NATIONAL OPEN UNIVERSITY OF NIGERIA

SCHOOL OF SCIENCE AND TECHNOLOGY

COURSE CODE: BIO 416

COURSE TITLE: INDUSTRIAL MICROBIOLOGY

INTRODUCTION

Industrial microbiology has been used in manufacture of products through the use of microorganisms. Microorganisms have been of immerse benefit to humanity through their role in food production and processing, the use of their products to improve human and animal health, in agriculture and for the maintenance and improvement of environmental conditions.

The study of industrial microbiology combines different areas such as foods and beverages, antibiotics and vaccines, agriculture and allied etc,.

Industrial microbiology is a three-credit course for students in the B Sc. Biology programme. The course if made up of three modules with 10 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.

This course guide provides you with what to expect in the course, how to work through the course material as a distance learner saddled with the responsibility of studying on your own, and your overall responsibilities and expectations. Tutorial sessions are also linked up with the course to provide the needed support you require.

WHAT YOU WILL LEARN IN THIS COURSE

The overall aim of this course BIO 416. Industrial Microbiology is to 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.

COURSE AIMS

This course aims at providing the learners with in depth understanding of industrial microbiology. It will help you to appreciate the various processes that microorganisms help in carrying out, their desirable effects as well as their contributions to the balance of nature

COURSE OBJECTIVES

To achieve the aims set out above, the course sets the overall objective. In addition, each unit has specific objectives stated at the beginning of a unit. Learners are advised to read them carefully before going through the unit. You will have to refer to them during the course of your study to monitor your progress. You are encouraged to always refer to the unit objectives after completing a unit.

WORKING THROUGH THIS COURSE

To complete this course, you are required to study through the units, the recommended textbooks and other relevant materials. Each unit contains some

self assessment exercises and tutor-marked assignments that, you will be required to submit. This will be followed by an end of term examination.

COURSE MATERIALS

The following are the components of this course.

1. The course guide
2. Study units
3. Textbooks
4. Assignment file
5. Presentation schedule

STUDY UNITS

This course material is made up of two modules of four units. These are:
Module 1: Basics of industrial microbiology

Unit 1: Nature of industrial microbiology

Unit 2: Microbes in industrial microbiology

Module 2: Techniques in industrial microbiology

Unit 1. Culture techniques

Unit 2. Maintenance of selected cultures

Module 3 Optimization of fermentations

Unit 1 Fermentation processes

Unit 2 Fermenter design and operations

TEXTBOOKS AND REFERENCES

Rittmann, B.E and Mcarly P.c (2001)Environmental Biotechnology Principles and Applications.New york MCGraw hill.

James M.J. (2000): Modern Food Microbiology 6th Edition.

Law B.A. (1997): Microbiology and Biochemistry of Cheese and Fermented Milk 2nd Edition...New York Chapman and Hall

Becton, Dickson and Co. (2005): Difco and BBL Manual. Manual of Microbiological Culture Media. 1st Ed. of Franklin Lakes N.J.BD.

Gottschall, J.C, Harder W, and Prins. R.A. (1992): Principles of Enrichment, Isolation, Cultivation and Preservation of Bacteria. In the Prokaryotes, 2nd Ed. A Balows et al. Editors New York. Spinger – Verlag.

ASSIGNMENT FILE

The assignment file will contain the Tutor-Marked Assignment (TMA) which will constitute part of the Continuous Assessment (CA) of the course. There are 15 assignments in this course with each unit having an activity/exercise for you to do to facilitate your learning as an individual.

PRESENTATION SCHEDULE

This presentation schedule in this course provides with important dates for completion of each tutor marked assignment. Please try to meet the deadlines.

ASSESSMENT

There are two aspects to the assessment of the course. These are the Tutor-Marked Assignments and written examination. In tackling the assignments, you are expected to apply information, knowledge and strategies gathered during the course. The assignments must be turned in to your tutor for formal assessment in accordance with the stated presentation schedules. The works you submit to your tutor for assessment will count for 30% of your total course work.

At the end of the course you will need to sit for a final written examination of three hour's duration. This examination will also count for 70% of your total course mark.

TUTOR-MARKED ASSIGNMENT

There are 4 Tutor-Marked Assignments to be answered in this course, and you are advised in your own interest to submit the assignments at the stipulated time in your study centre. You will be able to complete the assignments from the information and materials contained in your reading and study units. There are other self-assessment activities contained in the instructional material to facilitate your studies. Try to attempt them all. Feel free to consult any of the references to provide you with broader view and a deeper understanding of the course. Extensions will only be granted for submission after deadline on exceptional cases.

FINAL EXAMINATION AND GRADING

The final examination of NSS 213 will be of 3 hours duration and have a value of 60% of the total course grade. The examination will consist of questions which have bearings with the attempted self-assessment exercises and Tutor-Marked Assignments that you have previously encountered. Furthermore, all areas of the course will be evaluated. Make sure you give enough time to revise the entire course.

COURSE OVERVIEW

This table indicates the units, the number of weeks required to complete the assignments.

Unit	Title of Work	Week Activity	Assessment
	Course Guide	1	
	Module 1. Basics of industrial microbiology		
Unit 1	Nature of industrial microbiology	2	
Unit 2	Microbes in industrial microbiology	3	
	Module 2. Techniques in industrial microbiology		
Unit 1.	Culture techniques	4&5	
Unit 2.	Maintenance of selected cultures	6&7	
	Module 3 Optimization of fermentations		
Unit 1	Fermentation processes	8-10	
Unit 2	Fermenter design and operations	11&12	

HOW TO GET THE MOST OUT OF THE COURSE

In distance learning, the study units replace the university lecture. This is one of the greatest advantages of distance learning. You can read and work through specially designed study materials at your own pace, at time and place that suit you best. Think of it as reading the lecture notes instead of listening to a lecturer. In the same way that a lecturer might set you some reading task, the study units tell you when to read your other material. Just as a lecturer might give you an in-class exercise, your study units provide exercise for you to do at appropriate points.

The following are Practical Strategies for Working through the Course:

- Read the course guide thoroughly.
- Organize a study schedule.
- Stick to your own created study schedule.
- Read the introduction and objectives very well.
- Assemble your study materials
- Work through the units.
- Keep in mind that you will learn a lot by doing all your assignments carefully.
- Review the stated objectives.
- Don’t proceed to the next unit until you are sure you have understood the previous unit.

- Review the course and prepare yourself for the final examination.

FACILITATORS/TUTORS AND TUTORIALS

There are 12 hours of effective tutorial provided in support of this course. Details will be communicated to you together with the name and phone number of your tutor(s) through the study centre.

Your tutor(s) will mark and comment on your assignments, keep a close watch on your progress and any difficulties you might encounter and also provide assistance to you during the course. Ensure that you submit your assignments on schedule. You will get a feedback from your tutor(s) as soon as possible to the assignments.

Do not hesitate to contact your tutor(s) or study centre on phone or email in case of any of the following circumstances:

- You do not understand any part of the study units or the assigned reading.
- You have difficulty with the self test or exercises.
- You have questions or problems with an assignment, tutor's comments or grading of an assignment.

You are encouraged to attend the tutorials to allow for face to face contact with your tutor(s) and ask questions which you needed answers immediately. It is also an opportunity to discuss any grey area with your tutor(s). You can equally prepare questions to the tutorial classes for

meaningful interactions. You are sure to gain a lot from actively participating in the discussion. BEST OF LUCK

BIO 416 INDUSTRIAL MICROBIOLOGY

MODULE 1: BASICS OF INDUSTRIAL MICROBIOLOGY

UNIT 1: NATURE OF INDUSTRIAL MICROBIOLOGY

UNIT 2: MICROBES IN INDUSTRIAL MICROBIOLOGY

UNIT 1: NATURE OF INDUSTRIAL MICROBIOLOGY

CONTENTS

1.0 INTRODUCTTION

Industrial microbiology involves the use of microorganism to achieve specific goals. Industrial microbiology, however, generally focuses on products such as pharmaceutical and medical compounds (e.g. antibiotics, hormones, and transformed steroids), solvents organic acids, chemical feedstock's, 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 mutation-selection procedures. Genetic engineering has replaced this more traditional approach to developing microbial strains of industrial importance.

2.0 OBJECTIVES

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

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

3.0 MAIN CONTENT

3.1 NATURE OF INDUSTRIAL MICROBIOLOGY

Microorganisms are used in industrial microbiology to create a wide variety of products and to assist in maintaining and improving the environment. Most work in industrial microbiology has traditionally been carried out using microorganisms isolated from nature or modified through mutations. Microorganisms with specific genetic characteristic are now more commonly genetically engineered to meet the desired objectives.

3.1.1 MICROORGANISM OF INDUSTRIAL IMPORTANCE

The use of microorganisms in industrial microbiology follows a logical sequence. It is necessary to first identify or create a microorganism that carried out the desired process in the most efficient manner. This microorganism or its cloned genes are then used, either in a controlled environment such as a fermenter or in complex natural systems, such as in soils, or water, to achieve specific goals.

The first task of an industrial microbiologist is to find a suitable microorganism that has the following characteristics.

(a) Genetically stable

(b) Easy to maintain and grow

(c) Well suited for extraction or separation of desired products

3.2 FINDING MICROORGANISMS IN NATURE

Until relatively recently, microbial cultures used in industrial microbiology were 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.

3.3 GENETIC MANIPULATION OF MICROORGANIMS

Genetic manipulations are used to produce microorganism with new and desirable characteristics. The classical methods of genetic exchange coupled with recombinant DNA technology play a vital role in the development of cultures for industrial microbiology.

Table 1: Estimates of the Percent "Cultured" Microorganisms in Various Environments.

ENVIRONMENT	ESTIMATED PERCENT CULTURED
Sea water	0.001 – 0.100
Fresh water	0.25
Mesotrophic lake	0.1 – 1.0
Unpolluted estuarine waters	0.1 – 3.0
Activated sludge	1 – 15
Sediments	0.25
Soil	0.3

Source: D.A. Cowan, 2000. Microbial Genomes the Untapped Resource. Tibtech 18: 14 – 16.

3.3.1 MUTAGENES

Once a promising microorganism is found a variety of techniques can be used for its improvement, including chemical mutagens, ultraviolet light, and transposon mutagenesis. For example, the first cultures of *penicillium notatum*, which could be grown only under stationary conditions, yielded low concentration of penicillium.

In 1943, a strain of *Penicillium chrysogenum* was isolated – strain NRRL 1951 – which was further improved through mutation. Today most penicillin is produced with *Penicillium chrysogenum*, grown in aerobic stirred fermenter, which gives 55-fold higher penicillium yield than the original static cultures.

3.3.2 PROTOPLAST FUSION

Most yeast and molds are asexual or of a single mating type, which decrease the chance of random mutation that would lead to strain degeneration. Protoplast fusion can be used in genetic studies with the microorganism. Protoplast cells lacking a cell wall – are prepared by growing the cells in an isotonic solution while treating them with enzymes, including cellulose and beta-galacturonidase. The protoplasts are then generated using osmotic stabilizers such as sucrose. After regeneration of the cell wall, the need protoplasm fusion product can be used in further studies.

A major advantage of protoplast fusion technique is that protoplasts of different microbial species can be fused, even if they are closely linked taxonomically. For example, protoplasts of *Penicillium roquefortii* have been fused with those of *P. chrysogenum*. Even yeast protoplasts and erythrocytes can be fused.

3.3.3 TRANSFER OF GENETIC INFORMATION BETWEEN DIFFERENT ORGANISMS

The transfer and expression of genes between different organisms can give rise to novel metabolic processes and products. This is part of the rapidly developing field of combinatorial biology.

An important early example of this approach was the creation of the "super bug" patented by A.M. Chakarabarty in 1974, which had an increased capability of hydrocarbon degradation other examples are the expression, in *E. coli*, of the enzyme cretininase from *Psuedomonas putida* and the production of pediocin, a bacteriocin, in a yeast used in wine fermentation for the purpose of controlling bacteria contaminants. Genetic information transfer allows the production of specific proteins and peptides without contamination by other products.

Table 2: Combinatorial Biology in Biotechnology. The Expression of Genes in Other Organisms to Improve Processes and Products

Property or Product Transferred	**Micro organism Used**	**Combinatorial Process**
Ethanol production	*Eschericia 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	Penicillium	Production of 7 – ADC and 7

synthesis	chrtysogenum	– 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 sweetner in food industries
Creatininase[b]	*E. coli*	Expression of the creatininase gene from *Pseudomonas putida* R565. Gene inserted in a plasmid vector
Pediocin[c]	*S. cerevisiae*	Expression of bacteriocin from *Pediococcus acidilactici* in *S. cerevisiae* to inhibit wine contaminants
Acetone and butanol	*Clostridium*	Introduction of a shuttle vector

production	*acetobutylicum*	into *C. acetobutyliccum* 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 Engineering of *Sacchromyces cerevisiae*. Microbial Mol. Biol. Rev. 64 (1) 34 – 50.

3.3.4 MODIFICATION OF GENE EXPRESSION

Apart from inserting new genes in organisms, it is also possible to modify gene regulation by modifying regulatory molecules or the DNA sites to which they bind. The approaches make it possible to overproduce a wide variety of products.

A recent development is the use of modified gene expression to produce variants of the antibiotic erythromycin. Blocking specific biochemical steps in pathway for the synthesis of an antibiotic precursor results in modified final products. These altered products, which have slightly different structures, are tested for their possible antimicrobial effects. In addition, this approach enables a better understanding of the structure – function relationships of antibiotics.

3.3.5 PROTEIN EVOLUTION

One of the newest approaches for creating novel metabolic capabilities in a given microorganism is protein evolution, which employs forced evolution, adaptive mutation and in vitro evolution. Forced evolution and adaptive

mutation involves the application of specific environmental stresses to “force” microorganism to mutate and adapt, thus creating microorganisms with new biological capabilities. The mechanisms of these adaptive mutational processes include DNA rearrangement in which transposable elements and various types of recombination play critical roles.

Invitro evolution start with purified nucleic acids rather than a whole organism, DNA templates (e.g. mutagenized version of genes whose product is of interest) are transcribed in vitro by a phage RNA polymerase into RNA molecules that are selected based on their capacity to perform a specific function. The enzyme reverse transcriptase is then used to copy the selected RNA molecules in DNA, which can be amplified by PCR after a number of such cycles; a gene that might be of industrial importance will evolve.

3.4 PRESERVATION OF MICROORGANISMS

Once a microorganism or virus has been selected or created to serve a specific purpose, it must be preserved in its original form for further use and study. Periodic transfer of cultures has been used in the past, but this can lead to mutation and phenotypic changes in microorganism. To avoid these problems, a variety of culture preservative techniques may be used to main desired culture characteristics. Lyophilization, or freeze drying and storage in liquid nitrogen are frequently employed with microorganism.

Although storage are complicated and require expensive equipment, they allow microbial cultures to be stored for years without loss of viability or an accumulation of undesirable mutations.

Table 3: Methods Used to Preserve Cultures of Interest for Industrial Microbiology and Biotechnology

METHOD	COMMENTS
Periodic transfer	Variables of periodic transfer to new media include transfer frequency, medium used and holding temperature, this can lead to increased mutation rates, and production of variants.
Mineral oil slants	A stock culture is grown on a slant and covered with sterile mineral oil; the slant can be stored at refrigerator temperature.
Minimal medium, distilled water or water agar	Washed cultures are stored under refrigeration; these cultures can be viable for 3 – 5months or longer.
Freezing in growth media	Not reliable; can result in damage to microbial structures, with some microorganisms however, this can be a useful mean of culture maintenance.
Drying	Cultures are dried on sterile soil (soil stocks), on sterile filter paper disks, or in gelatin drops; these can be stored in a desiccators at refrigeration temperature, or frozen to improve viability.
Freeze-drying (lyophilization)	Water is removed by sublimation, in the presence of a cryoprotective agent; sealing in an ampoule can lead to long-term

	viability, with 30years have been reported.
Ultra freezing	Liquid nitrogen at -196^{o}C is used, and cultures of fastidious microorganisms have been preserved for more than 15years.

4.0 CONCLUSION

Industrial microbiology is mainly the use of microorganism of industrial importance in achieving a desired goal/ objectives to produce products of economic importance. These microorganisms are primarily sources for, reengineered, modified to yield a better result with selection and mutation continues to be important approaches for identifying new microorganism. These well-established procedures are now being complemented by molecular techniques including metabolic engineering and combinatorial biology. With combinatorial biology, it is possible to transfer genes from one organism to another, and to form new products.

Site-directed mutagenesis and protein engineering are used to modify gene expression. These approaches are leading to new and often different products with new properties.

5.0 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, modification and then their preservation. 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 mutagenesis, protoplast fusion, 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.

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.

6.0 TUTOR MARKED ASSIGNMENTS

(1) Explain the various methods used in manipulating microorganisms genetically for desired characteristics.

7.0. REFERENCES/FURTHER READINGS

Cowan, D.A. (2000): Microbial Genomes – the Untapped Resources. Tibtech 180.

MODULE 2: TECHNIQUES IN INDUSTRIAL MICROBIOLOGY

UNIT 1. CULTURE TECHNIQUES

UNIT 2. MAINTENANCE OF SELECTED CULTURES

CONTENTS

6.0 Tutor marked assignment

7.0 References/further readings

1.0 INTRODUCTION

The medium used to grow a microorganism is critical because it can determine the level of microbial growth and product formation. In order to maximize competitiveness, lower cost crude materials are used as sources of carbon, nitrogen and phosphorus. Crude plant hydroxyl salts often are used as complex sources of carbon, nitrogen and growth factors.

By products from the brewing industry frequently are employed because of their lower cost and greater availability. Other useful sources include molasses and whey from cheese manufacture.

2.0 OBJECTIVES

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.
- Explain the methods used in isolating pure cultures of microorganisms.

3.0 MAIN CONTENT

3.1 CULTURE MEDIA

A combination of nutrients used for the cultivation of microorganisms is called culture medium. Culture media may be liquid (broth) or solid agar. Media can be synthetic, semi synthetic or natural. There are different types of media. They are:

(i) Enrichment media

(ii) General purpose media

(iii) Selective media

(iv) Differential media

3.1.1 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.

3.1.2 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.

3.1.3 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.

3.1.4 DIFFERENTIAL MEDIA

This 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.

3.2 MEDIA FORMULATION

All microbes require water, sources of carbon, mineral elements and probably vitamin and oxygen if aerobic on a large scale, manufacturers normally use sources of cheap nutrient to make a medium which will need the following criteria.

(i) It must produce the maximum yield of products or biomass per gram of substrate used.

(ii) It must permit the maximum rate of product formation.

(iii) The yield of undesirable products must be minimal.

(iv) It must be cheap and of consistent quality.

(v) It must cause minimum problems in the production process particularly other aspects as aeration, purification extraction and waste management.

3.3 ISOLATION AND IDENTIFICATION OF CULTURE

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 plate and streak. Plate techniques have become indispensable tools of the microbiologist in isolating and subsequent identification of a microbial species.

3.3.1 SPREAD PLATE METHOD

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

3.3.2 STREAK PLATE

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.

TABLE 4. : TYPES OF MEDIA

PHYSICAL	**CHEMICAL**	**FUNCTIONAL**
Nature	Composition	Type
Liquid	Defined (synthetic)	Supportive (general purpose)
Semi solid	Complex	Enriched
Solid		Selective differential

TABLE 5: MAJOR COMPONENTS OF GROWTH MEDIA USED IN INDUSTRIAL PROCESSES

SOURCE	**RAW MATERIALS**

Carbon and energy	Molasses, whey, grains, agricultural waste (corn cobs)
Nitrogen	Corn-steep liquor, soy bean meal, stick liquor (slaughter house products), ammonia and ammonium salts, nitrates, dishler's soluble
Vitamins	Crude preparations of plant and animal products
Iron, trace salts	Crude inorganic chemicals
Buffers	chalk or crude carbonates

An organism must be kept by repeated sub culturing into fresh medium, but at each cell division, there is a small probability of mutation and strain degeneration. To avoid this, cultures can be stored by the following procedures.

(1) Storage at reduced temperature

(a) Storage on agar slope

Culture grown on agar slope may be stored in a refrigerator ($5^{o}C$) or a freezer ($-20^{o}C$) and sub cultured at approximately 6months to 1year. Interval especially if the slopes are covered with sterile medicinal grade mineral oil.

(b) Storage of slopes in water

Fungal spores can be preserved by suspending in sterile distilled water.

(c) Storage under liquid nitrogen

The metabolic activities of microorganisms may be reduced considerably by storing at very good temperature (-100^{o}C to -160^{o}C) which may be achieved using a liquid nitrogen refrigerator. A culture is grown to the maximum stationary phase; resuspended in a cryoprotective agent e.g. 10% glycerol and freezing the suspension in sealed glass ampoules before storage under liquid nitrogen.

(2) Storage in a dehydrated form

Moist sterile soil may be inoculated with culture and incubated for several days for some growth to occur and then allow to dry out at room temperature for approximately two weeks. The dried soil may be stored in a refrigerator. This technique has been used widely in fungal and actinomycetes storage.

(3) Lyophilisation

Lyophyilisation or freeze-drying involves the freezing of a culture followed by its drying under vacuum. The technique involves growing the culture to the maximum stationary freeze and resuspending the cells in a protective medium such as blood, serum, skin, milk, sodium few drops of the suspension is transferred to an ampoule which is then frozen and subjected to an ampoule which is sealed. The cell may remain viable for 10years and more.

3.3.3 POUR PLATE METHOD

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.

4.0 CONCLUSION

Culture media are classified based on function and composition as supportive media, selective media and differential media. Supportive media are used to culture wide variety of microbes. Enrichment/enriched media are supportive media that contain components that allow microbes to be differentiated from each other, usually based on some metabolic capability. Pure cultures usually are obtained by isolating individual cells with any of the three plating techniques. The spread-plate, the streak-plate and pour plate methods. Different methods are used in maintaining isolated cultures to make them viable for subsequent use.

5.0 SUMMARY

Much of the study of microbiology depends on the ability to grow and maintain microorganism in the laboratory, and this is possible only if suitable culture media are available. 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.

Although all microorganisms needs 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 vary greatly. Knowledge of a microorganisms

normal habitat often is used in selecting an appropriate culture medium because its nutrient requirements reflects its natural surroundings. Frequently a medium is used to select and grow specific microorganism or help identify a particular species. In such cases, the function of the medium also will determine its composition.

Culture media can be classified on the basis of several parameters. The chemical constituent from which they are made, their physical nature, and their function. Microbes are maintained in various ways such as storage at reduced temperature, storage at dehydrated forms and lyophilization in order to eliminate contamination, genetic mutation and retain viability for long period of time.

6.0 TUTOR – MARKED ASSIGNMENT

Describe the various methods used in isolation pure cultures of microbes stating the advantages of each.

7.0 REFERENCES/FURTHER READINGS

Becton, Dickson and Co. (2005): Difco and BBL Manual. Manual of Microbiological Culture Media. 1st Ed. of Franklin Lakes N.J.BD.

Gottschall, J.C, Harder W, and Prins. R.A. (1992): Principles of Enrichment, Isolation, Cultivation and Preservation of Bacteria. In the Prokaryotes, 2nd Ed. A Balows et al. Editors New York. Spinger – Verlag.

UNIT 2.MAINTENANCE OF SELECTED CULTURES

CONTENTS

1.0. 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.

2.0 OBJECTIVES

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

- Explain what natural selection is.
- Explain the methods of strain improvements.
- Explain what mutation

3.0. MAIN CONTENT

3.1. MUTATION

This is a change in the sequence in DNA (or RWA) in a few RNA viruses). It is obvious that since it is the sequence of these bases which is responsible for the type of proteins and hence enzymes synthesized, and change in the sequence will ultimately lead to a change in property. The basic procedure for producing microbial mutants involves exposure of cells to the action of mutagens. Mutagenic agents can either be physical or chemical.

Examples of physical are ionizing radiation, ultraviolet light. Chemical mutagents are nitrous acid, M – methyl – N – nitro – na – guanine **(NTG)**

Mutation and natural selection approach is hitor mix, whereas, genetic engineering permits purposeful manipulation or genetic information to engineer a microorganism that can produce high yields of variety of products.

3.2. STRAIN SELECTION

The selection of microorganism for industrial process begins with the screening to find the right candidates out of the many species of microbes, relatively few possess the genetic information needed to produce economically useful products. The first stage in the screening of microbes is the isolation i.e. obtaining either pure or mixed culture followed by the assessment to determine which of the microbes carry out the desired reaction or produce the desired product.

Due to the ubiquity of microbes, they can be isolated from natural sources e.g. rotten vegetable, sewage, soil, water etc. The isolate considered for industrial application must carry out the process economically and therefore the selection of the culture to be used is a compromise between the productivity of the organism and the economic constraint of the product. Some of the criteria used in selection the desired cultures are:-

Hybridization:- This is the artificial construction of a double stranded nucleic acid by complementary base pairing of two single-stranded nuclei acid.

It is an important method used in recombinant DNA technology for the identification of specific gene. Genetic recombination in fungi can be accomplished by haploid strains for ascopores or basidiospores and then made into providing new strains.

In some lower fungi, hybridization takes place by the use of pure sexual cycle which also ensures that strains have improved performance. The hybridization of yeasts and higher fungi requires three main steps.

(i) Sporulation

(ii) Spore formation

(iii) Hybridization

3.3. RECOMBINANT DNA TECHNOLOGY

This is the invitro incorporation of segments of genetic material from one cell to another cell. The technology of producing a genetically engineered bacterium is summarized below:

a) The nutritional requirement of the organism must be from a very cheap source.
b) The strain should be able to protect itself against contamination.
c) It must be stable and amenable for genetic manipulation.
d) It must have a high productivity i.e the rate at which substrates are converted to products must be high. Moreover, it must give a yield of product per unit time.
e) It must easy to extract the desired product after the bioconversion process.
f) It must be suitable for the type of process to be employed
g) It must not react with the equipment.

3.4. METHODS OF NATURAL SELECTION

It may be possible to design the isolation procedures in such a way that producers may be recognized at the isolation stage for example in assay for antibiotic production, where as in other cases, random isolation must be made and producers recognized at the subsequent stage. Some methods of natural selections are-

1) ***Enrichment culture:*** This is a technique resulting in an increase in the number of target organisms relating to the number of other types in the original medium. The process involves taking a mixed population and providing conditions either suitable for the growth of the desired organism by the provision of a particular substrate or by inclusion of certain inhibitors. The growth of the desired organisms result in modification of the medium and thus changes the selective force which may allow growth of other organisms resulting in succession. The selective force may be re-established by inoculating the enriched culture into identical fresh medium. Such sub culturing may be repeated several times before the dominant, organism is isolated by spreading a small innoculum of the enriched culture on solid medium. The time of sub culturing should correspond to the time when desired organism is dominant.

2) ***Screening for antibiotic production-*** The detection of antimicrobial action may be achieved by growing a potential producer on an agar plate in the presence of organisms against which antimicrobial action is required production of the antibiotic is indicated by inhibition of the test organism. Alternatively, the isolate may be grown in liquid culture and the cell-free broth can be obtained by centrifugation or filteration.

3) ***Screening for polysaccharide production-*** microbes producing polysaccharide (dextranxanthan) have been isolated from diverse environment.

However, the property of exopolysccharide production is difficult to apply in selective force.

It is customary to isolate for carbohydrate industrial waste which is expected to be rich in the desired organism. Isolates from such environment may then be grown on suitable media and producers recognized by mucilageneous appearance of the isolate. The suspected isolate may then be grown in liquid culture and the property of polysaccharide assessed.

(a) Source of donor genetic material:- DNA containing the genetic code for the property to be transferred into a bacterium from cells, or it may be synthesized.

(b) Production of hybrid DNA molecule:- The donor genetic material of a bacteriophage or plasmid. This is accomplished by the use of two enzymes; restriction endonucleases and ligase.

Restriction endonuclease cuts double strand DNA molecule and thus produce a well defined DNA segment of a given enzyme.

In this process, both the donor DNA and the agent into which the fragment of the donor DNA is to be incorporated are treated with the same endonuclease. The fragment can be connected by the addition of an enzyme called DNA ligase.

(c) Incorporation of hybrid DNA into host cell: - This is known as transformation or transfection. It involves introduction of phage hybrid DNA into the host cell. The most common technique for transformation depends on treating the recipient bacterium with $CaCl_2$ to make the membrane permeable to the DNA. The recipient bacteria are capable of receiving recombinant DNA molecules on the basis of only one bacterium per molecule. When bacteria are

transformed, a mixture of bacteria of various genotypes is produced. Each bacterial cell is capable of binary fission, yielding a colony of identical cells possessing equivalent genetic and therefore physiological traits once a colony with the proper phenotype is identified, the bacteria in it can be grown in limitless quantity to identify the gene.

3.5 CULTURE MAINTENANCE

Industrial microbes must be maintained in such a way as to eliminate

- Contamination
- Genetic change
- And retain viability

4.0. CONCLUSION:

Industrially useful microorganisms are unique and quite specific subsets of all microorganisms that are available on this planet, whereas microbes isolated from nature exhibit cell growth on their main physiological property. Industrial microbes' are organisms which have been selected carefully so that they manufacture one or more specific products. Even if the industrial microbe is one which has been isolated by traditional techniques, it can be highly modified before it enters large scale industry.

5.0. SUMMARY.

Industrially useful organisms are subjected to some conditions in order to achieve high metabolic specialization, industrial strain are genetically altered by mutation or recombination. Mutations a change in the sequence in DNA (or RNA) in a few RNA viruses). Hybridization is the artificial construction of a

double stranded nucleic acid by complementary base pairing of two single-stranded nucleic acid .It is useful in genetic engineering for the identification of specific genes. Recombinant DNA technology is the invitro incorporation of segments of genetic material from one cell to another cell. It may be possible to design the isolation procedures in such a way that producers may be recognized at the isolation stage for example in assay for antibiotic production, where as in other cases, random isolation must be made and producers recognized at the subsequent stage. Some methods of natural selections are- Enrichment culture: Screening for antibiotic production screening for polysaccharide production.

6.0 Tutor Marked Assignment:

Explain in details how an industrial microbe can be made more productive through strain selection and developments.

7.0 References/Further Readings.

Rittmann, B.E and Mcarly P.c (2001)Environmental Biotechnology Principles and Applications.New york MCGraw hill.

MODULE 3 OPTIMIZATION OF FERMENTATIONS

UNIT 1 FERMENTATION PROCESSES

UNIT 2 FERMENTER DESIGN AND OPERATIONS

UNIT 1 FERMENTATION PROCESSES

CONTENT

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. The fermentation process also is to create new, pleasing food flavours and odours. The major fermentation is used in microbiology are the lactic, propionic and ethanolic fermentations. These fermentations are carried out with a wide range of cultures, many of which have not been characterized.

2.0 OBJECTIVES

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

- Describe the various means of fermentation fruits, vegetable e.t.c.
- Explain in detail the process of bread making.
- Differentiate between the different types of beer produced from fermentation.

3.0 MAIN CONTENT

3.1 DRINKS

3.1.1 ALCOHOLIC BEVERAGES

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:

(i) Beer production

The brewing process starts with the malting of the grain followed by conversion of the malted grain to soluble extract which is fermented by yeast to yield beer. The methods employed determine the quality and the type of beer produced. Careful control must therefore be exercised at each stage of production to produce a beer of acceptable standard.

Barely beer can be divided into two broad groups:

(1) Top-fermented beer (ales)

(2) Bottom-fermented beer (lager)

This distinction is based on whether the yeast remains at the top of the brew (top-fermented beer) or sediment to the bottom (bottom-fermented beer) at end of fermentation.

Brewing yeast strains are of two major types. The top-fermenting and the bottom fermenting yeast. The top fermenting yeast remains uniformly distributed in the fermenting wort and are carried to the top by the CO_2 gas generated during fermentation whereas bottom yeast settle to the bottom. Top yeasts are used in the brewing of ales and bottom yeasts are used to make lager.

The bottom yeasts are usually given the species designation *Saccharomyces carlbergensis* and the top yeast are called *Saccharomyces cerevisiae*.

Fermentation by top yeasts usually occurs at high temperature, 14 – 23^{o}C while fermentation by bottom yeast occurs at 6 – 12^{o}C.

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.

(i) Malting: - This is carried out to promote synthesis of hydrolytic enzymes such as alpha amylase, endo-β gluconase and peptidases to solubulize the endosperm wall of the grain and secure the enzymytic 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 it's growth. Malting involves 3 processes. These are steeping, germination and kelming.

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 develop 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).

Kelming: This is drying of germinated grain at temperature between 50 – 90^{o}C to arrest growth and enzyme activity. The kelming 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.

After kelming, the kelmed malt can then be grind in a mill in readiness for marshing.

(2) Marshing:- This is the central process of brewing and consist of mixing the malt with adjuncts at temperature which are optimum for the saccharomytic and proteolytic enzymes present in the malt. The particular marshing procedure employed is of optimus importance to the character of the resulting beer.

Marshing extracts, those materials from the malt and adjuncts which can be solubilized under the particular conditions employed and the liquid resulting from mashing is known as the wort. Marshing is influenced by various factors such as temperature, time, concentration of malt, starch and protein.

The most importance objective of marshing is to produce fermentable sugar largely through the amenolytic degradation of solubilized starch. Two enzymes are mainly responsible for this α and β amylase. Hops give beer the bitter taste.

There are 3 widely contrasting mashing processes.

(i) Infussion marshing: - This is characterized by the biochemical conversion of the grist (malt) at a single temperature ($65^{o}C$) in one vessel which serves for extraction and filteration. The marsh is not boiled in this process.

(ii) Decoction marshing: - In this case, starch conversion starts at a lower temperature which is eventually raised sometimes by the removal, boiling and return of part of the marsh and any adjunct used.

(iii) The double-marsh method: - In this case, the starchy adjuncts are boiled and added to the malt. The warm marsh is finally transferred to a lauter turn where it is filtered.

(3) Fermentation: - The filtered-cooled wort (15^oC) is pumped into fermentation vessels which are often made of stainless steel. It is pitched with yeast (0.2kg/hl on it's 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 CO_2, glycerol, acetate and ethanol. Fermentation eventually cease due to exhaustion of nutrients and inhibition by ethanol.

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

(4) Lagering and ageing: - At the end of fermentation, the product is beer and it has a harsh taste and referred to as 'green beer'. 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 it's taste and appearance. Priming is done by small amount of sucrose, invert sugar or a mixture of cereal starch hydroxylase and invests sugar caramel. Primary serves as substrate fro secondary fermentation as well as beer sweetner.

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.

(5) 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.

(6) Pasteurization: **-** Filled and cannel bottles are pasteurized before labeling.

3.1.2 WINE PRODUCTION

The term wine refers to the alcoholic beverage made from the juice of variety of fruits by the fermentative action of selected yeast adapted to the particular type of wine followed by the ageing process. The term is frequently used to demote the alcoholic beverage made from grape fruit. The yeast used in the fermentation of grape wine is *Saccharomyces cerevisiae* var. *ellipsoideus* which have been selected over the years. There are two types of grape wine viz: Red wine which are fermented with the skin (red, purple) which contain the authocyanin pigment while white wine are produced from white grape or the juice of other grapes fermented without their skin.

3.1.3 SPIRIT

They are distilled liquor obtained from fermentation of yeast of various carbohydrates containing several raw materials e.g. sugarcane, grains and other

plant materials. Distilled liquor includes whiskey, from barley, malt water, rice and oats. Bourbon from corn, gin from grain, malt flavoured with jumper berries, rum from grains aquavit from grains flavoured with caraway seed, vodka from grain. The manufacturing step in the spirit industry consists of raw material preparation, yeast propagation, fermentation, and treatment of fermented liquor and in some cases maturation/ageing.

3.2 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. In modern baking practice, pure cultures of selected strains of *Saccharomyces cerevisiae* are mixed with bread dough to make about desire changes.

Desirable characteristics of *S. cerevisiae* selected from commercial production of bakers yeast includes the ability to ferment sugar in the dough vigorously and to grow rapidly. This is as well as other characteristics for which the strain was selected should be relatively stable. The CO_2 produced during the fermentation is responsible for the leavening or rising of the dough. The quality of the product depends on the proper selection of yeast and the incubation conditions as well as on the choice of materials. The major unit operations involved in baking are weighing, mixing, molding, fermentation, baking, cooling and packing.

3.3 FERMENTED MILKS

The majority of fermented milk products rely on Lactic Acid Bacteria (LAB), fermented milks were produced for thousands of years before Louis Pasteur discovered lactic acid fermentation. LAB includes species belonging to the genera lactobacillus, lactococcoccus, leuconostoc and streptococcus.

These bacteria are low G + C gram-positives that tolerate acidic conditions, are non sporing and are aero tolerant with a strictly fermentative metabolism.

3.3.1 CHEESE PRODUCTION

This is one of the oldest human foods. Cheese is classified based on texture or hardness as soft cheese (cottage, cream, brie) semi soft cheese (muenster, limburger, blue) hard cheese (cheddar, Colby, swiss) or very hard cheese (parmesan). All these result from a lactic acid fermentation of milk, which results in coagulation of milk proteins and formation of a curd. Rennin, an enzyme from calf stomach but now produced by genetically engineered microorganisms can also be used to promote curd formation.

After the curd is formed, it is heated and pressed to remove the watery part of the milk (called the whey), salted and then usually ripened. The cheese curd can be packed for ripening with or without additional microorganisms. In some cases, molds are used to further enhance cheese.

3.3.2 PROBOTICS

Microorganisms such as Lactobacillus and Bifidobacterium are being used in the rapidly developing area of probotics, the benefit beyond basis nutritive value. Probotics is the oral administration of either living or substances to promote health and growth, has the potential to re-establish the natural balance and return the host normal health and nutrition. The possible health benefits of the use of such microbial dietary adjurants include immunomodulation, control of diarrhea, anticancer effect and possible improvement of Crohn’s disease.

Acidophilus milk is produced by *Lactobacillus acidophilus, L. acidophilus* may modify the microbial flora in the lower intestine thus improving general health. It is often in use as a dietary adjunct, especially for lactose intolerant persons.

Another interesting group used in milk fermentation are bifidobacteria. The genus bifisobacterium contain irregular, nonsporing, gram-positive rods that may be club-shaped or forked at the end. Bifidobacteria are thought to help maintain the normal intestinal balance while improving lactose tolerance. Bifidobacterium – amended fermented milk products, including yoghurt are now available in various parts of the world.

3.4 MEAT AND FISH

Beside the fermentation of diary products, a variety of meat products especially sausage can be ferment. Country-cured hams, summer sausage, salami cervelat, fish sauces (processed by halophilic bacillus species) etc izushi and katsuobusji *Pediococcus cerevisiae* and *Lactobacillus plantarum* are most often involved in sausage fermentation. Izushi is based on the fermentation of fresh fish, rice and vegetable by *Lactobacillus sp*; katsuobushi results from the fermentation of tuna by *Aspergillus glaulus*. Both meat fermentation originated from Japan.

3.5 OTHER FERMENTED FOODS

Many other plant products can be fermented, as summarized in table 1. These include sufu, which is produced by the fermentation of tofu, a chemically coagulated soybean milk product. To carry out the fermentation, the tofu curd is cut into small chuncks and dipped into a solution of salt and citric acid. After the cubes are heated to pasteurize the surfaces, the fungi *Actinimucor elagans*

and some mucor species are added when a white mycelium develops, the cubes now called pehtze are aged in salted rice wine.

This product has achieved the status of a delicacy in many parts of the western world. Another popular product is tempeh, a soybean mash fermented by *Rhizopus*.

Table: 6 Major Categories and Examples of Fermented Milk Products

	Category	**Typical Examples**
(i)	Lactic fermentations mesophilic	Butter milk, cultured buttermilk, longofil, tetmjolk, ymer
	Thermophilic	Yoghourt, laban, zabadi, labnela, skyr, Bulgarian, buttermilk
	Probiotic	Biogarde, bifighurt, acidophilus milk, yakult, cultural – AB
(ii)	Yeast – lactic fermentation	Kefir, koumiss, acidophilus-yeast milk
(iii)	Mold – lactic fermentation	Villi

Source: Microbiology and biochemistry of cheese and fermented milk.

Table: 7.Fermented Food Produced from Fruits, Vegetables, Beans and Related Substrate

Foods	**Raw ingredients**	**Fermenting microorganism**	**Area**

Coffee	Coffee beans	*Erwinia dissolvens*, *Saccharomyces spp*	Brazil, Congo
Garri	Cassava	*Corynecbacterium manihot*, *Geotrichum spp*	West Africa
Kenkry	Corn	*Aspergillus spp*, *Penicillium spp*, *Lactobacilli*, yeast	Ghana, Nigeria
Ogi	Corn	*Lactobacillus plantarum*, *Lactococcus lactis*, *Zygosaccharomyces rou* x11	Nigeria
Sufu	Soyabeans	*Actinimucor elegans*, *Mucor spp*	China
Tempeh	Soybeans	*Rhizopus oligosporus*, *R. Oryzae*	Indonesia New Guinnea Surinam
Sauericraut	Cabbage	*L. mesenteroides*, *L. plantarum*, *L. brevis*	Worldwide
Olives	Green olives	*Leuconostoc mesenteroides*, *L. plantarum*	Worldwide

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

4.0 CONCLUSION

Dairy products, grains, meats, fruits and vegetables can be fermented. Lactic acid bacteria are the principal microbes involved in milk fermentation, fungi are also used. Wines are produced by the direct fermentation of fruit juices or musts. For fermentation of cereals and grains, starches and protein contained in these substances must first be hydrolysed to provide substrate for alcoholic fermentation.

The making of bread, sauerkraut, sufu and many other foods also involves the use of complex fermentation processes.

5.0 SUMMARY

Beer and ale are produced from cereal and grains. The starches in this substrate are grains. The starches in this substrate are hydrolyzed, in the process of malting and mashing, to produce fermentable wort. *Saccharomyces cerevisisiae* is major yeast used in the production of beer and ale.

Wines are produced from pressed grapes and can be dry or sweet depending on the level of free sugar that remains at the end of alcoholic fermentation.

6.0 TUTOR MARKED ASSIGNMENT

Describe in details the production of larger beer and ale.

7.0. REFERENCES/FURTHER READINGS

James M.J. (2000): Modern Food Microbiology 6th Edition.

Law B.A. (1997): Microbiology and Biochemistry of Cheese and Fermented Milk 2nd Edition...New York Chapman and Hall

UNIT 2: FERMENTER DESIGN AND OPERATION

CONTENT

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.

2.0 OBJECTIVES

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.

3.0 MAIN CONTENT

3.1 FERMENTER DESIGN AND OPERATION

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

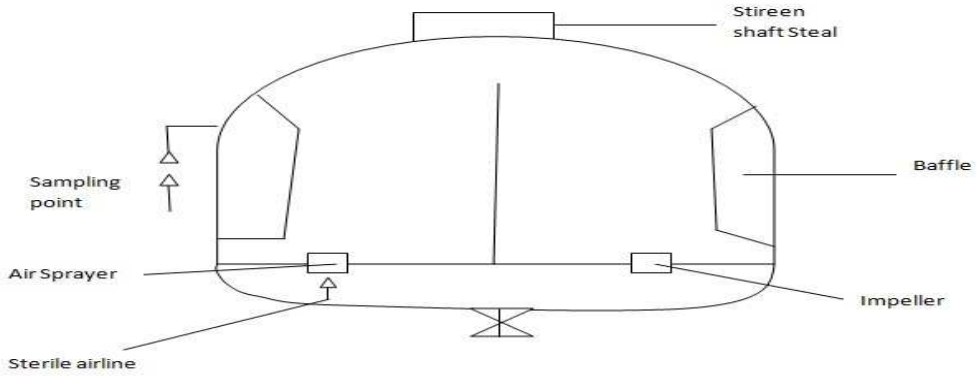
(10) It should have smooth internal surface.

(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.

The diagram showing a typical mechanically agigated and aerated fermenter.



In a fermenter with strict aseptic requirement, it is important to select materials that can withstand repeated steam sterilization. On a small scale, glass stainless steel can be used. Glass gives smooth surfaces; it is not toxic and corrosion-free. It is usually easy to examine the process while it is going on.

Temperature can be achieved by placing a fermenter in a thermostatically controlled bath or by use of internal cooling coil through which cold water is circulated to achieve the correct temperature. The sparger is the aeration system. The primary purpose of aeration is to provide microbes in submerged cultures with sufficient O_2 for metabolic requirements while agitation will ensure a uniform suspension of microbial cell is achieved in a homogeneous nutrient medium. Agitation may be avoided if aeration provides enough agitation for example in processes where broth of low viscosity and low focal solids are used.

In order to achieve and maintain aseptic condition during fermentation, the following operations should be performed:

- Sterilization of the fermenter
- Sterilization of the air supply
- Addition of inoculums, nutrients and other supplements must be done aseptically.
- There must be a sampling point
- Foam control
- Correct monitoring and control of various parameters such as pH, dissolved O_2, temperature e.t.c.

3.2 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 scale-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.

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.

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

Fermenter has much volume for a given surface area. Since gas transfer and mixing depend 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 & 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 thermo 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 1^{st} 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.

3.3 ANTIFOAMS

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 down stream 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.

Properties of Antifoams

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

(2) It must have no effect on the taste & odour.

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

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

3.4 Patents and Patent Laws.

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 r 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 it's 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 indentified as patentable. Such items includes vaccines, bacteria, insecticides, mycoherbicides etc.Microbes parse are not patentable except when they are used as part of a useful process.

4.0. CONCLUSION

Microorganisms can be grown in controlled environments of various types using fermenters and other culture systems. If defined constituents are used, growth parameters can be chosen and varied in the curse of growing a microorganism. This approach is particularly for the production of amino acids, organic acids and antibiotics. A patent is a privileged granted by letter to an inventor to protect a new invention.

5.0.SUMMARY

A fermenter is a vessel which does not permit contamination but provides conditions necessary for the maximum production of the desired product. In order to achieve and maintain aseptic condition during fermentation, the following operations should be performed:

Sterilization of the fermenter, sterilization of the air supply, addition of inoculums, nutrients and other supplements must be done aseptically, there must be a sampling point, foam control, correct monitoring and control of various parameters such as pH, dissolved O_2, temperature e.t.c. . 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. 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.

6.0. Tutor Marked Assignment

Explain the processes involved in a fermenter design

7.0 References/Further Readings.

Rittmann, B.E and Mcarly P.c (2001)Environmental Biotechnology Principles and Applications.New york MCGraw hill.