Module 1

1.5 Fermentation

1.5.1 Yeast Handling
1.5.2 Principle Fermentation Variables
1.5.3 Fermentation Technology

DIPLOMA IN DISTILLING

Contents

Abstract

In this final unit of Module 1 of the Diploma in Distilling, 1.5 Fermentation, we will explore the practice of distillery fermentation management.

Firstly, in 1.5.1 Yeast Handling, we will look at the different yeast sources available and their use in the distillery, including storage and laboratory testing.

In 1.5.2 Principle Fermentation Variables, the timeline of biochemical change during fermentation is explained, and the implications of yeast alcohol sensitivity discussed.

Finally, in 1.5.3 Fermentation Technology, different designs of fermentation vessel are examined.

Learning Outcomes

On completion of this section you should be able to:

1. *Understand the various types of yeast preparations commonly used in distilleries.*
2. *Outline a typical timeline for a distillery fermentation, including changes in sugar and alcohol concentrations.*
3. *Specify an appropriate fermentation vessel design for a given distillery environment.*

Prerequisite Understanding

Basic scientific knowledge and terminology. Unit 1.4, Yeast and Bacteria.

1.5.1 YEAST HANDLING

Yeast handling in the distillery

Five sources of yeast are available to the distiller: natural inoculum, yeast cake, yeast cream, dried yeast and brewery yeast. The naturally occurring yeast flora of grape juice and molasses can be used either with or without a supplementary cultured yeast. In either situation a low level of SO_2 treatment is often used, insufficient to harm fermentative yeasts but hopefully inhibitory to bacteria and aerobic yeasts.

Yeast for on-site brandy, rum or whisky fermentations should meet the following requirements (this does not concern brandy distillers using wine feedstock)::

- complete and rapid fermentation of sugars
- good flavour production
- tolerance of the osmotic stress of the initial 16 – 20% sugar concentration
- ability to continue fermentation to 8 – 10% alcohol
- no flocculence or chain formation (clumps foul the heating coils of pot stills and plates of continuous stills)
- ability to grow well above 30°C.

For industrial alcohol, growth up to 45 – 50oC is desirable for a significant saving of energy for continuous distillation, but for potable spirits 34 -35oC is the usual maximum. In addition, for each batch of yeast inoculum, it is essential to confirm that the yeast is the correct strain, is free from bacterial and wild yeast contamination, and has high vitality and % viability (see below).

Although some distilleries grow their own, the majority purchase their yeast from a specialist yeast culture company. Commercial yeast is sold as yeast cake (concentrated by filtration to about 28% dry weight), cream yeast (about 18% dry weight) and dried in an inert atmosphere to about 95% by weight and sealed in a vacuum pack. Dried yeast can be stored up to about 12oC for at least 1 year but if chilled storage is already available that is commonly used. Against the advantage of long storage is the nuisance of the routine required for pitching. Yeast from different manufacturers varies in rehydration procedure: one may require reconstitution in sugar solution of precise concentration and temperature (e.g. 35oC), another may more conveniently be scattered dry into the fermentation vessel when filling commences.

Yeast with significant moisture content must be stored in the range 2 – 4oC. Any lower risks freezing which would kill the yeast by ice crystals puncturing cell membranes, and higher accelerates deterioration, also encourages growth of any bacterial or wild yeast contaminants present. Cream yeast must be used as soon as possible after delivery, certainly within one week. Yeast cake can survive in chilled conditions for longer, but even so, use within one week is advisable. At one time yeast recovered from brewery fermentations was widely used in the whisky distilling industry but seldom, if at all, nowadays, largely because of its variable quality. Again, chilled storage and use within a few days were essential to minimise microbiological problems. Yeast cream stored in bulk is pumped in the calculated quantity to the fermentation vessel at the start of filling; yeast cake and brewer's yeast must first be diluted to yeast cream consistency in a mixing vessel before inoculation of the fermentor.

Testing a yeast culture for contamination should not normally be necessary if it comes from a reliable commercial culture plant, but a rapid test of viability and vitality is advisable, especially after more than a few days'storage. The simplest test for viability is staining with methylene blue (MB), a redox dye which is blue in its oxidized state and colourless when reduced. In a mixture of MB and diluted yeast culture examined under the microscope, living cells either fail to take up the dye, or if they do, reduce it to the colourless form. Either way, live cells are unstained. On death, cells rapidly become permeable to MB, and lacking metabolic activity, remain blue. A calibrated slide (haemacytometer, originally designed for counting blood cells, which are the same size as yeasts) allows the percentage of dead cells

to be counted quickly and accurately. However, the method does not actually distinguish dead from alive (i.e. capable of reproduction); the distinction is presence or absence of metabolic activity. Above about 90% this is unimportant, but.becomes increasingly significant with lower % "viability".

Although other redox dyes have been recommended as more reliable than MB, that is debatable, and measurement of yeast "vitality", analogous to fitness in humans, is now regarded as more meaningful. Various methods have been suggested, but the simplest is measurement of "acidification power". Active yeast added to unbuffered 20% glucose solution will cause a rapid fall in pH by the efflux of H+ associated with transport of the sugar across the cell membrane, to below pH 4 within 20 min. Like the MB test, it is
sufficiently rapid to justify testing before using a batch of yeast. In an alternative version using the same principle, efflux of Mg ions is measured, but that requires more complex equipment than a pH meter. Using a different principle, but also a rapid method, measurement of ATP content (obviously an indicator of activity) by its stimulation of bioluminescence of the firefly extract used in detection of microbial contamination (see the section on detection of contaminants) is also possible, but perhaps is an extravagant use of expensive reagents.

1.5.2 Principle Fermentation Variables

The sugar of grape juice is an approximately equal mixture of glucose and fructose, with smaller amounts of sucrose and raffinose. Cane molasses is mainly sucrose, with small amounts of fructose, glucose and raffinose. For both of these fermentable media, uptake of the sugars is rapid since fructose and glucose permeases are constitutive, i.e. always present, in the cell membrane and the wall-bound enzymes which hydrolyse sucrose and raffinose are formed rapidly in response to the presence of these sugars.

Therefore all sugars presented to the transport system of the cell membrane are monosaccharide. In many strains of Saccharomyces cerevisiae fructose and glucose are transported simultaneously and metabolised at approximately the same rate, but others show some preference for fructose transport or metabolism.

The composition of cereal wort is more complex. Carbohydrate constitutes about 90% of the total solids of all-malt wort. Typical composition, expressed as % of total carbo-hydrate, is maltose 46%, maltotriose 15%, glucose and maltotetraose each 10%, sucrose 5%, fructose 1%. The remainder of the 100% total is accounted for by 13% of malto-pentaose and higher dextrins, which are not fermentable by distilling yeast at the time of collection of the wort, but are hydrolysed to fermentable sugars by continuing enzyme action during the fermentation. The ratio of these sugars is similar in grain distillery worts from maize or wheat.

The sequence of uptake of the sugars discussed in Unit 1.4 is important: glucose (and fructose) first, then successively the increasingly larger saccharides.

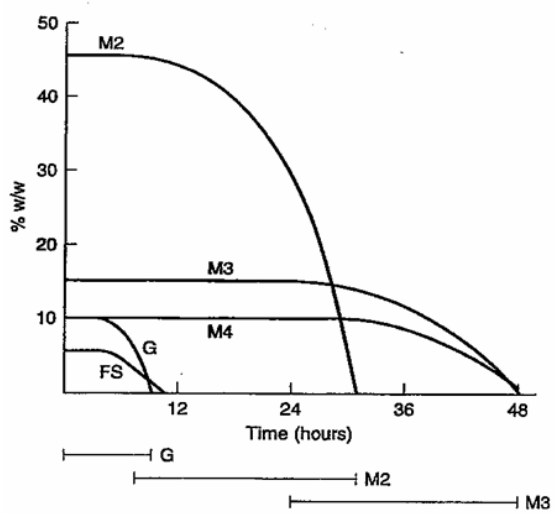


Figure 1 Sequence of utilisation of cereal wort sugars by distillery yeast.

G= glucose, FS = fructose and sucrose, M2, M3, M4 = maltose, maltotriose and malto- tetraose. The horizontal bars show the duration of G, M2 and M3 permease activity.

Although an increase of temperature during fermentation is inevitable, many distillery fermentations have either no temperature control at all or only a spray of cold water over the vessels: an unusual situation since almost all other biotechnology industries operate with precise attemperation of fermentations. So the only control measure may be the temperature of the wort at the time of filling the fermentor. In Scotch whisky distilleries, if the wort is cooled to 18–20°C, the natural increase during fermentation is unlikely to exceed 33°C, which distilling yeast can tolerate. Ideally, the temperature profile should be the same in successive fermentations otherwise variable amounts of flavour congeners are produced as metabolic by-products, particularly esters and higher alcohols. The effect of temperature and fermentation variables on production of flavour congeners by the yeast is covered in section 1.4, Metabolism, and so is not repeated here.

Progress of fermentation

Microbial growth in general can be described in terms of the graph in Figure 2. Immediately after inoculation (pitching) into the culture medium, there is a period of adjustment to the new conditions.

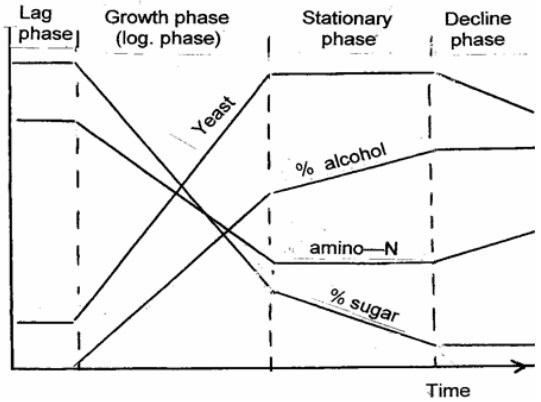


Figure 2 Simplified diagram of the principal changes during fermentation.

Cultured yeast, grown aerobically in a low concentration of sugar, requires a period of adjustment to the new conditions of high sugar concentration in fresh wort. This is the lag phase, named because there is no detectable increase in cell numbers, but nevertheless it is a period of intense biochemical activity to prepare for active growth. Its duration depends on temperature, concentration of nutrients, previous history of the yeast culture and various other factors, but usually active yeast growth begins within 6 – 12 hours. In theory, the doubling cell numbers with each cell division gives a straight line if the logarithms of cell numbers are plotted against time, and this is true at constant temperature, but since rum or whisky fermentations can vary over as much as 15°C, this causes some distortion of the graph. However, it is convenient to regard the growth/logarithmic/log phase as a straight line as in Figure 1.

Normally growth eventually ceases because of lack of nutrients, but in *Saccharomyces*

fermentations, growth ceases when further multiplication is impossible because of lack of unsaturated fatty acids and sterols to synthesise cell membranes. This is usually after about 2 generations (when stationary phase begins, because there is no further increase in cell numbers). Actually, this is lack of nutrient (oxygen), required for synthesis of essential lipid material. However, the enzyme systems remain active, continuing alcohol production for some time longer, but since there is no growth, there is no further need for amino acids, etc. So the simplified graphs of Figure 2.3 show yeast growth and amino-N graphs as mirror images, also falling sugar content and rising alcohol as mirror Images of each other. Finally, a decline phase begins with death and autolysis of cells, and release of amino-N, which may happen with a rum or whisky fermentation extended to 72 h.

Figure 3 shows the actual progress of a typical fermentation of cereal wort. Similar figures apply to molasses except that amino-N is much lower and a supplement of NH + would be necessary. However the shape of the N graph would be essentially the same. Brandy fermentations are likely to last longer at lower temperature in order to produce stronger estery flavours, but otherwise the same general pattern applies over the longer time scale. A standard quantity of pitching yeast is required, since significant variation alters the amount of yeast growth during the fermentation, and therefore also the amount of excreted flavour congeners. The graph shows yeast as millions per ml, as measured in the laboratory, but in practice the yeast is added by weight (number of 25 kg bags). Note a slight rise in amino-N towards the end of fermentation. Part of the reason is that active growth has ceased, recycling of unnecessary enzymes has begun, and part of the resulting amino acid pool is excreted. However, the graph of yeast cell count shows a slight drop; if that is due to cell death and autolysis, amino acids would be released. Since structural components of the yeast cells also contribute to the distillate, in an extreme case variation in fermentation conditions could cause their composition to vary enough to affect flavour.

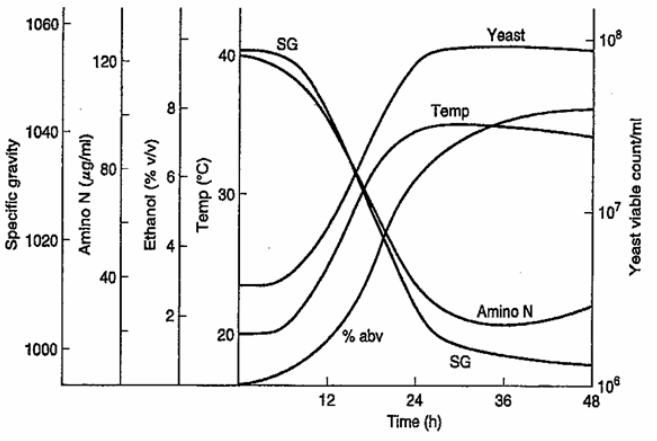


Figure 3 Progress of fermentation.

The graphs refer specifically to whisky. For rum, ^{O}Balling figures are more appropriate: SG 1060 = 15^{O}B, 1040 = 10^{O}B, 1020 = 5^{O}B.

In a pure culture fermentation, the falling pH is related to efflux of H+ ions with sugar transport, or excretion of metabolites such as pyruvate and succinate as acids. In Figure 3 the pH graph falls quickly with sugar transport, then rises slightly with the buffering effect of amino acids excreted when active growth ceases but falls again with continuing growth of acid-forming bacteria. In Scotch malt whisky fermentations, a low level of contamination by lactic acid bacteria from the malt is inevitable, but unusually heavy contamination can cause a significant alteration to the normal pattern and, by competition with the yeast for the available carbohydrate, reduce spirit yield.. In molasses worts the pH changes are more pronounced because the N supply is mainly NH +, with little or no buffering effect. In both malt whisky and dark rum fermentations, a low level of such contamination is often welcomed for its contribution to the complexity of spirit flavour. The situation with natural fermentations of molasses or grape must is

much more variable, and unpredictable, according to the range of bacteria and yeasts present.

Figures 3 and 4 both show fermentation over 48 h, but some rum and whisky distilleries ferment for longer, up to 72 h. Assuming the same original gravity and temperature, the progress of fermentation will be the same over the first 48 h and subsequently the changes associated with amino acid excretion and lactic acid production could reasonably be

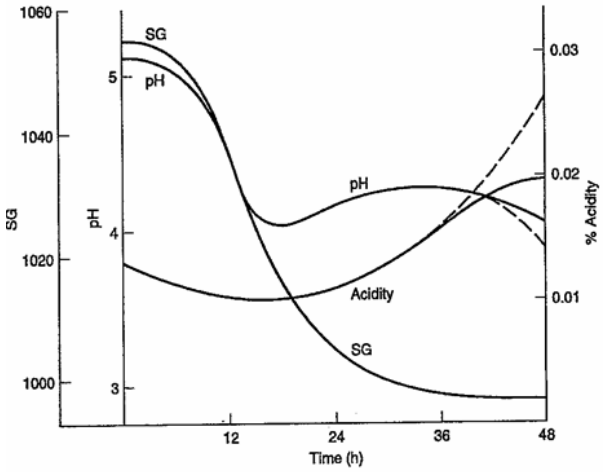


Figure 4 Effect of bacterial contamination on pH during fermentation.

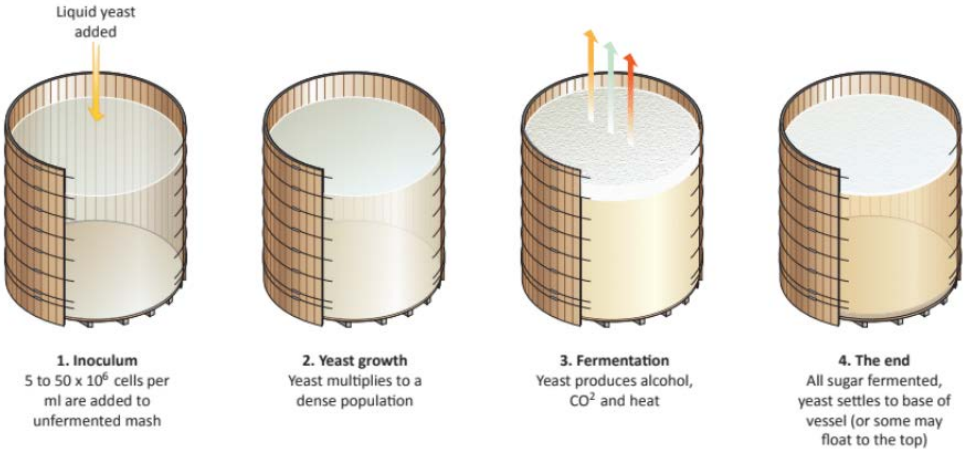
expected to be more pronounced, giving further development of flavour complexity.

Alcohol sensitivity and tolerance in yeast

Although ethanol is the stress factor mentioned in the syllabus, high osmotic pressure from the initial sugar concentration is equally important, and many of the adverse effects are similar for both. However, a specific problem from the organic solvent ethanol is its damage to the phospholipids of the cytoplasmic and other cell membranes.

Fermentations for potable spirits seldom exceed a final alcohol content of about 10% abv. Table wines are now commonly in the range 12 – 14.5% abv, fino sherry, which is produced entirely by fermentation (no brandy addition as with other types of sherry) is about 17% and sake, also by fermentation alone, is at least 20%. The Saccharomyces spp. involved do not appear to suffer any ill effects from high final alcohol concentration, but these are specialized strains. Distilling yeast was bred for other properties; and tolerance of unusually high % alcohol was not one of them.

Much of our present knowledge of the stress effects on yeast of high initial sugar and high final % abv comes from the interest of the brewing industry over the past 20 years in fermenting high gravity worts for subsequent dilution to standard sales gravity. In the yeast cell, stress is counteracted by increased production of glycerol, glycogen and trehalose, the latter two in the larger than



normal vacuole, but under the microscope the most obvious effect of pitching into too strong wort is plasmolysis (shrinkage) of the yeast cells. The osmotic effects appear to be too strong for the cytoplasmic membrane to control. High external alcohol concentration has equally drastic effects, partly since alcohol can not escape and more remains within the cell, affecting enzyme activity, and causing other damage. In particular, high alcohol concentration damages (dissolves?) membrane structure. Again, loss of intracellular material causes plasmolysis and ultimately cell death.

1.5.3 Fermentation Technology

Fermentation Vessels

The usual construction material of fermentation vessels (washbacks in Scotland) is wood or stainless steel. Epoxy-lined mild steel is very rare now but was more common previously. Stainless steel is virtually standard for fermentation vessels for continuous distillation, for which a capacity of 100,000 litres or more is not unusual. Many distilleries using batch distillation now have stainless steel fermentors, but the traditional appeal of wooden vessels is an important marketing consideration. The size of their fermentors is based on still capacity, and if the distillery has only one pair of stills, it is normal to have fermentors of twice the working volume of the wash still. For malt whisky, that volume of wort is produced by one mashing cycle of the mash tun.

Wooden vessels are normally of pine since the greater strength of oak and contribution to flavour, discussed in the maturation section of these notes, are irrelevant for the 2 - 3 day cycle of fermentation vessels. The usual design is a flat-bottomed cylinder of vertical planks bound by metal hoops. A wooden lid with a removable hatch protects against contamination by insects, rodents and airborne micro-organisms, and an electrically driven rotating blade ("switcher") is filled just below the lid as a mechanical foam breaker. Nowadays the lid may carry a spray-ball for cleaning purposes, and also for subsequent sterilization if live steam is not used.

Steel vessels are of similar design, except for the dished bottom (or a sloping or conical bottom which gives the same result) to facilitate drainage of fermented wash, cleaning solutions and rinses, and a dished top to avoid a right-angled corner which is difficult to clean. Unfortunately, construction of these desirable features would be impossibly difficult with wood.

Since the individual plates forming a steel vessel are unlikely to extend the full height as the planks of a wooden vessel must do, joins must be butt-welded and ground down to a smooth surface, at least on the inside riveted or overlapping welded joints create ledges which allow accumulation of residues and microbial contamination and are unacceptable. The same requirement applies to pipework. Attemperation is not normally fitted, but if it is, must be an external jacket, since internal cooling coils create obstruct cleaning and sterilization procedures. One or more sprayballs, or a rotating jet, would be expected as standard for cleaning.

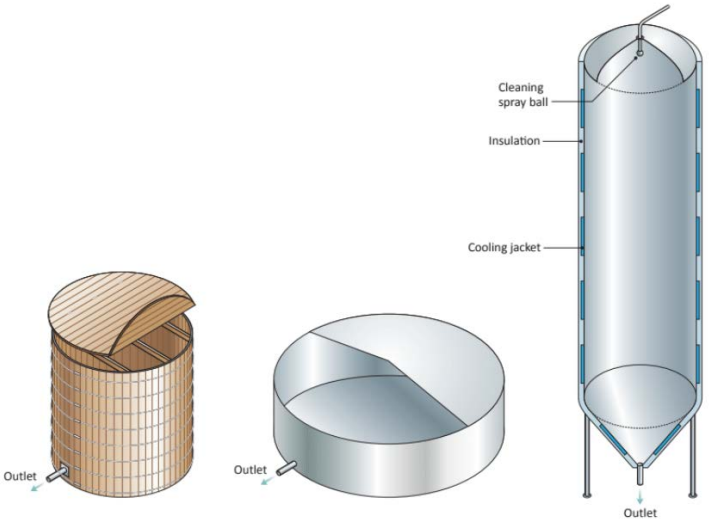


Figure 5 A traditional washback, flat-bottomed fermenter and cylindro-conical fermentation vessel

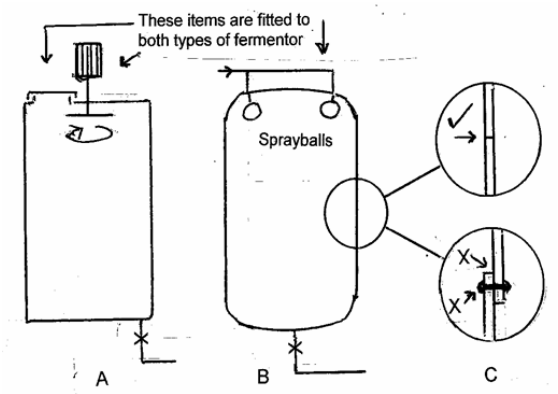


Figure 6 Fermentor design.

A, traditional wooden vessel, **B**, mild steel or stainless steel. **C** shows acceptable butt-welding of plates, and an unacceptable overlapping joint. The rivet makes it worse, but even with welding the overlap is a microbiological hazard.