# Department of Biosciences and Bioengineering
## BT 644
## Biorefineries

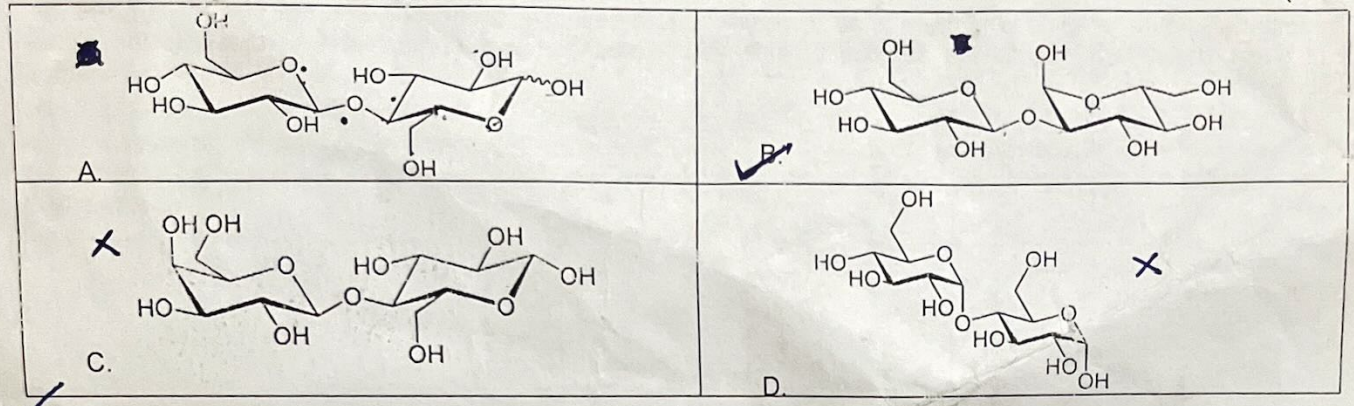
**Date:** 19/09/2023 **Mid Semester Examination** **Total Marks:** 30

### Answer ALL Questions

1. In spite of fewer by-products, dry milling is favoured over wet milling process for ethanol production. Why? (1 mark)

2. Pretreatment of hardwood with 1% sulphuric acid yielded 53.8, 21.11 and 6.01 (wt%) of cellulose, hemicellulose and lignin, respectively. Determine the holocellulose content of the pre-treated hardwood is exactly (in wt%). (1 mark)

3. A sample containing cellulose was digested using cellulase enzyme. From the structures given below, choose the correct dissacharide obtained by the enzyme action: (1 mark)




4. Match enzymes in Column A with their actions in Column B. (1 mark)

| A | B |
|---|---|
| Cellobiohydrolases | attack the chain ends of the cellulose polymers, liberating glucose (4) |
| Endoglucanases | hydrolyze soluble 1,6-β-d-glucans and cellobiose to glucose |
| β-glucosidases | attack the chain ends of the cellulose polymers, liberating the disaccharide cellobiose (1) |
| Cellodextrinases | decrease the degree of polymerization of macromolecular cellulose by breaking the linear cellulose chain (2) |
| | hydrolyze soluble 1,4-β-d-glucans and cellobiose to glucose (3) |

5. Liquid hot water pretreatment of rice straw is being studied in the laboratory. Suggest any three operation modes to carry out the pretreatment using a suitable reactor for scaling up the process. Show schematic in each case. (2 marks)

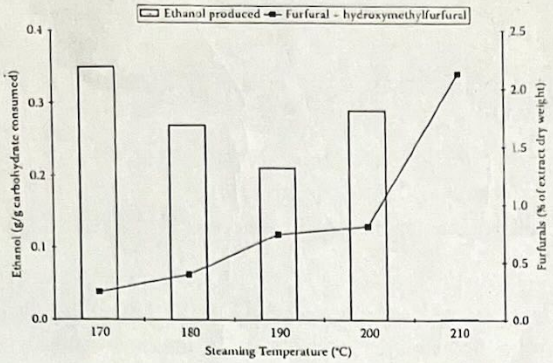
6. Briefly describe chemical method of ethanol production and mention some of its advantages over the biological method. (2 marks)

7. What causes stuck fermentation when using very gravity worts for ethanol production? How do you prevent and fix it? (2 marks)
   ↳ High sugar 15-16% by weight

8. Briefly discuss how production of ethanol can benefit from a multistage bioreactor system rather than a single stage chemostat system. (2 marks)

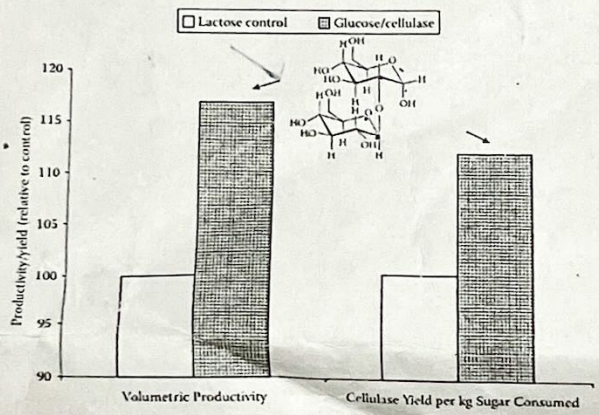
9. Mention at least four main features of the IOGEN Corporation process for bioethanol production. (2 marks)

10. The following figure shows the effect of steam pre-treatment of wood and furfurals generated on ethanol production by *Fusarium oxysporum*

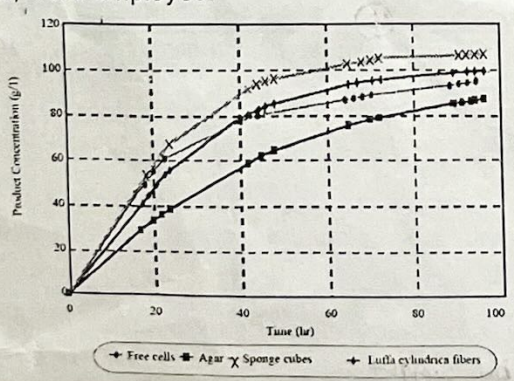



a) Why is ethanol production drastically low at a high temperature of 210 °C, whereas it is maximum at 170 °C? b) Mention how furfurals are generated from lignocellulosic biomass and their mode of action on the activity/metabolism of ethanologens. (2 marks)

11. In the following figure, explain why there is an increase in both cellulase production and the yield of enzyme per unit of sugar consumed due to the addition of cellulase-treated glucose, compared with that due to lactose addition. (2 marks)




12. The following figure compares the results of ethanol production by free or immobilized cells of *Saccharomyces cereveciae* under batch operated conditions. For cell immobilization three different supports, viz. agar, sponge cubes and luffa cylindrical fibres, were employed.




Changes in product concentrations against time using free and immobilized cells for batch processes. Agar, sponge cubes and luffa cylindrica fibers were used as immobilization materials.

Explain why ethanol concentration due to cells immobilized using sponge cubes or luffa cylindrical fibers is higher than that due to agar immobilized cells or free cells. Give valid reasons in support of your answer. (2 marks)

n ethanol

13. Size analysis was carried out on a sample of ground biomass. The data on mass fraction and average particle diameter of the fraction is given in the table below:

| **Weight fraction** | 0.2 | 0.4 | 0.4 |
|---|---|---|---|
| **Average particle diameter of the fraction (mm)** | 5 | 10 | 20 |

Calculate the mass mean diameter of the biomass sample in mm. (2 marks)

14. It is desired to degrade hemicellulose and lignin present in wheat straw by pretreatment employing the steam explosion technique with a severity factor of 3.94. Determine the incubation time required to pretreat the biomass at 200 °C temperature. (2 marks)

15. Estimate the theoretical growth and product yield coefficients for ethanol fermentation by *S. cerevisiae* as described by the following reaction:

$$C_6H_{12}O_6 \longrightarrow 2\,C_2H_5OH + 2\,CO_2$$

Assume that the $Y_{X/ATP}$ is 10.5 gdw/mol ATP and glycolysis yields 2 ATP/mole of glucose in yeast. (2 marks)

16. *Zymomonas mobilis* is used to convert cellulose derived from lignocellulose to ethanol in a batch fermenter under anaerobic conditions. The yield of biomass from substrate ($Y_{XS}$) is 0.06 g/g and $Y_{PX}$ is 7.7 g/g. The maintenance coefficient due to substrate ($m_s$) is 2.2 g/g·h, the specific rate of product formation ($q_p$) is 3.4 $h^{-1}$. The maximum specific growth rate of *Z. mobilis* is approximately 0.3 $h^{-1}$. 5 g bacteria are inoculated into 50 L of medium containing 12 g/L glucose. Determine batch culture time required (in hours) to produce (a) 10 g biomass and (b) 100 g ethanol. (4 marks)

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$\ln = \text{lag}\left(1 + e^{\cdot \frac{1 - 100}{14 \cdot 95}}\right)$