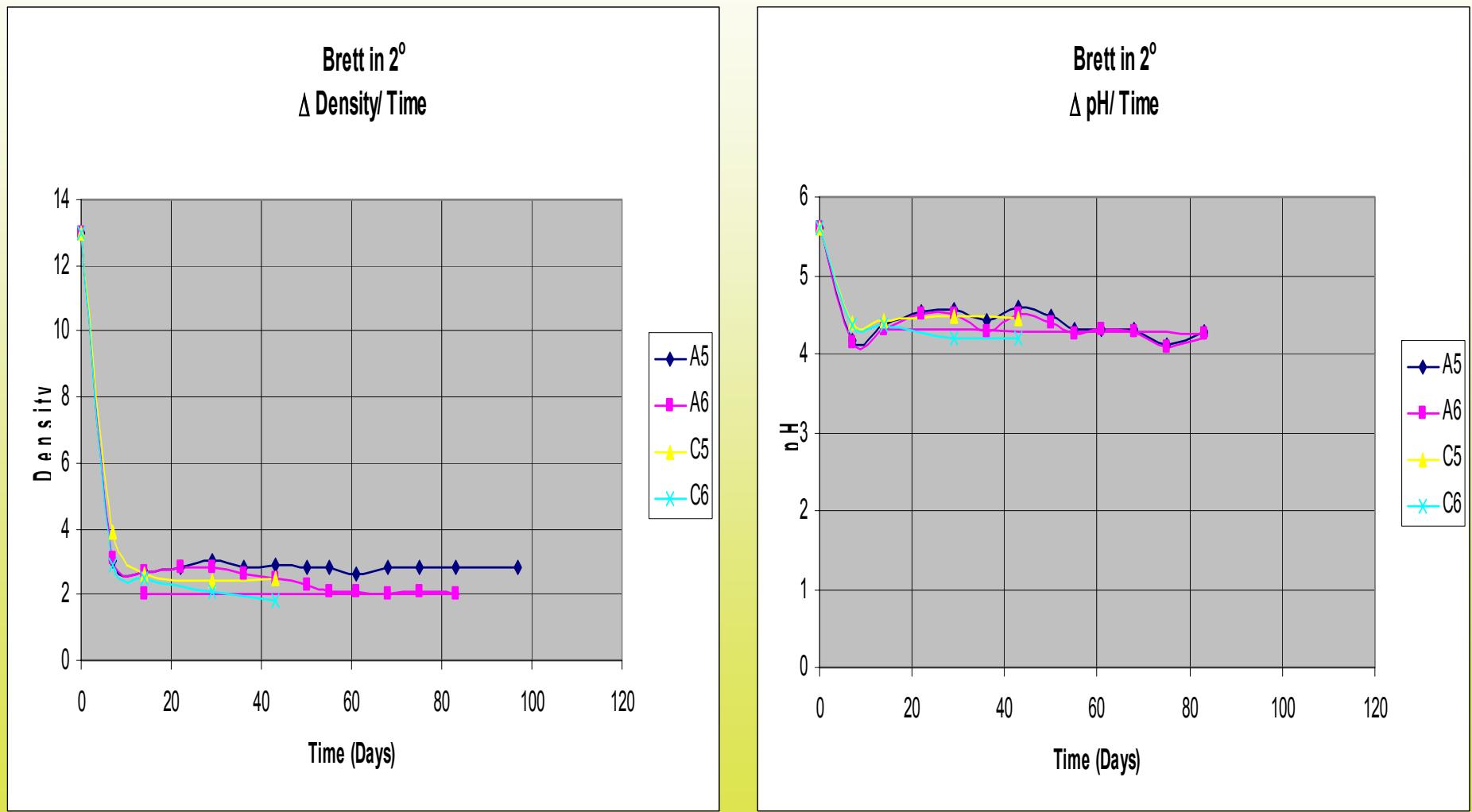
Fermentation Data

Brett Only



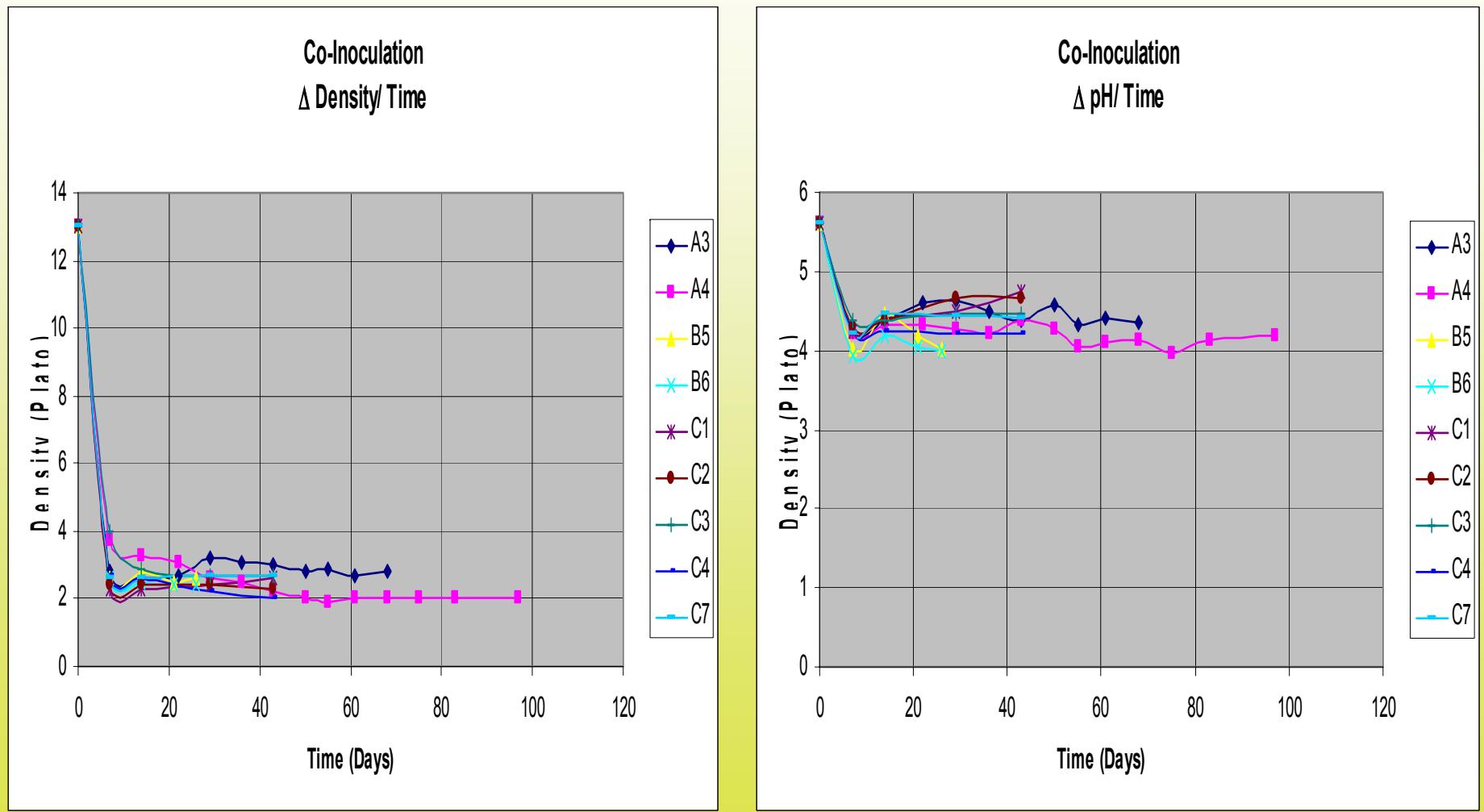
Fermentation Data

Brett in 2°



Fermentation Data

Co-Inoculation



Conclusions

- 2° inoculation preferred beer
 - Possible CO₂ inhibition with Co-inoculation
- Saccharomyces strain does make a difference.
 - Competition for metabolites
 - Production of Ethyl phenol pre-cursors
- Can produce nice Brett beer relatively quick.
 - Onset of Brett character in 3-5 weeks.
- TG stabilized as Brett flavors continued
 - TG 1.004-1.008
 - All Brett 60-80 days
 - Co-inoc & 2° inoc 30-50 days
 - May be different with different strain, grist, mash, OG.
- This is only one trial of 1 experiment!!
- Don't be afraid to try this at home.

Tasting

- 6 samples
- Beware of “Palate Fatigue”
- Go back frequently to “Control”
- Samples changed with every tasting.

Tasting #1

- Control (A7) Saccharomyces Only
 - 1007 German Alt
 - 14 weeks
 - TG 2.8°P
 - pH 4.54
- Nose
 - Slight fruit, red apple, pear
- Flavor
 - Fairly neutral
 - Slight fruit
 - Slight yeast
 - Malty finish

Tasting #2

- Co-inoculation (C2)
 - 1056-5526
 - 6 weeks
 - TG 2.3
 - pH 4.67
- Nose
 - Slight Sweetart/Smarties
 - Med Smoke
 - Slight Band-aid
 - Med-High Horsey
 - Med Burnt Rubber
- Flavor
 - Med Smoke
 - Med Band-aid
 - Med Horsey
 - Slight Acetic
 - Full on palate
 - Bready
- Overall
 - Not preferred

Tasting #3

- Co-inoculation (C4)
 - 3787-5526
 - 6 weeks
 - TG 2.0
 - pH 4.21
- Nose
 - Med Acetic
 - Med Fruit - Juicy Fruit
 - Mild Horsy
 - Slight Burnt Rubber
- Flavor
 - Med Acetic
 - Med Horsy
 - Slightly Asrtingent
- Overall
 - OK

Tasting #4

- Brett in 2° (C6)
 - 3787 1°, 5526 2°
 - 6 weeks
 - TG 1.8
 - pH 4.19
- Nose
 - Med Acetic
 - Med Horsy
- Flavor
 - Med Acetic
 - Med Horsy
 - Nice “Lambic” Character
 - Med Clove
- Overall
 - Preferred

Tasting #5

- Brett only (A2)
 - 5526 1°
 - 14 weeks
 - TG 2.0
 - pH 4.20
- Nose
 - Mild Acetic
 - Mild Horsey
 - Mild Fruit - Juicy Fruit
 - Mild Stone fruit
 - Dirty Socks
- Flavor
 - Mild Acetic
 - Mild Horsey
 - Mild Fruit - Juicy Fruit
 - Mild Stone fruit
 - Dirty Socks
- Overall
 - A lot cleaner than expected

Tasting #6

- Co-inoculation (A4)
 - 1007-5526
 - 14 weeks
 - TG 2.0
 - pH 4.18
- Nose
 - Slight Acetic
 - Mild Fruit - Pear
 - Mild Bubblegum
- Flavor
 - Slight Acetic
 - Mild Fruit - Pear
 - Mild Bubblegum
- Overall
 - A lot cleaner than expected

Thanks

- Jess Caudill - Wyeast Laboratories
- Lucy Joseph – UC Davis
- Roger Musche – Belgian Fine Technologies Intl.
- Vinnie Cilurzo – Russian River Brewing
- Shaun Hill – The Shed – Norrebro Bryghus
- Michael Tonsmiere – Mad Fermentationist
- David Osborne - Wesmar Chemicals
- Ron Jefferies – Jolly Pumpkin

Questions?

Contact Info:

Greg Doss

Wyeast Laboratories

greg@wyeastlab.com



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