INDIAN INSTITUTE OF TECHNOLOGY, KHARAGPUR

Time : 2 Hrs.

Date of Examination.....FN/AN
Mid- Semester Exam (Autumn) 2016

Full Marks : 30
4th yr. B.Tech./Dual Degree
(Biotech. & Biochem. Eng.)

Subject No. BT 40009 37
Subject Name: **Bioprocess Technology**
No. of Students : ~~45~~ of the Department of Biotechnology
Instruction: Answer all questions. Assume suitable data, if required.

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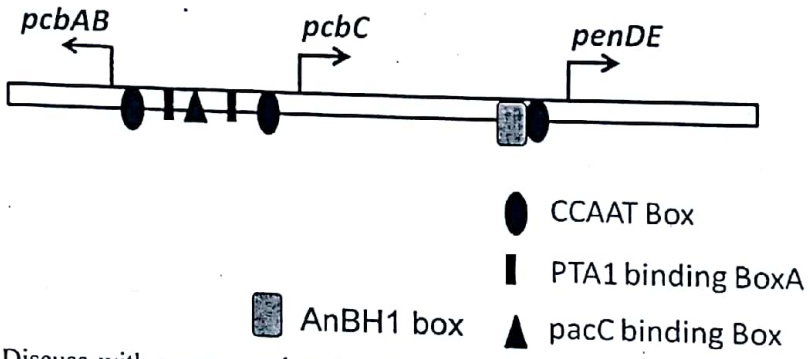
1. A) Using a flow diagram, briefly state the stages in penicillin production and recovery from fermentation broth. (2)

B) Given below is the penicillin gene cluster in *P.chrysogenum*. The intergenic regions are marked and important control elements are labelled.



Discuss with proper explanation, how the penicillin biosynthesis will be affected in each of the following hypothetical cases: (0.5x4=2)

I. penDE and pcbAB coding gene positions are interchanged, keeping the intergenic regions same and the pH of the growth medium is raised to 8.5 and AnBH1 is overexpressed.

II. Phosphorylation site on the response regulator is mutated and glucose is withdrawn from the growth medium.

III. Isolated pacC N-terminal domain is overexpressed in the given strain of *P.chrysogenum* and the cells were grown at a pH 8.

IV. AnBH1 binding to the AnBH box is reduced due to a point mutation and PENRI is overexpressed.

2. A) Disruption of glyceraldehyde-3-phosphate dehydrogenase gene or, addition of Lysine to the fermentation medium can improve production of clavulanic acid from *S. clavuligerus*. With proper explanation, justify the above statement.

B) Briefly write the steps involved in industrial production of Cephalosporin C from *A.chrysogenum*.

C) What enzymatic steps are involved in conversion of cephalosporin C to 7-amino cephalosporin C? (1+1.5+1 = 3.5)

3. (A) Write at least 4 bullet points to justify that the microbial cell factory (MCF) has advantages for commercial production of value-added compounds and industrial chemicals? (1)

(B) Elucidate at least 4 different strategies for improvement of microbial strain for industrial biotechnology. (1)

(C) (i) Mention the "conceptual strategies" in design & engineering of the pathway for MCFs. (ii) Mention at least 4 advantages of systems metabolic engineering for MCFs. (0.5+0.5)

(D) (i) Elucidate at least 4 different genetic regulations in MCFs to maximize the yield/ titer of the target product. (ii) Explain how the "chemically induced chromosomal evolution" helps in increasing the product yield/ titer in MCFs. (1+1)

(E) (i) MCFs can produce the building block monomers for eventual synthesis of industrially useful biopolymers. Mention four such acid monomers and the respective microbial strains that are used to produce them. (ii) What is the importance of polyhydroxyalkanoate (PHA)? (iii) Explain how the PHA can be produced in *Escherichia coli* using cheese industry waste by-product as substrate. (1+0.5+1)

4. (a) One Baker's yeast industry produces 1 MT of active dry yeast (containing 6% w/w moisture) per day using cane molasses as a raw material in a Chemostat. Cane molasses contains 50% (w/w) of sucrose. Concentration of the yeast in the fermentation broth is 10% w/v. Compute the followings

(i) volume of the fermenter;
(ii) total amount of cane molasses required;
(iii) write a block flow diagram of the process. (2+2+2)

Following date are given:

$\mu_{max} = 0.5\ h^{-1}$; $K_S = 2$ g/L; $Y_{x/S} = 0.5$ g cell mass/g sucrose

(b) Write are the characteristics of the industrial microorganism. Discuss the effect of chromium in the stainless steel. (1+0.5)

5. (i) What is 2^{nd} generation bioethanol? For 2^{nd}–Gen–bioethanol production, schematically illustrate the three major process steps. (1+1½ = 2½)

(ii) What is wet air oxidation in case of depolymerizatioon and delignification of biomass? How can its performance be improved by using alkaline peroxide? (1)

(iii) In immobilized enzyme reactors involved in SSF or SHF process for 2^{nd} Gen-bioethanol production, analyze the effect of external mass transfer on reaction and accordingly, recommend the best probable method of immobilization to mimic homogenous system behaviour in case of enzyme catalyzed biotransformations. (1 + 1 = 2)

(iv) In a typical bioethanol production facility, a medium containing 10 g L^{-1} of glucose was inoculated with Ho-Yeast. Glucose fermentation by yeast is described by the following stoichiometric equation: $C_6H_{12}O_6 \longrightarrow 2C_2H_6O + 2CO_2$. At the end of log phase, the residual glucose concentration was found to be 1 g L^{-1}, while the concentration of bioethanol was 3 g L^{-1}. Determine: (a) Fractional conversion of glucose to bioethanol and (b) Yield of bioethanol. (2)