COURSE GUIDE

BIO 416
INDUSTRIAL MICROBIOLOGY

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Introduction

Industrial microbiology covers the study of the large-scale and profit motivated production of microorganisms or their products for direct use, or as inputs in the manufacture of other goods. Industrial microbiology, motivates on products such as pharmaceutical and medical compounds (antibiotics, hormones, transformed steroids), solvents, organic acids, chemical feedstocks, amino acids, and enzymes that have direct economic value. The microorganisms used by industry have been isolated from nature, and in several ways, were improved upon using classic mutation-selection procedures. Industrial microbiology is clearly a branch of biotechnology and includes the traditional and nucleic acid parts.

Industrial microbiology with the course code of BIO 416 is a three-credit course for students in the B Sc. Biology programme. The course is made up of four modules with 5 study units. It will introduce the learner to industrial microbiology. At the end of the course, the learner is expected to demonstrate clear understanding of industrial microbiology and its applications

The work of an Industrial microbiologists is hinged on the Basics of industrial microbiology, Techniques in industrial microbiology, Fermentation processes; Fermenter design and operations.

The course guide tells you briefly what the course is all about, how to work through the course material and the responsibility of studying on your own, and your overall responsibilities and expectations. The tutorial sessions are also linked up with the course to provide the needed support you require. It gives you some directions on your Tutor- Marked Assignments.

Course Competencies

This course will provide the basics of Industrial microbiology, Techniques in industrial microbiology, Fermentation processes; Fermenter design and operations.

Course Objectives

The aim of this course which is bring to your understanding of industrial microbiology as the study of large-scale profit motivated production of microorganisms or their products for direct use or as inputs in the manufacture of other goods. The course overall objective which must be achieved. In addition to the course objectives, each of the units has its own specific objectives. You are advised to read carefully the specific

objectives for each unit at the beginning of that unit. This will help you to ensure that you achieve the objectives.

As you go through each unit, you should from time to time go back to these objectives to determine the level at which you have advanced. By the time you have finished going through this course, you should be able to:

- Describe the microorganism involved in industrial microbiology.
- Mention the methods of sourcing for microorganism of industrial importance.
- Describe how microorganisms of industrial importance are preserved
- Describe the modern classification trends in microbiology.
- Explain the different methods of strain improvement
- Describe how a laboratory fermenter can be scaled up to a commercial one.
- Explain what antifoams are and the work of antifoam in fermenters
- Understand the importance of scale up and the steps involved in scale up process.

Working Through This Course

In this course, you will be advised to devote your time in reading through the material. It expedient that you do all that has been stipulated in the course: study the course units, read the recommended reference textbooks and do all the unit(s) self- assessment exercise (s) and at some points, you are required to submit your assignment (TMAs) for assessment purpose. You should therefore avail yourself of the opportunity of being present during the tutorial sessions so that you would be able to compare knowledge with your colleagues.

Study Units

This course is divided into **4 modules** with a total of **twenty-one units** which are divided as follows:

Module 1: Basics Of Industrial Microbiology

Unit 1: Nature/ Microbes of industrial microbiology
Unit 2: Some organisms commonly used in industrial microbiology
Unit 3: Taxonomic grouping of micro-organisms important in industrial microbiology and biotechnology

Unit 4: Characteristics important in microbes used in industrial microbiology and biotechnology
Unit 5: Strain improvement

Module 2: Techniques In Industrial Microbiology

Unit 1. Culture techniques
Unit 2. Isolation and Identification of Culture
Unit 3. Criteria for the choice of raw materials used in industrial media
Unit 4. Raw materials used in compounding industrial media
Unit 5 Maintenance of selected cultures

Module 3: Fermentation Processes

Unit 1. Alcoholic beverages
Unit 2. Raw Materials for Brewing.
Unit 3. Wine Production
Unit 4. Distilled alcoholic (or spirit) beverages
Unit 5. Fermented Foods I: Bread Making and Milk products
Unit 6. Fermented Foods II: Some local fermented foods

Module 4: Fermenter Design And Operations

Unit 1. Fermenter I: Types and its construction
Unit 2. Fermenter II: Anaerobic Fermenters and Continuous Culture
Unit 3. Antifoams
Unit 4. Scale up process of the fermentation process
Unit 5. Intellectual Property Rights

References And Further Readings

You would be required to do all that has been stipulated in the course: study the course units and read and refer to t h e recommended reference textbooks/ journals in each unit of the course material.

Presentation Schedule

Presentation schedule for this course will be uploaded on the online course page (Moodle page).

Assessment

You are required to submit your assignment (TMAs) for assessment purpose.

How To Get The Most From The Course

The course comes with a list of recommended textbooks/Journals. These textbooks/Journals are supplement to the course materials so that you can take the advantage of reading further. Therefore, it is advisable you acquire some of these textbooks and read them to broaden your scope of understanding.

Online Facilitation

Online facilitation for this course will hold once in a week for the period of eight weeks. The time and day for the online facilitation will be one hour as indicated in the time table

Course Information

Course Code:	BIO 416
Course Title:	Industrial Microbiology
Credit Unit:	Three (3)
Course Status:	Elective
Course Blub:	This course is designed to provide the students with basics of Industrial microbiology, Techniques in industrial microbiology, Fermentation processes; Fermenter design and operations which motivates on production of products such as pharmaceutical and medical compounds solvents, organic acids, chemical feedstocks, amino acids, and enzymes that have direct economic value
Semester:	Second Semester
Course	Duration: 13 weeks

Required Hours for Study: 91 hours

Ice Breaker

I am Prof. Obinna C. Nwinyi, Professor of Microbiology at Covenant University, Ota and external facilitator in National Open University. I facilitate and coordinate courses online such as BIO 408 and BIO 217, supervise project, moderate examination questions, review courses and mark exam scripts for National Open University. The links below are my research ID URL:

nwinyi - Nucleotide - NCBI (nih.gov)
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Module 1: Basics Of Industrial Microbiology

Unit 1: Nature/ Microbes of industrial microbiology
Unit 2: Some organisms commonly used in industrial microbiology
Unit 3: Taxonomic grouping of micro-organisms important in industrial microbiology and biotechnology
Unit 4: Characteristics important in microbes used in industrial microbiology and biotechnology
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Module 1:	**Basics of industrial microbiology**
Unit 1:	Nature/ Microbes of industrial microbiology
Unit 2:	Some organisms commonly used in industrial microbiology
Unit 3:	Taxonomic grouping of micro-organisms important in industrial microbiology and biotechnology
Unit 4:	Characteristics important in microbes used in industrial microbiology and biotechnology
Unit 5:	Strain improvement

In this module we will discuss about the Basic of the industrial Microbiology with the following units:

UNIT1: NATURE/MICROBES OF INDUSTRIAL MICROBIOLOGY

1.1 Introduction

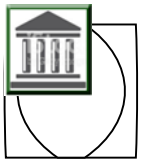
The industrial microbiology has been undertaking rapid change in recent times. Before now, Industrial microbiology has been used in manufacture of products through the use of microorganisms based on understanding of their physiology. Currently, powerful new tools and technologies especially genetic engineering, genomics, proteomics, bioinformatics bring on board new vistas for man's continued exploitation of microorganisms' processes. Some areas where these tools and technologies have been applied include food production and processing, human and animal health research, in agriculture and microbial ecology. Also, new approaches have become available for the utilization of some traditional microbial products such as immobilized enzymes and cells, site-directed mutation and metabolic engineering. Currently microbiology has been directed towards solving issues of

cancer by production of anti-tumor antibiotics and drug candidates. Exploration of new organisms towards producing new products have been directed to include unculturable organisms which are isolated mainly on genes isolated from the environment.

1.2 Intended Learning Outcomes (ILOs)

At the end of this unit, you should be able to:

- Name and describe the microorganism involved in industrial microbiology.
- Mention the methods of sourcing for microorganism of industrial importance.
- Describe how microorganisms of industrial importance are preserved.



1.3 Title of the main section

1.3.1 Nature of Biotechnology and Industrial Microbiology

Biotechnology has been defined according to United Nations Conference on Biological Diversity (also called the Earth Summit) at the meeting held in Rio de Janeiro, Brazil in 1992 as "**any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.**" Many examples abound of living things being used to make or modify processes for specific uses. Some of these include the use of microorganisms to make the antibiotic, penicillin or the dairy product, yoghurt; the use of microorganisms to produce amino acids or enzymes. Microorganisms are used in industrial microbiology to create a wide variety of products and to assist in maintaining and improving the environment. Industrial microbiology may therefore be defined as the study of the large-scale and profit motivated production of microorganisms or their products for direct use, or as inputs in the manufacture of other goods. Thus, yeasts may be produced for direct consumption as food for humans or as animal feed (single cell protein), or for use in bread-making; their product, ethanol, may also be consumed in the form of alcoholic beverages, or used in the manufacture of perfumes, pharmaceuticals, etc. Industrial microbiology is clearly a branch of biotechnology and includes the traditional and nucleic acid aspects.

In what areas can microorganisms be used in industrial microbiology?

Self-Assessment Exercises 1

Attempt the exercise to determine what you have learnt so far. 1min

i. 1. Describe industrial Microbiology?

1.3.2 Characteristics of Industrial Microbiology

The characteristics of industrial microbiology can be highlighted by comparing its features with those of another sub-division of microbiology, medical microbiology.

1.3.3 Industrial vs medical microbiology

The sub-disciplines of industrial microbiology and medical microbiology differ in at least three different ways.
First is the immediate motivation: In industrial microbiology the immediate motivation is profit and the generation of wealth. In medical microbiology, the immediate concern of the microbiologist or laboratory worker is to offer expert opinion to the doctor. The generation of wealth is of course at the back of the mind of the medical microbiologist but restoration of the patient to good health is the immediate concern.
The second difference is that the microorganisms in itself used in routine medical microbiology have little or no direct economic value, outside the contribution which they make to ensuring the return to good health of the patient who may then pay for the services. In industrial microbiology the microorganisms involved or their products are very valuable and the most important for the existence of the industrial microbiology establishment.

The third difference is the scale at which the microorganisms are handled. In industrial microbiology, the scale is large and the organisms may be cultivated in fermentors as large as 50,000 liters or larger. In routine medical microbiology the scale at which the pathogen is handled is limited to a loopful or a few milliliters. If a pathogen which normally would have no economic value were to be handled on the large scale used in industrial microbiology, it would most probably be to prepare a vaccine against the pathogen. Under that condition, the pathogen would then acquire an economic value and a profit-making potential; the operation would properly be termed industrial microbiology.

The microbiologist in an industrial establishment does not function alone. The microbiologist needs to interact constantly with other fields in the industrial setting. These include but not limited to chemical or production engineers, biochemists, economists, lawyers, marketing

experts, and other high-level functionaries. They all cooperate to achieve the purpose of the firm, which is not generate profit or wealth. The roles of a microbiologist include:

- the selection of the organism to be used in the processes;
- the choice of the medium of growth of the organism;
- the determination of the environmental conditions for the organism's optimum productivity i.e., pH, temperature, aeration, etc.
- during the actual production the microbiologist must monitor the process for the absence of contaminants, and participate in quality control to ensure uniformity of quality in the products;
- the proper custody of the organisms usually in a culture collection, so that their desirable properties are retained;
- the improvement of the performance of the microorganisms by genetic manipulation or by medium reconstitution. Mention any two ways that industrial Microbiology differ from medical microbiology.?

Self-Assessment Exercises 2

Attempt the exercise to determine what you have learnt so far. (1min).
i. The first task of an industrial microbiologist is to find a suitable microorganisms with the following characteristics except
A. Genetically stable B. Easy to maintain C. Well suited for the desired process D. highly unstable organism
ii. Which of the following is not a desired characteristic of the organism to be used for industrial application?
A. should produce less amount of product B. should be readily available C. should grow rapidly D. should be nonpathogenic

1.4 Nature of Industrial Microbiology

1.4.1 Obsolescence in Industrial Microbiology

Profit making is the motivating factor in the pursuit of industrial microbiology. Thus, less efficient methods are discarded as better ones are discovered. Indeed, a microbiological method may be discarded entirely in favor of a cheaper chemical method. For instance, ethanol which up till about 1930 was produced by fermentation however when cheaper chemical methods using petroleum as the substrate became available in about 1930, fermentation ethanol was virtually abandoned. From the mid-1970s the price of petroleum has climbed steeply. It has once again become profitable to produce ethanol by fermentation. Several countries notably Brazil, India and the United States have

officially announced the production of ethanol by fermentation for blending into gasoline as gasohol.

1.4.2 Fermentation in Industrial Microbiology

The word 'fermentation' in industrial microbiology, is any process in which micro-organisms are grown on a large scale, even if the final electron acceptor is not an organic compound (i.e. even if the growth is carried out under aerobic conditions). Thus, the production of penicillin, and the growth of yeast cells which are both highly aerobic, and the production of ethanol or alcoholic beverages which are fermentations in the physiological sense, are all referred to as fermentations.

1.5 Summary

In this unit, we have learnt some introductory concepts that will help us to the nature of industrial microbiology. Furthermore, the characteristics of industrial microbiology were highlighted. The roles microbiologists/Biologists which is to find a suitable microorganism that is of desired quality and with the unique qualities such as genetic stability and easy to maintain were discussed Also, the obsolescence in microbiology were briefly discoursed.

1.6 References/Further Readings/Web Sources

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https://www.youtube.com/watch?v=V0BzQQCCwgo
https://www.youtube.com/watch?v=IffrniNCDVk

1.7 Possible Answers to Self -Assessment Exercises

Self -Assessment Exercises 1 Answer

Industrial microbiology *may be defined as the study of the large-scale and profit motivated production of microorganisms or their products for direct use, or as inputs in the manufacture of other goods.*

Self -Assessment Exercises 2 Answer
i D
ii A

UNIT2: SOME ORGANISMS COMMONLY USED IN INDUSTRIAL MICROBIOLOGY

2.1 Introduction

Industrial microbiology, however, generally focuses on products such as pharmaceutical and medical compounds (e.g., antibiotics, hormones, and transformed steroids), solvents organic acids, chemical feedstocks, amino acids and enzymes that have economic value. The microorganism employed by industry have been isolated from nature, and in many cases, were modified using classic techniques such as mutation-selection procedures and the modern biotechnology tools. For instance, the Genetic engineering has replaced the traditional approach to developing microbial strains of industrial importance. Thus, it is of utmost importance to understand the basic nature of cells used in industrial microbiology.

2.2 Intended Learning Outcomes (ILOs)

At the end of this unit, you should be able to:

- Understand the basic nature of living things.
- Understand the modern classification trends in microbiology.
- Describe how microorganisms of industrial importance are preserved.

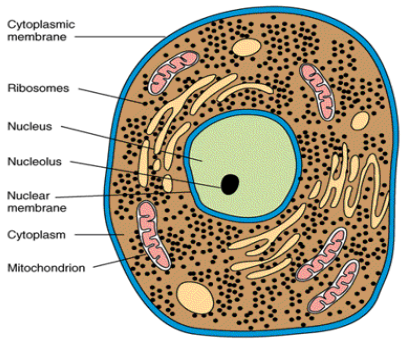
2.3 Title of the main section

2.3.1 Basic nature of cells of living things

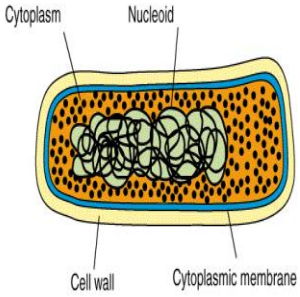
Living things are composed of cells. There are two basic types namely, prokaryotic cell and the eucaryotic cell **Figure 2.1**. The main features of typical cells of the two types include:

Cell wall: Procaryotic cell walls contain glycopeptides; these are absent in eucaryotic cells. Cell walls of eucaryotic cells contain chitin, cellulose and other sugar polymers. These provide rigidity where cell walls are present.

Cell membrane: The cell membrane is composed of a double layer of phospholipids. The cell membrane completely surrounds the cell. It is not a passive barrier, but enables the cell to actively select the metabolites it wants to accumulate and to excrete waste products.



Procaryotes



Eucaryotes

Figure 2.1 Cells of Eucaryotes and Procaryotes

***Ribosomes*:** These are the sites of protein synthesis. They consist of two sub-units. Procaryotic ribosomes are 70S and have two sub-units: 30S (small) and a 50S (large) sub-units. Eucaryotic ribosomes are 80S and have sub-units of 40S (small) and a 60S (large). (The unit S means Svedberg units, a measure of the rate of sedimentation of a particle in an ultracentrifuge, where the sedimentation rate is proportional to the size of the particle.

Mitochondria are membrane-enclosed structures where the processes of respiration and oxidative phosphorylation occur in energy release. Procaryotic cells lack mitochondria and the processes of energy release take place in the cell membrane.

Nuclear membrane surrounds the nucleus in eukaryotic cells, but is absent in procaryotic cells. In procaryotic cells only one single circular macromolecule of DNA constitutes the hereditary apparatus or genome. Eucaryotic cells have DNA spread in several chromosomes.

Nucleolus is a structure within the eucaryotic nucleus for the synthesis of ribosomal RNA. Ribosomal proteins synthesized in the cytoplasm are transported into the nucleolus and combine with the ribosomal RNA to form the small and large sub-units of the eucaryotic ribosome. What does the Unit Svedberg mean?

Self-Assessment Exercises 1

Attempt the exercise to determine what you have learnt so far. (1min).
i.What is the function of Ribosome?

2.4 Classification of living things: three domains of living things

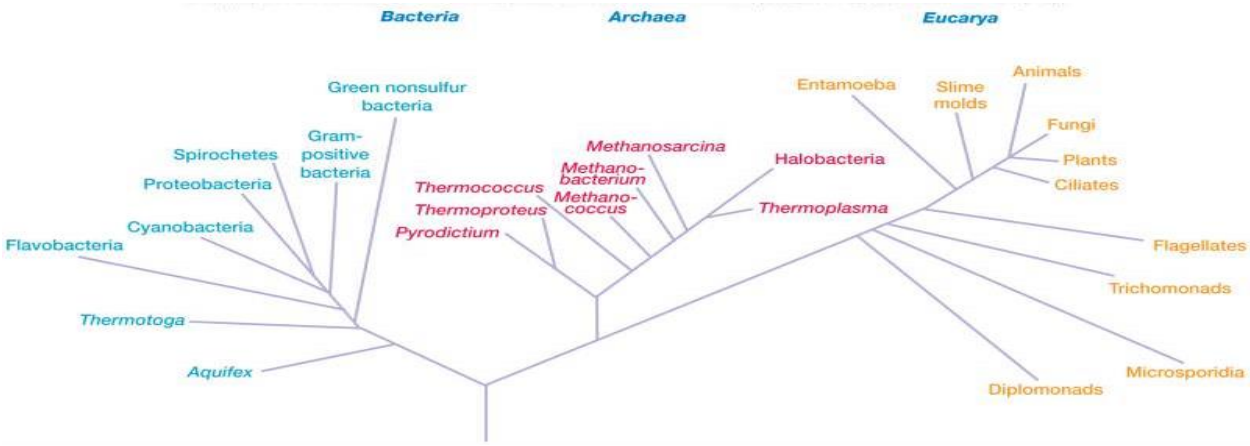
The classification of living things has evolved over time. The earliest classification placed living things into two simple categories, plants and animals. When the microscope was discovered in about the middle of the 16th century it enabled the observation of microorganisms for the first time. Living things were then divided into plants, animals and protista (microorganisms) visible only with help of the microscope. This classification subsisted from about 1866 to the 1960s. From the 1960s and the 1970s Whittaker's division of living things into five groups was the accepted grouping of living things. The basis for the classification were cell-type: procaryotic or eucaryotic; organizational level: single-

celled or multi-cellular, and nutritional type: heterotrophy and autotrophy. On the basis of these characteristics living things were divided by Whitakker into five groups: Monera (bacteria), Protista (algae and protozoa), Plants, Fungi, and Animals.

The current classification of living things is based on the work of Carl R Woese. While earlier classifications were based to a large extent on morphological characteristics and the cell type, with our greater knowledge of molecular basis of cell function, today's classification is based on the sequence of ribosomal RNA (rRNA)in the 16S of the small sub-unit (SSU) of the procaryotic ribosome, and the 18S ribosomal unit of eucaryotes. The use of the 16 S rRNA is because of the following:

- 16S (or 18S) rRNA is essential to the ribosome, an important organelle found in all living things (i.e., it is universally distributed);
- its function is identical in all ribosomes;
- its sequence changes very slowly with evolutionary time, and it contains variable and stable sequences which enable the comparison of closely related as well as distantly related species.

The classification is **evolutionary and attempts to link all livings things with evolution from a common ancestor**. For this approach, an evolutionary time-keeper is necessary. Such a time-keeper must be available to, or used by components of the system, and yet be able to reflect differences and changes with time in other regions appropriate to the assigned evolutionary distances. The 16S ribosomal RNAs meet these criteria as ribosomes are involved in protein synthesis in all living things. They are also highly conserved (remain the same) in many groups and some minor changes observed are proportionate with expected evolutionary distances.



According to the currently accepted classification living things are placed into three groups: ***Archaea, Bacteria, and Eukarya***.

Fig. 2.2 The Three Domains of Living Things Based on Woese's Work

In-Text Question (ITQ)

How long did the classification of into plants, animals and protista (microorganisms) last?
Self-Assessment Exercises 2

Answer: 1866-1960s.

Attempt the exercise to determine what you have learnt so far. (1min).
i. The current classification of living things is based on the work…… using what molecule….

ii. In 1970s Whittaker's division of living things into five groups were based on ……..

2.5 Summary:

In this unit, we have learnt the basic components of cells that are used predominantly in industry microbiology. In addition, the evolution of their classification system was discussed.

2.6 References/Further Readings/Web Sources

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https://www.youtube.com/watch?v=vwAJ8ByQH2U&t=291s

2.6 Possible Answers to Self -Assessment Exercises

Self -Assessment Exercises 1 Answer
Ribosomes: *These are the sites of protein synthesis*

Self -Assessment Exercises 2 Answer
i. Carl R Woese • 16S (or 18S) rRNA
ii. on cell-type: procaryotic or eucaryotic; organizational level: single-celled or multi-cellular, and nutritional type: heterotrophy and autotrophy

UNIT3 TAXONOMIC GROUPING OF MICRO-ORGANISMS IMPORTANT IN INDUSTRIAL MICROBIOLOGY AND BIOTECHNOLOGY

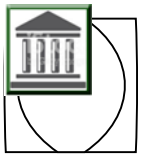
3.1 Introduction

The microorganisms currently used in industrial microbiology and biotechnology are found mainly among the bacteria and eukarya; the Archaea are not used. However, the processes used in industrial microbiology and biotechnology are dynamic and could change of over time.

3.2 Intended Learning Outcomes (ILOs)

At the end of this unit, you should be able to:

- Understand the various classes of organisms used in industrial microbiology.
- Understand the importance of microorganism over plant and animals



3.3 Title of the main section

3.3.1 Taxonomic grouping of micro-organisms important in Industrial Microbiology and Biotechnology

One of the criteria supporting the use of a microorganism for industrial purposes is the possession of properties which will enable the organism to survive and be productive in the face of competition from

contaminants.
Many organisms in Archaea are able to grow under extreme conditions of temperature or salinity and these conditions may be exploited in industrial processes where such physiological properties may put a member of the Archaea at an advantage over contaminants. Plants and animals as well as their cell cultures are also used in biotechnology.

Microorganisms have the following advantages over plants or animals as inputs in Biotechnology:

i). Microorganisms grow rapidly in comparison with plants and animals. The generation time (the time for an organism to mature and reproduce) is about 12 years in man, about 24 months in cattle, 18 months in pigs, 6 months in chicken, but only 15 minutes in the bacterium, *E. coli*. The consequence is that biotechnological products which can be obtained from microorganisms in a matter of days may take many months in animals or plants.

ii). The space requirement for growth microorganisms is small. For instance, A 100,000 litre fermentor can be housed in about 100 square yards of space, whereas the plants or animals needed to generate the equivalent of products in the 100,000 fermentor would require many acres of land.

iii). Microorganisms are not subject to the problems of the variations of weather which may affect agricultural production especially among plants.

iv). Microorganisms are not affected by diseases of plants and animals, although they do have their peculiar scourges in the form phages and contaminants, but there are procedures to contain them. Despite these advantages there are occasions when it is best to use either plant or animals; in general, however microorganisms are preferred.

Bacteria. The bacterial phyla used in industrial microbiology and biotechnology are found in the Proteobacteria, the Firmicutes and the Actinobacteria.

The Proteobacteria: The proteobacteria are a major group of bacteria. Proteobacteria include a wide variety of pathogens, such as *Escherichia*, *Salmonella*, *Vibrio* and *Helicobacter*, as well as free-living bacteria some of which can fix nitrogen. The group also includes the purple bacteria, so-called because of their reddish pigmentation, and which use energy from sun light in photosynthesis. All Proteobacteria are Gram-

negative, with an outer membrane mainly composed of lipopolysaccharides. Many moves about using flagella, but some are non-motile or rely on bacterial gliding. There is also a wide variety in the types of metabolism. Most members are facultatively or obligately anaerobic and heterotrophic, but there are numerous exceptions.

Proteobacteria are divided into five groups: (alpha), (beta), (gamma), (delta), (epsilon). The only organisms of current industrial importance in the Proteobacteria are *Acetobacter* and *Gluconobacter*, which are acetic acid bacteria and belong to the Alphaproteobacteria. An organism also belonging to the Alphaproteobacteria, and which has the potential to become important industrially is *Zymomonas*.
The bacterial phyla used in industrial microbiology and biotechnology are found in which group?

Self-Assessment Exercises 1

Attempt the exercise to determine what you have learnt so far. (1min).
i. The proteobacteria are divided into five groups namely .. …

The Acetic Acid Bacteria

The acetic acid bacteria are *Acetobacter* (peritrichously flagellated) and *Gluconobacter* (polarly flagellated). They have the following properties:

i. They carry out incomplete oxidation of alcohol leading to the production of acetic acid, and are used in the manufacture of vinegar.
ii. *Gluconobacter* lacks the complete citric acid cycle and cannot oxidize acetic acid;
Acetobacter on the on the other hand, has all the citric acid enzymes and can oxidize acetic acid further to CO_2.
iii. They tolerate acid conditions of pH 5.0 or lower.
iv. Their property of 'under-oxidizing' sugars is exploited in the following:

a. The production of glucoronic acid from glucose, galactonic aicd from galactose and arabonic acid from arabinose; The production of sorbose from sorbitol by acetic acid bacteria an an important stage in the manufacture of ascorbic acid (also known as Vitamin **C)**

The Firmicutes

The Firmicutes are a division of bacteria, all of which are Gram-positive, in contrast to Proteobacteria which are all Gram-negative. The

mycoplasmas belong to this group however they lack cell walls altogether and so do not respond to Gram staining, but still lack the second membrane found in other Gram-negative forms; consequently, they are regarded as Gram-positive. The Firmicutes tend to be restricted to a core group of related forms, called the low G+C group. The G+C ratio is an important taxonomic characteristic used in classifying bacteria. It is the ratio of Guanine and Cytosine to Guanine, Cytosine, Adenine, and Thymine in the cell. Thus, the GC ratio = G+C divided by G+C+A+T x 100. It is used to classify Gram-positive bacteria: low G+C Gram-positive bacteria (ie those with G+C less than 50%) are placed in the Firmicutes, while those with 50% or more are in Actinobacteria (the high G+C group). **Firmicutes contain many bacteria of industrial importance and are divided into three major groups**: i. spore-forming, - Spore-forming Firmicutes form internal spores, The group is divided into two: *Bacillus* spp, which are aerobic and *Clostridium* spp which are anaerobic.
ii. Non-spore forming- The non-spore forming low G+C members of the firmicutes group are very important in industry as they contain the lactic acid bacteria -The lactic acid bacteria are rods or cocci placed in the following genera: *Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus* and *Streptococcus* and are among some of the most widely studied bacteria because of their importance in the production of some foods, industrial and pharmaceutical products. Lactic acid bacteria are divided into two major groups: The **homofermentative group**, which produce lactic acid as the sole product of the fermentation of sugars, and the **heterofermentative,** which besides lactic acid also produce ethanol, as well as CO_2. and iii) wall-less (this group contains pathogens and no industrial organisms).

Use of Lactic Acid Bacteria for Industrial Purposes:

The desirable characteristics of lactic acid bacteria as industrial microorganisms include:
a. their ability to rapidly and completely ferment cheap raw materials,
b. their minimal requirement of nitrogenous substances
c. they produce high yields of the much-preferred stereo specific lactic acid
d. ability to grow under conditions of low pH and high temperature, and
e. ability to produce low amounts of cell mass as well as negligible amounts of other byproducts.
The choice of a particular lactic acid bacterium for production primarily depends on the carbohydrate to be fermented.

The Actinobacteria: The Actinobacteria are the Firmicutes with G+C content of 50% or higher. They derive their name from the fact that many members of the group have the tendency to form filaments or hyphae

(*actinis*, Greek for ray or beam).
The Lactic acid bacteria are divided into two major groups?

Self-Assessment Exercises 2

Attempt the exercise to determine what you have learnt so far. (1min).

ii. ***Lactic acid bacteria are divided into two major groups namely.***

The Actinomycetes

They have branching filamentous hyphae, which somewhat resemble the mycelia of the fungi, among which they were originally classified. In fact, they are unrelated to fungi, but are regarded as bacteria for the following reasons. First, they have petidoglycan in their cell walls, and second, they are about 1.0µ in diameter (never more than 1.5µ), whereas fungi are at least twice that size in diameter. As a group the actinomycetes are unsurpassed in their ability to produce secondary metabolites which are of industrial importance, especially as pharmaceuticals. The best-known genus is Streptomyces, from which many antibiotics as well as non-anti-microbial drugs have been obtained. The actinomycetes are primarily soil dwellers.

Fungi

Fungi are members of the Eucarya which are commonly used in industrial production. The fungi are traditionally classified into the four groups namely: Phycomycetes, Ascomycetes, Fungi Imperfecti, and Basidiomycetes. Among these the following are those currently used in industrial microbiology Phycomycetes (Zygomycetes) *Rhizopus* and *Mucor* are used for producing various enzymes Ascomycetes. Yeasts are used for the production of ethanol and alcoholic beverages *Claviceps purperea* is used for the production of the ergot alkaloids.

3.4 Summary:

In this unit, we have learnt the basic components of cells that are used predominantly in industry microbiology. In addition, the evolution of their classification system was discussed.

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https://www.youtube.com/watch?v=vwAJ8ByQH2U&t=291s

3.6 Possible Answers to Self -Assessment Exercises

Self -Assessment Exercises 1 Answer

i)Proteobacteria are divided into five groups: (alpha), (beta), (gamma), (delta), (epsilon).

Self -Assessment Exercises 2 Answer

i) homofermentative group, which produce lactic acid as the sole product of the fermentation of sugars, and ii) the heterofermentative, which besides lactic acid also produce ethanol, as well as CO_2.

UNIT4: CHARACTERISTICS IMPORTANT IN MICROBES USED IN INDUSTRIAL MICROBIOLOGY AND BIOTECHNOLOGY

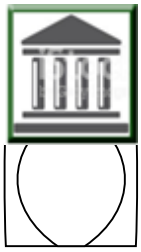
4.1 Introduction

The characteristics important in microbes used in industrial microbiology and biotechnology are topical in their selection for industrial uses.

4.2 Intended Learning Outcomes (ILOs)

At the end of this unit, you should be able to:

- Learn some of the characteristics of microorganisms used in industrial microbiology.
- Understand the reasons why some characters a preferred than others.



4.3 Title of the main section

4.3.1 Characteristics important in microbes used in Industrial microbiology and biotechnology

Microorganisms which are used for industrial production must meet certain requirements.

i) The organism must be able to grow in a simple medium and

should preferably not require growth factors (i.e. pre-formed vitamins, nucleotides, and acids) outside those which may be present in the industrial medium in which it is grown. It is obvious that extraneous additional growth factors may increase the cost of the fermentation and hence that of the finished product.

ii). The organism should be able to grow vigorously and rapidly in the medium in use. A slow growing organism no matter how efficient it is, in terms of the production of the target material, could be a liability. The slow rate of growth of some microorganisms exposes it, in comparison to other equally effective producers which are faster growers, to a greater risk of contamination. Second, the rate of the turnover of the production of the desired material is lower in a slower growing organism and hence capital and personnel are tied up for longer periods, with consequent lower profits.

iii. Not only should the organism grow rapidly, but it should also produce the desired materials, whether they be cells or metabolic products, in as short a time as possible.

iv. Its end products should not include toxic and other undesirable materials, especially if these end products are for internal consumption.

v. The organism should have a reasonable genetic, and hence physiological stability. An organism which mutates easily is an expensive risk. It could produce undesired products if a mutation occurred unobserved. The result could be reduced yield of the expected material, production of an entirely different product or indeed a toxic material. None of these situations is a help towards achieving the goal of the industry, which is the maximization of profits through the production of goods with predictable properties to which the consumer is accustomed.

vi. The organism should lend itself to a suitable method of product harvest at the end of the fermentation. If for example a yeast and a bacterium were equally suitable for manufacturing a certain product, it would be better to use the yeast if the most appropriate recovery method was centrifugation. This is because while the bacterial diameter is approximately 1µ, yeasts are approximately 5µ. Assuming their densities are the same, yeasts would sediment 25 times more rapidly than bacteria. The faster sedimentation would result in less expenditure in terms of power, personnel supervision etc which could translate to higher profit.

vii. Wherever possible, organisms which have physiological requirements which protect them against competition from contaminants should be used. An organism with optimum productivity at high temperatures, low pH values or which is able to elaborate agents inhibitory to competitors has a decided advantage over others. Thus, a thermophilic efficient producer would be preferred to a mesophilic one.

viii. The organism should be reasonably resistant to predators such as *Bdellovibrio* spp or bacteriophages. It should therefore be part of the fundamental research of an industrial establishment using a phage-susceptible organism to attempt to produce phage-resistant but high yielding strains of the organism.

ix. Where practicable the organism should not be too highly demanding of oxygen as aeration (through greater power demand for agitation of the fermentor impellers, forced air injection etc) contributes about 20% of the cost of the finished product.

x. The organism should be fairly easily amenable to genetic manipulation to enable the establishment of strains with more acceptable properties.

In industrial microbiology an organism which mutates easily is an expensive risk? True or False

Attempt the exercise to determine what you have learnt so far. (1min).
i Why is it important that any selected microorganisms for industrial purpose should have a reasonable genetic, and hence physiological stability?

Self-Assessment Exercises 1

4.4 FINDING MICROORGANISMS IN NATURE

Microbial cultures used in industrial microbiology was often obtained from natural materials such as soil samples, waters and spoiled bread and fruit. Culture from all areas of the world continues to be examined to identify new strains with desirable characteristics. Hunting for new microorganisms is known as bioprospecting.
Organisms can then be picked out especially if some means has been devised to select them. Selection could, for instance, be based on the ability to cause clear zones in an agar plate as a result of the dissolution of particles of the substrate in the agar. In the search for -amylase producers, the soil may be enriched with starch and subsequently suitable soil dilutions are plated on agar containing starch as the sole carbon source. Clear halos form around starch-splitting colonies against

a blue background when iodine is introduced in the plate.
Microbial cultures used in industrial microbiology was often obtained from……

Self-Assessment Exercises 2

Attempt the exercise to determine what you have learnt so far. (1min).
i. What is bioprospecting?

4.5 Summary:

In this unit, brought to fore the characteristics and reasons why some attributes of some microorganisms are important in industrial microbiology.

4.6 References/Further Readings/Web Sources

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4.7 Possible Answers to Self -Assessment Exercises

Self -Assessment Exercises 1 Answer
An organism which mutates easily is an expensive risk. It could produce undesired products if a mutation occurred unobserved

Self -Assessment Exercises 2 Answer
Bioprospecting is when cultures from all areas of the world continues to be examined to identify new strains with desirable characteristics

UNIT5: STRAIN IMPROVEMENT

5.1 Introduction

The strain improvement appears to be the one single factor with the greatest potential for contributing to greater profitability in any industrial processes.

5.2 Intended Learning Outcomes (ILOs)

At the end of this unit, you should be able to:

- The different methods of strain improvement.
- Understand the strategies for strain improvement

1.3 Title of the main section

5.3.1 Strain improvement

To recognize the importance of strain improvement it is important to remember that the ability of any organism to make any particular product is predicated on its capability for the secretion of a particular set of enzymes. The production of the enzymes, themselves depends ultimately on the genetic make-up of the organisms. Improvement of strains can therefore be:

(i) regulating the activity of the enzymes secreted by the organisms;
(ii) in the case of metabolites secreted extracellularly, increasing the permeability of the organism so that the microbial products can find this way more easily outside the cell;
(iii) selecting suitable producing strains from a natural population;
(iv) manipulation of the existing genetic apparatus in a producing organism;
(v) introducing new genetic properties into the organism by recombinant DNA technology or genetic engineering.

Selection from Naturally Occurring Variants

Selection from natural variants is a regular feature of industrial microbiology and biotechnology. For example, in the early days of antibiotic production the initial increase in yield was obtained in both penicillin and griseofulvin by natural variants producing higher yields in submerged rather than in surface culture. Another example is lager beer manufacture where the constant selection of yeasts that flocculate eventually gave rise to strains which are now used for the production of the beverage. Similarly in wine fermentation yeasts were repeatedly taken from the best vats until yeasts of suitable properties were obtained.

5.4 Manipulation of the Genome of Industrial Organisms in Strain Improvement

The manipulation of the genome for increased productivity may be done in one of two general procedures:
(a) manipulations not involving foreign DNA;
(b) manipulations involving foreign DNA.

5.4.1. Methods not involving foreign DNA

1. Conventional mutation

5.4.2. Methods involving DNA foreign to the organism (i.e. recombination)

2. Transduction
3. Conjugation
4. Transformation
5. Heterokaryosis
6. Protoplast fusion
7. Genetic engineering
8. Metabolic engineering
9. Site-directed mutation

5.4.1 Genome manipulations not involving Foreign DNA or Bases:

Conventional Mutation

The properties of any microorganism depend on the sequence of the four nucleic acid bases on its genome: adenine (A), thymine (T), cytosine (C), and guanine (G). arrangement of these DNA bases dictates the distribution of genes and hence the nature of proteins synthesized. A mutation can therefore be described as a change in the sequence of the bases in DNA (or RNA, in RNA viruses). It is clear that since it is the sequence of these bases which is responsible for the type of proteins (and hence enzymes) synthesized, any change in the sequence will lead ultimately to a change in the properties of the organism.
Mutations occur spontaneously at a low rate in a population of microorganisms. It is
this low rate of mutations which is partly responsible for the variation found in natural
populations. An increased rate can however be induced by mutagens, (or mutagenic
agents) which can either be physical or chemical.
Physical agents
(i) ionizing radiations:
(ii) ultraviolet light

(i) Ionizing radiations: X-rays, gamma rays, alpha-particles and fast neutrons are ionizing radiations and have all been successfully used to induce mutation. Ionizing radiations are so called because they knock off the outer electrons in the atoms of biological materials (including DNA) thereby causing ionization in the molecules of DNA. As a result, highly reactive radicals are produced and these cause changes in the DNA.

(ii) Ultraviolet light: The mutagenic range of ultraviolet light lies between wave length 200 and 300 nm. The main effect of ultraviolet light on DNA is the formation of covalent bonds between adjacent pyrimidine (thymine and cytosine) bases. Thymine is mainly affected, and hence the major effect of UV light is thymine dimerization, although it can also cause thymine-cytosin and cytosin-cytosin dimers.

Chemical mutagens
These may be divided into three groups:
(i) Those that act on DNA of resting or non-dividing organisms;
(ii) DNA analogues which may be incorporated into DNA during replication;

(iii) Those that cause frame-shift mutations.

i. Chemicals acting on resting DNA

Some chemical mutagens, such as nitrous acid and nitrosoguanidine work by causing chemical modifications of purine and pyrimidine bases that alter their hydrogen bonding properties. For example, nitrous acid converts cytosine to uracil which then forms hydrogen bonds with adenine rather than guanine. These chemicals act on the non-dividing cell and include nitrous acid, alkylating agents and nitrosoguanidine (NTG)

ii. Base analogues

These are compounds which because they are similar to base nucleotides in composition may be incorporated into a dividing DNA in place of the natural base.

(**iii) Frameshift mutagens (also known as intercalating agents) Frameshift or intercalating agents** are planar three-ringed molecules that are about the same size as a nucleotide base pair. During DNA replication, these compounds can insert or intercalate between adjacent base pairs thus pushing the nucleotides far enough apart that an extra nucleotide is often added to the growing chain during DNA replication. A mutation of this sort changes all the amino acids downstream and is very likely to create a nonfunctional product since it may differ greatly from the normal protein.

Choice of mutagen

Mutagenic agents are numerous but not necessarily equally effective in all organisms. When one agent fails to produce mutations then another should be tried. Other factors besides effectiveness to be borne in mind are

(a) the safety of the mutagen: many mutagens are carcinogens,
(b) simplicity of technique, and
(c) ready availability of the necessary equipment and chemicals.

Among physical agents, UV is to be preferred since it does not require much equipment, and is relatively effective and has been widely used in industry.

In-Text Question (ITQ)

Improvement of strains can therefore be explained as…..

Answer: (i) regulating the activity of the enzymes secreted by the organisms;

(ii) in the case of metabolites secreted extracellularly, increasing the permeability of the organism so that the microbial products can find this way more easily outside the cell;

(iii) selecting suitable producing strains from a natural population;
(iv) manipulation of the existing genetic apparatus in a producing organism;
(v) introducing new genetic properties into the organism by recombinant DNA technology or genetic engineering

Self-Assessment Exercises 1

Attempt the exercise to determine what you have learnt so far. (1min).

i. What strain improvement?

5.4.2 Improvement Methods Involving Foreign DNA or Bases

Transduction

Transduction is the transfer of bacterial DNA from one bacterial cell to another by means of a bacteriophage. In this process a phage attaches to, and lyses, the cell wall of its host. It then injects its DNA (or RNA) into the host.

Transformation

Transformation is a change in genetic property of a bacterium which is brought about when foreign DNA is absorbed by, and integrates with the genome of, the donor cell. Cells in which transformation can occur are 'competent' cells**.**

Conjugation

Conjugation involves cell to cell contact or through sex pili (singular, pilus) and the transfer of plasmids. Conjugation involves a donor cell which contains a particular type of conjugative plasmid, and a recipient cell which does not. The donor strain's plasmid must possess a sex factor as a prerequisite for conjugation; only donor cells produce pili. The sex factor may on occasion transfer part of the hosts' DNA.

Parasexual recombination

Parasexuality is a rare form of sexual reproduction which occurs in some fungi. In parasexual recombination of nuclei in hyphae from different strains fuse, resulting in formation of new genes. Parasexuality is important in those fungi such as *Penicillium chrysogenum* and *Aspergillus niger* in which no sexual cycles have been observed. It has been used to select organisms with higher yields of various industrial product such as phenoxy methyl penicillin, citric acid, and gluconic acid.

Protoplast fusion

Protoplasts are formed from bacteria, fungi, yeasts and actinomycetes when dividing cells are caused to lose their cell walls. Protoplasts may be produced in bacteria with the enzyme lysozyme, an enzyme found in tears and saliva, and capable of breaking the α-1-4 bonds linking the building blocks of the bacterial cell wall. Protoplast fusion enables recombination in strains without efficient means of conjugation such as actinomycetes. It has also been used previously to produce plant recombinants. The technique involves the formation of stable protoplasts, fusion of protoplasts and subsequent regeneration of viable cells from the protoplasts. Fusion from mixed populations of protoplasts is greatly enhanced by the use of polyethylene glycol (PEG).
Protoplast fusion has been successfully done with *Bacillus subtilis* and *B. megaterium* and among several species of *Streptomyces* (*S. acrimycini, S. olividans, S. pravulies*) has been done between the fungi *Geotrichum* and *Aspergillus*. The method has great industrial potential and experimentally has been used to achieve higher yields of antibiotics through fusion with protoplasts from different fungi.

Site-directed mutation

The outcome of conventional mutation which we have discussed so far, is random, the result being totally unpredictable. Recombinant DNA technology and the use of synthetic DNA now make it possible to have mutations at specific sites on the genome of the organism in a technique known as Site-Directed Mutagenesis. The mutation is caused by in vitro change directed at a specific site in a DNA molecule.

Metabolic engineering

Metabolic engineering is the science which enables the rational designing or redesigning of metabolic pathways of an organism through the manipulation of the genes so as to maximize the production of biotechnological goods. In metabolic engineering, existing pathways are modified, or entirely new ones introduced through the manipulation of the genes so as to improve the yields of the microbial product, eliminate or reduce undesirable side products or shift to the production of an entirely new product.

Genetic engineering

Genetic engineering, also known as recombinant DNA technology, molecular cloning or gene cloning. has been defined as the formation of new combinations of heritable material by the insertion of nucleic acid

molecules produced by whatever means outside the cell, into any virus, bacterial plasmid or other vector system so as to allow their incorporation into host organisms in which they do not naturally occur but in which they are capable of continued propagation.

Plasmids

Plasmids are circular DNA molecules with molecular weights ranging from a few million to a few hundred million Daltons. Plasmids appear to be associated with virtually all known bacterial genera. They replicate within the cell. Some of the larger plasmids, known as conjugative plasmids, carry a set of genes which promote their own transfer in a sexual process known as conjugation. plasmids usually carry genes for antibiotic or heavy metal resistance. They often also carry genes for the production of toxins, bacteriocins, antibiotics, and unusual metabolites.

Table 1 Products that have improved using DNA Manipulations

Property or Product Improved	**Microorganism Used**	**Process used**
Ethanol production	*Escherichia coli*	Integration of pyruvate decarboxylase and alcohol dehydrogenase II from *Zymomonas mobilis*
1, 3 – propanediol Production	*E. coli*	Introduction of genes from the *Klebsiella pneumonia* dha region into E. coli make possible anaerobic 1, 3 – propanediol production
Cephalosporin precursor synthesis	*Penicillium chrysogenum*	Production of 7 – ADC and 7 ADCA precursors by incorporation of expandase gene of *Cephalosoporin acrenomium* into *Penicillium*

		by transformation
Lactic acid production	*Saccharomyces Cerevisiae*	A muscle borine lactate dehydrogenase gene (LDH-A) expressed in *S. cerevisiae*
Xylitol production	*S. cerevisiae*	95% xylitol conversion from xylose was obtained by transforming the XYL/ gene of Pichia stipitis encoding a xylose reductase into *S. cerevisiae* making this organism for the production of xylitol, which serves as sweetener in food industries
Creatininase	*E. coli*	Expression of the creatininase gene from *Pseudomonas putida* R565. Gene inserted in a plasmid vector
Pediocin	*S. cerevisiae*	Expression of bacteriocin from *Pediococcus acidilactici* in *S. cerevisiae* to inhibit wine contaminants
Acetone and butanol production	*Clostridium acetobutylicum*	Introduction of a shuttle vector into *C. acetobutylicum* results in acetone and butanol formation.

7 – ACA = 7 – aminocephalosporanic acid,7 – ADCA = 7 – aminodecatoxycephalosporanic acid

Adapted from S. Ostergard, L. Olsson, and J. Nelson 2000. Metabolic

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5.4.3 Molecular Biology and Bioinformatics relevance in Industrial Microbiology and Biotechnology

New approaches anchored on developments in molecular biology have been followed in many industrial microbiology processes and products such as vaccines, the search for new antibiotics, and the physiology of microorganisms.
In conjugation only donor cells produce pili True or False?

Self-Assessment Exercises 2

Attempt the exercise to determine what you have learnt so far. (1min).
i. Briefly explain the how strain improvement can be carried out?

5.5 Summary

In this unit we have learnt some introductory concepts that will help us to understand industrial microbiology. These are sourcing for the microorganisms of interest and its modification/improvement. The first task of an industrial microbiologist is to find a suitable microorganism that is of desired quality and with the following characteristics genetically, stable, easy to maintain, well suited for extraction or separation of desired products.

Microbial cultures are obtained from natural materials such as soil, samples, water, spoiled food and fruits. The sourcing for new strains of microbes with desired of character is known as bioprospecting. Various methods are used in manipulating microorganisms genetically which are Transduction Conjugation, Transformation, Heterokaryosis, Protoplast fusion, and mutagenesis, transfer of genetic information between different organisms, modification of gene expression and protein evolution. Each of these manipulative methods have unique advantages on each microorganism undergoing the method.

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5.7 Possible Answers to Self -Assessment Exercises

Self -Assessment Exercises 1 Answer

Improvement of strains can therefore be put down in simple term as follows:

(i) regulating the activity of the enzymes secreted by the organisms;

(ii) in the case of metabolites secreted extracellularly, increasing the permeability of the organism so that the microbial products can find this way more easily outside the cell;

(iii) selecting suitable producing strains from a natural population;

(iv) manipulation of the existing genetic apparatus in a producing organism;

(v) introducing new genetic properties into the organism by recombinant DNA technology or genetic engineering.

Self -Assessment Exercises 2 Answer

Briefly explain the how strain improvement can be carried out?

Answer: *The manipulation of the genome for increased productivity may be done in one of two general procedures:*

(a) manipulations not involving foreign DNA;

(b) manipulations involving foreign DNA.

A. Methods not involving foreign DNA

- *Conventional mutation*

B. Methods involving DNA foreign to the organism (i.e. recombination)

- *Transduction*
- *Conjugation*
- *Transformation*
- *Heterokaryosis*
- *Protoplast fusion*
- *Genetic engineering*
- *Metabolic engineering*
- *Site-directed mutation.*

Glossary

7 – ACA = 7 – aminocephalosporanic acid

7 – ADCA = 7 – aminodecatoxycephalosporanic acid

CO_2 = Carbon dioxide

DNA = Deoxyribonucleic acid

O_2 = Oxygen

rRNA = ribosomal RNA

SSU = small sub-unit,

End of the module Questions

1). rDNA Technology procedure has a great application in strain improvement? (**True or False**)

2). Protoplast fusion is a technique where the whole genome is transferred from one organism to another one **(True or False)**

3). The organism to be used must be able to produce appreciable amount of the product. It should be readily available and should grow rapidly and vigorously. It should be nonpathogenic **(True or False)**

4). Restriction endonuclease cut double-stranded DNA molecules at particular nucleotide sequences and thus produce a well-defined DNA fragment for a given enzyme and a given DNA. **(True or False)**

5) Transfection involves the introduction of plasmid hybrid DNA into the host cell **(True or False)**.

6) Explain how industrial microbiology and medical microbiology differ in at least three different ways.

7) Discuss the attributes that make acetic acid bacteria are *Acetobacter* (peritrichously flagellated) and *Gluconobacter* essential in industrial microbiology

8) Explain the criteria used by Whittaker's in classifying living things into five groups

Module 2:	**Techniques in industrial microbiology**
Unit 1.	Culture techniques
Unit 2.	Isolation and Identification of Culture
Unit 3.	Criteria for the choice of raw materials used in industrial media
Unit 4.	Raw materials used in compounding industrial media
Unit 5	Maintenance of selected cultures

UNIT 1. Culture techniques

Contents

1.0 Introduction

The medium used for growing a microorganism is critical because it can determine the extent of microbial growth and product formation. Thus, the use of a good, adequate, and industrially usable medium is as important as the deployment of a suitable microorganism in industrial microbiology. Unless the medium is adequate, no matter how distinctively productive the organism is, it will not be possible to harness the organism's full industrial potentials. Indeed, not only might the production of the desired product be reduced but toxic materials may be produced. Liquid media are generally employed in industry because they require less space, are more amenable to engineering processes, and eliminate the cost of providing agar and other solid agents.

1.2. Intended Learning Outcomes (ILOs)

At the end of this unit, you should be able to:

- Explain the types of media used in microbiology.
- Describe the ways of formulating these media.
- Explain the sources of these media.

1.3 Main content

1.3.1 Culture medium/History.

A culture medium is a substrate containing nutrients that can support the growth of microorganisms. Culture media may be liquid (broth) or solid agar. Media can be synthetic, semi synthetic or natural. There are different types of media

History
The original media used by Louis Pasteur – urine or meat broth. Growth in a Liquid medium usually exhibit diffuse growth while solid medium exhibits discrete colonies.
A medium could be of:

- **Natural culture media**: are prepared from biological materials e.g. potatoes, yam or meat. The chemical compositions of natural media are unknown.
- **Synthetic culture media:** referred to as artificial media are specially compounded. So, their chemical compositions are known. They are prepared with known quantities of each chemical constituent eg Sabouraud dextrose broth.
- **Semi-synthetic culture media**: are those whose chemical compositions are known but any biological material e.g. agar whose chemical composition is unknown is added to the medium.

1.3.2 Types of culture media.

Culture media can be classified based on their consistency, constituents/ingredients/ special media, and Based on oxygen requirements.

Based on their consistency
a) solid medium
b) liquid medium
c) semi solid medium

Based on the constituents/ ingredients
a) simple medium
b) complex medium
c) synthetic or defined medium
d) Special media

Special media
- Enriched media

- Enrichment media
- Selective media
- Indicator media
- Differential media
- Sugar media
- Transport media
- Media for biochemical reactions

Based on Oxygen requirement

- Aerobic media
- Anaerobic media

Based on consistency:

The Solid media – contains 2% agar. On it the colony morphology, pigmentation, hemolysis can be appreciated: Nutrient agar, Blood agar
Liquid media contains no agar. Eg: Nutrient broth
Semi solid medium contains 0.5% agar. Eg: Motility medium
What is the original media used by Louis Pasteur?

Self-Assessment Exercises 1

Attempt the exercise to determine what you have learnt so far. (1min).
i A culture media can be made of

ii. Differentiate between a. Liquid medium and solid medium ?

Nutrient Agar (NA). The NB consists of peptone, meat extract, NaCl, while the Nutrient Agar comprises of Nutrient broth and 2% agar.

Complex media: These are media other than basal media. They have added ingredients. It provides special nutrients.

Synthetic or defined media: The media is prepared from pure chemical substances and its exact composition is known. Eg: peptone water – 1% peptone + 0.5% NaCl water.

Special Media include the following:

Enrichment media

Blood and other special nutrients may be added to general purpose media to encourage the growth of fastidious microbes. These specially fortified media (e.g. blood agar) are called enrichment media.

General purpose media
Media such as tryptic soy broth and tryptic soy agar are called general purpose media because they sustain the growth of many microorganisms.

Selective media

This media favours the growth of particular microorganisms. For example, bile salt or dyes like basic fucshin and crystal violets favour the growth of gram –ve bacteria by inhibiting the growth of gram +ve bacteria. Others are endo agar, eosin, methylene blue agar and Mac Conkey agar.

Differential media

These media are those that distinguish among different groups of microbes and ever permit tentative identification of microorganisms based on their biological characteristics. Blood agar is both a differential medium and an enriched one. It distinguishes between hemolytic and non-hemolytic bacteria.

Indicator media

These media contain an indicator which changes its colour when a bacterium grows in them. Eg: Blood agar; Mac Conkey's medium; Christensen's urease medium.

1.4 Summary

In this unit we have learnt about the history, the types of culture media used in industrial microbiology. Culture media can be classified based on their consistency, constituents/ingredients/ special media, and based on oxygen requirements. These media are used to culture wide variety of microbes.

1.5 References/further readings/Web Sources

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1.6 Possible Answers to Self -Assessment Exercises

Self -Assessment Exercises 1 Answer

Natural, semi-synthetic and synthetic media.

Self-Assessment Exercise 2: Solid media – contains 2% agar. It elaborates Colony morphology, pigmentation, and hemolysis. Eg: Nutrient agar, Blood agar

Liquid media – no agar. For inoculum preparation, Blood culture, for the isolation of pathogens from a mixture.

Unit 2: Isolation and Identification of Culture

2.1 Introduction:

This unit covers the isolation and identification techniques cultures within the laboratory. Basic nutrients required for industrial media were discussed.

2.2. Intended Learning Outcomes (ILOs)

At the end of this unit, you should be able to:

- Explain the types of culture techniques used in microbiology.
- The basic nutrient requirements of industrial media.

2.3 Main content: Isolation and identification techniques cultures

The isolation and identification of microbes in natural sources such as food, water, blood and medical samples often require the use of complex media of selective, differential etc in most case, **the pour, spread and streak plates**. Plate techniques have become indispensable tools of the microbiologist in isolating and subsequent identification of a microbial species.

2.3.1 Spread plate technique

If a mixture of cells is spread out on an agar surface, using a specially shaped rod every cell grows into a completely separate colony. Because

each colony arises from a single cell, each colony represents a pure culture. The spread plate is an easy, direct way of achieving this result.

2.3.2 Streak plate technique

The streak plate techniques use an inoculating loop to spread cells across an agar surface. The microbial mixture is transferred to the edge of an agar plate with an inoculating loop or swab and then streaked out over the surface in one of several patterns. After the first sector is streaked, the inoculating loop is sterilized and inoculums for the second sector are obtained from the first sector this is done for the other sectors. Eventually very few cells will be on the loop and single cells will drop from it as it is rubbed along the agar surface.

2.3.3 The pour plate technique:

The original sample is diluted several times to reduce the microbial population sufficiently to obtain separate colonies when plating. Then small volumes of several diluted samples are mixed with liquid agar that has been cooled to about 45^{o}C, and the mixtures are poured immediately into sterile culture plates. Like the spread plates, the pour plates can be used to determine the number of cells in a population.
The streak plate technique involves the use of an inoculating loop to spread cells across an agar surface? True or False

Self-Assessment Exercises 1

Attempt the exercise to determine what you have learnt so far. (1min).
i Mention the types of plates techniques you know?

2.4 The basic nutrient requirements of industrial media.

All microbiological media, whether for industrial or for laboratory purposes must satisfy the needs of the organism in terms of carbon, nitrogen, minerals, growth factors, and water. In addition, they must not contain materials which are inhibitory to growth. The three major groups of heterotrophic organisms usually grown on an industrial scale include the bacteria, yeast and molds.

Carbon or energy requirements are usually met from carbohydrates, notably (in laboratory experiments) from glucose. It must be borne in mind that more complex carbohydrates such as starch or cellulose may be utilized by some organisms. Furthermore, energy sources need not be limited to carbohydrates, but may include hydrocarbons, alcohols, or even organic acids. In composing an industrial medium, the carbon content must be adequate for the production of cells.

Nitrogen is found in proteins including enzymes as well as in nucleic acids hence it is a key element in the cell. Most cells would use ammonia or other nitrogen salts. The quantity of nitrogen to be added in a fermentation can be calculated from the expected cell mass and the average composition of the micro-organisms used. Any nitrogen compound which the organism cannot synthesize must be added.

Minerals form component portions of some enzymes in the cell and must be present in the medium. The major mineral elements needed include P, S, Mg and Fe.

Trace elements required include manganese, boron, zinc, copper and molybdenum.

Growth factors include vitamins, amino acids and nucleotides and must be added to the medium if the organism cannot manufacture them.

Under laboratory conditions, it is possible to meet the organism's requirement by the use of purified chemicals since microbial growth is generally usually limited to a few liters. However, on an industrial scale, the volume of the fermentation could be in the order of thousands of liters. Therefore, pure chemicals are not usually used because of their high expense, unless the cost of the finished material justifies their use. Pure chemicals are however used when industrial media are being developed at the laboratory level. The results of such studies are used in composing the final industrial medium, which is usually made with unpurified raw materials. The extraneous materials present in these unpurified raw materials are not always a disadvantage and may indeed be responsible for the final and distinctive property of the product.

2.5 Summary

In this unit we have learnt about the history, the types of culture media used in industrial microbiology. Culture media can be classified based on their consistency, constituents/ingredients/ special media, and based on oxygen requirements. These media are used to culture wide variety of microbes. A culture medium is a solid or liquid preparation used to grow, transport and store microorganism. To be effective, the medium must contain all the nutrients the microorganism requires for growth. Specialized media are essential in the isolation and identification of microorganism, the testing of antibiotic sensitivities, water and food analysis, industrial microbiology and other activities.

2.6 References/Further Readings/Web Sources

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2.7 Possible Answers to Self -Assessment Exercises

Self -Assessment Exercises 1 Answer

a. Pour plate, streak plate or spread plate techniques

Unit 3: Criteria for the choice of raw materials used in industrial media

3.1 Introduction:

This unit covers the choices that determine the type of raw material used in industrial media.

3.2. Intended Learning Outcomes (ILOs)

At the end of this unit, you should be able to:

- Explain the criteria for the choice of raw materials used in industrial media and the factors that influence their selection

3.3 Main content

Criteria for the choice of raw materials used in industrial media

In consideration of the type of raw materials to be used in the production of given products using selected microorganism(s), the following factors should be noted.

✓ Cost of the material
✓ Ready availability of the raw material
✓ Transportation costs
✓ Ease of disposal of wastes resulting from the raw materials
✓ Uniformity in the quality of the raw material and ease of standardization
✓ Adequate chemical composition of medium
✓ Presence of relevant precursors

✓ Satisfaction of growth and production requirements of the microorganisms

Cost of the material:
The cheaper the raw materials the more competitive the selling price of the final product will be. No matter, therefore, how suitable a nutrient raw material is, it will not usually be employed in an industrial process if its cost is so high that the selling price of the final product is not economic. Due to these economic considerations the raw materials used in many industrial media are usually waste products from other processes. Corn steep liquor and molasses are, for example, waste products from the starch and sugar industries, respectively.

Ready availability of the raw material
The raw material must be readily available in order not to halt production. If it is seasonal or imported, then it must be possible to store it for a reasonable period in a conducive warehouse where microorganisms will not destroy it.

Transportation costs
The closer the source of the raw material to the point of use the more suitable it is for use, if all other conditions are satisfactory.

Ease of disposal of wastes resulting from the raw materials:
Waste materials often find use as raw materials for other industries. Thus, spent grains from breweries can be used as animal feed. But in some cases, no further use may be found for the waste from an industry. When choosing a raw material therefore the cost, if any, of treating its waste must be considered.

Uniformity in the quality of the raw material and ease of standardization
The quality of the raw material in terms of its composition must be reasonably constant in order to ensure uniformity of quality in the final product and the satisfaction of the customer and his/her expectations. In a raw material with extremes of variability in quality is clearly undesirable as extra costs are needed, not only for the analysis of the raw material, but for the nutrients which may need to be added to attain the usual and expected quality in the medium.

Adequate chemical composition of medium
The medium must have adequate amounts of carbon, nitrogen, minerals and vitamins in the appropriate quantities and proportions necessary for the optimum production of the commodity in question. In addition, the demands of the microorganisms must also be met in terms of the compounds they can utilize.

Presence of relevant precursors

The raw material must contain the precursors necessary for the synthesis of the finished product. Precursors often stimulate production of secondary metabolites either by increasing the amount of a limiting metabolite, by inducing a biosynthetic enzyme or both.

Satisfaction of growth and production requirements of the microorganisms

Many industrial organisms have two phases of growth in batch cultivation: the phase of growth, or the trophophase, and the phase of production, or the idiophase. In the first phase cell multiplication takes place rapidly, with little or no production of the desired material. It is in the second phase that production of the material takes place, usually with no cell multiplication and following the elaboration of new enzymes. Often these two phases require different nutrients or different proportions of the same nutrients. The medium must be complete and be able to cater for these requirements.

The cheaper the raw materials the more competitive the selling price of the final product? True or False

Self-Assessment Exercises 1

Attempt the exercise to determine what you have learnt so far. (1min).
i Mention the criteria to consider when selecting industrial media?

ii. Many industrial organisms have two phases of growth in batch cultivation. Wha they?

3.5 References/Further Readings/Web Sources

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2.7 Possible Answers to Self -Assessment Exercises

Self -Assessment Exercises 1 Answer

Answer:

- *Cost of the material*
- *Ready availability of the raw material*
- *Transportation costs*
- *Ease of disposal of wastes resulting from the raw materials*
- *Uniformity in the quality of the raw material and ease of standardization*
- *Adequate chemical composition of medium*
- *Presence of relevant precursors*
- *Satisfaction of growth and production requirements of the microorganisms*

i. *the phase of growth, or the trophophase, and the phase of production, or the idiophase*

Unit 4: Raw materials used in compounding industrial media

4.1 Introduction:

This unit covers the raw materials used in compounding industrial media. A raw material may be cheap in one country or even in a different part of the same country but may not be cheap in another, especially if it has already found use in some production process. In such cases suitable substitutes must be found if the goods must be produced. The use of local substitutes where possible is advantageous in reducing the transportation costs and even creating some employment in the local population.

4.2. Intended Learning Outcomes (ILOs)

At the end of this unit, you should be able to:

- Know some of the raw materials used in compounding industrial media

4.3 Main content: Some raw materials used in compounding industrial media

The raw materials should be cheap and available to the industry that would require to make use of it. This reduces the cost of transportation and overhead cost.

(a) Corn steep liquor

This is a by-product of starch manufactured from maize. Corn steep liquor is considered adequate, being rich in carbohydrates, nitrogen, vitamins, and minerals. Its composition is highly variable and would

depend on the maize variety, conditions of steeping, extent of boiling. Corn steep liquor is highly acidic, it must be neutralized (usually with $CaCO_3$) before use.

(b) Pharmamedia

This is a yellow fine powder made from cotton-seed embryo. It is used in the manufacture of tetracycline and some semi-synthetic penicillin. It is rich in protein, (56% w/v) and contains 24% carbohydrate, 5% oil, and 4% ash, the last of which is rich in calcium, iron, chloride, phosphorous, and sulfate.

(c) Distillers soluble

This is a by-product of the distillation of alcohol from fermented grain. It is rich in nitrogen, minerals, and growth factors.

(d) Soya bean meal

Soya beans (soja) (*Glycine max*), is an annual legume which is widely cultivated throughout the world in tropical, sub-tropical and temperate regions. The seeds are heated before being extracted for oil that is used for food, as an antifoam in industrial fermentations, or used for the manufacture of margarine. The resulting dried material, soya bean meal, has about 11% nitrogen, and 30% carbohydrate and may be used as animal feed.

(e) Molasses

Molasses is a source of sugar, and is used in many fermentation industries including the production of potable and industrial alcohol, acetone, citric acid, glycerol, and yeasts. It is a by-product of the sugar industry. There are two types of molasses depending on whether the sugar is produced from the tropical crop, sugar cane (*Saccharum officinarum)* or the temperate crop, beet, (*Beta alba).*

(f) Sulfite liquor

Sulfite liquor (also called waste sulfite liquor, sulfite waste liquor or spent sulfite liquor) is the aqueous effluent resulting from the sulfite process for manufacturing cellulose or pulp from wood.

(g) Other Substrates

Other substrates used as raw materials in fermentations are alcohol, acetic acid, methanol, methane, fractions of crude petroleum and Barley.

(h) Growth factors

Growth factors are materials which are not synthesized by the organism and therefore must be added to the medium. They usually function as cofactors of enzymes and may be vitamins, nucleotides etc. The pure forms are usually too expensive for use in industrial media and materials containing the required growth factors are used to compound the medium. Growth factors are required only in small amounts.

Water

Water is a raw material of vital importance in industrial microbiology, although this is often overlooked. It is required as a major component of the fermentation. medium, as well as for cooling, and for washing and cleaning. It is therefore used in rather large quantities, and measured in thousands of liters a day depending on the industry. In some industries such as the beer industry the quality of the product depends to some extent on the water. In order to ensure constancy of product quality the water must be regularly analyzed for minerals, color, pH, etc. and adjusted as may be necessary.

Is use of local substitutes where possible is advantageous in reducing the transportation costs and even creating some employment in the local population.? True or False

Self-Assessment Exercises 1

Attempt the exercise to determine what you have learnt so far. (1min).
i Mention some raw materials required as industrial media?

4.4 Some potential sources of components of Industrial Media Carbohydrate Sources

Cassava (manioc)

The roots of the cassava-plant *Manihot esculenta* Crantz serve mainly as a source of carbohydrate for human (and sometimes animal) food in many parts of the tropical world. Its great advantage is that it is high yielding, requires little attention when cultivated, and the roots can keep in the ground for many months without deterioration before harvest. The inner fleshy portion is a rich source of starch and has served, after hydrolysis, as a carbon source for single cell protein, ethanol, and even beer.

Sweet potato

Sweet potatoes *Ipomeia batatas* is a warm-climate crop although it can be grown also in subtropical regions. There are a large number of cultivars, which vary in the colors of the tuber flesh and of the skin; they also differ in the tuber size, time of maturity, yield, and sweetness. They are widely grown in the world and are found in South America, the USA, Africa and Asia. They are regarded as minor sources of carbohydrates in comparison with maize, wheat, or cassava, but they have the advantage that they do not require much agronomic attention. They have been used as sources of sugar on a semi-commercial basis because the fleshy roots contain saccharolytic enzymes. The syrup made from boiling the tubers has been used as a carbohydrate (sugar) source in compounding industrial media. Butyl alcohol, acetone and ethanol have been produced from such a syrup, and in quantities higher than the amounts produced from maize syrup of the same concentration.

Yams

Yams (*Dioscorea* spp) are widely consumed in the tropics. Yams have been employed in producing various products such as yam flour and yam flakes. When the production of yam is carried out on a sufficiently large scale it is to be expected that the waste materials resulting from peeling the yams could yield substantial amounts of materials which on hydrolysis will be available as components of industrial microbiological media.

Cocoyam

Cocoyam starch has been found to be of acceptable quality for pharmaceutical purposes. Should it find use in that area, starchy by-products could be hydrolyzed to provide components of industrial microbiological media.

Millet: Millets are hardy and will tolerate great drought and heat, grow on poor soil and mature quickly. Attention is being turned to them for

this reason in some parts of the world. It is for this reason also that millets could become potential sources of cereal for use in industrial microbiology media**.**

Rice: Rice, *Oryza sativa* is one of the leading food corps of the world being produced in all five continents, but especially in the tropical areas. Although it is high-cost commodity, it has the advantage of ease of mechanization, storability, and the availability of improved seeds. Crop would yield substrates cheap enough for industrial microbiological use. Rice is used as brewing adjuncts and has been malted experimentally for beer brewing.

Sorghum

Sorghum, *Sorghum bicolor*, is the fourth in term of quantity of production of the world's cereals, after wheat, rice, and corn. It is used for the production of special beers in various parts of the world. It has been mechanized and has one of the greatest potentials among cereals for use as a source of carbohydrate in industrial media in regions of the world where it thrives.

Protein Sources

Peanut (groundnut) meal

Some leguminous seeds may be used as a source for the supply of nitrogen in industrial media. The groundnut cake left after the nuts have been freed of oil is often used as animal feed. But with soya bean, oil from peanuts may be used as anti-foam while the press-cake could be used for a source of protein. The nuts and the cake are rich in protein.

Blood meal

Blood consists of about 82% water, 0.1% carbohydrate, 0.6% fat, 16.4% nitrogen, and 0.7% ash. It is a waste product in abattoirs although it is sometimes used as animal feed. Drying is achieved by passing live steam through the blood until the temperature reaches about 100°C. This treatment sterilizes it and also causes it to clot. It is then drained, pressed to remove serum, further dried and ground. The resulting blood-meal is chocolate-colored and contains about 80% protein and small amounts of ash and lipids.

Fish Meal

Fish meal is used for feeding farm animals. It is rich in protein (about 65%) and, minerals (about 21% calcium 8%, and phosphorous 3.5%) and may therefore be used for industrial microbiological media production. Fish meal is made by drying fish with steam either aided by vacuum or by simple drying. Alternatively hot air may be passed over the fish placed in revolving drums. It is then ground into a fine powder. Sweet potatoes have been used as sources of sugar on a semi-commercial

basis because the fleshy roots contain…….

Self-Assessment Exercises 2

Attempt the exercise to determine what you have learnt so far. (1min).
i Mention other potential sources of carbohydrates and proteins that can used in industrial microbiology

4.5 The use of plant waste materials in industrial microbiology media

Serious consideration has therefore been given, in some studies, to the possibility of deriving industrial microbiological raw materials not just from wastes, but from crops grown deliberately for the purpose. However, plant materials in general contain large amounts of polysaccharides which are not immediately utilizable by industrial microorganisms and which will therefore need to be hydrolyzed or saccharified to provide the more available sugars.

Starch
Starch is a mixture of two polymers of glucose: amylose and amylopectin. Amylose is a linear (1 →4) ∞ D glucan usually having a degree of polymerization (D.P., i.e., number of glucose molecules) of about 400 and having a few branched residues linked with (1 → 6) bonding. Starches from various sources differ in their proportion of amylopectin and amylose. The more commonly grown type of maize, for example, has about 26% of amylose and 74% of amylopectin.

Saccharification of starch
Starch occurs in discrete crystalline granules in plants, and in this form is highly resistant to enzyme action. However, when heated to about 55°C – 82°C depending on the type, starch gelatinizes and dissolves in water and becomes subject to attack by various enzymes.
Before saccharification, the starch or ground cereal is mixed with water and heated to gelatinize the starch and expose it to attack by the saccharifying agents. The starch-containing material to be hydrolyzed is ground and mixed with dilute hydrochloric acid, sulfuric acid or even sulfurous acid.

Use of enzymes
Enzymes hydrolyzing starch used to be called collectively diastase but recently now called amylases. Enzymatic hydrolysis has several advantages over the use of acid: (a) since the pH for enzyme hydrolysis is about neutral, there is no need for special vessels which must stand the high temperature, pressure, and corrosion of acid hydrolysis; (b) enzymes are more specific and hence there are fewer side reactions

leading therefore to higher yields; (c) acid hydrolysis often yields salts which may have to be removed constantly or periodically thereby increasing cost; (d) it is possible to use higher concentrations of the substrates with enzymes than with acids because of enzyme specificity, and reduced possibility of side reactions.

Cellulose, Hemi-celluloses and Lignin in Plant Materials

Cellulose

Cellulose is the most abundant organic matter on earth. Unfortunately, it does not exist pure in nature and even the purest natural form (that found in cotton fibres) contains about 6% of other materials. Three major components, cellulose, hemi-cellulose and lignin occur roughly in the ratio of 4:3:3 in wood.

Hemi-celluloses

They are very easily hydrolyzed by chemical or biological means. The nature of the hemicellulose varies from one plant to another. In cotton the hemicelluloses are pectic substances, which are polymers of galactose.

Lignin

Lignin is a complex three-dimensional polymer formed from cyclic alcohols. It is important because it protects cellulose from hydrolysis.

4.6 Summary

In this unit we have learnt about the raw materials used in compounding industrial media and some potential sources of components of Industrial media were discussed. Although all microorganisms need sources of energy, carbon, nitrogen, phosphorus, sulphur, and various minerals, the precise composition of a satisfactory medium will depend on the species one is trying to cultivate because nutritional requirement varies greatly. Knowledge of a microorganism's normal habitat often is used in selecting an appropriate culture medium because its nutrient requirements reflect its natural surroundings.

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4.8 Possible answers to Self-Assessment Exercises

Self-Assessment Exercise 1
Answer: *Corn steep, molasses, pharmamedia, sulfite liquor, distiller solubles.*

Self-Assessment Exercise 2
Answer: *Carbohydrate Sources- These include yam, sweet potato, cocoyam,yam, cassava,millet, sorghum, rice*

- *Protein sources- These include the fish meal, blood meal, peanut*

Unit 5: Maintenance of selected cultures

5.1. Introduction

Industrially-used microbes are unique and quite specific subset of all the microbes available on earth whereas microbes isolated from nature exhibit cell growth as their main physiological property, industrial microbes have been selected carefully so that they manufacture one or more specific products. Even the industrial microbe is one which had been isolated by traditional techniques. It becomes a highly "modified" organism before it enters large scale industries. To a great extent, industrial microbes are metabolic specialist capable of producing specifically, and to high yield particular metabolites. In order to achieve this, high metabolic specialization, industrial strains are genetically altered by mutation or recombination.

For most industrial microbiologist, the methods of mutation and recombination DNA technology are the common methods of strain improvement. The most dramatic examples of strain improvement come from the applications of recombinant DNA technology which has resulted in organisms producing compounds which they were not able to produce previously. Furthermore, the advances in these techniques have resulted in very significant improvements in the production of conventional fermentation products.

5.2 Intended Learning Outcomes (ILOs)

- At the end of this unit, you should be able to:
  Explain how cultures are collected and maintained
- Explain the methods of strain improvements.

5.3. Main content

Maintenance of selected cultures.

The gene pool of organisms with desirable properties must be preserved and be constantly available.
In industrial microbiology, the strain is often more valuable than the species as the ability to produce the unique characteristics of a product resides in the strain. Industrial microbiological establishments usually keep a collection of the microorganisms which possess the gene pools for producing the goods manufactured by the establishment. This stock of organisms is known as a **culture collection** and ensures a regular supply of organisms to be used in the manufacturing process. Organisms in a culture collection are maintained in a low metabolic state in which replication of the cells is kept to a minimum or even entirely restricted. Industrially important microorganisms are often mutants, and the condition of low metabolism in which they are kept, limits their tendency to revert to their low-yielding ancestors. In some circumstances organisms are maintained for comparatively short periods of days in an active state in which they are immediately ready for use in fermentations; such organisms are called **working stock**.
Why is it important to keep industrial microorganisms at low metabolism…….

Self-Assessment Exercises 1

Attempt the exercise to determine what you have learnt so far. (1min).
i What is a culture collection?

Collections handle a wide variety of organisms, of whatever kind. The best known in this category is the American Type Culture Collection (ATCC). Other collections are specialized and may handle only pathogenic microorganisms, such as the National Collection of Type Cultures (NCTC) in Colindale, London, UK or industrial microorganisms, such as National Collection of Industrial Bacteria (NCIB) in Aberdeen, Scotland. Still others almost exclusively handle one type of organism such as Center vor Braunsveitzer (CBS) in Holland, which handles fungi exclusively. Many universities all over the world have culture collections which reflect their range of microbiological interests.
Culture Collections around the world are linked by the World Federation of Culture Collections (WFCC). The WFCC is an affiliate of the

International Union of Microbiological Societies (IUMS) the organization which links national microbiological societies worldwide. The WFCC is concerned with the collection, authentication, maintenance, and distribution of cultures of microorganisms and cultured cells. Its aim is to promote and support the establishment of culture collections and related services, to provide liaison and set up an information network between the collections and their users, and work to ensure the long-term perpetuation of important collections. Culture Collections are organized on regional and international basis for the exchange of cultures and ideas and include the Asian Network on Microbial Research (ANMR), BCCCM (Belgium Co-ordinated Collections of Microorganisms), ECCO (European Culture Collection Organization), JFCC (Japanese Federation of Culture Collections), MICRO-NET (Microbial Information Network of China), MSDN (Microbial Strain Data Network, UK), UKNCC (United Kingdom National Culture Collection),USFCC (United States Federation of Culture Collections, USA).

Handling Culture Collections

Cultures are expensive to purchase. Universities can however build their own cultures collections by preserving cultures arising from their research. No matter what the source of a valuable organism, it is important that several replicates are stored immediately for fear of contamination while tests are carried out to ascertain its potential for fulfilling the expected activity.

5.3.2 Methods of preserving microorganisms

Several methods have been devised for preserving microbial cultures. None of them can be said to apply exclusively to industrial microorganisms. Furthermore, no one method is suitable for preserving all organisms. Methods employed in the preservation of microorganisms all involve some limitation on the rate of metabolism of the organism.

The principle involved in preserving microorganisms are:

(a) reduction in the temperature of growth of the organism;
(b) dehydration or desiccation of the medium of growth;
(c) limitation of nutrients available to the organism.

Microbial Preservation Methods Based on the Reduction of the Temperature of Growth

Preservation on agar with ordinary refrigeration (4 – 10°C)

a) Aerobic organisms

Agar slants: Aerobic organisms may be grown on agar slants and refrigerated at 4 – 10°C as soon as they have shown growth.

Petri dishes: Aerobic organisms may also be stored on Petri dishes. The plates may be sealed with special tapes to prevent the plates from drying out on account of evaporation. The special tapes of different colors may be used to identify special attributes or groups among the cultures.

b) Anaerobic organisms: Anaerobic organisms may be stored on agar stabs which are then sealed with sterile molten petroleum jelly. Storage using the agar methods has advantages and disadvantages. The advantage is that agar storage methods are inexpensive because they do not require any specialized equipment.

The disadvantages are:

- The organisms must be sub-cultured at intervals which have to be worked for each organism, medium used, laboratory practice, etc. This is because the temperature of the refrigeration is not low enough to limit growth completely.
- Consequent on regular sub-culturing is the possibility that contaminations and or mutations may occur.
- Petri dishes occupies a lot of space in the refrigerator when compared with agar slants.
- The process of sub-culturing is tedious apart from the possibility of contamination and mutation.
- When petroleum jelly is used as a seal, the arrangement can be messy.

Oil overlay

Oil overlay function to limit oxygen diffusion thus many bacteria, particularly anaerobes and facultative, and fungi survive for up to three years, and most of them for at least one year.

Medium for storing organisms on agar

The nature of the agar medium on which organisms are stored is of importance. A medium prepared from natural components rather than a chemically defined material is preferable, since a defined medium may, because it lacks some components present in the natural components, select for organisms specifically capable of growing on it. A stock culture medium should also not be unduly rich in carbohydrates such as glucose which could lead to early production of acid and hence possible early microbial death. Where glucose is used, such as for lactic bacteria, the medium should be buffered with calcium carbonate.

Popularity of agar storage methods

The storage on agar is however very popular and is the most widely used after lyophilization.

Preservation in Deep Freezers at about -20°C, or between -60°C and -80°C

The regular home freezer attains a temperature of about -20 °C.
Laboratory deep freezers used for molecular biology work range in temperature
between -60 °C and -80 °C. It is possible to store microorganisms in either type of deep freezers in the form of agar plugs or on sterile glass beads coated with the organism to be stored.

Preservation on glass beads
The bacteria to be preserved are placed in broth containing cryoprotective compounds such as glycerol, raffinose, lactose, or trehalose. Sterile glass beads are placed in the glass vials containing the bacterial cultures. The vials are gently shaken before being put in the deep freezers.

Advantages of the above freezing methods

(a) the methods are simple to use and require a minimum of equipment;
(b) they save space as many hundreds of cultures can be stored in a small space;
(c) beads thaw rapidly and hence the method saves time,
(d) differently bead colors can represent different bacteria and so recognizing them is easy;
(e) the methods can be adapted for both aerobic and anaerobic organisms;
(f) the methods are suitable for situations or countries where power outages occur, as the freezer can remain cold for some time during power failures.

Storage in low temperature liquid or vapor phase nitrogen (-156°C to -196°C)

The liquid or vapor phase of nitrogen at -156°C to -196°C is widely used for preserving microorganisms and cultured cells. Fungi, bacteriophages, viruses, algae, protozoa, bacteria, yeasts, animal and plant cells, and tissue cultures have all been successfully preserved in it. It is a major method for organisms which will not survive freeze-drying. The period of survival and the number of surviving organisms is higher for most organisms than when freeze drying is used.
Some of the most commonly used cryoprotectants are (vol/vol) 10-20% glycerol and 5-10% dimethyl sulfoxide (DMSO) in broth culture of the organism in vials which are then frozen in liquid nitrogen. Vials for storing organisms in low temperature nitrogen may be made of glass or

fashioned from ordinary polypropylene (plastic) drinking straws.

Freezing at –156°C to -196°C has the following disadvantages:

(a) As liquid nitrogen evaporates, it has to be replenished regularly; if not replenished the cultures may be lost.

(b) A risk of explosion exists when cultures are frozen in liquid nitrogen in improperly sealed glass vials which permit entry of liquid nitrogen into the vials. Such vials may explode when warmed to thaw them. Discarding poorly sealed glass vials removes such risks; vapor phase storage removes such dangers.

(c) Although it is not labor intensive the equipment is expensive.

(d) Finally, it is not a convenient method for transporting organisms. What is the full meaning of ATCC, JFCC

Self-Assessment Exercises 2

Attempt the exercise to determine what you have learnt so far. (1min).
i In microorganisms' preservation what is the purpose of oil overlay?

Microbial Preservation Methods Based on Dehydration

Reduction in temperature limits the metabolism of the organism, dehydration removes water a necessity for the metabolism of the organism.

Drying on sterile silica gel

Many organisms including actinomycetes and fungi are dried by this method. Screw-cap tubes half-filled silica gel are sterilized in an oven. On cooling a skim-milk suspension of spores and the cells of the fungus or actinomycetes is placed over the silica gel and cooled. They are dried at 25°C, cooled and stored in closed containers containing desiccants.

Preservation on sterile filter paper

Spore-forming microorganisms such as fungi, actinomycetes, or *Bacillus* spp may be preserved on sterile filter paper by placing drops of broth containing the spores on sterile filter paper in a Petri dish and drying in a low temperature oven or in a dessicator. Alternatively, sterile filter paper may be soaked in the broth culture of the organism to be dried, placed in a tube, which is then evacuated and sealed. After drying the filter paper may be placed in sterile screw caps bottles and stored either at room temperature or in the refrigerator.

Preservation in sterile dry soil

The most commonly used form of storage in a dry state is the use of dry sterile soil. In this method dry soil is sterilized by autoclaving. It is then inoculated with a broth or agar culture of the organism. The soil is

protected from contamination and allowed to dry over a period of time. Subsequently it may be refrigerated. The method has been widely and successfully used to store sporulating organisms especially clostridia and fungi; it has also been used for bacilli and *Azotobacter* sp. Some non-sporulating bacteria which do not survive well under Lyophilization, may be stored in soil.

Freeze-drying (drying with freezing), lyophilization

Freeze-drying or lyophilization is widely employed. The principle of the method is that the organism is first frozen. Subsequently, water is removed by direct vaporization of the ice with the introduction of a vacuum. The suspension is not in the liquid state, distortion of shape and consequent cell damage is minimized. At the end of the drying the ampoule containing the organism may be stored under refrigeration although survival for many years has also been obtained by storage at room temperature. Lyophilization is preferred for the preservation of most organisms because of its success with a large number of organisms, the relatively inexpensive equipment, the scant demand on space made by ampoules, but above all, the longevity (up to 10 years or more in some organisms) of most organisms stored by lyophilization.

L-drying (liquid drying, drying without refrigeration)

This is considered a modification of drying methods, since unlike freeze-drying, the organisms are not frozen, but dried from the liquid state. It has been used to preserve non-spore formers sensitive to freeze-drying, such as Cytophaga, Spirillum and Vibrio. Liquid drying has been effectively used to preserve organisms such as anaerobes that are damaged by freezing.

Microbial Preservation Methods Based on the Reduction of Nutrients

Storage in distilled water

Many organisms die in distilled water because of water absorption by osmosis. However, some have been known to survive for long periods in sterile distilled water. Usually, such storage is accompanied by refrigeration; some organisms are however, harmed by refrigeration. Among organisms which have been stored for long periods with this method are *Pseudomonas solanaceanum, Saccharomyces cerevisiae,* and *Sarcina lutea*. The attractiveness of this method is its simplicity and inexpensiveness.

5.4 The Need for Experimentation to Determine the Most Appropriate Method of Preserving an Organism

No one method can be said to suitable for the preservation of all and

every organism. The appropriate method must be determined for each organism. The preservation method must retain the characteristics which are desirable in the organism and this is crucial for industrial microorganisms.
No one method can be said to suitable for the preservation of all and every organism? True or False

Self-Assessment Exercises 3

Attempt the exercise to determine what you have learnt so far. (1min).
i Using one example explain the principle involved in preserving microorganisms by

5.6. Summary

Industrially useful organisms are subjected to some conditions in order to achieve high metabolic specialization, industrial strains are genetically altered by mutation or recombination. Different methods are used in preserving sourced microbes of interest. The methods ranged from simple to complex and expensive ones. These are periodic transfer, mineral oil slant, minimal medium, distilled water, or water agar, growth media freezing, drying, the more sophisticated method such as freeze drying (lyophilization) and ultra-freezing.

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5.8. Possible answers to Self-Assessment Exercises

Self-Assessment Exercise 1
Answer: Industrial microbiological establishments that usually keep a collection of the microorganisms which possess the gene pools for producing the goods manufactured by the establishment

Self-Assessment Exercise 2
Answer 2 Oil overlay function to limit oxygen diffusion thus many bacteria, particularly anaerobes and facultative, and fungi survive for up to three years, and most of them for at least one year.

Self-Assessment Exercise 3
Preservation on agar with ordinary refrigeration (4 – 10°C)
a) Aerobic organisms
Agar slants: Aerobic organisms may be grown on agar slants and refrigerated at 4 – 10°C as soon as they have shown growth.
Petri dishes: Aerobic organisms may also be stored on Petri dishes. The plates may be sealed with special tapes to prevent the plates from drying out on account of evaporation. The special tapes of different colors may be used to identify special attributes or groups among the cultures.
b) Anaerobic organisms: Anaerobic organisms may be stored on agar stabs which are then sealed with sterile molten petroleum jelly

Glossary
ANMR=Asian Network on Microbial Research
ATCC =American Type Culture Collection.
DMSO = dimethyl sulfoxide
NCTC =National Collection of Type Cultures

End of the module Questions

1). What is the concentration of agar in solid media?
2). Oil overlay function to limit oxygen diffusion **(True or False)**
3). In industrial microbiology, the gene pool of organisms with desirable properties must be preserved and be constantly available **(True or False)**
4). Why is Lyophilization preferred for the preservation of most organisms?
5). Write on potential sources of components of Industrial Media of carbohydrate and protein origin.

Module 3:	**Optimization of fermentations**
Unit 1.	Alcoholic beverages
Unit 2.	Raw Materials for Brewing.
Unit 3.	Wine Production
Unit 4.	Distilled alcoholic (or spirit) beverages
Unit 5.	Fermented Foods I: Bread Making and Milk products
Unit 6.	Fermented Foods II: Some local fermented foods

Unit 1. Alcoholic beverages.

1.0 Introduction

For several thousand years, fermentation has been a major way of preserving food. Microbial growth, either of natural or inoculated population, causes chemical and/or textural changes to form a product that can be stored for extended periods.

1.1 Intended Learning Outcomes (ILOs)

At the end of this unit, you should be able to:

- Explain in detail the process of production of Alcoholic beverages.
- Differentiate between the different types of beer produced from fermentation.

1.2 MAIN CONTENT

Alcoholic beverages

The fermentation process also is to create new, pleasing food flavours and odours.'Fermentation' is derived from the Latin verb *fervere* to boil, thus describing the appearance of the action of yeast on extracts of fruit

or malted grain. The boiling appearance is due to the production of carbon dioxide bubbles caused by the anaerobic catabolism of the sugar present in the extract. To a biochemist or industrial microbiologists, the term fermentation has different meanings. Its biochemical meaning relates to the generation of energy by the catabolism of organic compounds, whereas its meaning in industrial microbiology is broader. Industrial microbiologists have extended the term fermentation to describe any process for the production of products by the mass culture of a micro-organisms. There are five major groups of commercially important fermentations:

(i) Those that produce microbial cells (or biomass) as the product.
(ii) Those that produce microbial enzymes.
(iii) Those that produce microbial metabolites.
(iv) Those that produce recombinant products.
(v) Those that modify a compound which is added to the fermentation the transformation process.

Microbes play prominent role in the production of alcoholic beverages such as beer, wine, vodka, brandy, whisky e.t.c. The production of these beverages is discussed below:

1.2.1 Beer production

Barley beers
The word beer derives from the Latin word *bibere* meaning to drink. The process of producing beer is known as brewing. Beer brewing from barley was practiced by the ancient Egyptians as far back as 4,000 years ago of which it was reported that the art was learnt from the peoples of the Tigris and Euphrates where man's civilization is said to have originated.

Types of Barley Beers
Barley beers can be divided into two broad groups: **top-fermented beers** and **bottom-fermented beers**. This distinction is based on whether the yeast remains at the top of brew (top-fermented beers) or sediments to the bottom (bottom-fermented beers) at the end of the fermentation.

Bottom-fermented beers:
Bottom-fermented beers are also known as lager beers because they were stored or 'lagered' (from German lagern = to store) in cold cellars after fermentation for clarification and maturation. Yeasts used in bottom-fermented beers are strains of *Saccharomyces uvarum* (formerly *Saccharomyces carlsbergensis*). Several types of lager beers are known. They are ***Pilsener, Dortumund*** and ***Munich***, and named after Pilsen

(former Czechoslovakia) ***Dortmund*** and ***Munich*** (Germany), the cities where they originated. Most of the lager (70%-80%) beers drunk in the world is of the Pilsener type.

Pilsener beer: This is a pale beer with a medium hop taste. Its alcohol content is 3.0-3.8% by weight. Traditionally it is lagered for two to three months, but modern breweries have substantially reduced the lagering time, which has been cut down to about two weeks in many breweries around the world. The water for Pilsener brew is soft, containing comparatively little calcium and magnesium ions.

Dortmund beer: This is a pale beer, but it contains less hops (and therefore is less bitter) than Pilsener. However, it has more body (i.e., it is thicker) and aroma. The alcohol content is also 3.0-3.8%, and is classically lagered for slightly longer: 3-4 months.

Munich: This is a dark, aromatic and full-bodied beer with a slightly sweet taste, because it is only slightly hopped. The alcohol content could be quite high, varying from 2 to 5% alcohol.

Weiss: Weiss beer of Germany made from wheat and steam beer of California; USA are both bottom fermented beers which are characterized by being highly effervescent.

Barley beers can be divided into two broad groups: top-fermented beers and bottom-fermented beers (True or False)

Self-Assessment Exercises 1

Attempt the exercise to determine what you have learnt so far. (1min).
i Bottom fermented beers are referred to …….

1.2.2. Top-fermented beers

Top fermented beers are brewed with strains of ***Saccharomyces cerevisiae***.

(i) ***Pale Ale***: English ale is a pale, highly hopped beer with an alcohol content of 4.0 to 5.0% (w/v) and sometimes as high as 8.0%. Hops are added during and sometimes after fermentation. It is therefore very bitter and has a sharp acid taste and an aroma of wine because of its high ester content. Mild ale is sweeter because it is less strongly hopped than the standard Pale ale.

(ii) ***Porter:*** This is a dark-brown, heavy bodied, strongly foaming

beer produced from dark malts. It contains less hops than ale and consequently is sweeter. It has an alcohol content of about 5.0%.

(iii) ***Stout***: Stout is a very dark heavily bodied and highly hopped beer with a strong malt aroma. It is produced from dark or caramelized malt; sometimes caramel may be added. It has a comparatively high alcohol content, 5.0-6.5% (w/v) and is classically stored for up to six months, fermentation sometimes proceeding in the bottle. Some stouts are sweet, being less hopped than usual.

Fermentation by top fermenter yeasts usually occurs at high temperature, 14 – 23ºC while fermentation by bottom fermenter yeast occurs at 6 – 12ºC. Fermentation is accomplished in a shorter period of time (5 – 7days) for top fermentation while it takes 8 – 14days for bottom fermentation. The brewing process, specifically production of barley beer involves the following unit operations.
Hops are not bitter nor has a sharp acid taste and never add aroma of wine (True or False)

Self-Assessment Exercises 2

Attempt the exercise to determine what you have learnt so far. (1min).
i Mention the types of bottom fermented beer?
ii. Top fermented beers are brewed with strains of……

1.3. Summary.

Beer and ale are produced from cereal and grains. The starches in this substrate are fermented given rise to the different types of beer. *Saccharomyces cerevisiae* is major yeast used in the production of beer and ale.

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1.4. Possible answers to Self-Assessment Exercises

Answers Self-Assessment Exercise 1

lager beers

Answers Self-Assessment Exercise 2

i.They are Pilsener, Dortumund and Munich, and named after Pilsen (former Czechoslovakia) Dortmund and Munich (Germany),Weiss

ii. Saccharomyces cerevisiae

Unit 2 Raw Materials for Brewing.

2.0 Introduction

The raw materials used in brewing are: barley, malt, adjuncts, yeasts, hops, and water. The benefits of these raw materials will be discussed in details.

2.1 Intended Learning Outcomes (ILOs)

At the end of this unit, you should be able to:

- Explain in detail the raw materials for brewing.
- The advantages and disadvantages of each major raw materials for brewing.

2.2 MAIN CONTENT

Raw Materials for Brewing
The raw materials used in brewing are: **barley, malt, adjuncts, yeasts, hops, and water.**

The barley has the following advantages. Its husks are thick, difficult to crush and adhere to the kernel. This makes malting as well as filtration after mashing, much easier than with other cereals, such as wheat. The second advantage is that the thick husk is a protection against fungal attack during storage. Thirdly, the gelatinization temperature (i.e., the temperature at which the starch is converted into a water-soluble gel) is 52-59°C much lower than the optimum temperature of alpha-amylase (70°C) as well as of beta-amylase (65°C) of barley malt. Finally, the

barley grain even before malting contains very high amounts of beta-amylase unlike wheat, rice and sorghum.
Two distinct barley types are known. One with six rows of fertile kernel (*Hordeum vulgare*) and the other with two rows of fertile kernels (*Hordeum distichon*). These differ in many other properties and as a result there are thousands of varieties. The six-row varieties are richer in protein and enzyme content than the two-row varieties.

2.2.1 Adjuncts

Adjuncts are starchy materials which were originally introduced during the brewing process. It includes materials other than would be hydrolyzed by amylase. For example, the term now includes sugars (e.g., sucrose) added to increase the alcoholic content of the beer. Starchy adjuncts, which usually contain little protein contribute, after their hydrolysis, to fermentable sugars which in turn increase the alcoholic content of the beverage. Adjuncts thus help bring down the cost of brewing because they are much cheaper than malt. They do not play much part in imparting aroma, color, or taste. Starch sources such as sorghum, maize, rice, unmalted barley, cassava, potatoes can or have been used, depending on the price.

2.2.2 Hops

Hops are the dried cone-shaped female flower of hop-plant *Humulus lupulus* (synomyn:*H. americanus, H. heomexicams, H. cordifolius*). It is a temperate climate crop and grows wild in northern parts of Europe, Asia and North America. Hop extracts are becoming favored in place of the dried hops. The importance of hops in brewing lies in its resins which provide the bitter taste in beer and the essential (volatile) oils which provide the hop aroma.

The addition of hops has several effects:
(a) It replaces the flat taste of unhopped beer with the characteristic bitterness and pleasant aroma of hops.
(b) Hops have some anti-microbial effects especially against beer sarcina (*Pediococus damnosus*) and other beer spoiling bacteria.
(c) Due to the colloidal nature of the bitter substances they contribute to the body, colloidal stability and foam head retention of beer.
(d) The tannins in the hops help precipitate proteins during the boiling of the wort; these proteins if not removed cause a haze (chill haze) in the beer at low temperature.

2.2.3 ***Water***

The mineral and ionic content and the pH of the water have profound

effects on the type of beer produced. Some ions are undesirable in brewing water: nitrates slow down fermentation, while iron destroys the colloidal stability of the beer. In general calcium ions lead to a better flavor than magnesium and sodium ions. Water is so important that the natural water available in great brewing centers of the world lent special character to beers peculiar to these centers. Water of a composition ideal for brewing may not always be naturally available. It is treated in one of the following ways:

(a) By the addition of calcium sulfate (gypsum).
(b) An acid may be added: lactic acid, phosphoric, sulfuric or hydrochloric.
(c) The water may be decarbonated by boiling or by the addition of lime calcium hydroxide.
(d) The water may be improved by ion exchange, which may if it is so desired remove all the ions.

What are adjuncts?

Self-Assessment Exercises1

Attempt the exercise to determine what you have learnt so far. (1min).
i Mention the barley types …
ii. What are adjuncts?

2.2.4 Brewer's yeasts

Yeasts in general will produce alcohol from sugars under anaerobic conditions, but not all yeasts are necessarily suitable for brewing. Brewing yeasts are able, besides producing alcohol, to produce from wort sugars and proteins a balanced proportion of esters, acids, higher alcohols, and ketones which contribute to the peculiar flavor of beer.

A number of characteristics distinguish the two types of brewers' yeasts (i.e., the top and the bottom-fermenting yeasts).

(a) Under the microscope *Sacch. uvarum* (*Sacch. carlsbergensis*) usually occurs singly or in pairs. *Sacch. cerevisiae* usually forms chains and occasionally cross-chains as well.
(b) *Sacch. cerevisiae* sporulates more readily than does *Sacch. uvarum*.
(c) Perhaps the most diagnostic distinction between them is that *Sacch. uvarum* is able to ferment the trisaccharide, raffinose, made up of galactose, glucose, and fructose. *Sacch. cerevisiae* is capable of fermenting only the fructose moiety; in other words, it lacks the enzyme system needed to ferment melibiose which is formed from galactose and glucose.

(d) *Sacch. cerevisiae* strains have a stronger respiratory system than *Sacch uvarum* and this is reflected in the different cytochrome spectra of the two groups.
(e) Bottom-fermenters are able to flocculate and sink to the bottom of the brew, a characteristic lacking in most strains of *Sacch. cerevisiae*. Bottom ferments are classified into rapid settling or slow-settling (powdery); settling characteristics affect the rate of production, some secondary yeast metabolites, and hence beer quality.

2.3 Brewery Processes

The processes involved in the conversion of barley malt to beer may be divided into the
following:
1. Malting
2. Cleaning and milling of the malt
3. Mashing
4. Mash operation
5. Wort boiling treatment
6. Fermentation
7. Storage or lagering
8. Packaging

Malting: Malting is specialized and is not carried out in the brew house. Rather, breweries purchase their malt from specialized maltsters (or malt producers). The purpose of malting is to develop amylases and proteases in the grain. These enzymes are produced by the germinated barley to enable it to break down the carbohydrates and proteins in the grain to nourish the germinated seedling before its photosynthetic systems are developed enough to support the plant. However, the development of the seedling is halted by drying, but at temperatures which will not completely inactivate the enzymes in the grain. These enzymes are reactivated during mashing and used to hydrolyze starch and proteins and release nutrients for the nourishment of the yeasts.
Malting process: - This is carried out to promote synthesis of hydrolytic enzymes such as alpha amylase, endo-β gluconase and peptidases to solubilize the endosperm wall of the grain and secure the enzymatic breakdown of soluble component to low molecular weight.
The malting process thus provides sugar for the yeast from which it obtains energy and amino acids for its growth. Malting involves 3 processes. These are **steeping, germination and kilning**.
Steeping: Involves the soaking of the grains in water to a moisture level of 42 – 46% for a period of up to 48 hours.
Germination: After soaking, the chatted grain is allowed to grow for 4 – 5 days. This process develops the endosperm enzyme which will modify the starch, proteins and cell wall of the endosperm into useful

extract (soluble materials released from malt during mashing).

Kilning: This is drying of germinated grain at temperature between 50 – 90^{o}C to arrest growth and enzyme activity. The kilning process also reduces the moisture content of the grain to about 3 – 5%. It can thus be stirred, develop flavour and characteristic, and colour forming potentials. kilning, which consists of heating the 'green' malt in an oven, first with a relatively mild temperature until the moisture content is reduced from about 40% to about 6%. Subsequently the temperature of heating depends on the type of beer to be produced. For beer of the Pilsener type the malt is pale and has no pronounced aroma and kilning takes 20-24 hours at 80–90°C. For the darker Munich beers with a strong aroma drying takes up to 48 hours at 100 – 110°C. For the caramelized malts used for stout and other very dark beers, kilning temperature can be as high as 120°C.

Cleaning and milling of the malt

At the top of the brewing tower, the barley malt is cleaned of dirt and passed over a magnet to remove pieces of metals, particularly iron. It is then milled.

The purpose of milling is to expose particles of the malt to the hydrolytic effects of malt enzymes during the mashing process. The finer the particles therefore the greater the extract from the malt. However, very fine particles hinder filtration.

Mashing:

Mashing is the central part of brewing. It determines the nature of the wort, hence the nature of the nutrients available to the yeasts and therefore the type of beer produced. The purpose of mashing is to extract as much as possible the soluble portion of the malt and to enzymatically hydrolyze insoluble portions of the malt and adjuncts. The aqueous solution resulting from mashing is known as **wort**.

Mashing is influenced by various factors such as temperature, time, concentration of malt, starch and protein. The most importance objective of mashing is to produce fermentable sugar largely through the amylolytic degradation of solubilized starch. Two enzymes are mainly responsible for this α and β amylase. Starch forms about 55% of the dry weight of barley malt. Of the malt starch 20-25% is made up of amylose. The key enzymes in the breakdown of malt starch are the alpha and beta-amylases. The Proteolytic activity in wort is however dependent on pH and for this reason wort pH is maintained. The progress of mashing is affected by a combination of temperature, pH, time, and concentration of the wort. When the temperature is held at 60-65°C for long periods a wort rich in maltose occurs because beta amylase activity is at its optimum and this enzyme yields mainly maltose. On the other hand, when a higher temperature around 70°C is employed dextrins predominate. Dextrins contribute to the body of the beer but are not utilized by yeast. Mash exposed to too high a temperature will therefore be low in alcohol due to insufficient maltose production.

The concentration of the mash is important. The thinner the mash the higher the extract (i.e., the materials dissolved from the malt) and the maltose content.

Mashing methods

There are three broad mashing methods:

(a) **Decoction methods**, where part of the mash is transferred from the mash tun to the mash kettle where it is boiled.

(b) **Infusion methods**, where the mash is never boiled, but the temperature is gradually raised.

(c) **The double mash method** in where the starchy adjuncts are boiled and added to the malt.

(a) **Decoction methods**

In these methods the mash is mixed at an initial temperature of 35-37°C and the temperature is raised in steps to about 75°C. About one-third of the initial mash is withdrawn, transferred to the mash kettle, and heated slowly to boil, and returned to the mash tun, the temperature of the mash becoming raised in the process. The enzymes in the heated portion become destroyed but the starch grains are cooked, gelatinized and exposed. Another portion may be removed, boiled and returned. In this way the process may be a **one, two or three-mash process**. In a three-mash process the initial temperature of 35-40°C favors proteolysis; the mash is held for about half hour at 50°C for full proteolysis, for about one hour at 60-65°C for saccharification and production of maltose, and at 70-75°C for two or three hours for dextrin production.

(b) Infusion method: The method involves grinding malt and a smaller amount of unmalted cereal, which may sometimes be precooked. The ground material, or grist, is mixed thoroughly with hot water (2:1 by weight) to produce a thick porridge-like mash and the temperature is carefully raised to about 65°C. It is then held at this temperature for a period varying from 30 minutes to several hours. On the average the holding is for 1-2 hours. The enzyme acts principally on the starch and its degradation products in both the malted and unmalted cereal, and only a little protein breakdown occurs. Further hot water at 75-78°C is sprayed on the mash to obtain as much extract as possible and to halt the enzyme action.

(c)The double Mash: In a typical double mash method ground malt is mashed with water at a temperature of 35°C. It is then held for an hour during the 'protein rest' for proteolysis. Adjuncts are then cooked in an adjunct cooker for 60-90 minutes. Sometimes about 10%malt is added during the cooking. Hot cooked adjunct is then added to the mash of ground malt to raise the temperature to 65-68°C for starch hydrolysis and maintained at this level for about half hour. The temperature is then increased to 75°C-80°C after which the mashing is terminated. During

starch hydrolysis completion of the process is tested with the iodine test.

Mash operation: This involves husks and other insoluble materials are removed from the wort.

Wort boiling:

The wort is boiled for 1-1½ hours in a brew kettle. The corn syrup or sucrose is used as an adjunct it is added at the beginning of the boiling. Hops are also added, some before and some at the end of the boiling.

The purpose of boiling is as follows.

(a) To concentrate the wort, which loses 5-8% of its volume by evaporation during the boiling;

(b) To sterilize the wort to reduce its microbial load before its introduction into the fermentor.

(c) To inactivate any enzymes so that no change occurs in the composition of the wort.

(d) To extract soluble materials from the hops, which not only aid in protein removal, but also in introducing the bitterness of hops.

(e) To precipitate protein, which forms large flocs because of heat denaturation and complexing with tannins extracted from the hops and malt husks. Unprecipitated proteins form hazes in the beer, but too little protein leads to poor foam head formation.

(f) To develop color in the beer; some of the color in beer comes from malting but the bulk develops during wort boiling. Color is formed by several chemical reactions including caramelization of sugars, oxidation of phenolic compounds, and reactions between amino acids and reducing sugars.

(g) Removal of volatile compounds: Volatile compounds such as fatty acids which could lead to rancidity in the beer are removed.

Fermentation: - The filtered-cooled wort (15^{o}C) is pumped into fermentation vessels which are often made of stainless steel. It is pitched with yeast (0.2kg/hl on its way to the fermentation vessel). The fermentation proceeds for the desired number of days depending on the type of beer, during which some fermentable sugars are transformed to CO2, glycerol, acetate and ethanol. Fermentation eventually ceases due to exhaustion of nutrients and inhibition by ethanol.

$$C_6H_{10}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

Lagering and ageing: - At the end of fermentation, the product is beer and it has a harsh taste and referred to as ‘green beer’. It has a yeasty taste arising probably from higher alcohols and aldehydes. Materials which might undesirably affect flavor and which are present in green beer e.g., diacetyl, hydrogen sulfide, mercaptans and acetaldehyde are decreased by evaporation during secondary fermentation. An increase occurs in the desirable components of the beer such as esters. Any tannins, proteins, and hop resins still left are precipitated during the lagering period. In England, where ale or top beer is consumed, the beer is not processed further but drawn at this stage. It is however, primed to

improve its taste and appearance. Priming is done by small amount of sucrose, invert sugar or a mixture of cereal starch hydroxylase and invests sugar caramel. Priming serves as substrate for secondary fermentation as well as beer sweetener. After 'priming', the beer is 'fined' by the addition of isinglass. Isinglass, a gelatinous material from the swim bladder of fish, precipitates yeast cells, tannins and protein-tannin complexes. Lagering gives the beer its final desirable organoleptic qualities, but it is hazy due to protein-tannin complexes and yeast cells. The beer is filtered through kieselghur or through membrane filters to remove these. The beer is thereafter pasteurized and distributed.

In the case of lager beer, a fermented beer is transferred to maturation tank and in the process, it is aerated and this facilitates a 2^o fermentation. Lagering occurs at 0 – 3^oC for 2weeks to several months. The CO_2 produced during the 2^o fermentation purges out dissolved O_2, H_2S and other unwanted violates. Moreover, the beer acquires additional properties which make it harsh taste mellow. Diacetyl vicinyl diketones formed during yeast fermentation are taken by yeast preventing formation of odours. All suspended and undissolved solid gradually settle to the bottom of the tank and the beer is decanted free of yeast cells and other solids. It is filtered either with filter mass or filter sleet to remove its haze with the aid of diatomaceous earth filter. In some breweries, ascorbic acid is added to prevent oxidation of some of the beer components.

Packaging:

The beer is transferred to pressure tanks from where it is distributed to cans, bottles and other containers. The beer is not allowed to come in contact with oxygen during this operation; it is also not allowed to lose CO_2, or to become contaminated with microorganisms. To achieve these objectives, the beer is added to the tanks under a CO_2, atmosphere, bottled under a counter pressure of CO_2, and all the equipment is cleaned and disinfected regularly.

Bottles are thoroughly washed with hot water and sodium hydroxide before being filled. The filled and crowned bottles are passed through a pasteurizer, set to heat the bottles at 60°C for half hour**.**

Bottling: - The filtered beer is pumped to the bottling hall for bottling. Some breweries, the beer is carbonated to the extent of 0.45 – 0.50% by weight. In some other breweries, no CO_2 is added, it is the CO_2 generated during secondary fermentation that is used for carbonation.

Pasteurization: - Filled and cannel bottles are pasteurized before labeling.

Beer Defects

The most important beer defect is the presence of haze or turbidity, which can be of biological or physico-chemical origin.

Biological turbidities

Biological turbidities are caused by spoilage organisms and arise because of poor brewery hygiene (i.e. poorly washed pipes) and poor pasteurization. Spoilage organisms in beer must be able to survive the following stringent conditions found in beer: low pH, the antiseptic substances in hops, pasteurization of beer, and anaerobic conditions. Yeasts and certain bacteria are responsible for biological spoilage because they can withstand these. Wild or unwanted yeasts which have been identified in beer spoilage are spread into many genera including *Kloeckera*, *Hansenula*, and *Brettanomyces*, but *Saccharomyces* spp appear to be commonest, particularly in top-fermented beers.

Physico-chemical turbidities

Non-biological hazes developing beer may be due to one or more of the following:

(i) Hazes induced by metals: Tin, iron, copper have all been identified as causing hazes in beer.

(ii) Protein-tannin hazes: Beer tannings or polyphenols are derived from hops and barley husks. They react with proteins to form complex molecules which become insoluble in the form of haze.

Hazes contain polypeptides, polyphenols, carbohydrates and a small amount of minerals.

Beer hazes are divided into two: ***Chill hazes*** (0.1–2 nm diameter particles) form at O°C and re-dissolve at 20°C. ***Permanent hazes*** (1.0–10 nm) remains above 20°C.

Protein-tannin hazes may be removed by:

(a) addition of papain which hydrolyzes the polypetides to low molecular weight components which cannot form hazes;
(b) adsorption of the polypeptides by silica gel and bentonite;
(c) precipitation of polypetides by tannic acid;
(d) adsorption of the polyphenols by polyamide resins e.g. Nylon 66.

(iii) Polysaccharide sediments: Freezing and thawing of beer may cause an unpredictable haze which can appear in the form of flakes. This haze differs from chill haze in being distinctly carbohydrate in nature.

(iv) Oxalate hazes and sediments. Oxalate sediments may appear after several week's storage inbeers rich in oxalate as a result of a low calcium content.

v) Other beer defects: Wild or gushing beer is a defect observed as a violent over foaming when a bottle of beer is opened. The taste is unaffected. Gushing is due to the formation of micro-bubbles; excess pressure may force the micro-bubbles back into solution. Gushing beers have been identified with malt made from old barley and trial brews have shown them to be associated with the presence of mycelia of Fusarium during the steeping. The off-flavor developed when beer is exposed to sunlight is due to the formation of mercaptans by photochemical reaction in the blue-green region (420-520 nm) of visible light. What is the essence of carrying out the malting process?

Self-Assessment Exercises 2

Attempt the exercise to determine what you have learnt so far. (1min).
i Mention the three mashing methods
ii. What is green beer?

2.4. Summary.

The raw materials for brewing have been discussed. In addition the brewing processes have been highlighted with their defects enunciated.

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2.6. Possible answers to Self-Assessment Exercises

Answers Self-Assessment Exercise 1
-(Hordeum vulgare) and (Hordeum distichon).

-Adjuncts are starchy materials which were originally introduced during the brewing process. It includes materials other than would be hydrolyzed by amylase

Answers Self-Assessment Exercise 2
i.(a) Decoction methods, where part of the mash is transferred from the mash tun to the mash kettle where it is boiled.
(b) Infusion methods, where the mash is never boiled, but the temperature is gradually raised.
(c) The double mash method in where the starchy adjuncts are boiled and added to the malt.

ii. It has a yeasty taste arising probably from higher alcohols and aldehydes. Materials which might undesirably affect flavor and which are present in green beer e.g. diacetyl, hydrogen sulfide, mercaptans and acetaldehyde are decreased by evaporation during secondary fermentation.

Unit 3. Wine Production

3.0 Introduction

Wine is defined as a product of the normal alcoholic fermentation of the juice of sound ripe grapes.

3.1 Intended Learning Outcomes (ILOs)

At the end of this unit, you should be able to:

- Explain in details the process of production of Wine
- Understand the steps in wine making.

3.2 MAIN CONTENT

Wine production

Wine is defined as a product of the "normal alcoholic fermentation of the juice of sound ripe grapes". Nevertheless, any fruit with a good proportion of sugar may be used for wine production. Thus, citrus, bananas, apples, pineapples, strawberries etc., may all be used to produce wine. Such wines are always qualified as fruit wines. The production of wine is simpler than that of beer in that no need exists for malting since sugars are already present in the fruit juice being used. This however exposes wine making to greater contamination hazards.

3.2.1 Processes in Wine Making

***Crushing of Grapes*:**

The selected ripe grapes of 21° to 23° Balling (*density scale, named for*

its developer Karl Balling and used for measuring sugar content in water-based solutions) are crushed to release the juice which is known as '**must**', after the stalks which support the fruits have been removed. These stalks contain tannins which would give the wine a harsh taste if left in the must. The skin contains most of the materials which give wine its aroma and color. For the production of red wines, the skins of black grapes are included, to impart the color. Grape juice has an acidity of 0.60-0.65% and a pH of 3.0-4.0 due mainly to malic and tartaric acids with a little citric acid. During ripening both the levulose content and the tartaric acid contents rise.

Fermentation:

(i) Yeast used: The **must** is partially 'sterilized' by the use of sulphur dioxide, a bisulphate or a metabisulphite which eliminates most microorganisms in the must leaving wine yeasts. Yeasts are then inoculated into the must. The yeast which is used is *Saccaromyces cerevisiae* var, *ellipsoideus*. Other yeasts which have been used for special wines are *Sacch. fermentati, Sacch. oyiformis* and *Sacch. bayanus.*

(ii) Control of fermentation:

(a)***Temperature***: The fermentation is cooled and the temperature is maintained at around 24°C with cooling coils mounted in the fermentor.

(b) ***Yeast Nutrition***: To produce sweet wine glucose-fermenting wine yeasts are used leaving the fructose which is much sweeter than glucose. Most nutrients including macro- and micro-nutrients are usually abundant in must; occasionally, however, nitrogenous compounds are limiting.

(c) ***Oxygen***: Oxygen is required in the earlier stage of fermentation when yeast multiplication is occurring. In the second stage when alcohol is produced the growth is anaerobic and this forces the yeasts to utilize such intermediate products.

(iii) Flavor development: Although some flavor materials come from the grape most of it come from yeast action. Flavor of wine has been elucidated with gas chromatography and has been shown to be due to alcohols, esters, fatty acids, and carbonyl compounds, the esters being the most important. Diacetyl, acetonin, fusel oils, volatile esters, and hydrogen sulfide have received special attention. Autolysates from yeasts also play a role.The fermentation is usually over in three to five days. At this time 'pomace' formed from grape skins (in red wines) will have risen to the top of the brew. At the end of this fermentation the wine is allowed to flow through a perforated bottom if pomace had been allowed. When the pomace has been separated from wine and the fermentation is complete or stopped, the next stage is 'racking'. The wine is allowed to stand until a major portion of the yeast cells and other fine suspended materials have collected at the bottom of the container as sediment or 'lees'. It is then 'racked', during which process the clear wine is carefully pumped or siphoned off without disturbing the lees.

The wine is then transferred to wooden casks (100-1,000 gallons), barrels (about 50 gallons) or tanks (several thousand gallons). The wood allows the wine only slow access to oxygen. During ageing desirable changes occur in the wine.

Clarification

The wine is allowed to age in a period ranging from two years to five years, depending on the type of wine. At the end of the period some will have cleared naturally. For others artificial clarification may be necessary. The addition of a fining agent is often practiced to help clarification. The usual fining agents for wine are gelatin, casein, tannin, isinglass, egg albumin, and bentonite. In some countries the removal of metal ions is accomplished with potassium ferrocyanide known as 'blue fining'; it removes excess ions of copper, iron, manganese, and zinc from wines.

Packaging

Wine from various sources is sometimes blended and then pasteurized. In some wineries, the wine is not pasteurized, rather it is sterilized by filtration. In many countries the wine is packaged and distributed in casks.

Wine Defects

The most important cause of wine spoilage is microbial; less important defects are acidity and cloudiness. Factors which influence spoilage by bacteria and yeasts include the following (a) wine composition, specifically the sugar, alcohol, and sulfur dioxide content; (b) storage conditions e.g., high temperature and the amount of air space in the container; (c) the extent of the initial contamination by microorganism during the bottling process. When proper hygiene is practiced bacterial spoilage is rare. When it does occur the microorganisms concerned are acetic acid bacteria which cause sourness in the wine. Lactic acid bacteria especially *Leuconostoc*, and sometimes *Lactobacillus* also spoil wines. Various spoilage yeasts may also grow in wine.

Wine Preservation

Wine is preserved either by chemicals or by some physical means. The chemicals used include bisulphites, diethyl pyrocarbonate and sorbic acid. Physical means include pasteurization and sterile filtration. Pasteurization is avoided, when possible, because of its deleterious effect on wine flavor.

When selected ripe grapes are crushed to release the juice which is known as……?

Self-Assessment Exercises 1

Attempt the exercise to determine what you have learnt so far. (1min).
i Flavor of wine has been elucidated with gas chromatography and has been shown to be due to……...

3.2.2 Classification of Wines

Grape wines may be classified in several ways. **Some of the criteria include place of origin, color, alcohol content and sweetness**. The two major groups are the natural and fortified wines.

(A.) Natural wines: Contains 9-14% alcohol; nature and keeping quality mostly dependent on 'complete' yeast fermentation and protection from air

1. ***Still wines*** (known as 'Table' wines intended as part of meal); **no carbon dioxide added.**

(a) Dry table wines: (no noticeable sweetness) (i) White; (ii) Rose (pink); (iii) Red

(b) Sweet table wines (i) White (ii) Rose

Further naming of above depends on grape type, or region of origin.

2. ***Sparkling wines*** (**appreciable CO_2 under pressure**)

(a) White (Champagne); (b) Rose (Pink Champagne); (c) Red (Sparkling burgundy; cold duck)

(B). Fortified (**Dessert and appetizer**) wines: Contain 15 to 21% alcohol; nature and keeping quality depends heavily on addition of alcohol distilled from grape wine.

1. *Sweet wines*

(a) White (Muscatel, White port, angelica)

(b) Pink (California tokay, tawny port)

(c) Red (Port, black Muscat)

2. *Sherries*: (White sweet or dry wines with oxidized flavors)

(a) Aged types

(b) Flor types

(c) Baked types

3. *Flavored specialty wines* (usually white Port base)

(a) Vermouth (pale dry, French; Italian sweet types)

(b) Proprietary brands

The natural wines

These result from complete natural fermentation. Further fermentation is prevented because the sugar is to a large extent exhausted. Spoilage organisms such as acetic acid bacteria do not grow if air is excluded. Owing to the natural limit of sugar in grapes, the alcohol content does not usually exceed 12%. They are sub-divided into still (without added CO_2) and sparkling (with added CO_2). Color and sweetness also subdivide the wines. The color, pink or red, is derived from the color of the grape; white wine comes from a grade whose skin is light-green, but whose juice is clear.

Table wines: The natural wines are usually consumed at one sitting once they are opened. For this reason, they are called 'table' wines and intended to be part of a meal.

Dessert and appetizer wines: The dessert or appetizer wines are served at the beginning (appetizers) or at the end (dessert) of meals. They

contain extra alcohol from distilled wines, partly to make them more potent, but also to preserve them from yeast spoilage.

Sparkling Wines: Sparkling wines contain CO_2 under pressure before they are opened. They are called sparkling because the gentle release of carbon dioxide from the wine after the bottle is opened gives the wine a sparkle. The best known of the sparkling wines is produced in Champagne. Champagne is produced either in a bottle or in bulk.

3.2.3 Fruit wines: cider and perry

Cider is derived from apples, (*Malus pumila*) and perry from pears or a mixture of pears and apples. They differ from other fruit wines in that their alcohol content is low (4-5% with a maximum of 7-8% v/v) because sugar is not usually added. The basic processes are similar to those of grape wine: pressing out the juice, fermenting, maturing, and bottling. Fruit wines have been made from cashew, pineapples, and other fruits.

3.3 Palm Wine

Palm wine is a general name for alcoholic beverages produced from the saps of palm trees. It differs from the grape wines in that it is opaque. It is drunk all over the tropical world in Africa, Asia, South America. Palm wine is usually a whitish and effervescent liquid both of which properties derive from the fact that the fermenting organisms are numerous and alive when the beverage is consumed. The sap produced from *Elaeis guiniensis* contains about 12% sucrose, about 1% each of fructose, glucose, and raffinose, and small quantities of protein and some vitamins and is a clear, sweet, syrupy liquid. To produce palm wine a succession of microorganisms occurs roughly: Gram-negative bacteria, lactic acid bacteria and yeasts and finally acetic acid bacteria. Yeasts in palm wine have been identified as coming from various genera. The great problem with palm wine is that its shelf life is extremely short. It is best consumed within about 48 hours, but certainly not beyond about five days after tapping.

3.4 Other African alcoholic beverages

(i) ***Bouza*** is an alcoholic beverage produced in Egypt since the time of the Pharoahs. It is drunk by all classes, presently among the lower income groups. Egyptian Bouza is prepared either from wheat or maize but the most popular is from wheat.

(ii) ***Talla*** (tella) is an Ethiopian small-producer beer with a smokey flavor derived from inverting the fermentation containers and talla collection pots over smouldering olive wood. Talla also acquires some smoke flavor from the toasted, milled and boiled cereal grains. During the toasting the grains are roasted until they begin to smoke slightly. In the production of talla, of which various types exist, powdered hop leaves and water are put in a fermentation vessel and allowed to stand for about three days.

(iii) ***Busaa*** is an acidic alcoholic beverage drunk among the Luo. Abuluhya and Maragoli ethnic groups of Kenya. It is porridge-like and

light-brown in color and is warmed to 35- 40°C before being consumed. A stiff dough made from maize flour and water is incubated at room temperature for three to four days.

(iv) ***Merissa*** is a sour Sudanese alcoholic (up to 6%) beverage made from sorghum. It has a pH of about four (4) and a lactic acid content of about 2.5%. Sorghum grains are malted, dried, and ground into a coarse powder.

(v)***Tej*** is a mead (i.e., a wine made by fermenting honey) of Ethiopian origin. It is yellow,
sweet, effervescent, and cloudy due to its yeast content.

(vi)***Agadagidi*** wines are made from bananas and plantains and have the opaque, effervescent sweet-sour nature typical of African traditional alcoholic beverages. In Nigeria the best-known agadagidi is found in the cocoa-growing areas of south western Nigeria where plantains provide shade for the young cocoa trees. The ripe fruits are peeled and soaked in water where the sugars dissolving from the preparation permit the development of yeasts and lactic acid and giving rise to a typical opaque effervescent wine. The alcohol content is about l%.

(vii) ***Mbege*** is consumed in Tanzania mainly by those living near Mount Kilimanjaro. It is produced from a mixture of malted millet and fermented banana juice. The juice is produced by boiling the ripe banana followed by decantation. The banana infusion is mixed with cooked and cooled millet malt and allowed to ferment for four to five days.

During the production of table wine, does it involve addition of carbon dioxide?

Self-Assessment Exercises 2

Attempt the exercise to determine what you have learnt so far. (1min). *i What is Agadagidi made from ……...*

3.5. Summary.

In this study various types of wines produced from various fruits were discussed. Also palm wine which from sap of palm trees were discussed. Furthermore, other forms of African alcoholic beverages such as Bouza, Tella, Agadagidi, Tej, Merrisa and Mbege were discussed.

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3.7 Possible answers to Self-Assessment Exercises

Answers Self-Assessment Exercise 1

....alcohols, esters, fatty acids, and carbonyl compounds, the esters

Answers Self-Assessment Exercise 2

...wines are made from bananas and plantains

Unit 4. Distilled alcoholic (or spirit) beverages

4.0 Introduction

The distilled alcoholic or spirit beverages are those potable products whose alcohol contents are increased by distillation.

4.1 Intended Learning Outcomes (ILOs)

At the end of this unit, you should be able to:

- Explain in details the process of spirit beverages
- Understand the principles of spirit beverages.

4.2 MAIN CONTENT

Distilled alcoholic (or spirit) beverages

The distilled alcoholic or spirit beverages whose alcohol contents are increased by distillation. In the process of distillation volatile materials emanating directly from the fermented substrate or after microbial (especially yeast) metabolism introduce materials which have a great influence on the nature of beverage. The components of spirit beverages which confer specific aromas on them are known as **congeners**.

4.2.1 Principles in the Production of Spirit Beverages

(i) ***Preparation of the medium***: In the grain beverages (whisky, vodka, gin) the grain starch is hydrolyzed to sugars with microbial enzymes or with the enzymes of barley malt. In all the others no hydrolysis is necessary as sugars are present in the fermenting substrate as in brandy (grape sugar) and rum (cane sugar).

(ii) ***Propagation of yeast inoculum***: Large distilleries produce hundreds of liters of spirits daily for which fermentation broths many more times

in volume are required. These broths are inoculated with up to 5% (v/v) of thick yeast broth.

(iii) ***Fermentation***: When the nitrogen content of the medium is insufficient nitrogen is added usually in the form of an ammonium salt.

(iv) ***Distillation***: Distillation is the separation of more volatile materials from less volatile ones by a process of vaporization and condensation. Three systems used in spirit distillation are *a. Rectifying Stills b. Pot Still c.Coffey still.*

(v) ***Maturation***: Some of the distilled alcoholic beverages are aged for some years, often prescribed by legislation.

(vi) ***Blending***: Before packaging, samples of various batches of different types of a given beverage are blended together to develop a particular aroma.

Spirit Beverages include: Whisky, brandy, rum, vodka, kai-kai (or akpeteshi), schnapps, and cordials.

In-Text Question (ITQ)

The components of spirit beverages which confer specific aromas on them are known as?

Answer: Congeners

Self-Assessment Exercises 1

Attempt the exercise to determine what you have learnt so far. (1min).
i What is the three systems used in spirit distillation ……...

Whisky

Whisky is the alcoholic beverage derived from the distillation of fermented cereal. Various types of whiskies are produced; they differ principally in the cereal used. In all whisky-producing countries the alcoholic content, the materials and the method of preparation are controlled by government regulations. For instance, the Scottish Malt Whisky the barley is malted just as in beer making, but during the kilning smoke from peat is allowed to permeate the green (fresh) malt, that the whisky made from the malt has a strong aroma of peat smoke, derived mainly from phenol. In the United States the principal types of whisky are rye and bourbon whiskies. Rye whisky is prepared from rye and rye malt, or rye and barley and barley malt. Bourbon whisky is prepared from preferably yellow maize, barley malt or wheat malt.

Brandy

Brandy is a distillate of fermented fruit juice. Thus, brandy can be produced from any fruit-strawberries, paw-paw, or cashew. The word brandy refers to the distillate from fermented grape juice. It is subject to a distillation limitation of 170° proof (85%). The fermented liquor is double distilled, without previous storage, in pot stills. A minimum of two years maturation in oak casks is required for maturation.

Rum

Rum is produced from cane or sugar by products especially molasses or cane juice. Rum production is associated with the Carribean especially Jamaica, Cuba, and Puerto Rico. It is also produced in the eastern USA. Rum with a heavy body is produced from molasses; while light rum is produced from cane syrup using continuous distillation. During the fermentation the molasses is clarified to remove colloidal material which could block the still by the addition of sulphuric acid. The pH is adjusted to about 5.5 and a nitrogen source ammonium sulphate or urea may be added.

The word brandy refers……?

Self-Assessment Exercises 2

Attempt the exercise to determine what you have learnt so far. (1min).
i Various types of whiskies are produced; they differ principally in the ……..

Gin, Vodka, and Schnapps

Gin, vodka, and schnapps are water clear.The congenerics derived from fermentation are removed and flavoring is provided (except in vodka) with plant parts. The raw materials for their production is usually a cereal but potatoes or molasses may be used. For gin, maize is used, while for vodka rye is used. The cereals are gelatinized by cooking and mashed with malted barley. Schnapps are gin flavored with herbs.

Kai-kai, Akpeteshi, or Ogogoro

Kai-kai is an alcoholic beverage widely drunk in West Africa. It is produced by distilling fermented palm-wine. It is the base for preparing some of the better known brands such as
schnapps.

4.3. Summary.

In this section the various spirit beverage production has been highlighted. In addition, the steps involved in the production were discussed.

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4.5 Possible answers to Self-Assessment Exercises

Answers Self-Assessment Exercise 1

....a)*Rectifying Stills b. Pot Still c.Coffey still*

Answers Self-Assessment Exercise 2

... the cereal used

Unit 5. Fermented Foods I: Bread Making and Milk products

5.0 Introduction

Fermented foods are derived through the activities of microorganisms in which the weight of the microorganisms in the food is usually small. The influence of microbial activity on the nature of the food, especially in terms of flavor and other organoleptic properties, is profound.

5.1 Intended Learning Outcomes (ILOs)

At the end of this unit, you should be able to:

- Understand the process of bread making.

5.2 MAIN CONTENT

Fermented foods

Fermented foods may be defined as foods which are processed through the activities of microorganisms. The influence of microbial activity on the nature of the food, especially in terms of flavor and other organoleptic properties, is profound. Fermented foods are influenced mainly by the nature of the substrate and the organisms involved in the fermentation, the length of the fermentation and the treatment of the food during the processing.

Fermented foods have several advantages:

(a) Fermentation serves as a means of preserving foods in a low-cost manner; thus, cheese keeps longer than the milk from which it is

produced;
(b) The organoleptic properties of fermented foods are improved in comparison with the raw materials from which they are prepared; cheese for example, tastes very different from milk from which it is produced;
(c) Fermentation sometimes removes unwanted or harmful properties in the raw material; thus fermentation removes flatulence factors in soybeans, and reduces the poisonous cyanide content of cassava during garri preparation;
(d) The nutritive content of the food is improved in many items by the presence of the microorganisms; thus the lactic acid bacteria and yeasts in garri and the yeasts in bread add to the nutritive quality of these foods;
(e) Fermentation often reduces the cooking time of the food as in the case of fermented soy bean products, or ogi the weaning West African food produced from fermented maize.

5.2.1 BREAD BAKING

The use of yeast as a leavening agent in baking dates back to the very early history of the Jews, Egyptians, Greeks and Romans. Bread has been known to man for many centuries and excavations have revealed that bakers' ovens were in use by the Babylonians, about 4,000 B.C. Today, bread supplies over half of the caloric intake of the world's population including a high proportion of the intake of Vitamins B and E. The basic ingredients in bread-making are **flour, water, salt, and yeasts**. In modern breadmaking however a large number of other components and additives are used as knowledge of the baking process has grown. These components depend on the type of bread and on the practice and regulations operating in a country. They include 'yeast food', sugar, milk, eggs, shortening (fat) emulsifiers, anti-fungal agents, anti-oxidants, enzymes, flavoring, and enriching ingredients. The ingredients are mixed together to form dough which is then baked.

Flour

Flour is the chief ingredient of bread and is produced by milling the grains of wheat, various species and varieties of which are known. For flour production most countries use *Triticum vulgare*. The chief constituents of flour are starch (70%), protein (7-15%), sugar (1%), and lipids (1%). In bread-making from *T. vulgare* the quality of the flour depends on the quality and quantity of its proteins. Flour proteins are of two types. The first type forming about 15% of the total is soluble in water and dilute salt solutions and is non-dough forming. It consists of albumins, globulins, peptides, amino acids, and enzymes. The remaining 85% are insoluble in aqueous media and are responsible for dough formation. They are collectively known as gluten. It also contains lipids. Gluten has the unique property of forming an elastic structure when moistened with water. It forms the skeleton which holds the starch, yeasts, gases and other components of dough. Gluten can be easily

extracted, by adding enough water to flour and kneading it into dough. After allowing the dough to stand for an hour the starch can be washed off under a running tap water leaving a tough, elastic, sticky and viscous material which is the gluten.

Yeast

The yeasts used for baking are strains of *Saccharomyces cerevisiae*. The ideal properties of yeasts used in modern bakeries include but not limited to:

(a) Ability to grow rapidly at room temperature of about 20-25°C;
(b) Easy dispersibility in water;
(c) Ability to produce large amounts of CO_2 rather than alcohol in flour dough;
(d) Good keeping quality i.e., ability to resist autolysis when stored at 20°C;
(e) Ability to adapt rapidly to changing substrates such as are available to the yeasts during dough making.

The roles of yeasts in bread-making are leavening, flavor development and increased nutritiveness.

The amount of yeasts used during baking depends on the flour type, the ingredients used in the baking, and the system of baking used. Very 'strong' flours (i.e., with high protein levels) require more yeast than softer ones. High amount of components inhibitory to yeasts e.g., sugar (over 2%), antifungal agents and fat) usually require high yeast additions. Baking systems which involve short periods for dough formation, need more yeast than others. In general, however yeast amounts vary from 2-2.75% (and exceptionally to 3.0%) of flour weight.

The roles of yeasts in bread-making are leavening, flavor development and increased nutritiveness.

Sugar

Sugar is added to provide:

(a) carbon nourishment for the yeasts; additional to the amount available in flour sugar
(b) to sweeten the bread;
(c) to afford more rapid browning (through sugar caramelization) of the crust and hence greater moisture retention within the bread. Sugar is supplied by the use of sucrose, fructose corn syrups (regular and high fructose), depending on availability.

Shortening (Fat)

Animal and vegetable fats are added as shortenings in bread-making at about 3% (w/w) of flour in order to yield (a) increased loaf size; (b) a more tender crumb; and c) enhanced slicing properties.

Emulsifiers (Surfactants)

Emulsifiers are used in conjunction with shortening and ensure a better distribution of the fat in the dough.

Milk

Milk to be used in bread-making must be heated to high temperatures before being dried. Milk is added to make the bread more nutritious, to help improve the crust color, presumably by sugar caramelization and because of its buffering value.

Salt

About 2% sodium chloride is usually added to bread. It serves the following purposes:

(a) It improves taste;

(b) It stabilizes yeast fermentation;

(c) As a toughening effect on gluten;

(d) Helps retard proteolytic activity, which may be related to its effect on gluten;

(e) It participates in the lipid binding of dough.

Due to the retarding effect on fermentation, salt is preferably added towards the end of the mixing.

Water

Water is needed to form gluten, to permit swelling of the starch, and to provide a medium for the various reactions that take place in dough formation.

Enzymes

Sufficient amylolytic enzymes must be present during bread-making to breakdown the starch in flour into fermentable sugars.

Mold-inhibitors (antimycotics) and enriching additives

The chief anti-mycotic agent added to bread is calcium propionate. Others used to a much lesser extent are sodium diacetate, vinegar, mono-calcium phosphate, and lactic acid. It is used for controlling spoilage fungi: *Rhizopus*, *Mucor*, *Aspergillus* and *Penicillium.*

Baking

Bread is baked at a temperature of about 235°C for 45–60 minutes. As the baking progresses and temperature rises gas production rises and various events occur as below:

• At about 45°C the undamaged starch granules begin to gelatinize and are attacked by alpha-amylase, yielding fermentable sugars;

• Between 50 and 60°C the yeast is killed;

• At about 65°C the beta-amylase is thermally inactivated;

• At about 75°C the fungal amylase is inactivated;

• At about 87°C the cereal alpha-amylase is inactivated;

• Finally, the gluten is denatured and coagulates, stabilizing the shape and size of the loaf.

5.2.2 Systems of Bread-making

Large-scale bread-making is mechanized. The processes of yeast-leavened bread-making

may be divided into:

(a) Pre-fermentation (or sponge mixing): At this stage a portion of the ingredients is mixed with yeast and with or without flour to produce an

inoculum. During this the yeast becomes adapted to the growth conditions of the dough and rapidly multiplies. Gluten development is not sought at this stage.
(b) Dough mixing: The balance of the ingredients is mixed together with the inoculum to form the dough. This is the stage when maximum gluten development is sought.
(c) Cutting and rounding: The dough formed above is cut into specific weights and rounded by machines.
(d) First (intermediate) proofing: The dough is allowed to rest for about 15 minutes usually at the same temperature as it has been previous to this time i.e., at about 27°C. This is done in equipment known as an overhead proofer.
(e) Molding: The dough is flattened to a sheet and then molded into a spherical body and placed in a baking pan which will confer shape to the loaf.
(f) Second proofing: This consists of holding the dough for about 1 hour at 35-43°C and
in an atmosphere of high humidity (89-95°C).
(g) Baking: During baking the proofed dough is transferred, still in the final pan, to the oven where it is subjected to an average temperature of 215-225°C for 17-23 minutes. Baking is the final of the various baking processes. It is the point at which the success or otherwise of all the previous inputs is determined.
(h) Cooling, slicing, and wrapping: The bread is depanned, cooled to 4-5°C sliced (optional in some countries) and wrapped in waxed paper, or plastic bags.

5.2.3 The Three Basic Systems of Bread-making

There are three basic systems of baking. All three are essentially similar and differ only in the presence or absence of a pre-fermentation. Where pre-fermentation is present, the formulation of the pre-ferment may consist of a broth or it may be a sponge (i.e., includes flour). All three basic types may be sponge i.e includes flour. All three basic types may also be batch or continuous.
(i) Sponge doughs: This system or modification of it is the most widely used worldwide.
In the sponge-dough system of baking a portion (60-70%) of the flour is mixed with water, yeast and yeast food in a slurry tank (or 'ingridator') during the pre-fermentation to yield a spongy material due to bubbles caused by alcohol and CO_2. If enzymes are used, they may be added at this stage. The sponge is allowed to rest at about 27°C and a relative humidity of 75-80% for 3.5 to 5 hours. During this period the sponges rises five to six times because of the volatile products released by this yeast and usually collapses spontaneously. During the next (or dough) stage the sponge is mixed with the other ingredients.
(ii) ***The liquid ferment system***. In this system water, yeast, food, malt, sugar, salt and, sometimes, milk is mixed during the pre-fermentation at

about 30°C and left for about 6 hours. After that, flour and other ingredients are added in mixed to form a dough.
(iii) ***The straight dough system***: In this system, all the components are mixed at the same
time until a dough is formed. The dough is then allowed to ferment at about 28-30°C for 2- 4 hours. During this period, the risen dough is occasionally knocked down to cause it to collapse. Thereafter, it follows the same process. The straight dough is usually used for home bread making.
The basic ingredients in bread-making are……?

Self-Assessment Exercises 1

Attempt the exercise to determine what you have learnt so far. (1min).
i Mention the three Basic Systems of Bread-making

5.2.4 Role of Yeasts in Bread-making

Methods of Leavening: Leavening is the increase in the size of the dough induced by gases
during bread-making. Leavening may be brought about in a number of ways.
(a) Air or carbon dioxide may be forced into the dough; this method has not become popular.
(b) Water vapor or steam which develops during baking has a leavening effect. This has not been used in baking; it is however the major leavening gas in crackers.
(c) Oxygen has been used for leavening bread. Hydrogen peroxide was added to the dough and oxygen was then released with catalase.
(d) It has been suggested that carbon-dioxide can be released in the dough by the use of decarboxylases, enzymes which cleave off carbon dioxide from carboxylic acids.
This has not been tried in practice.
(e) The use of baking powder has been suggested. Baking powder consists of about 30% sodium bicarbonate mixed in the dry state with one of a number of leavening acids, including sodium acid pyrophosphate, monocalcium phosphate, sodium aluminum phosphate, monocalcium phosphate, glucono-delta-lactone. CO_2 is evolved on contact of the components with water: part of the CO_2 is evolved during dough making, but the bulk is evolved during baking. Baking powder is suitable for cakes and other high-sugar leavened foods, whose osmotic pressure would be too high for yeasts. Furthermore, weight for weight yeasts is vastly superior to baking powder for leavening.
(f) Leavening by microorganisms, may be done by any facultative organism releasing gas under anaerobic conditions such as heterofermentative lactic acid bacteria, including *Lactobacillus*

plantarum or pseudolactics such as *Escherichia coli*. In practice however yeasts are used; even when it is desirable to produce bread quickly such as for the military or for sportsmen and for other emergency conditions the use of yeasts recommends itself over the use of baking powder.

5.2.5 Factors which effect the leavening action of yeasts

(i) The nature of the sugar available: When no sugar is added to the dough such as in the traditional method of bread-making, or in sponge of sponge-doughs and some liquid
ferments, the yeast utilizes the maltose in the flour.
(ii) Osmotic pressure: High osmotic pressures inhibit yeast action. Baker's yeast will produce CO_2 rapidly in doughs up to a maximum of about 5% glucose, sucrose or fructose or in solutions of about 10%. Beyond that gas production drops off rapidly. Salt at levels beyond about 2% (based on flour weight) is inhibitory on yeasts.
iii) Effect of nitrogen and other nutrients: short fermentations require no nutrients but for
longer fermentation, the addition of minerals and a nitrogen source increases gas
production.
(iv) Effect on fungal inhibitors (anti-mycotic agents): Anti-mycotics added to bread are all
inhibitory to yeast.
(v) Yeast concentration: The weight of yeast for baking rarely exceeds 3% of the flour weight. A balance exists between the sugar concentration, the length of the fermentation and the yeast concentration.

5.3 Fermented foods made from milk

Milk is the fluid from the mammary glands of animals which is meant for feeding the young of mammals. It is a complex liquid consisting of several hundred components of which the most important are proteins, lactose, fat, minerals, enzymes, and vitamins in which emusified fat globules and casein micelles are present. Milk proteins are divided into two: **caseins** and **whey** proteins.
Lactose: The main carbohydrate in milk is lactose, which is found only in milk. It is a dissacharide of glucose and galactose and has a low sweetening ability, as well as low solubility in water.
Fat: Fat consists of one molecule of glycerol and three of fatty acids. Over 60 different acids are known in butter, many of them, being of low molecular weight of about 10 carbon atoms or less, and include saturated and unsaturated fatty acids.
Enzymes: Enzymes found in milk include proteases, carbohydrases,

esterases, oxidases/ reductases.

Minerals: Milk is a major source of calcium; other minerals in milk are phosphorous, magnesium, sodium, potassium, as well as sulphate and chloride ions. When fat is removed from milk such as during butter making, the remnant is **skim milk**. When casein is removed such as during cheese manufacture, the remnant is known as **whey**.

Cheese

Cheese is a highly proteinaceous food made from the milk of some herbivores. Cheese is believed to have originated in the warm climates of the Middle East some thousands of years ago, and is said to have evolved when milk placed in goat stomach was found to have curdled. Cheese made from the milk of goat and sheep has a much stronger flavor than that made from cow's milk. This is because the fat in goat and sheep milk contain much lower amounts of the lower fatty acids, caproic, capryllic, and capric acids. About a thousand types of cheese have been described depending on the properties and treatment of the milk, the method of production, conditions such as temperature, and the properties of the coagulum, and the local preferences.

Stages in the manufacture of cheese

(a) ***Standardization of milk***: The quality of the milk has a decided effect on the nature of cheese. Cheese made from skim milk is hard and leathery; the more fat a cheese contains the smoother its feel to the palate.

(b***) Inoculation of pure cultures of lactic acid bacteria as starter cultures***: In the past, lactic acid was produced by naturally occurring bacteria. Nowadays they are inoculated artificially, by specially selected bacteria termed starters. Indeed, lactic acid formation is necessary in all kinds of cheese.

Lactic acid has the following effects:

(i) It causes the coagulation of casein at pH 4.6, the isoelectric point of that protein, which is used in the manufacture of some cheeses, e.g., cottage cheese.

(ii) It provides a favorably low pH for the action of rennin the enzyme which forms the curd from casein in other types of cheeses.

(iii) The low pH eliminates proteolytic and other undesirable bacteria.

(iv) It causes the curd to shrink and thus promotes the drainage of whey.

(v) Metabolic products from the lactic acid bacteria such as ketones, esters and aldehydes contribute to the flavor of the cheese.

Problems of lactic acid bacteria in cheese-making

(i) *Attack by bacteriophages*: Bacteriophages sometimes attack the lactic acid starters and besides choosing strains that are resistant to phages, rotations (i.e., using different lactic mixtures every three or four days) helps eliminate them.

(ii) *Inhibition by penicillin and other antibiotics*: Lactic acid bacteria, being Gram-positive

are particularly susceptible to penicillin used to treat diseased udder in

mastitis if the antibiotic finds its way into the milk; other antibiotics also have an inhibitory effect on them.

(iii) *Undesirable strains*: Some strains of lactic acid bacteria are undesirable in cheese making because they produce too much gas, undesirable flavors, or produce antibiotics against other lactic acid bacteria. They arise by mutation.

(iv) *Sterilant and detergent residues*: Sterilant and detergent residues may inhibit the growth of starter bacteria. The minimum concentration required for inhibition varies with the different anti-microbial agents and between different strains of starter bacteria. Residues gain entry to milk at the (a) farm, (b) during transportation to the factory, and (c) the factory due to careless use of sterilants or detergents, incomplete draining or inadequate rinsing of equipment. The inhibitory effects of sterilant and detergent residues are prevented by the correct and ethical use of these materials. Proper use includes the use of the chemical at the correct concentration and adequate rinsing and draining.

(c) Adding of rennet for coagulum formation: The classical material used in the formation of the coagulum is 'rennet' which is derived from the fourth stomach, abomasum or veal of freshly slaughtered milk-fed calves. Rennin (chymosin) is the enzyme responsible for the coagulation of the milk.

(d) Shrinkage of the curd: The removal of whey and further shrinkage of the curd is greatly facilitated by heating it, cutting it into smaller pieces, applying some pressure on it and lowering the pH. Acid produced by the lactic starters introduce elasticity in the curd, a property desirable in the final qualities of cheese.

(e) Salting of the curd and pressing into shape: Salt is added to most cheese varieties at some stage in their manufacture. Salt is important not only for the taste, but it also contributes to moisture and acidity control.

(f) Cheese ripening: The ripening or maturing of cheese is a slow joint microbiological and biochemical process which converts the brittle white curd or raw cheese to the final full-flavored cheese. The agents responsible for the final change are enzymes in the milk, in the rennet and those from the added starter microorganisms as well as other micro-organisms which confer the special character of the cheese to it.

Fermented milk foods

Yoghurt: Many types of fermented milks are produced and drunk around the world (Table 1). Yoghurt is a fermented milk traditionally believed to be an invention of the Turks of Central Asia. Yoghurt has been present for many years; it is only recently (within the last 30-40 years) that it has become popular. This is due to many factors including the introduction of fruit and other flavorings into yoghurt, the convenience of it as a ready-made breakfast food and the image of yoghurt as a low-fat healthy food. In the manufacture of yoghurt, two kinds of lactic acid bacteria, *Lactococcus* spp. and *Lactobacillus* spp., are generally used with usually unpasteurized milk. Most commonly used are *Lactococcus*

salivarius and *thermophilus*, and *Lactobacillus* spp., such as *Lacto. acidophilus*, *bulgaricus* and *bifidus*. See Tables 1and 2.

The chief anti-mycotic agent added to bread is……...?

Self-Assessment Exercises 2

Attempt the exercise to determine what you have learnt so far. (1min).
i What are the effects of Lactic acid during cheese making?

Table 1 Fermented milk and their presumed countries of origin

Name	**Presumed country of origin**	**Description**	**Cultures**
Yoghurt Acid	Asia, Balkans	Acidic, set or stirred, characteristic aroma	*S. thermophilus, Lb. bulgaricus*
Acidophilus milk	USA	Set stirred or liquid mild flavor	*Lb. acidophilus*
Kafir	Caucasus	Stirred beverage, creamy consistency, characteristic taste and aroma (CO_2)	*Lc. Lactis, Lc cremoris,Lb.Kefir, Lb.casei, Lb.*
Lassi	India	Sour milk, drink diluted with water, consumed salted, spicy or sweet	*Lactococcus spp. Lactobacillus spp.Leuconostoc*
Dahi	India	Set, stirred, or liquid beverage pleasant	*S. thermophilus, Lb. bulgaricus, Lc.diacetylactis*
Leben	Middle East	Set or stirred product, pleasant taste and aroma	*S. thermophilus, Lb. bulgaricus, Lb acidophilus*
Filmjilk	Sweden	Viscous stirred beverage, clean acid taste	*Lc.lactis, Lc.cremoris,Lc.diacetylactics,*
Villi	Finland	Viscous stirred product, mildly sour	*Lc.lactis, Lc cremoris.Lc diacetylactics*

Foods	**Raw ingredients**	**Fermenting microorganism**	**Area**
Coffee	Coffee beans	*Erwinia dissolvens, Saccharomyces* spp	Brazil, Congo
Garri	Cassava	*Corynebacterium manihot, Geotrichum spp*	West Africa
Kenkry	Corn	*Aspergillus* spp, *Penicillium* spp, Lactobacilli, yeast	Ghana, Nigeria
Ogi	Corn	*Lactococcus lactis, Zygosaccharomyces rou x11*	Nigeria
Sufu	Soyabeans	Actinimucor elegans, Mucor spp.	China
Tempeh	Soyabeans	*Rhizopus oligosporus, R. oryzae*	Indonesia New Guinnea Surinam
Sauerkrant	Cabbage	*L. mesenteroides, L. plantarum, L. brevis*	World wide,
Olives	Green olives	*Leuconostoc mesenteroides,* L. plantarum	World wide

Table 2 Fermented food produced from fruits, Vegetables, Beans and Related Substrates

Adapted from James M. Jay (2000): Modern Food Microbiology

5.4. Summary.

In this section bread making and cheese production were discussed with the necessary steps involved highlighted.

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5.6 Possible answers to Self-Assessment Exercises

Answers Self-Assessment Exercise 1

....a) Sponge doughs b The liquid ferment system c. The straight dough system:

Answers Self-Assessment Exercise 2

... (i) It causes the coagulation of casein at pH 4.6, the isoelectric point of that protein, which is used in the manufacture of some cheeses, e.g., cottage cheese.

(ii) It provides a favorably low pH for the action of rennin the enzyme which forms the curd from casein in other types of cheeses.

(iii) The low pH eliminates proteolytic and other undesirable bacteria.

(iv) It causes the curd to shrink and thus promotes the drainage of whey.

(v) Metabolic products from the lactic acid bacteria such as ketones, esters and aldehydes contribute to the flavor of the cheese

Unit 6. Fermented Foods II: Some local fermented foods

6.0 Introduction

Local fermented foods are derived through the activities of microorganisms however this section covers various foods produced regionally or nationally in the different geographical locations in Africa and Asia.

6.1 Intended Learning Outcomes (ILOs)

At the end of this unit, you should be able to:

- Understand the process of production of some local fermented foods consumed in densely populated localities in Africa

6.2 MAIN CONTENT Local Fermented foods

6.2.1 Fermented foods from corn

Corn is a tropical crop, but grows in the summer in temperate climates. It is known as maize (*Zea mays* L) in some parts of the world. it is used to make important fermented foods in west Africa and it is sometimes mixed with sorghum, *Sorghum bicolor* Linn for this purpose.

Ogi, also known as akamu, is a Nigerian sour gruel made from maize. In Ghana the equivalent foods are known as koko. For ogi preparation,

corn is soaked in water for about two days. Thereafter the cereal is wet-milled and sieved to remove the fibrous portions of the maize. The starchy sediment is allowed to settle and to ferment for another two days. The water is decanted off and the starchy sediment is ogi. Studies of the microbiology of ogi production show that in the early stages of fermentation, fungi such as *Fusarium* and *Cephalosporium* which are acquired from the field, and which form the bulk of the organisms in the first 24 hours, soon disappear to be replaced with lactic acid bacteria especially (*Lactobacillus plantarum* and *Lact mesenteroides*) and yeasts (*Saccharomyces cerevisiae*, *Rhodotorula* spp., and *Candida mycoderma* which become the dominant organisms at the time of the milling. The flavor of ogi has been shown to be due to the activities of lactic acid bacteria and occasionally to yeasts and acetic acid bacteria. The following acids were identified in that quantitative order by gas chromatography: acetic, butyric, pentanoic, isohexanoic, and isobutyric. The nutritional quality of ogi suffers from its method of preparation: the soaking of the grains and the discarding of the over tails before milling leads to loss of minerals as well as a portion of the already low quantity of protein and amino acids present in the cereals.

Mahewu, also known as mogou, is a South African sour food. The main organism found in locally-made mahewu is *Streptococcus lactis*, mahewu is made on an industrial scale by inoculating *Leuconostoc delbruckii* into autoclaved 8-10% maize slurry, fermenting the mixture for about 12 hours and spray-drying the slurry. It is an acid food of about pH 3.5, and in order to ensure that the proper level of lactic acid is produced to attain this pH level, buffering salts such as $CaHPO_4$ are sometimes added. It is a convenient food consumed by miners and the dry powder needs only to be reconstituted in cold water to get the food ready for consumption.

6.2.2 Fermented foods from cassava

Cassava is an important source of food all over the tropical world in South America, Africa, India and the Far East. It is a member of the family Euphorbiaceae and is classified as *Manihot esculenta Crantz* (formerly *Manihot utillisaima* Pohl). It has been with various names around the world: manioc (Madagascar and French-speaking Africa) tapioca (India, Malaysia), ubi scetlela (Indonesia) manioca or yucca (Latin America).

The plant tolerates low soil fertility and drought, better than most crops and needs little maintenance once planted. It has also been claimed to be a higher producer of carbohydrate then commonly cultivated cereals and tuber crops and under favorable conditions will yield above 90 ton/hectare. It is therefore not surprising that it is the staple food in densely populated areas of the tropics such as Central Java in Indonesia, the State of Kerala in India, south-eastern Nigeria and north-eastern

Brazil and is consumes by an estimated 400 million people around the world. Nigeria is currently the world's largest producer followed by Brazil, Indonesia, Zaire, Thailand, and Tanzania. Cassava may be processed by boiling, roasting, drying, leaching with cold water, or by fermentation. By far the most popular method of processing cassava is by fermentation. In producing fermented cassava products, the roots may first be grated before fermentation, the whole root may be cut into large pieces and fermented in water (retted). The best-known example of foods produced from cassava pulp is garri, while those produced from the retting of whole roots include foo-foo, chikwuangue, kokonte, and cinguada.

Garri

Garri is a popular food for about 100 million people in West Africa.

Preparation of Garri: Garri is currently prepared mostly on small, house-hold scales. The
first stage is the peeling to remove the brownish thin outer covering to reveal the white fleshy inner portion which is grated on a hand-held rasper or crushed in a grating machine. The central pith and primary xylem provide some fibers in the grated material some of which is removed by sieving, but which is appreciated by some garri consumers. The mash resulting from the grating is placed in cloth bags for between 18 and 48 hours and fermented. During the period of fermentation, the mash is dewatered by placing heavy objects on the cloth bags. At the end of the fermentation period, the mash is sieved through a coarse sieve and heated, sometimes with a little palm oil, in a flat iron pot with stirring.

Microbiology of the fermentation of Garri: The microbiological processes of cassava fermentation for garri production is that when cassava roots are grated to produce the mash which is bagged and fried to produce garri, the indigenous linamarase present in the roots is released and makes contact with the cyanogenic glucosides in the roots. The glucosides, mostly linamarin and some lotaustralin (about 5% of the total glucosides) are then broken down into glucose and HCN. The HCN release is characteristically noticed by the pungent smell in evidence whenever cassava is being grated. As the amount of the linamarase is insufficient to hydrolyze all the cyanogenic glucosides or because the particles are not fine enough to ensure complete contact between the enzyme and the substrate, there is always residual glucoside which enters the garri as cyanide. Many of the organisms encountered in fermenting cassava mash are lactic acid bacteria and yeasts. *Lactobacillus, Leuconostoc*, the yeast Candida and various other yeasts are encountered in fermenting cassava mash, and many strains of these have been found to produce linamarase. The main organism found in locally-made mahewu is……?

Self-Assessment Exercises 1

Attempt the exercise to determine what you have learnt so far. (1min).
i …… characteristically noticed by the pungent smell in evidence whenever cassava is being grated?

Other cassava fermented foods: Foo-Foo, Chikwuangue, Lafun, Kokonte,
Bikedi, and Cinguada

The preparation of these foods, although eaten in different parts of Africa, is similar. Foo-foo is eaten in parts of Eastern Nigeria, while Lafun is eaten in Western Nigeria. Chikwuangue is eaten in Democratic Republic of the Congo, Bikedi in Congo (Brazzaville), kokonte and Cinguada are eaten in Ghana and East Africa respectively. In the preparation of these foods, cassava roots are cut into large pieces and immersed in still water in pots or in running stream water and allowed to ret for one to five days; for foo-foo or chikwuangue fermentation retting takes between three and six days so that the starch can be extracted from the retted roots by macerating with the hands. For kokonte and cinguada, retting is only partial and hardly lasts more than two days; the material is then sun-dried and pounded into a flour when it is known as lafun in Nigeria. For foo-foo and bikedi the retted roots are macerated to extract the starch. The retting is a result of the breakdown of the pectin in the cell walls of the cassava root brought about by pectinases produced by bacteria of the genus *Bacillus* spp., while the lactic acid bacteria are responsible for the flavor of these foods.

6.2.3 Fermented vegetables

Vegetables have been preserved by fermentation from ancient time by lactic bacterial action. A wide range of vegetables and fruits including cabbages, olives, cucumber, onions, peppers, green tomatoes, carrots, okra, celery, and cauliflower have been preserved.

Sauerkraut

Sauerkraut is produced by the fermentation of cabbages, *Brassica oleracea*, and has been known for a long time. Specially selected varieties which are mild-flavored are used. The cabbage is sliced into thin pieces known as slaw and preserved in salt water or brine containing about 2.5% salt. The slaw must be completely immersed in brine to prevent it from darkening. Kraut fermentation is initiated by *Leuconostoc mesenteroides*, a heterofermentative lactic acid bacterium (i.e., it produces lactic acid as well as acetic acid and CO_2.) It grows over a wide range of pH and temperature conditions. CO_2 creates anaerobic conditions and eliminates organisms which might produce enzymes which can cause the softening of the slaw. CO_2 also encourages the growth of other lactic acid bacteria. Gram negative coliforms and pseudomonads soon disappear, and give

way to a rapid proliferation of other lactic acid bacteria, including *L. brevis*, which is heterofermentative, and the homofermentative *L. plantarum*; sometimes *Pediococcus cerevisiae* also occurs. Compounds which contribute to the flavor of sauerkraut begin to appear with the increasing growth of the lactics. These compounds include lactic and acetic acids, ethanol, and volatile compounds such as diacetyl, acetaldehyde, acetal, isoamyl alcohol, n-hexanol, ethyl lactate, ethyl butarate, and iso amyl acetate.

Cucumbers (pickling)

Cucumber (*Cucumis sativus*) is eaten raw as well after fermentation or pickling. Cucumbers for pickling are best harvested before they are mature. Mature cucumbers are too large, ripen easily and are full of mature seeds. Cucumbers may be pickled by dry salting or by brine salting. Dry salting is also generally used for cauliflower, peppers, okra, and carrots. It consists of adding 10 to 12% salt to the water before the cucumbers are placed in the tank. This prevents bruising or other damage to the vegetables. Brine salting is more widely used. A lower amount of salt is added, between 5 and 8% salt being used. Higher amounts were previously used to prevent spoilage. It has been found that at this salt concentration, the succession of bacteria is similar to that in kraut. However, *Leuconostoc* spp. never dominate. During the primary fermentation lasting two or three days, most of the unwanted bacteria disappear allowing the lactics and yeasts to proliferate. In the final stages, after 10 to 14 days, *Lactobacillus plantarum* and *L. brevis*, followed by *Pediococcus*, are the major organisms.

6.2.4 Fermentations for the production of the stimulant beverages

Tea, coffee, and cocoa are produced mainly in the rainforest zones of the Indian subcontinent and in South America and West Africa respectively. Tea can also grow in the cooler temperatures of mountains. The beverages are stimulating on account of their content of caffeine and theobromine.

Tea Production

Tea (*Camellia sinensis*; previously Thea) is believed to have originated from south-east China. It has now spread to many parts of the world The young tea leaves are harvested by hand and spread on trays to wither. Thereafter, the leaves are rolled to squeeze out juices from the leaves and spread the juices over the surface of the leaves. This exposes the polyphenols to oxidation, and the green color gradually begins to turn brownish. Rolling also breaks the leaves into smaller
pieces. The ‘fermentation’ involves the polyphenols, afterward the tea is fired subjected to hot air of between 80 and 90°C. After firing, the tea is sorted and graded.

Coffee Fermentation

Coffee (*Coffea arabica* and *C robusta*) originated from Ethiopia

It takes from three to five years of growth before the coffee tree is ready to bear fruit. The fruits grow slowly, taking from 8 to 12 months to reach maturity (when they are bright red in color). Each coffee fruit or berry contains two seeds covered by pulp. There are two methods of processing coffee: *the wet method* and *the dry method*. In the wet method, the fruits are passed through a pulping machine which removes the pulp leaving by mucilage which is removed by pectinolytic enzymes of microbiological origin.

The coffee may also be dried by exposure to sunlight. When dry, the fruits are dehulled to remove the dry outer portions. The studies carried on the microbiology of the coffee fermentation showed that many of the organisms were pectinolytic organisms, including spore-forming and non-spore forming ones. Other workers found lactic acid bacteria (*Leuconostoc* spp. and *Lactobacillus* spp.) and yeasts (*Saccharomyces* spp and S*chizosaccharomyces* spp.), and it would appear that these developed from the release of the pectinolytic organisms.

Cocoa Fermentation

Cocoa (*Theobroma cacao*) is a native of South America, but today the major producers are Ghana, Nigeria, Ivory Coast, Cameroon, and Malaysia. The tree produces pods which contain from 40 to 60 seeds. The pods are opened and the seeds heaped and allowed to ferment, often in baskets which permit liquid to drain out. During fermentation the mucilaginous outer covering of the seeds is broken down by microbial action, while the seeds themselves change from pinkish to black.

6.2.5 Fermented foods derived from legumes and oil seeds

Fermented Foods from Soybeans

The soybean plant itself Glycine max is a legume believed to have originated from Eastern Asia. It is now grown around the world. The soybean seed has an unusual composition. It is rich in protein and oil, and comparatively low in carbohydrates. Its average composition is 42% protein 17% carbohydrate, 18% oil, and 4.6 ash. Sucrose, raffinose, stachyose and pentosans are among the carbohydrates. The beans are rich in phospholipids, nucleic acids, and vitamins especially thiamin, riboflavin, and niacin. It should be noted that the composition of soybeans varies from place to place. Soybean is a very nutritious food. However, it has shortcomings which are ameliorated by fermentation. Soybeans contain compounds which make the legume unattractive until they are removed by the various stages involved in their processing by fermentation.

First, they contain carbohydrates, which are not absorbed until they reach the colon, where the gases produced when they are broken down by microorganisms give rise to flatulence. These carbohydrates include the oligosacharides, raffinose and stachyose and the polysaccharide, arabinogalactan. Second, soybeans have a bitter and ‘beany’taste when

crushed. This is because the lipoxygenase enzyme which helps produce this taste and the substrate (oil) are held in separate compartments in the tissues of the seeds until the latter are broken or crushed.
Third, soybeans contain anti-nutritional factors such as trypsin inhibitor, hemagglutinins and saponins. Finally even after cooking, about 1/3 of the protein of soybeans cannot be digested.
The soaking of the soybean preceding cooking leaches out a large proportion of the flatulence producing carbohydrates. The 'beany' flavor is due to the presence of several carbonyl compounds such as hexanol and pentanol. These are removed by the action of microorganisms.
Mention the two methods of processing coffee.?*Self-Assessment Exercises 2*

Attempt the exercise to determine what you have learnt so far. (1min).
i The 'beany' flavor in soybean is due to……

Soy sauce
Soy sauce known as shoyu in Japan is a salty pleasantly tasting liquid with a distinct aroma and which is made by fermenting soybeans, wheat, salt with a mixture of molds, yeasts and bacteria. Five different types of shoyu are recognized by the Japanese Government, depending on the proportions of the ingredients used and the method of preparation.

6.2.6 Fermented Foods from Protein-rich Oil-seeds

Stew condiments made from oil-rich seeds are eaten in parts of West Africa, while condiments from fish and fish products appear to be common in parts of Asia. Stew condiments eaten in parts of Nigeria include **dawadawa**, know as **iru** in the Southwest geopolitical zone of Nigeria which is produced from the seeds of *Parkia biglobosa*. A condiment very similar to dawadawa is **okpeyi** and comes from the Nsukka area of the Southeast and is made from the seeds of *Prosopsis africana*. Another major soup condiment popular in the Southeast zone is **ogili** which may be made from the seeds of castor-oil seeds (*Ricinus communis*) or egusi (*Citrullus lanatus* sub-species colocynthoides). Egusi, pumpkins and squashes are members of the family Cucurbitaceae or cucurbits and their seeds contain about 50% oil and 35% protein after dehulling. Besides egusi, another well-known cucurbit in Nigeria is ugu or Telfaria sp.; its seeds and those of soybeans are sometimes used for making ogili. A fermented delicacy and meat substitute, **ugba** or **ukpaka** is made from the seeds of the African locust bean, *Pentaclethra macrophylla* Benth). This is generally eaten with stockfish and it is very popular in the South-east zone of Nigeria.

6.3 SUMMARY

The making of local fermented foods also involves the use of fermentation processes.

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6.6 Possible answers to Self-Assessment Exercises

Answers Self-Assessment Exercise 1

...HCN release

Answers Self-Assessment Exercise 2

the presence of several carbonyl compounds such as hexanol and pentanol

Glossary

CO_2 = Carbondioxide

O_2 = Oxygen

End of the module Questions

1). In Brewing Technology which process stops germination of grains? (**Kilning**)

2) Water is not an example of adjuncts **(**True or **False)**

3). Sherry is a type of ……. (**Wine** or Brandy)

4). Racking refers to removing clear liquid from sediments (**True** or False)

5). Explain ways that Grape wines may be classified in several ways.

6) Write on the production of Sauerkrant and Pickles

7) What is lagering

8) Write on Microbiology of Garri fermentation.

Module 4: Fermenter design and operation

Unit 1. Fermenter I: Types and its construction
Unit 2. Fermenter II: Anaerobic Fermenters and Continuous Culture
Unit 3. Antifoams
Unit 4. Scale up process of the fermentation process
Unit 5. Intellectual Property Rights

UNIT1: FERMENTER I: TYPES AND ITS CONSTRUCTION

1.0 Introduction

Microorganisms can be grown in culture tubes, shake flasks and stirred fermenters or other mass culture system stirred fermenters can range in size from 3 or 4 liters to 100,000 liters for larger, depending on production requirement. Not only must the medium be sterilized but aeration, pH adjustment, sampling and process monitoring must be carried out under rigorous controlled conditions. When required, foam control agent must be added, especially with high-protein media. Environmental conditions can be changed or held constant over time, depending on the goals for the particular process.

1.1 Intended Learning Outcomes (ILOs)

At the end of this unit, you should be able to:

- Explain the set-up of a fermenter.
- Describe how a laboratory fermenter can be scaled up to a commercial one.
- Explain what antifoams are.
- Explain the work of antifoam in fermenters.

1.2 Main content: Fermenter I:

Types and its construction

A fermentor (or fermenter) is a vessel for the growth of microorganisms which, while not permitting contamination, enables the provision of conditions necessary for the maximal production of the desired products. In other words, the fermentor ideally should make it possible to provide the organism growing within it with optimal pH, temperature, oxygen, and other environmental conditions. A fermenter is a vessel which does not permit contamination but provides conditions necessary for the maximum production of the desired product. Fermentors are therefore also known as **bioreactors**. Fermentors may be **liquid**, also known as **submerged or solid state**, also known as surface. Most fermentors used in industry are of the submerged type, because the submerged fermentor saves space and is more amenable to engineering control and design. Depending on the purpose, a fermentor can be as small as 1 liter or up to about 20 liters in laboratory-scale fermentors and range from 100,000 liters to 500,000 liters (approximately 25,000 – 125,000 gallons) for factory or production fermentors. Several types of fermentors are known and they may be grouped in several ways: shape or configuration, whether aerated or anaerobic and whether they are batch or continuous. The most commonly used type of fermentor is the **Aerated Stirred Tank Batch Fermentor**.

In-Text Question (ITQ)

A fermenter is a vessel which does not permit contamination but provides conditions necessary for the maximum production of the desired product. True or false?

Answer: True

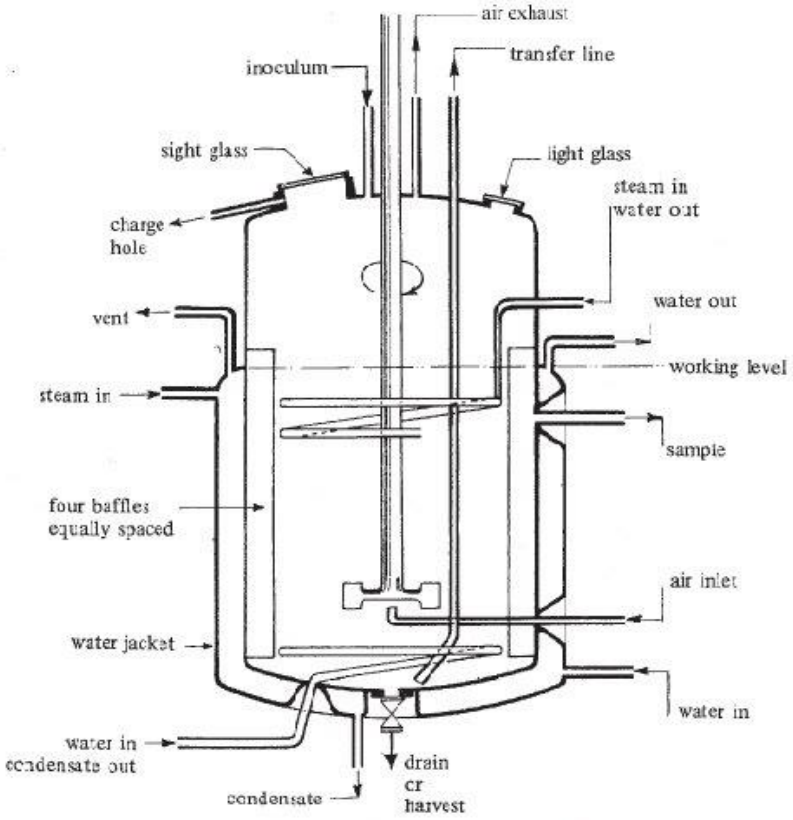
1.2.1 Aerated stirred Tank Batch Fermentor

A typical fermentor of this type is an upright closed cylindrical tank fitted with four or more baffles attached to the side of the wall, a water jacket or coil for heating and/or cooling, a device for forcible aeration (known as sparger), a mechanical agitator usually carrying a pair or more impellers, means of introducing organisms and nutrients and of taking samples, and outlets for exhaust gases. Modern fermentors are highly automated and usually have means of continuously monitoring, controlling or recording pH, oxidation-reduction potential, dissolved oxygen, effluent O_2 and CO_2, and chemical components of the fermentation broth (or fermentation beer as the broth is called before it is extracted). However, the fermentor need not have all these gadgets and many automated activities can also be handled manually.

It is important that one knows the type of fermentation required and when a fermentor is being planned; a fermentor is expensive and once

installed it may be unnecessarily expensive to drastically remodify it.

Design of Stirred Tank Fermenter



Self-Assessment Exercises 1

Attempt the exercise to determine what you have learnt so far. (1min).

i. What is a fermenter?

1.2.2. Construction Materials for Fermentors

A simple batch fermentor may consist of no more than an open tank made of wood, concrete or carbon-steel if contamination is not a serious problem and provided that no need exits for strict pH and temperature control, or that the temperature is controlled in the building.

Serious contamination is restricted because of the acidity of the medium usually used. However, for fermentations with strict sterility requirements and closely controlled environmental needs, such as in the antibiotic industry, a material which can withstand regular steam sterilization is necessary. Stainless steel is therefore normally used for pilot and production fermentors. Laboratory scale fermentors are usually made of Pyrex glass to

enable autoclaving. Where a highly corrosive material is fermented, e.g. citric acid, the fermentor should definitely be made of stainless steel. It is inevitable that small quantities of the material of which the

fermentor is made will dissolve in the medium. Some materials, e.g., iron may inhibit the productivity of organisms in certain fermentations. It is for this reason that carbon-steel fermentors are often lined with glass, or 'plastic' materials e.g. a phenolic epoxy coating. The material used for lining depends on the expected abrasion on the wall of the fermentor by medium constituents. Glass lining is employed only for small fermentors because of the high cost and the possibility of breakage. In order to avoid contamination, fermentor vessels of all types should be of welded construction throughout. The welds should be free of pinpoints where organisms can develop in small bits of old media, and shielded from sterilization. The joint inlets and outlets of the fermentor should be designed so as to provide smooth surfaces and eliminate pockets difficult to sterilize. If gaskets are used at joints these should be nonporous.

Aeration and Agitation in a Fermentor

Oxygen is essential for growth and yield of metabolites in aerobic organisms. In fermentations where aerobic organisms are used, the supply of oxygen is therefore critical. For the oxygen to be absorbed by microorganisms it must be dissolved in aqueous solution along with the nutrients. In other fermentations, the aeration requirement need not be as intense but must be presented to the organisms at a controlled level. It has been shown that oxygen control in industrial fermentations is as important as pH, temperature and other environmental controls. However, in some fermentations where sterility is not necessary such as in yeast fermentation, the air is merely scrubbed by passing it through glycerol. The air used in fermentation, whether, sterile or not, is forced under pressure into the bottom of the fermentor just below the lowest impeller the air enters through a sparger which is a pipe with fine holes. The smaller the holes the finer the bubbles and the more effective the supply of oxygen to the microorganisms. However, if the holes are too small, then a greater pressure will be required to force the air through, with consequent higher consumption of energy and therefore of costs. A balance must be struck between wide holes which may become plugged and holes small enough to release fine bubbles.

For many fermentations especially where filamentous fungi and actinomycetes are involved, or the broth is viscous, it is necessary to agitate the medium with the aid of impellers. In large-scale operations, where aeration is maintained by agitator-created swarms of tiny air bubbles floating through the medium, the cost is very high. Agitators with their attached impellers serve a number of ends. They help to distribute the incoming air as fine bubbles, mix organisms uniformly, create local turbulence, as well as ensure a uniform temperature. The optimal number and arrangement of impellers have to be worked out by engineers using information from pilot plant experiments. The viscosity of the broth affects the effectiveness of the impellers. Since the viscosity of the broth may alter as fermentation proceeds, a satisfactory compromise of size, shape, and number of impellers must be worked out. In unbaffled fermentors a vortex or inverted pyramid of

liquid forms and liquid is thrown up on the side of the fermentor. The result is that heavier particles sediment and thorough agitation is not achieved. The insertion of baffles helps eliminate the formation of a vortex and interferes with the upward throw of liquid against the side of the fermentor. The use of baffles thus ensures not only a more thorough mixing of the nutrient and air but also the breakup of the air bubbles. In order to appreciate the importance of oxygen, there are two film model gas must pass through to get to the organisms.
These barriers include the following:
(i) Gas-film resistance between gas and interface;
(ii) Gas-liquid interfacial resistance;
(iii) Liquid-film resistance between interface and the bulk of the liquid;
(iv) Liquid-path resistance characterized by oxygen gradient in bulk;
(v) Liquid-film resistance around cell or cell-clump;
(vi) Inter-cellular or intra-clump resistance;
(vii) Resistance to reaction ('absorption') of oxygen with the cell respiratory enzymes.
The function of agitation of the fermentation may be taken as follows:
(i) Gas dispersion or the creation of a large air-liquid interfacial area;
(ii) Reduction in the thickness, and hence to resistance to oxygen diffusion of the liquid film which surrounds each bubble;
(iii) Bulk mixture of the culture;
(iv) Control of clump size.
Provisions for agitation and aeration are thus very important components of an Aerated Stirred Tank Fermentor. In large-scale operations, where aeration is achieved by swarms of tiny air bubbles floating through thousands of liters of medium, the cost of aeration and agitation could be high.
The exhaust air from the fermentor is passed through a filter which is sterilized with steam from time to time. This is especially necessary if the organism being grown is pathogenic (e.g., for vaccines). The exhaust pipe is positioned away from the incoming sterile air to avoid any chance of contamination. Furthermore, the agitation shaft which is impelled by a motor is fitted with a special seal at the point where it enters the fermentor in order to avoid contamination.
Sterile air is needed in some aerobic fermentations and it is produced in several ways including irradiation, electrostatic absorption of particles, the use of heat resulting from the compression of the gas. But the most commonly used method is the passage of the air through filters either made of materials such as cellulose nitrate, or more commonly of cotton and sometimes other materials.

Temperature Control in a Fermentor

Many fermentations processes release heat, which must be removed so as to maintain the optimum temperature for the productivity of the organism. In small laboratory fermenters
temperature control may be achieved by immersing the tank in a water bath; in medium sized ones control may be achieved by a jacket of cold water circulating outside the tank or merely by bathing the unjacketed cylinder with water. In large fermenters, temperature is maintained by

circulating refrigerated water in pipes within the fermentor and sometimes outside it as well. A heating coil is also provided to raise the temperature when necessary.

Foam Production and Control

Foams are dispersions of gas in liquid. In fermentation they usually occur as a result of agitation and aeration. However, in most industrial fermentations, foam has undesirable microbiological, economic and chemical engineering consequences, as follows:

(i) The need to accommodate foams means that a substantial head space is left in industrial fermentations. By reducing foaming, it has been possible to increase the total fermentation by 30-45%.

(ii) If the fermentation medium is such that it encourages rapid foaming, then the maximum aeration and agitation possible cannot be introduced because of excessive foaming. The effect of this is that the oxygen transfer rate is reduced.

(iii) If the foam escapes, then contamination may be introduced when foam bubbles coalesce and fall back into the medium after wetting the filters and other non-sterile portions of the fermentor.

(iv) Organic nutrients or inorganic ions with complex organic compounds may be removed from the medium by foam floatation, a phenomenon well known in beer fermentation, when proteins, hop-resins, dextrin's, etc., concentrate in the foam layer. A loss of nutrient from fermentations in this way could lead to reduced yield.

(v) It can be seen that the fermentation product may also be removed should it be amenable to foam floatation. Such a loss has actually been observed in a laboratory experiment with the antibiotic, monamycin.

(vi) Loss of microorganisms could also easily occur by floatation thereby leading also to reduced yields.

The more stable surface foams are the most troublesome. The unstable ones breakdown in

about 20 seconds and cause no further havoc. In contrast with surface foams are the so-called fluid foams which occur within the broth. These are common in highly viscous mycelial fermentations and in unbaffled vortex fermentors.

Foaming patterns

Fermentation media are usually made up of complex materials whose compositions are not always precisely known. Of the compounds which give rise to foams, proteins produce the most stable foams. A medium consisting of only inorganic compounds will not foam unless suitable metabolites are produced by the organisms. It is sometimes possible to reduce foaming by altering the medium composition of the fermentation. Thus, it was possible to use a larger broth volume by reducing foam from a yeast fermentation following the absorption of caramel and organic acids with bentonite from a sample of molasses. Furthermore, the concentration of nutrients, the pH, the method of preparing the medium components e.g. sterilization time, etc., can all affect foam formation and stability.

The pattern of peak foam formation and disappearance during the

course of fermentation depends on the composition of the medium and the nature and the activity of microorganisms taking part in the fermentation. Four or five foaming patterns have been noted:

In the first type the foam remains constant throughout the fermentation. This is not common in media made of complex materials and is more frequent in defined media consisting mainly of inorganic components.

In the second type the foam falls from a fairly high level to a low but constant level, following the utilization of foam stabilizers in the nutrients by micro-organisms. In this type the microorganisms themselves produce neither foam stabilizers nor defoamers.

In the third type foam life-time falls at first, but then rises. Under this condition the foam stabilizers in the original medium are metabolized but the organism also produces foam-stabilizing metabolites.

In the fourth type the medium initially contains only a low amount of foam stabilizers. These increase as autolysis of the mycelium sets in. If these are later metabolized the foaming may once more drop resulting in a fifth pattern.

1.2.3 Process Control in a Fermentor

The course of a fermentation may be followed by monitoring various operational parameters within the fermentor e.g., pH, air input, effluent gases, temperature; factors such as cell yield, or the output of metabolites may also be followed. The degree of accuracy of the monitoring depends of course on the instruments being used for the purpose.

pH measurement and control

The importance of the control of pH in microbial growth cannot be overemphasized. In some industrial fermentations, good yield depends on accurate control (and hence accurate measurement) of the pH of the fermentation broth. Sometimes the control of pH is achieved by natural buffers present in the medium such as phosphates and calcium. The buffering effect of these compounds is however usually temporary. The broth must therefore be sampled and the pH adjusted as desired with either acid or base. This method is laborious and may not accurately reflect the continuous change taking place in the pH of the broth. Sterilizable pH probes have become available and these are inserted in the fermentor or in a suitable projection in which the broth bathes the electrode. With these electrodes it is now possible to use an arrangement which will monitor pH changes and automatically induce the introduction into the medium of either acid or alkali.

Carbon dioxide measurement

Water and carbon dioxide are two of the most common end-products of aerobic fermentations. The measurement of CO_2 therefore helps determine the course of the fermentation as well as the carbon balance. At least three principles are employed in current equipment for CO_2 determination. The first method, which is the most widely used,

depends on the ability of CO_2 to absorb infrared rays. A sensitive sensor translates this absorption to a gauge or record, from which it can be read off. In another principle, the effluent gas emerging from the broth is bubbled through a dilute solution of NaOH containing phenol red. The change in color of the phenol red is reflected in a photocell and the amount of CO_2 may be calculated from a standard curve. The third method depends on the thermal conductivities of the various gases in a mixture.

Oxygen determination and control

A number of methods are available for determining the oxygen concentration in a fermentation broth. Of the chemical methods, the best known is that of Winkler which is routinely used to determine the biochemical oxygen demand (B.O.D) of water. This method relies on the back-titration, using iodine and starch, of unoxidized manganous salt added to the liquid to be analyzed. Interfering substances are usually present in fermentation broths. Modern dissolved oxygen probes are autoclavable and are based on one or the other of two principles: the polarographic or the galvanic method.

Pressure

It is important to know the pressure of gases in order to ensure that a positive pressure is maintained. A positive pressure helps eliminate contamination and contributes to the maintenance of proper aeration. Pressure may be determined with aid of a manometer.

Computer control

Automation is an engineering problem and the expected advantages of computerization have been given as follows:

(i) It should reduce labor by eliminating manual intervention. The use of a computer should render an operator's work easier and reduce human error; it is alsp possible to make changes while fermentation is on.

(iii) Automatic recording of all aspects of the fermentation is possible with a computer and is useful in meeting any regulatory requirements as well as in improving fermentation operations.

(iv) Experimentation should be easier as it should be much easier to study the effect of altering any variables such as dissolved oxygen, temperature, pH, air flow,nutrient addition, etc.

(v) Quality control should be easier to carry out.

(vi) In the event of power failure, and other emergencies, the system should be able to shut up itself and restart and gradually build up to the original level of activity.

What are foams.?

Self-Assessment Exercises 2

Attempt the exercise to determine what you have learnt so far. (1min).

i. Mention factors that are monitored during process control?

1.3 SUMMARY

Various forms of fermenters and their process control have been discussed in this section.

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1.4 Possible answers to Self-Assessment Exercises

Answers Self-Assessment Exercise 1

Answer: A fermentor (or fermenter) is a vessel for the growth of microorganisms which, while not permitting contamination, enables the provision of conditions necessary for the maximal production of the desired products.

Answers Self-Assessment Exercise 2

The course of a fermentation may be followed by monitoring various operational parameters within the fermentor e.g., pH, air input, effluent gases, temperature; factors such as cell yield, or the output of metabolites.

UNIT2: FERMENTER II: ANAEROBIC FERMENTERS AND CONTINUOUS CULTURE

2.0 Introduction

Microorganisms can be grown in culture tubes, shake flasks and stirred fermenters or other mass culture system stirred fermenters can range in size from 3 or 4 liters to 100,000 liters for larger, depending on production requirement. Not only must the medium be sterilized but aeration, pH adjustment, sampling and process monitoring must be carried out under rigorous controlled conditions. When required, foam control agent must be added, especially with high-protein media. Environmental conditions can be changed or held constant over time, depending on the goals for the particular process.

2.1 Intended Learning Outcomes (ILOs)

At the end of this unit, you should be able to:

• Explain the set-up of a fermenter.
• Describe how the continuous cultures

2.2 Main content:

Fermenter II: Anaerobic Fermenters and Continuous Culture
Anaerobic fermentors, whether strict or micro-aerophilic (i.e., requiring small amounts of oxygen) are not commonly used in industry. When they do (i.e., require oxygen), they are essentially the same as (aerobic) fermentor. They, however, differ in the construction and operation as listed below.
(i) Vigorous aeration through air sparging is absent, as oxygen is not

required.
(ii) Agitation when done is aimed only at achieving an even distribution of organisms,
nutrients and temperature, but not for aeration. In some cases, agitation may be essential only initially; the evolution of CO_2 and H_2 in anaerobic fermentors may stir the medium.
(iii) The medium is introduced into the fermentor while hot to prevent the absorption of
gases; and usually it is also introduced at the bottom of the fermentor.
(iv)The fermentor itself is filled as much as possible, in order to avoid an airspace which would introduce oxygen.
(v) If strict anaerobiosis is desirable, then an inert gas such as nitrogen may be blown
through the fermentation, at least initially, to remove oxygen.
(vi) Some low redox compounds, such as cysteine, may be introduced into the medium.
It is especially important that it be possible for aerobic or anaerobic fermentations to be carried in the same vessel as some fermentations such as alcohol manufacture require an earlier aerobic stage in which cells are produced in large numbers and a later stage in which alcohol is produced anaerobically. But even the strictly anaerobic fermentations can be carried out in the stirred tank batch fermentor.

Anaerobic fermentors, whether strict or micro-aerophilic (i.e., requiring small amounts
of oxygen) are not commonly used in industry True or False.?

Fermentor configurations

Fermentors have been grouped into four:
(i) **Batch fermentors**
(ii) **Continuous stirred tank fermenters**: The tank used in this system is essentially similar to that of the batch fermentor. It differs in the inlet of medium and the outlet of broth. The
(iii) **Tubular fermentors**: The tubular fermentor was named because it resembled a tube. In general tubular fermentors are continuous unstirred fermentors in which the reactants move in a general direction. Reactants enter at one end and leave from the other and no attempt is made to mix them. Due to the absence of mixing, there is a gradual fall in the substrate concentration between the entry point and the outlet while there is an increase in the product in the same direction.
(iv) **The fluidized bed fermentor**: This is essentially similar to the tubular fermentor. In both the continuous stirred fermentor and the tubular fermentor there is a real danger of the organisms being washed out. The fluidized bed reactor is an answer to this problem because it is intermediate in nature between the stirred tank and the tubular fermentor. The microorganisms which are in a fluidized bed fermentor are kept in suspension by a medium flow rate whose force just balances the gravitational force.

Self-Assessment Exercises1

Attempt the exercise to determine what you have learnt so far. (1min).

i. Fermenters have been grouped into four types, List them?

Continuous Fermentations

Continuous fermentations are those in which nutrients are continuously added, and products are also continuously removed. Continuous fermentations contrast with batch fermentations in which the products are harvested, the fermentor cleaned up and recharged for another round of fermentation.

The advantages of continuous fermentation include:

i. More intensive use of the equipment, especially the fermentor, and therefore greater return on the initial capital outlay made in installing them.
ii. savings in labor
iii. Continuous processes are more easily automated. Helps eliminate human error and thus ensures greater uniformity in the quality of the products.

Theory of continuous fermentation

In a batch culture four or five phases of growth are well recognized: the lag phase, the phase of exponential or logarithmic growth, the stationary phase, the death or decline phase. Some others add the survival phase. In the lag phase individual cells increase somewhat in size but there is no substantial increase in the size of the population. In the **exponential phase**, the population doubles at a constant rate, in an environment in which the various nutritional requirements are present in excess. As the population increases, various nutrients are used up and inhibitory materials, including acids, are produced; in other words, the environment changes. The change in the environment soon leads to the death of some organisms. In the stationary phase the rate of growth of the organisms is the same as the rate of death. The net result is a constant population. In the death phase, the rate of death exceeds the growth rate and the population declines at an exponential rate.

If, however during the exponential phase of growth, a constant volume is maintained by ensuring an arrangement for a rate of broth outflow which equals the rate of inflow of fresh medium, then the microbial density (i.e., cells per unit volume) remains constant. This is the principle of one method of the continuous culture in the laboratory, namely, the turbidostat.

In a **batch culture** the various nutrients required by an organism are usually initially present in excess. If all but one of the nutrients are present in adequate amount, then the rate of growth of the organisms will depend on the proportion of the limiting nutrient that is added.

It is then possible to control the growth at any given rate but which rate is less than the maximum possible, by letting in fresh nutrient at the

same rate as broth is released and also supplying one of the nutrients at a level slightly less than the maximum. This principle is employed in the chemostat method of continuous growth. In both the **chemostat and the turbidostat** the rate of nutrient inflow and broth outflow must relate to the generation time or growth rate of the organism. If the rate of nutrient addition is too high, then sufficient time is denied to the organism to develop an adequate population. The organisms are then washed out in the outflow. If on the other hand the rate of nutrient addition is too low, a stationary phase may set in and the population may begin to decline.

Classification of continuous microbial cultivation

Single-state continuous fermentations

There are fermentations in which the entire operation is carried out in one vessel, the nutrient being added simultaneously with broth outflow. This system is suited for growth related fermentations such as yeast, alcohol, or organic acid production.

Multiple-stage continuous fermentation

This consists of a battery of fermentation tanks. The medium is led into the first and the outflow into the second, third, or fourth as the case may be. This is most frequently used for the fermentation involving metabolites. The first tank may be used for the growth phase and subsequent tanks for production, depending on the various requirements identified for maximal productivity.

In-Text Question (ITQ)

In the stationary phase the rate of growth of the organisms is the same as the rate of death. True or False.?

Answer: True

Recycled single or multiple stage continuous fermentation

The out flowing broth may be freed of the organisms by centrifugation and the supernatant returned to the system. This system is particularly useful where the substance is difficult to degrade or not easily miscible with water such as in hydrocarbons. Recycling can be applied in a single stage fermentor. In a multiple stage fermentor, recycling may involve all or some of the fermentation vessels in the series depending on the need.

Semi-continuous fermentations

In semi-continuous fermentations, simultaneous nutrient addition and outflow withdrawal are carried out intermittently, rather than continuously. There are two types of semi-continuous fermentation, namely;

(i) ‘cyclic-continuous’; (ii) ‘cell reuse’

In Cyclic-continuous, a single vessel is usually employed, although a series of vessels may be used. Fermentation proceeds to completion or near completion and a volume of the fermentation broth is removed. Fresh medium of a volume equivalent to that withdrawn is introduced into the vessel. As the size of the fresh medium is reduced, the time taken to complete the fermentation cycle is reduced until eventually the intermittent feeding becomes continuous. This system has been said to ensure a compromise, between the desirable and undesirable

features of batch and continuous fermentation; productivity has however been shown theoretically and experimentally to be lower than in continuous fermentation. In cell reuse, cells are centrifuged from the fermentation broth and used to reinoculated fresh medium. It is continuous only in the sense that cells are reused; in essence it is a batch fermentation.

ANAEROBIC BATCH FERMENTORS

Anaerobic fermentors, whether strict or micro-aerophilic (i.e., requiring small amounts of oxygen) are not commonly used in industry. They, however, differ in the construction and operation. These include:

(i) Vigorous aeration through air sparging is absent, as oxygen is not required.

(ii) Agitation when done is aimed only at achieving an even distribution of organisms,
nutrients and temperature, but not for aeration.

(iii) The medium is introduced into the fermentor while hot to prevent the absorption of gases; and usually it is also introduced at the bottom of the fermentor.

(iv) The fermentor itself is filled as much as possible, in order to avoid an airspace
which would introduce oxygen.

(v) If strict anaerobiosis is desirable, then an inert gas such as nitrogen may be blown
through the fermentation, at least initially, to remove oxygen.

It is especially important that it be possible for aerobic or anaerobic fermentations to be carried in the same vessel as some fermentations such as alcohol manufacture require an earlier aerobic stage in which cells are produced in large numbers and a later stage in which alcohol is produced anaerobically.

In designing and constructing a fermenter, a number of factors must be considered.

(1) The vessel must be capable of being operated aseptically for a number of days and should be reliable in long term operations.

(2) Adequate aeration and agitation should be provided to meet the metabolic requirements of microorganisms.

(3) Power consumption should be as low as possible.

(4) It must have a system of temperature control.

(5) It must have a system of pH control.

(6) Sampling facilities should be provided.

(7) Evaporation losses from the fermenter should not be excessive.

(8) The vessel should be designed to require the minimal use of labour in operation cleaning, harvesting and maintenance.

(9) The vessel should be suitable for a range of processes.

(10) It should have smooth internal surface.

(11) The cheapest material which enables satisfactory result to be achieved should be used.

(12) The vessel should be of similar geometry to both smaller and larger vessels in the pilot plant to facilitate scale-up.

(13) There should be adequate service provision for industrial parts.

Self-Assessment Exercises 2

Attempt the exercise to determine what you have learnt so far. (1min).

i. Mention any three factors that are considered during design of fermenter?

2.3 SUMMARY

In this unit anaerobic fermentation design and the continuous cultivation system have been discussed.

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2.4 Possible answers to Self-Assessment Exercises

Answers Self-Assessment Exercise 1
i. Batch Fermenters ii. Continuous stirred tank fermenter iii. Tubular fermenter iv. Fluidized bed fermenter

Answers Self-Assessment Exercise 2
Answer: . (1) The vessel must be capable of being operated aseptically for a number of days and should be reliable in long term operations.
(2) Adequate aeration and agitation should be provided to meet the metabolic requirements of microorganisms.
(3) Power consumption should be as low as possible.
(4) It must have a system of temperature control.
(5) It must have a system of pH control. (6) Sampling facilities should be provided.
(7) Evaporation losses from the fermenter should not be excessive

UNIT3: ANTIFOAMS

3.0 Introduction

The pattern of peak foam formation and disappearance during the course of fermentation depends on the composition of the medium and the nature and the activity of microorganisms taking part in the fermentation.

3.1 Intended Learning Outcomes (ILOs)

At the end of this unit, you should be able to:
• Explain what antifoams are.
• Explain the work of antifoam in fermenters.

3.2 Main content:

ANTIFOAMS

Four or five foaming patterns have been recognized. In the **first type** the foam remains constant throughout the fermentation. This is not common in media made of complex materials and is more frequent in defined media consisting mainly of inorganic components. In the **second type** the foam falls from a fairly high level to a low but constant level, following the utilization of foam stabilizers in the nutrients by micro-organisms. In this type the microorganisms themselves produce neither foam stabilizers nor defoamers. In the **third type foam life**-time falls at first, but then rises. Under this condition the foam stabilizers in the original medium are metabolized but the organism also produces foam-stabilizing metabolites. In the **fourth type** the medium initially contains only a low amount of foam stabilizers. These increase as autolysis of the mycelium sets in. If these are later metabolized, the foaming may once more drop resulting in a **fifth pattern**. In practice combinations of all or some of these may occur simultaneously.

Foams in industrial fermentations are controlled either by chemical or mechanical means. Chemicals controlling foams have been classified

into antifoams, which are added in the medium to prevent foam formation, and defoamers which are added to knock down foams once these are formed.

Foams are formed via froths which are temporary dispersals of gas bubbles in a liquid of no foam formation ability. Bubbles in a froth coalesce as they rise to the surface. In a foam however, they do not coalesce. Rather, the liquid film between two bubbles thins to a lamella. Materials which yield foam forming aqueous solutions such as proteins, peptides, synthetic detergents, soaps, and natural products such as saponin, lower the surface tension of the solution and permit foam formation.

Chemicals controlling foams have been classified into **antifoams or defoamers**. Defoamers are once they are formed. Most of the media used in culturing organisms contain protein which is susceptible to foam formation due to the fine bubble which easily induce foam. The problem of antifoam is widespread in fermentation process and can be counteracted in a number of ways. One possibility is to ensure that there:

(1) Is a sufficient space available in the fermenter for the foam produced. However, this reduces the effective volume of the fermenter as well as the additional changes of contamination. Foaming can also be hindered/counteracted using chemical & mechanical measures.

(a) Chemical antifoam agents such as animal vegetable oil reduce the surface tension of the broth and at the same time, they reduce the solubility of oxygen which in turn affects the aeration requirement. It may also make downstream processing more difficult.

b) Mechanical defoamers can be employed instead of chemical agents. Mechanical elements mounted on the agitation shaft are that it will affect the speed of rotation for effective defoaming.

To rectify this disadvantage, some defoamers are separately driven. It has a disadvantage in that it increases the danger of contamination because of the additional shaft installation through the fermenter.

What is the function of defoamers.?

Self-Assessment Exercises 1

Attempt the exercise to determine what you have learnt so far. (1min).

i. Foams in industrial fermentations are controlled either…..?

(1) It must be non-toxic to microorganism and higher animals.

(2) It must have no effect on the taste & odour; a change in the usual organoleptic properties of the finished goods due to the antifoam or other components of the medium may result in consumer rejection of the goods.

(3) It must not serve as a source of contamination.

(4) It must not be metabolized (i.e. organisms)

(5) Must be autoclavable.

(6) be active in small concentrations, cheap, and persistent.

(7) not impair oxygen transfer.

Table 1. Examples of antifoams which have been used in industry

Category	Example	Chemical nature	Remarks
Natural oils and Fats	Peanut oil and soybean oil	Esters of glycerol and long chain mono-basic acids	Not very efficient . Used as carriers for other antifoam; may be metabolized.
Alcohol	Sorbitan alcohol	Mainly alcohols with 8-12 carbon atoms	Not very efficient; may be toxic; or may be metabolized
Sorbitan derivatives	Sorbitan monolaurates (Span 20-Atlas)	Derivatives of sorbitol produced by reacting it with H_2SO_4 or ethylene	Span 20-active in extremely small amounts
Polyethers	P200, P1200, P2000	Polymers of ethylene oxide propylene oxide	Active but varies with fermentation
Silicones	Antifoam A	Polymers of polydimethyl-siloxane fluids	Very active; inert, highly dispersable; low toxicity; expensive

Antifoams may be added manually when foam is observed. This will involve close watch and could be expensive. Automatic antifoam additions are now very common and depend on a probe which is activated when foams rise and make contact with the probe. One of the earliest is the ***wick defoamer*** in which the foam drew some antifoam on making contact with a wick. Modern methods are electrically activated systems. Other systems which have been used include antifoam introduction via the sparging air, or continuous drip-feeding.

Why is it important that antifoam should not have taste & odour?

3.3 SUMMARY

In this unit the antifoam types, properties and the various forms used in the industry have been highlighted.

3.4. References/Further Readings/Web Sources

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3.5 Possible answers to Self-Assessment Exercises

Answers Self-Assessment Exercise 1

… Foams in industrial fermentations are controlled either by chemical or mechanical means

UNIT4: SCALE UP PROCESS OF THE FERMENTATION PROCESS

4.0 Introduction

When the microorganism used in a fermentation is new, experimentation must be carried out to determine conditions for its maximum productivity. It is usual to initiate the studies in a series of conical flasks of increasing size and to progress through a 10–20-liter fermentor to a pilot plant (100-500 liter) and finally to a production plant (10,000-200,000 liters). The processes involved in the increasing scale of operation culminating in the production plant is known as ***scaling up***.

4.1 Intended Learning Outcomes (ILOs)

At the end of this unit, you should be able to:
- Understand the importance of scale up.
- The steps involved in scale up process.

4.2 Main content:

Scale up process of the fermentation process

One of the most important and complicated aspect of industrial microbiology is the transfer of a process from small scale laboratory equipment to large scale commercial equipment, a process called **scaling-up**. In reality, a microbial process does not behave the same in large scale fermenter as a small-scale laboratory equipment. Hence a proper understanding of the problems of scale-up is extremely important. In a well-established fermentation procedure, any change to be introduced must be experimented on and tested out in a pilot plant whose function is to simulate the conditions and structures of the production plant. This procedure is often referred to as **scaling down**. The processes of scaling up and scaling down are essentially in the domain of the chemical engineer who depends on data supplied by the microbiologist. Information gathered at the **shake flask stage** is used

to predict requirements in the pilot plant which itself serves a similar purpose for the production plant. The optimum requirements of medium composition, aeration, temperature, redox potential, pH, foaming, etc., are determined and extrapolated for the next higher scale.

Mixing and aeration are much easier to accomplish in the small laboratory flask than in the large industrial fermenter. As the size of the equipment is increased, the surface/volume ratio changes, the larger antifoam agents.

Examples

- Higher alcohol
- Silicones
- Natural esters
- Lard and vegetable oils, palm oil
- Fatty acids and derivatives
- Castor oil

The transfer of a process from small scale laboratory equipment to large scale commercial equipment, is……...?

Self-Assessment Exercises1

Attempt the exercise to determine what you have learnt so far. (1min).

i. Information gathered at the shake flask stage is used for ……..?

Fermenter has much volume for a given surface area. Since gas transfer and mixing depend on more surfaces exposed than on fermenter volume. It is obviously more difficult to mix the big can than the small flask. Oxygen transfer is much more difficult to obtain in a large fermenter due to different surface/volume ratio. Most commercial fermentations are aerobic; hence effective O_2 transfer is essential. With the rich culture media, used in industrial

processes, high biomass is obtained leading to high oxygen demand. If aeration is reduced even for a short period, the culture may experience partial anaerobiosis with serious consequences in terms of product yield and time. If there are pockets within the large fermenter where mixing is less efficient, microbial cell in such pockets will experience different environment conditions than the ones in the bulk fluid. In laboratory flask, such pockets do not exist. Scale up of an industrial process is the task of biochemical engineer, who is familiar with gas transfer, fluid dynamics, mixing and thermos-dynamics. The role of the industrial microbiologist is to work closely with the biochemical engineer to ensure that all parameters needed for a successful operation are understood and microbial strains appropriate for large scale fermentation are available. In transferring an industrial process from the laboratory to the commercial fermenter stage, several steps can be envisioned as listed below:

(1) Experiment in the laboratory flask which is generally the 1st indication that a process of commercial interest is possible.

(2) Testing some parameters such as variations in medium, temperature, pH etc in laboratory fermenter, the lab fermenter is a small fermenter generally of glass of 5 to 10L size.

(3) The pilot plant stage usually carried out in equipment of 300 to 3000L size. The conditions in this case closely approach a commercial scale. In the pilot plant fermenter, careful instrumentation and computer control is introduced so that in the lab fermenter can be obtained.
(4) The commercial fermenter itself generally of 10,000 to 400,000L.

Inoculum preparation

The conditions needed for the development of industrial fermentations often differ from those in the production plant. This is because except in a few examples where the cells themselves are the required product, e.g., in single cell protein, or in yeast manufacture, most fermentation products are metabolites. Cells to be used must be actively growing, young and vigorous and must therefore be in the phase of logarithmic growth. The inoculum usually forms 5-20% of the final size of the fermentation. By having an inoculum of this size, the actual production time is considerably shortened. The initial source of the inoculum is usually a single lyophilized tube. If the content of such a tube were introduced directly into a 100,000-liter pilot fermentor, the likelihood is that it would take an intolerably long time to achieve a production population, during which period the chance of contamination is created.

In-Text Question (ITQ)

Scale up of an industrial process is the task of biochemical engineer, who is familiar with gas transfer, fluid dynamics, mixing and thermos-dynamics True or False.?

Answer: True

4.3 SUMMARY

. In this unit, scale up, which is a transfer process from small scale laboratory equipment to large scale commercial equipment have been discussed.

4.4. References/Further Readings/Web Sources

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4.5 Possible answers to Self-Assessment Exercises

Answers Self-Assessment Exercise 1

Information gathered at the shake flask stage is used to predict requirements in the pilot plant which itself serves a similar purpose for the production plant.

UNIT5: INTELLECTUAL PROPERTY RIGHTS

5.0 Introduction

Intellectual property rights are the rights given to persons over the creations of their minds. They usually give the creator an exclusive right over the use of his/her creation for a certain period of time.

5.1 Intended Learning Outcomes (ILOs)

At the end of this unit, you should be able to:
- Understand the importance intellectual property rights
- Types of works that can secure intellectual property rights

5.2 Main content

Intellectual property rights: They are exclusive rights given by law to an individual s' data resulting from one's work that requires protection.

- Ideas, thoughts or products of one's intellectual efforts. Intellectual properties are intangible and unavailable to others as long as they are undisclosed. Ones expressed in tangible form; it can be legally protected.

Why intellectual property rights: Intellectual property rights are created to prevent others from using one's invention or artistic work without one's express permission.
It could be enforced by
- Patenting
- Copyrights
- Trademarks
- Trade secrets

How does it work?
It involves protection at specific periods: Owners of intellectual property

are granted protection under state/Federal/International law under varying conditions and periods of time. The protection includes the right to

- Defend their rights to the property the created
- Prevent others from taking advantage of their ingenuity
- Encourage their continuity innovativeness and creativity
- Assure the world a flow of useful, informative and intellectual works.

Example of intellectual properties include:

a) Written material
b) Computer software
c) Word or phrase
d) Symbol
e) Invention
f) Biotechnology innovation
g) New plant variety

There are several intellectual properties laws in Nigeria

- Patents and design decree No.60 of 1970
- National office of Industrial decree No.70 of 1979
- Trade Marks Act 29 of 1965
- Copyright decree no.47 of 1988

Nigerian intellectual properties laws membership

- ✓ Paris Convention since 1963
- ✓ Patent law treaty since 2000
- ✓ Rome convention since 1993
- ✓ World intellectual property organization

Government agencies responsible for Intellectual property rights include:

- Federal ministry of commerce. Commercial law department
- Federal ministry of commerce, Registry of Trade marks, Patents and Design
- National office for Technology Acquisition and promotion (NOTAP)
- National Biotechnology Development Agency (NABDA)

Intellectual properties are intangible and available to others as long as they are undisclosed True or False.?

Patent

Patents give one the right to limited monopoly of an idea or invention. Ones patented, it cannot be publicly disclosed.

The following cannot be patented i) Literary, dramatic, musical and artistic works

ii) Inventions which are offensive to public morality.

Trade secrets

Information that is not published or publicly disclosed. It may be highly valuable, depending on how well the secret is kept. The protection may last as long as secrecy is maintained. The knowledge usually is limited to a few individuals with need to know.

Copyrights:

Is a government granted right that protects against plagiarism. The work

may be in print, electronic form, film or photograph etc**.** It protects writings, software, art, music, drama etc the protection begins at time of creation. Works are marked with (C). Registration provides more protection.

What can be copyrighted?

- Original works of authorship
- Literary works
- Pantomime and choreographic works
- Pictorial graphic and sculptural works
- Motion picture and audiovisuals
- Architectural works
- Sound recording

Ones such works are copyrighted it gives one exclusive right to

- Reproduce copies of work
- Distribute copies of work to public by sale

Copyrights can be allowed for criticisms, comments, news, reporting and teaching. The extent of copy rights will be determined based on

- Purpose and character of the use, including whether such use is for commercial or non-profit or educational works
- The nature of the copyrighted work
- The amount and sustainability of the portion used in relation to copyrighted work as a whole.

Trademarks: is a brand name that describes a product belonging to a given company or organization.

3.5 Patent and Patency

A patent is a privileged granted by letter to an inventor to protect a new invention. It is a form of protection issued by a government to an inventor of a new product or process who publicly disclose the details of his or her invention and in return is granted for a limited period a legally enforceable right to exclude others from commercially exploiting it. Patent laws are set up for two reasons

(1) To induce the inventor to disclose something of his invention

(2) To ensure that an inventor is not exploited without some reward to the invention for his innovations

An invention is patentable if it is new, useful and obvious from what is already known in 'prior art' and in the 'state of art'. The current patent law in Nigeria is the Patent and Design Decree 1970.This decree states an invention as patentable

(a) If it is new, results from inventive activity and is capable of industrial application

(b) If it constitutes an improvement upon a patented invention and it is capable of industrial application

Patents cannot be validly obtained in respect of

(1) Plant or animal varieties are essentially biological process for the production of plants and animals (other than microbial process, and their products.)

(2) Inventions whose publications or exploitations will be contrary to public order or morality are not patented merely because its application/exploitation is prohibited.

Principles and discoveries of a scientific invention for the purpose of this decree patent are valid in Nigeria and some other countries for 20 years and 7 years in USA. A wide range of microbiological inventions are generally identified as patentable. Such items include vaccines, bacteria, insecticides, mycoherbicides etc. Microbes parse are not patentable except when they are used as part of a useful process.

What is Copyright?

Self-Assessment Exercises1

Attempt the exercise to determine what you have learnt so far. (1min).

What do you understand by intellectual property rights?

5.3. SUMMARY

This Unit discussed the concept of intellectual property rights and some of the laws in Nigeria governing the administration of the rights.

5.4. References/Further Readings/Web Sources

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5.5 Possible answers to Self-Assessment Exercises

Answers Self-Assessment Exercise 1
They are exclusive rights given by law to an individual s'

- *Data resulting form one's work that requires protection*
- *Ideas, thoughts or products of ones intellectual efforts. Intellectual properties is intangible and unavailable to others as long as they are undisclosed. Ones expressed in tangible form, it can be legally protected*

Glossary
CO_2 = Carbondioxide
NaOH = Sodium hydroxide
O_2 = Oxygen

End of the module Questions

1.Why is fermenters used in the industry submerged type? **Answer: Its because the submerged fermentor saves space and is more amenable to engineering control and design**

2. The most commonly used type of fermentor is ……..:**Aerated stirred Tank Batch**
Fermentor

3. . Stainless steel is therefore normally used for pilot and production fermenters. **True** or False?

4. In fermentation foam usually occur as a result of ……and …….. **Answer: agitation and aeration.**

5. Water and carbon dioxide are not two of the most common end-products of aerobic fermentations. True or **False.**

6. Why is fludized bed fermentor preferred over batch fermentor?

7. Provide the examples of chemical antifoam