BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI – DUBAI CAMPUS
DUBAI INTERNATIONAL ACADEMIC CITY
SECOND SEMESTER 2012 – 2013
BIOT C337 INDUSTRIAL MICROBIOLOGY & BIOPROCESS ENGINEERING
COMPREHENSIVE EXAMINATION (CLOSED BOOK)

Duration: 3hours. **Date: 3.6.2013** **Max. Marks: 30**

Note: a) Answer all the questions b) answer to the point and c) draw suitable diagrams if required.

1. Why microbes are important in bioprocess technology? Mention any one example each for a gram positive and gram negative microbe with significance in bioprocess applications. [2.0]
2. Why batch fermentations are most preferred over continuous fermentation using microbes? Briefly explain with advantages and disadvantages. [2.0]
3. What are the major components of a bioreactor and how the bioreactor is useful in the fermentation technology? Briefly explain with a suitable diagram. [2.0]
4. Why glucose is considered as the major substrate for industrial fermentations? Write a short note on different sources for glucose, end products and microbes which prefer glucose as the preferential substrate. [2.0]
5. Why the enrichment cultures are preferred in isolation of industrial microbes? Mention the method of isolation using this procedure and strain improvement methods. [1.5]
6. Why alternative sources of substrates for industrial fermentation are required? Give any two each for carbon and nitrogen sources, and mention the significance of yield coefficient. [2.0]
7. What are the major physico-chemical parameters mostly affect the optimum performance of a bioreactor? Briefly explain any two parameters for each with suitable diagrams.[3.0]
8. How the industrially important products expressed intra- and extracellular environments in a bacterial system are processed for industrial applications? Explain with suitable methods and examples. [3.0]
9. What are the major industrial enzymes produced in large scale for biotechnological applications? Briefly explain with any four examples and their applications. [2.0]
10. How microbes are used to produce fuels and industrial chemicals? Briefly explain with any one example for each. [2.0]
11. Why microbial steroid biotransformation is preferred? Give two examples of steroids and microbes employed with suitable explanations. [2.0]
12. Mention any one example each for microbially produced therapeutic peptides for cancer treatment, hormones, neurological disorder and vaccines. [2.0]
13. Write a short note on production of vinegar with the microbes involved, the method and mention applications. [1.5]
14. What are food additives and supplements? Give any two examples for each. [1.5]
15. Why microbial biomass production is important? Name any one method for large scale production of microbial biomass for biotechnological applications. [1.5]

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COMPREHENSIVE EXAMINATION (CLOSED BOOK)

Duration: 3hours. **Date: 3.6.2013** **Max. Marks: 30**

Note: a) Answer all the questions b) answer to the point and c) draw suitable diagrams if required.

1. Why microbes are important in bioprocess technology? Mention any one example each for a gram positive and gram negative microbe with significance in bioprocess applications. [2.0]
   Bacillus, pseudomonas, e.coli, proteins and fine chemicals.
2. Why batch fermentations are most preferred over continuous fermentation using microbes? Briefly explain with advantages and disadvantages. [2.0]
   Minimum contamination, pharmaceutical are produced in excess, economical.
3. What are the major components of a bioreactor and how the bioreactor is useful in the fermentation technology? Briefly explain with a suitable diagram. [2.0]
   Temperature controls; Blender; pH controls; aantifoam controls; harvesting module; sampling module; O2 controls; Aeration controls; Inoculation port; Nutrient input Cooling; Sterilization port/lamps
4. Why glucose is considered as the major substrate for industrial fermentations? Write a short note on different sources for glucose, end products and microbes which prefer glucose as the preferential substrate. [2.0]
   a. Pyruvate to lactic acid; lactate dehydrogenase; *Lactobacillus lactis*
   b. Pyruvate to ethanol; alhohol dedyrogenase; *Zymomonas*
   c. Pyruvate to 2, 3-butanediol; *Enterobacter, Erwinia; Klebsiella*
   d. Pyruvate to acetic acid; *E. coli*
5. Why the enrichment cultures are preferred in isolation of industrial microbes? Mention the method of isolation using this procedure and strain improvement methods. [1.5]
   Repress the common microbes, specific, allow target organism to grow using batch or preferentially continuous system, genetic engg, traits improvements.
6. Why alternative sources of substrates for industrial fermentation are required? Give any two each for carbon and nitrogen sources, and mention the significance of yield coefficient. [2.0]
   Malt, whey; y=biomass produced/substrate utilized
7. What are the major physico-chemical parameters mostly affect the optimum performance of a bioreactor? Briefly explain any two parameters for each with suitable diagrams.[3.0]
   a. Control of chemical and physical conditions by agitation and different methods.
   agitation, STR, Hydrodynamic
   b. Mass transfer.
   O2 transfer
8. How the industrially important products expressed intra- and extracellular environments in a bacterial system are processed for industrial applications? Explain with suitable methods and examples. [3.0]
   Centrifugation, filtration
9. What are the major industrial enzymes produced in large scale for biotechnological applications? Briefly explain with any four examples and their applications. [2.0]
   Protease; lipase; B. subtilis; A. oryzae

10. How microbes are used to produce fuels and industrial chemicals? Briefly explain with any one example for each. [2.0]
    Butanol, ethanol, aminoacids.
11. Why microbial steroid biotransformation is preferred? Give two examples of steroids and microbes employed with suitable explanations. [2.0]
    Inflammation, contraceptives, skin treatments, chemical-microbial, cheap transformation, eg. diosgenin from Mexican yam/stigmasterol from soy bean. Eg. aspergillus, fusarium, rhizopus, androgens, corticosteroids, oestrogens etc.
12. Mention any one example each for microbially produced therapeutic peptides for cancer treatment, hormones, neurological disorder and vaccines. [2.0]
    IL, IF, insulin, endorphins, neuropeptides, hep-b vaccines.
13. Write a short note on production of vinegar with the microbes involved, the method and mention applications. [1.5]
    Acetobacter sp., aeration, methods, additives
14. What are food additives and supplements? Give any two examples for each. [1.5]
    Stabilizers, extend shelf life, enzymes, emulsifiers, vitamins, acids
15. Why microbial biomass production is important? Name any one method for large scale production of microbial biomass for biotechnological applications. [1.5]
    Protein, animal fodder, proteins, biopesticides, bel/symba process.

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SECOND SEMESTER 2012 – 2013
BIOT C337 INDUSTRIAL MICROBIOLOGY & BIOPROCESS ENGINEERING
TEST-II (OPEN BOOK)

Duration: 50 min. **Date: 28.4.2013** **Max. Marks: 20**

Note: *Answer all the questions*
Answer to the point and draw suitable diagrams if required

1. Briefly explain on any four factors affecting the diffusion of oxygen in a bioreactor? How the oxygen diffusion is efficiently achieved in an industrial type bioreactor with suitable diagram. [3.0]

2. What are the major differences between the solid-substrate and suspended type bioreactor? Give any four applications in industry and pharmaceutical applications. List the advantages and disadvantages of each fermentation methods. [3.0]

3. Explain the principles of α-amylase production from *Bacillus subtilis* and develop a method for the large scale production strategies. Explain in detail on the methods to be selected for gram positive and gram negative bacteria. Provide suitable schematic diagrams. [4.0]

4. What are the methods employed for soluble and insoluble protein production? The objective is to obtain soluble protein component for pharmaceutical applications and how it is made finally as soluble proteins? [3.0]

5. Why the GMM are required in industrial microbiology and bioprocess engineering technology? What are the different methods, regulations and control mechanisms adopted for the GMM? [2.0]

6. How industrial enzymes are produced? Explain any one method of recycling of industrial enzymes with suitable diagram. [2.0]

7. How the enzymes activity is preserved in detergents though the detergents denature the proteins? Explain with your reasons and suitable diagrams. [3.0]

Ans. key.

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Duration: 50 min. **Date: 28.4.2013** **Max. Marks: 20**

Note: *Answer all the questions*
Answer to the point and draw suitable diagrams if required

1. Briefly explain on any four factors affecting the diffusion of oxygen in a bioreactor? How the oxygen diffusion is efficiently achieved in an industrial type bioreactor with suitable diagram. [3.0]
   temperature, pH, agitation etc, refer to agitation methods.

2. What are the major differences between the solid-substrate and suspended type bioreactor? Give any four applications in industry and pharmaceutical applications. List the advantages and disadvantages of each fermentation methods. [3.0]
   Solid state: enzyme production, use mostly low cost materials, ease of operation and conserve energy
   Suspended: biomass, enzymes, sensitive products, expensive, requires huge quantities of water for both upstream and downstream processing

3. Explain the principles of α-amylase production from *Bacillus subtilis* and develop a method for the large scale production strategies. Explain in detail on the methods to be selected for gram positive and gram negative bacteria. Provide suitable schematic diagrams. [4.0]
   Plasma membrane, cell wall, recombinant procedures difficult as well as the selection methods; screening for extra cellular and intracellular enzyme production, optimization for different parameters, and scale up studies using bioreactors
4. What are the methods employed for soluble and insoluble protein production? The objective is to obtain soluble protein component for pharmaceutical applications and how it is made finally as soluble proteins? [3.0]
   Culturing-harvesting-processing for cell lysis-addition of components for prevention of proteolysis-temperature control (+4°C)-suitable methods (mechanical/ enzymatic)-incubation- centrifugation/ ultrafiltration-concentration of proteins- (lyophilization/ precipitation)-dialysis to remove salts-concentration-addition of components to prevent oxidation-finishing-storage conditions.
5. Why the GMM are required in industrial microbiology and bioprocess engineering technology? What are the different methods, regulations and control mechanisms adopted for the GMM? [2.0]
   Containment; regulations; sterilization methods; QC; QA [refer text]
6. How industrial enzymes are produced? Explain any one method of recycling of industrial enzymes with suitable diagram. [2.0]
   6 classess of enzymes, key industrial enzymes, immobilization-entrapment methods-eg. Polyacrylamide gel, alginate, gelatin, glutaraldehyde.
7. How the enzymes activity is preserved in detergents though the detergents denature the proteins? Explain with your reasons and suitable diagrams. [3.0]
   Use of halophiles, thermophiles enzymes, enzyme stabilizers and carriers, added salts.

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TEST-I (CLOSED BOOK)

Duration: 50 min. **Date: 10.3.2013** **Max. Marks: 20**

Note: *Answer all the questions*
Answer to the point and draw suitable diagrams if required

1. Write a short note on the following with justification and aspects of industrial importance/products derived. [4.0]
   a. Archaeans
   b. Eubacteria-Gram negative
   c. Eubacteria-Gram positive
   d. Fungi-Filamentous

2. How heavy molecular materials are utilized by the industrial microbes? Briefly explain with suitable examples. [2.0]

3. Explain the following: (a) specific growth rate constant (μ) and (b) yield coefficient (Y). [2.0]

4. Write a short note on critical dilution rate (D_{crit}) with a suitable diagram. [2.0]

5. How the microbial growth in culture is monitored by using ATP bioluminometry? Give advantages and disadvantages of this method. [2.0]

6. Why control of microbial growth in industrial fermentations is required and how this is achieved? Give any two methods, advantages and disadvantages. [2.0]

7. What are secondary metabolites? Name any four secondary metabolites produced by fungi and the biosynthetic pathways involved. [2.0]

8. Name four fermentation products with name the substrate, enzyme and the microbes. [2.0]

$$S \xrightarrow{E} P$$

9. How the glyoxylate cycle is important in industrial microorganisms. [2.0]

Ans. key.

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TEST-I (CLOSED BOOK)

Duration: 50 min. **Date: 10.3.2013** **Max. Marks: 20**

Note: *Answer all the questions*
Answer to the point and draw suitable diagrams if required

1. Write a short note on the following with justification and aspects of industrial importance/products derived. [4.0]
   a. Archaeans: extremophiles; eg. Methanobacterium, methanococcus, pyrococcus, sulfococcus, thermoproteus; industrial enzymes.
   b. Eubacteria-Gram negative: Acetobacter, acinetobacter, agrobacterium, alcaligenes, Erwinia, E. coli, Thermus, thiobacillus. Molecular biology, enzymes, nostoc, spirulina.
      E.coli: model organism, heterologous proteins expression.
   c. Eubacteria-Gram positive: Actinomyces, Bacillus, Clostridium, Lactobacillus, Leuconostoc; industrial enzymes, lipases, proteases, amylases.
      B. subtilis: spore forming, enzymes, antibiotics eg. Bacitracin, gramicidin, polymyxin, insecticides
   d. Fungi-Filamentous: Aspergillus, Claviceps, Fusarium, Mucor, Rhizopus; enzymes hydrolytic. Mainly for the enzymes.
      Fungi-Yeasts: Candida, Pichia, Saccharomyces, Yarrowia; Enzymes, Fermentation technology.
2. How heavy molecular materials are utilized by the industrial microbes? Briefly explain with suitable examples. [2.0]
   Engulfment, phagocytosis, Carbon sources: Starch waste, molasses, whey, n-alkanes, gas oil, sulfite waste liquor, sewage, cellulose waste, carbon bean.
   Nitrogen sources: Soya meal, yeast extract, cottonseed extract, dried blood, corn steep liquor, fish soluble, groundnut meal.
   Carbohydrates-amylases
   Proteins-proteases
   Lipids-lipases
3. Explain the following: (a) specific growth rate constant (μ) and (b) yield coefficient (Y). [2.0]
   (a) specific growth rate constant (μ): measures how fast the cells are growing in turn the product formation/biomass [$\mu=1/x*dx/dt$; where x=concentration of biomass (g/L); μ=specific growth rate (per hour)
   (b) yield coefficient (Y): the yield coefficient relates to the quantity of biomass produced per gram of substrate utilized.
4. Write a short note on critical dilution rate (D_{crit}) with a suitable diagram. [2.0]
   The dilution rate, if increased above maximum specific growth rate, complete wash-out of the cells occurs, as the cells have insufficient time to double before being washed out of the reactor via the overflow. The point at which this is just avoided is referred to as critical dilution rate.
5. How the microbial growth in culture is monitored by using ATP bioluminometry? Explain with principle and give advantages and disadvantages of this method. [2.0]
   ATP bioluminometry

6. Why control of microbial growth in industrial fermentations is required and how this is achieved? Give any two methods, advantages and disadvantages. [2.0]
Irradiation, chemical, contamination.
7. What are secondary metabolites? Name any four secondary metabolites produced by fungi and the biosynthetic pathways involved. [2.0]
Ergot alkaloids-mixed biosynthetic origin
Griseofulvin-derived from 7-acetate units via polyketide pathways
Gallic acid-derived from shikimate pathways intermediates
Kojic acid-derived directly from glucose

8. Name four fermentation products with name the substrate, enzyme and the microbes. [2.0]

$$S \xrightarrow{E} P$$

a. Pyruvate to lactic acid; lactate dehydrogenase; *Lactobacillus lactis*
b. Pyruvate to ethanol; alhohol dedyrogenase; *Zymomonas*
c. Pyruvate to 2, 3-butanediol; *Enterobacter, Erwinia; Klebsiella*
d. Pyruvate to acetic acid; *E. coli*

9. How the glyoxylate cycle is important in industrial microorganisms. [1.0]
Utilize acetate units; link to gluconeogenesis; allow TCA cycle to continue

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QUIZ-I (CLOSED BOOK)

Duration: 20 min. **Date: 26.3.2013** **Max. Marks: 5**

Note: *Answer all the questions*
Answer to the point and draw suitable diagrams if required

1. What are enrichment cultures and mention the advantages of this method for isolation of industrial microbes? [1.0]

2. List any four ideal properties of an industrial strains for fermentation. [0.5]

3. What are eukaryotic hosts in fermentation technology and why it is required? Give any two examples and their applications. [1.0]

4. What are the different factors which affect selection of raw materials for fermentation and list any four? [1.0]

5. Give three examples for C and N sources as substrates? [0.5]

6. Differentiate inducers and elicitors. Give examples for each. [1.0]

Ans. key

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QUIZ-I (CLOSED BOOK)

Duration: 20 min. **Date: 26.3.2013** **Max. Marks: 5**

Note: *Answer all the questions*
Answer to the point and draw suitable diagrams if required

1. What are enrichment cultures and mention the advantages of this method for isolation of industrial microbes? [1.0]
   Isolation of microbes, specific

2. List any four ideal properties of an industrial strains for fermentation. [0.5]
   Substrate utilization, growth, toxin, separation

3. What are eukaryotic hosts in fermentation technology and why it is required? Give any two examples and their applications. [1.0]
   Yeast, pichia, PTM

4. What are the different factors which affect selection of raw materials for fermentation and list any four? [1.0]
   Cost, availability, etc

5. Give three examples for C and N sources as substrates? [0.5]
   Molasses, whey, etc

6. Differentiate inducers and elicitors. Give examples for each. [1.0]
   Secondary metabolite production, GMM