



Yeast Management

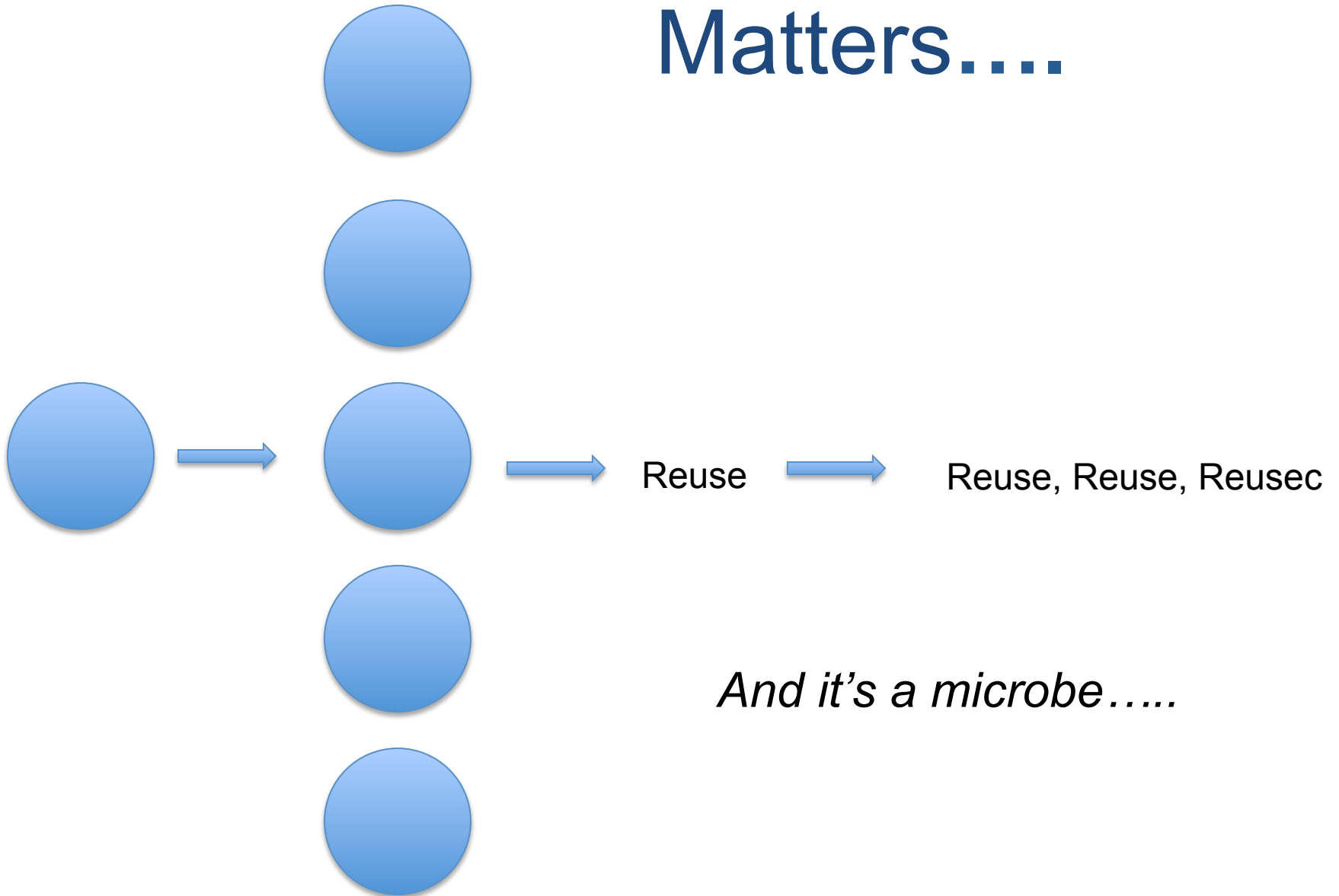
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Yeast Management

- What does it mean?
- We often say yeast can be reused 5-10 times.
- With the best possible yeast management, could yeast be re-used forever?
- For this seminar: Laboratory storage, yeast propagation, yeast handling

To Further Complicate Matters....



Something to Consider....

**NOT ALL YEAST HANDLING IS
THE SAME**

It all Starts in the Lab

Yeast must be handled in a way to:

- 1. Prevent mutations**
- 2. Maximize physiology**
- 3. Maximize cell count is not
number one priority**
- 4. Flavor and stability of the yeast
are most important.**

Yeast Propagation

What is propagation?

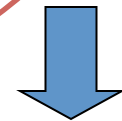
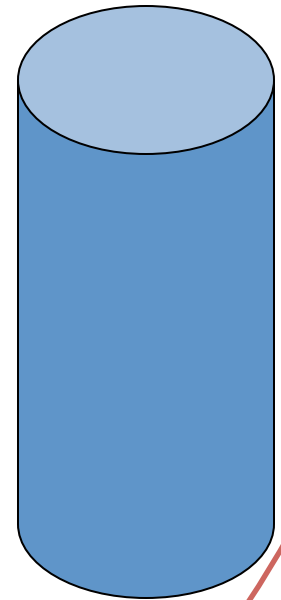
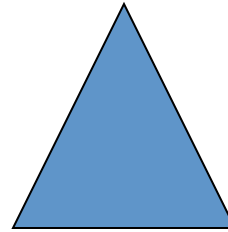
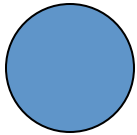
Definition:

- 1. The act of propagating; continuance or multiplication of the kind by generation or successive production; as the the propagation of animals or plants.**
- 2. The act of producing offspring or multiplying by such production.**

Propagation starts in the Laboratory

Basic concept:

 **-80C Freeze**



Agar slant or Petri dish

**Shake-flasks
100ml ->1L**

**Propagation vessel
10 - 20 L**

Brewery

Laboratory propagation: typical example, ale yeast

Slope/slant/plate culture

↓ Strain selection/maintenance

10mL wort (8°P)

↓ 25°C, 24-48h, aeration/shaking

100mL wort (8°P)

↓ 25°C, 24-48h, aeration/shaking

1000mL wort (12-16°P)

↓ 22°C, 24-48h, aeration/shaking

10L wort (12-16°P)

↓ 22°C, 48-72h, aeration/shaking

To the Brewery (e.g. 1barrel of wort)

Laboratory propagation - key considerations

- Aseptic technique!
- Sterile growth media
- Do not exceed 1->10 volume increments
- Aeration (but remember Crabtree effect - may be best to use weak wort <math><10^{\circ}\text{P}</math>)
- Temperature (higher than in plant, 20-25°C)

Lab Propagation Environment



Important that propagation room is kept clean. Ideally completely tiled. Walls, floor, ceiling sprayed with suitable sanitizer at regular intervals. Extractor fan and ideally a de-humidifier.

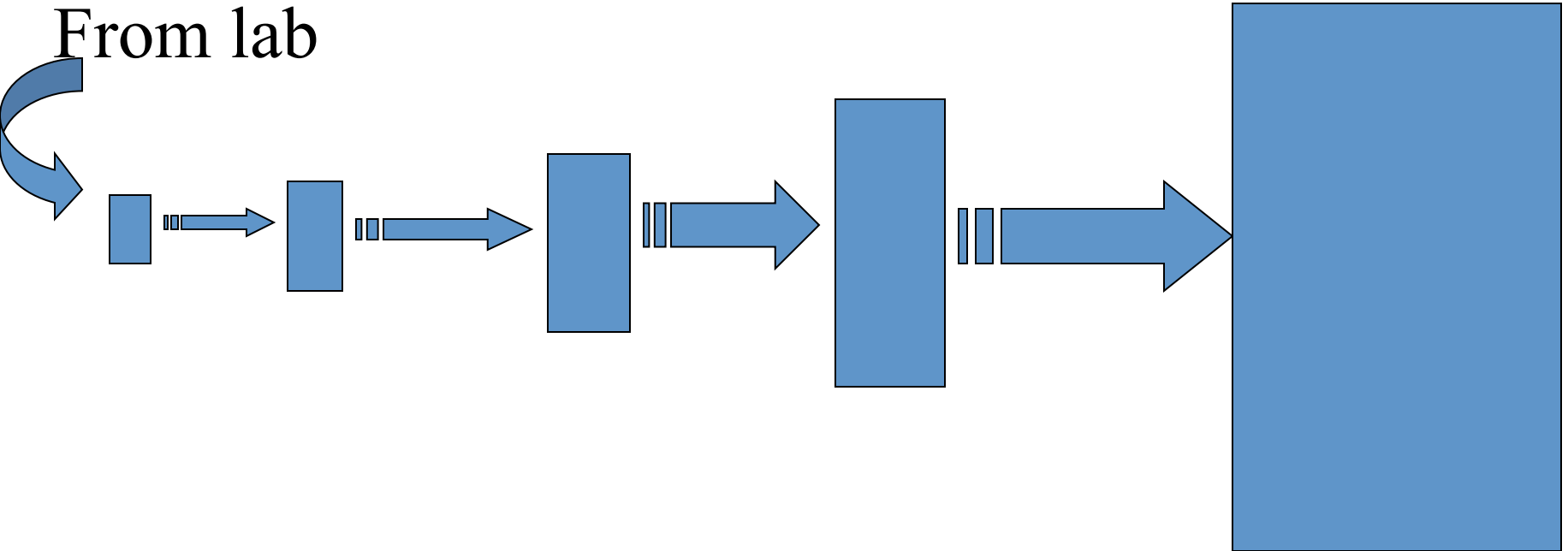
Further precautions :

UV lights, foot baths, double doors, positive pressure.

From the Lab into the Brewery

- **Objectives**
 - Sufficient yeast biomass in good physiological condition
- **Targets**
 - Rapid growth (minimal lag phase)
 - Require enough yeast for pitching, typically 1×10^7 cells/ml for normal gravity wort

Brewery propagation -example



	1Bbl	>10Bbl	>60Bbl	>300Bbl	>900Bbl
Ale	22°C	20°C	20°C	20°C	20°C
Lager	18°C	18°C	16°C	14°C	12°C

Brewery propagation - key considerations

- **WORT**- sterility, type, gravity?
- **CONDITIONS** - hygiene, aeration/agitation, pure O₂?
- **CONTAMINATION**-vessel, wort, air,CIP system, valves
- **TEMPERATURE** - gradual decreases
- **TIME** - transfer yeast in log phase (stat phase later in prop)
- **BATCH** or **FED-BATCH**?

Brewery Propagation Environment



Floor is very important. Especially with restaurant breweries.

Sanitation is paramount. Have regular protocols. Walls, floor, ceiling sprayed with suitable sanitizer at regular intervals.

Environmental testing is necessary.

Further precautions :

UV lights, foot baths, double doors, positive pressure.

What kind of propagation should *you* do?

Brewpub?

**No lab, do
one step propagation,
brewery propagation.**

Microbrewery?

**If no lab, do
one step propagation.**

If lab, more options

Recommendation for Brewpubs and most Microbreweries

If your ale brew size is:	If your lager brew size is:	Start with:
7 to 10 bbl		1 bbl
11 to 21 bbl	7 to 10 bbl	2 bbl
22 to 50 bbl	11 to 21 bbl	7 bbl
51 to 80 bbl	22 to 50 bbl	10 bbl
81 to 120 bbl	51 to 80 bbl	15 bbl
121 to 150 bbl	81 to 120 bbl	21 bbl

Regional Brewery?

Must have well equipped and well staffed lab.

Lab propagation and brewery plant propagation.

Macro Brewery?

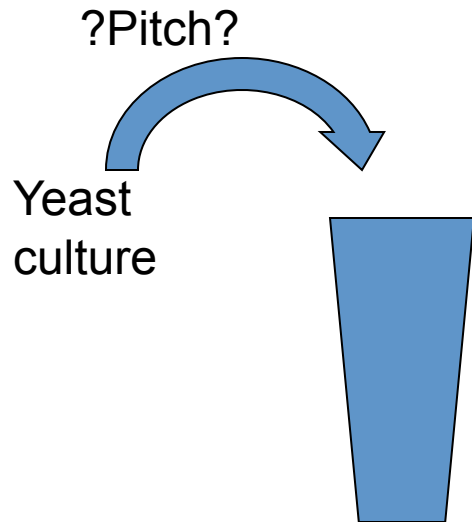
Must have well equipped and well staffed lab.

Lab propagation and brewery plant propagation.

Yeast Pitching

- Add a specific amount of yeast to freshly oxygenated wort, at the correct fermentation temperature
- Yeast can be new, first generation, or reused from previous fermentation
- Yeast can be reused 5-10 times.
- Pitch more yeast for high gravity beers

Yeast Pitching Numbers



A)
1 million cells/ml/plato
10 plato = 10 million cells/ml
20 plato = 20 million cells/ml

B)
1 lb per bbl
= 0.5 million cells/ml/plato

2 Main Rules of Thumb for calculating pitching rate

Notes: These are based on re-pitching rates

Laboratory grown 1st generation cultures
can be 50% less cells

Yeast Pitching Calculations Worksheet

Example:

Parameter	Value	Unit
Batch size	20	HL (Hectoliter)
Strenght of wort	12	Degrees Plato
Wanted re-pitching rate	1	Million cells / ml / degree Plato
Concentration of slurry	0.8	Billion cells / ml

Question: How much yeast slurry (volume) should the brewer add to achieve the wanted pitching rate?

First, let's calculate the total number of cells we want to add to the full batch of wort

We have 20 HL wort. 20 HL is $(20 * 100 \text{ L}) = 2,000 \text{ L}$

2,000 L equals $(2,000 * 1,000 \text{ ml}) = 2,000,000 \text{ ml} = 2 * 10^6 \text{ ml}$ of wort

Goal: We want 1 million cells / ml / degree Plato. With a 12 Plato wort, we therefore want 12 million cells / ml or $12 * 10^6 \text{ cells/ml}$

We have just calculated that we have 2 million ml wort ($2 * 10^6$). Therefore our total goal of how many cells to add is

$$2 * 10^6 \text{ml} * (12 * 10^6 \frac{\text{cells}}{\text{ml}}) = 24 * 10^{12} \text{ or } 2.4 * 10^{13} \text{ cells}$$

Slurry concentration obtained by cell count is

$$0.8 \text{ billion cells / ml, or } 0.8 * 10^9 \text{ cells /ml}$$

How much slurry to add?

Our goal was to add $2.4 * 10^{13}$ cells to the tank and we have $0.8 * 10^9$ cells/ml in our slurry. To calculate this we must use the formula for pitching

$\text{Volume of slurry to pitch (ml)} = \frac{\text{total cells wanted (cells)}}{\text{concentrations of slurry (cells/ml)}}$
--

So, now we have all we need:

$$\text{Volume to pitch (ml)} = \frac{2.4 * 10^{13} \text{ cells}}{0.8 * 10^9 \text{ cells/ml}}$$

$$\text{Volume to pitch (ml)} = 30,000 \text{ ml}$$

$$\underline{\underline{\text{Volume to pitch (L) = 30 L}}}$$

Yeast Pitching: Can you Estimate the Density?



A)
Centrifuge a 10ml sample of slurry.
Calibrate volume of packed cells to
cell count via microscope

B)
Estimate cell count by packed cell
volume. 1/3 cell pack is approx.
1 billion cells per ml.

What would you estimate the cell count to be?

Using the bars, the cell pack is 63% by volume, so this would be 2.7 billion cells/ml

Yeast Pitching: A Common Brewery Practice



Craft Brewers often collect yeast into 5 gallon yeast brink, such as a Cornelius keg. They then store and Re-pitch by total volume

1 liter of yeast slurry per 1 HL of beer
Is a good rule of thumb
In a 10HL batch:
10 liters of yeast at 1 billion/ml, would
Result in 10 million/ml in 1000 L

It is common to see twice this rate, 20
Liters (5 gallons), which would be 20
Million per ml, too high for most beers

Yeast Pitching Rate Experiment

	5 mil cells	10mil cells	20ml cells	30mil cells	40mil cells
Time (hours)					
0	18.7	18.7	18.7	18.7	18.7
24	17.8	17.6	16.8	16.5	16
48	13.6	13.2	12.5	12.1	12
72	10.9	10.9	9.3	9.7	9.8
96	9.3	9.1	8.7	8	8.2
120	7.4	7.3	6.8	6.6	6.6
144	5.9	5.9	5.9	5.8	5.8
168	4.8	4.7	4.8	5.1	4.9

Dissolved Oxygen



Dissolved Oxygen Experiment

**Gravity (plato) vs. Time in
20 L Homebrew size**

	2.71ppm	5.12ppm	9.2ppm	14.08ppm
	shake	30 seconds	1 min	2 min
Time (hours)				
0	18.7	18.7	18.7	18.7
24	17.6	17.3	17.5	16.9
48	13.5	12.8	12.7	11.9
72	11.7	10.7	9.9	9.5
96	10	9	8.8	7.8
120	7.8	7.3	6.5	6.2
144	6.4	6.3	5.5	5.2
168	5.3	5	4.3	4.3

Dissolved Oxygen

Brewery	Brewery Flow Rate	Length of Time In-line	Original Gravity (P°)	Actual DO (ppm)
1	6L/min	40 min for 40bbl	12.5	5
2	7L/min	30-40min for 10bbl	12	8.25
3	7L/min	20min for 8bbl	12.8	9
4	12L/min	75-80 min for 15bbl	25.5	5.5
5	6L/min	25-30min for 10bbl	12.8	35.8
6	5L/min	90min for 15bbl	12.7	24.4
7	7L/min	40min for 40bbl	12.3	6.2
8	6L/min	30min for 10bbl	14.4	8.1
9	6L/min	45min for 15bbl	13.2	5.42
10	7L/min	35min for 10bbl	12.5	7.2
11	7L/min	40min for 15bbl	12.7	6.54
12	6L/min	35min for 10bbl	12.3	5.85

Table 1. Summary of current craft brewery oxygen delivery and dissolved oxygen levels. Parker 2008

Dissolved Oxygen

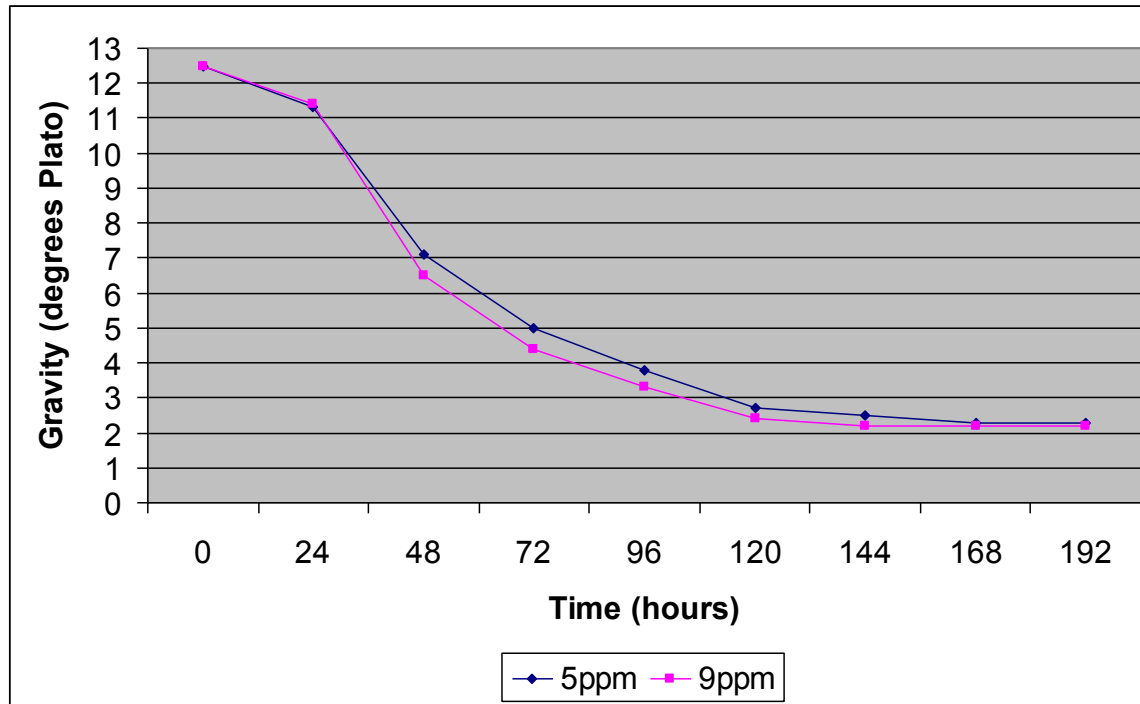


Figure 1. Fermentation speed of one brewery showing current versus oxygen-enhanced wort. Parker 2008.

Dissolved Oxygen

Time (hours)	19.15ppm	10.15ppm	5.48ppm
0	30.0%	30.0%	30.0%
12	74.3%	62.8%	54.2%
24	70.6%	58.1%	51.4%
36	53.1%	44.5%	48.1%
48	26.9%	31.0%	36.8%
60	27.2%	24.4%	24.6%
72	26.8%	20.7%	15.0%
84	24.5%	20.3%	14.6%
96	22.2%	19.4%	14.1%
120	12.0%	12.6%	11.2%
144	9.2%	9.4%	5.4%

Table 2. Budding percentage of yeast during fermentation with varying dissolved oxygen levels. Parker 2008.

Dissolved Oxygen

Time (hours)	19.15ppm	10.15ppm	5.48ppm
0	30.0%	30.0%	30.0%
12	74.3%	62.8%	54.2%
24	70.6%	58.1%	51.4%
36	53.1%	44.5%	48.1%
48	26.9%	31.0%	36.8%
60	27.2%	24.4%	24.6%
72	26.8%	20.7%	15.0%
84	24.5%	20.3%	14.6%
96	22.2%	19.4%	14.1%
120	12.0%	12.6%	11.2%
144	9.2%	9.4%	5.4%

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Dissolved Oxygen

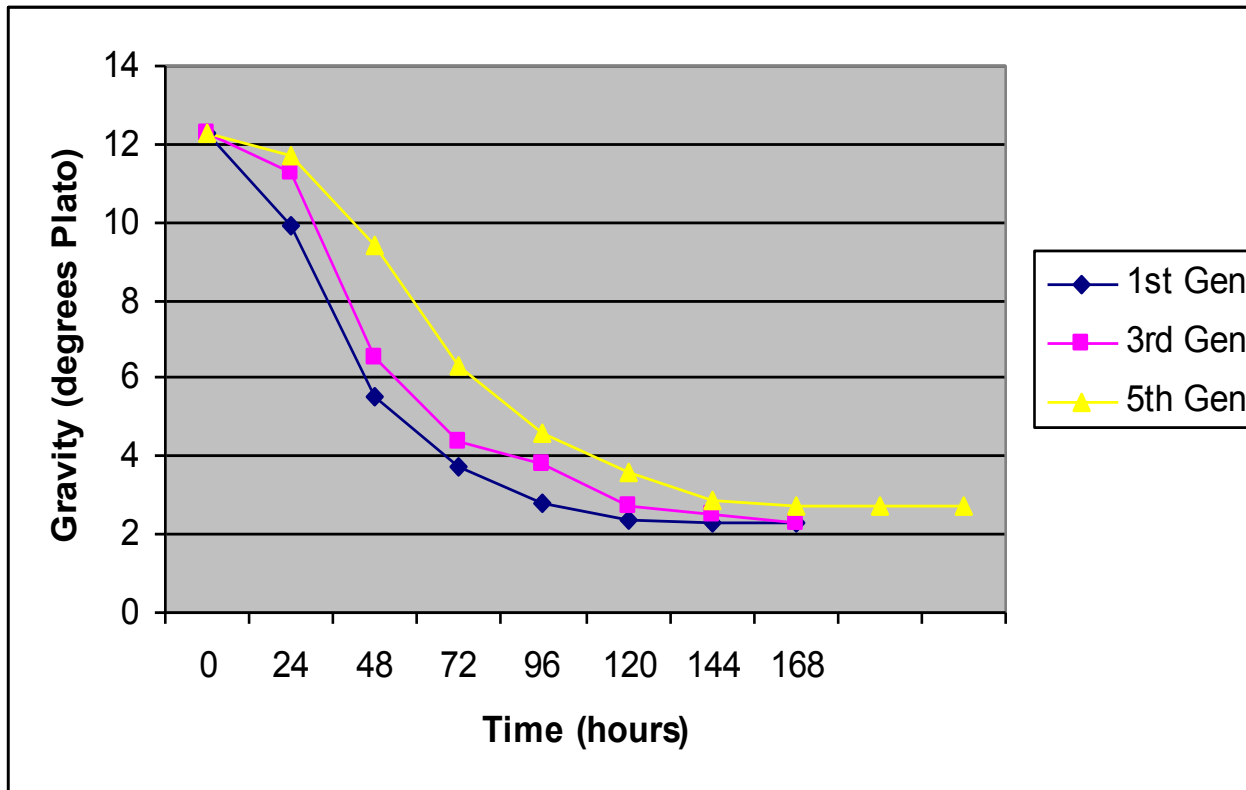


Figure 3. Fermentation performance of worts with various yeast generations with depleted oxygen resources. Parker 2008