PROJECT REPORT ON BREWING PROCESS AND REDUCTION OF OFF-FLAVOUR(DIACETYL) FROM BEER BY YEAST MANAGEMENT SYSTEM

Dissertation submitted in fulfillment

Master of Science in M.Sc BIOTECHNOLOGY

Submitted By

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UB GROUP

UNITED BREWERIES Ltd, Khurda , Odisha

CERTIFICATE

This is to certify the dissertation entitled " ***Brewing Process and Reduction of off flavour (diacetyl) from beer by yeast management system***" submitted by Mukesh Kumar Samal in partial fulfilment of the requirement for the degree of Master of Science in Biotechnology, KIIT School of Biotechnology, KIIT University,Bhubaneswar bearing Roll No. 1661015 & Registration No. 16646451461 is a bonafide research work carried out by him under my guidance and supervision from 18th December 2017 to 11th April 2018.

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SUMMARY

- ❖ Claculations
- ❖ Discussion
- ❖ Conclusion
- ❖ Reference

INTRODUCTION

ABOUT THE COMPANY

United Breweries Limited, Khuda Unit, Odisha

United breweries group

United Breweries Group or UB Group is an Indian conglomerate company headquartered in UB City, Bangalore in the state of Karnataka. The Company has annual sales of over US$4 billion and a market capitalization of approximately US$12 billion. Its core business includes beverages, aviation, electrical and chemicals. The company markets beer under the Kingfisher brand, and owns various other brands of alcoholic beverages. . United Breweries is India's largest producer of beer with a market share of around 48% by volume.

The logo:- **The Pegasus, which is the symbol of the United Breweries, first found its place as the Group logo in 1940. Then, the Helladic horse**

associated with beer and nectar in Greek mythology carried a beer cask between the wings, ostensibly because beer formed the core operations of the Group. Later, the beer cask was removed to represent the Group's multifaceted operations.

The group owns the Mendocino Brewing Company in the United States. United Spirits Limited has 144 brands. Under its umbrella, including White Mischief Vodka, the market leading brand in India. Some products are listed below as..

Beer

Kingfisher Blue
Kingfisher Strong
Kingfisher Premium
Kingfisher Ultra
UB Premium Ice
Kalayani Black Label Premium
Kingfisher Draught
Kingfisher Red

Vodka

White Mischief
Pinky

Wines

Bouvet Ladubay
Four Seaso*ns*

Whisky

Royal Challenger
Whyte & Mackay
Black Dog Whisky
Signature
McDowell's No.1

Rum

Celebration Rum
McDowell's No.1

ABOUT THE INDUSTRY

Current beer production in India is still very low compared to most of the countries with a per capita consumption of less than 52 L per annum. As Indian economic development continues at its present place, the country's beer consumption is expected to grow sizable, in near future. The industry is concentrated with United Brewers (UB) accounting for 45%of total beer sales.

Beer is classified into strong & mild segments, the basic difference being the alcohol content. Mild contains 4.5-5.0% alcohol by volume while strong beer contains close to 7-8% alcohol & accounts form 65%of the beer market.

There has been a gradual shift towards strong beer .This began in the 1990's & continues because of the tax structure of the industry which favours hard liquor. Thus, regular drinker look for value for money purchases in the form of hard liquor rather than beer. SAB Miller latched on to this trend quite early.

Its Hayward's 5000 sells close to 10 million cases per annum compared to Hayward's 2000, a mild beer that sells 2.5 million ceases per annum. The competition has affected UB's popular brand Kingfisher. To counter this UB launched Kingfisher Strong. In addition, overseas investment in the Indian Brewing industry is beginning albeit slowly! The Stroh Brewing Company in a Brewery in Rajasthan but the plant closed when Stroh ceased operations in the United States. The Fosters Brewing Group has invested in brewery located at Aurangabad. Yuksom Breweries Sikkim located at Melli Sikkim & Denzong Breweries, Khurda, Orissa.

BREWING PROCESS

What is a Beer?

In its most basic form, beer is produced from only three ingredients: Water, Malt and Hops. Using these ingredients beer goes through a basic process called fermentation. During this process microscopic organism called "yeast" breaks down the sugars into alcohol and carbon dioxide gas. Two nutrients in the malt are required for this purpose, they are: Nitrogen and Phosphorus. About half of the sugars is converted into alcohol and the other half escapes to the surface as carbon dioxide gas.To give the beer flavour, aroma and the body other ingredients are used during the brewing process. The two main ingredients used are malt and hops. In addition, some carbon dioxide gas must be retained to give the beer carbonation and a nice foamy head.

MATERIALS AND METHODS

REQUIREMENTS FOR PRODUCTION-:

MALT:

What is malt?

Barley malt grains are partially-germinated seeds which have been heated and dried. Contrary to expectation, they are not necessarily dead. Many can re-germinate, given the opportunity (water, air, time), though they are seldom capable of forming a healthy plant.Malts fall into two broad types: standard malts and specialty malts. Standard malts, which include those used for the bulk of the grist of both lagers and ales, provide extract, flavour, colour, and nutrients for yeast. Specialty malts are used primarily to supply colour and flavour, while sacrificing extract yield.

One very important aspect of malt is that it is substantially inhomogeneous. There are considerable differences between individual corns. This has a major impact on processing, and on prediction of the performance of a single batch.

What does malt contain?

Like most commodities, malt is taken for granted. However, the myriad of components it provides us with is remarkable. Important chemicals contributed to the brewing process by pale malt include **starch**, protein, amino acids and peptides, phosphate, polyphenols, melanoidins, O- and N-heterocyclic compounds, lipids and sterols, beta-glucan, vitamins, metal ions, and enzymes. In addition, malt husk material provides the filter medium for mash tun and lauter tun separation systems.

Starch

The starch granules comminute from the malt make up the greatest portion of the extract. This cereal starch is in granular form with two forms:

Amylopectin (70-80%)

Amylose (20-30%)

Malt Adjuncts:

Barley contain considerable protein, if these barley are employed in the medium as only source of carbon and nitrogen the resulting beer is dark coloured, somewhat unstable and too filling for the average taste. This situation is correct by diluting the malt proteins with additional starch, which has added in the form of malt adjuncts.

Therefore adjuncts are anything that is not malt, yeast, hops and water. Adjuncts may be any carbohydrate source other than malted barley which contributes sugars to the wort.

Brewing Adjuncts:

Basic raw cereals:	Barley, Wheat
Raw Grits:	Corn (maize), rice, sorghum
Flaked:	Corn, Rice, Barley, Oats
Torrefied or Micronized:	Corn, Barley, Wheat
Flour or Starch:	Corn, Wheat, Rice, Potato, Cassava, Soya, Sorghum
Syrup:	Barley, Potato, Sucrose
Malted cereal other than barley:	Wheat, Oat, Rye, Sorghum[6]

Water:

Large amount of water are employed in the production of lager beer, in fact, each barrel of beer requires, an average of 10-12 barrels of water. Obviously only a small portion of this water ends up in the finished product, the rest is utilized in various fermentation and processing steps.

The characteristics of good water for brewing are a pH of 6.5-7 less than 100 ppm calcium and magnesium carbonates, trace amounts of magnesium, 250 to 500 ppm calcium sulphate, 200-300 ppm sodium chloride and 1 ppm or less of Iron. Water containing higher levels in carbonates produces a heavy flavoured, dark coloured product.[3]

In contrast, the absence of carbonates but presence of calcium sulphate allows the production of light beers and pale ales with a light flavoured and absence of harshness

Hops- the bitter maker:

Hops were originally introduced by brewers to help inhibit the growth of beer spoiling bacteria, to maintain flavour stability, and to help retain the head (foam) of the beer. There are over 50 different varieties of hops which are grown commercially in Germany, southern England, southern Australia, Tasmania and Washington State. They are selected for their unique bitterness in flavour or aroma.

Alpha acids and Beta acids are the resins contributing to bitterness. They are conventionally measured on a mass percent basis. Of the two, alpha acids are the most dominant in bitterness contribution. Both alpha and beta acids are not soluble in water, in order to allow them to contribute to the bitterness of the beer, they must be modified. The bitterness, flavour and aroma of hops are released into the wort through boiling. The timing of additions of hops to the wort is called a hopping schedule. The amount of bitterness imparted to the wort in called utilization. There are four basic classifications of hop use. Bittering , flavouring, finishing and dry hopping. These terms refer to how the selected hop variety is used.

Sugars:

Sugar is also an additional carbon substance to the yeast and it is also a malt adjuncts. It doesn't require any enzymatic activity because yeast can sue directly. Approximately 80-90% of the weight of these malt adjuncts is extracted in to the wort.

CHEMICALS AND ENZMES

Termamyl,Ultraflow,Ceram,Proteases,Fungamyl BrewQ,Biofoam,$CaCl_2$,Caramel,STABIQUICK

MALTING AND MILLING

Malting:

In most of the world, malt and barley malt are synonymous, although in some areas other grains are malted for brewing use. Malt is prepared from germinated barley seeds and contains natural enzymes that digests the starch of the grains and converts it into sugars. Since brewing yeasts are unable to digest starch, the malting process is essential for the preparation of a fermentable[10] material from cereal grains.

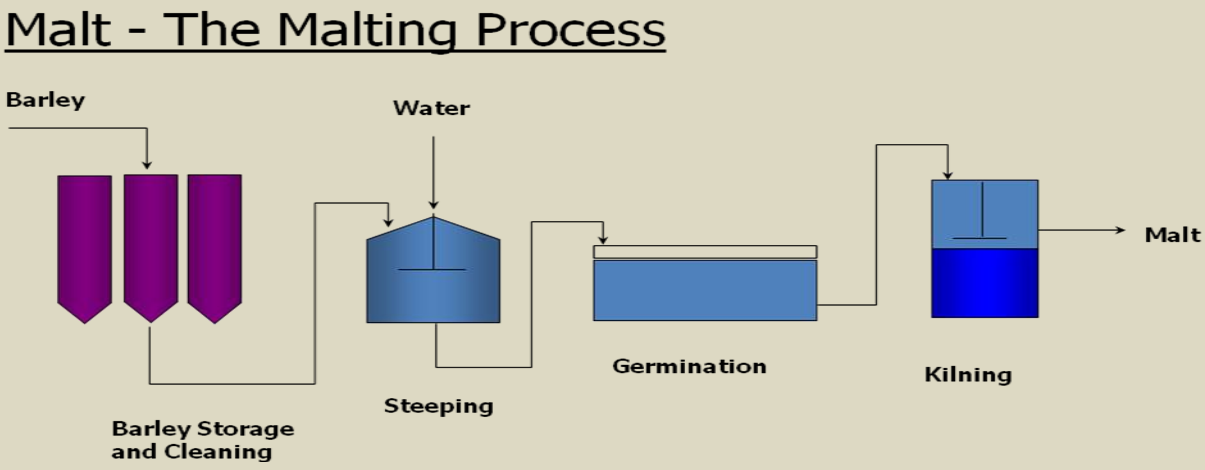
Malting is a controlled natural process rather than a manufacturing operation. Malting process involves the following steps:

-Cleaning and grading

-Stepping

-Germination

-Kilning



(MALTI NG PROCESS)

Milling:

Milling is the first brewing step taken in the brew house or kitchen of the brewery. Malt and adjunct grain, where used, are milled to render the grain mass more wet table and to facilitate the extraction of digested grain extracts into the brewing water. Wetting the crushed malt activates the malt enzymes and promotes their interaction with the grain starch and protein materials.

Milling of grains occurs throug0h a multitude of mechanical methods. The selection of mills and employment of a brewery specific milling process is at the discretion of the brewer. Grist preparation is usually done by either dry or wet milling.

The objectives of milling are:

To split the husk longitudinally to expose the endosperm.

To bring about the crushing complete disintegration of the endosperm to make all its grist available to enzymatic action and conversion to saccharides.

MASHING

INTRODUCTION

Next process point in the production of the beer is to convert the malted barley into a nutritional extract essential for fermentation performance and optimum beer quality.

The purpose of mashing is to extract the soluble sugars, dextrins and inorganic substances from the malt grains in addition to converting the insoluble material (starch and protein) to a fermentable form. The extract quality of a mash, like any biochemical reaction, is also influenced by environmental conditions. These conditions include temperature, pH, mash thickness, enzyme content, and substrate composition.

The mash tun is pre-filled with hot liquor prior to mashing in. This liquor is most commonly referred to as foundation liquor. Foundation liquor is also used in cereal cookers. This serves several purposes:

It pre-heats and maintains mash tun temperature.
Prevents blockage of the tun filter plates (if present).
Minimises any oxidation of the mash.
It prevents excessive damage to the husk as it hits the base of the tun.

Why is necessary to agitate the mash?

As the grist particles hydrate they swell and the heat forces gelatinisation of the starch. This uncoiling of the starch structure gives the mash a very viscous consistency. If the mash is not agitated, because of its viscous nature, it would stick and potentially burn on to the sides of the vessel. If agitation of the mash is too severe there is a risk of imparting shear stresses upon the mash. Most importantly there is a possibility of shearing and extracting -glucans from the malt, as well as very fine cereal particles that can hinder wort separation and extract recovery.

Mash agitation has the benefit of evenly distributing the enzymes and substrate. It is essential to have good mixing of the mash to provide maximum enzyme substrate contact. Without this the extract recovery can be impaired.

Barley starch gelatinises at around 61 -62°C, whereas rice and maize starch gelatinise at temperatures between 70 -80°C.

MASHING BIOCHEMISTRY

what is happening to the malt extract during mashing? The principle changes that occur are as follows:

Soluble sugars and proteins are leached from the grist particles.
Enzymatic degradation of some of the insoluble grist substances.
Enzyme denaturation and inactivation.
Chemical interaction of wort constituents.
A decrease in wort pH principally due to the presence and interaction of Ca2+ ions.

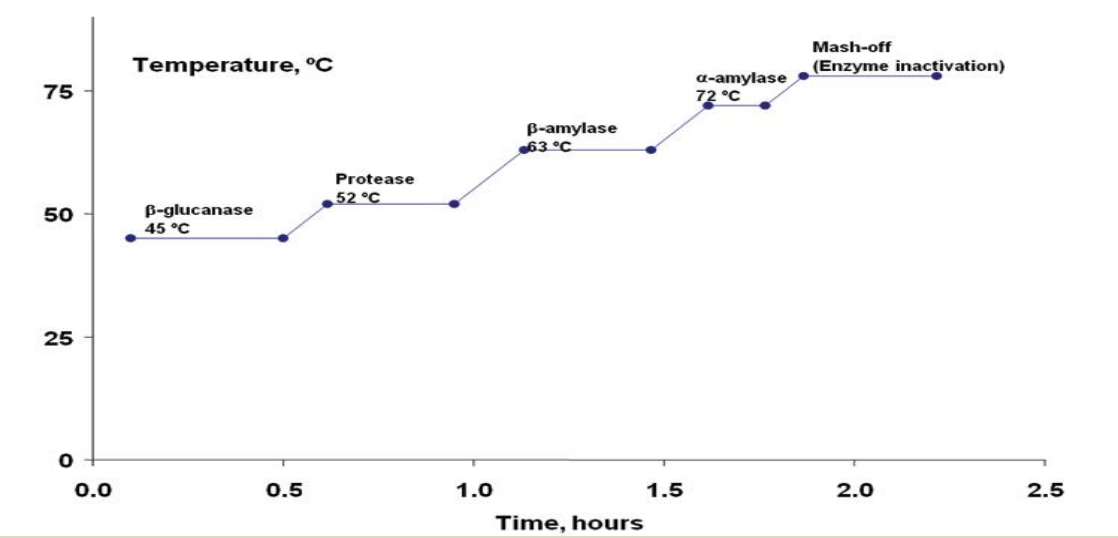
Enzymatic Rests:

In step infusion and decoction mashing, the mash is heated to different temperature, at which specific enzymes works optimally. The table below shows the optimal temperature for the enzyme. Brewers mostly pay attention to and what material those enzyme breakdown. A thicker mash acts as a buffer for enzymes. Once a step is passed, the enzymes active in that step are denatured and permanently inactive.

The time between rest is preferably as short as possible but if the temperature is raised more than 1°C per minute, enzymes may prematurely denatured in the transition layers near heating elements(temperature rate should be 1°C per minute.

Optimal rest temperature for major mashing enzymes:

Temperature	Enzymes	Breakdown
45°C	β-glucanase	β-glucans
52°C	Proteases	Proteins
62°C	β-amylase	Starch
72°C	α-amylase	Starch



(Mashing Profile)

Extract Composition

The nature of the extract composition is obviously dependant upon the grist recipe and mashing conditions. Approximately 75 - 80% of the total grist weight is extracted during mashing, and the remainder (the insoluble material) is removed from the
process along with the spent grains. The carbohydrate fraction of this extract mainly comprises starch degradation products and soluble components of the malt modified during malting. The predominant simple sugars of the wort are:

_ Monosaccharides (glucose and fructose)
_ Disaccharides (sucrose and maltose)
_ Trisaccharides (maltotriose)

The estimate of wort fermentability is calculated using the composition of the Mono-, Di-, and Trisaccharides as a percentage of the total. This usually gives a fermentability of
approximately 75%. This can then be linked to the attenuation limit.

LAUTERING

Introduction:

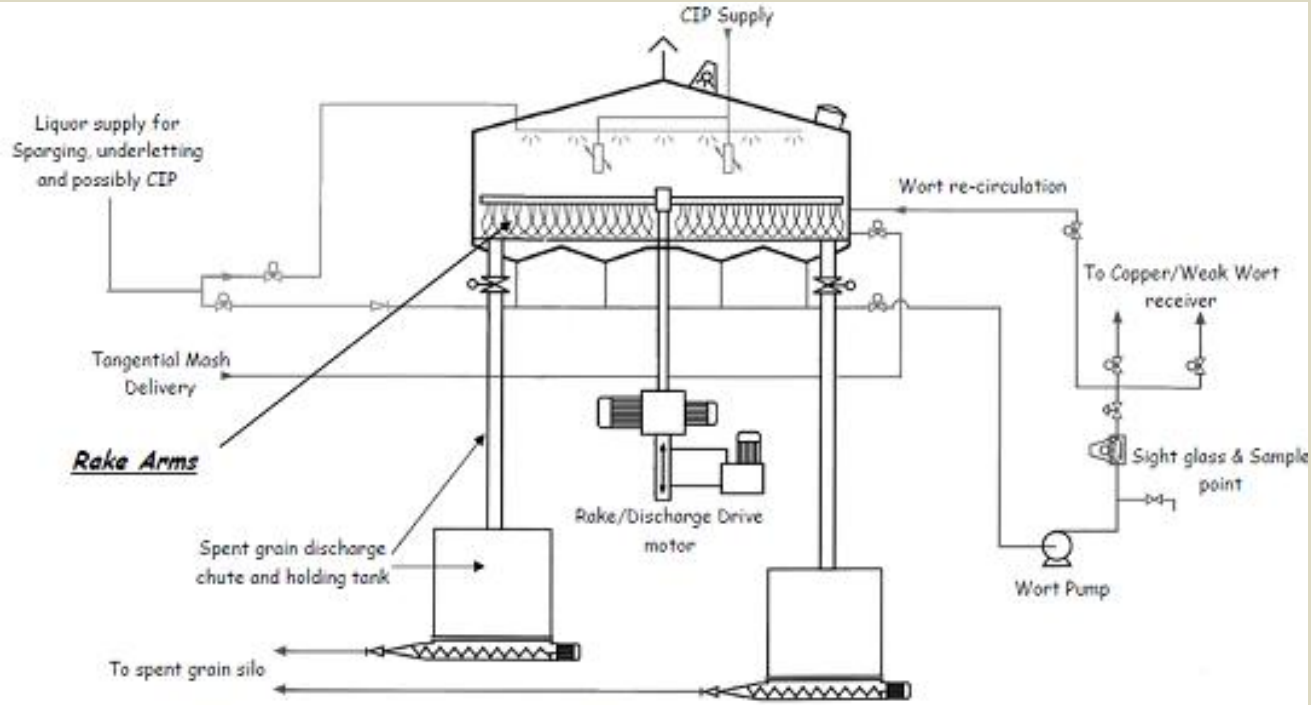
Lautering is a complex screening procedure that retains the malt residue from mashing on screens or perforated tube surfaces so that it forms a filtering mass. This mass allows the clear grain digested called wort to flow through while it retains essentially all particulate matters. Sparging devices introduce water into the grain mash to displace as much wort as possible without excessive dilution of the wort.

The husk and other grain residue in the mash as well as precipitated proteins and other solids are removed from the mash by passing it through a lauter tun and this process is known as lautering. This trub is staining tank with a false bottom slotted or perforated to allow passage of wort.

Once mash conversion is complete and starch degradation has occurred, the extract now in solution must be separated from the remaining cereal solids. This process of wort separation should produce clear, sweet, unhoped wort The mash is then transferred to the lauter tun or mash filter. Which is a kind of filters.

The lauter tun is effectively a big filter. The vessel has a false bottom, which is a slatted floor plate that creates a sieve through which the wort is run-off. The cereal mass or spent grains are trapped for removal.

When preparing the lauter tun the bottom of the vessel is layered with hot liquor. This can be referred to as underlet or foundation liquor. The underlet helps maintain the lauter tun temperature and prevent excessive husk damage when filling.



A simplified schematic diagram of a modern rapid batch lauter tun. (Adapted with kind permission of Briggs of Burton).

Under-letting is also a practical measure to raise the temperature of the mash . this liquor layer above the false floor, stops the slots in the plate becoming blocked by the mash and stopping wort separation. Floating the bed of mash above the false bottom throughout the filtration cycle means that the required volume and rate of wort run-off from the lauter tun is maintained.

Prior to first worts collection a volume of wort is recycled through the lauter tun. This ensures that the wort is bright before collection to the copper(s). The water used for mashing in, which contains the majority of the sugar extracted, is collected first. These are known as the first or strong worts.

After strong wort collection, the grain bed is flushed with fresh, hot liquor during a process known as sparging. This purges residual extract from the grain bed. Sparging continues until the required wort volume and gravity is achieved.

The cereal bed acts as a filter to clarify the wort. As the wort is drawn off, the pressure differential above the bed and below the false bottom increases. This results in dragging the bed down, and compacting it. To overcome this the brewer can use two corrective measures. They can either insert a second underlet to re-float the bed, and or they use rakes to separate the bed.

After the majority of the strong worts have been collected, it is essential to recover the residual extract trapped within the grain particles. 2nd Spraying or sparging the bed with hot liquor to purge out the remaining sugars achieves this.

Sparging is then stopped and the residual weak worts drained from the bed. These weak worts are either destroyed or in some cases recycled and used for the next brew. This is one method of minimising brew house losses.

The spent grains are removed from the tun. In some instances this material may be used to produce animal feeds, hence recovering some of the raw material costs.

WORT BOILING

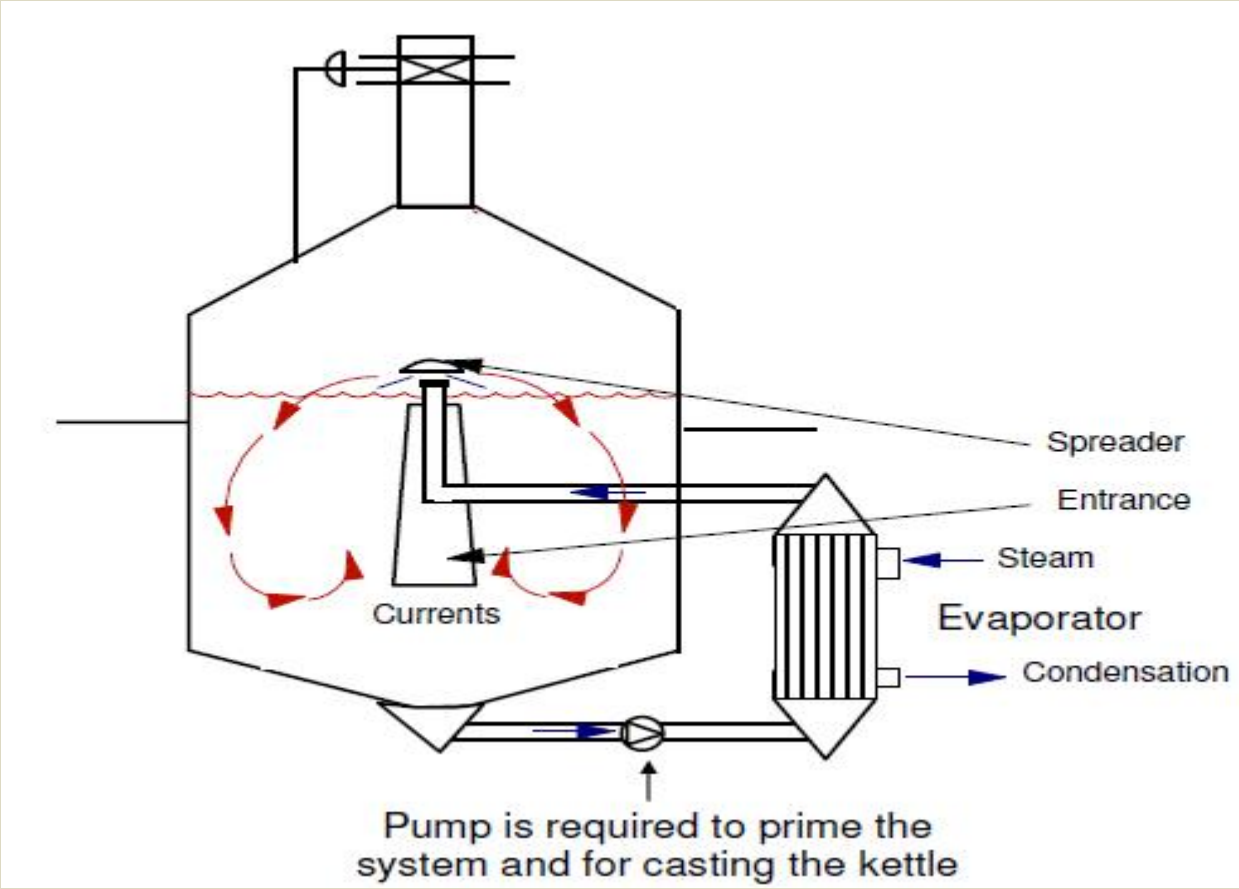
Wort boiling may be regarded as the turning point in the brewing of beers, many complex reactions take place during wort boiling. As the wort is heated the residual amylases and other enzymes are inactivated which terminates the mashing process and fixes the carbohydrates composition of the wort. At the boiling point, the wort is sterilized and the micro flora of the malt, hops and other adjuncts used are destroyed

Wort boiling is a key step in the production of quality beer. This process stage is missing in the production of whisky, where sweet wort is passed direct to fermentation without being boiled.

The main purposes of boiling are:
1. To halt enzyme activity.
2. To sterilise the wort.
3. To concentrate the wort by evaporation.
4. To develop colour and flavour.
5. To remove unwanted volatile flavour compounds.
6. To achieve the required colloidal stability.
7. To extract bitterness from the hops (isomerisation of bitter substances).

Wort boiling is a major usage of energy in the brewery and can account for 39% of the brewery's total energy. Steps are taken to make this process as energy efficient as possible by using heat recovery systems.

Boiling carried out in a wort kettle also known as a wort copper because early vessels were made of copper.



(BOILING PROCESS IN WORT KETTLE WITH EXTERNAL WORT BOIL

ACTION OF TRUB SEPARATION N WHIRLPOOL

The most common system for trub removal is the whirlpool separator. It has the advantage of being suitable for use with hop pellets and hop extracts and in situations where whole hop removal does not also remove trub. It is a simple, elegant design, with very few moving parts, so once optimised, there is very little to be maintained or serviced[7].

The whirlpool is a circular vessel, usually of stainless steel construction, into which wort is introduced tangentially. This tangential inlet supplies the centrifugal force that accelerates the trub particles towards the outside wall of the vessel, and then downwards and inwards to the centre of the vessel The transfer rate of the wort from kettle to whirlpool is very high, up to 4,000 hl/hr, but care is taken to prevent shear forces that could break up the coagulated trub particles. The design of the tangential

entry port is therefore important in keeping the velocity low but with a high volume flow rate.

The trub will eventually form a cone at the centre of the vessel. After a suitable circulation period, say 20 - 30 minutes, the clear wort is run off from above the trub cone through outlets at various heights on the vessel side.

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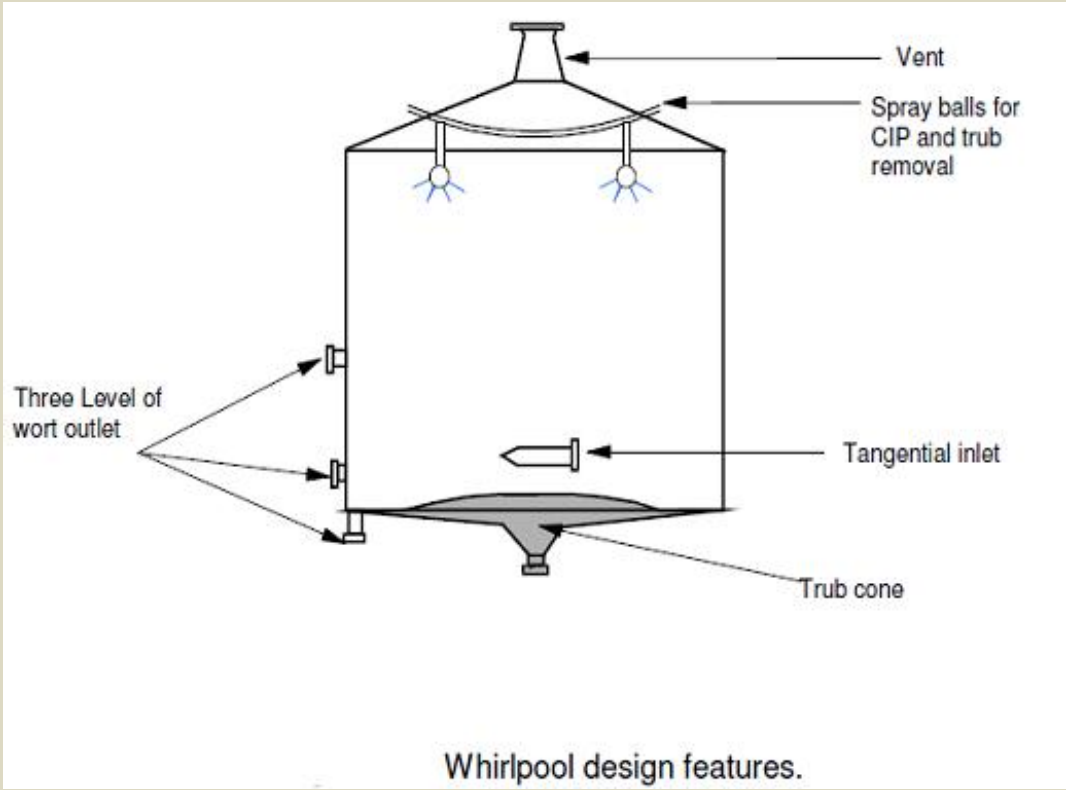
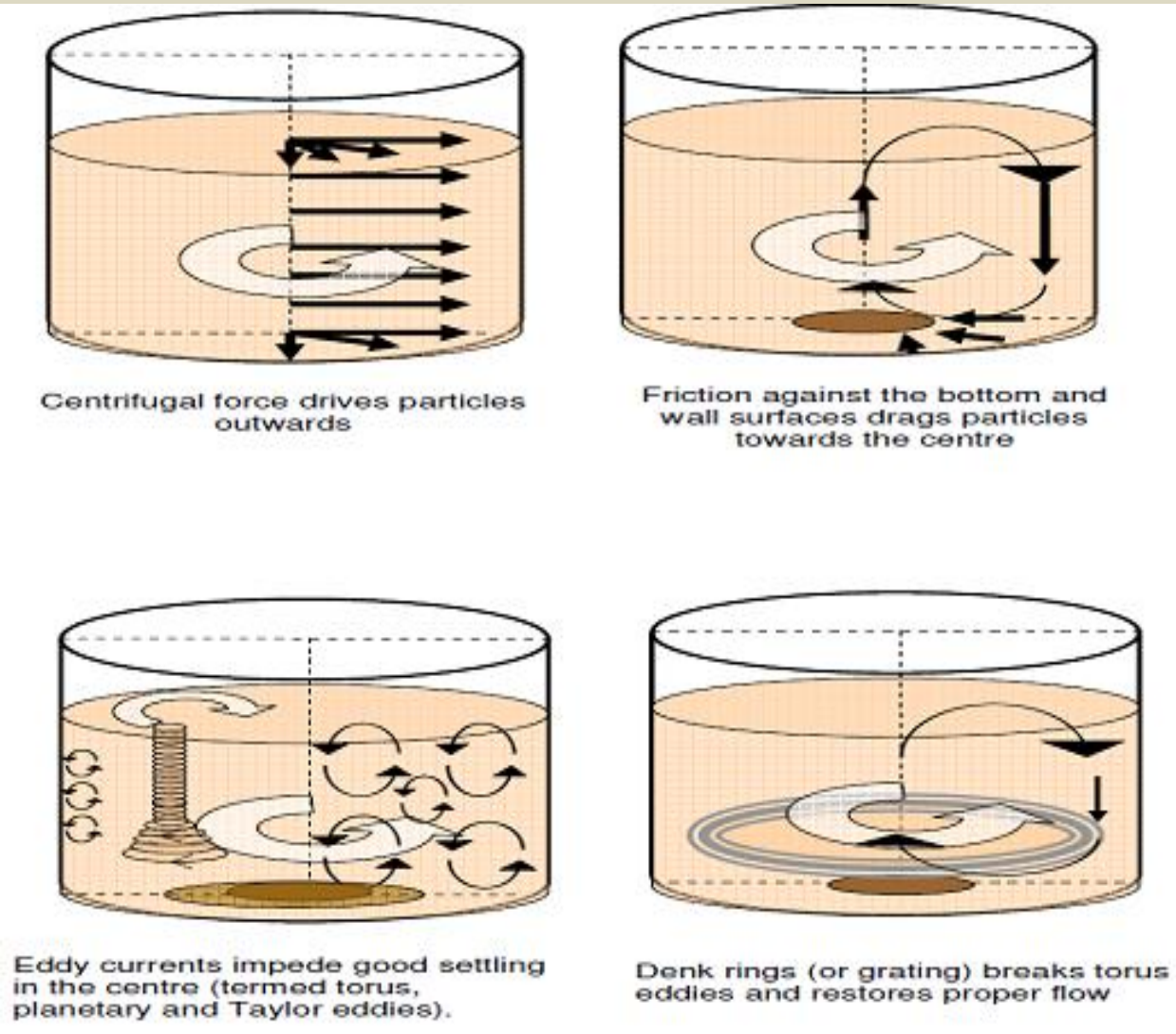


Whirlpool design features.

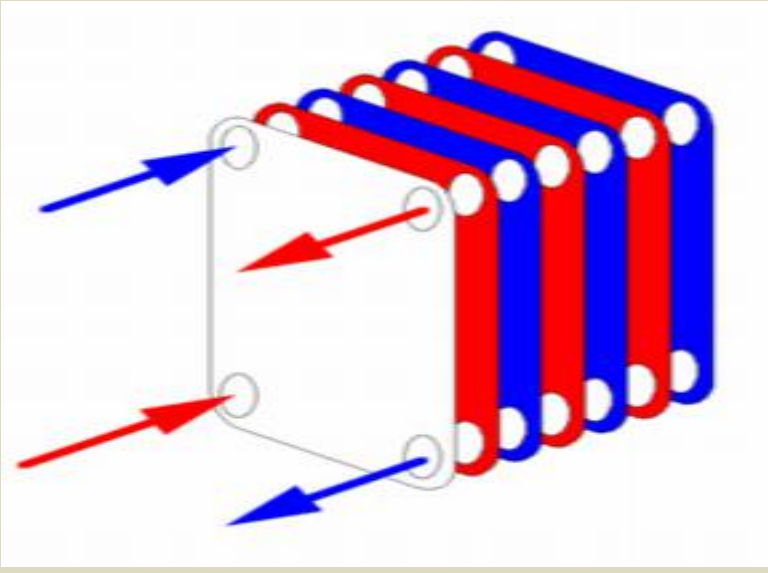


(Whirlpool design features)

WORT COOLING

Wort needs to be cooled to 12^0C - 16^oC for fermentation. This is the optimum temperature for the yeast to function and for the required flavour development . Most breweries now use plate heat exchangers to cool the wort.[10]

PLATE HEAT EXCHANGERS



(PLATE HEAT EXCHANGERS SHOWING DIRECTION OF WORT FLOW)

Most breweries cool their wort through enclosed stainless steel tubular or plate heat exchanges. The heat exchangers are designed to operate with counter current flow for optimum heat transfer with the principal cooling media being brewing water. It is usual to collect approximately an equal volume of water, between 90^oC - 95^oC, to the volume of wort cooled. The usual wort exit temperature from a normal water stage of the chillier is between 12^oC - 16^oC (depending on the incoming water temperature). If lower collection temperatures are required, then the water used in the cooling can be pre-cooled to around 4^oC or a glycol cooling section can be added to the wort cooler.

ADDING DILUTION WATER

The wort coming from the whirlpool will probably be at a higher strength than required for the fermentation. Water is therefore added to dilute the wort to the correct specific gravity. There are alternatives for how and where this dilution water can be added:

It can be added in-line whilst wort flows to the hot side of the wort heat exchanger or the cold side.

It can be added batch wise to the fermenter before the wort is added or after the wort has been added.
If dilution water is added before cooling, then:

Sterility is easy to guarantee.
There is good mixing through the heat exchanger.
There is easy control on a feedback loop.
The energy from the hot water is totally recovered.

WORT AERATION

Optimum aeration of the wort is critical for uniform fermentation process. Aeration can be done either through the injection of sterile air or through the use of oxygen. and the addition point can be on either the hot or the cold side of the wort heat exchanger.It is important to provide oxygen in the wort so that yeast can replicate in the early stages of fermentation and thus ensure an adequate fermentation. If there is not enough DO in the wort, then there will be insufficient yeast replication and this will lead to an inadequate fermentation.

It is normal to add the oxygen to the cold wort, since it rapidly reacts with hot wort to produce darker colours and encourage oxidation.The level of oxygen required depends on the yeast strain, and is influenced by the wort gravity - high gravity wort requires a higher oxygen level. The amount of DO required is usually in the range of 7 - 18 mg/l oxygen.

YEAST PROPAGATION:

Yeast is a facultative anaerobe which is just a fancy way of saying that it can survive and grow in the presence (aerobic) or absence (anaerobic) of oxygen.The presence of oxygen determines the metabolic fate of the cell. In terms of the yeast cell, its survival, growth and metabolism is optimal in the presence of oxygen. In this case, yeast will rapidly grow to high densities and will convert sugar (glucose) to carbon dioxide and water. Under anaerobic conditions, yeast grows much more slowly and to lower densities and glucose is incompletely metabolized to ethanol and carbon dioxide. It is important to realize that optimal yeast growth is distinct from fermentation. Therefore, the conditions and methodologies used for propagating and maintaining yeast need not be identical to those

used for fermenting wort. The purpose of a yeast starter is not to produce an enjoyable fermented beverage but rather to produce a sufficient quantity of yeast for subsequent fermentation. Propagation conditions should be such that a maximal amount of yeast is produced which provides optimal fermentation performance once pitched.

FERMENTATION PROCESS

Fermentation is the all important step of brewing. It involves pitching the yeast to the wort and letting the yeast do all the work under ideal conditions. The wort is allowed to cool and is transferred to the fermenters.

The main biochemical reaction which converts sugar in wort to alcohol to produce beer which represents as equation:

SUGAR ⟶ ETHYL ALCOHOL+ CO_2+ HEAT (Exothermic reaction)

The main factors that affect the fermentation rate and influence beer quality:-
The amount of yeast used to inoculate or pitch the fermentation.
Yeast cell viability and yeast quantity.
The proper level of dissolved oxygen in wort at pitching.
Wort soluble nitrogen concentration.
Wort fermentable carbohydrate concentration and
Temperature.

Lagering: or Maturation and Cold Storage:

After fermentation beer is transferred to storage tanks and held approximately at about -1^{o}C to 2^{o}C for longer time. This process is called Lagering.

Beer which has completed primary fermentation is set to be ‘green', it contains little CO_2 and its taste and aroma are given to those of mature beer. The maturation process (sometimes called ‘conditioning', ‘lagering', or ‘run storage') is carried out in closed containers and up to recent times was process occupying weeks and in some cases, months.

Traditionally, maturation involves a secondary fermentation and is brought about by the action of the small charge is yeast remaining in the beer often racking from the fermentation vessels. The yeast attacks either:

Fermentable carbohydrate which have escaped degradation in the primary ferment of priming sugars.

Small qualities of fermentable carbohydrate added in the form of ‘Priming Sugars'. Added wort and added actively fermenting wort, a process called ‘Krausening'.

FILTRATATION

Matured beer will still have particles in suspension, mainly yeast but also smaller particles, unless it has been fined. There are three types of filtration:-

The purpose of ‘rough' filtration is to remove all the particles that would make the beer cloudy.
The purpose of ‘polishing' filtration is to remove all yeast and bacteria so that the beer is sterile.
The purpose of ‘stabilising' filtration is to prevent non-biological haze formation in package, that is the haze formed by the protein/tannin particles

Objective:

To remove solid particles from the beer stream to produce a bright stable beer free of yeast and (most) bacteria. Primary filtration can remove particles in 1 micron range. Fine or sterile filtration removes particles above 0.45 micron.

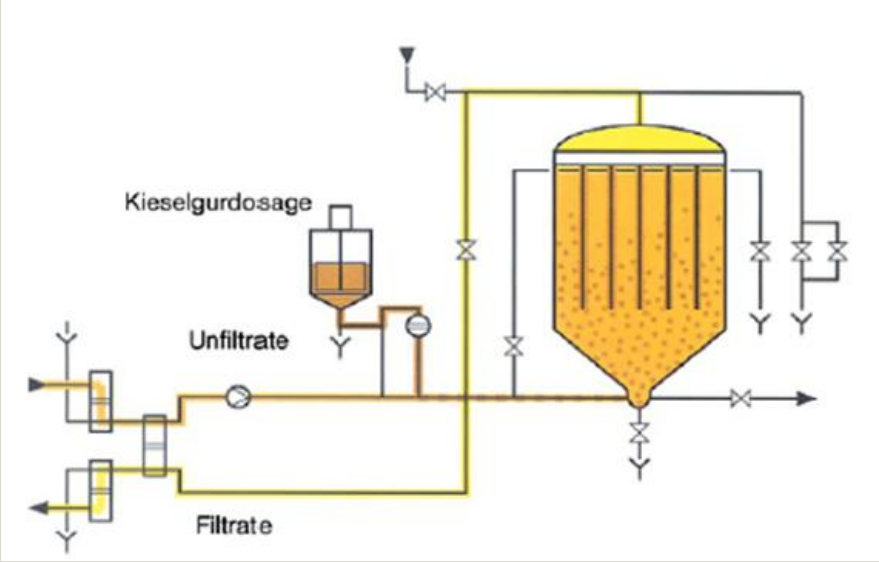
Types of filter:

Plate & Frame
Candle
Horizontal leaf - can also be used for PVPP
Sterile filters - sheet or cartridge filters.

(PHOTO OF DELLA TOFFOLA FILTER USED FOR FILTRATION)

Filtration Process:

Bed porosity is maintained by the continual dosing of a filter aid like kieselguhr. The bed has to be build up carefully to ensure adequate porosity[11]. The first precoat will be a coarse powder followed by a second precoat usually the same powder as the bottom feed. As soon as filtration commences powder is dosed into the beer flow to continue to build the bed up and keep it porous. The pressure will rise as the filter blinds so operators need to increase the dosing rate if the pressures starts to rise more than normal. Similarly if the pressure build more slowly, more powder is being used than necessary and the filter run will be shorter than expected. The filter will need washing off once the void area is full of powder. It is possible to dose in silica hydro gel stabiliser at this stage. Yeast and haze particles will be trapped but most bacteria will pass through. Filter runs can be prolonged by ensuring the beer is bright to the eye by using finings or centrifugation before the filter.



(Filtration Process)

BBT(Bright beer tank)

Tanks must be clean and sterile. They should have smooth internal surfaces. Tanks should be counter pressured with inert CO2 or nitrogen. Beer flow to the tanks should be at the correct flow rate without sharp bends which can promote fobbing. Inlet rate should be smooth to avoid gas break out in the bottom of the tank. Correct high CO2 and DO levels by purging with oxygen free nitrogen through a sinter at the base of the vessel. Degassing to correct dissolved gas levels will cause some fobbing in the tank. Fob will remain on the vessel walls during emptying and could dry and cause a haze in the next filling if vessel rinsing is ineffective. Sometimes dried bits of collapsed fob fall back into the parent beer and cause similar rejects.

Tanks should be lagged or cooled using jackets. Usually insulation is sufficient but temperature will rise so a maximum residence period of 2 -3 days will be stipulated. Beer warming up will tend to lose dissolved CO2 and N2, becomes more prone to micro infection and packaging warm beer leads to fobbing which may allow oxygen pick up and difficulties attaining the required fill levels. It is essential to recheck haze and dissolved gases, CO2, N2 and O2 every shift if the tank is still waiting to be packaged.

Objectives of Bright Beer Tank

Carbonate the beer
Childproof & stabilize the beer
Modify & improve beer flavor balance
Clarify the beer
Blend & standardize the product

Bright Beer Tank

RESULT AND DISCUSSION

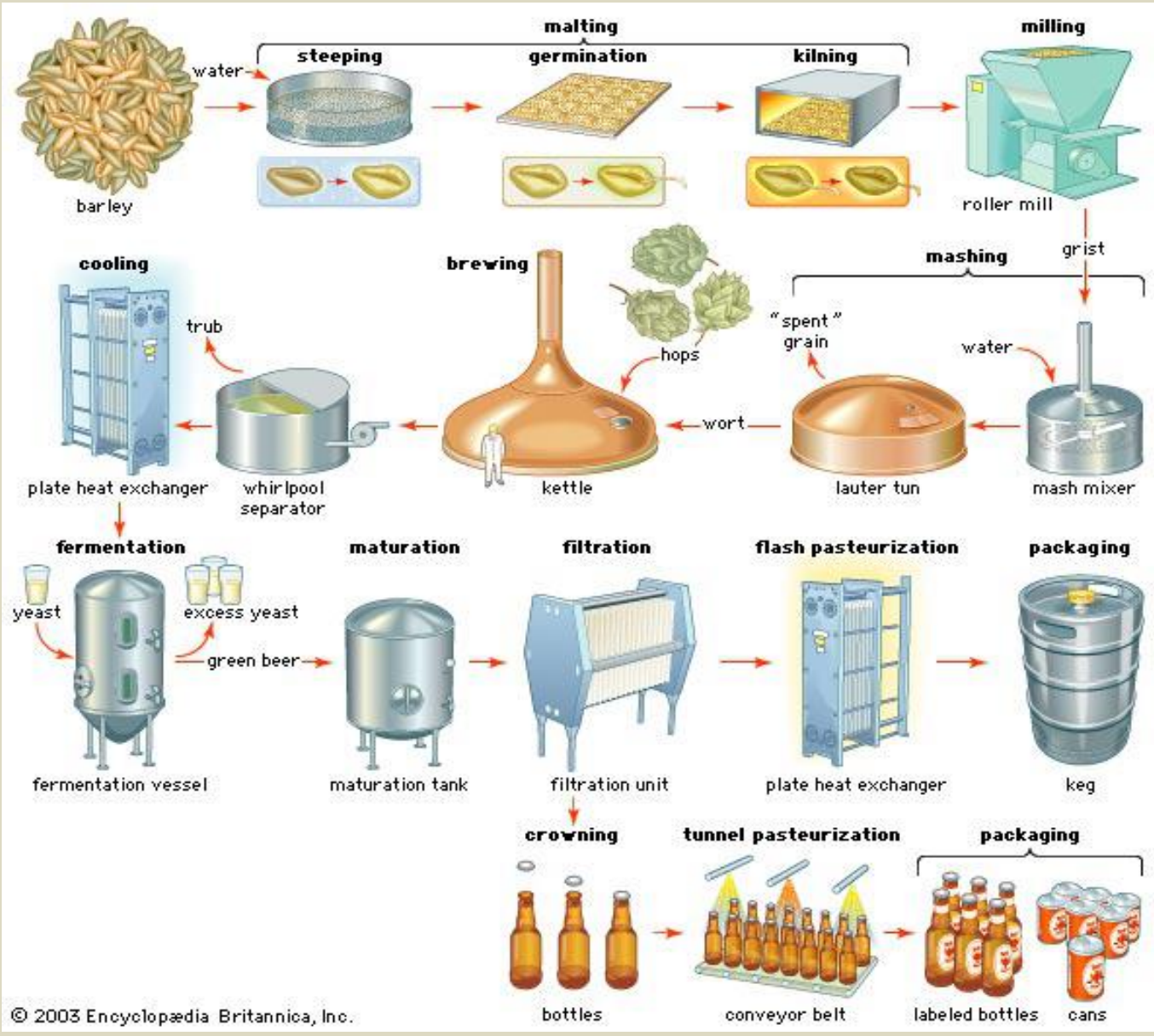
For Natural enzyme activation in barley, Malting process is done.

For high recovery Exogenous enzymes are used that provides that helps maintaining the body of the beer, that improves quality of beer. Because the natural enzymes in Malt is not capable to give good recovery and due to use of Adjuncts used in brewery,

adjuncts have less enzyme activity. Proper boiling of wort can develop good colour and flavour to the beer

Yeast recovery is done after the fermentation process for recovery of yeast after each generation for reuse of yeast up to sixth generation , so that yeast pitching cost can be reduced . and It is use to six generation only because after sixth generation yeast is not that much viable that it can produce required amount of alcohol in beer processing. If we will use that yeast it will affect quality of beer like change in flavour.

SUMMARY



(The figure above is presenting the brief summary of the total process)

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REDUCTION OF OFF -FLAVOUR(DIACETYL))FROM BEER BY YEAST MANAGMENT SYSTEM

1. Introduction

In the theatre of brewing, yeast is viewed as a “supporting actor”. But unlike the more commercial leading actors such as water, malt and hops , yeast appears not only once but in many successive productions. Further, with fermentation being the longest chunk of the process, yeast is on stage longer. Finally and critically, as an agent of change, yeast facilitates the most dramatic event in the process of transformation of wort to beer.

Yeast is a single celled microorganism that reproduces by budding. They are biologically classified as fungi and responsible for converting fermentable sugars into alcohol and other by products. There are literally hundreds of varieties and strains of yeast. In the past, there were two types of beer yeast: ale yeast (the "top-fermenting" type, *Saccharomyces cerevisiae*) and lager yeast (the "bottom-fermenting" type, *Saccharomyces uvarum*, formerly known as *Saccharomyces carlsbergensis*). *Saccharomyces* is one of about 40 ascosporo genous yeasts (Barnett et al., 1992).

Currently 10 species of *Saccharomyces* are recognized: *S. bayanus, S. castelli, S. cerevisisae, S. dairensis, S. exiguous, S. kluyveri, S. paradoxus, S. pastorianus, S. servazii* and *S. unisporus.* Two types of *Saccharomyces* yeasts are involved in beer fermentation, top fermenting (or ale) yeasts and bottom fermenting (or lager) yeasts. Since the last century, ale yeasts have been classified as *S. cerevisiae*, whereas lager yeasts have been known under several names such as *S. carlsbergensis, S. uvarum* and *S. cerevisiae.* Both yeast species belong to the closely related *Saccharomyces*, *sensu stricto* species (Jakobson et al., 1980). In breweries, *S. pastorianus* is well known as a beer spoiling wild yeast. From a brewer’s point of view, there are several differences between ale and lager yeast with vital consequences for the brewing process.

Thus, it is still sensible to maintain these two kinds of yeasts as separate types. Since a lot of these top fermenting yeasts are employed in cylindro-conical tanks now a day and are also cropped from the cone like bottom fermenting yeast, the segregation becomes blurred when using modern fermentation technology. Most differences between these two types of yeast are in their fermentative ability, rate of sugar utilization, tolerance

to temperature, flocculation characteristics and profile of volatiles. Top fermentation produces beers that are more fruity and estery, whereas lager fermentation provides beers with a purer and partly sulfurous aroma (Walker et al., 2004). Morphological differences between ale and lager yeasts are small. Shape and size of the cells cannot be used for differentiation. Under the microscope both types of yeast can only be distinguished by their budding characteristics. Lager yeasts separate very soon after budding, and mother and daughter cells then bud again. This results in single or pairs of cells in the microscope image. The cells of top fermenting yeasts still stick together when they bud again. As a result of multilateral budding, a small complex cell cluster is formed. Side branches break away and build new clusters. A differentiation can be made under the microscope. At the end of fermentation the disintegration of the clusters leads to complications in differentiation. The most important differences for the brewer are visible in fermentation management. The temperature profile differs significantly. Lager yeast naturally ferments between 7 and 15°C. Ale yeast is employed at higher temperatures between 18 and 25°C. This leads to a 2 - fold greater yeast crop for ale yeast. Although lager yeast prefers higher temperatures (optimum growing temperature around 28°C), it is able to maintain the metabolism under much colder conditions.

This characteristic makes lager yeast suitable for cold fermentation with its typical flavour profile. Ale and lager yeast can also be separated according to temperature requirements. Only ale yeast still grows at cultivation temperatures over 34°C. Despite the widespread use of top and bottom fermenting yeast of the genus *Saccharomyces,* other yeasts are involved in the brewing of some specialty beers. Most of these beers are fermented spontaneously; at later stages of fermentation yeasts such as *Dekkera* spp. (perfect form of *Brettanomyces* spp.) are involved (Postam et al., 1989). *B. bruxellensis* and *B. lambicus* are the most common species. These *Brettanomyces* yeasts are weak fermenting, but they are able to utilize dextrins. A slow growth during conditioning is characteristic. In most of these special beers the *Brettanomyces* yeast is socialized with brewing yeast or acid - producing bacteria. This results in totally different flavor profiles because of high amounts of ethyl acetate and acetic or lactic acid.

1.1 Yeast Life Cycle

The life cycle of yeast is activated from dormancy when it is added (pitched) to the wort. Yeast growth follows four phases, which are somewhat arbitrary because all of the phases may overlap in time:

1)Lag period

2)Growth phase

3)Fermentation phase

4)Sedimentation phase

1.1.1 Lag Phase

Reproduction is the first great priority upon pitching, and the yeast will not do anything else until food reserves are built up. This stage is marked by a drop in pH because of the utilization of phosphate and a reduction in oxygen. Glycogen, an intracellular carbohydrate reserve, is essential as an energy source for cell activity since wort sugars are not assimilated early in the lag phase. Stored glycogen is broken down into glucose, which is utilized by the yeast cell for reproduction the cell's first concern. Low glycogen levels produce abnormal levels of vicinal diketones (especially diacetyl) and result in longer fermentations.

1.1.2 Growth Phase

The growth phase often referred to as the respiration phase, follows the lag phase once sufficient reserves are built up within the yeast. This phase is evident from the covering of foam on the wort surface due to the liberated carbon dioxide. In this phase, the yeast cells use the oxygen in the wort to oxidize a variety of acid compounds, resulting in a significant drop in pH. In this connection, some yeast strains will result in a much greater fall in pH than others within the same fermenting wort.

1.1.3 Fermentation Phase

The fermentation phase quickly follows the growth phase when the oxygen supply has been depleted. Fermentation is an anaerobic process. In fact, any remaining oxygen in the wort is "scrubbed," i.e. stripped out of solution by the carbon dioxide bubbles produced by the yeast. This phase is characterized by reduction of wort gravity and the production of carbon dioxide, ethanol, and beer flavors. During this time period, yeast is mostly in suspension, allowing itself dispersal and maximum contact with the beer wort to quickly convert fermentables. Most beer yeasts will remain in suspension from 3 to 7 days, after which flocculation and sedimentation will commence.

1.1.4 Sedimentation Phase

The sedimentation phase is the process through which yeast flocculates and settles to the bottom of the fermenter following fermentation. The yeast begins to undergo a process that will preserve its life as it readies itself for dormancy, by producing a substance called glycogen. Glycogen is necessary for cell maintenance during dormancy and, as mentioned, is an energy source during the lag phase of fermentation.

1.2 Flocculation

The flocculation characteristics of yeast are of great importance. The term "flocculation" refers to the tendency to form clumps of yeast called flocs. The flocs (yeast cells) descend to the bottom in the case of bottom-fermenting yeasts or rise with carbon dioxide bubbles to the surface in the case of top-fermenting yeasts. The flocculation characteristics need to be matched to the type of fermentation vessel used a strongly cropping strain will be ideal for skimming from an open fermenter but unsuitable for a cylindro-conical fermenter.

A phenomenon shown by brewing yeast is very important for the fermentation performance and following beer production steps flocculation. The ability of yeast to build cell clusters and settle to the bottom of the tank can be positive or negative for the process. If the yeast settles down too early, not enough yeast cells remain active to reduce diacetyl. If the flocculation is too weak and too many yeast cells stay in suspension, filtration and haze problems may occur. At present, the exact mechanism behind flocculation remains uncertain. Several theories have been proposed and some factors influencing the process have been determined (e.g. zinc and calcium). The cell wall seems to play a major role in flocculation; its composition is discussed below. Some of the proteins found in the cell wall are coded by genes (Flo1p, Flo5p, Flo9p, and Flo10p) and an influence of these genes in flocculation has been shown (Flikweert et al., 1996). Carbohydrate binding domains were also found on the cell wall. The domains are cell wall - linked proteins, which are called zymolectins. These zymolectins seem to bind to carbohydrate receptors of adjacent cells (Verstrepen et al., 2003). Based on these results, the lectin hypothesis for flocculation has been widely accepted. The zymolectins bind to the terminal non-reducing mannose, glucose and fructose residues of saccharides of the

respective receptors. Calcium is suspected of being an essential part of the sugar binding center, keeping up its structure. The zymolectins are first synthesized at the end of the exponential growth, activated by calcium (Dufour et al., 2003). Although the mechanism of flocculation is becoming clearer, strategies for directly influencing flocculation are few and of empirical character.

1.3 Attenuation

Attenuation refers to the percentage of sugars converted to alcohol and carbon dioxide, as measured by specific gravity. Most yeasts ferment the sugars glucose, sucrose, maltose, and fructose. To achieve efficient conversion of sugars to ethanol (good attenuation) requires the yeast to be capable of completely utilizing the maltose and maltotriose. Brewing yeasts vary significantly in the rate and extent to which they use these sugars. Lager strains are often better at utilizing maltotriose than there are counterparts. The degree of attenuation obtainable exerts a great influence on the organoleptic properties of the resultant beer and, consequently, is one of the determinant factors in the process of yeast selection.

1.4FlavourComponent

The selection of the yeast strain itself is perhaps one of the most important contributors to beer flavor. Different strains will vary markedly in the byproducts they produce: esters, higher alcohols, fatty acids, hydrogen sulfide, and dimethyl sulfide. The yeast strain must also be capable of reproducible flavor production.

1.5 The Propagation of yeast

The objective of propagation is to produce large quantities of yeast with known characteristics for the primary role of fermentation, in as short a time as possible. Most brewers use a simple batch system of propagation, starting with a few milliliters of stock culture and scaling up until there is enough yeast to pitch a commercial brew. Scale-up introduces actively growing cells to a fresh supply of nutrients in order to produce a crop of yeast in the optimum physiological state. It was a batch growth system with 5 to 10× multiplication per stage, usually with simple pulsed aeration and without agitation. These

systems had a lengthy growth period, reaching typically 100 × 106 cells/ml, and often required three stages to pitch production wort volumes, with a total cycle time of up to two weeks 7-33. Recent developments have concentrated on rapid growth and production of high viability yeast suitable for pitching production wort volumes, with representative beer quality and good yeast growth during production, Flow profiled oxygen sparging is used, linked with controlled intensive agitation to produce effective and extremely rapid yeast growth, while controlling foam generation.

1.6 Yeast Morphology and Chemical Composition

Brewing yeast is mostly round or ovoid. Partly elliptical or cylindrical cells can also be observed. They are of very regular shape and size. Cells measure 5–10μm in diameter, 3–1μm in width, and 4–14μm in length. The values are imprecise because the size of the cell depends greatly on the physiological state (e.g. prior to budding, yeast cells can reach 3 times the volume of regular cells). Like other living cells, yeast is composed mainly of water. The non-aqueous cell material is polymeric. Carbohydrates, proteins and nucleic acids made up of the six elements carbon, hydrogen, oxygen, nitrogen, phosphorous and sulfur form the bulk of the material with a large range of low molecular weight organic compounds and inorganic ions making up the remainder.

2. REVIEW OF LITERATURE

2.1 DIACETYL

The compound responsible for buttery or butterscotch flavors that sometimes arise in beer can be controlled if you understand the mechanisms that contribute to its production. This review of the basic processes behind diacetyl formation and reduction will help to understand how to keep the diacetyl level in the beer at or below the acceptance threshold for the style. Diacetyl and 2,3-pentanedione are important contributors to beer flavor and aroma. Organic chemists classify both as ketones, and diacetyl is usually called 2,3-butanedione in the literature (John Wiley et al., 1984). Sometimes these two ketones are grouped and reported as the vicinal diketone (VDK) content of beer (G.J Fix et al., 1989). Brewers' awareness and acceptance of

both diacetyl and 2,3-pentanedione have changed dramatically over the past four to five decades. A

1952 report, for example, stated that the average diacetyl level of American commercial beer was 0.33 mg/l, more than three times the flavor threshold of 0.10 mg/l (O.B. West et al., 1952). Today the average is near 0.05 mg/l. Some notable exceptions exist. Some stouts can have levels as high as 0.60 mg/l, and a few British pale ales have diacetyl levels near 0.30 mg/l. Diacetyl levels in beers brewed by microbreweries and brewpubs tend to vary considerably, ranging from 0.03 mg/l to more than 1.0 mg/l in cases I have investigated. The presence of diacetyl is usually indicated by a buttery or butterscotch tone. In fresh beer the flavor can be confused with that of caramel malts. Given time it is easy to distinguish the two diacetyl tends to be unstable in most beers and can take on raunchy notes. The flavoring imparted by caramel malts, on the other hand, tends to be stable. The flavor threshold of 2,3-pentanedione is near 1.0 mg/l; it usually imparts flavors that recall honey. This compound can be found well above threshold levels in some Belgian ale, where it is considered a natural flavor constituent for this style. It occurs less often in other beer styles and is regarded as a defect.

2.1.1 Diacetyl Formation

The study of diacetyl and beer began with Pasteur's fundamental work in the 1870s (L. Pasteur et al., 1876). Using microscopy, Pasteur found that what we know today as lactic acid bacteria were responsible for many off-flavors in beer. The term sarcina sickness is used to describe this effect. Apparently, the involvement of diacetyl in sarcina sickness was discovered early, but it was not until 1939 that Shimwell linked this compound with the taste and smell of butter (J.L. Shimwell et al., 1939). Earlier studies got the organic chemistry right but were wide of the mark in terms of flavor chemistry (A. Zimmermann et al., 1904). Even today it is estimated that 20% of beer drinkers do not detect the presence of diacetyl even at rather high concentrations (M.C. Meilgaard et al., 1991). During the early period, the only known mechanism of diacetyl formation was bacterial infection caused by unsanitary conditions. Practical brewers believed that some other factor must be involved, because buttery tones occasionally showed up in beer brewed in impeccably clean environments. Major breakthroughs occurred during the 1950s and early 1960s. J. Owades developed an effective technique for measuring diacetyl and used this method to study the fate of the compound in brewing (J.L. Owades et al., 1959). This work pointed to culture yeast as a major player in both the production and the reduction of diacetyl which shown in figure1. Inoue and his colleagues at the Kirin Research Laboratory in Japan

also made a major contribution by identifying acetolactic acid as the precursor to diacetyl (T. Inoue et al., 1973). This work was followed by a large number of papers in which various facets of diacetyl formation and reduction were studied. Wainwright's excellent 1973 review article contains 149 references (T. Wainwright et al., 1973). Although a number of factors affect diacetyl formation in beer, the basic middle pathway shown in Figure 1 is the one used for each. The dominant carbon flows down the left most branches, leading to ethanol production. Most beers contain between 30,000 and 50,000 mg/l of ethanol, so a significant amount of pyruvate is processed in this manner. Because the flavor threshold for diacetyl is 0.10 mg/l, a slight diversion of the carbon flow to the middle pathway can profoundly affect the flavor of finished beers. Note that this pathway also competes with yeast assimilation and the utilization of the amino acid valine. The practical significance of this is discussed in the section "Wort and Proteins" below. A similar pathway is involved in the production of 2,3-pentanedione, except that different compounds are involved. The precursor is acetohydroxybutyrate, and the competing amino acid is leucine. A number of factors lead to diacetyl formation, but only one reliable method can reduce diacetyl levels, enzymatical reduction by yeast. Acetone, the intermediate product, has a rather unpleasant, musty taste, but because it has a flavor threshold of 3.0 mg/l its effect is not nearly as damaging to beer flavors as an equivalent amount of diacetyl. The final product, butanediol, is neutral as far as beer flavor is concerned.

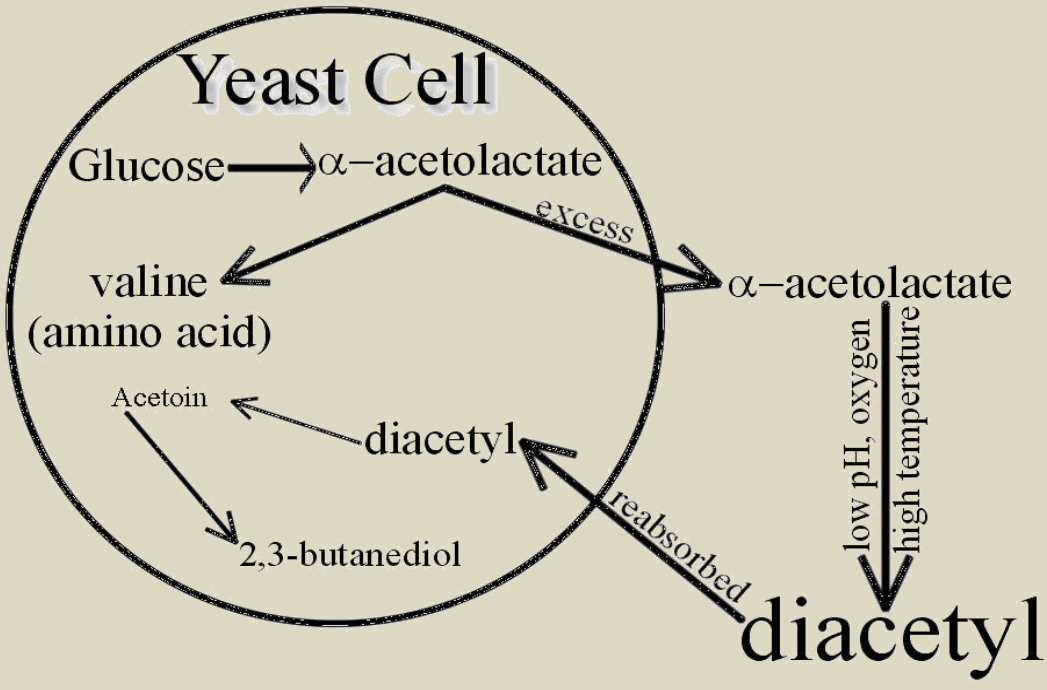

Figure 1: Pathway of Diacetyl

2.1.2 The Effect of Yeast

As noted above, brewer's yeast contains enzymes for both producing and reducing diacetyl. Various yeast strains differ dramatically in this regard. Three lager strains were tested. The strains W-206 and W-34/70 are regarded as excellent reducers. The third strain, W-308, is less reliable (G.J. Fix et al., 1993). Sterile wort was fermented in each case at 10 degrees C (50 degrees F). Diacetyl formation in three yeast strains. Highly flocculent yeast usually behave much like W-308 and can leave perceptible levels of diacetyl in beer, which is one reason why most commercial yeast strains are powdery and fully flocculate only after chilling. Yeast behavior for a given strain can also vary with reuse. These mutants are strong producers of diacetyl and have lost their ability to reduce diacetyl. The presence of Gram positive bacteria also cannot be ruled out as contributors to diacetyl formation. Yeast that is free of those two defects usually displays better performance with reuse. Diacetyl production and reduction are strongly influenced by temperature, and the rates for both increase as temperature increases. Thus, an ale fermented at 20 degrees C (68 degrees F) typically has a higher diacetyl peak than, say, a lager fermented at 10 degrees C (50 degrees F).

The rate of diacetyl reduction, however, is much higher in the ale than in the lager, which is why most lager brewers prefer to get diacetyl levels below 0.10-0.15 mg/l at the end of

9

the main fermentation. Some additional reduction occurs in cold storage, but at a very slow rate. For this reason, some brewers raise the temperature of a cold-fermented beer to 20 degrees C (68 degrees F) for a brief period following the end of the main fermentation, a practice that is usually called diacetyl rest. One alternative is the so called Narziss fermentation. In this procedure the first two thirds of the fermentation is done at 8-10 degrees C (46-50 degrees F). During the final third of fermentation, the temperature is allowed to increase to 20 degrees C (68 degrees F), after which the beer is transferred to cold storage. Another alternative is to add freshly fermenting wort (kraeusen) to diacetyl-laden beer in cold storage.

2.1.3 Bacterial Influences

The bacteria that can directly promote diacetyl production consist of Gram positive cocci (Pediococci) and select strains of Gram positive rods (Lactobacillus). The effect of using these

bacteria is easy to identify both bacteria also produce lactic acid, and the net effect is a rather raunchy butter tone with an unmistakable acid after taste. In both amateur and commercial brewing of yesteryear, infections from unsanitary equipment were not uncommon. Today, with the availability of highly effective sanitizers, infections tend to occur only in sloppy and poorly managed operations. It has been my experience that in modern operations, infections, when they occur, happen through pitching yeast. It is unnecessary for fully developed pitching yeast to be sterile. Practical experience has shown that as long as bacteria relevant to beer are kept below the level of 1-10 cells per

10 million yeast cells, the finished beer will remain unaffected. The situation is dramatically different in the initial stages of yeast propagation. Here, sterile conditions are needed, as is pure culture yeast. This is particularly true when propagating yeast from slants, but it also applies to starting up semidormant liquid yeast. It is relatively easy to measure bacterial levels, particularly for lactic acid bacteria (G.J. Fix et al., 1992), and hence there is little justification for leaving these matters to chance.

Care should be taken when using yeast that has been held in bulk storage. If culture yeast goes dormant, they tend to excrete amino acids (M.J. Lewis et al., 1963), which bacteria can use as a source of nitrogen. Low levels of bacteria can grow to unacceptably high levels by this means. The safest course is to store the yeast under a sterile wort cover, at a temperature as close to 0 degrees C (32 degrees F) as possible, and for as brief a period as possible.

2.1.4 Wort and Proteins

The basic diacetyl formation pathway shows clearly the major role that amino acids play. Worts deficient in valine tend to lead to elevated diacetyl levels. As long as a sufficient amount of valine is present, there will be a net reduction of diacetyl after it reaches a peak level. If the valine content is depleted prematurely, however, net diacetyl production will resume, leading to what has been called the "second diacetyl peak." Some of this extra diacetyl ultimately will be reduced, but invariably the finished beer will contain a higher diacetyl content than it would had the wort contained adequate valine. The same remarks apply to 2,3- pentanedione and leucine. Both leucine and valine are regarded as critical amino acids because yeast usually cannot metabolize adequate replacements from other nitrogen sources if leucine and valine are missing. If sufficient amounts of proteins are to be available in the fermentation, they must come from wort; this

in turn depends on the amount of malted grains used. High quality malt used with reasonable mashing systems will yield wort that is rich in all the relevant amino acids.

Brewers use a single number to characterize the size of their wort's amino acid pool namely, its free amino nitrogen (FAN) level. This number is to wort amino acids what specific gravity (or percent extract) is to wort carbohydrates. High FAN levels mean adequate leucine and valine pools. Conversely, low FAN levels result in inadequate levels of leucine and valine. An all malt wort at 12 degrees P (SG =1.048), for example, typically has a FAN level in the range of 300-325 mg/l. This level is considered ideal. A 10 degrees P (SG =1.040) all-malt wort, on the other hand, has a FAN level near 250-270 mg/l generally regarded as adequate. However, if a third of the malt were replaced with an equivalent amount of unmalted cereal grains or sugar (or both), then the FAN level would fall to 165-180 mg/l, which is generally regarded as inadequate. Apart from economic considerations, this is one of the main reasons why large industrial brewers using high adjunct levels tend to favor high gravity brewing. If the 10 degrees P wort with 33% adjuncts were concentrated to, say, 14 degrees P wort, then the FAN level would increase to 230-250 mg/l. This is a major improvement over the dilute adjunct wort.

Reports have been published of various deficiencies in certain brands of malt extracts (J. Paik et al., 1991). It appears that, in general, the protein levels of wort produced from extracts are lower than those obtained from grain worts. These and related issues are discussed in an excellent article by Lodahl (M. Lodahl et al., 1993). Although it is important that our wort have a sufficient amino acid pool, it is also important that our culture yeast be able to use this nitrogen source. The situation for leucine and valine is particularly critical because their uptake by brewing yeast is rather slow in general and incomplete in the case of dysfunctional yeast. Respiratory deficient mutants represent an extreme example of this. In all such cases the inevitable result is elevated diacetyl levels. One cannot overstate the importance of using appropriate media in propagating yeast. The instruction sheet that accompanies yeast slants sold by Siebel (Chicago, Illinois) states that propagation should be done with wort that is "of a similar original gravity and composition to the major production brand." Many brewers use dilute, unhopped wort having a specific gravity of 1.020. Although opinions may differ about these two options, totally artificial media should be avoided. I recently did some tests with what is often called "baker's media." It consisted of a dextrose sucrose solution enriched with mineral nutrients (G. Reed et al., 1991). Yeast propagated on this media showed excellent cell growth rates during propagation. Their

performance in the main fermentation, however, was unacceptable. A major defect was an extremely slow and incomplete valine uptake. .

2.2 YEAST MANAGEMENT

2.2.1 Nutrient Requirements and Intake

Wort represents a rich source of nutrients for yeast. It contains fermentable sugars, assimilable nitrogen, minerals and vitamins, as well as minor growth factors. The important oxygen must be supplied by aeration. During yeast propagation and fermentation, the concentration of various nutrients changes. By careful brew house work all malt worts can be created containing all essential nutrients in ample amounts. Only zinc and, rarely, biotin can be critical.

2.2.2 Carbohydrates and Fermentable Sugars

Fermentable sugars are the main carbon source for the yeast. After being pitched, the yeast (in an appropriate physiological condition) immediately injects the monosaccharide. They enter the cell via the membrane by facilitated diffusion (Slaughter et al., 2003). Regular wort contains mainly the following sugars: fructose, glucose,sucrose, maltose, maltotriose and dextrins. Except for the dextrins, all sugars can be utilized for the generation of energy and biosynthesis. The uptake of sucrose proceeds simultaneously. It is cleaved by an invertase in the periplasm, the resulting monosaccharides are introduced into the cell as mentioned above (Menese et al., 2008). Following the monosaccharides, maltose and maltotriose are injested via active transport. Specific energy consuming transport systems import the saccharides into the cytoplasm, where they are split into glucose and metabolized. The initial glucose concentration in the wort plays a key role in the order of the sugar consumption. As long as glucose is still present in the fermentation media, the yeast will take in no other sugar. This is due to a glucose repression. The transcription of enzyme coding genes (e.g. maltase) is repressed (Reitenberger et al., 1997). This phenomenon can be important when employing high amounts of glucose (crystals or liquid) as additional extract. The yeast enzymatic system becomes adapted to the high glucose amounts and reduces or even halts the ensuing maltose intake. Insufficient fermentation results occur because of the inadequate maltose metabolism. Another important carbohydrate source for yeast is glycogen. It serves as a reserve carbohydrate and is generated in the cell during the later anaerobic stages of fermentation. After repitching the yeast to aerated wort, glycogen is utilized for initializing the enzyme activities and yeast

metabolism. It is important to preserve the glycogen content during yeast storage to ensure a fast fermentation start.

2.2.3 Oxygen

Under brewing conditions, oxygen can be considered as a nutrient for yeast. Although yeast goes through long anaerobic phases during fermentation and most of the respiratory pathways are blocked due to the Crabtree effect, yeast needs oxygen for a sufficient yeast growth. Oxygen seems to be the limiting factor for yeast growth during propagation and fermentation. An amount of 7–9mg/l dissolved oxygen is considered sufficient for proper fermentation, but it must be mentioned that the oxygen demand of the yeast is very strain dependent (Klein et al., 1998). The oxygen enters the cell via facilitated diffusion and at the beginning of fermentation the oxygen dissolved in wort is consumed within hours. At the same time, the glycogen reserves are depleted. The yeast uses the oxygen for certain growth maintaining hydroxylations. It is involved in the synthesis of sterols and unsaturated fatty acids. Both components are scarcely represented in wort, but are essential for yeast growth. Therefore, a synthesis by the yeast is imperative. Studies have shown that the addition of these substances to wort can replace aeration (Hammond et al., 1993). The importance of the synthesized components becomes visible if their disposition is determined. Both sterols and unsaturated fatty acids are essential parts of the cell membrane. As mentioned earlier, they are responsible for the fluidity and integrity of membranes. If there is a lack of sterols or unsaturated fatty acids, no functioning cell membrane exists and, for example, no nutrient intake is possible. After the synthesis of sterols they are rapidly esterized and a sterol pool is built. When the oxygen is used up, these sterol pools are employed for yeast growth. They limit the yeast growth. Other influences of oxygen can also be observed. It regulates several genes and influences the building of mitochondria (Tenge et al., 2002).

2.2.4 Nitrogen Sources

In terms of nitrogen sources, wort also provides yeasts with readily utilizable sources of nitrogen (amino acids and low molecular weight peptides) essential for cellular biosyntheses, enzyme and nucleic acid function. They are present in abundance.

S. cerevisiae is non-diazotrophic (cannot fix nitrogen) and non-proteolytic (being unable to utilize proteins as nitrogen sources) (Walker et al., 2000). Without a certain yeast growth,

sufficient fermentation performance is not possible. Therefore, nitrogen sources are essential. For all malt wort (12°P) an amount of 900–1200 mg/l total soluble nitrogen and 200–240mg/l free amino nitrogen are considered adequate. It should be taken into account that the required amount is also strain dependent some strains show perfect fermentation results despite a much lower nitrogen supply.

2.2.5 Minerals and Trace Elements

Metals are very important for yeast cell physiology. They are needed to maintain the cell's structural integrity, flocculation, gene expression, cell division, nutrient intake, enzyme activity and more. The most important metals that influence yeast fermentation are potassium, magnesium, calcium, manganese, iron, copper, and Potassium is the most abundant cation in wort. It is the main electrolyte essential for osmoregulation, and additionally acts as cofactor for enzymes involved in oxidative phosphorylation, protein and carbohydrate metabolism. Magnesium is absolutely essential for yeast growth. It acts as an essential cofactor for over 300 enzymes (e.g. synthesis of DNA, glycolytic enzymes). Magnesium deficient cells will not complete cell division; generally, it maintains cell viability and vitality. Magnesium is also involved in the stress response of the cell and is important for high gravity brewing where the ion is involved in protection against ethanol stress. Calcium main role for brewing purposes is in flocculation. It binds to yeast cell walls and stabilizes the lectin binding center of other yeast cells. On the other hand, calcium can affect yeast physiology by suppressing magnesium dependent enzymes. Zinc is one of the nutrients that can be deficient even in carefully prepared wort. Zinc is found in abundance in the mash, but most of it remains in the spent grains after lautering. A critical limit for yeast physiology is reached below 0.12 mg zinc per liter wort. More important is the zinc pool in the yeast; if it is maintained, even zinc deficient worts can be fermented well. Zinc plays a major role in yeast's fermentative metabolism because it is essential for alcohol dehydrogenase activity, and also stimulates the intake of maltose and maltotriose. In addition, it promotes yeast flocculation and maintains protein structures. A zinc deficiency in yeast definitely leads to slower fermentation rates with poor results. Other trace elements that influence fermentation include manganese, copper and iron. These are required for yeast metabolism as enzyme cofactors (e.g. manganese) and in yeast respiratory pathways as components of redox pigments (iron and copper). In conclusion, minerals and trace elements play an important role in yeast propagation and fermentation.

2.2.6 Vitamins and Other Growth Factors

This group contains mainly organic compounds that are needed in very low concentrations. For yeast, these are vitamins, purines, pyrimidines and fatty acids. Vitamins are used as components of cofactors. Biotin is an absolute necessity for most yeast strains; pantothenate and inositol are sometimes required, whereas pyridoxine and thiamin seem only to be needed by top fermenting brewer's yeast (Annem et al., 1999). Sulfur and phosphorus are needed as inorganic compounds. Sulfur participatesin the synthesis of sulfur containing amino acids. Methionine and inorganic sulfur are the main sources for yeast. Phosphorus is essential for phospholipids,in the phosphorous bonds of the nucleic acids and for the numerous phosphorylationsin the yeast metabolism (Litzenburger et al.,1996). Due to the low amounts in which the yeast needs these substances, they do not play a critical role in regularly prepared worts.

2.3 Metabolic Pathways during Propagation and Fermentation

2.3.1 Carbohydrate Metabolism for Cell Growth and Energy Generation

The dominating metabolic pathway of brewer's yeast during beer production is the formation of ethanol by consumption of wort carbohydrates. In general, alcoholic fermentation is an energy generation under anaerobic conditions, whereby glucose is metabolized to ethanol and CO_2. The balance of energy is 2ATP/mol glucose.

Acetaldehyde serves as the hydrogen acceptor. In detail, glucose is transferred via glycolysis to pyruvate. The enzyme pyruvate decarboxylase secretes CO_2 and acetaldehyde remains. Then alcohol dehydrogenase transforms the aldehyde to ethanol. Under strictly aerobic conditions the same pathways are taken up to pyruvate, but pyruvate is then changed to acetyl-CoA by the pyruvate dehydrogenase. In the following tricarboxylic acid cycle the acetyl-CoA is used for energy generation. The hydrogen acceptor here is oxygen and 38ATP/mol glucose can be gained. A very important regulatory phenomenon interacts in this pathway. The Crabtree effect refers to the occurrence of alcoholic fermentation (rather than respiration) of glucose under aerobic conditions because of higher sugar concentrations (Geiger et al., 2004). These sugar concentrations are always found under brewing conditions (e.g. working with wort). Thus, the Crabtree effect occurs constantly during propagation and the start of fermentation, and is totally independent of aeration. Despite the low level of aerobic metabolism, yeast should also grow under brewing conditions. Acetyl-CoA as a precursor for biomass production has to be generated in a different way. Yeast

metabolism takes a devious route. The acetyl-CoA is built via the so called pyruvate dehydrogenase bypass. Here, pyruvate is transferred to acetaldehyde. This is metabolized to acetate and then to acetyl–CoA via two enzymes: (i) acetaldehyde dehydrogenase and (ii) acetyl-CoA synthetase (Lustig et al., 1999) (see Figure 2).

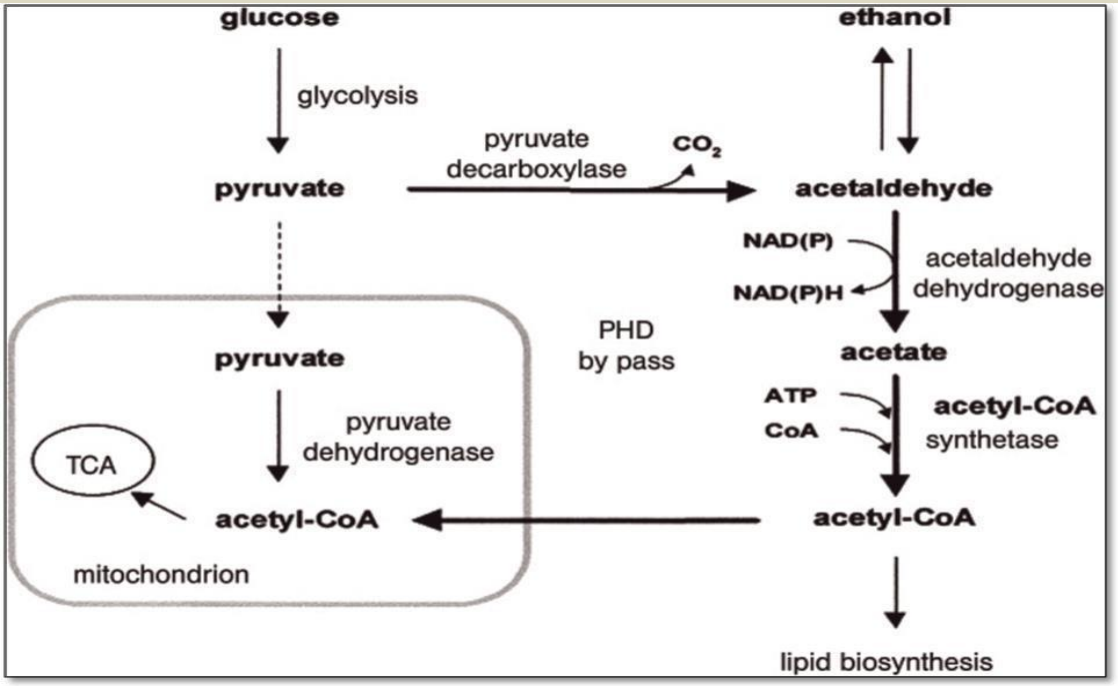

Figure 2: Aerobic and anaerobic fate of glucose. PDH = pyruvate dehydrogenase; TCA = tricarboxylic acid (Hand Book of Brewing Page No.131 Fig No.-5.3).

2.3.2 Phenolic Compounds

A special flavor attribute of some German wheat beers is reminiscent of the aroma of cloves. The responsible substances behind this aroma are 4-vinylguaiacol and 4-vinylphenol. For this type of beer the flavor of 4-vinylguaiacol and 4-vinylphenol is of utmost important. The synthesis is regulated by the POF (phenolic off-flavor) gene. This gene can also be found in lager yeasts and even in wild yeasts, but it is only expressed in ale and wild yeast. A contamination with wild yeast results in a phenolic off-flavor in lager beers. For wheat beers, this flavor attribute is desired to a certain degree. It is synthesized by yeast out of the precursor's ferulic acid and coumaric acid. These acids are extracted out of the malt during mashing.

2.3.3 Formation of Sulfur Dioxide

Sulfur dioxide is generated during primary fermentation by yeast. It is very important as a natural antioxidant and enhances flavor stability of the finished beer. The component seems to

be formed from intermediates in the pathway that reduces sulfate taken out of the wort. The sulfate enters the yeast by active transport and is reduced enzymatically to sulfite. After another reduction, the resulting sulfide is used for the synthesis of sulfur containing amino acids. When yeast growth ceases, no further sulfur containing amino acids are used and the sulfite is excreted out of the cell. Four phases of sulfur dioxide formation during primary fermentation can be distinguished. Phase 1: No sulfur dioxide excretion because sulfite metabolism is repressed due to a high level of sulfurs containing amino acids. Phase 2: Sulfur dioxide is used for the synthesis of amino acids. Phase 3: Yeast growth ceases, the synthesized sulfur dioxide is excreted. Phase 4: The total metabolism and the sulfur dioxide production come to a standstill.

The sulfur dioxide synthesis is directly linked to yeast growth more yeast growth means less SO_2, however, reduction of yeast growth to enhance flavor stability has to be assessed carefully.

3. Materials and Methods

3.1 INSTRUMENTS

UV-Spectrophotometer, Distillation Apparatus (Condenser, Round bottom flask, collector, bent tube, air tight glass wares), Pipettes, Volumetric flasks, 25ml graduated cylinders, Oil bath, Yeast slope, Zink sulphate heptahydrate, Fermenter, Incubator, Autoclave, Slides, Cover Slip, Microscope, Petri plates, Bottle Turner.

3.2 REAGENTS

1. Hydroxylamine hydrochloride ($NH_2.OH.HCl$) 8% aqueous solution.
2. Disodium hydrogen phosphate heptahydrates (Na_2HPO_4) 8.5% aqueous solution.
3. Saline water(0.85% NaCl).
4. $CuSO_4$ Solution.
5. Y.M.C.A (Yeast Malt Agar with Copper Sulphate) Media.
6. W.L.N(Wallerstein Laboratory Nutrient) Media.

3.2.1 Stock Solution

Dimethyl glyoxime: 0.1349gm of pure dimethyl glyoxime was dissolved in distilled water and the volume was made up to 1000ml by adding distilled water, such that the resulting solution has the concentration equivalent to 100mg per liter of diacetyl.

3.2.2 Working Solution

From the stock solution, 20ml was taken and diluted with distilled water and the volume was made up to 100ml, such that the resulting solution has the concentration equivalent to 20mg/liter of diacetyl.

3.3METHODS

3.3.1Starter Culture Preparation

3.3.1.1 Yeast Propagation

1. The yeast cells from the slope were removed by using sterile saline water.
2. It was inoculated in two flasks containing 500ml sterile wort each.
3. It was incubated at 25-27^{0}C for 48hrs with constant oxygenation.
4. Then the two 500ml cultures were inoculated in to two flasks containing 5 liters sterile wort and incubated at 20-22^{0}C for 48hr with constant oxygenation.
5. The two 5 liters culture were inoculated to first yeast propagator containing 1HL of wort heated with 100^{0}C and was cooled to 16-18^0 C and incubated at 16-18^{0}C for 24-36hrs.
6. The 1HL cultured was transferred to second yeast propagator containing 10HL wort heated with 100^{0}C and was cooled to 13-14^{0}C with constant oxygenation.

3.3.2 Yeast budding study

A drop of yeast sample was taken over a glass slide. A piece of cover slip was kept over it and viewed fewer than 100X binocular Olympus microscope, for the morphological study.

3.3.3 Fermentation

10HL cultured from yeast propagator was pitched to culture brew of 100HL at 10-12^{0}C. Online aeration was given during wort collection and the temperature was maintained 10-12ºC during fermentation. The fermentation was carried out 7-8 days at temperature 10-12^{0}C till the end gravity and pH 5.5. The fermentation medium composed of amino acids, carbohydrates and proteins.

3.3.4WildYeast Study

3.3.4.1 Y.M.C.A Media:

24gms of Y.M.C.A was taken in to 1000ml and was autoclaved at 121^{0}C for 15mins. 25ml of $CuSO_4$ was added to the media before pouring to the plates. The media was poured to plates and allowed for solidification. After the solidification of media the sample was poured over it and was kept in incubator for 24hrs.

3.3.5 Diacetyl Estimation

3.3.5.1 Standard Curve

1. 0, 1, 2,3,4,5 ml of dimethyl glyoxime working solution was taken in 25ml graduated cylinders.
2. 1ml of hydroxylamine hydrochloride and disodium hydrogen phosphate was added to each tube and the volume was made up to 10ml.
3. Absorbance was taken at 230nm and the graph was plotted between absorbance vs blank to get standard curve and the regression factor was calculated.

3.3.5.2 Sample Measurement

1. 100ml of undegassed beer was taken in 500ml round bottom flasks.
2. 3.5ml of 8.5% Na_2HPO_4 was added in to it.
3. Distillation was started with tip of the delivery tube dipping in to 2ml of distilled water.
4. The distillate was collected in 25ml graduated Pyrex cylinder to 18ml and the volume was made up to 20ml by distilled water.
5. 10ml of the solution was transferred to another 25ml graduated Pyrex cylinder and labeled one cylinder for sample and another for blank.
6. 1ml of 8% Na_2.OH.HCl was added to the cylinder labeled “sample”.

7. Both the cylinders were heated in oil bath at 80^0C for 15mins and were cooled in room temperature.
8. 1ml of 8% Na_2.OH.HCl was added to the cylinder labeled “blank.”
9. 8.5% Na_2HPO_4 solution was added to both the cylinders. The volume was adjusted to 10ml by distilled water and was mixed thoroughly.
10. The absorbance was taken of cylinder labeled sample vs cylinder labeled blank at 230nm.
11. The absorbance was multiplied with the regression factor to get the concentration of diacetyl in the beer.

3.3.6 Study on reduction of Microbial contamination during CIP

CIP(Cleaning In Place) plays a significant role in each and every Brewing Industry. It is basically a cleaning process of UTs which comes to play after every batch of fermentation. To reduce microbial load, a sterilant known as **Divoson Forte** is generally used as final rinse in CIP process.

3.3.6.1 Study on Antimicrobial Activity of Divoson Forte .

1.Five sample bottles were taken & labelled as Blank,0.1%,0.2% ,0.3%,0.4%.
2.400ml Soft water which is used for CIP was collected in five numbers of 500ml sample bottle.
3.To increase the microbial load in the water samples some different kinds of microbes such as Acetic Acid Bacteria, Lactobacillus Bacteria , Wild yeast has been added manually.
4. All the bottles were mixed thoroughly by using the bottle turner for 10 minutes.
5.0.5ml,1.0ml,1.5ml,2.0ml Divoson Forte were added to 0.1%,0.2% ,0.3%,0.4% labelled bottle respectively.
6.Again all the bottles mixed properly for 10 minutes.
7. Using the Membrane Filter technique , all five samples were filtered through the 0.4micron membrane filter using a filter funnel and vaccum system.
8.The filter paper were placed in respective WLN plates and incubated for 24hours at 30 degree Celsius.

4.RESULTS

4.1 Starter Culture & Yeast Propagation

Starter culture was selected and yeast was cultivated in Yeast Cellar with all sterile and optimal conditions for multiplication.

Figure 5: Genetically modified yeast strain

Figure 6: Yeast Cellar

The yeast propagation was carried out at temperature 16-18^{0}C in yeast propagator as shown in the following figure

Figure 7: Yeast Propagator

4.2 Yeast Budding Study

Morphological examinations were performed using the media wort as the nutrient for the yeast. The budding of yeast was observed under 100X magnification of Olympus binocular microscope.

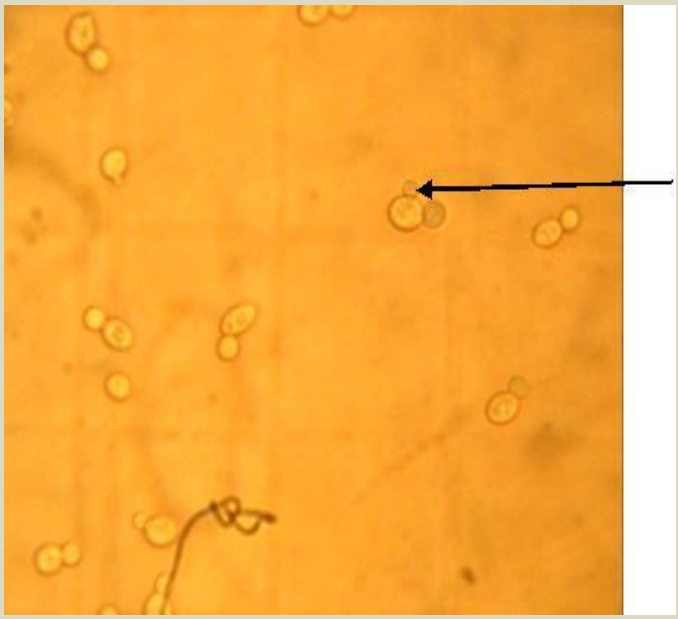

Figure 8: 100X magnified view of *Saccharomyces uvarum* under Olympus Binocular Microscope.After 96hrs growth period, the *Saccharomyces uvarum* exhibited multiplicity. It was noticed from the photograph that the yeast cells having budding.

4.3 Fermentation

Yeast from progator was introduced to YPT(Yeast Pitching Tank).

Figure 9: Yeast Pitching Tank

After 7-8 days of fermentation the substrate were converted into beer.

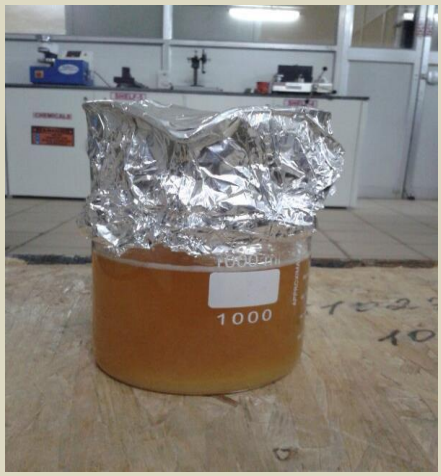

Figure 10: Sample collected from Fermentation Vessel

4.4 Wild Yeast Study

On completion of incubation, numerous colonies of *Saccharomyces* wild yeast observed on Y.M.C.A media .

11: Wild Yeast in YMCA medi**Figure** a

After fermentation, yeast batches harvested and stored in YST. A single batch of yeast can be cropped and harvested up to 6th Generation.

Figure 12: Yeast Storage Tank

4.5 Diacetyl

Table 2: Calculation For Regression Factor

Sl no.	Volume of standard solution (ml)	Concentration (ppm)		Distilled water (ml)	Absorbance at 230nm	Slope	Regression factor
1	0	0	1ml of 8% Na2HPO4	8	0.141	1.304	0.7663
2	1	0.1	+	7	0.278		
3	2	0.2	1ml of 8.0% NH2.OH.HCl	6	0.413		
4	3	0.3		5	0.552		
5	4	0.4		4	0.682		
6	5	0.5		3	0.812		

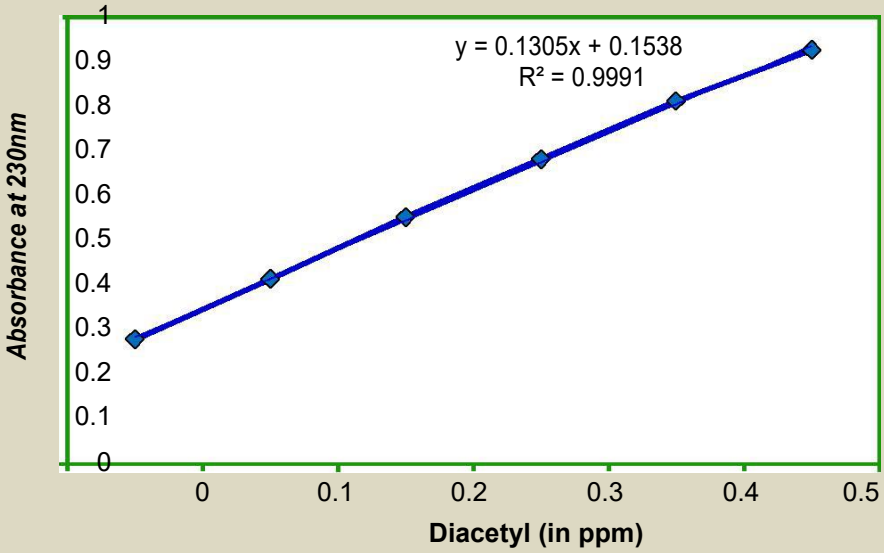

Figure 13: Standard Graph for diacetyl

4.6 Estimation of Diacetyl

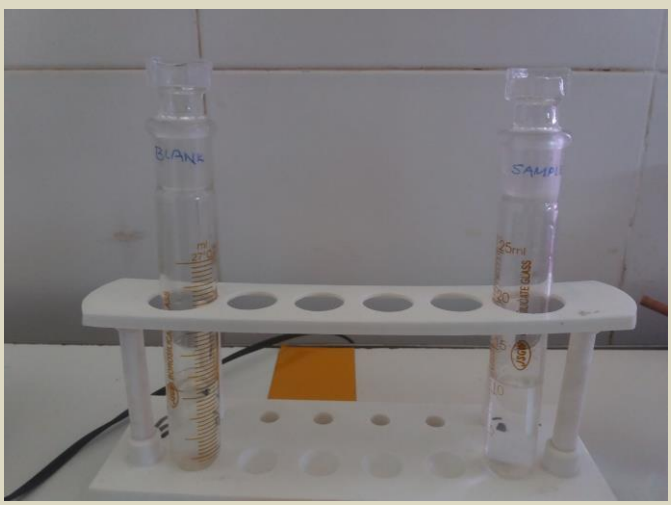

Figure 14: Blank and Sample tube

Figure 15: Oil Bath

CALCULATION

The sample was measured under UV spectrophotometer at 230nm and it was observed 0.0894nm. The absorbance was multiplied with the regression factor to get the concentration of diacetyl in the beer.

Diacetyl (ppm) = Absorbance X Regression Factor

0 0.0894 X 0.7663

0 0.0685 ppm

It was found that the diacetyl amount in beer was 0.0685 ppm.

4.7 Study on reduction of microbial contamination during CIP

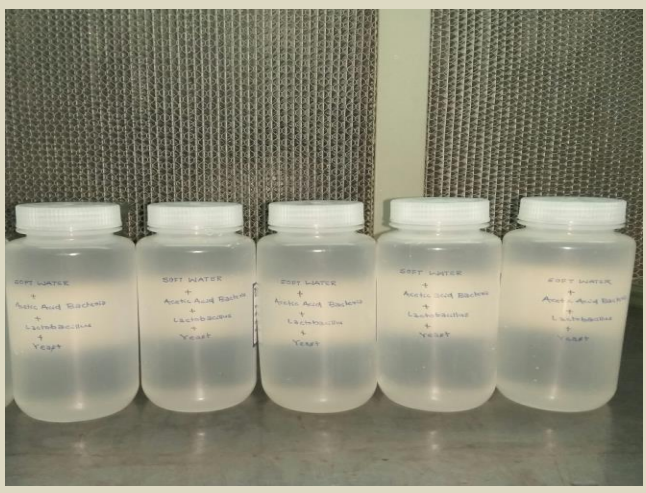

Figure 16: Sample bottles(containing softwater+Acetic Acid Bacteria+Lactobacillus+Wild Yeast+ Divoson Forte).

Figure 17: WLN plates of Blank,0.1%,0.2%,0.3%,0.4% after 24hours of incubation.

Hence from the above study it has been concluded that 0.3% of divoson forte is enough to minimise microbial load. Here the case study validates the percentage of Divoson forte which is used in CIP as final Rinse..

5. Discussion

Diacetyl is naturally produced by all yeast during fermentation and smells like Butterscotch. Diacetyl is naturally produced by all yeast during fermentation and is then “reabsorbed” by yeast cells. Increased diacetyl or diacetyl that is not reabsorbed may be a result of high flocculating yeast, weak or mutated yeast, over or under oxygenating, low fermentation temperatures and weak or short boils. It is generally regarded as a flaw when detected in lagers. Some brewers, and drinkers alike, desire small amounts in ales.

If diacetyl is more in beer then sickness in the mouth and tongue, savior headache and vomiting will arise after drunk. The ketone group increases toxicity in beer. The entire beer aroma will suppress by VDK. If VDK is more in beer then it cannot be consumable.

Yeast that is highly flocculent may fall out of suspension before it gets a chance to absorb the diacetyl, using medium flocculation yeast should give the yeast a good chance to absorb diacetyl. Always use high quality yeast and avoid weak or possibly mutated strands that may be incapable of handling diacetyl properly. Allow yeast to begin initial growth with the use of a yeast starter. Sufficient oxygen is to be supplied for yeast growth, but avoid over oxygenating especially after pitching yeast. Allow enough time for yeast to fully ferment at appropriate temperatures.

6. CONCLUSION

Beer is alcoholic beverage consumed all over the world. It reduces the risk of heart disease, helps the kidney, packed with nutrition containing vitamin B, Vitamin B12, Riboflavin and also rich in silicon which helps to strengthen the bones, reduces risk of diabetes, risk of cancer, and increases brain health if consumed in moderate. If diacetyl is there in beer then such health problems arises as savior headache, vomiting after drunk, and the ketone group increases toxicity in beer. The beer will smells like butter. The entire beer aroma will suppress by diacetyl.

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Thank
You