

JEPPIAAR ENGINEERING COLLEGE

DEPARTMENT OF BIOTECHNOLOGY

B.TECH /BATCH (2017-2021): II YEAR / III SEM



QUESTION BANK

BT8303– BASIC INDUSTRIAL BIOTECHNOLOGY (R-2017)

Compiled By

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OBJECTIVES:

1. To make the students aware of the overall industrial bioprocess so as to help them to manipulate the process to the requirement of the industrial needs.
2. The course prepares the students for the bulk production of commercially important modern Bioproducts, Industrial Enzymes, Products of plant and animal cell cultures.

UNIT I INTRODUCTION TO INDUSTRIAL BIOPROCESS 10

Fermentation - Bacterial, Fungal and Yeast, Biochemistry of fermentation. Traditional and Modern Biotechnology - A brief survey of organisms, processes, products. Basic concepts of Upstream and Downstream processing in Bioprocess, Process flow sheeting - block diagrams, pictorial representation.

UNIT II PRODUCTION OF PRIMARY METABOLITES 9

Primary Metabolites- Production of commercially important primary metabolites like organic acids, amino acids, alcohols and vitamins.

UNIT III PRODUCTION OF SECONDARY METABOLITES 9

Secondary Metabolites- Production processes for various classes of secondary metabolites: Antibiotics and Steroids.

UNIT IV PRODUCTION OF ENZYMES AND OTHER BIOPRODUCTS 9

Production of Industrial Enzymes, Biopesticides, Biofertilizers, Biopreservatives, Biopolymers, Biodiesel, Cheese, Beer, SCP & Mushroom culture. Bioremediation.

UNIT V PRODUCTION OF MODERN BIOTECHNOLOGY PRODUCTS 8

Production of recombinant proteins having therapeutic and diagnostic applications, vaccines. Bioprocess strategies in Plant Cell and Animal Cell culture.

TOTAL: 45 PERIODS

OUTCOMES:

At the end of the course, the students will be able

1. To explain the steps involved in the production of bioproducts and methods to improve modern biotechnology.
2. To apply basic biotechnological principles, methods and models to solve biotechnological tasks.
3. To identify and debate the ethical, legal, professional, and social issues in the field of biotechnology.
4. To design and deliver useful modern biotechnology products to the Society.

TEXT BOOKS:

1. Satyanarayana, U. "Biotechnology" Books & Allied (P) Ltd., 2005.
2. Kumar, H.D. "A Textbook on Biotechnology" 2nd Edition. Affiliated East West Press Pvt.Ltd, 1998.
3. Balasubramanian, D. et al., "Concepts in Biotechnology" Universities Press Pvt.Ltd., 2004.
4. Ratledge, Colin and Bjorn Kristiansen "Basic Biotechnology" 2nd Edition Cambridge University Press, 2001.
5. Dubey, R.C. "A Textbook of Biotechnology" S.Chand & Co. Ltd., 2006.

REFERENCES:

1. A.H. Patel " Industrial Microbiology" Macmillan
2. Prescott, S.C. and Cecil G. Dunn, "Industrial Microbiology", Agrobios (India), 2005.
3. Cruger, Wulf and Anneliese Crueger, "Biotechnology: A Textbook of Industrial Microbiology", 2nd Edition, Panima Publishing, 2000.
4. Moo-Young, Murrey, "Comprehensive Biotechnology", 4 Vols. Pergamon Press, (An Imprints of Elsevier) 2004.
5. C.F.A Bryce and EL.Mansi, Fermentation microbiology & Biotechnology, 1999.
6. K.G.Ramawat & Shaily Goyal, Comprehensive Biotechnology, 2009, S.Chand publications.

S. NO.	TITLE	REFERENCE BOOK	PAGE NO.
UNIT I - INTRODUCTION TO INDUSTRIAL BIOPROCESS (10)			
1.	Fermentation - Bacterial, Fungal and Yeast, Biochemistry of fermentation.	Prescott, S.C. and Cecil G. Dunn, "Industrial Microbiology", Agrobios (India), 2005.	Chapter 1
2.	Traditional and Modern Biotechnology -	Prescott, S.C. and Cecil G. Dunn, "Industrial Microbiology", Agrobios (India), 2005.	Chapter 12
3.	A brief survey of organisms, processes, products.	Cruger, Wulf and anneliese Crueger, "Biotechnology: A Textbook of Industrial Microbiology",	Pg.No 9-50, 64-107,111-121.
4.	Upstream and Downstream processing in Bioprocess.	Satyanarayana, U. "Biotechnology" Books & Allied (P) Ltd., 2005	Pg.No 239-267, 270-280
5.	Basic concepts of Process flow sheeting -block diagrams, pictorial representation.	Research Internet Papers	10 pages
UNIT II PRODUCTION OF PRIMARY METABOLITES (9)			
1.	Primary Metabolites -	Satyanarayana, U. "Biotechnology" Books & Allied (P) Ltd., 2005	Pg.No 254
2.	Production of commercially important primary metabolites like Organic acids, and Amino acids.	Satyanarayana, U. "Biotechnology" Books & Allied (P) Ltd., 2005	Pg.No 318-322
3.	Production of commercially important primary metabolites like Alcohols and Vitamins.	Satyanarayana, U. "Biotechnology" Books & Allied (P) Ltd., 2005	Pg.No 344-354
UNIT III PRODUCTION OF SECONDARY METABOLITES (9)			
1.	Secondary Metabolites -	Satyanarayana, U. "Biotechnology" Books & Allied (P) Ltd., 2005	Pg.No 255
2.	Production processes for various classes of secondary metabolites: Antibiotics	Satyanarayana, U. "Biotechnology" Books & Allied (P) Ltd., 2005	Pg.No 329-343

3.	Production processes for various classes of secondary metabolites: Steroids.	Cruger, Wulf and anneliese Crueger, "Biotechnology: A Textbook of Industrial Microbiology",	Pg.No 286-301
UNIT IV PRODUCTION OF ENZYMES AND OTHER BIOPRODUCTS (9)			
1.	Production of Industrial Enzymes, Biopesticides and Biofertilizers.	A.H. Patel " Industrial Microbiology" Macmillan and Satyanarayana, U. "Biotechnology" Books & Allied (P) Ltd., 2005	Pg.No137, Pg.No 188. And Pg.No 645-657, 598
2.	Production of Biopreservatives, Biopolymers, Biodiesel.	Satyanarayana, U. "Biotechnology" Books & Allied (P) Ltd., 2005	Pg. No 395-398,382-392, Internet Notes
3.	Production of Cheese, Beer, and SCP	Satyanarayana, U. "Biotechnology" Books & Allied (P) Ltd., 2005	Pg.No373-380, 362-370
4.	Production of Mushroom culture & Bioremediation.	Satyanarayana, U. "Biotechnology" Books & Allied (P) Ltd., 2005	Pg.No 380-381, 718, Pg No727
UNIT V PRODUCTION OF MODERN BIOTECHNOLOGY PRODUCTS (8)			
1.	Production of recombinant proteins having therapeutic and diagnostic applications: Vaccines.	Satyanarayana, U. "Biotechnology" Books & Allied (P) Ltd., 2005	Pg.No 199-212
2.	Bioprocess strategies in Plant Cell Culture	Satyanarayana, U. "Biotechnology" Books & Allied (P) Ltd., 2005	Pg.No 497-522
3.	Bioprocess strategies in Animal Cell culture.	Satyanarayana, U. "Biotechnology" Books & Allied (P) Ltd., 2005	Pg.No 407-414

PART A

UNIT I INTRODUCTION TO INDUSTRIAL BIOPROCESS 10

Fermentation - Bacterial, Fungal and Yeast, Biochemistry of fermentation. Traditional and Modern Biotechnology - A brief survey of organisms, processes, products. Basic concepts of Upstream and Downstream processing in Bioprocess, Process flow sheeting - block diagrams, pictorial representation.

PART-A

1. Define Fermentation with example Nov/Dec 2016, 2017

It is a metabolic process that converts sugar to acids, gases, or alcohol. It occurs in yeast and bacteria, and also in oxygen-starved muscle cells, as in the case of lactic acid fermentation. Fermentation is also used more broadly to refer to the bulk growth of microorganisms on a growth medium, often with the goal of producing a specific chemical product. French microbiologist Louis Pasteur is often remembered for his insights into fermentation and its microbial causes. The science of fermentation is known as zymology.

2. What is Modern Biotechnology?

Modern biotechnology refers to a number of techniques that involve the intentional manipulation of genes, cells and living tissue in a predictable and controlled manner to generate changes in the genetic make-up of an organism or produce new tissue. Examples of these techniques include: recombinant DNA techniques (r DNA or genetic engineering), tissue culture and mutagenesis

3. What is Traditional Biotechnology?

Traditional biotechnology refers to a number of ancient ways of using living organisms to make new products or modify existing ones. In its broadest definition, traditional biotechnology can be traced back to human's transition from hunter-gatherer to farmer. As farmers, humans collected wild plants and cultivated them and the best yielding strains were selected for growing the following seasons.

4. Comment on GRAS and GILSP

Some organism are termed **GRAS** *ie.* Generally Recognized As Safe. or Assessment of hazardous organism are known pathogenicity of organism, virulence level, number of organisms required to initiate infection, routes of infection, known incidence of infection, local existence of vectors and reserves of micro organisms, volume of organisms used in process, techniques used for cultivation and harvesting and prophylaxis and treatment facility. Good industrial large scale practice (**GILSP**) involves safe and highly productive organism for the process.

5. What are the general requirements of a bioreactor?

The design and construction of biochemical reactors must preclude foreign contamination (sterility). Furthermore, non-septic conditions should be maintained during the fermentation and ensure containment.

(2) Optimal mixing with low, uniform shear achieved by proper designing of agitator and aerator

(3) Adequate mass transfer (oxygen) achieved by monitoring the speed of agitator and agitator

(4) Clearly defined flow conditions that can be maintained by proper opening valves and monitoring devices

(5) Feeding of substrate with prevention of under or overdosing by proper feed ports and monitoring

(6) Suspension of solids

(7) Gentle heat transfer

(8) Compliance with design requirements such as: ability to be sterilized; simple construction; simple measuring, control, regulating techniques; scaleup; flexibility; long term stability; compatibility with up- downstream processes; antifoaming measures.

6. What is a Process Flow Diagram?

A **process flow diagram** (PFD) is a **diagram** commonly used in chemical and **process** engineering to indicate the general **flow** of plant **processes** and equipment. The PFD displays the relationship between major equipment of a plant facility and does not show minor details such as piping details and designations.

7. What is a process flow chart?

A flowchart is a picture of the separate steps of a process in sequential order.

Elements that may be included are: sequence of actions, materials or services entering or leaving the process (inputs and outputs), decisions that must be made, people who become involved, time involved at each step and/or process measurements.

The process described can be anything: a manufacturing process, an administrative or service process, a project plan. This is a generic tool that can be adapted for a wide variety of purposes.

8. When to Use a Flowchart?

To develop understanding of how a process is done.

To study a process for improvement.

To communicate to others how a process is done.

When better communication is needed between people involved with the same process.

To document a process.

When planning a project.

9. Comment on Batch Fermentations

A tank of fermenter is filled with the prepared mash of raw materials to be fermented. The temperature and pH for microbial fermentation is properly adjusted, and occasionally nutritive supplements are added to the prepared mash. The mash is steam-sterilized in a pure culture process. The inoculum of a pure culture is added to the fermenter, from a separate pure culture vessel. Fermentation proceeds, and after the proper time the contents of the fermenter, are taken out for further processing. The fermenter is cleaned and the process is repeated. Thus each fermentation is a discontinuous process divided into batches.

10. Comment on Continuous Fermentation

Growth of microorganisms during batch fermentation confirms to the characteristic growth curve, with a lag phase followed by a logarithmic phase. This, in turn, is terminated by progressive decrements in the rate of growth until the stationary phase is reached. This is because of limitation of one or more of the essential nutrients. In continuous fermentation, the substrate is added to the fermenter continuously at a fixed rate. This maintains the organisms in the logarithmic growth phase. The fermentation products are taken out continuously. The design and arrangements for continuous fermentation are somewhat complex.

11. Write notes on Yeast Fermentation

Fermentation does not necessarily have to be carried out in an anaerobic environment. For example, even in the presence of abundant oxygen, yeast cells greatly prefer fermentation to aerobic respiration, as long as sugars are readily available for consumption (a phenomenon known as the Crabtree effect). Fermentation reacts NADH with an endogenous, organic electron acceptor. Usually this is pyruvate formed from the sugar during the glycolysis step.

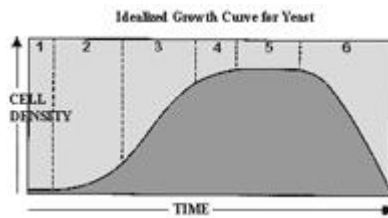
During fermentation, pyruvate is metabolized to various compounds through several processes:

ethanol fermentation, aka alcoholic fermentation, is the production of ethanol and carbon dioxide

lactic acid fermentation refers to two means of producing lactic acid:

1. **homolactic fermentation** is the production of lactic acid exclusively
2. **heterolactic fermentation** is the production of lactic acid as well as other acids and alcohols.

Sugars are the most common substrate of fermentation, and typical examples of fermentation products are ethanol, lactic acid, carbon dioxide, and hydrogen gas (H_2). However, more exotic compounds can be produced by fermentation, such as butyric acid and acetone. Yeast carries out fermentation in the production of ethanol in beers, wines, and other alcoholic drinks, along with the production of large quantities of carbon dioxide. Fermentation occurs in mammalian muscle during periods of intense exercise where oxygen supply becomes limited, resulting in the creation of lactic acid.



12. Comment on Fungal fermentation

Industrial fermentation with fungi is used to produce a number of commercial products. Fungal metabolism is exploited to manufacture ethanol, citric acid, steroids, antibiotics and other substances with applications in the food, fuel, chemical and pharmaceutical industries.

Fungi have been used for thousands of years to modify foods and beverages. Bread made without yeast fungi is flat. The addition of yeast to flat bread dough causes the dough to rise during baking. The result is the soft texture we associate with bread. Yeasts are used in different cultures to make other modified foods. Yogurt, beer, and wine were invented in Europe and the Middle East. Saki, soy sauce, miso, tempeh, ont-jom and similar products were invented in the Far East.

The distinctive flavors and textures of Camembert, Brie and blue cheeses are due to fungi.

13. Features of Upstream Process: (NOV/DEC)-2014, 2017

What do you mean by upstream & downstream process?

❖ **The upstream processing in biotechnology involves identifying and extracting the raw materials. This forms the initial process of fermentation.**

➤ **Upstream process**-it deals with the:

- Inoculum preparation which includes screening or microorganisms and selection of suitable strain and genetic modification of the organism if needed.
- Preparation of culture media having suitable growth parameters at laboratory scale
- Scale up of the entire process.
- Inoculation.



14. Features of Downstream Process:

Downstream process-

- ❖ When the products are subjected to a series of processes including separation and purification which are collectively known as **Downstream processing**.
- ❖ It is also known as product recovery.
- ❖ Materials –upstream-finished products

The downstream processing deals with:

- Solid-liquid separation
- Release of intracellular products
- Concentration
- Purification
- Formulation

St Paul's college of pharmacy 4

15. What are the stages in down stream processing? (NOV/DEC)-2015

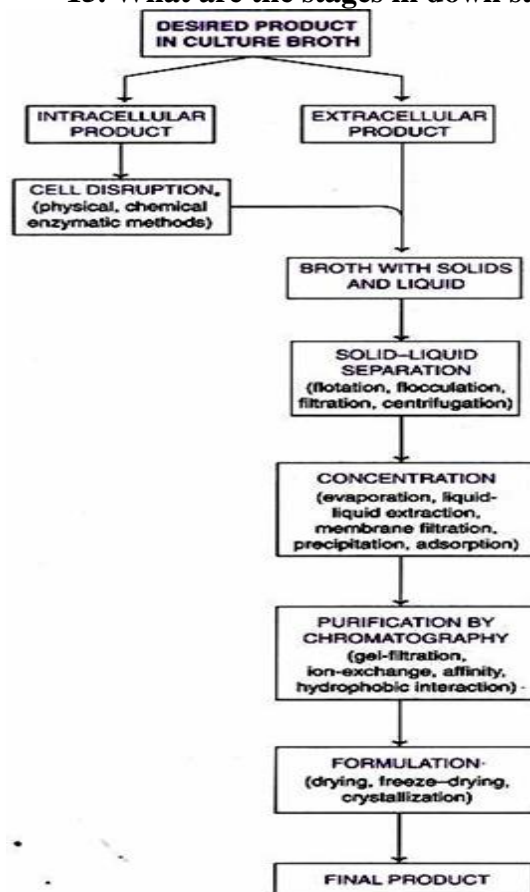


Fig. 20.1 : A summary of the major steps in downstream processing.

16. Write the advantages in using bacteria and fungi in the fermentation process?
(NOV/DEC)-2015

Mixed-culture fermentations offer a number of **advantages** over conventional single-culture fermentations:

- Product yield may be higher. Yogurt is made by the fermentation of milk with *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.
- The growth rate may be higher. In a mixed culture one microorganism may produce needed growth factors or essential growth compounds such as carbon or nitrogen sources beneficial to a second microorganism. It may alter the pH of the medium, thereby improving the activity of one or more enzymes. Even the temperature may be elevated and promote growth of a second microbe.
- Mixed cultures are able to bring about multistep transformations that would be impossible for a single microorganism. Examples are the *miso* and *shoyu* fermentations in which *Aspergillus oryzae* strains are used to make *koji*. Koji produces amylases and proteases, which break down the starch in rice and proteins in soybeans. In the *miso* and *shoyu* fermentations, these compounds are then acted on by lactic acid bacteria and yeast to produce flavor compounds and alcohol.

17. Write the disadvantages in using bacteria and fungi in the fermentation process?
(NOV/DEC)-2015

Mixed-culture fermentations also have some disadvantages.

- Scientific study of mixed cultures is difficult. Obviously, it is more difficult to study the fermentation if more than one microorganism is involved. That is why most biochemical studies are conducted as single-culture fermentations because one variable is eliminated.
- Defining the product and the microorganisms employed becomes more involved in patent and regulatory procedures.
- Contamination of the fermentation is more difficult to detect and control.
- When two or three pure cultures are mixed together, it requires more time and space to produce several sets of inocula rather than just one.
- One of the worst problems in mixed-culture fermentation is the control of the optimum balance among the microorganisms involved. This can, however, be overcome if the behavior of the microorganisms is understood and this information is applied to their control.

18. What is Flocculation?

In flocculation, the cells (or cell debris) form large aggregates to settle down for easy removal. The process of flocculation depends on the nature of cells and the ionic constituents of the medium. Addition of flocculating agents (inorganic salt, organic polyelectrolyte, mineral hydrocolloid) is often necessary to achieve appropriate flocculation.

19. Comment on Microbial Fermentation(NOV/DEC)-2014

Industrial fermentation is the intentional use of **fermentation** by **microorganisms** such as **bacteria** and **fungi** to make products useful to humans. The rate of fermentation depends on the concentration of microorganisms, cells, cellular components, and enzymes as well as temperature, pH and for **aerobic fermentation** oxygen. Product recovery frequently involves the concentration of the dilute solution. Nearly all commercially produced enzymes, such as **lipase**, **invertase** and **rennet**, are made by fermentation with **genetically modified microbes**.

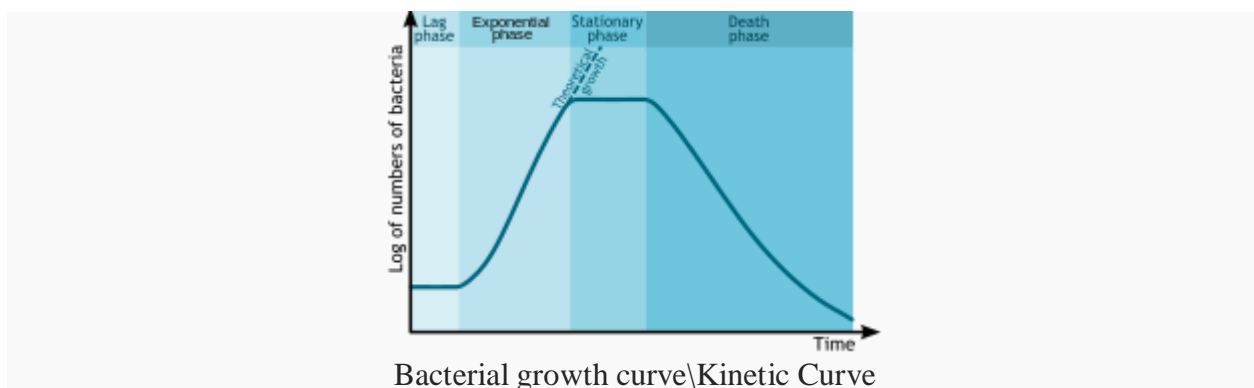
20. Comment on the four different types of fermentation

In general, fermentations can be divided into four types:

- Production of biomass (viable cellular material)
- Production of extracellular metabolites (chemical compounds)
- Production of intracellular components (enzymes and other proteins)
- Transformation of substrate (in which the transformed substrate is itself the product)

These types are not necessarily disjoint from each other, but provide a framework for understanding the differences in approach. The organisms used may be bacteria, yeasts, molds, algae, animal cells, or plant cells. Special considerations are required for the specific organisms used in the fermentation, such as the dissolved **oxygen** level, nutrient levels, and **temperature**.

21. Plot the different Phases of microbial growth and comment.



When a particular organism is introduced into a selected growth medium, the medium is inoculated with the particular organism. Growth of the inoculum does not occur immediately, but takes a little while. This is the period of adaptation, called the lag phase.

Following the lag phase, the rate of growth of the organism steadily increases, for a certain period—this period is the log or exponential phase.

After a certain time of exponential phase, the rate of growth slows down, due to the continuously falling concentrations of nutrients and/or a continuously increasing (accumulating) concentration of toxic substances.

This phase, where the increase of the rate of growth is checked, is the deceleration phase. After the deceleration phase, growth ceases and the culture enters a stationary phase or a steady state.

The biomass remains constant, except when certain accumulated chemicals in the culture lyse the cells (chemolysis). Unless other micro-organisms contaminate the culture, the chemical constitution remains unchanged.

If all of the nutrients in the medium are consumed, or if the concentration of toxins is too great, the cells may become **senescent** and begin to die off. The total amount of biomass may not decrease, but the number of viable organisms will decrease.

22. What is submerged fermentation and give example? (NOV/DEC)-2014

Submerged fermentation (SmF) utilizes free flowing liquid substrates, such as corn steep liquor, molasses and nutrient broths. The enzymes and bioactive compounds are secreted into the fermentation broth. The substrates are utilized quite rapidly and hence need to be constantly supplemented with nutrients. This fermentation technique is best suited for microorganisms such as bacteria that require high moisture.

At **Karyotica** we have developed cutting edge SmF platform for production various enzymes including protease, Asperginase and many other biocatalysts. All our recombinant range of enzymes are mainly produced through SmF process.

23. What is Bioprocess Engineering?

Bioprocess – A series of physiological reactions or operations carried out for the production of specific substances. Eg. fermentation. Bioprocess Engineering – the modification and regulation of bioprocess in order to produce a large amount of the desired product

24. What are the Main components of a fermentor?

Base components like the drive motor, heaters, pumps, gas control. → Vessels → & accessories. Peripheral equipments such as reagent bottles. → Instrumentation → & sensors.

25. Explain each Component of a fermentor & its Uses

The components of the fermentor combine to perform the following operations: Provide operation free from contamination. → Maintain specific temperature. → Provide adequate mixing → & aeration. Control the pH of the culture. → Allow monitoring → & or the control of dissolved oxygen. Facilitates the growth of wide range of organisms →

26. Give the major types of bioreactor Nov/Dec 2016

Based on the designs of the bioreactors, they can be grouped into the following types.

- a) Continuous stirred tank bioreactors
- b) Bubble Column Bioreactors
- c) Airlift Bioreactors
- d) Fluidized Bioreactors**
- e) Packed Bioreactors
- f) Photobioreactors

27. What are Molasses?

It is a byproduct of sugar industry and is one of the cheapest source of carbohydrates. Sugarcane molasses (sucrose around 48%) and sugar beet molasses (sucrose around 33%) are commonly used. Molasses also contains nitrogenous substances, vitamins and trace elements. Variation in the composition of the molasses also occurs which is mostly dependent on the climatic conditions and production process.

28. What is Whey?

It is a byproduct of dairy industry and is produced worldwide. Most of it is consumed by humans and animals. Whey is a reasonably good source of carbon for the production of alcohol, SCP, Vitamin B12, Lactic acid and gibberelic acid. Storage of Whey is a limiting factor for its widespread use in fermentation industry.

29. What is Batch Sterilization?

The culture media are subjected to sterilization at 121°C in batch volumes, in the bioreactor. Batch Sterilization can be done by injecting the steam into the medium (direct method) or injecting the steam into the interior coils (indirect method). For the direct batch sterilization, the steam should be pure and free from all chemical additives.

30. What is Continuous Sterilization?

This is carried out at 140°C for a very short period of time ranging from 30-120 secs. (This in contrast to the batch fermentation done at 121°C for 20-60mins). This is based on the principle that the time required for killing microorganisms is much shorter and at higher temperature. Continuous Sterilization is carried out by directly injecting the steam or by means of heat exchangers. The main advantage with Continuous Sterilization is that about 80-90% of the energy is conserved.

PART –B

1. Explain in detail the traditional and modern biotechnology outlook with suitable examples. (Nov/Dec 2015, **Nov/Dec 2016**).

Ans: Text Book U.Satyanarayana , Pg No- 3-5 and Research Article from Internet source.

2. Comment on Historical overview of Industrial Fermentation Process some few methods and their respective advantages and disadvantages. (May/Jun 2013, May/Jun 2011, April/May 2015).

Ans: Text Book U.Satyanarayana , Pg No- 4-5 and Research Article from internet source.

3. Write in detail about upstream and downstream processing for obtaining product of your interest through modern biotechnology. Present the above process in the form of flow chart/block diagram or pictorial representation.(May/Jun 2013, May/Jun 2011, April/May 2015, **Nov/Dec 2016, Nov/Dec 2017**).

Ans: Biotechnology by U.Satyanarayana Pg.No: UPS – 252-254 and DSP 270-271

4. Write in detail about downstream processing for obtaining product of your interest through modern biotechnology. Present the above process in the form of process flow chart/block diagram or pictorial representation.

(May/Jun 2013, May/Jun 2012, April/May 2015, Nov/Dec 2015, **Nov/Dec 2017**).

Ans: Biotechnology by U.Satyanarayana Pg.No: 270-280

5. What are the main utilities of Fermentor? Describe functions of main components of the fermentor.(May/Jun 2013, May/Jun 2011, April/May 2015).

Ans: Biotechnology by U.Satyanarayana Pg.No: 239-254: Text Book of Industrial Fermentation by Wulf crueger: Pg No: 64-107

6. What is the main role of microorganism in fermentation? Explain in detail the design and selection of a strain for the fermentation process. (May/Jun 2013, May/Jun 2011, April/May 2015).

Ans: Biotechnology by U.Satyanarayana Pg.No: 254-269: Text Book of Industrial Fermentation by Wulf crueger: Pg No:4-7, 9-20, 111-121

PART –C

1. Comment on Process flow sheeting –Elaborate the block diagrams, draw a pictorial representation for any one product of your interest. (Nov.Dec 2015, **Nov/Dec 2016**)

Answer: Pg No: 67-72, K.G Ramawat & Shailey Goyal, Comprehensive Biotechnology

2. Write a detailed note on the Biochemistry of Fermentation by the microbes. . (Nov.Dec 2015)

Answer: Pg No: 123-128, Dubey, R.C. Text Book of Biotechnology

3. Write short note on the different types of Batch fermentation & Fed Batch Fermentation
Nov.Dec 2015

Answer: Pg No: 675-679, Prescott & Dunn, Industrial Biotechnology

UNIT II PRODUCTION OF PRIMARY METABOLITES 9

Primary Metabolites- Production of commercially important primary metabolites like organic acids, amino acids, alcohols and vitamins.

PART - A

1. What are metabolites?

Metabolites – The cells have the ability to produce certain metabolic products when they are cultured in a specific nutrient medium. The metabolites are grouped into 2 categories: secondary & primary metabolites. The classification of which is mainly based upon the utility of the metabolites for the growth of the organism.

2. What are Primary essential metabolites? Nov/Dec 2014, 2015,2016

Primary metabolites are involved in growth, development, and reproduction of the organism. The primary metabolite is typically a key component in maintaining normal physiological processes; thus, it is often referred to as a central metabolite. Primary metabolites are typically formed during the growth phase as a result of energy metabolism, and are deemed essential for proper growth.

3. Comment on the examples of Primary metabolites Nov/Dec 2014, Nov/Dec 2017

It includes alcohols such as ethanol, lactic acid, and certain amino acids. Within the field of industrial microbiology, alcohol is one of the most common primary metabolites used for large-scale production. Specifically, alcohol is used for processes involving fermentation which produce products like beer and wine. Additionally, primary metabolites such as amino acids-- including L-glutamate and Llysine, which are commonly used as supplements-- are isolated via the mass production of a specific bacterial species, *Corynebacteria glutamicum*. Another example of a primary metabolite commonly used in industrial microbiology includes citric acid. Citric acid, produced by *Aspergillus niger*, is one of the most widely used ingredients in food production.

4. What is growth curve?

Growth curve – a graphic representation of the growth of the bacteria (or population changes) in a culture medium. Exponential phase – period of culture growth when cells divide steadily at a constant rate. Also called as log phase / logarithmic phase. Stationary phase – the interval directly following a growth phase when the number of viable bacteria remains constant.

5. Comment on the production of lactic acid.

Lactic acid and its production by lactic acid bacteria have a long history in the food industry and microbial processes for lactic acid production were established early in the past century. However, the large-scale commercial production of the purified acid by microorganisms is

relatively new. The production of the biodegradable plastic polylactide (used, for instance, in food containers) led to increased interest in optically pure lactic acid. This accounts for the recent shift from chemical to microbial production processes. The filamentous fungus *Rhizopus oryzae* is another natural producer that has the advantage of growing on mineral medium and carbon sources such as starch or xylose.

6. How is ethanol produced commercially?

Ethanol fermentation, also referred to as alcoholic fermentation, is a biological process in which sugars such as glucose, fructose, and sucrose are converted into cellular energy and thereby produce ethanol and carbon dioxide as metabolic waste products. Because yeasts perform this process in the absence of oxygen, ethanol fermentation is classified as anaerobic. Ethanol fermentation occurs in the production of alcoholic beverages and ethanol fuel, and in the rising of bread dough.

7. Write any two importance of production medium in antibiotic production by microorganisms. Nov/Dec 2014

Antibiotic production employs a variety of media, a different one for each stage of operation. A typical medium has about 10% (w/v) solids. Generally, yields are much higher on complex media. In some cases, a suitable precursor for the antibiotic is also provided as in the case of penicillin G production, where phenylacetic acid or phenoxyacetic acid is used as precursor. As antibiotics are secondary metabolites, the production medium is so designed that a key nutrient becomes limiting at a critical stage to initiate the secondary metabolism in the organism (e.g., glucose for penicillin production and phosphate for several antibiotics produced by *Streptomyces*).

8. Comment on Vitamin B₁₂

Vitamin B₁₂ (cyanocobalamin) is a water soluble vitamin with complex structure. The empirical formula of cyanocobalamin is C₆₃H₉₀N₁₄O₁₄PCO. The structure of vitamin B₁₂ consists of a corrin ring with a central cobalt atom. The corrin ring is almost similar to the tetrapyrrole ring structure found in other porphyrin compounds e.g. heme (with Fe) and chlorophyll (with Mg). The corrin ring has four pyrrole units. Cobalt present at the centre of the corrin ring is bonded to the four pyrrole nitrogen's. Cobalt also binds to dimethylbenzimidazole and amino isopropanol. Thus, cobalt atom present in vitamin B₁₂ is in a coordination state of six.

9. Write notes on genetically engineered strains for vitamin B₁₂ production:

By employing modern techniques of genetic engineering, vitamin B₁₂ production can be enhanced. A protoplast fusion technique between *Protaminobacter ruber* and *Rhodospseudomonas spheroides* resulted in a hybrid strain called *Rhodospseudomonas protamicus*. This new strain can produce as high as 135 mg/l of vitamin B₁₂ utilizing carbon source.

10. What are the factors affecting Beta carotene Production?

Trisporic acid which can act as a microbial sexual hormone improves production yield of β -carotene. β -ionones enhance p-carotene synthesis by increasing the activity of enzymes, and not by their direct incorporation into β -carotene. When the fermentation medium is supplemented with purified kerosene, β -carotene production is almost doubled. Kerosene increases the solubility of hydrophobic substrates.

11. Comment on the Microbial Production of Gibberellins

So far only one microorganism, the fungus namely *Gibberella fujikuroi* has been found to produce gibberellins. This is actually a pathogenic fungus of rice seedlings. Gibberellin production can be carried out by using a glucose-salt medium at pH 7.5 and temperature 25°C for 2-3 days. The fermentation process is conducted in aerated submerged process. After the growth of the fungus is maximum, the production of gibberellins commences.

12. Comment on the applications of Citric Acid

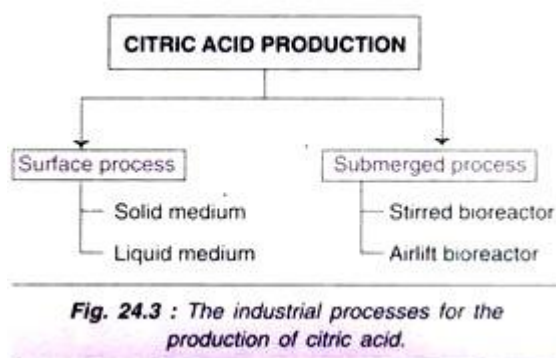
1. Citric acid, due to its pleasant taste and palatability, is used as a flavoring agent in foods and beverages e.g., jams, jellies, candies, desserts, frozen fruits, soft drinks, wine. Besides brightening the colour, citric acid acts as an antioxidant and preserves the flavors of foods.
2. It is used in the chemical industry as an antifoam agent, and for the treatment of textiles. In metal industry, pure metals are complexed with citrate and produced as metal citrates.
3. In pharmaceutical industry, as trisodium citrate, it is used as a blood preservative. Citric acid is also used for preservation of ointments and cosmetic preparations. As iron citrate, it serves as a good source of iron.
4. Citric acid can be utilized as an agent for stabilization of fats, oils or ascorbic acid. It forms a complex with metal ions (iron, copper) and prevents metal catalysed reactions. Citric acid is also used as a stabilizer of emulsions in the preparation of cheese.
5. In detergent/cleaning industry, citric acid has slowly replaced polyphosphates.

13. Write note on Microbial Strains for Citric Acid Production

Many microorganisms can produce citric acid. The fungus *Aspergillus Niger* is most commonly used for industrial production of citric acid. The other organisms (although less important) include *A. clavatus*, *A. wentii*, *Penicillium luteum*, *Candida catenula*, *C. guilliermondii* and *Corynebacterium* sp. For improved industrial production of citric acid, mutant strains of *A. Niger* have been developed. The strains that can tolerate high sugar concentration and low pH with reduced synthesis of undesirable byproducts (oxalic acid, isocitric acid and gluconic acid) are industrially important.

14. Comment on the Production Processes for Citric Acid:

There are two processes by which citric acid can be industrially produced — the surface process and submerged process (Fig. 24.3).



15. Write a note on the Production of Citric Acid from Alkanes:

Both yeasts and bacteria can be used for citric acid production from n-alkanes (C₉-C₂₃ hydrocarbons). The citric acid yield is better from hydrocarbons compared to sugars i.e. 145% of citric acid from paraffin. The most commonly used organism is *Candida lipolytica*. The fermentation can be carried out in batch, semi-continuous or continuous modes. The pH should be kept above 5. The major limitations of citric acid production from alkanes are—very low solubility of alkanes and increased production of unwanted isocitric acid.

16. Write note on applications of Gluconic acids

1. Gluconic acid is used in the manufacture of metals, stainless steel and leather, as it can remove the calcareous and rust deposits.
2. It is used as an additive to foods and beverages.
3. Gluconic acid has pharmaceutical applications — calcium and iron therapy.
4. Sodium gluconate is used as a sequestering agent in many detergents.
5. Gluconate is used for desizing polyester or polyamide fabrics.
6. It is utilized in the manufacture of highly resistant (to frost and cracking) concrete.

17. Comment on Microorganisms used for producing Acetic acid

The commercial production of acetic acid is carried out by a special group of acetic acid bacteria, which are divided into two genera. *Gluconobacter* that oxidizes ethanol exclusively to acetic acid. *Acetobacter* that oxidizes ethanol first to acetic acid, and then to CO₂ and H₂O. These over-oxidizers are Gram-negative and acid tolerant e.g. *A. aceti*, *A. peroxidans*, *A. pasteurianus*.

18. Comment on L-Ascorbic Acid and its applications (NOV/DEC)-2015

L-Ascorbic acid is the commonly used chemical name for the water soluble vitamin C. This vitamin forms a redox system and participates in several biological processes. It is intimately involved in the biosynthesis of collagen, the most abundant protein in the human body. Vitamin C also protects the body against carcinogenic nitrosamines and free radicals. The deficiency of ascorbic acid causes scurvy.

Applications of Ascorbic Acid:

Because of the wide range of physiological and beneficial functions of ascorbic acid, its commercial production assumes significance. Vitamin C is mainly used in food and pharmaceutical industries.

19. Write note on two-step fermentation process of L-Ascorbic acid (NOV/DEC)-2015

In this, D-glucose is converted to 2, 5-diketogluconic acid by *Erwinia*, *Acetobacter* or *Gluconobacter* sp. In the second step, *Corynebacterium* sp converts 2, 5-diketogluconic acid to 2-keto-L-gluconic acid, (Fig. 24.10A). It is also possible to involve *Bacillus megaterium* for converting L-sorbose to 2-keto-L- gluconic acids. The latter, by chemical reactions, can be converted to ascorbic acid.

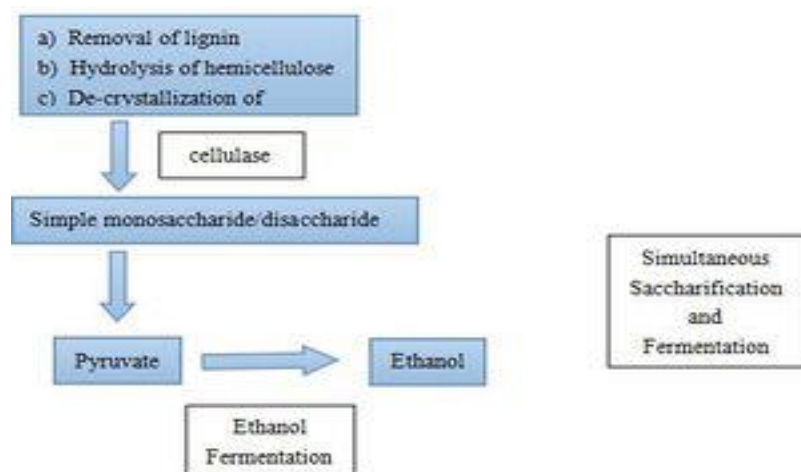
20. Write note on production process of Acetic acid

For every molecule of ethanol oxidised, one molecule of acetic acid is produced. Thus, high-yielding strains can produce 11-12% acetic acid from 12% alcohol. For optimal production, adequate supply of oxygen is very essential. Insufficient O₂, coupled with high concentration of alcohol and acetic acid result in the death of microorganisms. Surface fermentation or submerged fermentation processes can be carried out to produce acetic acid. Trickling generation process, a type of surface fermentation, is very commonly used.

21. Comment on *Saccharomyces cerevisiae* and ethanol production

Saccharomyces cerevisiae is an unicellular yeast, capable of utilizing glucose but not xylose as energy source because it lacks xylose reductase(XR) and xylitol dehydrogenase(XDH). Since xylose is derived from lignocellulose, not being able to ferment xylose is energetically and economically inefficient.

22. Comment on microbial biofuel production via ethanol fermentation



23. Comment on Beer production

Beer is the most consumed alcoholic beverage in the world. It is made most often of malted barley and malted wheat. Sometimes a mixture of starch sources can be used, such as rice. Unmalted maize can be added to the barley or wheat to lower cost. Potatoes, millet and other foods high in starch are used in different places in the world as the primary carbohydrate source.

24. Write four carbon sources used for production of aminoacids by fermentation. Nov/Dec 2017

Amino acids can be produced by microorganisms by utilizing several carbon sources e.g. **glucose**, **fructose**, alkanes, **ethanol**, glycerol, propionate. Certain industrial byproducts like molasses and starch hydrolysate can also be used.

25. Write notes on Wine production

Wine is made from grapes or other fruit. The grapes are first cleaned of leaves and stems and the fruit is crushed into must that is ready for fermentation. The yeasts used for the fermentation grow a film on the fruit or in the environment. These wild strains play an important role in the final properties of the drink. However, cultivated strains of *Saccharomyces cerevisiae* are often added to improve the consistency of the final product. There are hundreds of commercially available yeast strains for wine fermentation.

26. What are some of the uses of Ethanol? Nov/Dec 2016

Drinks: The "alcohol" in alcoholic drinks is simply ethanol.

Industrial methylated spirits (meths): Ethanol is usually sold as industrial methylated spirits which is ethanol with a small quantity of methanol added and possibly some colour.

As a fuel: Ethanol burns to give carbon dioxide and water and can be used as a fuel in its own right, or in mixtures with petrol (gasoline). "Gasohol" is a petrol / ethanol mixture containing about 10 - 20% ethanol.

As a solvent: Ethanol is widely used as a solvent. It is relatively safe, and can be used to dissolve many organic compounds which are insoluble in water. It is used, for example, in many perfumes and cosmetics.

27. Write the three approaches of AA large scale production

For the large- scale production of amino acids, microbiological methods are employed. There are three different approaches.

1. Direct fermentation methods:
2. Conversion of metabolic intermediates into amino acids:
3. Direct use of microbial enzymes or immobilized cells:

28. What is meant by Auxotrophic Mutation?

These mutants are characterized by a lack of the formation of regulatory end product (i.e. repressor or regulatory effector). The intermediates of the metabolic pathways accumulate and get excreted.

29. Write the major molecular ways of strain development for AA

The following are the major ways of strain development. In fact, several methods are combined to successfully develop a new strain for producing amino acids.

Auxotrophic mutation

Genetic recombination

Recombinant DNA technology

30. Write note on genetically engineered strains for vitamin B₁₂ production

By employing modern techniques of genetic engineering, vitamin B₁₂ production can be enhanced. A protoplast fusion technique between *Protaminobacter ruber* and *Rhodopseudomonas spheroides* resulted in a hybrid strain called *Rhodopseudomonas protamicus*. This new strain can produce as high as 135 mg/l of vitamin B₁₂ utilizing carbon source.

PART B

1. Comment on the commercial importance or uses of amino acids (**Nov/Dec 2016**).
2. Explain primary metabolite production and the steps involved in the production process of any one or two amino acids. Comment on the commercial uses of amino acids (May/Jun 2013, May/Jun 2011, April/May 2015,).

Ans: Biotechnology by U.Satyanarayana Pg.No:344-361: Text Book of Industrial Fermentation by Wulf crueger Pg.No: 150-169

3. Elaborate the steps involved in the production process of any one commercially important alcohol and any one vitamin.(May/Jun 2013, May/Jun 2011, April/May 2015).
Ans: Biotechnology by U.Satyanarayana Pg.No:311-316, 355-361: Text Book of Industrial Fermentation by Wulf crueger Pg.No:124-131, 219-226.

4. How will you increase the production of riboflavin? Explain with neat process Flow sheet. (May/Jun 2013, May/Jun 2011, April/May 2015).
Ans: Biotechnology by U.Satyanarayana Pg.No:357-358.

5. Elaborate the steps involved in the production process of any one commercially important alcohol. Ans: Biotechnology by U.Satyanarayana Pg.No:357-358311-312.

6. Elaborate the steps involved in the production process of any one commercially important Acetic Acid (**Nov/Dec 2016**)

Ans: Biotechnology by U.Satyanarayana Pg.No:344-354: Text Book of Industrial Fermentation by Wulf crueger Pg.No:15-169

PART C

1. Explain the commercial importance and production process of any one organic acid (citric acid by submerged fermentation). (May/Jun 2013, May/Jun 2011, April/May 2015, **Nov/Dec 2016, Nov/Dec 2017**).

Ans: Biotechnology by U.Satyanarayana Pg.No: 318-324: Text Book of Industrial Fermentation by Wulf crueger Pg.No: 134-148

2. Write a brief account on the Primary Metabolites and Primary Essential Metabolites and draw a graph explaining the different phases of growth

Ans: Biotechnology by U.Satyanarayana Pg.No:

3. Write a detailed overview of Microbial Overproduction of Vitamins and the strain improvement process **Nov/Dec 2017**

Ans: Biotechnology by U.Satyanarayana Pg.No

UNIT III PRODUCTION OF SECONDARY METABOLITES 9

Secondary Metabolites- Production processes for various classes of secondary metabolites: Antibiotics and Steroids.

PART A

1. Write notes on Antibiotics and the screening Process

Useful antibiotics are often discovered using a screening process. To conduct such a screen, isolates of many different microorganisms are cultured and then tested for production of diffusible products that inhibit the growth of test organisms. Most antibiotics identified in such a screen are already known and must therefore be disregarded. The remainder must be tested for their selective toxicities and therapeutic activities, and the best candidates can be examined and possibly modified.

A more modern version of this approach is a rational design program. This involves screening directed towards finding new natural products that inhibit a specific target, such as an enzyme only found in the target pathogen, rather than tests to show general inhibition of a culture.

2. Define Secondary Metabolites & its Characteristics Nov/Dec 2014, 15,16

Secondary metabolites are typically organic compounds produced through the modification of primary metabolite synthases. Secondary metabolites do not play a role in growth, development, and reproduction like primary metabolites do, and are typically formed during the end or near the stationary phase of growth. Examples of secondary metabolites with importance in industrial microbiology include atropine and antibiotics such as erythromycin and bacitracin.

3. Write any two importance of production medium in antibiotic production by microorganisms. Nov/Dec 2014,15

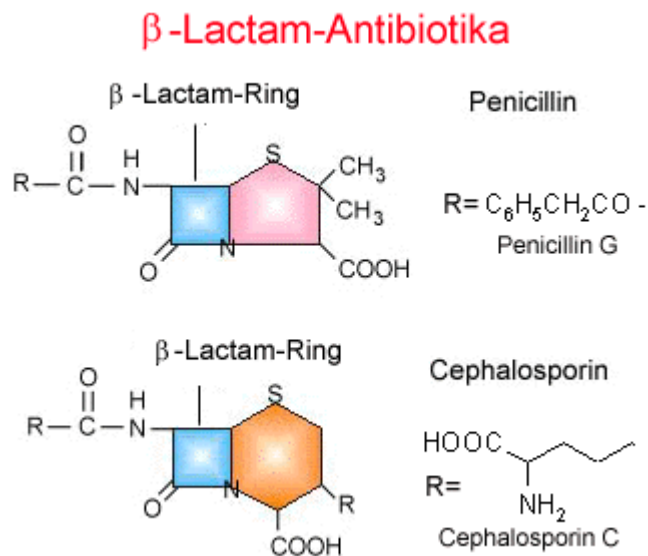
Antibiotic production employs a variety of media, a different one for each stage of operation. A typical medium has about 10% (w/v) solids. Generally, yields are much higher on complex media. In some cases, a suitable precursor for the antibiotic is also provided as in the case of penicillin G production, where phenylacetic acid or phenoxyacetic acid is used as precursor. As antibiotics are secondary metabolites, the production medium is so designed that a key nutrient becomes limiting at a critical stage to initiate the secondary metabolism in the organism (e.g., glucose for penicillin production and phosphate for several antibiotics produced by *Streptomyces*).

4. Write the importance of precursors in secondary metabolite production.

Inducer induces the production of a secondary metabolite, for eg., the presence of starch induce the production of Amylase enzyme, because during lag phase the limiting reactant

like glucose level will be low, it is necessary for bacteria to survive so it will release amylase enzyme to break starch and yield glucose from that, thus the presence of Starch only induce the amylase enzyme which is the secondary metabolite.. Similarly precursors are used for the production of a particular metabolite, eg., for Penicillin G production phenylethylamine is needed , which only incorporated into the penicillin to yield Penicillin G, corn steep liquor contains phenylethylamine which is acting as a precursor for Penicillin G production.

5. Draw the structures of β -lactam ring. Nov/Dec 2014,15



6. Are enzymes secondary metabolites? Give two examples of polysaccharide degrading enzymes.

Enzymes belong to primary metabolite because they are directly involved in normal growth, development and reproduction. In other words, an organism would die without enzymes.

Two polysaccharide degrading enzymes include alginate lyase and cellulase.

7. Differentiate between sterols and steroids. Nov/dec 2014,15

Sterols are an important class of organic molecules. They occur naturally in plants, animals and fungi, with the most familiar type of animal sterol being cholesterol. Cholesterol is vital to cellular function, and a precursor to fat-soluble vitamins and steroid hormones.

A steroid is a terpenoid lipid characterized by its sterane core and additional functional groups. The core is a carbon structure of four fused rings: three cyclohexane rings and one cyclopentane ring. The steroids vary by the functional groups attached to these rings and the oxidation state of the rings.

8. What are aminoglycoside antibiotics? Nov/Dec 2014, 2015

Aminoglycoside antibiotics – a class of antibiotics, which disrupt the normal synthetic sequence of protein synthesis. Aminoglycosides have several potential antibiotic mechanisms, some as protein synthesis inhibitors, although their exact mechanism of action is not fully known:

They interfere with the proofreading process, causing increased rate of error in synthesis with premature termination. Also, there is evidence of inhibition of ribosomal translocation where the peptidyltRNA moves from the A-site to the P-site. They can also disrupt the integrity of bacterial cell membrane.

9. What are the different types of penicillin?

There are 4 classes of penicillins, based upon their ability to kill various types of bacteria.

From narrow to broad range of effectiveness they include:

Natural Penicillins (Penicillin G, Procaine, Penicillin G, Penicillin V, • Benzathine).

Penicillinase-Resistant Penicillins (Cloxacillin, Dicloxacillin, Methicillin, • Nafcillin, Oxacillin).

Aminopenicillins (Ampicillin, Amoxicillin, Bacampicillin).

The • aminopenicillins were the first penicillins discovered to be active against gram-negative bacteria (such as E. coli and H. influenzae).

Extended Spectrum Penicillins (sometimes called anti-pseudomonal • penicillins).

10. Give the uses of Antibiotics. Nov/Dec 2014, 2015

Cell wall synthesis inhibitors usually stop bacteria from forming their cell walls. They kill bacteria and not human cells because human cells do not form cell walls. Examples of cell wall synthesis inhibitors are beta lactams, semisynthetic penicillins, and bacitracin.

Cell membrane inhibitors kill bacterial cells by disorganizing the outer membranes of bacteria. An example of a cell membrane inhibitor is polymyxin.

Protein synthesis inhibitors interfere with the process of translation in protein synthesis. Their action is usually on the ribosomes. Examples of protein synthesis inhibitors are tetracyclines, chloramphenicol, macrolides, and aminoglycosides.

Chemotherapeutic agents affecting the synthesis of nucleic acids block the division and growth of cells by inhibiting synthesis of DNA and RNA. Most of these agents affect both animal and bacteria cells, so they cannot be used as an antibiotic. However, nalidixic acid and rifamycins are selectively active towards bacteria.

Competitive inhibitors are mostly synthetic. These drugs work by disrupting the metabolic rate of bacteria. Some examples include sulfonamides, isoniazid, para aminosalicylic acid, and ethambutol

11. Comment on Biotransformation in steroids Nov/Dec 2014, 2015, Nov/Dec 2017

Biotransformation (regiospecific and stereospecific bioconversion) is a biological process whereby an organic compound is modified into reversible product. It involves simple, chemically defined reactions catalyzed by enzymes present in the cell.

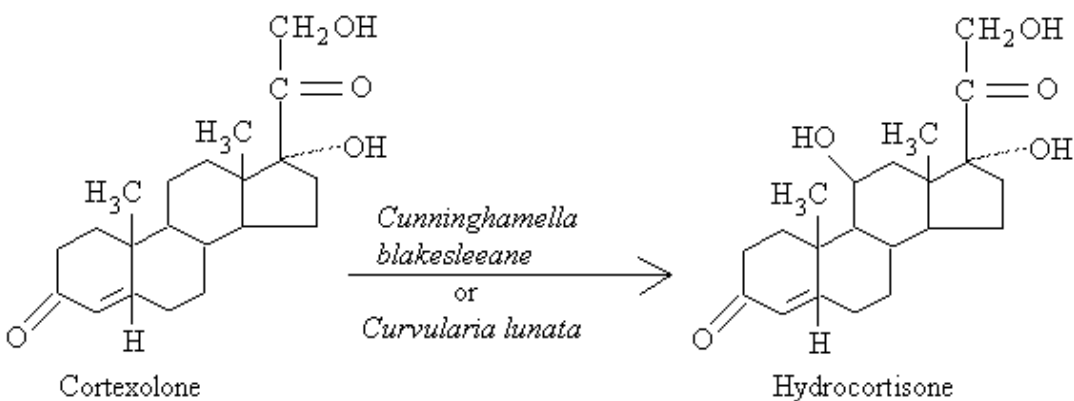
Microbial transformation

- When the transformation of the organic compounds is carried out by microorganism then the process is called as microbial transformation.

12. Write note on Hydroxylation Nov/Dec 2014, 2015

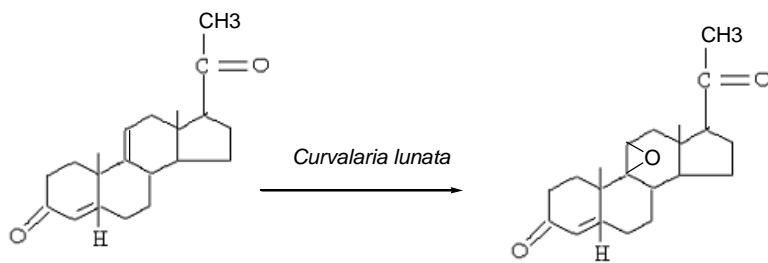
- Hydroxylation involves the substitution of hydroxyl group directly for the hydrogen at the position, be it α or β , in the steroid with a retention of configuration. The oxygen atom in the hydroxyl group is derived from molecular oxygen (gaseous), not from water, and the hydroxyl group thus formed always retains the stereochemical configuration of the hydrogen atom that has been replaced.

Example: Certain microorganisms can introduce hydroxyl groups at any of several of the carbon atoms of the steroid molecule.



13. Write note on Epoxydation

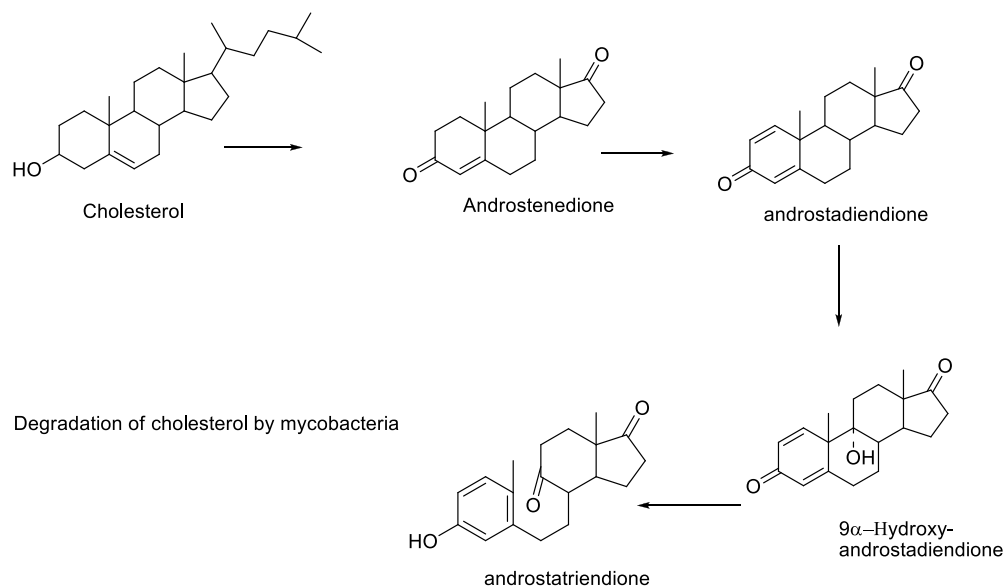
The epoxidation of steroidal double bonds is a rare example of biological epoxidation. The 9,11-epoxidation of 9(11)-dehydro-compounds, and the 14, 15-epoxidation of 14(15)-dehydrocompounds, using *Curvularia lunata*.



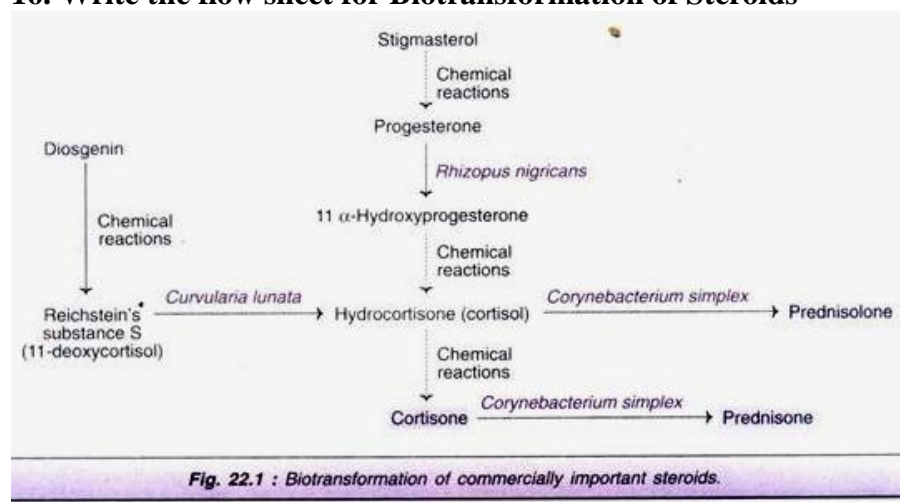
14. Comment on Fermentation condition of steroids

M/O	Steroid substrate	Steroid product	Length of incubation , temperature , aeration
<i>Alcaligenes faecalis</i>	Cholic acid	Ketocholic acids (90-100%)	2 days (monoketo acid) 4 days (diketo acid) 6 days (triketo acid) 37-39° ,surface culture
<i>Fusarium solani</i>	Progesterone	1,4- androstadiene-3, 17-dione(85%)	4 days , 25° C , rotary shaker (100 rpm)
<i>Corynebacterium mediolanum</i>	21-acetoxy -3 β - hydroxy -5-pregnen-20-one	21-hydroxy-4-pregnene-3, 20-dione (30%)	6 days , 36-37° C , pure oxygen with agitation

15. Comment on steroid degradation



16. Write the flow sheet for Biotransformation of Steroids



17. Comment on the Industrial production of Antibiotics

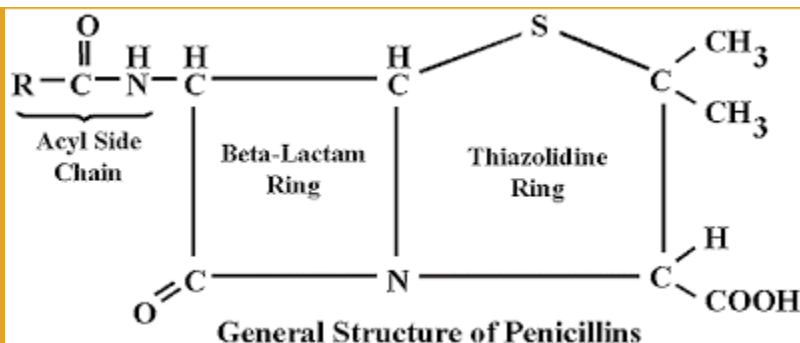
Antibiotics are produced industrially by a process of fermentation, where the source microorganism is grown in large containers (100,000–150,000 liters or more) containing a liquid growth medium. Oxygen concentration, temperature, pH and nutrient levels must be optimal, and are closely monitored and adjusted if necessary. As antibiotics are secondary metabolites, the population size must be controlled very carefully to ensure that maximum yield is obtained before the cells die. Once the process is complete, the antibiotic must be extracted and purified to a crystalline product. This is easier to achieve if the antibiotic is soluble in organic solvent. Otherwise it must first be removed by ion exchange, adsorption or chemical precipitation.

18. Write note on strains used for microbial production

Microorganisms used in fermentation are rarely identical to the wild type. This is because species are often genetically modified to yield the maximum amounts of antibiotics. Mutation is often used, and is encouraged by introducing mutagens such as ultraviolet radiation, x-rays or certain chemicals. Selection and further reproduction of the higher yielding strains over many generations can raise yields by 20-fold or more. Another technique used to increase yields is gene amplification, where copies of genes coding for enzymes involved in the antibiotic production can be inserted back into a cell, via vectors such as plasmids. This process must be closely linked with retesting of antibiotic production.

19. Note on the structure of Penicillin

Penicillin was the first naturally occurring antibiotic discovered. It is obtained in a number of forms from *Penicillium* moulds. Penicillin is not a single compound but a group of closely related compounds, all with the same basic ring-like structure (a β -lactam) derived from two amino acids (valine and cysteine) via a tripeptide intermediate. The third amino acid of this tripeptide is replaced by an acyl group (R) and the nature of this acyl group produces specific properties on different types of penicillin.



20. What are the types of Penicillin?

There are two different types of penicillin.

Biosynthetic penicillin is natural penicillin that is harvested from the mould itself through fermentation.

Semi-synthetic penicillin includes semi synthetic derivatives of penicillin - like Ampicillin, Penicillin V, Carbenicillin, Oxacillin, Methicillin, etc.

These compounds consist of the basic Penicillin structure, but have been purposefully modified chemically by removing the acyl group to leave 6-aminopenicillanic acid and then adding acyl groups that produce new properties.

These modern semi-synthetic penicillins have various specific properties such as resistance to stomach acids so that they can be taken orally, a degree of resistance to penicillinase (or β -lactamase) (a penicillin-destroying enzyme produced by some bacteria) and an extended range of activity against some Gram-negative bacteria. Penicillin G is the most widely used form and the same one we get in a hypodermic form.

21. Penicillin Upstream process-Comment

PENICILLIN:

1. Up stream process

❖ Inoculum development: micro organism-**P.chrysogenum**

Strain development: **P.chrysogenum** NRRL 1951

planting & selection

NRRL 1951 B.25

x-ray, uv, mutagen

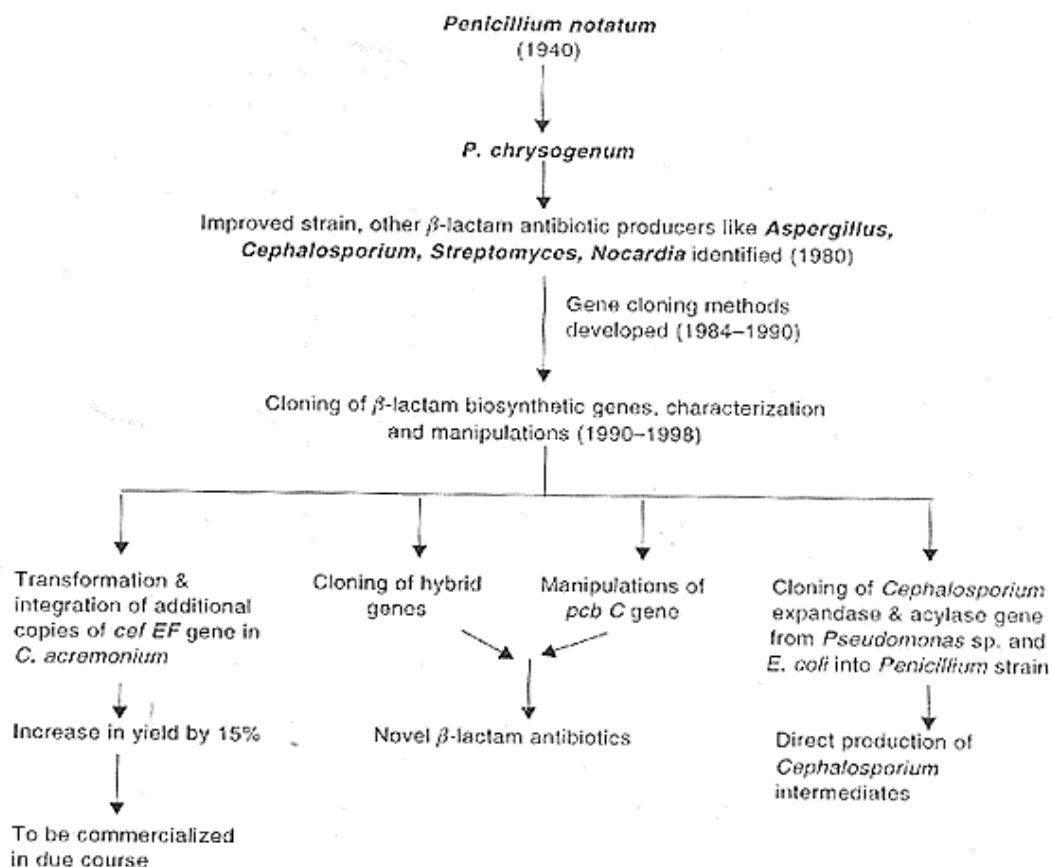
commercial strain



22. List any four common antibiotics produced by industrial fermentation Nov/Dec 2017

<i>Antibiotic</i>	<i>Producing microorganism</i>
Cephalosporin	<i>Cephalosporium acremonium</i>
Chloramphenicol	<i>Streptomyces venezuelae</i>
Erythromycin	<i>Streptomyces erythreus</i>
Griseofulvin	<i>Penicillium griseofulvin</i>
Penicillin	<i>Penicillium chrysogenum</i>
Streptomycin	<i>Streptomyces griseus</i>
Tetracycline	<i>Streptomyces aureofaciens</i>
Gentamicin	<i>Micromonospora purpurea</i>

23. Manipulation of Genes for *Penicillium Notatum* Nov /Dec 2013



24. Comment on SSf for Cephalosporin

Solid state fermentation systems were developed for the production of cephalosporins with *Streptomyces clavuligerus* and *Cephalosporium acremonium*. *S. clavuligerus* NRRL 3585 was grown on moistened barley under optimum solid state fermentation conditions for 7 days;

approximately 300 micrograms cephalosporins per g substrate were extracted from the kernels. *C. acremonium* C-10 produced approximately 950 micrograms cephalosporin C per g substrate after 10 days of solid state fermentation.

25. Comment on production of Cephalosporin by *Acremonium* Nov /Dec 2013

Production of cephalosporin C employing *Acremoniumchrysogenum* ATCC 48272 under solid state fermentation was optimized. Different substrates like wheat bran, wheat rawa, bombay rawa, barley and rice bran were studied to optimize the best substrate. Wheat rawa showed the highest antibiotic yield. Physical and chemical parameters were optimized. The maximum productivity of cephalosporin C (22 281 µg/g) was achieved by employing wheat rawa and with optimized process parameters including 1% w/w soluble starch and 1% w/w yeast extract as additives, incubation period of 5 days, incubation temperature at 30 °C, 1.5:10 (v/w) ratio of salt solution to weight of wheat bran, inoculum level 10% v/w, moisture content of solid substrate 80% and pH 6.5.

26. Give the main classes of antibiotics? Nov /Dec 2016

The main classes of antibiotics are:

- (a) Beta-Lactams. Penicillins. Cephalosporins.
- (b) Macrolides.
- (c) Fluoroquinolones.
- (d) Tetracyclines.
- (e) Aminoglycosides.

27. Are beta lactams bacteriostatic or bactericidal?

Bactericidal antibiotics kill bacteria; bacteriostatic antibiotics slow their growth or reproduction. Antibiotics that inhibit **cell wall synthesis**: the Beta-lactam antibiotics (**penicillin derivatives (penams), cephalosporins (cephems), monobactams, & carbapenems**) and **vancomycin**.

28. Write note on new steroid production.

New steroid fermentation processes that produce a variety of intermediates from sterols such as cholesterol and phytosterols have recently been developed. Especially, two fermentation processes for producing intermediates ADD and 4 AD, respectively, have been put into practice for the production of sex hormones and a diuretic drug, spironolactone.

29. Comment on Steroid Uses

Steroids of plant origin can be transformed into steroids like those of animal origin. This transformation is done by using the fungus *Rhizopus stolonifer* through the process of hydroxylation and dehydrogenation. Such steroids can then be used for man and other animals.

Such steroids are used

1. As anti-inflammatory drugs.
2. As anti-cancer drugs.
3. For developing immunity against asthma.
4. For organ transplantation.
5. For family planning.

30. Comment on the types of reaction in the Biotransformation of Steroids

The microbial transformation of steroids broadly involves oxidation (introduction of hydroxyl groups, splitting of side chains, production of epoxides etc.) reduction (conversion of aldehydes or ketones to alcohols, hydration of double bonds), hydrolysis and ester formation.

PART B

1. Explain the upstream and downstream processing of penicillin (Antibiotics) with the help of a flow sheet. (May/Jun 2013, May/Jun 2011, April/May 2015, **Nov/Dec 2016**).

Ans: Biotechnology by U.Satyanarayana Pg.No:332-334

2. Explain the upstream and downstream processing of streptomycin with the help of a flow sheet.

Ans: Biotechnology by U.Satyanarayana Pg.No:336-337

3. Write a detailed flow sheet with significance in the commercial production process of any one or two steroids from plant sources (**Nov/Dec 2016**).

Ans: Biotechnology by U.Satyanarayana Pg.No:306-310; Text Book of Industrial Fermentation by Wulf crueger Pg.No: 286-301

4. Write note on β -lactam antibiotics. Describe the fermentation of β -lactam antibiotics along with the bioparameters to be controlled.

Ans: Biotechnology by U.Satyanarayana Pg.No:329-335

5. Describe Secondary metabolites & its classes and compare the same with primary metabolites. **Nov/Dec 2017**

Ans: Biotechnology by U.Satyanarayana Pg.No:255-257; All classes of Sec.Metabolites mentioned above.

6. Describe genetic manipulations of streptomyces along with good antibiotic manufacturing practices?

Ans: Biotechnology by U.Satyanarayana Pg.No:342-343

PART C

1. Discuss about the various secondary metabolites which are commercially produced for human use and also compare the primary and secondary metabolites. **Nov/Dec 2017**

Ans: Biotechnology by U.Satyanarayana Pg.No:255-257;

2. Explain the Antibiotics production process with an example and discuss its advantages .
Nov/Dec 2017

Ans: Biotechnology by U.Satyanarayana Pg.No:336-337

3. Describe the importance of the production of biotransformed Steroid production.

Ans: Biotechnology by U.Satyanarayana Pg.No:306-310; Text Book of Industrial Fermentation by Wulf crueger Pg.No: 286-301

UNIT IV PRODUCTION OF ENZYMES AND OTHER BIOPRODUCTS 9

Production of Industrial Enzymes, Biopesticides, Biofertilizers, Biopreservatives, Biopolymers, Biodiesel, Cheese, Beer, SCP & Mushroom culture. Bioremediation.

PART A

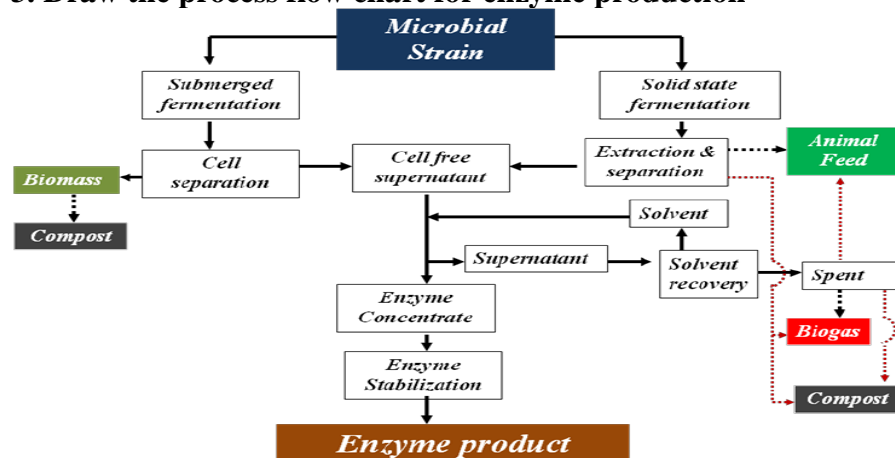
1. Comment on the methods of Fermentation by Enzymes

Mainly, there are two methods of fermentation which are used to produce enzymes. First is submerged fermentation and second is solid-state fermentation. In Submerged fermentation, the production of enzymes is done by microorganisms in a liquid nutrient media. Whereas in Solid-fermentation is carried out by cultivation of microorganisms and production of enzyme is done on a solid substrate. Compounds containing carbon in or on the substrate are busted down by the micro organisms thus producing the enzymes either extracellular or intracellular. The enzymes are isolated by various methods such as centrifugation, and for extracellular produced enzymes and lysing of cells for intracellular enzymes.

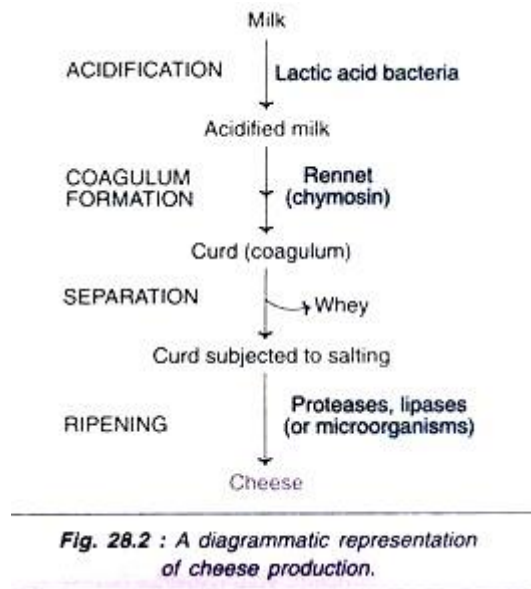
2. What are the substrates used for enzyme production

Agro-industrial residues are generally considered the best substrates for the SSF processes, and use of SSF for the production of enzymes is no exception to that. A number of such substrates have been employed for the cultivation of microorganisms to produce host of enzymes. Some of the substrates that have been used included sugar cane bagasse, wheat bran, rice bran, maize bran, gram bran, wheat straw, rice straw, rice husk, soyhull, sago hampas, grapevine trimmings dust, saw dust, corncobs, coconut coir pith, banana waste, tea waste, cassava waste, palm oil mill waste, aspen pulp, sugar beet pulp, sweet sorghum pulp, apple pomace, peanut meal, rapeseed cake, coconut oil cake, mustard oil cake, cassava flour, wheat flour, corn flour, steamed rice, steam pre-treated willow, starch, etc. Wheat bran however holds the key, and has most commonly been used, in various processes.

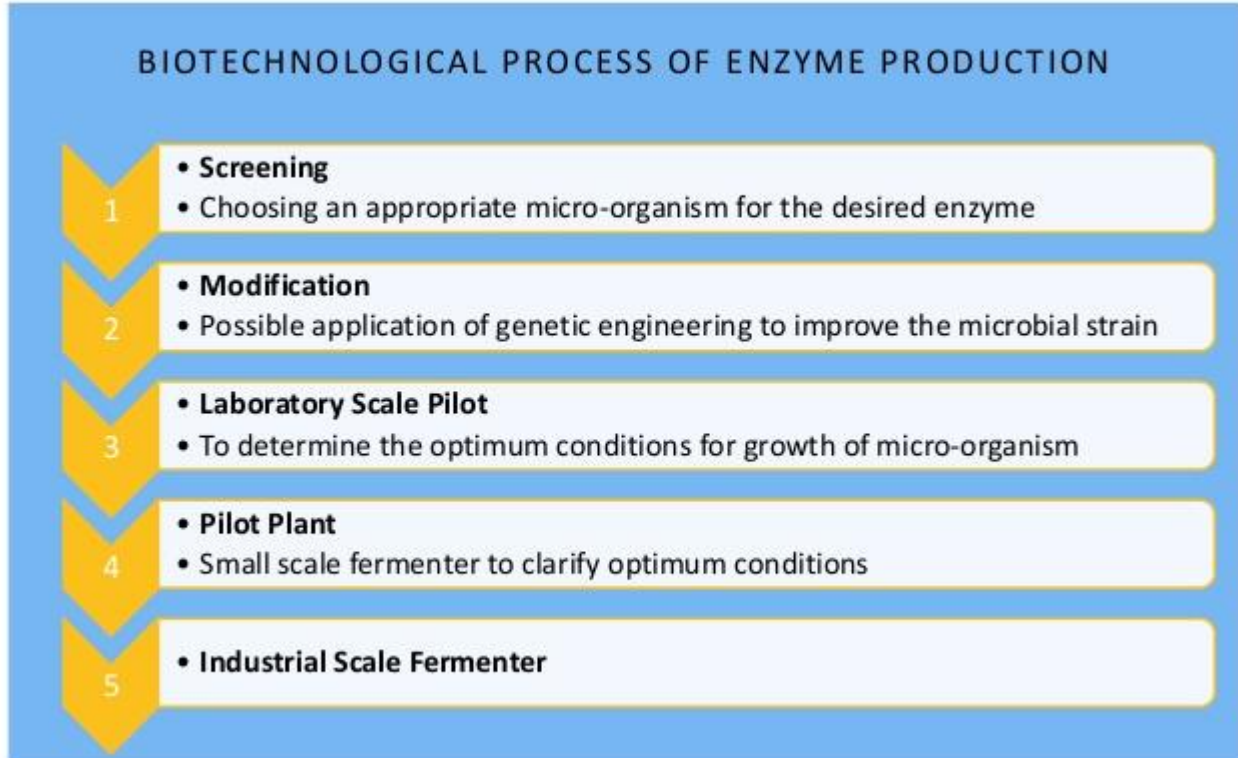
3. Draw the process flow chart for enzyme production



4. Write the flow chart for Cheese production Nov /Dec 2014



5. Comment on process of Enzyme Production



6. Write note on Enzyme formulation?

ENZYME FORMULATION

Enzymes are sold as stabilized liquid concentrates or as particulate solids

WHY FORMULATION?

Primary task of formulation is to minimize losses in enzymatic activity during transport, storage and use. Secondary purposes include, prevention of microbial contamination, avoidance of precipitation or haze formation, minimizing formation of sensitizing dust or aerosols and improving color and odor

HOW IT WORKS?

By preventing denaturation, catalytic-site deactivation and proteolysis i.e. PREVENT UNFOLDING by altering the protein's environment so as to induce a compact protein structure. There are several ways to accomplish this.

Careful selection of packaging materials. One should use tight bottles and stoppers to prevent access to moisture and should not release any traces of heavy metals or other enzyme-inactivating substances into the enzyme solution or suspension. In some cases, enzymes must be protected from light and packaged in brown glass bottles.

PACKAGING

7. Comment on Biopesticide categories

Biopesticides fall into three major classes: **Microbial** pesticides which consist of bacteria, **entomopathogenic fungi** or viruses (and sometimes includes the metabolites that bacteria or fungi produce). Entomopathogenic **nematodes** are also often classed as microbial pesticides, even though they are multi-cellular. Biochemical pesticides or herbal pesticides are naturally occurring substances that control (or monitor in the case of **pheromones**) pests and microbial diseases. Plant-incorporated protectants (PIPs) have genetic material from other species incorporated into their genetic material (*i.e.* **GM crops**). Their use is controversial, especially in many European countries. RNAi pesticides, some of which are topical and some of which are absorbed by the crop.

Reason for Biopesticides: Biopesticides have usually no known function in photosynthesis, growth or other basic aspects of plant physiology; however, their biological activity against insect pests, **nematodes**, fungi and other organisms is well documented. Every plant species has developed a built-in unique chemical complex structure that **protects it from pests**.

8. Write note on *Bacillus thuringiensis* Nov /Dec 2013

Bacillus thuringiensis, a bacterial disease of Lepidoptera, Coleoptera and Diptera, is a well-known insecticide example. The toxin from *B. thuringiensis* (Bt toxin) has been incorporated directly into plants through the use of genetic engineering. The use of Bt Toxin is particularly controversial. Its manufacturers claim it has little effect on other organisms, and is more environmentally friendly than synthetic pesticides. However, at least one scientific study has suggested that it may lead to slight histopathological changes on the liver and kidneys of mammals with Bt toxin in their diet.

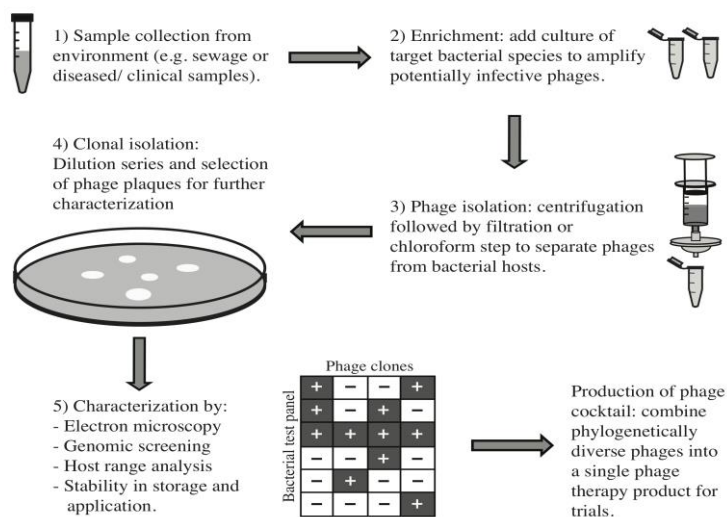
9. Write note on Microbial Insecticides

Microbial insect control utilizes pathogenic microorganisms isolated from diseased insects during naturally occurring epidemics. Typically, such epidemics only occur when pest population densities are high and usually after appreciable damage have been done to crops. Over 400 species of fungi and more than 90 species of bacteria which infect insects have been described including *Bacillus thuringiensis*, varieties of which are manufactured and sold throughout the world primarily for the control of caterpillar pests and more recently mosquitoes and black flies.

Among fungal pesticides, five have been introduced since 1979, and three in 1981. Many countries with centrally planned economies have been using fungal pesticides successfully for many years. So far, more than 40,000 species of *Bacillus thuringiensis* have been isolated and identified as belonging to 39 serotypes. These organisms are active against either Lepidoptera, or Diptera or Coleoptera.

10. Comment on phage biopesticide production

Typical production of phage biopesticide



11.

WHAT IS BIO-PESTICIDE?

- A compound that kills organisms by virtue of specific biological effects rather than as a broader chemical poison
- Differ from **biocontrol** agents in being passive agents, whereas biocontrol agents actively seek the pest
- The rationale behind replacing conventional **pesticides** with bio-pesticides is that the latter are more likely to be **selective** and **biodegradable** (FAO, 2005)



WHY BIO-PESTICIDES?

- **Human and environmental safety**
- **Alternatives to conventional pesticides:**
 - 25 million cases of acute occupational pesticide poisoning in developing countries each year (WHO, 1990)
 - 14% of all known occupational injuries and 10% of all fatal injuries are caused by pesticides (ILO, 1996)
 - Obsolete pesticides stored in developing countries - 20,000 tonnes in Africa alone
- **Amenable to small-scale, local production in developing countries**
- **Address increased public awareness of environmental and food safety**
- **Fundamental component of Integrated Pest Management**
 - Natural enemies protected
 - Controls pests resistant to conventional pesticides
- **Products available in small, niche markets that are typically unaddressed by large agrochemical companies**

12. Comment on Biofertilizers

A bio-fertilizer provides the following benefits:

1. Since a bio-fertilizer is technically living, it can **symbiotically** associate with plant roots. Involved microorganisms could readily and safely convert complex organic material into simple compounds, so that they are easily taken up by the plants. Microorganism function is in long duration, causing improvement of the soil fertility. It maintains the natural habitat of the soil. It increases crop yield by 20-30%, replaces chemical **nitrogen** and **phosphorus** by 25%, and stimulates plant growth. It can also provide protection against drought and some soil-borne diseases.
2. Bio-fertilizers are cost-effective relative to chemical fertilizers. They have lower manufacturing costs, especially regarding nitrogen and phosphorus use.

13. Comment on some of the important groups of Bio-fertilizers

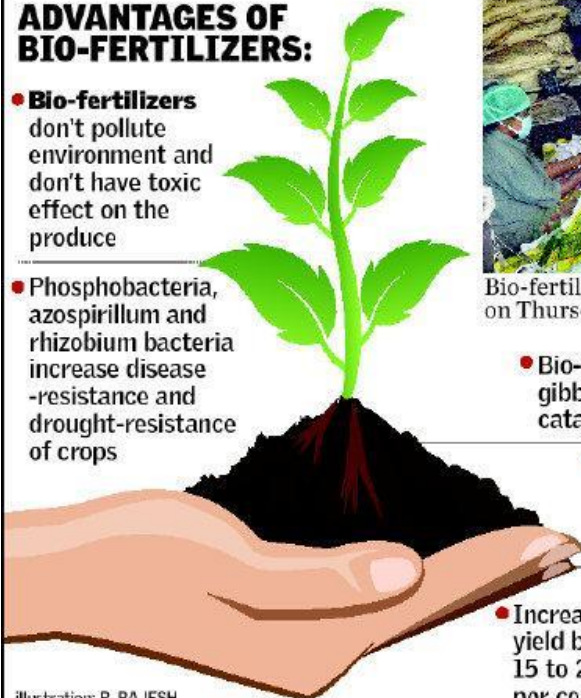
1. **Azolla-Anabena symbiosis:** Azolla is a small, eukaryotic, aquatic fern having global distribution. Prokaryotic blue green algae Anabena azolla resides in its leaves as a symbiont. Azolla is an alternative nitrogen source. This association has gained wide interest because of its potential use as an alternative to chemical fertilizers.
2. **Rhizobium:** Symbiotic nitrogen fixation by Rhizobium with legumes contributes substantially to total nitrogen fixation. Rhizobium inoculation is a well-known agronomic practice to ensure adequate nitrogen.


14. Comment on the advantages of Biofertilizers

ADVANTAGES OF BIO-FERTILIZERS:

- **Bio-fertilizers** don't pollute environment and don't have toxic effect on the produce
- Phosphobacteria, azospirillum and rhizobium bacteria increase disease -resistance and drought-resistance of crops
- Bio-fertilizers produce indole acetic acid, gibberellins, biotin and vitamin B that catalyse growth of crops and yield
- Cheaper than chemical fertilizers; 200 gram of solid bio-fertilizer is available for **Rs. 6** while a liquid bio-fertilizer costs **Rs. 280** per litre
- Can be used for paddy, pulses, small foodgrains, vegetables, coconut, sunflower, sesame, groundnut, cotton, banana and applied in orchards
- Increase yield by 15 to 20 per cent

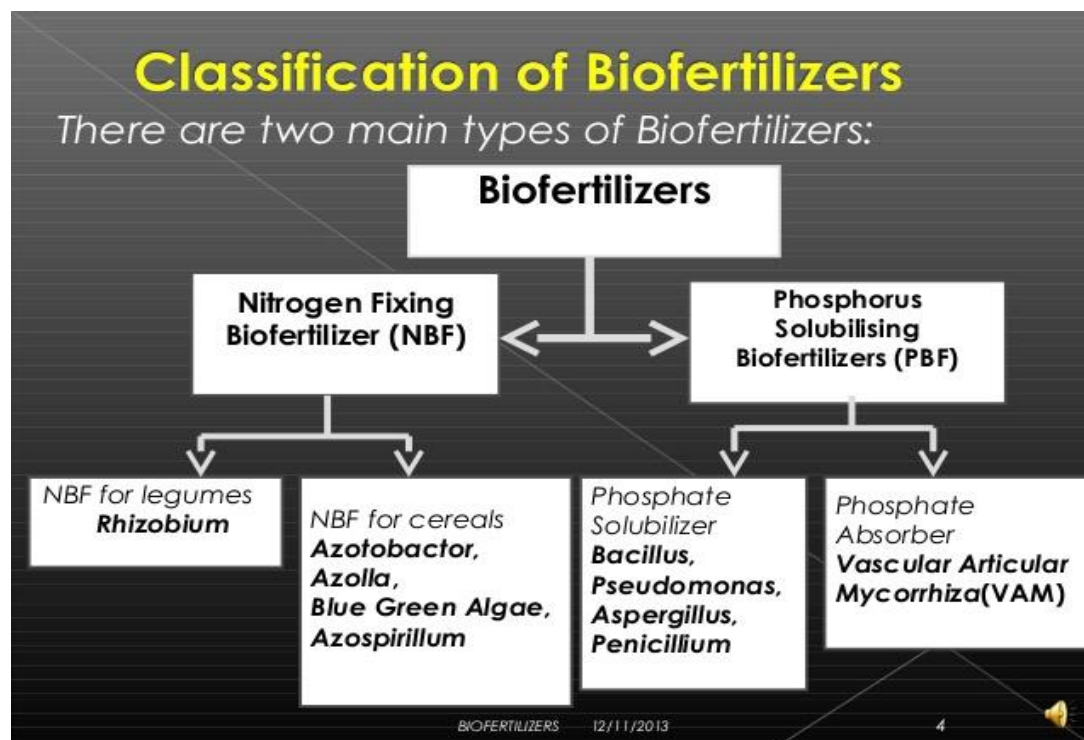
illustration: R. RAJESH





Bio-fertilizers being packed in Tuticorin on Thursday.-PHOTO: N. RAJESH

15. Comment on the classification of Biofertilizers




16. What is Xanthan Gum?

Xanthan Gum

"Xanthan Gum is a microbial polysaccharide derived from the bacterium *Xanthomonas campestris*"

Phew! Now that was one hard sentence. As listed earlier, Xanthan gum falls under two functions; as a Thickener, Stabilizer, texturizer and a Fat replacer.

It can be used with or as a substitute for Gelatine and Guar Gum. This ingredient is considered safe, and there doesn't seem to be any thing that suggests otherwise. Xanthan gum is great for people who are allergic to gluten. However there are some people who are allergic to Xanthan gum itself, and they may experience some mild symptoms upon digestion.



17. Comment on Biopreservatives Nov /Dec 2013

A **preservative** is a substance or a chemical that is added to products such as food, beverages, pharmaceutical drugs, paints, biological samples, cosmetics, wood, and many other products to prevent decomposition by microbial growth or by undesirable chemical changes. In general, preservation is implemented in two modes, chemical and physical. Chemical preservation entails adding chemical compounds to the product. Physical preservation entails processes such as refrigeration or drying. Preservative food additives reduce the risk of foodborne infections, decrease microbial spoilage, and preserve fresh attributes and nutritional quality. Some physical techniques for food preservation include dehydration, UV-C radiation, freeze-drying, and refrigeration. Chemical preservation and physical preservation techniques are sometimes combined.

18. Comment on the modes of action of preservatives

Preservatives generally offer limited protection against viral contamination. Bactericides and fungicides may evince their effects on a variety of microbial cellular targets, for example; the cell wall, the cytoplasmic membrane or the cytoplasm. It is often difficult to assign a precise target for a specific class of preservative; the target can and does change with preservative concentration. As a consequence, preservatives can often interfere with several different microbial cellular mechanisms (Table 2). Such cytotoxicity may also affect mammalian cells. Hence inclusion levels should be minimal, consistent with adequate preservation. There is a

regulatory expectation that the reason for preservative inclusion, proof of efficacy, safety information, control methods in finished product and details of labeling in the finished product should all be addressed by the applicant.

Table 2. Site of Preservative Activity in Microbial Cell		
Cell Wall	Cytoplasmic membrane	Cytoplasm
Phenols	2-Phenoxyethanol	2-Phenoxyethanol and other organic alcohols
Aryl and alkyl acids	Parabens	Aryl and alkyl acids
Organo mercurials	Organo mercurials	Halogenated preservatives
EDTA (edetic acid)	EDTA	
Chlorhexidine, cetrimide	Chlorhexidine, hexachlorophene	Chlorhexidine (high concentrations)
Glutaraldehyde	Formaldehyde donators e.g. bronopol, imidurea	Formaldehyde donators e.g. bronopol, imidurea
Anionic surfactants	Benzalkonium chloride (BKC)	

19. Comment on the reason for biopolymers

Synthetic polymers have become an essential part of our life due to their properties of durability, strength, lightness and cost. These very desirable properties have also made the plastics a source of environmental and waste management problem. Also these polymers are primarily derived from non renewable fossil (petrochemical) which are disappearing fast. Ideally the polymer should not only be biodegradable but also be produced from renewable resources. As a solution to this, biodegradable plastics (mainly polyhydroxyalkanoates (PHA)) have been developed through biotechnological routes. These are polyesters of various hydroxyalkanoates which are synthesized by numerous microorganisms as energy reserve materials when an essential nutrient such as nitrogen or phosphorus is limited in presence of excess carbon source. They are also completely degraded to water and carbon dioxide under aerobic conditions and to methane under anaerobic conditions by microorganisms in soil, sea, lake water and sewage. But the main property which sets them apart from other polymers is their similar mechanical properties to the synthetically produced polymers like polypropylene. They can be used for the development of disposable items, packaging films, and also as biodegradable carriers.

20. What is PHB? Nov /Dec 2014

Poly- β -hydroxybutyrate (PHB), the most widespread and best characterized member of PHAs, is a homopolymer consisting of 3-hydroxybutyrate (HB). Organisms producing PHB include a wide variety of taxonomically different groups. Among all, *Wautersia eutropha* (formerly known

as *Ralstonia eutropha* and *Alcaligenes eutrophus*) has been most extensively studied due to its ability to accumulate large amount of PHB from inexpensive sources. It features accumulation of biopolymer when there is a limitation of an essential nutrient such as nitrogen, phosphorous, magnesium or sulfur in the presence of excess carbon source. There is a need to understand the kinetics of growth and product formation by *Wautersia eutropha* under batch cultivation mode so that a mathematical description of the biological process can be established. This model will be highly instrumental in identification of right bioreactor configuration and appropriate cultivation strategy so that the biopolymer concentration and productivity by microbial cultivation can be enhanced.

21. Why do we need biofuels?

Sustainable biofuels are essential to ensure a constant, secure supply of energy for individuals and industry. Advanced biofuels will reduce our dependency on fossil fuels and limit our impact on the environment. It is also argued that investing in biofuel production may boost the economy of developing countries.

22. Comment on new approaches to biofuels

‘Second-’ and ‘third-generation’ biofuels are generated from non-food crops. Microbes play a key role in the development of these biofuels. They are more sustainable than first-generation biofuels as they produce higher yields, reduce greenhouse gas production and do not compete with crops grown for food.

23. Comment on two major areas of biofuel research

Two major areas of research are lignocellulosic biofuels and algae. Microbiologists are currently working in a number of areas to make biofuel production more efficient. These include: – Scaling-up the production of microbial cellulase that will break down celluloses into fermentable sugars. – Engineering yeast to tolerate higher concentrations of alcohol to increase bioethanol production. – Genetically modifying micro-organisms to ferment sugars more efficiently to increase bioethanol yields. – Optimizing microbial strains that will convert sugars into biobutanol as an alternative to bioethanol. – Finding algae that produce high yields of oils or are otherwise well-adapted for biodiesel production.

23. Expand and define SCP Nov /Dec 2014, Nov/Dec 2017

Single Cell Protein: A variety of microorganisms and substrate are used to produce single cell proteins. Yeast is suitable for single cell protein production because of its superior nutritional quality. The supplementation cereals with single cell proteins, especially yeast, make them as good as animal proteins. The necessary factor considered for use of SCP is the demonstration of the absence of toxic and carcinogenic compounds originated from the substrates, biosynthesized by the microorganisms or formed during processing. High nucleic acid content and low cell wall digestibility are two of the most important factors limiting nutritional and toxicological value of yeast for animal or human consumption.

24. What are the uses of biopolymers? Nov/Dec 2017

Sugar based polymers, such as Polyactides, naturally degenerate in the human body without producing any harmful side effects. This is the reason why they are used for medical purposes. Polyactides are commonly used as surgical implants.

Starch based biopolymers can be used for creating conventional plastic by extruding and injection molding.

Biopolymers based on synthetic are used to manufacture substrate mats.

Cellulose based Biopolymers, such as cellophane, are used as a packaging material.

These chemical compounds can be used to make thin wrapping films, food trays and pellets for sending fragile goods by shipping.

25. Draw a flow chart on Mushroom Cultivation

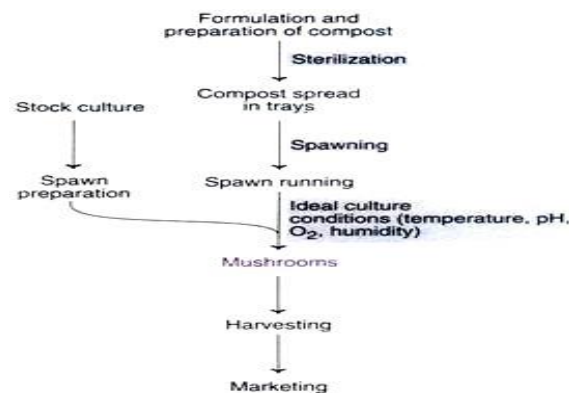


Fig. 29.5 : An overview of edible mushroom production.

26. What are the four main types of Biopolymers? (Nov/Dec 2016)

There are four main types of biopolymer based respectively on:

1. Starch
2. Sugar
3. Cellulose
4. Synthetic materials

Starch based polymers. Sugar based biopolymers. Sugar based biopolymers. Cellulose based biopolymers. Cellulose based biopolymers. Synthetic based biopolymers. Synthetic based biopolymers.

27. What are the advantages of using MO for SCP production? (Nov/Dec 2016)

Large-scale production of microbial biomass has many advantages over the traditional methods for producing proteins for food or feed.

1. Microorganisms have a much higher growth rate (algae: 2–6 hours, yeast: 1–3 hours, bacteria: 0.5–2 hours). This also allows to select for strains with high yield and good nutritional composition quickly and easily compared to breeding.

2. Whereas large parts of the crop, such as stems, leaves and roots are not edible, single-cell microorganisms can be used entirely. Whereas parts of the edible fraction of crops contains is undigestible, many microorganisms are digestible at a much higher fraction.^[4]
3. Microorganisms usually have a much higher protein content of 30–70% in the dry mass than vegetables or grains.^[20] The amino acid profiles of many SCP microorganisms often have excellent nutritional quality, comparable to a hen's egg.

28. What are the different types of Bioremediation Process?

- **Microbial bioremediation** uses microorganisms to break down contaminants by using them as a food source.
- **Phytoremediation** uses plants to bind, extract, and clean up pollutants such as pesticides, petroleum hydrocarbons, metals, and chlorinated solvents.
- **Mycoremediation** uses fungi's digestive enzymes to break down contaminants such as pesticides, hydrocarbons, and heavy metals.

29. What is LAB?

Of special interest are lactic acid bacteria (LAB). Lactic acid bacteria have antagonistic properties which make them particularly useful as biopreservatives. When LABs compete for nutrients, their metabolites often include active antimicrobials such as lactic and acetic acid, hydrogen peroxide, and peptidebacteriocins. Some LABs produce the antimicrobial nisin which is a particularly effective preservative. These days LAB bacteriocins are used as an integral part of hurdle technology. Lactic acid bacteria and propionibacteria have been extensively studies for their efficacy against spoilage causing yeasts and molds in food spoilage.

30. What is the process of making cheese?

Starter cultures, or good bacteria, are added to start the cheese making **process**. They help determine the ultimate flavor and texture of the **cheese**. Next, a milk-clotting enzyme called rennet is added to coagulate the milk, forming a custard-like mass.

PART B

1. Comment on the biosynthesis of biopesticides, biofertilizers (production formulation) and biodiesel with the help of a flow chart. Nov Dec 2013, 2014
Ans: Biotechnology by U.Satyanarayana Pg.No:644-645, 645-647,393,399: Industrial Biotechnology by A.H.Patel. Pg.No: 188-202
2. Give a detailed account on Biopreservatives, Biopolymers, and **Bioremediation** their characteristics, stages in their biosynthetic processes and their advantages and limitations.**Nov/Dec 2016**
Ans: Biotechnology by U.Satyanarayana Pg.No:382-392, 718-728
3. Describe in detail the large scale production of amylase.
Ans: Biotechnology by U.Satyanarayana Pg.No:281-286.

4. Discuss in detail Bioremediation with examples. **Nov/Dec 2017**

Ans: Biotechnology by U.Satyanarayana Pg.No:727-728

5. Elaborate the important criteria for selection of microorganisms, basic production process of SCP in a detailed manner. **Nov/Dec 2016**

Ans: Text Book of Industrial Fermentation by Wulf crueger Pg.No: 306-315;
Biotechnology by U.Satyanarayana Pg.No:373-380

PART C

1. Elaborate the important criteria for selection of microorganisms, basic production process of Cheese & Beer, from any microorganism in a detailed manner. **Nov/Dec 2016.**

Ans: Biotechnology by U.Satyanarayana Pg.No:362-381, Text Book of Industrial Fermentation by Wulf crueger Pg.No: 306-315

2. Describe the biodiesel production by fermentation process in the industries (**Nov/Dec 2017**)

Ans: Text Book : U. Satyanarayana – Biotechnology Page No.(398 to 399)

3. Explain in detail the Industrial production of Enzymes and write in detail the commercial applications of the same

Ans: Text Book : U. Satyanarayana – Biotechnology Page No.(1398 to 1399)

4. Describe the production process of Mushroom culture. (**Nov/Dec 2017**)

UNIT V PRODUCTION OF MODERN BIOTECHNOLOGY PRODUCTS (8)

Production of recombinant proteins having therapeutic and diagnostic applications, Vaccines. Bioprocess strategies in Plant Cell and Animal Cell culture.

PART A

1. Comment on protein expression and purification

Protein Expression and Purification

- Isolation of genes.
- Insertion of isolated gene to expression vector.
- Transfer of recombinant vector into host cell through Transformation.
- Identification and isolation of cells containing recombinant vector.
- Growth of cells through fermentation.
- Isolation and purification of protein.



2. Write notes on production of recombinant DNA

Production of Recombinant protein

- There are basically two methods for producing **recombinant proteins**.
- One is the molecular Cloning a laboratory method used to make **recombinant** DNA.
- The other method is the Polymerase chain reaction used to proceed the replication of any specific DNA sequence selected .
- The basic difference between the two methods is that molecular cloning incorporates the replication of the DNA within a living cell, whereas PCR replicates DNA in the test tube, without living cells.

7

3. Comment on the methods used to produce recombinant proteins

2. METHODS USED TO PRODUCE RECOMBINANT PROTEINS

(i) Production of recombinant proteins in microbial bioreactors

Examples

- *E.coli* expression system
- *Saccharomyces cerevisiae*

(ii) Mammalian cell derived bioreactors

- E.g. Chinese Hamster Ovary cell (CHO) bioreactors.

➤ (iii) Animal Bioreactors “Pharming”

Production of Recombinant Therapeutic Proteins in the Milk of Transgenic Animals Eg, Cows, sheep, pigs etc.

4. Comment on the applications of recombinant DNA

APPLICATIONS

- Several proteins are created from recombinant DNA (recombinant proteins) and are used in medical applications.
- Hematopoietic growth factor.
- Interferon's
- Hormones
- Recombinant protein vaccines
- Tissue/bone growth factors and clotting factors
- Biological response modifiers
- Monoclonal/Diagnostic/Therapeutic antibodies
- Recombinant proteins is extensively used in biotechnology, medicine and research.



15

5. Comment on Recombinant Protein Production

Recombinant Protein Production

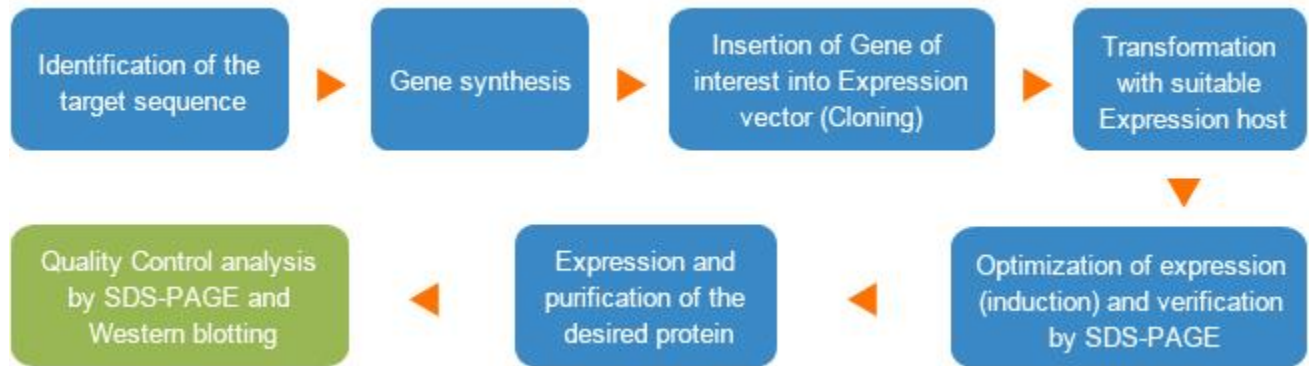
-Why?

- over-expression to get enough amount
- easy purification

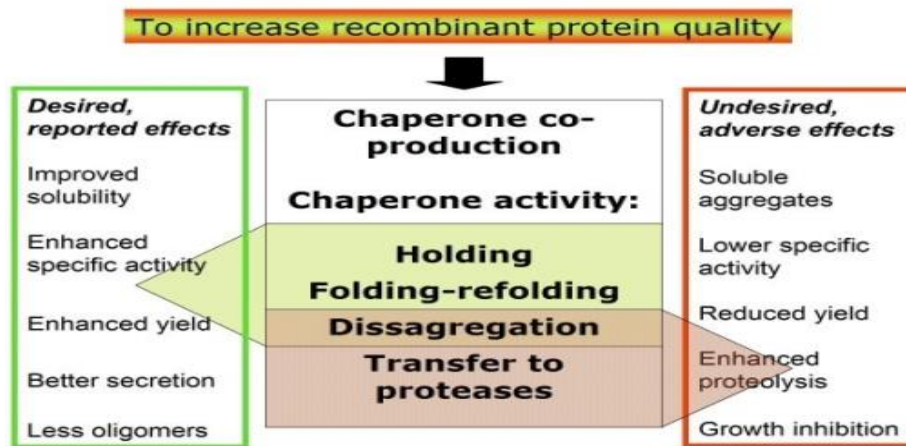
-Application

- functional studies
- structural studies
- vaccine/antigen/antibodies
- therapeutic drug
- industrial enzymes for reaction

6. Steps of Recombinant protein production- Comment



7. Write notes to increase recombinant protein quality

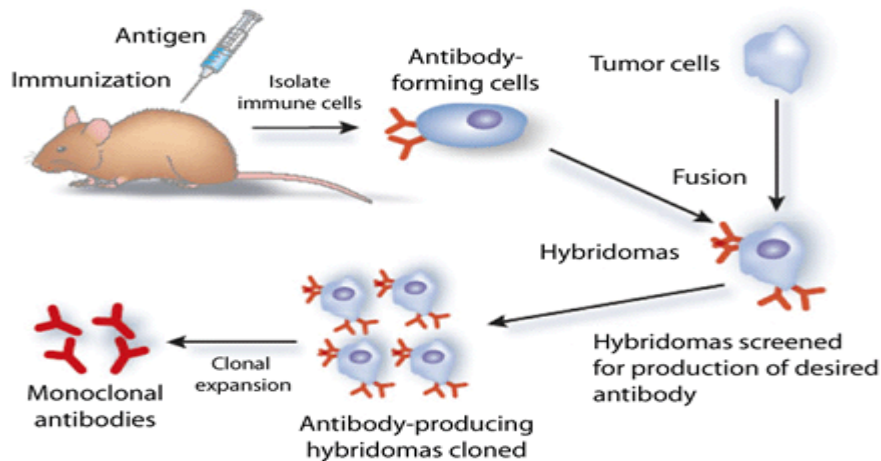


8. What are the potential problems in using microorganisms

There are some considerations and potential hazards when we consider the manipulation of the genetic material of microorganisms, for example:

- Risk of uncontrolled dispersal into the natural environment.
- Microorganisms are highly adaptable to different ecological niches and could disrupt those environments.
- Sideways transfer of genetic material to different species could occur.
- Unforeseen metabolic modifications could be hard to control.
- Creation of new pathogenic microorganisms is a possibility.
-

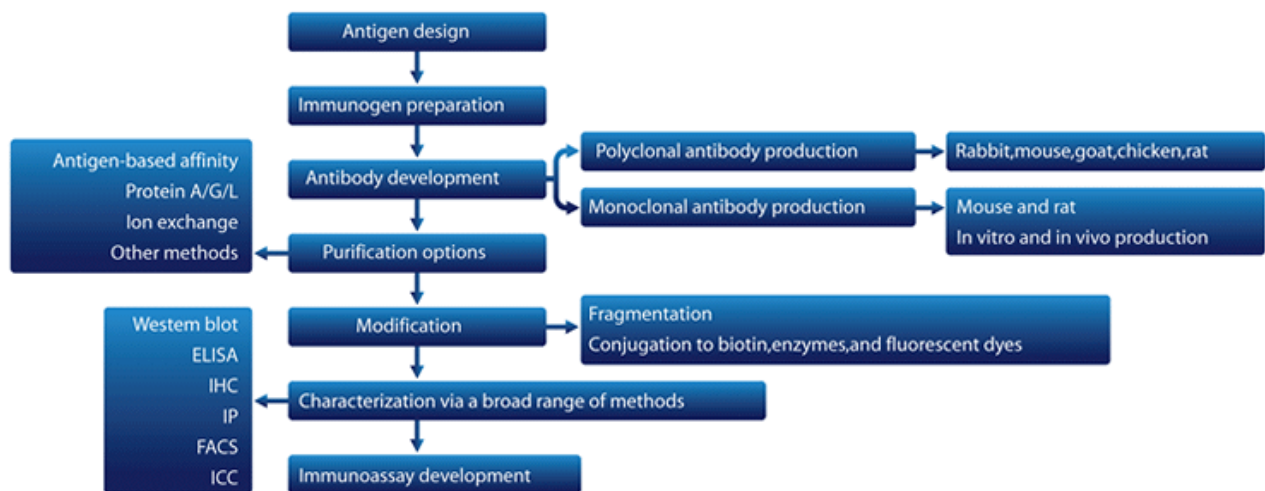
9. Draw a neat representation of production of Monoclonal antibodies



10. What is the need for recombinant protein biopharmaceuticals?

Biopharmaceuticals currently represent the fastest-growing sector of the pharmaceutical industry, driven by a rapid expansion in the manufacture of recombinant protein-based drugs. Consequently, the efficient expression and production of these valuable biomolecules face challenges in improving their quantity and quality while minimizing time and cost. To meet these demands, an increasing variety of recombinant production platforms are being developed. Unfortunately, there is no “universal” production system which can guarantee high yields of recombinant protein, particularly as every biomolecule itself causes its own issues in terms of expression. To meet the demand, it is crucial to increase the throughput of expression, production and purification processes and systems.

11. Comment on various antibody design flow chart



12. Comment on the Production of recombinant protein therapeutics in cultivated mammalian cells.

Cultivated mammalian cells have become the dominant system for the production of recombinant proteins for clinical applications because of their capacity for proper protein folding, assembly and post-translational modification. Thus, the quality and efficacy of a protein can be superior when expressed in mammalian cells versus other hosts such as bacteria, plants and yeast. Recently, the productivity of mammalian cells cultivated in bioreactors has reached the gram per liter range in a number of cases, a more than 100-fold yield improvement over titers seen for similar processes in the mid-1980s. This increase in volumetric productivity has resulted mainly from improvements in media composition and process control. Opportunities still exist for improving mammalian cell systems through further advancements in production systems as well as through vector and host cell engineering.

13. Write notes on Protein Therapeutics and its advantages

In comparison to small-molecule drugs protein therapeutics have several very important advantages:

- specific directing and higher efficacy with low number of side effects,
- better PK and PD,
- the specific interactions with the molecular target cannot be imitated by any chemical compounds,
- they are well tolerated and, as they are naturally produced by body, it is less probable that they elicit immune response,
- the clinical development and approval time can be shorter than in case of small-molecule drugs,
- their unique structure and functions allow the comprehensive patent protection,
- the recombinant DNA technology allows to choose the expression system (e.g. bacteria, yeast, insect cells, mammalian cells) dictated by the costs or the need of modification within the structure.

14. Specify few Monoclonal antibodies

Brand	Generic	Company	Therapeutic category	Indications
ReoPro	Abciximab	Eli Lilly	Blood modifier	Acute coronary syndrome
Rituxan	rituxumab	Genentech	Cancer	Non-Hodgkin's lymphoma
Herceptin	Trastuzumab	Genentech	Cancer	Breast cancer
Synagis	Palivizumab	MedImmune	Respiratory	Respiratory syncytial virus
Campath	Alemtuzumab	Schering AG	Cancer	Non-Hodgkin's lymphoma
Humira	Adalimumab	Abbott Labs	Anti-arthritis	Rheumatoid arthritis
Xolair Omalizumab	Omalizumab	Genentech	Respiratory diseases	Paediatric asthma, peanut allergies
Erbitux	Cetuximab	Imclone Systems	Cancer	Colon cancer
Avastin	Bevacizumab	Genentech	Cancer	Colon cancer

15. Draw a flow chart for rec. therapeutic proteins

This flow chart below shows critical stages of an upstream production platform that relate to success of a therapeutic product.



16. Comment on the Expression of recombinant DNA

Following transplantation into the host organism, the foreign DNA contained within the recombinant DNA construct may or may not be expressed. That is, the DNA may simply be replicated without expression, or it may be transcribed and translated at a recombinant protein is produced.

Expression of a foreign gene requires restructuring the gene to include sequences that are required for producing an mRNA molecule that can be used by the host's translational apparatus (e.g. promoter, translational initiation signal, and transcriptional terminator). Specific changes to the host organism may be made to improve expression of the ectopic gene. In addition, changes may be needed to the coding sequences as well, to optimize translation, make the protein soluble, direct the recombinant protein to the proper cellular or extracellular location, and stabilize the protein from degradation.

17. Justify the need for the industrial production of recombinant proteins Nov/Dec 2017

The most notable applications of the recombinant technology having direct impact on humanity have been:

1. Large scale production of therapeutic protein such as insulin, hormones, vaccine and interleukins using recombinant microorganisms.
2. Production of humanized monoclonal antibodies for therapeutic application
3. Production of insect resistant cotton plant by incorporation of insecticidal toxin of *Bacillus thuringiensis* (Bt cotton plant).
4. Production of golden rice (rice having vitamin A) by incorporating three genes required for its synthesis in rice plant.
5. Bioremediation by the use of recombinant organisms &
6. Use of genetic engineering techniques in forensic medicine.

18. Comment on the types of biomolecules produced through recombinant DNA technology Recombinant Hormones

Insulin (and its analogs), growth hormone, follicle stimulating hormone, salmon calcitonin.

Blood products

Albumin, thrombolytics, fibrinolytics, and clotting factors (Factor VII, Factor IX, tissue plasminogen activator, recombinant hirudin)

Cytokines and growth factors

Interferons, interleukins and colony stimulating factors (Interferon, α , β and γ , erythropoietin, interleukin-2, GM-CSF, GCSF)

Monoclonal antibodies and related products Mouse, chimeric or humanized; whole molecule or fragment; single chain or bispecific; and conjugated (rituximab, trastuzumab, infliximab, bevacizumab)

Recombinant Vaccines Recombinant protein or peptides, DNA plasmid and anti-idiotypic (HBsAg vaccine, HPV vaccine).

Recombinant Enzymes Dornase- α (Pulmozyme), Acid glucosidase (Myozyme), α -L-iduronidase (Aldurazyme) and Urate Oxidase.

Miscellaneous products Bone morphogenic protein, conjugate antibody, pegylated recombinant proteins, antagonist.

19. Comment on few value added transgenic crops

Some of the value added transgenic crops include:

- (a) Golden rice: containing beta carotene to overcome vitamin A deficiency in regions where rice is the staple food (b) Canola containing high levels of oleic acids and laurate (c) Barley containing feed enzymes (d) tomatoes which does not rot in room temperature (e) Other vegetables and fruits with delayed ripening as well as modified flavour characteristics.

Transgenic crops with improved nutrition quality have already been produced by introducing genes involved in the metabolism of vitamins, minerals and amino acids.

20) Write note on the application of Recombinant DNA in Environment

A vast majority of applications of environmental biotechnology use naturally occurring microorganisms (bacteria, fungi, etc.) to identify and filter manufacturing waste before it is introduced into the environment.

For example when gene such as the mercury resistance gene (**mer**) or the toluene degradation (tol) gene is linked to genes that code for bioluminescence within living bacterial cells, the biosensor cells can signal extremely low levels of inorganic mercury or toluene that are present in contaminated waters and soils by emitting visible light, which can be measured with fiber-optic flurometers.

21. Comment on the bioreactor as a tool for large-scale culture of animal cells.

Bioreactors play a key role in the field of biologics, where they are used for the production of recombinant therapeutic proteins by large-scale cultivation of animal cells. There are several types of bioreactors, including stirred-tank, airlift, hollow-fiber, and Rotary Cell Culture System (RCCS) designs. The stirred-tank bioreactor is one of the most commonly used types, and is used both for industrial applications and laboratory research. Important improvements have been made in the design of traditional bioreactors, and new types of bioreactor are also being developed such as Couette-Taylor bioreactor, multifunctional-membrane bioreactor, and shaking bioreactor.

Two main goals will be pursued: firstly, to increase output by high density cultivation of animal cells to produce high value protein pharmaceuticals or viral vectors for clinical gene therapy; and secondly, to create a three-dimension space similar to that of an in vivo environment to regenerate tissue or organ and to reproduce valuable cells that are hard to culture in the traditional culture system.

22. What are anchorage dependent cells?

- **Anchorage Dependent cells**
 - Require surface attachment to grow
 - They include mostly primary cells and cell lines such as :
 - Chinese Hamster Ovary cells (CHO),
 - Baby Hamster Kidney Cells (BHK) and
 - Human Fibroblast cells (FS-4)

23. What is meant by Passaging cells?

Passaging (also known as subculture or splitting cells) involves transferring a small number of cells into a new vessel. Cells can be cultured for a longer time if they are split regularly, as it avoids the senescence associated with prolonged high cell density. Suspension cultures are easily passaged with a small amount of culture containing a few cells diluted in a larger volume of fresh media. For adherent cultures, cells first need to be detached; this is commonly done with a mixture of trypsin-EDTA; however, other enzyme mixes are now available for this purpose. A small number of detached cells can then be used to seed a new culture. Some cell cultures, such as RAW cells are mechanically scraped from the surface of their vessel with rubber scrapers.

24. Comment on the applications of cell culture

Biological products produced by recombinant DNA (rDNA) technology in animal cell cultures include enzymes, synthetic hormones, immunobiologicals (monoclonal antibodies, interleukins, lymphokines), and anticancer agents. Although many simpler proteins can be produced using rDNA in bacterial cultures, more complex proteins that are glycosylated (carbohydrate-modified) currently must be made in animal cells. An important example of such a complex protein is the hormone erythropoietin.

25. Comment on Tissue Cultures

Tissue culture is the general term for the removal of cells, tissues or organs from an animal or plant and their subsequent placement into an artificial medium environment for maintaining cell viability.

26. Comment on Organ Culture

The culture of whole organs or intact organ fragments with the intent to use cells as machinery to produce biological is called **Organ Culture**. When the cells are removed from the organ fragments prior to or during cultivation thus disrupting their normal relationships with neighboring cell, the technology is called **Cell Culture**.

27. Comment on Primary Culture

When cells are individually dissociated from an organism and placed into a suitable medium and support culture environment, they will attach, divide and grow. This cell culture is named **Primary Culture**. Cell culture may be initiated from normal, embryonic or malignant

28. Define Totipotency Nov /Dec 2016

Totipotency is the ability of a single cell to divide and produce all of the differentiated cells in an organism. Spores and zygotes are examples of **totipotent** cells. In the spectrum of cell potency, **totipotency** represents the cell with the greatest differentiation potential.

29. What are 3 major applications of Genetic Engineering technology in antibiotic production?

Directed mutation and selection, protoplast fusion, and both semirandom and specific recombinant DNA methods are examples of alternative procedures for manipulating the biosynthetic pathways of microorganisms for strain improvement and for new hybrid antibiotic synthesis.

30. What is Pluripotency?

Pluripotent cells can give rise to all of the cell types that make up the body; embryonic stem cells are considered **pluripotent**. Multipotent cells can develop into more than one cell type, but are more limited than **pluripotent** cells; adult stem cells and cord blood stem cells are considered multipotent.

31. Name any two media used in plant and animal cell culture respectively Nov/Dec 2017

Plant tissue culture media should generally contain some or all of the following components: macronutrients, micronutrients, vitamins, amino acids or nitrogen supplements, source(s) of carbon, undefined organic supplements, growth regulators and solidifying agents.

Animal cell culture media attempts at serum-free **culture** by using serum substitutes (eg, several hormones and growth factors, transferrin, and selenite) grew in number and a variety of serum-free **media** was developed, with each **medium** tailored to researchers' **cell** type of interest.

PART B

1. Describe in detail the therapeutic and diagnostic applications of recombinant proteins.
Ans: Biotechnology by U.Satyanarayana Pg.No:213-226 **Nov /Dec 2017**
2. Write detailed notes on production and purification of insulin by r-DNA technology.**Nov/Dec 2016**
Ans: Biotechnology by U.Satyanarayana Pg.No:189-192
3. Write the detailed steps and processes involved in the production of Vaccines **Nov/Dec 2017**
Ans: Biotechnology by U.Satyanarayana Pg.No: 411-413
4. What are the strategies followed for bioprocessing of plant cell culture mass production?
Nov /Dec 2014,15
Ans: Biotechnology by U.Satyanarayana Pg.No:552-564
5. Discuss the Characterization of cultured cells & measurement of growth parameters of cultured cells. Nov /Dec 2014,15
Ans: Biotechnology by U.Satyanarayana Pg.No:428-435
6. Explain in detail the various techniques in plant transformation. (**Nov/Dec 2016**)
Ans: Biotechnology by U.Satyanarayana Pg.No:506-517.

PART C

1. Discuss the application & production of secondary metabolites and application of plant tissue culture. Nov /Dec 2014

Ans: Biotechnology by U.Satyanarayana Pg.No:806-817.
2. Comment on the contemporary challenges involved in the production of modern biotechnology products. Also provide the possible solutions to address them. **Nov /Dec 2017**

Ans: Biotechnology by U.Satyanarayana Pg.No:325-328.
3. Describe the bioprocess strategies involved in Animal Cell Culture Nov /Dec 2014
Ans: Biotechnology by U.Satyanarayana Pg.No:113-18