Fermentation Management

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Good Beer – Balancing Act

Volatile Aroma Compounds
Residual Sugar
Hop Flavor
Color
Hop Aroma

Bitterness
Malt Character
Clarity
Negative Flavor Compounds
Ethanol
Brewing Control Panel

Beginning Brewing Control

Experienced Brewing Control
It’s All About Control

Brewer’s Goal
1. Complete control over all process variables.
2. Manipulate the variables to achieve the desired beer.

Control Points in Brewing

Ingredient Selection + Good Processing + Successful Fermentation + Good Finishing = Good Beer

- Malt
- Hops
- Water
- Adjuncts
- Misc.

- Milling
- Mash
- Sparge
- Boil
- Cooling

- Desired Culture
- Aeration
- Yeast Pitch Rate
- Culture Health
- Temperature
- Yeast Nutrition

- Conditioning
- Dry Hopping
- Clarification
- Kegging/Bottling
Successful Fermentation

• Rapid onset of fermentation
• Complete reduction of fermentable carbohydrates. Attenuation.
• Proper levels of Flavor and Aroma compounds.
• Expected flocculation and sedimentation of yeast.
General Metabolism

Lag Phase
- O₂ Uptake
- Cell Membrane Synthesis
- Sugar Uptake
- Nutrient Uptake

Log Phase
- Sugar Uptake
- Nutrient Uptake
- Cell Growth
- Fermentation
- Higher Alcohols

Stationary Phase
- Sugar Uptake
- Nutrient Uptake
- Fermentation
- Higher Alcohols
- Esters
Successful Fermentation

• Rapid onset of fermentation
• Complete reduction of fermentable carbohydrates. Attenuation.
• Proper levels of Flavor and Aroma compounds.
• Expected flocculation and sedimentation of yeast.
Attenuation

Apparent Attenuation (ASBC Apparent Degree of Fermentation)

\[%AA = \frac{\text{OG} - \text{TG}}{\text{OG}} \times 100\]

Example:
OG = 1.056, TG = 1.012

\((56-12)/56 \times 100 = 78.57\%\)
Attenuation Testing

1. Available sugar
   - Different densities
     • 1.040, 1.048, 1.060
   - Different Fermentability (Sucrose Addition)

2. Particular strain’s ability to metabolize available sugar
   - Shaken Fermentation
   - Different Strains
     • Ales
     • Lagers

3. Yeast contact with sugar (Flocculation)
   - Static Fermentation
   - Strains with different flocculation characteristics
     • Low Flocculation (1007, 2124)
     • Med Flocculation (1056)
     • High Flocculation (1968, 2206)
Available Sugar

• Density Not a Factor
  – Increase in OG – Increase TG
  – Attenuation % the same

• Fermentability is a Factor
  – Grist Bill
    • Malts
    • Adjuncts
  – Mash
Strain’s Ability to Metabolize Available Sugar

- Strain not really a factor
  - Ale strains
    - All very close
  - Lager and Wheat
    - Slightly less (1-1.5% < Ales)
Yeast Contact with Sugar

• Difference between shaken vs. static
  – Shake always **slightly** higher attenuation
    • (+1-2%)
  – Possible O$_2$

• Flocculation characteristics
  – Not a factor
Attenuation Testing

- Available sugar
  - Density
    - Not a Factor
  - Fermentable Sugar
    - Factor

- Particular strain’s ability to metabolize available sugar
  - Not a factor

- Yeast contact with sugar (Flocculation)
  - Not a factor
Attenuation Testing

- Available sugar
  - Density
    - Not a Factor
  - Fermentable Sugar
    - Factor

- Particular strain’s ability to metabolize available sugar
  - Not a factor

- Yeast contact with sugar (Flocculation)
  - Not a factor

- Fermentation Management
  - Pitch Rate, Temperature, Aeration, Health, Nutrition
Attenuation

• Balance beers with Sugar not Strain
• BU:TG
• Keep mash profile the same
• Adjust grist to achieve expected terminal
  – Decrease BU:TG
    • + Fermentable
      – Add Sugar
  – Increase BU:TG
    • Unfermentable
      – Dextrin
      – Increase starting Density
<table>
<thead>
<tr>
<th>BU</th>
<th>TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2.50 2.14 1.88 1.67 1.50 1.36 1.25 1.15 1.07 1.00 0.94 0.88 0.83 0.79 0.75</td>
</tr>
<tr>
<td>17.5</td>
<td>2.92 2.50 2.19 1.94 1.75 1.59 1.46 1.35 1.25 1.17 1.09 1.03 0.97 0.92 0.88</td>
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<td>20</td>
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<tr>
<td>90</td>
<td>15.00 12.86 11.25 10.00 9.00 8.18 7.50 6.92 6.43 6.00 5.63 5.29 5.00 4.74 4.50</td>
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<tr>
<td>95</td>
<td>15.83 13.57 11.88 10.56 9.50 8.64 7.92 7.31 6.79 6.33 5.94 5.59 5.28 5.00 4.75</td>
</tr>
</tbody>
</table>
Successful Fermentation

• Rapid onset of fermentation
• Complete reduction of fermentable carbohydrates. Attenuation.
• Proper levels of Flavor and Aroma compounds
• Expected flocculation and sedimentation of yeast.
**Esters**

- Responsible for fruity character (Banana, Apple) in beer.
- Crucial to flavor profile of beer. May be considered positive or negative.
- Product of enzyme driven reaction of a fatty acid and an alcohol.

<table>
<thead>
<tr>
<th>Ester</th>
<th>Typical Conc.</th>
<th>Sensory Threshold</th>
<th>Flavor</th>
<th>Alcohol</th>
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<tbody>
<tr>
<td>Ethyl Acetate</td>
<td>18 ppm</td>
<td>25</td>
<td>Solvent, Fruity</td>
<td>Ethanol</td>
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<tr>
<td>Isoamyl Acetate</td>
<td>2.7 ppm</td>
<td>2 ppm</td>
<td>Banana, Pear, Sweet</td>
<td>Isoamyl alcohol</td>
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<tr>
<td>Ethyl Caproate</td>
<td>0.14</td>
<td>0.2 ppm</td>
<td>Apple, Anise</td>
<td>Ethanol</td>
</tr>
<tr>
<td>Ethyl Caprylate</td>
<td>0.17</td>
<td>0.5 ppm</td>
<td>apple</td>
<td>Ethanol</td>
</tr>
<tr>
<td>Phenyl ethyl acetate</td>
<td>0.54</td>
<td>3.8 ppm</td>
<td>Roses, honey</td>
<td>Phenyl ethyl alcohol</td>
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</tbody>
</table>
Higher/ Fusel Alcohols

• Responsible for fruity alcoholic character in beer.
• Product of amino acid assimilation or carbohydrate metabolism
• Precursor for Ester synthesis

<table>
<thead>
<tr>
<th>Higher Alcohol</th>
<th>Threshold</th>
<th>Flavors</th>
<th>Amino Acid</th>
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</thead>
<tbody>
<tr>
<td>Isoamyl alcohol</td>
<td>110 ppm</td>
<td>Banana, solvent</td>
<td>Leucine</td>
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<tr>
<td>Isobutanol</td>
<td>200 ppm</td>
<td>alcohol, rough</td>
<td>Valine</td>
</tr>
<tr>
<td>n-Propanol</td>
<td>800 ppm</td>
<td>alcohol, rough</td>
<td>A-Aminobutyric</td>
</tr>
<tr>
<td>Phenyl ethyl alcohol</td>
<td>125 ppm</td>
<td>Alcohol, rose</td>
<td>Phenyl alanine</td>
</tr>
</tbody>
</table>
Phenolics

- Clove, Earthy
- May be desirable in some styles
  - German Hef, Belgian Style Ales
- Strain must be POF+ (Phenolic Off Flavor Gene)
  - German Wheat & Belgian Strain
  - Wine
  - Brett/Wild Yeast Strains & Bacteria
- Decarboxilation of Phenolic Acids to Vinyl Phenols
  - Ferulic Acid - 4 Vinyl Guaiacol (Clove)
  - Increased Ferulic Acid Extraction - Mash Rest @ 43-45 C (110-111F)
  - Level of function of POF
  - Overshadowed by Esters
Undesirable Flavor Compounds

• Sulfur $\text{H}_2\text{S}$
  – Rotten Egg
  – Low Sensory Threshold 0.05 – 5 ppm
  – Possible stress indicator
  – Natural production
    • Lagers, German Wheat, Wit

• Diacetyl (VDK)
  – Butter, Butterscotch
  – Low Sensory Threshold 0.15 – 0.9 ppm
Successful Fermentation

- Rapid onset of fermentation
- Complete reduction of fermentable carbohydrates. Attenuation.
- Optimal production of desirable fermentation byproducts.
- Minimal production of unwanted byproducts.
- Expected Flocculation and Sedimentation of yeast.
Sedimentation vs. Flocculation

- Sedimentation
  - the motion of particles in suspension in response to an external force such as gravity
- Flocculation
  - The aggregation of cells to form clumps (flocs) of 100-1000 cells
Cells adhere to each other through highly specific binding of Lectin receptors on one cell to Mannan ligands on the adjacent cell surface.

Zymolectin Binding

Zymolectins

Mannan

Ca^{2+}

Ca^{2+}

Ca^{2+}

Ca^{2+}
Factors that Influence Flocculation

- Strain genetics
- Attenuation (Depletion of sugars)
- Cell Surface Hydrophobicity
- Turbulence
- Age of cell
- Factors affecting electrostatic charges on cells
It’s All About Control

Brewer’s Goal
1. Complete control over all process variables.
2. Manipulate the variables to achieve the desired beer.

Control Points in Brewing

Ingredient Selection + Good Processing + Successful Fermentation + Good Finishing = Good Beer

- Malt
- Hops
- Water
- Adjuncts
- Misc.

- Milling
- Mash
- Sparge
- Boil
- Cooling

- Desired Culture
  - Aeration
- Yeast Pitch Rate
- Culture Health
- Temperature
- Yeast Nutrition

- Conditioning
- Dry Hopping
- Clarification
- Kegging/Bottling
Desired Culture

Choose a culture that will deliver the desired results.

• Flavor Production
  – Volatile Aroma Compounds
    • POF+
    • Ester Production
    • Malt character
    • Hops
• Temperature Tolerance.
  – Ales – 58-101F (Optimal >85F)
  – Lagers – 45-93F (Optimal <85F)
• Alcohol tolerance.
  – Ales – 10-11%
  – Lagers – 9-10%
  – Belgian – 12-13%
• Flocculation
Aeration

• Typical requirements 10-15 ppm
  – Strain dependant.
  – Max level with air 8 ppm
  – Higher levels require pure oxygen

• Inadequate Oxygen
  – Low cell growth
  – Incomplete attenuation

• Too much?
  – Can cause longer lag times
  – Attenuation is fine

• Required in synthesis of membrane components
  Sterols & Unsaturated fatty acids
Aeration - Sterols

- Increase membrane permeability and pliability
- Increase ability to adapt to changing conditions
  - Sugar levels (Osmotic), EtOH, pH Drop, Temperature
- Cell Growth Regulation
  - 50% reduction of sterol with each bud
  - Stops budding when sterol reduced to minimum level for cell function
- Reduces Flavor and Aroma Compounds
  - Reduces Ester synthesis
## Aeration Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Dissolved Oxygen</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splashing during run-in</td>
<td>2-4 ppm</td>
<td>0 sec.</td>
</tr>
<tr>
<td>Splashing and Shaking</td>
<td>8 ppm</td>
<td>40 sec.</td>
</tr>
<tr>
<td>Aquarium Pump*</td>
<td>8 ppm</td>
<td>5 min.</td>
</tr>
<tr>
<td>Pure Oxygen with stone**</td>
<td>0-26 ppm</td>
<td>60 sec (12 ppm)</td>
</tr>
</tbody>
</table>

*2 micron stone
**2 micron stone 3.5 LPM (Vigorous bubbles)

### Results

- Pumping air through stone is not efficient
  - 8 ppm, foaming
  - Surface area to volume bad
- Shaking is efficient but difficult
- Pure oxygen through stone is not efficient but easy.
  - Surface area to volume bad

### Alternative Methods

- Purge headspace with O2 and shake 20 seconds. Repeat
- Inline O2
  - Expensive but easy and efficient
  - Surface area to volume good
- Pretreatment
  - Aerate culture (small amount of wort)
  - Unsaturated Fatty Acids (Olive oil)
  - Wyeast nutrient packet
Pitch Rates

• Defined as
  – Viable cells per ml. of wort in fermenter

• Critical for successful fermentation
  – Too low
    • Long lag and incomplete attenuation
  – Too high
    • Not too much of a concern. Possible sulfur off-flavors

• Affects Ester/Higher alcohol production
  – Increase pitch rates decreases ester production.
  – Wheat beer keep pitch rates low
  – Lagers increase pitch rates
Pitch Rates

How much yeast do I need?

   - Standard Ale (<1.060, >60°F) 6 million cells per ml.
   - Increase gravity or Decrease in temperature requires additional yeast

2. Cell counts
   - Yeast Supplier
     - Wyeast Activator 95 ml. @ 1.2 x 10⁹ Cells/ml.
   - Sedimentation Estimate
   - Hemacytometer

<table>
<thead>
<tr>
<th>STYLE</th>
<th>GRAVITY</th>
<th>PITCHING TEMPERATURE (°F)</th>
<th>FERMENTATION TEMPERATURE (°F)</th>
<th>PITCH RATE (Million Cells/ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ale</td>
<td>&lt;1.060 (15P°)</td>
<td>&gt;65</td>
<td>&gt;65</td>
<td>6.00</td>
</tr>
<tr>
<td>Ale</td>
<td>1.061-1.076 (15-19P°)</td>
<td>&gt;65</td>
<td>&gt;65</td>
<td>12.00</td>
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<tr>
<td>Ale</td>
<td>&gt;1.076 (19P°)</td>
<td>&gt;65</td>
<td>&gt;65</td>
<td>&gt;18.00</td>
</tr>
<tr>
<td>Lager*</td>
<td>&lt;1.060 (15P°)</td>
<td>&gt;65</td>
<td>&lt;60</td>
<td>6.00</td>
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<tr>
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<td>&lt;60</td>
<td>&lt;60</td>
<td>&gt;24.00</td>
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</table>
Allow Yeast to settle under refrigeration

Estimate Yeast Pack

Take into consideration Trub and Flocculation (Powdery strains don’t pack)
Calculating Pitch Rates

1. Decide how many cells/ ml wanted in fermenter
   - eg. 5 gal., 1.050 OG, Pitch and Ferment @ 50F
   - 12x10^6 cells/ ml

2. Calculate Total Cells Needed
   - (Desired Cells/ ml) x (Total mls In fermenter)
   - (5 gal=1.89x 10^4 mls)
   - eg. (12x10^6 cells/ml) x (1.89x10^4 mls) = 2.27x10^{11} cells

3. (Total cells)/(Cell count of slurry)= mls slurry to pitch
   - eg. (2.27x10^{11} total cells)/(1.2x10^9 cells/ ml)

Answer 189 mls slurry
Starter

- 1 Activator into 2L starter = 1 Doubling
- 1.040 @ 70F, 8 ppm DO
- Stir plate increases growth 25-50%
- Pitching at extremely high level. (>60 million cells.ml.)
- Completed in 18-24 hr.
- Check density
- Pitch or Refrigerate soon after reaching TG

Pitch Rate and Growth Calculator  http://www.wyeastlab.com/hb_pitchrate.cfm
Culture Health

• Yeast is perishable
  – Treat it like your milk and eggs
  – Over time viability decreases
    • Accelerated by exposure to warm temperatures
    • Keep it cold
    • If it warms up, use it.

• Poor Condition
  – Long lag time
  – Incomplete attenuation
  – Increased production of undesirable byproducts
    • Sulfur
    • Diacetyl
Culture Health

**Viability** – % Live/Dead (Ability of cell to reproduce)

**Vitality** – How alive (Fermentation performance)

Viability

- Dead or Alive

Vitality

- Increasing Vitality
Viability/ Vitality

- **Viability Assessment Methods**
  - Staining (Methylene Blue, Fluorescent, etc)
  - Plate counts (CFU)
  - Capacitance
  - ATP content

- **Vitality Assessment Methods**
  - \(O_2\) Uptake
  - Acidification Power (AP)
  - Intracellular pH
  - \(CO_2\) Production
Testing Viability

Or

Good-
Signs of CO₂ production in 4-6 hr.
Temperature

- **Hot**
  - Rapid glycolytic flux
  - Can affect sensory
    - Increase in Higher Alcohols and Esters
  - Ethanol toxicity increases at elevated temperatures
    - Big Beers

- **Cold**
  - Affect fluidity of membrane
  - Reduce nutrient uptake and by-product excretion
  - Increases $H_2S$ production
  - Reduces ester and higher alcohols (bad or good?)
  - Poor attenuation
Temperature

• Pitch yeast within 10F of wort temperature
• Lager strains
  – Temperature tolerant
  – Typical working range 46-60F
• Ales
  – Tolerant to cool conditions
  – Typical working range 58-72F
• Belgians and Wheats (German)
  – Sensitive to cool conditions
  – Typical working range 65-75F
Basic Temp Control

• Control Environment
  – Heater or Cooler
  – May rise 5-10 degrees

• Evaporation
  – Wet Tshirt Fan

• Water Bath (Best)
  – Large thermal Mass
  – Minimize temp shifts
  – Add Ice or hot water to maintain/change temp
Advanced Temp Control

Control fermentation temperature based on beer temperature

- Glycol Cool
- Peltier Cool
- Fermwrap Heat
Nutrition

• Use of nutrient is cheap insurance
  – High gravity wort
  – High adjunct
• Nitrogen
  – Typically abundant in all malt beer
• Minerals (Inorganic Ions)
  – Mg and Zn
• Vitamins
  – Typically abundant in all malt beer
• Yeast Hulls
  – Lipid Source
  – Bind toxic med chain fatty acids
  – Nucleation sites
Magnesium

• Absolutely essential nutrient
• Role in stress response (EtOH, Temp)
• Limitation
  – Decreased Yeast Growth
  – Decreased fermentation rate
  – Decreased EtOH tolerance
• Membrane stability (Structural integrity)
• Enzyme Cofactor
  – Sugar metabolism
• Inhibited by Ca$^{+2}$
  – Ca$^{+2}$ typically available 2x [Mg$^{++}$]
• Bioavailability – Historically considered adequate
  – Chelation
Zinc

• Optimal level 0.4-2.0 ppm
• Essential cofactor for many enzymes
  – Alcohol dehydrogenase
• Structural stability of ~3% all yeast proteins
• Cellular integrity - regulating membrane fluidity
• Bioavailability
  – Chelation by proteins and polyphenols
Troubleshooting

- Density
  - Starting
  - Current
- Wort Volume
- Wort Fermentability
- Strain
- Pitch Rate
- Culture Health

- Aeration
- Temperature
  - Pitching
  - Current
- Byproducts (Sulfur)
- Nutrition
Tools

- Refractometer*
- Hydrometer*
- Thermometer*
  - Mason Jar
  - PET Bottle
  - Thief

- Nose
- Eyes
- Tongue
- pH Meter*
  - Nalgene Bottles

*Calibrate frequently.
Monitor Fermentation

• Take density readings often.
  – Do not rely on visual airlock assessment
  – Density readings affected by
    • Temperature
    • Gas
      – Shake to degas sample
    • Yeast in suspension
      – +0.002-0.004 S.G.
      – More important close to TG
• Temperature
• pH Meter
  – pH will drop before density drops
Fermentability

• Where should this fermentation finish?
• Brewing process or fermentation?
  – Unfermentable sugar
  – Unhealthy fermentation
• Forced Fermentation Test
  – Large inoculum 60-75E6 cells per ml
  – Every batch
  – Results in 24-36 hours
Forced Fermentation

- Decant 250 ml. (50% headspace) wort into Mason jar.
- Add 15 ml. (1 Tbs.) liquid yeast* or 3 g (1 tsp) dried yeast
- Cover loosely with foil or lid.
- Incubate 75ºF 24-36 hours
- Agitate frequently
- Allow yeast to settle
- Measure density = Terminal

*Added liquid with slurry can drop density -0.001
Forced Fermentation Results

• FF High Terminal Density
  – Unfermentable sugar
    • Incomplete saccharification in mash
    • High proportion of unfermentable in grist

• FF Lower Terminal than Actual Fermentation
  – Fermentation Problem
Diacetyl (VDK)

- Natural byproduct of fermentation
- Pediococcus
- Bi-product of Amino acid biosynthesis
- May be reduced by yeast following fermentation with proper diacetyl rest (24-72 hours warm)
- High flocculent strains less efficient at reducing

\[
\alpha\text{-acetolactate} \rightarrow \text{Valine} \rightarrow \text{Isoleucine} \rightarrow \text{O}_2 \rightarrow \text{Diacetyl}
\]
Diacetyl Testing

• Check post fermentation prior to cooling
• Collect Two 200 ml. samples from the fermentation. Cover loosely
• Store one sample at room temperature
• Heat other sample to 140-160°F in a water bath for 10 min
• Cool the heated sample to room temp in an ice bath
• Smell both samples
# Diacetyl Testing

<table>
<thead>
<tr>
<th>Sample</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Heated</td>
<td>Diacetyl rest is complete. Beer can be cooled.</td>
</tr>
<tr>
<td>Clean Clean</td>
<td>Beer still has precursors. Continue rest. Re-test in 24-48 hr.</td>
</tr>
<tr>
<td>Clean Butter</td>
<td>Beer still has diacetyl. Continue rest. Re-test in 48 hr.</td>
</tr>
<tr>
<td>Butter Butter</td>
<td></td>
</tr>
</tbody>
</table>
Thank You

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