Chapter 10

Bioprocessing and Biomanufacturing

EXERCISES

1. Differentiate between primary and secondary metabolites on the basis of their functions with example.

Ans: Here's a differentiation between primary and secondary metabolites, emphasizing their functions and examples:

Primary Metabolites

Function:

*Essential for basic growth, development, and reproduction of an organism.

*Involved in vital processes like energy production, nutrient synthesis, and structural maintenance.

Examples:

***Carbohydrates:** Glucose (energy source), cellulose (cell wall component)

Proteins: Enzymes (catalyze reactions), structural proteins (form cytoskeleton)

***Lipids:** Fatty acids (energy storage), phospholipids (cell membrane structure)

***Nucleic acids:** DNA (genetic material), RNA (protein synthesis)

Secondary Metabolites

Function:

*Not directly involved in growth, development, or reproduction.

*Have diverse roles in defense, communication, adaptation, and interaction with the environment.

Examples:

***Pigments:** Chlorophyll (photosynthesis), anthocyanins (flower color)

***Alkaloids:** Caffeine (defense against herbivores), nicotine (insecticide)

***Terpenoids:** Menthol (fragrance), limonene (citrus flavor)

***Phenolics:** Flavonoids (antioxidants), tannins (defense against pathogens)

Key Differences:

Additional Insights:

*Secondary metabolites often have medicinal properties, leading to the development of drugs and nutraceuticals.

*They can be used in agriculture for pest control, flavor enhancement, and food preservation.

*Research on secondary metabolites continues to reveal their ecological roles and potential applications.

2. Explain the challenges encountered during the development of a bioprocess.

Ans: Developing a bioprocess is no walk in the park. It involves navigating a complex landscape of challenges across various stages, from upstream to downstream and scale-up. Here's a breakdown of some key hurdles you might encounter:

Upstream Challenges:

***Selecting the right organism or cell line:** Finding the best organism or cell line for efficient production of your desired biomolecule (e.g., protein, enzyme) can be tricky. Factors like yield, growth rate, and susceptibility to contamination need careful consideration.

***Developing optimal culture conditions:** Fine-tuning temperature, pH, oxygen levels, and nutrient composition in the growth medium is crucial for maximizing cell growth and product yield. This often involves extensive experimentation and optimization.

***Maintaining sterility:** Contamination with bacteria, viruses, or other microbes can ruin your entire bioprocess. Rigorous aseptic techniques and robust sterilization protocols are essential to prevent contamination.

***Scaling up from lab to production:** Moving from small-scale lab cultures to large-scale production bioreactors presents scaling challenges. Factors like oxygen transfer, nutrient distribution, and waste removal need to be carefully addressed to maintain efficient production at larger volumes.

Downstream Challenges:

***Developing efficient purification processes:** Isolating and purifying your desired biomolecule from the complex mixture of cellular products can be tricky and expensive. Choosing the right chromatography, filtration, or other purification techniques is crucial for obtaining high-purity product.

***Handling large volumes of bioproducts:** Efficiently processing and storing large volumes of bioproducts can pose logistical and infrastructure challenges. Considerations include temperature control, sterility maintenance, and potential degradation of the product over time.

***Meeting regulatory requirements:** Biopharmaceutical products must meet stringent regulatory standards for purity, safety, and efficacy. Navigating complex regulatory pathways and complying with Good Manufacturing Practices (GMP) can be a lengthy and expensive process.

***Developing cost-effective processes:** Bioprocesses can be expensive to set up and run. Optimizing processes for efficiency, minimizing waste, and finding cost-effective materials and equipment are crucial for commercial viability.

Additional Challenges:

***Bioprocess variability:** Biological systems are inherently variable, leading to fluctuations in product yield and quality. Implementing robust process control and monitoring systems is essential to ensure consistency and product quality.

***Emerging technologies:** Advances in fields like bioinformatics, automation, and microfluidics offer exciting opportunities for bioprocess development, but integrating and adapting these technologies can be challenging.

These are just some of the many challenges encountered during bioprocess development. Overcoming these hurdles requires a combination of scientific expertise, engineering know-how, regulatory awareness, and business acumen. By understanding these challenges and implementing effective strategies to address them, researchers and engineers can pave the way for successful development and commercialization of biopharmaceutical products.

3. Describe briefly the design and components of a typical bioreactor and their applications.

Ans: A typical bioreactor is a sophisticated yet essential tool in biotechnology, mimicking living conditions to promote the growth of cells or microorganisms for various purposes. Let's explore its design and components, diving into some diverse applications:

Design and Components:

***Vessel:** The core container usually made of stainless steel, glass, or plastic, ensuring sterility and temperature control. Size varies depending on the scale of the process (lab-scale to massive industrial bioreactors).

***Agitator:** A stirring mechanism providing mixing and aeration, crucial for oxygen delivery, nutrient distribution, and preventing cell sedimentation. Different types exist, like impellers, paddles, or airlift systems.

***Aerator:** Provides oxygen, essential for most aerobic organisms. Common methods include air spargers, membrane oxygenation, or cascade systems.
***Temperature control:** Ensures optimal temperature for cell growth and activity, often achieved through jackets surrounding the vessel or internal coils.
***pH control:** Maintains desired pH level, vital for enzymatic activity and cell health, using acids, bases, or CO2 injection.
***Monitoring and control system:** Tracks parameters like temperature, pH, oxygen levels, and biomass concentration, allowing for real-time process control and adjustments.

Applications:

***Production of biopharmaceuticals:** From recombinant proteins and enzymes for medical therapy to vaccines and antibodies.
***Biofuel production:** Fermentation of microorganisms to generate ethanol, biodiesel, or other biofuels.
***Wastewater treatment:** Utilizing microorganisms to breakdown organic pollutants and purify water.
***Food production:** Culturing microorganisms for enzymes used in food processing, vitamins, and amino acid production.
***Tissue engineering:** Growing cells for therapeutic applications like skin grafts or cartilage regeneration.

Additional features:

***Sterilization system:** Ensures a contaminant-free environment, critical for successful bioprocesses.
***Sampling ports:** Allow for sterile extraction of samples for monitoring and analysis.
***Feed ports:** Add nutrients and other essential components during the process.
***Remember:** Bioreactor design and components can be customized based on specific applications and organisms used. The key is to create a controlled environment optimized for efficient growth, production, and desired outcomes

4. Explain the basic operational stages of a bioprocess using concept map.

Ans: Bioprocess Operational Stages - A Concept Map

Central Theme: Bioprocess Flow Diagram

Upstream Stages:

.Strain/Cell Line Selection:

*Desired product

*Yield, growth rate

*Susceptibility to contamination

.Media Preparation:

*Nutrients, pH, oxygen levels

*Sterilization

.Inoculum Preparation:

*Seed culture growth optimization

*Scalability considerations

.Fermentation/Cultivation:

*Bioreactor conditions (temperature, agitation)

*Monitoring and control (pH, DO)

Downstream Stages:

.Harvesting:

*Cell separation & product recovery

*Centrifugation, filtration

.Product Purification:

*Chromatography, extraction techniques

*Concentration & polishing

.Quality Control:

*Purity, safety, and efficacy testing

*Regulatory compliance

Additional Links:

.Process Optimization:

*Scale-up, cost-effectiveness

*Waste reduction

.Data Acquisition & Analysis:

*Sensors, monitoring systems

*Process control & improvement

.Benefits of using a concept map:

*Visualizes the interconnectedness of bioprocess stages.

*Simplifies complex information flow.

*Identifies key decision points and dependencies.

*Provides a framework for further discussion and exploration.

5. Describe briefly the following:

(a) upstream processing

Ans: Here's a brief description of upstream processing in bioprocesses:

Upstream processing is the first major stage of a bioprocess, focusing on preparing cells or microorganisms for the production of a desired product. It encompasses several key steps:

1. Strain/Cell Line Selection:

***Carefully choosing the organism or cell line that will most efficiently produce the target product.**

***Factors considered include:**

.Yield of the desired product

.Growth rate

.Tolerance to process conditions

.Susceptibility to contamination

***Media Preparation:**

2. Formulating a nutrient-rich growth medium that supports optimal cell growth and product formation.

***Optimizing factors such as:**

.pH

.Temperature

.Oxygen levels

.Carbon source

.Specific nutrient requirements

.Sterilizing the medium to prevent contamination.

3. Inoculum Preparation:

*Growing a starter culture of the chosen organism or cell line to provide a seed for large-scale production.

*Ensuring optimal growth conditions to maximize cell density and viability.

*Scaling up the inoculum size appropriately for the production bioreactor.

4. Fermentation/Cultivation:

***Facilitating the growth of cells or microorganisms in a controlled environment (usually a bioreactor) to produce the desired product.**

***Maintaining optimal conditions for growth and production, including:**

.Temperature

.Agitation

.pH control

.Dissolved oxygen (DO) levels

.Nutrient supply

.Removal of waste products

(b) downstream processing

Ans: Downstream processing, the second major stage of a bioprocess, is all about isolating and purifying the valuable product from the complex mixture you created in upstream processing. Here's a quick breakdown:

1. Harvesting:

***Separating the cells from the broth, often using techniques like:**

.Centrifugation

.Filtration

.Precipitation

2. Product Recovery:

***Extracting the desired product from the cells, employing methods like:**

.Cell disruption (e.g., sonication, enzyme treatment)

.Solid-liquid separation (e.g., filtration)

.Solvent extraction

3. Product Purification:

***Removing impurities and contaminants from the extracted product, using techniques like:**

.Chromatography

.Dialysis

.Crystallization

4. Concentration and Polishing:

***Concentrating the purified product and finalizing its form for:**

.Storage

.Formulation
.Use in the intended application
5. Quality Control:
***Assessing the purity, safety, and efficacy of the final product to ensure it meets:**
.Regulatory standards
.Specifications for downstream applications
Think of it as refining gold from ore:
.Upstream processing is mining the ore (growing cells and producing the product).
.Downstream processing is extracting the gold (isolating and purifying the desired product).

6. Explain the recovery and purification process of an intracellular product with the help of a flow diagram.

Ans: Recovery and Purification of an Intracellular Product Flow Diagram:
Central Theme: Intracellular Product Recovery and Purification
Upstream Stages:
.Fermentation/Cultivation:
***Bioreactor culture of organism/cell line producing the intracellular product.
***Monitoring and control of growth conditions (temperature, pH, etc.).**
Harvesting & Cell Disruption:
***Separation: R**emoving cells from the broth (centrifugation, filtration).
***Cell Disruption:** Breaking open cells to release intracellular product (sonication, enzymatic lysis, mechanical methods).
Clarification & Crude Extract:
***Removal of Cell Debris:** Centrifugation to separate cell debris from intracellular extract.
***Crude Extract:** Initial solution containing the desired product alongside other cellular components.
Purification:
***Chromatography:** Separates molecules based on size, charge, or affinity. Multiple chromatography steps may be used for high purity.
***Membrane Filtration: Separates molecules based on size and pressure. Can be used for initial clarification or final polishing.**

***Precipitation:** Selective precipitation of the product using specific salts or pH changes.
***Dialysis:** Removes small molecules and salts from the product solution.
Concentration & Polishing:
***Concentration:** Techniques like ultrafiltration or evaporation to increase product concentration.
***Polishing:** Final purification steps like sterile filtration or crystallization to achieve desired product form and purity.
Downstream Stages:
***Quality Control:** Testing for purity, concentration, and activity/functionality.
***Product Formulation:** Preparing the product for its intended use (e.g., buffer, stabilizers).
***Storage & Distribution:** Proper storage and transportation of the final product.
Additional Notes:
*The specific recovery and purification steps will vary depending on the nature of the intracellular product and the desired level of purity.
*Optimization of each step is crucial for efficient and cost-effective production.
*Downstream processing can be a significant cost factor in biomanufacturing.

7. Write short notes on the following:
(a) reverse osmosis
(b) dialysis
Ans: (a) Reverse Osmosis:
Reverse osmosis (RO) is a water purification process that utilizes a semipermeable membrane to remove ions, molecules, and impurities from water. It is a type of filtration technique that operates by applying pressure to force water molecules through the membrane while blocking larger particles, contaminants, and ions. The process is called "reverse" osmosis because it involves the movement of water molecules from an area of higher solute concentration to an area of lower solute concentration.

Key Points:

Semipermeable Membrane: RO systems consist of a semipermeable membrane that allows water molecules to pass through while rejecting impurities.
Pressure Applied: External pressure is applied to overcome the osmotic pressure and facilitate the movement of water through the membrane.
Purification: RO is effective in removing a wide range of contaminants, including salts, minerals, bacteria, and other pollutants.
Applications: RO is commonly used for desalination of seawater, production of drinking water, wastewater treatment, and various industrial processes.

(b) Dialysis:

Dialysis is a medical treatment that replicates some functions of the kidneys when they are impaired or fail. It is commonly used to remove waste products, excess fluids, and electrolytes from the blood in individuals with kidney dysfunction. Dialysis works on the principle of diffusion and osmosis, using a semipermeable membrane to filter and purify the blood.

Key Points:

Semipermeable Membrane: Dialysis involves the use of a semipermeable membrane that allows small molecules, such as waste products and electrolytes, to pass through while retaining larger components like blood cells and proteins.
Diffusion and Osmosis: The process relies on the principles of diffusion (movement of solutes from an area of higher concentration to lower concentration) and osmosis (movement of water across a membrane to equalize concentrations).
Hemodialysis: In hemodialysis, blood is circulated through an external dialysis machine where it is filtered, and the purified blood is returned to the patient.
Peritoneal Dialysis: Peritoneal dialysis involves the introduction of a dialysis solution into the abdominal cavity, where it absorbs waste products, and then it is drained out.
Lifesaving Treatment: Dialysis is a vital and lifesaving treatment for individuals with end-stage renal disease or acute kidney injury.

Both reverse osmosis and dialysis leverage the properties of semipermeable membranes to achieve purification and separation in different contexts, whether for water treatment or medical interventions in patients with kidney disorders

8. Match the following:

(a) Agitator	**(i) Breaking the vortex formation**
(b) Sparger	**(ii) Provides area for circulation of water of desired temperature**
(c) Baffle	**(iii) Helps in mixing the contents**
(d) Jacket	**(iv) Provides adequate and continuous supply of air**

Ans: Here's the matched pairing:

(a) Agitator: (iii) Helps in mixing the contents
(b) Sparger: (iv) Provides adequate and continuous supply of air
(c) Baffle: (i) Breaking the vortex formation
(d) Jacket: (ii) Provides an area for circulation of water of desired temperature

9. A culture in a closed vessel to which no additional medium is added is called _____ culture.

(a) Continuous
(b) Batch
(c) Fed-batch
(d) Semi continuous

Ans: (b) Batch.

10. Assertion: Secondary metabolites are used in defense against pathogens, phytoplanktons, improving tolerance to abiotic, etc.
Reason: Secondary metabolites are intermediate or indirect products.

(a) Both assertion and reason are true and the reason is the correct explanation of the assertion.
(b) Both assertion and reason are true but the reason is not the correct explanation of the assertion.
(c) Assertion is true but reason is false.

(d) Both assertion and reason are false.

Ans: (c) Assertion is true but reason is false.

SUMMARY

• There are various metabolic processes in living systems which are responsible for the synthesis of many metabolites which can be classified into primary and secondary.

• Primary metabolites are essential for the growth and development of living organisms whereas, secondary metabolites have diverse functions in defense system, tolerance to abiotic stress, etc.

• Secondary metabolites are used in many industries, such as pharmaceuticals, cosmetics, drugs, food additives, etc. However, these compounds are synthesized in very small amounts in the natural system. Therefore, efforts are being made to scale up the production of these beneficial metabolites using bioprocess engineering.

• Bioreactor or fermenter is an engineered vessel which may provide optimum conditions for the product formation and based on the requirement , different types of bioreactors may be configured in bioprocessing.

• Bioprocessing can be operated through two stages: upstream processing and downstream processing.

• In upstream processing, formulation and sterilisation of the media and equipment take place along with the production of pure, healthy and active culture for inoculation.

• The growth of organisms under optimum conditions for desired product formation takes place in a bioreactor or a fermenter.

• There are three modes of operations in bioprocessing: (i) batch (closed vessel system), (ii) (fed-batch) (growth limiting substrate is fed intermittently or continuously) and (iii) continuous (growth limiting substrate is fed continuously).

• In downstream processing, the product is recovered and purified using various techniques, such as reverse osmosis, distillation, drying, etc.

• Many desired products of animals, plants and microbial origin have been commercialised till date.