CHEM-E3140 Bioprocess technology II, exam 8.12.2015 (choose **6** questions from **7** alternatives)

1. Describe how the active site of glycoside hydrolases is functioning (5p)

2. Describe Michaelis-Menten and Briggs-Haldane kinetics theories with their similarities and differences (5p)

3. Immobilization of enzymes (5p)

4. Answer shortly with 1-3 lines, (1p each):

a) Dimensionless operation time
b) Carrier free immobilization
c) What are the advantages of AlkOx process?
d) Mention three different functions of lignin in vascular plants
e) Mention three different applications for lignin
f) What is the difference between genomic and cDNA libraries?
g) Define high throughput screening.
h) What bonds are cleaved by proteases?
i) What does nucleophile in enzymatic catalysis?
j) Define deep eutectic solvent (DES).

5. Choose the right alternative. NB! The correct answer +0.5 p, wrong answer -0.5 p, no answer 0 p.

Reaction rate may decrease due to substrate desaturation, enzyme inactivation, equilibrium displacement or product inhibition	Yes	No
Industrial enzyme production in 2014 was worth about 7,2 billion euros	Yes	No
Cellulases and glucose isomerases are hydrolases	Yes	No
The temperature that maximizes enzyme activity can be harmful for the stability	Yes	No
pH affects strongly the enzymes ionization stage in nonaqueous media	Yes	No
The immobilization costs can hinder the use of immobilization	Yes	No
The largest share of the enzyme market comprises immobilized enzymes	Yes	No
Glucose isomerase is the most widely used (ton per year) immobilized enzyme	Yes	No
1 kg of immobilized penicillin G amidase can produce in its operational life-time 1-2 tons of 6-APA	Yes	No
Sugar alcohols like xylitol, sorbitol, arabitol and mannitol act as activators for glucose isomerase	Yes	No
AlkOx process side products are sulphur free lignin and organic acids	Yes	No
White rot fungi secrete lignin modifying enzymes: laccase, manganese peroxidase, and versatile glucose isomerases	Yes	No
The hydrophobic adsorption of enzymes to lignin induces denaturation of enzymes on lignin surfaces	Yes	No
The structure of lignin changes from one feedstock to another and during the lignocellulose pretreatment	Yes	No
Protein "mutagenesis" means in immobilization substitution of surface-exposed amino acids with new amino acids	Yes	No
Microporous immobilization support material has a minimum of 50 nm pore diameter	Yes	No
Difference between the temperature optimum of cellulolytic enzymes and that of the fermenting micro-organisms is usually insignificant	Yes	No
Almost all *Trichoderma reesei* cellulases have catalytic domain and cellulose binding domain	Yes	No
Biocatalysts that work efficiently in neat organic solvents are lipases and proteases	Yes	No
Disadvantages of using membrane reactors with lignocellulose material are e.g. low concentration of glucose in the permeate and continuous removal of products	Yes	No

6. *Mycobaterium smegnatis* bacterial L-lactate2-mono-oxygenase, catalyzing lactic acid oxygenation to acetic acid, is immobilized in spherical agarose beads:

$CH_3CH(OH)COOH + O_2 \rightarrow CH_3COOH + CO_2 + H_2O$

Beads of 4 mm in diameter are immersed in a well-mixed solution containing 0,8 mM oxygen. A high lactic acid concentration is provided so that oxygen is the rate limiting substrate. The effective diffusivity of oxygen in agarose is $2{,}1 \cdot 10^{-9}$ $m^2 s^{-1}$. K_M for the immobilized enzyme is 0,013 mM, activity is 0,11 mmol s^{-1} g_{enzyme}^{-1}. The beads contain 0,012 kg enzyme per m^3 agarose gel.

a) Estimate oxygenation reaction order from substrate concentration and K_M- value.
b) Determine maximal reaction rate per immobilized agarose-m^3
c) Determine the largest bead size that allows the maximum conversion rate i.e. oxygen level remains above zero inside the beads
d) Plot the oxygen concentration profile inside the beads (5 p)

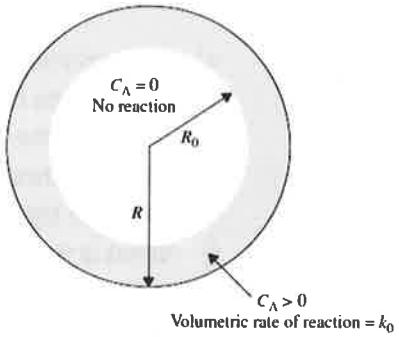

TABLE 13.1 Steady-State Concentration Profiles

First-order reaction: $r_A = k_1 C_A$	
Sphere[a]	$C_A = C_{As}\frac{R}{r}\frac{\sinh(r\sqrt{k_1/\mathscr{D}_{Ae}})}{\sinh(R\sqrt{k_1/\mathscr{D}_{Ae}})}$
Flat plate[b]	$C_A = C_{As}\frac{\cosh(z\sqrt{k_1/\mathscr{D}_{Ae}})}{\cosh(b\sqrt{k_1/\mathscr{D}_{Ae}})}$
Zero-order reaction: $r_A = k_0$	
Sphere[c]	$C_A = C_{As} + \frac{k_0}{6\mathscr{D}_{Ae}}(r^2 - R^2)$
Flat plate[c]	$C_A = C_{As} + \frac{k_0}{2\mathscr{D}_{Ae}}(z^2 - b^2)$

7. Assuming that you can use Michaelis-Menten kinetics and it's integrated formula:

$$\int_{s}^{s_i} \frac{K_M + s}{s} ds = \int_{0}^{t} k \cdot e \cdot dt \qquad (2.53)$$

$$K_M \cdot ln\frac{s_i}{s} + (s_i - s) = k \cdot e \cdot t \qquad (2.54)$$

Invertase is added at a level of 1 g/L to a 60mM sucrose solution. The kinetic parameters of the enzyme under the conditions of reaction are: K_M = 4 mM and V_{max} = 2000 µmoles/(min g_{enzyme}). Define the substrate concentration function f(s) and iterate how much the sucrose concentration is at the time point 20 minutes? You may start initial guess between with a value between 10-40 mM. (5p)

To solve the concentration level at different time points you can utilize Newton's iteration method. Consider the following equation:

$g(x) = a$

The aim of the Newton's method is to find the value of x for which the nonlinear function g(x) is equal to the constant a. For this purpose, equation is rewritten as its equivalent:

$f(x)=g(x) - a = 0$

To determine the value of x for which f(x) is equal to zero, the function f(x) is linearized and x can be calculated:

$x = x_0 - (f(x_0)*\Delta x) / (f(x_0+\Delta x)-f(x_0))$

The relative error (E) obtained in the nth iteration is estimated as follows: $E=x/(x-x_0)$