Industrial Microbiology
Six Steps to be followed up

Presented by

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Micro 566 Microbial biotechnology

Industrial Microbiology

I. Definitions
II. Fermentation Products
III. Environmental roles of microorganisms
IV. Microbiology and quality assurance
V. Identification of Microbes
VI. Microbial Indicator Species

I. Definitions

A. Industrial microbiology

1. The use of microorganisms to produce commercially valuable products
2. Industrial microbiology includes many areas, including food production, pharmaceuticals, fuel, bioremediation, and others

I. Definitions

B. Fermentation:

The term can be used in two contexts

1. In its broad context, "fermentation" means the growth of microorganisms for the purpose of manufacturing a product
2. In its narrow context, "fermentation" refers to a specific set of metabolic pathways in which pyruvic acid is reduced to form reduced waste products, with the regeneration of NAD for glycolysis

I. Definitions

C. Fermentation medium
   1. The medium or growth substrate on which the microorganism is grown
   2. Some processes may use crude organic components as media; others may required more purified substrates

I. Definitions

D. Producer microorganism
   1. The organism used for a particular process
   2. Some are naturally occurring strains; others have been modified through genetic manipulation

E. Trophophase
   1. The period of active growth of a microbe
   2. Equivalent to the logarithmic (exponential) growth period

I. Definitions

F. Idiophase
1. The period following trophophase, during which microbial biomass production has peaked and no new net biomass is produced
2. Equivalent to stationary phase

G. Primary metabolites
1. Microbial products produced during trophophase
2. Examples include amino acids, nucleotides, fermentation end products, and many types of enzymes

I. Definitions

H. Secondary metabolites
1. Products produced during idiophase
2. Examples include many antibiotics and mycotoxins

I. Fermenter
1. A vessel in which fermentation is carried out
2. The fermenter must include systems to regulate key growth requirements, such as nutrient addition, temperature, oxygen, and pH

I. Definitions

J. Upstream processing
   1. Components of the production system that occur prior to fermentation
   2. Includes cleaning, formulation of the medium, sterilization of the vessel and medium, adding the medium and organism to the vessel, etc.

I. Definitions

K. Downstream processing
   1. Components of the production system that occur after fermentation
   2. Includes harvesting and purification of the product, disposal or the waste, etc.
   3. Some products are intracellular, which means that the cells have to be harvested and lysed to release the product
   4. Other products are secreted into the medium, from which they may be purified

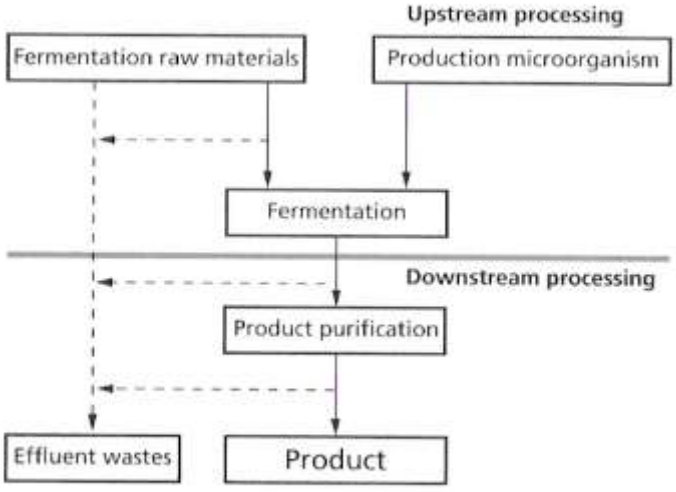

Fig. 1 Outline of a fermentation process.

II. Fermentation Products

- Foods, beverages, food additives, dietary supplements
  - Examples are alcoholic beverages, fermented dairy products and other fermented foods, yeast breads, organic acids used in food production (such as citric acid), amino acids and vitamins
  - Single-cell protein (SCP) is protein derived directly from microbial sources for animal or human consumption
  - Food manufacturers must adhere strictly to the "Generally Recognized as Safe" list (GRAS), which lists the ingredients and additives that are approved for foods & beverages in the US

II. Fermentation Products

B. Pharmaceuticals
   1. Antibiotics, alkaloids, steroids, vaccines
   2. Recombinant human proteins, such as insulin, growth hormone, and interferon

C. Microbial enzymes
   1. "Bulk"enzymes, such as hydrolytic enzymes, can be used with minimal DSP in partially purified form
   2. Other enzymes are highly purified for specialized purposes, such as restriction endonucleases

II. Fermentation Products

D. Industrial chemicals and fuels
   1. Alcohols, organic solvents such as acetone and butanol, organic acids, polysaccharides, and others
   2. Currently methane & ethanol are the main fuels from microbial sources, although there are other potential fuels that could be developed

III. Environmental Roles

A. Wastewater treatment
B. Biodegradation and bioremediation processes
C. Desulfurization of coal
D. Metal leaching
E. Microbe-based pest control

IV. Microbiology and Quality Assurance

A. Many different industries, such as food producers, pharmaceutical manufacturers, and hospitals, operate under very strictly controlled aseptic conditions
B. Quality assurance technologists routinely perform microbial testing to assure compliance with governmental regulations
C. Basic techniques of microbial isolation and identification are key components of microbiological QA

V. Identification of Microbes

A. Colony morphology
B. Cell shape & arrangement
C. Cell wall structure (Gram staining)
D. Special cellular structures
E. Biochemical characteristics

V. Identification of Microbes

F. Serological Tests
1. Use group specific antiserum isolated from the plasma of animals that have been sensitized to the organism
   a. The antiserum contains antibody proteins that react with antigens on the unknown organism.
   b. The reaction can be detected by examining agluttination or by using sera labeled with colorimetric or fluorescent labels

V. Identification of Microbes

F. Serological Tests (cont.)

1. Advantages:
   a. Highly specific
   b. Does not usually require the organism to be isolated into pure culture
   c. Can be used to identify organisms that can't be grown on medium

V. Identification of Microbes

G. Nucleic acid sequencing

1. Genes for specific enzymes
2. The nucleic acid sequence for the complete genome of several species is now available
3. 5S and 16S rRNA (ribosomal RNA) sequences; comparison of these sequences has been extensively used to determine the phylogenetic relationships of microbial groups

VI. Microbial Indicators

A. Microbial Indicators
1. Coliform bacteria are used as indicators of the presence of fecal contamination in water or food
2. *Staphylococcus aureus* is used as an indicator of contamination from human skin contact
3. Coliforms:
   a. Total Coliforms: Gram-negative, facultatively anaerobic, nonsporing, rod-shaped bacteria that ferment lactose with gas formation at 35°C
   b. Fecal Coliforms: Coliforms that can grow at 44.5°C

VI. Microbial Indicators

B. Methods for Detecting Coliforms
1. Most Probable Number (MPN) test
   a. Multiple dilution tubes of lactose or lauryl tryptose broth are inoculated with 10, 1, and 0.1 ml of a water sample, then incubated at 35°C for 24 hr
   b. Tubes that are positive for gas are used to inoculate brilliant green lactose bile broth tubes, which are incubated at 35°C for 48 hr
   c. Tubes that are positive for gas are further confirmed by streaking onto EMB or Endo agar
   d. Estimated value of Most Probable Number is determined from MPN tables

VI. Microbial Indicators

B. Methods for Detecting Coliforms

2. Membrane Filtration Technique
   a. Samples are filtered on 0.45 um filters and plated onto selective media at appropriate temp
   b. Total Coliforms: Endo medium at 35°C for 24 hr
   c. Fecal Coliforms: mFC medium at 44.5°C for 24 hr
   d. Fecal streptococci (enterococci): KFS medium at 35°C for 48 hr

VI. Microbial Indicators

B. Methods for Detecting Coliforms

3. Presence-absence (PA) test
   a. 100 ml of a water sample is cultured in a single bottle of lactose broth, lauryl tryptose broth, and bromocresol purple indicator
   b. Yellow color indicates a positive presumptive test & requires further confirmation

VI. Microbial Indicators

B. Methods for Detecting Coliforms
   4. Colilert MUG test
      a. 100 ml of sample added to MUG medium, containg ONPG and MUG
      b. Incubated for 24 hr at 35°C
      c. Yellow color indicates coliforms
      d. Examined under a long-wave UV lamp for fluorescence; this indicates presence of *E. coli*