MODULE 2: Yeast and Beer

UNIT 2.9: Beer Spoilage Organisms

SECTION 2.9.1: Types of Organisms

ABSTRACT: Of the micro-organisms from air, water and raw materials that enter the brewery, some are particularly well adapted to this environment. Growth of these organisms on raw materials and during fermentation can release metabolites that can seriously affect the stability and organoleptic properties of beer. Some bacteria and yeasts can proliferate in beer to produce hazes and off-flavours. This unit reviews these micro-organisms, their occurrence and effects in the brewery, and procedures for their isolation and identification.

LEARNING OUTCOMES: On completion and comprehension of this unit you will be able to:

1. Know the organisms which can spoil worts and beer and their typical growth conditions.
2. Understand the effects that these organisms can cause.

PREREQUISITE UNDERSTANDING: To have studied Section 2.1.
2.9 Beer Spoilage Organisms

2.9.1 Types of Organisms

2.9.1.1 Introduction

2.9.1.2 Bacteria

(a) Gram Positive
(b) Lactobacillus
(c) Pediococcus
(d) Gram Negative
(e) Acetic Acid Bacteria
(f) Zymomonas
(g) Enterobacteriaceae
(h) Miscellaneous Gram Negative Rods

2.9.1.3 Yeasts

(a) Brettanomyces
(b) Saccharomyces

2.9.1.4 Moulds

(a) Ascomycetes
(b) Fungi Imperfecti
2.9 BEER SPOILAGE ORGANISMS

2.9.1 TYPES OF ORGANISMS

2.9.1.1 INTRODUCTION

Of the micro-organisms from air, water and raw materials that enter the Brewery, some are particularly well adapted to this environment. Growth of these organisms on barley and malt during fermentation can release metabolites that can seriously affect the stability and organoleptic qualities of the finished product. Some bacteria and yeasts can proliferate in beer giving rise to hazes and off-flavours. This section reviews these micro-organisms, their occurrence and effects in the brewery; procedures for their isolation and identification are described in Section 2.9.2.

2.9.1.2 BACTERIA

Most bacteria are unicellular organisms that multiply by binary transverse fission. They are generally spherical (coccis) or rod-shaped, although the actinomycetes group form filaments analogous to the fungi. Bacteria are diverse and ubiquitous but fortunately few cause problems in the brewery.

A different staining procedure of prime importance in bacteriology is the Gram reaction. Gram-positive cells retain a crystal violet-iodine complex after washing with ethanol or acetone. Gram-negative cells do not. This property is correlated with other structural properties of the bacterial cell, making it of immense taxonomic significance.

Thus, the brewery bacteria will be described in two sections.

(a) Gram-positive

The Gram-positive bacteria encountered in the brewery are collectively described as the lactic acid bacteria. This includes both cocci and rods classified in the genera *Pediococcus* and *Lactobacillus* respectively.

These bacteria share many important properties; they are fermentative and characteristically use a variety of carbohydrates and other organic compounds as a source of carbon and energy.
Lactic acid is always a major fermentation product, sometimes the only one.

These bacteria grow on the surface of solid media exposed to air, but more readily in an atmosphere rich in carbon dioxide and many strains are micro-aerophilic (prefer a low oxygen tension).

The lactic acid bacteria lack respiratory metabolism and, even when growing on a rich medium, the colonies remain small and are chalky white in colour due to the lack of cytochromes.

They are typically acid-tolerant and grow optimally at low pH.

(b) *Lactobacillus*

The genus *Lactobacillus* contains some 28 species divided into four physiological groups.

Group I strains are homofermentative *i.e.* produce lactic acid as virtually the only metabolite from glucose. They are further divided into:

- I A (thermobacteria), those that grow at 45°C but not 15°C; and
- I B (streptobacteria) which have the opposite temperature relationships.

Group II (betabacteria) lactobacilli are hetero-fermentative and metabolise glucose to lactic acid, carbon dioxide, acetic and acid and/or ethanol.

II A contains the well-recognised, fermentatively active species; and II B comprises species which are inert to most carbohydrates, acidophilic and tolerate ethanol (15%). These species are less well studied and tend to occur in restricted habitats (e.g. malo-lactic fermentation of wine).

*Lactobacilli*, like most Gram-positive bacteria, are sensitive to hop resins, but selection in the brewery has led to the emergence of resistant strains.

The common brewery lactobacillus is the heterofermentative (Group 11A) *L. brevis* (Formerly *L. pastorianus*). The cells exhibit considerable pleo-morphism, forming long rod-shaped structures, and they grow optimally at about 30°C and PH4 to 5. Brewery strains are typically insensitive to hops but ethanol becomes inhibitory at around 5 %.

*Lactobacillus* strains that show slight differences have been given specific names in the past: *e.g.*

- *L. lindneri* has a lower optimal temperature for growth (19°C) and was isolated from lager;
L. diastaticus, an amylolytic organism, was isolated from super-attenuated stout. L. pastorianus var. brownii produces extracellular polysaccharide, thus causing ‘rope’ in beer. These features are not sufficiently significant to merit varietal status and these bacteria are accordingly included in L. brevis, but the metabolic diversity displayed by these species should be remembered.

L. frigidus and L. parvus may also be mentioned in the literature; these are now called L.Buchneri, a close relative of L. brevis.

Other lactobacilli that have been isolated in breweries include:
L. delbrueckii, a thermophilic member of group IA that has been found in sweet wort held at 50 – 60°C and
the homofermentative (Group 1B) species L. plantarum and L. casei; and L. fructivorans from Group IIB.

(c) Pediococcus
Although numerous Gram-positive cocci can be isolated from beer and particularly brewery plant, only one species is hop-tolerant and able to proliferate in beer, Pediococcus damnosus (formerly P. cerevisae).
This organism was originally associated with “sarcina sickness” in beer characterised by turbidity, acidity and diacetyl.
Varieties of P. damnosus that produce ‘rope’ and are amylolytic have been described.

(d) Gram-negative
The Gram-negative bacteria encountered in the brewery can be found at all stages within in the production process and include the following:

(e) Acetic acid bacteria
The most distinctive property of these bacteria is their ability to oxidise ethanol and acetic acid. They are aerobic rods, typically hop and acid-tolerant and cause spoilage of beers stored in the presence of air.

The genus Acetobacter comprises the “over-oxidising” strains that oxidise ethanol to acetic acid, and finally to carbon dioxide and water.
Gluconbacter (previously Actemonas) strains produce only acetic acid from ethanol. Common brewery species are A. pastorianus (previously A. rancens) and G. oxydans. Some strains (particularly G. oxydans) can grow in beer in the presence of very limited supplies of oxygen and produce voluminous quantities of rope.

(f) Zymomonas

These virtually anaerobic Gram-negative rods are peculiar amongst the bacteria in conducting an alcoholic fermentation of sugars. They are dangerous because they grow readily in beer, producing turbidity and serious off-flavours, but fortunately they tend to be uncommon. Their habitat includes the soil, water and brewing equipment.

There is only one species, Z. mobilis (previously Z. anaerobia), which accommodates both motile and non-motile strains. Two varieties are recognised: subsp. mobilis includes those strains isolated from breweries; and subsp. pommaceae comprises strains responsible for “cider sickness”.

(g) Enterobacteriaceae

This family include a variety of genera of Gram-negative rod-shaped bacteria, all phenotypically similar and differentiated on the basis of sugar fermentations and other metabolic attributes.

They are aerobic and facultatively anaerobic organisms, typically bile-salt tolerant but sensitive to ethanol and low pH, two factors that restrict their spoilage potential in the brewery. They are generally unable to grow in beer but grow rapidly in wort producing metabolites that can affect beer flavour adversely.

One example, apparently peculiar to breweries, is Obesumbacterium proteus (synonyms: Flavobacterium proteus and Hafnia protea). This bacterium is ubiquitous in top-fermenting yeasts and less common in lager yeasts.

Other enterobacteria isolated from wort and brewery plant include Hafnia alvei, Cirobacter freundii, Klebsiella and Enterobacter species. All these organisms develop in wort and during the early stages of fermentation.
(h) **Miscellaneous Gram-negative rods**

The following can be distinguished from *enterobacteria* by their inability to ferment sugars and/or the presence of cytochrome C (oxidase reaction), can be isolated from water and wort. They are classified in various genera, *e.g.* *Acinetobacter, Alcaligenes* and *Pseudomonas*.

2.9.1.3 **YEASTS**

Yeasts that are not deliberately used in the brewery and not under full control are designated “wild” yeasts. Not all are harmful nor cause spoilage; nevertheless their presence indicates that contamination has occurred and this can easily lead to spoilage.

Yeasts, classified in several genera, have been isolated in breweries and implicated in beer spoilage. These include species of *Brettanomyces, Hansenula, Candida, Klockera, Pichia* and *Saccharomyces*.

Of these, *Pichia, Hansenula* and most *Candida* strains are aerobic, thus limiting their spoilage potential to beers stored in the presence of air. Under suitable conditions they grow rapidly giving rise to films on the surface of the liquid and sometimes hazes and off-flavours.

(a) **Brettanomyces**

*Brettanomyces* species are aerobic but produce large quantities of acid and have been reported to affect seriously the flavour of draught beer.

(b) **Saccharomyces**

Probably the most important and widespread wild yeasts are *Saccharomyces* species, *e.g.* *S. uvarum* (formerly *S. carlbergensis*), *S. diastaticus* and *S. pastorianus*. These strains are responsible for hazes and turbidity and, in the case of *S. diastaticus*, over-attenuation.
2.9.1.4 Moulds

Moulds grow as branching filaments (hyphae), usually between 2 and 10µm wide which interlace to form a network or mycelium. The hyphae are coenocytic (have a multinucleate continuous protoplasm) and reproduce by the formation of various kinds or sexual and asexual spores.

Moulds are not directly involved in beer spoilage but they are common contaminants of barley and other cereals, and malt. Metabolites produced during growth on the grain can later give rise to off-flavours in beers and cause the rapid evolution of carbon dioxide upon release of pressure, known as ‘gushing’.

The fungi pertinent to the brewer and maltster are almost invariably Ascomycetes and fungi imperfecti (Deuteromycetes).

(a) Ascomycetes

The principal distinguishing features of the Ascomycetes are:
(a) normally haploid;
(b) asexual reproduction by non-motile spores (conidia) formed on hyphae or on modified hyphae (conidiophores);
(c) sexual reproduction by fusion of hyphae or some similar process. This gives rise to a diploid ascus and a fruiting body or ascocarp which may develop to enclose several asci.

(b) Fungi Imperfecti

Where sexual reproduction is rare, absent or unknown, the organisms are placed in the fungi imperfecti group. These are generally imperfect forms of Ascomycetes and dual names may be given to the same organism depending on the presence or absence of the sexual stage.

The fungal flora of barley in the field includes Fusarium, Helminthosporium and Alternaria, and during storage Aspergillus and Penicillium, all fungi imperfecti.
ABSTRACT: Of the micro-organisms from air, water and raw materials that enter the brewery, some are particularly well adapted to this environment. Growth of these organisms on raw materials and during fermentation can release metabolites that can seriously affect the stability and organoleptic properties of beer. Some bacteria and yeasts can proliferate in beer to produce hazes and off-flavours. This unit reviews these micro-organisms, their occurrence and effects in the brewery, and procedures for their isolation and identification.

LEARNING OUTCOMES: On completion and comprehension of this unit you will be able to:

1. Understand the principles of detection and quantification of organisms and the applications to specific spoilage organisms.
2. Describe the use of selective media, membrane filtration and forcing tests.

PREREQUISITE UNDERSTANDING: To have studied Section 2.9.1.
2.9 Beer Spoilage Organisms

2.9.2 Detection Methods

2.9.2.1 Introduction

2.9.2.2 General Procedures

(a) Liquids

(b) Solids

(c) Swabs

2.9.2.3 Culture Media

(a) Bacteria

(b) Yeasts

(c) Moulds

2.9.2.4 Identification

(a) Bacteria

(b) Wild Yeasts

(c) Moulds
2.9 BEER SPOILAGE ORGANISMS

2.9.2 DETECTION METHODS

2.9.2.1 INTRODUCTION
The previous section reviewed the micro-organisms capable of spoiling beer, in terms of producing off-flavours and haze instability. This section describes the procedures available for the isolation and identification of these micro-organisms.

2.9.2.2 GENERAL PROCEDURES

(a) Liquids
Liquid samples should be collected aseptically and examined as soon thereafter as possible. Viable organisms are generally estimated by diluting the sample (if necessary) in sterile physiological saline or buffer and inoculating into a suitable medium.

Inoculation may be spreading 0.1 ml onto the surface of a dry agar plate (spread plate), or by placing 1 ml into a Petri dish, adding cooled (45 °C) sterile media, mixing and allowing to solidify (pour plate).
If the sample contains very low numbers of organisms, e. g from a post-pasteurisation or sterilisation stage, it should be concentrated by membrane filtration. The filter can then be placed on the surface of the medium and colonies will develop.

(b) Solids
Solids should be thoroughly washed by vigorous agitation in sterile saline or buffer. After settlement, the washings are treated as for liquid samples.

(c) Swabs
Cotton wall swabs on wire or wooden sticks can be used to assess the microbiological populations on the walls of collection vessels and other accessible areas of brewery plant.
To obtain a quantitative estimate of contamination, a defined area, (e.g. 0.5 square metre), should be thoroughly covered.
The swabs are often kept in Ringer’s solution which is then plated out as mentioned above; alternatively the swab can be used to prepare an initial inoculation on a plate. Inaccessible parts of the plant and pieces of equipment can be sampled by rinsing with sterile saline.

2.9.2.3 Culture Media

(a) Bacteria
Numerous media have been developed for the growth of brewery micro-organisms. Each brewery tends to use its own methods which have been perfected over the years and are probably ideal for that site. Only general observations about the suitability of various media can therefore be made here.

For the inhibition of yeasts and moulds, and the selective growth of bacteria, actidione (cycloheximide) can be added to media at 20 to 100 µg/ml. The lower concentration will inhibit brewing yeasts and is ideal for general purposes, the higher concentration will prevent the growth of most wild yeasts.

The Enterobacteria, including O. proteus and some miscellaneous non-fermentative bacteria, grow readily on MacConkey agar incubated at 30°C. Entrobacteria generally form colonies after 24h, but O. proteus strains take longer, 36 to 48h, and are typically lactose-negative, i.e. form pink/colourless colonies as opposed to the red, lactose-fermenting colonies of most Citrobacter, Klebsiella and Enterobacter strains.

Acetic acid bacteria can be grown on wort, beer or malt extract agar supplemented with yeast extract (1%) and incubated at 25 to 30°C, the pH should be around 4.0 and additional agar may be added to assist solidification.

Zymomonas mobilis should be incubated anaerobically at 30°C on hopped wort agar supplemented with yeast extract, or alternatively, 0.5 yeast extract, 2% glucose and agar in distilled water.
The **Lactic acid bacteria** are typically fastidious organisms and their importance as beer spoilage organisms has led to the formulation of various media for their recovery and growth.

Sucrose agar which contains yeast extract, peptone, salts and a high concentration of sucrose (5 %) has been recommended, but the single carbohydrate source may be insufficient for the detection of all lactic acid bacteria able to spoil beer, particularly those inactive ones of group IIB.

These use of two carbohydrates, maltose and glucose in a nutrient base, preferably de Man, Rogosa and Sharpe (MRS) agar, or Universal Beer Agar (UBA), or Wallerstein Laboratories Nutrient (WLN) agar enables the recovery and growth of a greater range of lactic acid bacteria.

Plates are generally incubated aerobically at 25 to 30°C.

It is sometimes useful to conduct a total count of saprophytic bacteria in raw materials, water or on brewery plant. Suitable media include: WLN (a medium comprising yeast extract, casein hydrolyse and salts); plate count agar; and a glucose-tryptone-yeast extract agar.

Aerobic incubation at 25 to 30°C will allow the growth of most bacteria and the addition of actidione will inhibit culture yeasts.

### (b) Yeasts

Three selective media are generally advocated for suppression of brewing yeasts thus allowing the detection of wild yeasts.

The inclusion of a low concentration of actidione in malt extract or some other suitable medium is generally sufficient to prevent growth of brewing yeast, but allows *Pichia*, *Torulopsis*, *Brettanomyces* and other wild yeasts to grow. The optimal concentration should be determined experimentally but 10 to 20 µg/ml is in common usage.

Lysine medium contains L-lysine as the sole major nitrogen source. A wide range of wild yeasts are able to utilise this lysine and grow normally, but *Saccharomyces* strains cannot and their growth is prevented.

Wild *Saccharomyces* strains obviously escape detection on this medium, but can be isolated on crystal violet (20 µg/ml) medium which inhibits brewing yeast but not strains of *S. pastorianus*, *S. diastaticus* and other *Saccharomyces* species.

Unfortunately, most lysine-utilising yeasts are sensitive to crystal violet so the two media are used in conjunction to detect the variety of yeasts likely to be encountered in the brewery.
(c) Moulds
For the isolation of fungi from contaminated grains or malt, suitable media include potato dextrose agar and malt extract agar supplemented with penicillin and streptomycin to inhibit bacterial growth.

2.9.2.4 IDENTIFICATION

(a) Bacteria
Data for the identification of the principal bacteria likely to be found in the brewery are provided in Table 1. A crucial aspect of most bacterial systematics is the Gram strain, which should always be conducted on a young, preferably exponential-phase culture, since many Gram-positive bacteria become Gram-negative after prolonged growth.

The scheme for the Gram-positive bacteria is generally straightforward, but the main tests will be described:

Although the Lactic acid bacteria are catalase-negative and do not cause evolution of oxygen from hydrogen peroxide solution when it is poured over a colony, some strains, particularly pediococci, produce a ‘pseudocatalase’. This enzyme decomposes hydrogen peroxide but differs from catalase in not containing a haem prosthetic group.

Anaerobic growth and fermentation of glucose distinguishes between micrococci and staphylococci. The test is performed by inoculating two tubes of a tryptone, yeast extract medium containing glucose and bromocresol purple. One of the tubes is sealed with paraffin oil to provide anaerobic conditions. Fermentative bacteria (staphylococci) produce an acid indicator change in both tubes, oxidative bacteria (micrococci) produce acid only in the top of the open tube.

The Lactobacilli can be divided into homofermentative and heterofermentative groups by inoculating a tryptone, yeast extract semi-solid agar containing glucose and pouring an agar seal over the surface. Heterofermentation gives rise to carbon dioxide that accumulates beneath the seal. Homofermentation does not. Further identification of the lactobacilli is difficult and not normally necessary.
With regard to the Gram-negative bacteria, the source of the culture and the nature of the isolation conditions provide a valuable indication towards identity. Thus the formation of acid in a medium which comprises yeast extract agar, bromocresol green and ethanol, is specific for acetic acid bacteria and the ability to ‘over-oxidise’ the acetic acid to carbon dioxide and water is typical of Acetobacter.

Zymomonas mobilis can be distinguished from Enterobacteria by its ability to grow in beer enriched with yeast extract and glucose, in which the production of hydrogen sulphide accompanied by a “boiling fermentation” is typical of this bacterium. Other useful criteria are given in Table I.B. Z. mobilis var. pommacae can be differentiated from the typical beer spoilage strains by its inability to grow in a yeast extract glucose medium containing 0.5 % NaCl.

Finally, Obesumbacterium proteus can be distinguished from other Enterobacteria by growth on MacConkey agar. Enterobacteria generally form colonies after 24h and 30 °C which are either red, due to the fermentation of lactose, or pink, due to an inability to metabolise this sugar. O. Proteus strains from minute pink colonies after 24h that become readily visible after 48h incubation. They are typically lactose-negative.

Identification of Enterobacteria is complicated and generally unnecessary.
Table 1. Identification tables for some genera of common brewery bacteria.

A. Gram-positive bacteria

<table>
<thead>
<tr>
<th></th>
<th>Lactobacillus</th>
<th>Pediococcus</th>
<th>Micrococcus</th>
<th>Staphylococcus</th>
<th>Bacillus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape</td>
<td>R</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>R</td>
</tr>
<tr>
<td>Formation of Endospores</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Fermentation of glucose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Catalase</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

B. Gram-negative bacteria

<table>
<thead>
<tr>
<th></th>
<th>Enterobacteria</th>
<th>O.proteus</th>
<th>Acetobacter</th>
<th>Gluconobacter</th>
<th>Zymomonas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid production from ethanol</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CO₂ + H₂O from ethanol</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anaerobic growth</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Key

R = rods
C = cocci
+ = > 90% strains positive
- = < 10% strains positive
± = 11 – 89 % strains positive
(b) Wild Yeasts

In most circumstances, identification of wild yeasts to genus and species level is unnecessary. However, Table 2 gives the principal taxa likely to be encountered.

Nevertheless, it is often essential to determine if a yeast is a contaminant and the selective media mentioned in 2.9.2.3 aid this problem. Further characterisation can include the recording of colonial morphology and colour on a standard medium, e.g., WLN agar and the development of a procedure for indexing the data.

Tests of actual brewing properties can be used to identify contaminants (or mutants) closely related to the culture yeast. Such tests include flocculation and head formation, performed in small scale fermentations, but are often tedious to conduct.

Table 2. Characteristics of some common yeast genera

<table>
<thead>
<tr>
<th></th>
<th>Brettanomyces</th>
<th>Candida</th>
<th>Hansenula</th>
<th>Pichia</th>
<th>Kloeckera</th>
<th>Saccharomyces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>ML</td>
<td>ML</td>
<td>ML</td>
<td>ML</td>
<td>A</td>
<td>ML</td>
</tr>
<tr>
<td>Fermentative/</td>
<td>F</td>
<td>F/O</td>
<td>O</td>
<td>O</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>aerobic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May use NO₃</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>as sole nitrogen source</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid produced</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Key

A = apiculate (polar budding only)
ML = multilateral budding (from any point on the cell wall) and may form Pseudomycelium
F = capable of fermentative growth
O = strict aerobe
+ = positive
- = negative
± = variable
(c) Moulds

Moulds are generally identified from a morphological examination of growth and spore formation.
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UNIT 2.9: Beer Spoilage Organisms

SECTION 2.9.3: Control Factors – Occurrence and Effects of Micro-organisms in the Brewery and Prevention of Contamination

ABSTRACT: Of the micro-organisms from air, water and raw materials that enter the brewery, some are particularly well adapted to this environment. Growth of these organisms on raw materials and during fermentation can release metabolites that can seriously affect the stability and organoleptic properties of beer. Some bacteria and yeasts can proliferate in beer to produce hazes and off-flavours. This unit reviews these micro-organisms, their occurrence and effects in the brewery, and procedures for their isolation and identification.

LEARNING OUTCOMES: On completion and comprehension of this unit you will be able to:

1. Describe the occurrence and effects of various micro-organisms at different stages during the brewing process.
2. Know how to prevent such contamination occurring.

PREREQUISITE UNDERSTANDING: To have studied Sections 2.9.1 and 2.9.2.
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2.9.3 Control Factors _____________________________________ 3

2.9.3.1 Occurrence and Effects of Micro-organisms in the Brewery _____________________________________________ 3
  (a) Raw Materials ______________________________________ 3
  (b) Wort ______________________________________________ 5
  (c) Fermentation _______________________________________ 5
  (d) Conditioning and Dispense ____________________________ 7

2.9.3.2 Prevention of Contamination __________________________ 8
2.9 BEER SPOILAGE ORGANISMS

2.9.3 CONTROL FACTORS

2.9.3.1 OCCURRENCE AND EFFECTS OF MICRO-ORGANISMS IN THE BREWERY

(a) Raw Materials

Water
The water used in the brewery should be microbiologically pure and conform to the standards set for public water supplies. The contamination of water is determined by screening for common intestinal bacteria, commensals that would not survive for more than a few days outside the intestine, e. g. *Escherichia coli*. These bacteria do not themselves constitute a serious hazard, but they indicate that faecal matter has entered the supply and that the water is liable to contamination with more dangerous organisms.

To search for the serious pathogens in the first instance is not practical because of their scarcity compared with *E. coli*. The presence of *E. coli* in a water supply immediately deems it unsatisfactory and the source of contamination should be traced, particularly in the case of deep well and other pure waters that are normally free of microbes.

The water should therefore be pure as it enters the brewery but thereafter there may be many sources of contamination (e. g. defective valves or taps, services reservoirs or cisterns, and packing in joints of pipes may all harbour coliforms). If a comparison of the water before and after distribution reveals wide differences in quality then the cause should be investigated and removed.

It is possible however that the water may contain a variety of saprophytic bacteria that are derived from the vegetation and the soil. These will include: pseudomonads, *Acinetobacter* and *Alcaligenes* species, and the free-living coliforms, e. g. *Klebsiella*, *Enterobacter* and *Hafnia* species.

It is necessary to distinguish these ‘atypical’ saprophytes from the ‘typical’ intestinal commensal *E. coli* during water examination. This is achieved by conducting differential counts in MacConkey broth at 44°C when only *E. coli* is able to grow, ferment the lactose and release gas at this temperature.
Although the ‘atypical’ bacteria do not represent a health hazard, they are a source of contamination in the brewery and can generally develop rapidly in wort (seldom in beer, the pH is too low and alcohol content too high) to produce phenolic and sulphur compounds that can seriously affect the final flavour of the beer.

**Barley and malt**

As cereal grains develop in the field they become colonized by a variety of bacteria and fungi. These ‘field fungi’ require the moisture content of the grain to be high (22-25% on a wet weight basis) for growth. Thus the longer the grain is subject to wet weather, the more profusely they develop, giving rise to discoloration or ‘weathered barley’. Such barley has long been implicated in the lowering of malt and beer quality. *Fusarium* species in particular have been associated with increased steeping and respiration loss, decreased rootlet growth and decreased malt recovery from barley. In the brewery, increased extract, increased soluble nitrogen in worts and beer, increased α-amylase activity and decreased beer stability (gushing) result from the use of weathered barley.

After harvest the grains are usually stored at below 13% moisture content preventing the growth of field fungi. If the moisture content rises above 13.2% spores from various xerophytic ‘storage’ fungi can germinate. *Aspergillus halophilicus* and *Aspergillus restrictus* are amongst the first to develop. The moisture resulting from their growth allows the proliferation of the less xerophytic species of *Aspergillus*. e.g. *A. amstelodami* and *A. repens* and *Penicillium* spp. Barley grain spoiled by these storage fungi becomes discoloured and presents the same problems to the maltster and brewer as those mentioned above in connection with field fungi. Fortunately, the mycotoxins secreted by many fungi during growth are largely degraded during the brewing process and there seems to be no risk of finding them in dangerous amounts in the finished beer.

**Cereal adjuncts**

These are generally grits or flakes of various cereals including wheat and maize. The mycoflora of wheat parallels that of barley: the storage flora includes *A. repens, A. amstelodami, P. cyclopium* and several other species of these genera. A similar flora has been reported on rye while members of the *A. glaucus* group often predominate on maize. Thus the use of highly contaminated cereal adjuncts could lead to some of the problems described above, although the lower rate at which they are used will reduce the effects.
Hops
The microflora of stored hops has not been studied to any extent. Hops tend to be bacteriostatic but it is possible that storage under moist conditions could give rise to fungal growth which would presumably have detrimental effects.

(b) Wort
Wort is an ideal growth medium for most Gram-negative bacteria and some hop-tolerant, Gram-positive bacteria. It should therefore never be stored at temperatures below 55°C for any length of time before pitching; even at 50 to 65°C thermophilic lactobacilli, e.g. *L. debrueckii*, and aerobic, spore-forming bacilli can grow, resulting in prompt acidification.

Even in the most scrupulously cleaned breweries, foci of bacterial infection persist in pipework, heat exchangers and collection vessels. If these bacteria, enterobacteria and miscellaneous Gram-negative rods, are allowed to develop in wort they have several detrimental effects on the flavour of the finished beer.

Most enterobacteria produce dimethyl sulphide (DMS) and other organo-sulphur compounds that have very low sensory threshold values (around 40 mg/litre) and produce objectionable, ‘parsnip-like’ odours in beer.

Furthermore, these bacteria are often associated with phenolic off-flavours arising from the decarboxylation of substituted 4-hydroxycinnamic acids from the cereals. These compounds are reduced by the yeast to yield 4-ethylphenol and 4-ethylguaiacol – and corresponding ‘smoky’ taints.

Finally, the level of fusel oils is often increased, but not significantly.

(c) Fermentation
The general practice is therefore to cool the wort as rapidly as possible and pitch with yeast. Unless the yeast is a pure culture, a variety of yeasts and bacteria will be added to the wort at this stage, despite a strict adherence to cleaning procedures.

Probably the most ubiquitous bacterium in British top-fermenting yeast is *O. proteus*. This organism presumably originates from water, but it is partly adapted to brewery conditions and is almost
invariably found amongst pitching yeasts after two or three consecutive fermentations. It grows during the early stages of the fermentation but is inhibited by the low pH and rising ethanol concentration 24-36 h after pitching. It tends to associate with the yeast, rises to the surface of the fermentation, and is consequently skimmed and repitched to the surface of the fermentation. In low numbers it has little effect, but as its population increases it has several detrimental effects, including slowing of the fermentation rate and an increase in the final pH of the beer. Flavour impairment is principally due to the production of DMS and other sulphur compounds (but not hydrogen sulphide). 

*O. proteus* is not so prevalent in larger pitching yeasts but *Ent. agglomerans* has been found and has similar effects.

Enterobacteria and other Gram-negative bacteria are rapidly inhibited during fermentation and, unless rampant growth has taken place prior to pitching, have little effect on the beer. These organisms do not typically associate with the yeast on the surface of the fermentation and are not therefore returned to subsequent fermentations.

Acetic acid bacteria can sometimes be isolated from the fermentation but the essentially anaerobic conditions preclude their growth.

Lactic acid bacteria, principally *L. brevis* and *P. damnosus*, are sometimes present in the fermentation. They do not constitute a serious threat at this stage because competition with the yeast retards their growth. Diacetyl, the most potent flavour compound they produce (taste threshold about 0.05 mg/litre), is reduced by the yeast to butane –2,3-diol (taste threshold about 500 mg/litre) and effectively removed.

The brewing yeast should be pure and free from stray, wild yeasts. This ideal state lasts for a short while and is upset by competition from two sources. First, the yeast may mutate, but any derivatives must have a selective advantage over the parent otherwise they will be rapidly lost. Such mutant strains can be a problem if they are non-flocculent or deranged in some fermentative capacity (e.g. maltotriose metabolism).

Second, contaminating yeasts originating from raw materials, the air, or brewery plant may compete with and outgrow the culture yeast. Often these yeasts are non-flocculent and give rise to troublesome hazes even when present in very low numbers. If the contaminating yeast is very small it can complicate filtration, again causing hazes.
S. diastaticus secretes glucoamylase, an exo-amylase that removes glucose residues from amylodextrins and can lead to over attenuation of beers. Several yeasts, including Bretanomyces and Candida species, can produce estery and phenolic off-flavours.

(d) Conditioning and Dispense

Beer that has been racked and is conditioning prior to packaging, or has been packaged but through some fault in filtration or pasteurisation contains viable organisms, is liable to spoilage.

The number of bacteria that can grow in the product is limited because of the low pH, presence of ethanol, absence of readily fermentable carbohydrate and anaerobic conditions. Nevertheless, the lactic acid bacteria, principally L. brevis and P. damnosus can grow in this environment, albeit slowly, and consequently affect the flavour (‘sarcina sickness’). The most important flavour compound produced is diacetyl and, since there is no yeast in suspension, it is not reduced but accumulates, imparting a sweet ‘buttery’ flavour to the beer. This can in fact be removed by the addition of yeast which reduces the diacetyl to butane-2,3-diol (krausening), a relatively innocuous compound. Some strains of L. brevis and P. damnosus can produce rope in badly contaminated beers.

Although enterobacteria do not generally grow in beer, Z. mobilis is a relatively rare contaminant of cask-conditioned ales that has become less frequent with the introduction of metal containers (rather than wood). It grows readily in beer, particularly if fermentable carbohydrate is present, and produces sulphurous, estery taints due to the release of acetaldehyde and hydrogen sulphide. Again, in the absence of yeast, these compounds accumulate, seriously impairing the organoleptic qualities of the beer.

Acetobacter and Gluconobacter strains that gain access to the beer during conditioning or storage are unlikely to cause problems so long as anaerobic conditions are maintained. Nevertheless, it should be emphasised that some strains of G. odydans require little air and reputedly grow given the air-space in the neck of a bottle. Growth, with the oxidation of ethanol into acetic acid obviously has dire consequences for the brewer and in addition voluminous rope can be produced.
Should wild yeasts, particularly Saccharomyces species, gain access to conditioning beer, films and hazes are the most common result, often with estery off-flavours.

Recent studies have shown that however microbiologically pure draught beer is at it leaves the brewery, the design and operation of dispense systems allows contamination in the pub. In one study, cask conditioned beers generally contained not only the pitching yeast but high counts (10^3 to 10^5 organisms/ml) of Candida and Torulopsis species. Moreover, virtually all samples revealed strains of Acetobacter, Gluconbacter and Zymomonas, although the levels were low and spoilage was not evident. Hansenula, Pichia and Torulopsis strains and acetic acid bacteria have been recovered from tank and keg beers. Although not all dangerous to health, these organisms could readily affect the quality of the beer if the turn-round was slow.

2.9.3.2 PREVENTION OF CONTAMINATION

Cleanliness is essential in the prevention of microbial infection. Wherever suitable nutrients (wort, sugar syrups, spent yeasts, etc) accumulate, micro-organisms will proliferate. This is particularly important in poorly designed or installed pipework, valves, coolers and other pieces of brewery equipment in which chronic contamination can occur and lead to the continual infection of wort and beer. This problem should be eliminated by attention to recommended cleaning procedures.

Cleaned and sterilised plant should contain a sufficiently low level of debris that:
(a) no residual flavours from ingredients or absorbed detergent are imparted to the brew; and
(b) a low level of micro-organisms remains on the surface.

This is generally achieved in a safe and economical way by combining a detergent and a sanitiser. Common detergents include alkalis (e.g caustic soda), complex phosphate (e.g sodium tripolyphosphate), organic compounds (e.g sodium gluconate, surfactants), and organic and mineral acids.

Each has its own characteristics, including wetting and penetration properties, organic dissolving power, rinsability and calcium salt dissolving power, and can be used in combination to a produce the desired effect.
However, they are not necessarily bacteriocidal and separate sanitiser or detergent/sanitiser is often used on plant which is likely to be contaminated by bacteria and is to be sterilised.
Sanitisers normally operate by:
(a) destruction of the micro-organism by chemical oxidation (chlorine, hydrogen peroxide, peracids, nitric acid);
(b) osmotic destruction of the cell wall (anionic surfactants, ampholytic surfactants and quaternary ammonium compounds; or
(c) poisoning (formaldehyde, sulphur dioxide, quaternary ammonium compounds and numerous organic chemicals).

Steam is expensive and dangerous but is used for difficult equipment that is inaccessible. Wet, rather than superheated steam is the most effective.

High temperature operation vessels, mash tuns, boiling coppers, lauter tuns, etc are not conducive to microbial growth and a hot caustic detergent is generally used without a sanitiser.
Wort receivers, coolers and whirlpools accumulate general debris and salts and should be cleaned with a detergent containing a high concentration of chelating agent followed by a separate sanitiser.

Collection, fermenting and lagering vessels are largely contaminated with yeast films and cleaned with a cold caustic solution followed by a sanitiser.
Similarly, bright beer tanks, keg racking units, road tankers, and cellar tank units may be treated with a mild detergent/sanitiser if the soil level is low, but the need for sterility is high if a stable product is to be maintained and a separate sanitiser may be necessary.

The application of detergents and sanitisers is as complex subject and there is no universal product for brewery plant. In-place cleaning systems have revolutionized plant hygiene but there is still a need for careful attention to detail if spoilage of beers by micro-organisms is to be prevented.