Yeast Biology

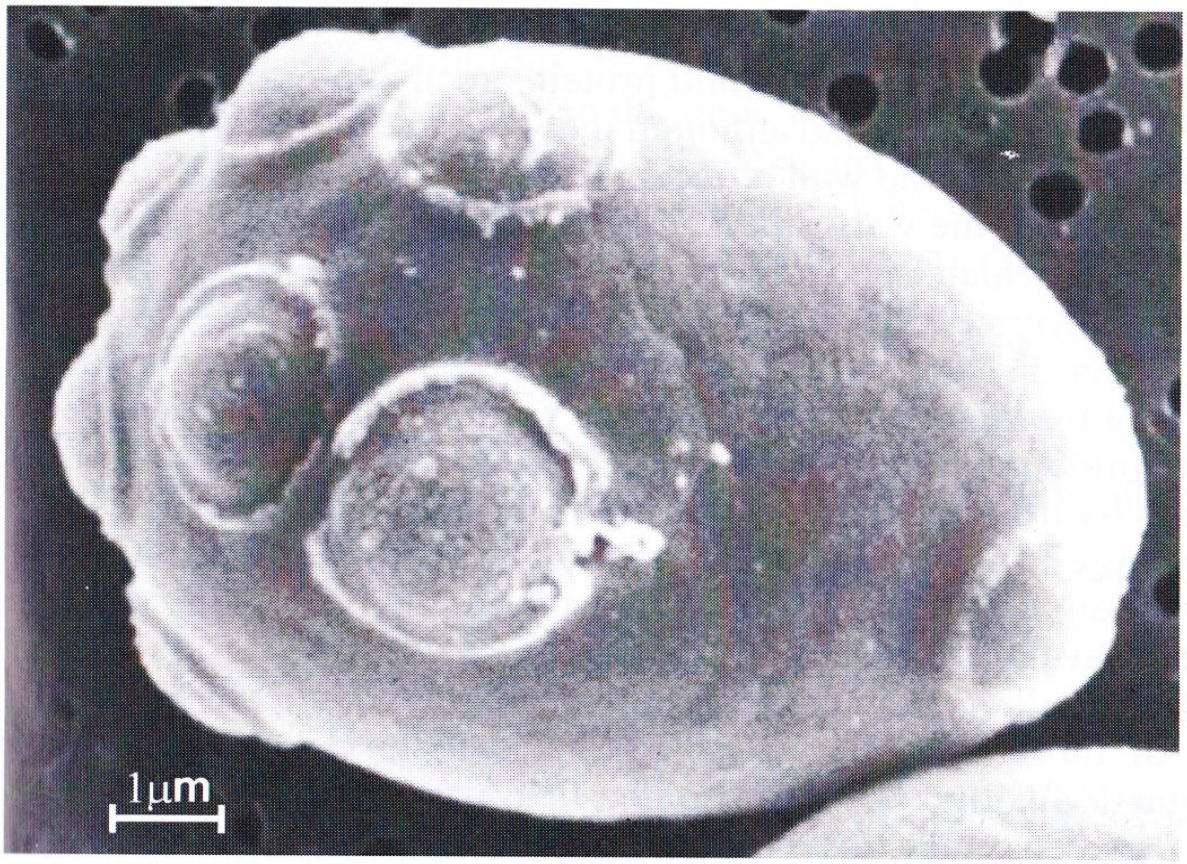


Figure 5. Electron micrograph of a yeast cell with multiple bud scars.

Malt	Hops	Yeast	H_2O
Barley	whole hops	strain specific	most will do -- or
any grain	pellets	slants/prop	treatment
Extract	extract	dried	R/O
anything fermentable	hopped extract	pitchable	filtration
			UV Light

Yeast Management
Keep it pure
Don't let it die -- but keep in a high vitality state
Keep the right amount of yeast

The cost of material management varies with the process or materials chosen and the size of operations. What are some of the costs of what equipment manufacturers or consultants specify for each ingredient?

What is the fifth ingredient? Aeration or oxygen

Used for sterol synthesis - critical for the health of yeast
Unsaturate bonds in fatty acids " "
Not for respiration

Typically 5-10 ppm dissolved O_2 is required

Sterile Air provides just ~ 8ppm dissolved O_2

Sterile O_2 - typically can dissolve to about 40 ppm in brewery setting

How much air is too much? That is difficult to say, it could be toxic to yeast when too high, but that is difficult to do in a brewery. It could oxidize wort compounds affecting beer flavor or stability - Maybe. Within 4 hours of O_2 is typically either taken up by the yeast or pushed out as CO_2 forms. Can degrade or remove foam forming proteins.

What kind of Oxygen? Medical grade or welding/industrial grade?
The difference of the two is that medical is tested and the purity certified. Both types filled out of the same tanks.

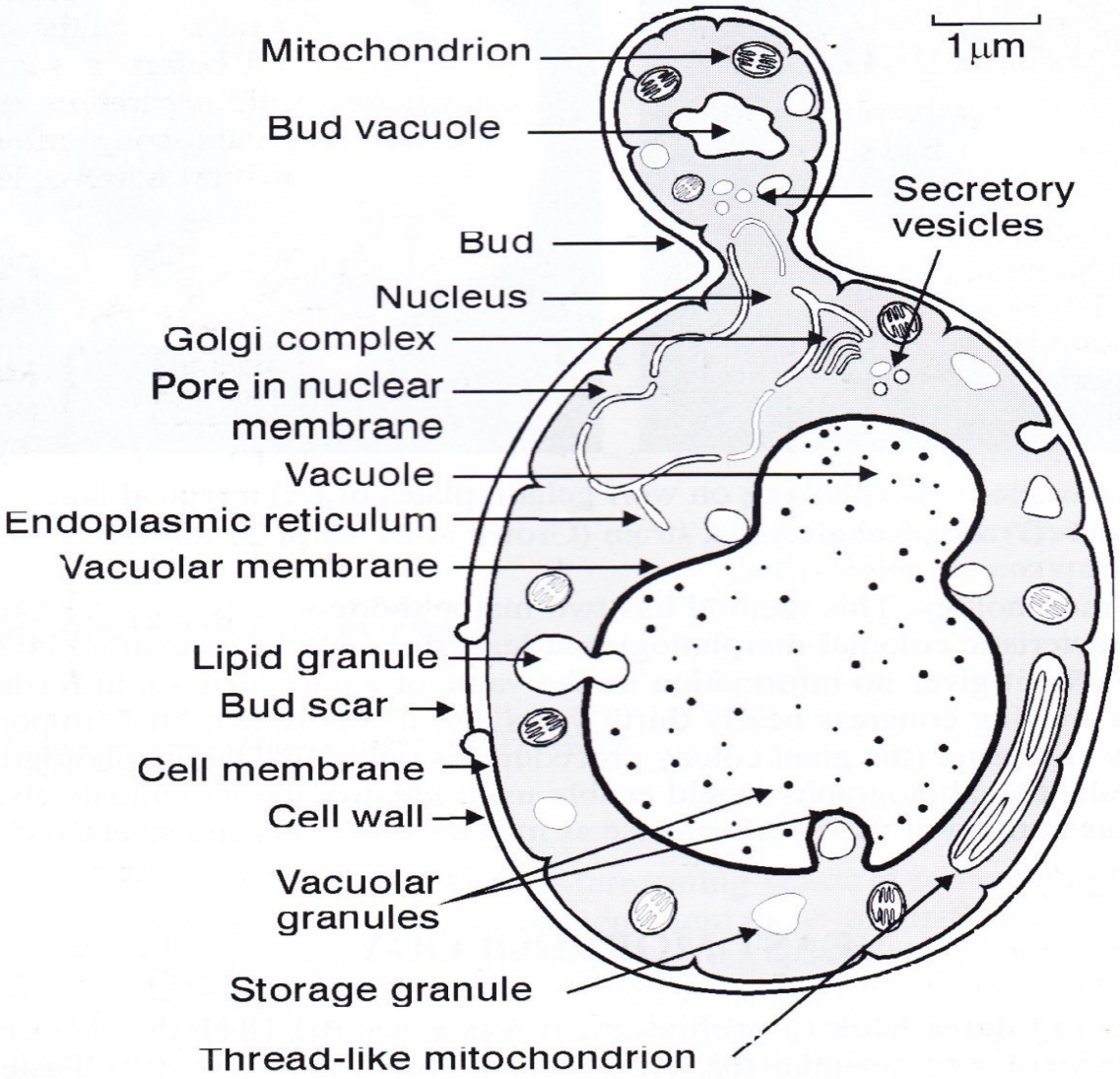


Figure 4. Main features of a typical yeast cell.

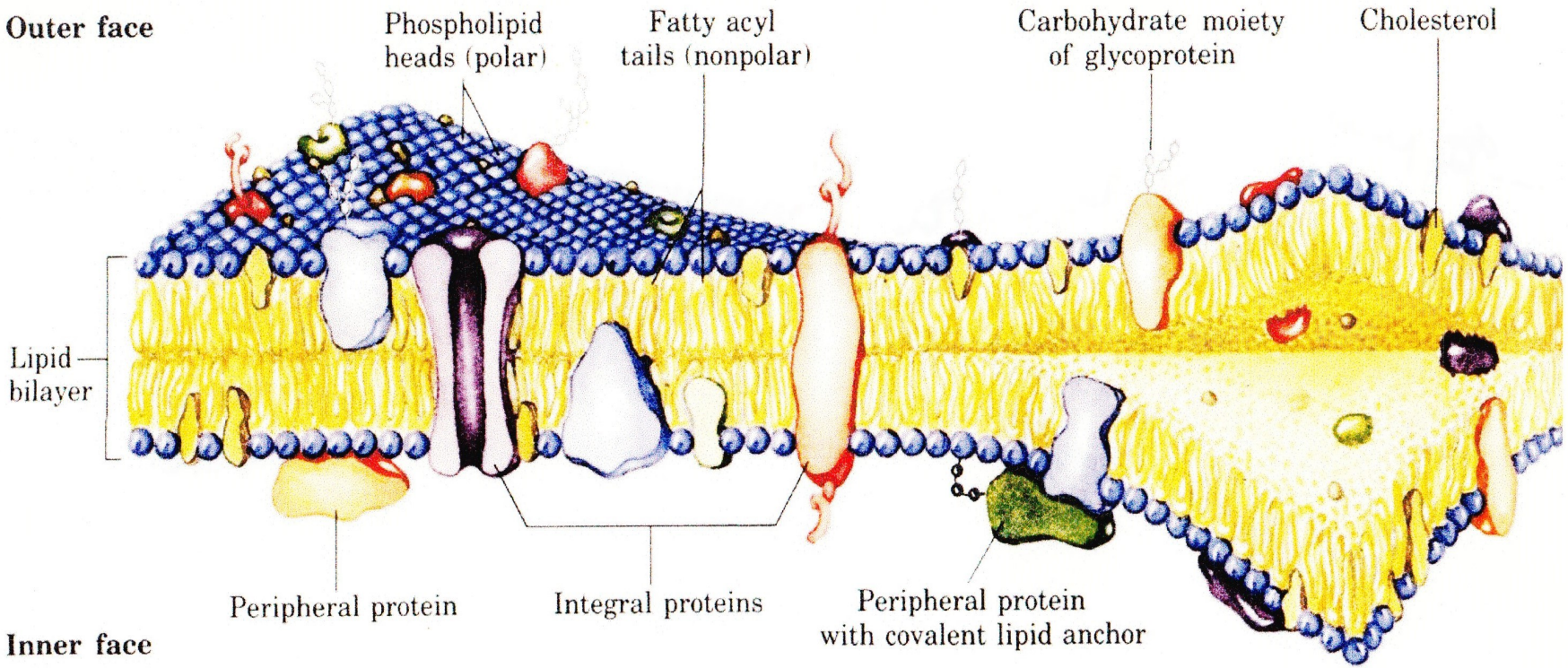
What is the purpose of the cell wall?

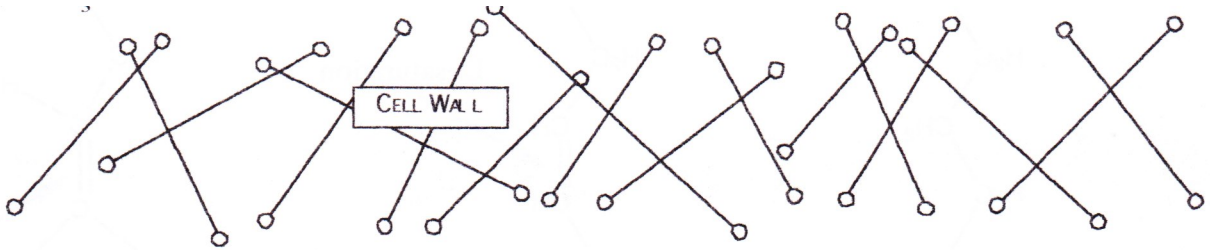
1. Barrier for the yeast cell keeping out other large objects.
2. Osmotic barrier - helps keep its shape under pressure
3. determines the flocculation characteristics. Maybe whether they are top cropping or bottom yeast. Calcium and sodium affect this.
4. Determines the charge on the yeast - which is a net negative charge

(Managing the lag phase)

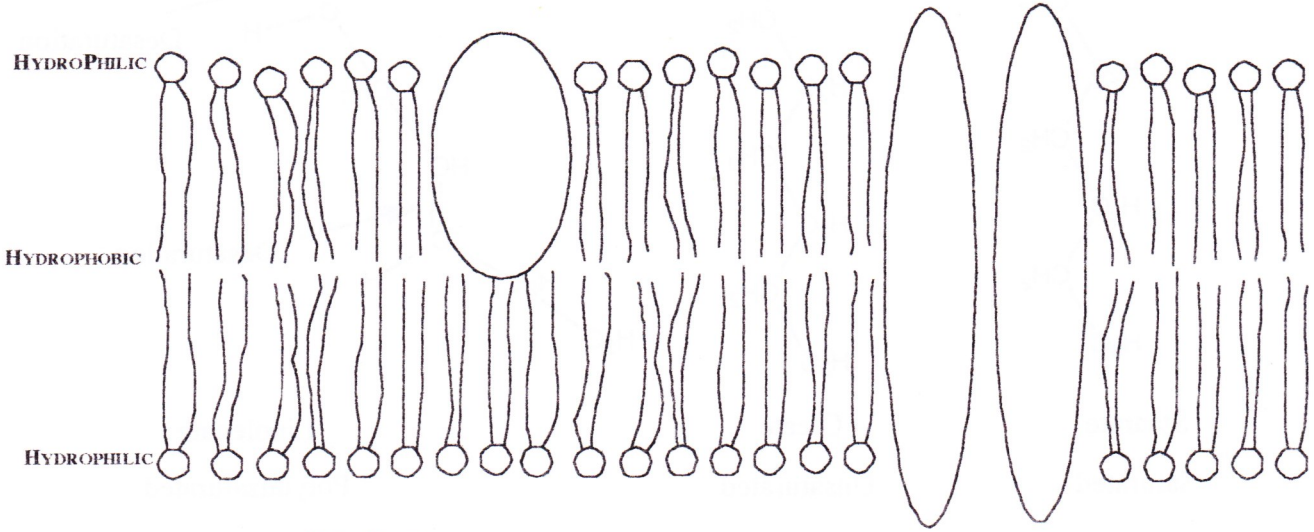
Depending on how the last fermentation ends up - will determine how long the lag phase is on the next generation - along with other factors; time stored between pitching, type of beer retrieved from.
Over all age of the culture.

Cell Wall Lipid Bilayer





PERIPLASM



ENDOPLASM

Yeast and Strain Selection – Some criteria

1. Provides flavor and aroma to beer
2. Alcohol tolerance – typically 10-15% ABV
3. Attenuation – the degree to which it can utilize fermentable sugars (real degree of fermentation – RDF)
4. Flocculation characteristics – can affect diacetyl management - filterability
5. Mutation potential ?????
6. Head Size
7. Open Fermentation – True top cropper preferred.
8. Beer Styles – Lager – Ale – Wheat(type) Belgian

Why Culture new Yeast?

1. **Purity**
2. **Viability**
3. **Harvest Affects**
4. **Storage Affects – time**
5. **Flavor Drift**
6. **Mutation**
7. **Beer Styles – High Gravity Beers**

Sources for yeast –

1. Laboratories - Theoretically Pure - Choice of volumes
2. Other Breweries –Questionable quality – May be limited availability
3. Liquid vs. Dry
- **purity ??**
- **consistency - lot to lot**
- **flavor characteristics – losses – additions or changes in flavor**
- **ease of use**
- **reuse potential**
- **overall cost when repitching**

Sources for yeast –

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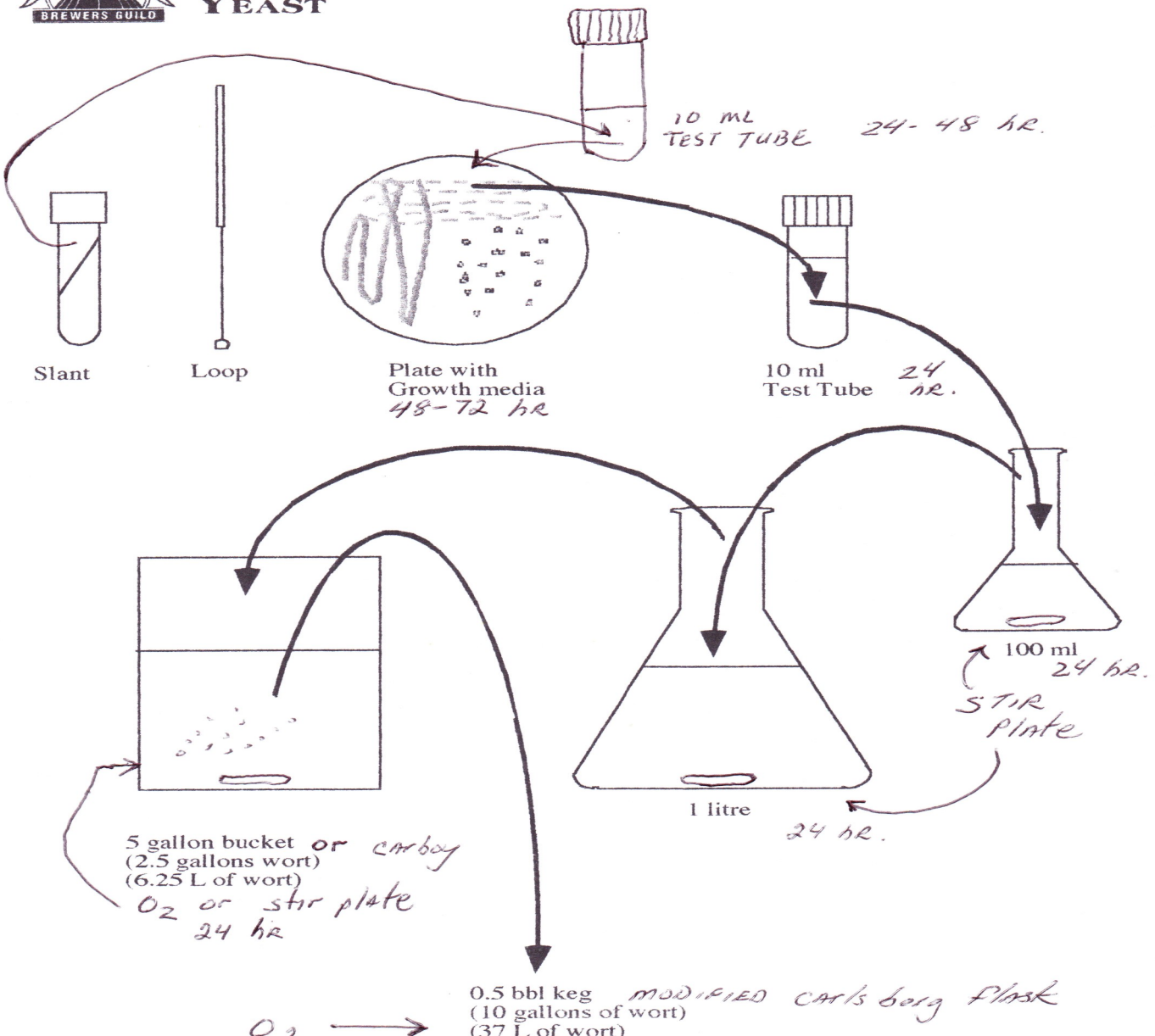
YEAST


10 ML
TEST TUBE
24- 48 hr.
Slant
Loop
Plate with
Growth media
48-72 hr
10 ml
Test Tube
24
hr.
100 ml
24 hr.
STIR
Plate
1 litre
24 hr.
5 gallon bucket or carboy
(2.5 gallons wort)
(6.25 L of wort)
O2 or stir plate
24 hr
O2
0.5 bbl keg MODIFIED Carlsberg flask
(10 gallons of wort)
(37 L of wort)
24 hr.

se of its formulation, this material has a tendency to lump in the bottle. It may noved easily by breaking with a spatula or other sharp instrument. The cultural of the medium is in no way impaired by this lumping.

TY CONTROL

	Identity Specifications
rated powder:	white to off-white, homogeneous with a tendency to clump
on of 5.25%* solution:	pH 6.7 ± 0.2 at 25°C
ed medium:	light to light-medium amber, clear, may have a slight precipitate

or Bacto Tryptophan Assay Medium

RENCES

l. Chem., 177:533, 1949.
l. Chem., 155:1, 1944.

AGING

Arginine Assay Medium	100 g	0466-15-0
Cystine Assay Medium	100 g	0467-15-9
Glutamic Acid Assay Medium	100 g	0961-15-0
Histidine Assay Medium	100 g	0992-15-3
Isoleucine Assay Medium	100 g	0437-15-6
Leucine Assay Medium	100 g	0421-15-4
Lysine Assay Medium	100 g	0422-15-3
Methionine Assay Medium	100 g	0423-15-2
Phenylalanine Assay Medium	100 g	0469-15-7
Threonine Assay Medium	100 g	0323-15-3
Tryptophan Assay Medium	100 g	0327-15-9
Tyrosine Assay Medium	100 g	0468-15-8
Valine Assay Medium	100 g	0991-15-4

WL NUTRIENT AND DIFFERENTIAL MEDIA

DED USE

WL Nutrient Broth and Bacto WL Nutrient Medium are recommended for the ion of yeasts, molds and bacteria encountered in brewing and industrial fer- ion processes.

WL Differential Medium, also used in the microbiological control processes in mentation industry, permits the unrestricted growth of bacteria and inhibits de- ent of yeasts and molds.

RY/PRINCIPLES

WL Nutrient media are prepared according to the formulae described by Green ay.[1,2] In their study of various fermentation processes, Green and Gray pointed e inadequacy of the microscopic count in fermentation control procedures. An tive study of the method of examination of worts, beers, and liquid yeast and fermentation products led to the development of two media; one containing no

selective agent and the other, a differential medium containing the antibiotic Actidione® (cycloheximide) as a selective agent.

Bacto WL Nutrient media permit the development of yeast. In those instances in which the number of yeast cells is comparatively small, certain bacteria can be detected. Green and Gray[2] reported that counts of viable bakers' yeast may be made on the WL nutrient medium at pH 5.5. If the reaction is adjusted to pH 6.5, the count of bakers' and distillers' yeast may be made. In making microbial counts using these media, the temperature and time of incubation will vary depending on the various materials under investigation. Temperatures of 25°C are generally employed with brewing materials and 30°C for bakers' yeast and alcohol fermentation mash analyses. Incubating periods run from 2 to 7 days, depending on the flora encountered. Incubation periods of 10 to 14 days may be used in some cases.

Bacto WL Differential Medium has the same formula as Bacto WL Nutrient Medium, with the addition of 0.004 g of Actidione® per liter. This inhibits the development of yeasts without interfering with the development of bacteria generally encountered in beers.

A reliable count of bacteria can be obtained at pH 5.5. To obtain estimations of beer cocci and lactic rods, plates should be incubated under anaerobic conditions. For estimation of acetic acid rods and termobacteria (very small rods occuring in wort as described by Linder in about 1900 as *Termobacterium lutescens, iridescens* and *erythrimum*) incubate under aerobic conditions. To analyze bakers' yeast and alcohol fermentation mashes, the reaction is adjusted to pH 6.5. Plates containing dilutions of bakers' yeast are incubated aerobically, while those from alcoholic fermentation mashes are incubated anaerobically.

FORMULAE

BACTO WL NUTRIENT BROTH
DEHYDRATED

Ingredients per liter

Bacto Yeast Extract	4 g	Calcium Chloride	0.125 g
Bacto Casitone	5 g	Magnesium Sulfate	0.125 g
Bacto Dextrose	50 g	Ferric Chloride	0.0025 g
Monopotassium Phosphate	0.55 g	Manganese Sulfate	0.0025 g
Potassium Chloride	0.425 g	Bacto Brom Cresol Green	0.022 g

Final pH 5.5 ± 0.2 at 25°C.

One pound will make 7.5 liters of final medium.
Rehydrate with 60 grams/liter.

BACTO WL NUTRIENT MEDIUM
DEHYDRATED

Ingredients per liter

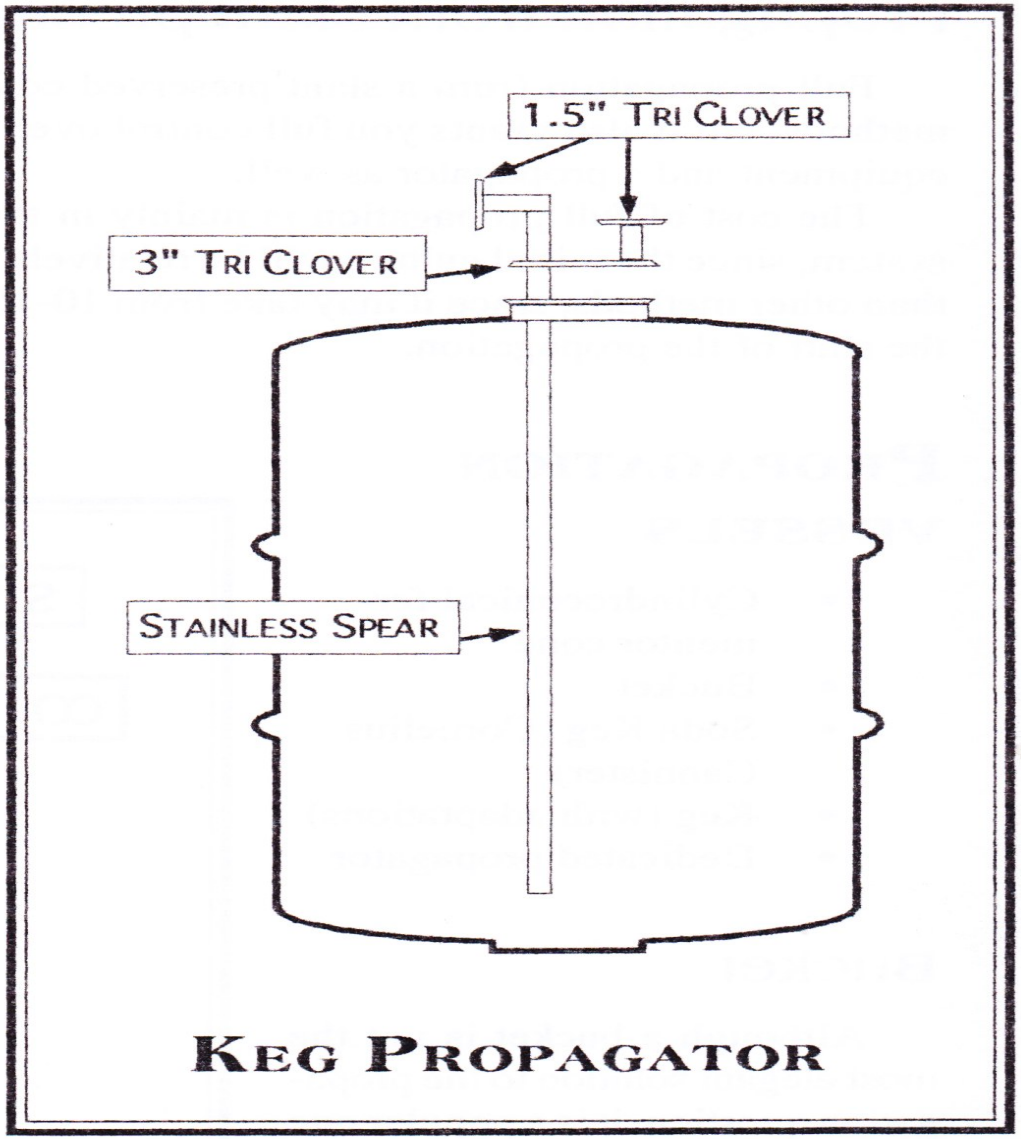
Bacto Yeast Extract	4 g	Magnesium Sulfate	0.125 g
Bacto Casitone	5 g	Ferric Chloride	0.0025 g
Bacto Dextrose	50 g	Manganese Sulfate	0.0025 g
Monopotassium Phosphate	0.55 g	Bacto Agar	20 g
Potassium Chloride	0.425 g	Bacto Brom Cresol Green	0.022 g
Calcium Chloride	0.125 g		

Final pH 5.5 ± 0.2 at 25°C.

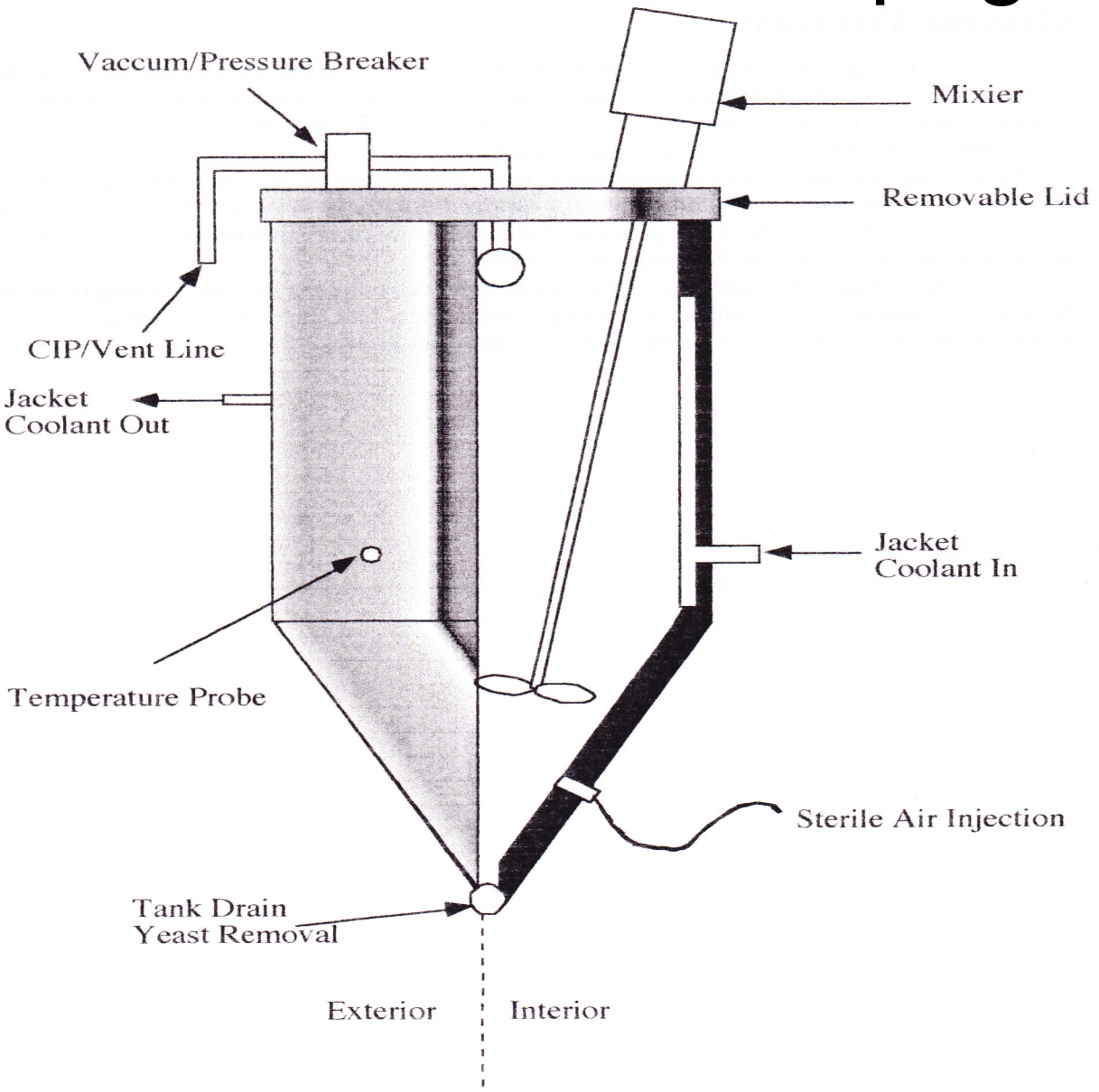
One pound will make 5.6 liters of final medium.
Rehydrate with 80 grams/liter.

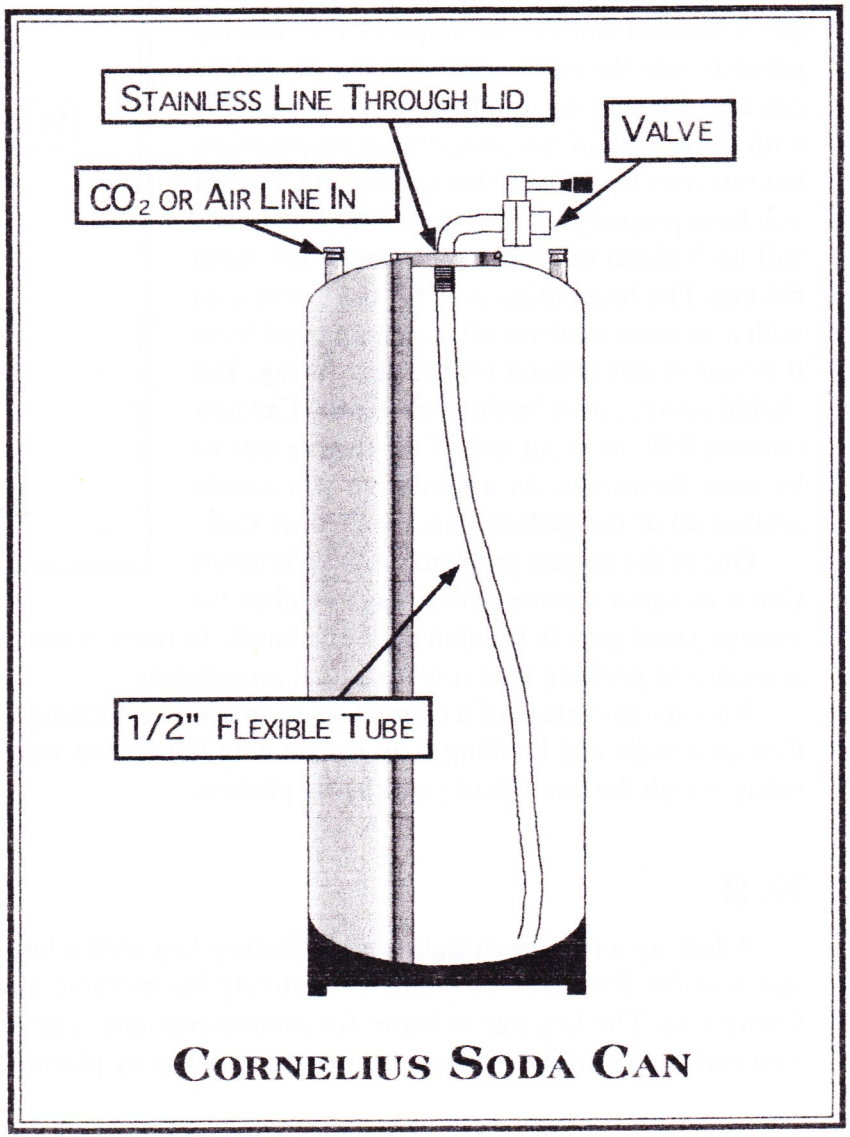
Carlsberg Flask

Modified Keg Propagator - Brink



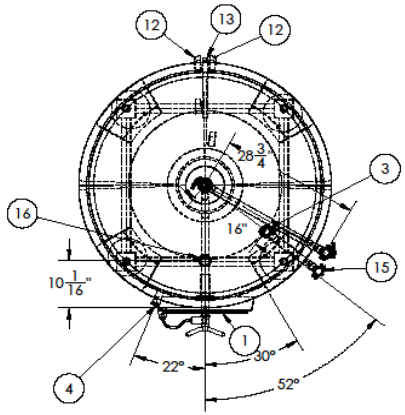
Features of a Yeast Propagator



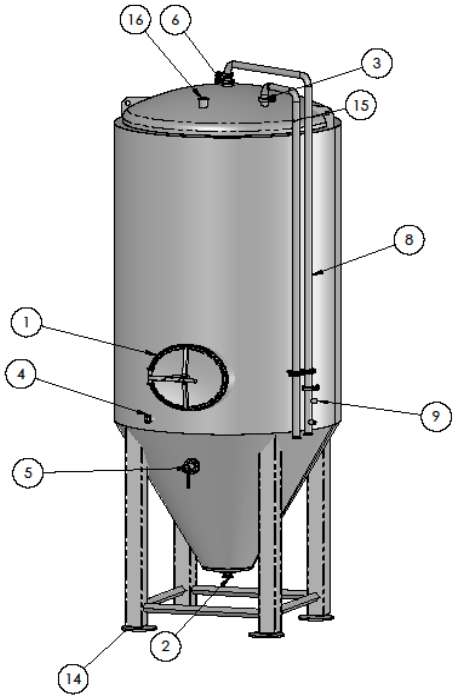


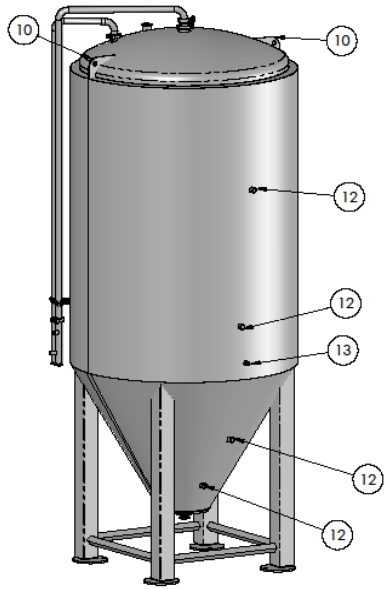
CORNELIUS SODA CAN

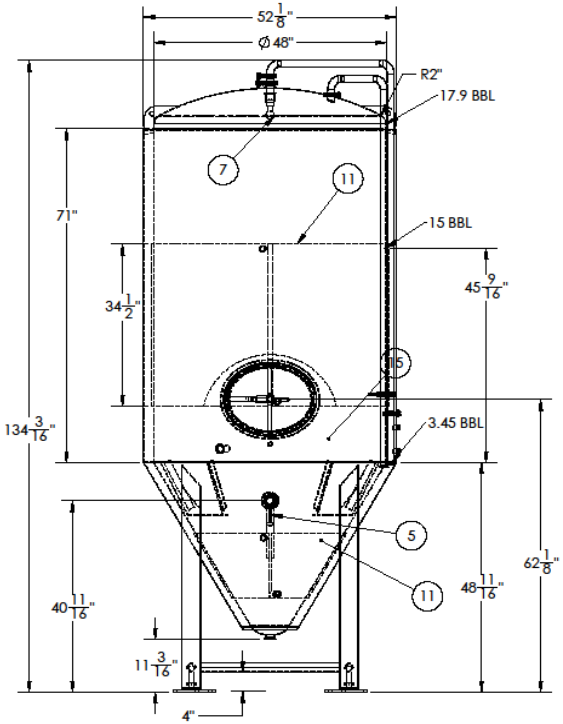
REVISIONS				
ZONE	REV.	DESCRIPTION	DATE	APPROVED
	A	INITIAL RELEASE	06/25/12	

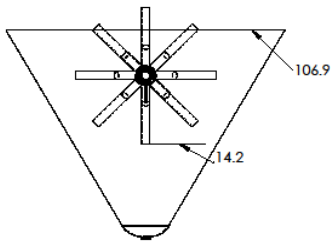


PRELIMINARY









RACKING ARM ROTATION

PRELIMINARY

ITEM	DESCRIPTION	FITTING/SIZE	QTY
1	ZORZINNI 190 MANWAY	447 mm X 546 mm	1
2	DRAIN	2.0 INCH TC	1
3	PRESSURE RELIEF PORT	2.0 INCH TC	1
4	SAMPLE PORT	1.5 INCH TC	1
5	RACKING ARM	1.5 INCH TC THROUGH 2" DIP	1
6	CIP PORT	2 INCH TC	1
7	REMOVABLE CIP ROTATING SPRAY BALL	1.5 INCH TC	1
8	CIP DOWN TUBE	1.5 INCH TC	1
9	REMOVABLE CIP GAS AND PRESSURE PORTS	3/8 NPT	1
10	LIFTING EAR	1 INCH HOLE	2
11	COOLING JACKETS	DIMPLE	2
12	JACKET PORTS	1 INCH NPT	4
13	TEMP WELL	1/2 INCH NPT	1
14	ADJUSTABLE FEET	8 INCH DIAMETER, BOLT DOWN	4
15	VPRV DOWNTUBE	1.5" TC	1
16	DRY HOP PORT	2 INCH TC	1

NOTES:
1. TANK WORKING VOLUME: 15 BBL, (465 GALS) ; MAXIMUM VOLUME: 22.5 BBL, (699GALS)
2. TANK AND JACKET WORKING PRESSURE: 15PSI
3. WEIGHT EMPTY: 1104 LBS
4. WEIGHT FULL (WATER): 6,944 LBS
5. TIP-UP HEIGHT: 132 9/16" INCHES

CUSTOMER: **SAMPLE**

UNLESS OTHERWISE SPECIFIED:
DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ±
ANGULAR: MACH ± 1° BEND ± 2°
TWO PLACE DECIMAL ± .01
THREE PLACE DECIMAL ± .005
INTERPRET GEOMETRIC TOLERANCING PER: ANSI-Y 14.5
MATERIAL: SEE NOTES
FINISH: SEE NOTES
DO NOT SCALE DRAWING

	NAME	DATE
DRAWN	DLP	06/25/12
CHECKED		
ENG APPR.		
MFG APPR.		
Q.A.		

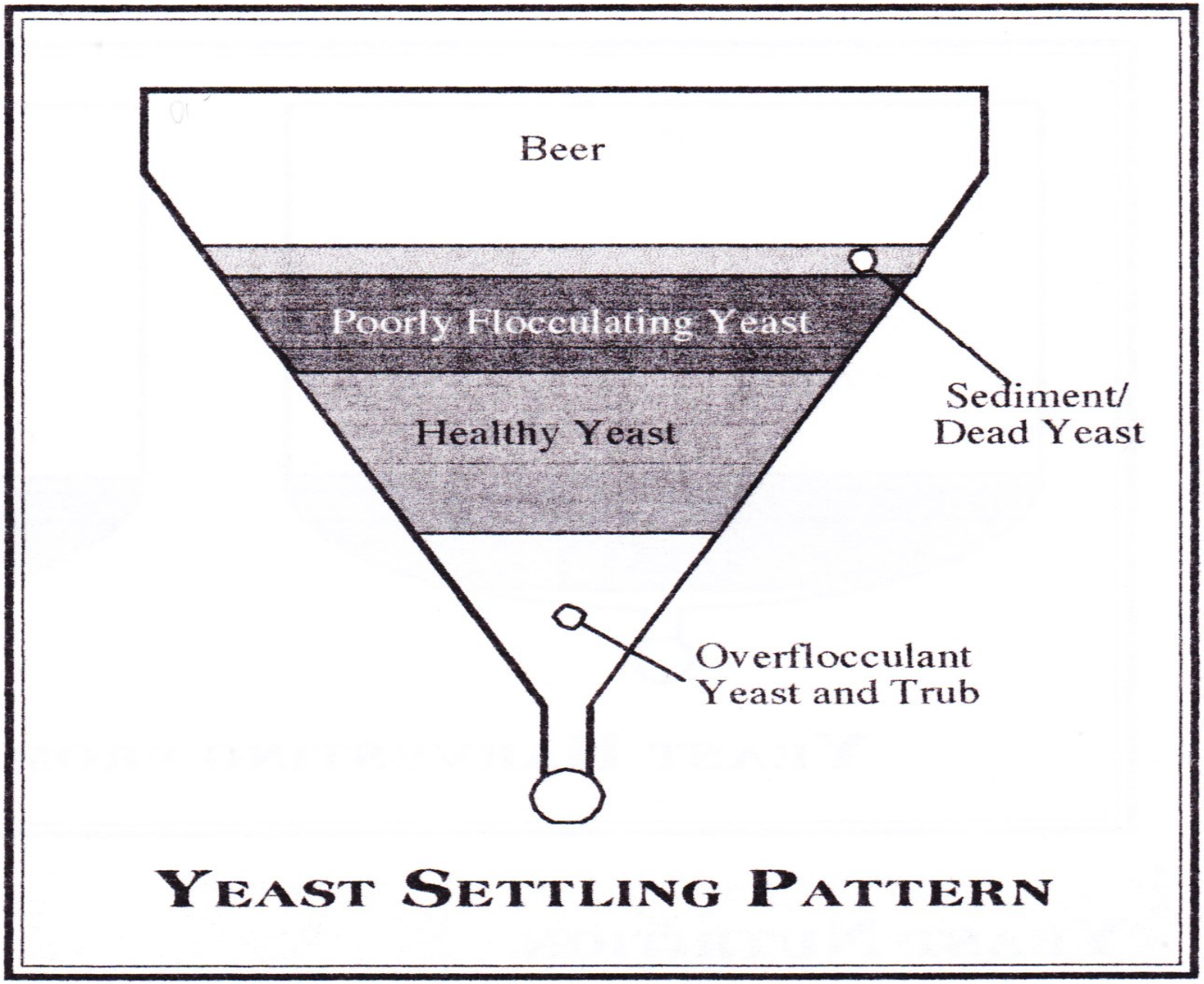
COMMENTS:

METALCRAFT FABRICATION LLC
PO BOX 13185, OR 97213 503-807-7726

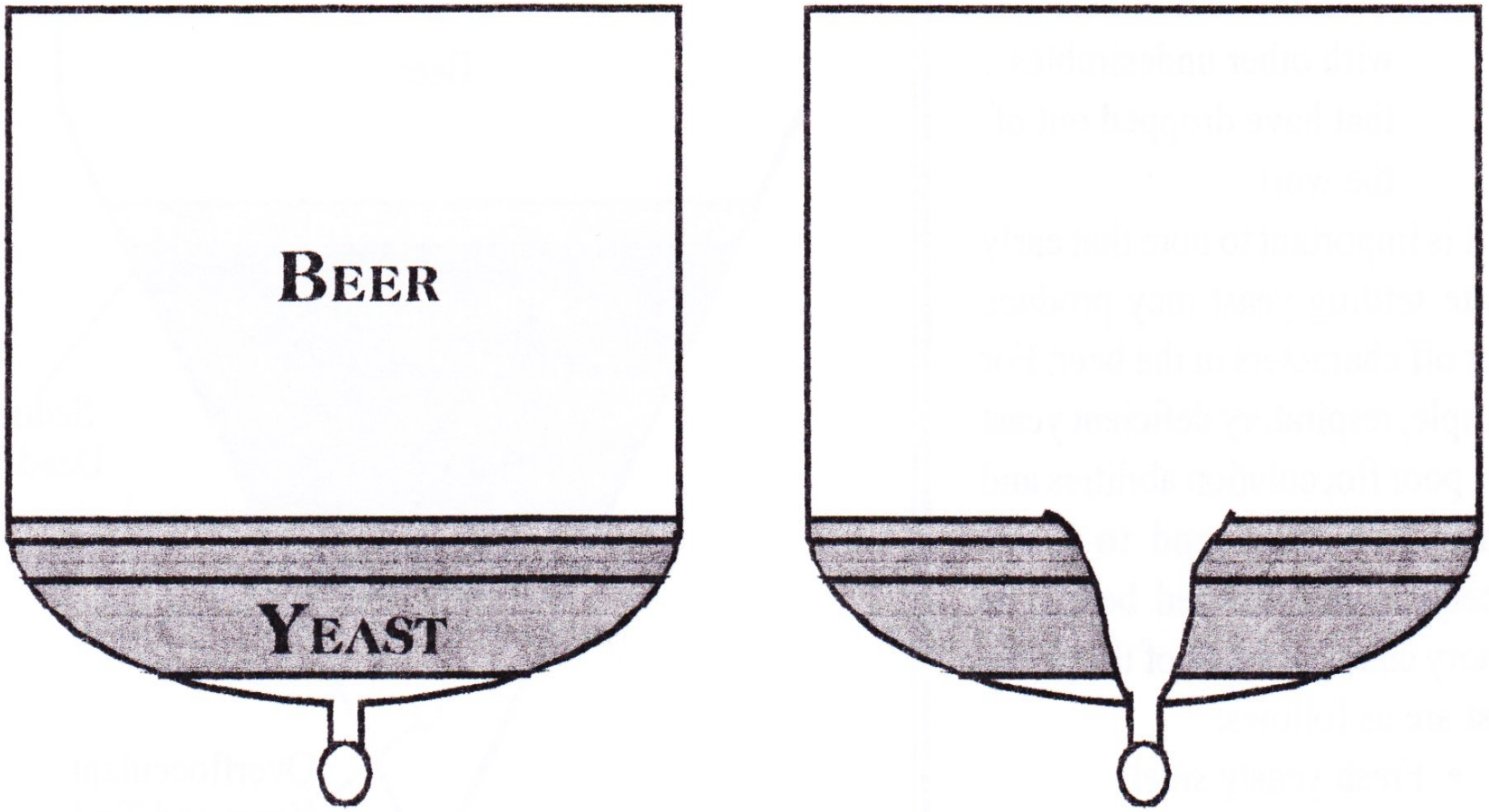
TITLE: 15 BBL FERMENTER

SIZE C | DWG. NO. 1061-07 | REV A

SCALE: 1:20 | SHEET 1 OF 1



YEAST SETTLING PATTERN



YEAST HARVESTING FROM A DISH BOTTOM

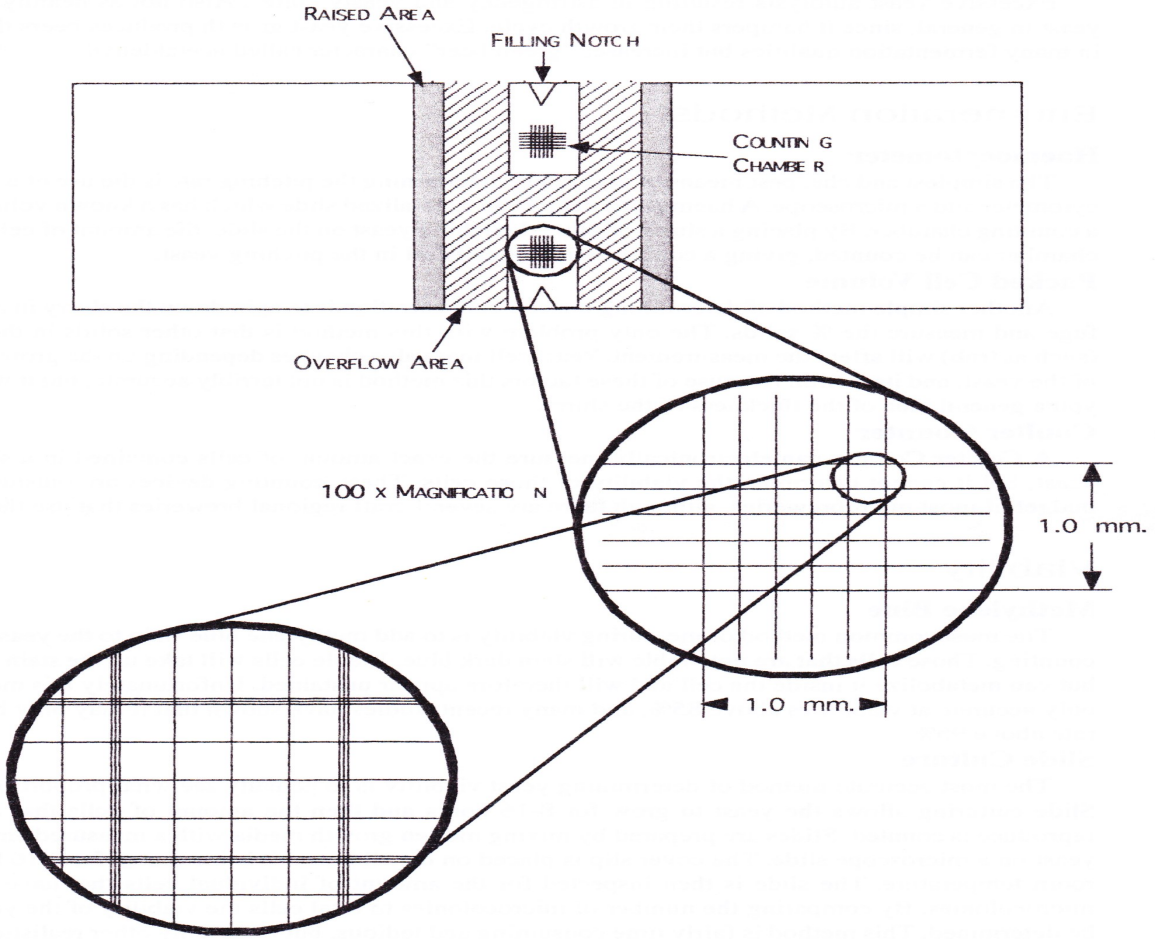
Open Fermenter with skimmer

Open Fermenter

Wooah Baby !

Pitching the proper amount of Yeast

- Hemacytometer
- Microscope slide with a fixed volume grid
- Requires Serial Dilution
- Count # cells on grid under Microscope
- Calculate cells per mL.
- Calculate amount to pitch for desired cell count



HAEMOCYTOMETER

Yeast Viability

- Addition of Methylene Blue Dye to Cells
- 0.01–0.1% soln. dye w/2% Sodium Citrate
- Dead Cells absorb dye Blue = dead
- 90% viability for relative accuracy
- Weak vitality cells hard to identify

Yeast – Packed Cell Volume

- Homogenous Sample w/ 0.5% NAOH
- Centrifuge
- Determine Cell Mass - % of cell (solids)
- 40% solids ~ 1 X 10^9 / ml
- 55% solids ~ 1.4 X 10^9 / ml
- Calculate Pitch Rate based on cell density
- Wt. or Vol. Yeast/ Wt. or Vol. Wort

Yeast Measurement Methods

- Slide Culture: Standard Method to measure other methods against
- Measures viability and vitality
- Coutler Counter – Measures Particles
- Aber Instrument – Measures Conductance
- Pitch Rates:
- 1 X 10^6 / ml X degrees plato of wort
- ~ 1 pint or 0.5 liter / Hl or bbl based on % viable
- Or - More for high gravity beer and lagers beer

Yeast Washing

- Traditional – disperse yeast over a fine shaking screen to settle yeast out and remove CO2
- Acid Washing - Add 50% by volume chilled acidified water to pH 2.2 2 hours continual agitation
- Chlorine Dioxide – 1 ml stabilized ClO2 / liter of yeast, agitate 1 hour.

Yeast Tracking

- **Strain ID and generation**
- **Harvested from where andwhen?**
- **Trub removal yeast removal. How muc?**
- **Beer style , volume, date**
- **Rate of fermentation, time, rate of gravity drop**
- **pH change over fermentation**
- **Temperature of fermenter at harvest**
- **Age of yeast at pitching, pH of yeast at pitching**
- **Quantity of yeast pitched**
- **Actual pitch rate**
- **Viability**
- **Sensory Properties of beer and yeast**
- **Visual Properties**
- **Lab results**