

April 12, 2011

ANSWER SHEET - Problem 1 All about beer

| Country: | |
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| Team: | |

| Student's name | Signature |
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A. Yeast and fermentation

TASK A.I: YEASTS

- A.I.1 Which of the following statements are true about yeast genome? Circle correct statements.
- A Yeast genome is localized on 1 circular chromosome
- B Yeast genes do not contain introns.
- C Yeast genome contains many genes homologous to the human genes.
- D Yeast genome is permanently haploidic

A.I.2 What is a prerequisite for *S. cerevisiae* to become an optimal model organism? Circle correct statements.

- A *S. cerevisiae* is small unicellular organism with a short generation time (doubling time 1.25–2 hours at 30 °C) and can be easily cultured.
- B S. cerevisiae can be transformed allowing for either the addition of new genes or deletion through homologous recombination.
- C As a eukaryote, *S. cerevisiae* shares the complex internal cell structure of plants and animals.
- D S. cerevisiae research is a strong economic driver, at least initially, as a result of its established use in industry.

TASK A.II: ETHANOL FERMENTATION

- A.II.1 Which from the following statements is true about glycolysis? Circle correct statements.
- A One of the waste products is water
- B Ten ADP molecules (per one glucose molecule) are converted to ten ATP molecules
- C Glycolysis is typical for eukaryotes
- D Glycolysis takes place in mitochondria, where pyruvic acid as the end product can directly enter the Krebs cycle

A.II.2 What is responsible for rising of the bread dough? Circle correct statements.

1 pt

- A During fermentation process baker yeast produces heat, water gets evaporated and form bubbles in the dough
- B Baker yeast produce carbon dioxide as waste product, which forms bubbles in the dough
- C Baker yeast produce ethanol and heat as waste product. Evaporated ethanol forms bubbles in the dough.
- D Bubbles inside the dough are empty spaces where yeast locally consumed all the dough material, the dough expands because yeast divides many times and form substantial part of the dough mass



- A.II.3 Which technology is used for transformation of starch to simple sugars in brewery? Circle correct statements.
- A Grain kernels that have been germinated are rich source of enzyme amylase which does the job.
- B Grain kernels are heated and high temperature treatment cleaves the starch to fermentable sugars
- C Enzyme amylase is produced in bacteria, isolated and are used for starch treatment
- D Grain kernels are pre-treated with starch splitting bacteria or yeast and then inoculated with ethanol producing yeast strain
- A.II.4 Write two chemical equations when sucrose is transformed into an ethanol via glucose and fructose first (than to ethanol and CO₂). 2 pts

 $C_{12}H_{22}O_{11} + H_2O \rightarrow 2 C_6H_{12}O_6$ 342 + 18 = 2x180

 $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$ $180 = 2 \times 46 + 2 \times 44$ 180 = 92 + 88

 $\textbf{A.II.5} \ \ \textbf{Write the summary chemical equation of sucrose transformation into an ethanol.}$

1 n

 $C_{12}H_{22}O_{11} + H_2O \rightarrow 4C_2H_5OH + 4CO_2$

A.II.6 How much ethanol can be theoretically produced from 1 kg of sugar-beet storage roots? Write your calculation. 2 pts

342 / 184 = 0.54 kg of ethanol from 1 kg of sucrose, x 0,2 (20% sucrose content) = 0,108 kg

- A.II.7 Why is not possible to reach the theoretical maximal sucrose transformation efficiency using fermenting microorganisms? Circle the correct answer. More than 1 answer could be true!
- A some carbon is released in a form of CO₂
- B some sucrose will stay in the solution unfermented, because ethanol will block fermentation process
- C some carbon will end up in the macromolecules allowing the microorganism to grow and divide
- D reactants are never 100% transformed into products



A.II.8 What percentage of the total land should be used as sugar-beet field, if Czech Republic, with energy consumption of 496 TWh per year, will decide to cover all the energy from the sugar-beet sucrose via ethanol production? Please, do not include into your calculation extra energy you have to spend for sugar-beet production. Write your calculations.

5 pts

 $0,\!108$ kg sucrose/1 kg of sugar-beet roots corresponds to $0,\!1125$ kg of 96% ethanol solution and therefore $0,\!143$ litres of 96% ethanol solution

from $10\ 000\ m2 - 60\ 000\ kg$ tubers

1m2 - 6 kg tubers - 0,858 litres of 96% ethanol solution - 18,447 MJ - 1,8447.107 J

Czech Republic needs per annum 496.1012x3600 J of energy - 1 785 600.1012 J - 1,785.1018 J

It corresponds to 0.968.1011 m2 - 0.968.105 km2 - 96 800 km2

We need an extra land – at least 23%!!! – result is 123%

A.II.9 What percentage of total land should be used as sugar-beet field, if Czech Republic decide to fuel all the cars with ethanol produced from sugar-beet sucrose? Please, do not include into your calculation extra energy you have to spend for sugar-beet production. Write your calculations.

2 pts

CR annual petrol/diesel consumption is $5.106 \times 103 \text{ kg} = 5.109 \text{ kg} = 6,37.109 \text{ litres}$

It contains 6,37.1010x3,2.107 J = 20,384.1017J energy

From 1m2 we can get 1,845 .107 energy – we need 1,105.1010 m2 of the land – 11 050 km2

It corresponds to 14% of the CR total area.

A.II.10 How many kilograms of matter and antimatter together you have to annihilate to obtain energy equivalent of energy annually spent in the Czech Republic? Write your calculations.

2 pts

E=m.c² E=1,785.10¹⁸ J, M=?, c= 3.10^8 m/s m=E/c² $178,5.10^{16}/9.10^{16}$ 19,8 kg is enough.



| A. II.11. !!! In a spare time only – you compete for the special prize!!! Imagine that you have the power to transform the whole Czech Republic into shape optimal to satisfy it's energy consumption with sugar-beet production. To make the task simpler - CR in your model is an island with square shape. You can perform any change you can imagine. |
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| Draw an image of the optimal shape in the box and describe the sizes in km units. |
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| There are plenty of correct answers! |
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TASK A.III: ETHANOL TOLLERANCE

| of cthanol A, 10% concentration of ethanol B and 20% concentration of ethanol C. Don't hurry with the decision, wait at least 60 min. Write down how long did you let the yeast ferment (in minutes). 3 pts A.III.2 Try to quantify the katabolic activity (as a measure of CO ₂ production in different solutions according the TASK C.II.) Katabolic activity in flask A count as a 100%. A.B. C. | A.III.1 | Observe and draw the results in the appropriate box. Mark the 0 concentration | |
|---|---------|--|----------------------|
| A.III.2 Try to quantify the katabolic activity (as a measure of CO ₂ production in different solutions according the TASK C.II.) Katabolic activity in flask A count as a 100%. | | of ethanol A, 10% concentration of ethanol B and 20% concentration of | ikahih 11/4/11 14·17 |
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| A III 3 | Which metabolites of sucrose catabolism will be generated in three different |
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| A.111.5 | growth conditions A-C (use chemical formulae) 1.5 pts |
| | |
| A | |
| В | |
| C | |
| | |
| A.III.4 | Which type/types of metabolism you expect at the end of your experiment in three different growth conditions A-C? Use abbreviations AE for aerobic and AN for anaerobic 1.5 pts |
| A | |
| В | |
| C | |
| | |
| A.III.5 | What will be major limitation for the growth of yeast in three different growth conditions A-C? Use abbreviation O for O ₂ , S for sucrose, C for CO ₂ , E for ethanol and T for temperature. 1.5 pts |
| A | |
| В | |
| С | |
| | |



TASK A.IV: YEAST DOMESTICATION

| A.IV.1 Identify the wild-type, domesticated and intermediately domesticated strains and | | | |
|---|--------------|--|--|
| draw typical examples of selected morphologies. Use the lowest magnification of | | | |
| the microscope and/or magnifying glass. 3 pts | | | |
| wild-type | domesticated | | |
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| A.IV.3 Identify the patterns corresponding to the wild colonies and domesticated ones and draw typical examples. 4 pts | | |
|---|--------------|--|
| wild-type | domesticated | |
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A.IV.4 Which statement/statements about domesticated vs. wild-type yeast cells and corresponding colonies is/are true? Circle correct statements. 1 pt

- A Domesticated colonies are more complex, because selection pressure in energy rich conditions drives cooperative behaviour between individual cells.
- B In wild-type colonies, grown from the yeast isolated from the real environment, cells differentiate into specialized subsets, optimized for particular duties. Colony is therefore more complex and structured.
- C Under optimal conditions organisms tend to lose some traits, in our situation ability to form complex structured colony, which is of no use in the liquid substrate or baker's dough
- D Domesticated colonies are smooth and lack structural complexity and are formed from cells without ability to differentiate into specialized subsets



B. Iodometric determination of reducing sugars

TASK B.I: STANDARDISATION OF 0.1 M Na₂S₂O₃ SOLUTION

| B.I.1 Record the volume of standardized 0.1 M sodium thiosulfate used. 5 pts. | | | | |
|---|----|----|----|-------|
| titration number | 1. | 2. | 3. | mean: |
| Na ₂ S ₂ O ₃ consumption (mL) | | | | |

| B.I.2 Calculate the concentration of Na ₂ S ₂ O ₃ solution. | 2 pts. |
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| Calculations: | |
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| Concentration of Na ₂ S ₂ O ₃ solution is: | |



TASK B.II: ANALYSIS OF GLUCOSE SAMPLE

| B.II.1 Record the volume of standardized 0.1 M sodium thiosulfate used. 20 pts. | | | | |
|---|----|----|----|-------|
| titration number | 1. | 2. | 3. | mean: |
| Na ₂ S ₂ O ₃ consumption* (mL) | | | | |

^{*} present the consumption before the subtraction of blank sample

| B.II.2 Calculate the content of glucose in the sample. | 3 pts. |
|--|--------|
| Calculations: | |
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| Content of glucose in sample is: | |

TASK B.III: SUPPLEMENTARY QUESTIONS

B.III.1 Write equations describing the reaction of iodine with the following compounds: 3 pts.

a) HCHO (formaldehyde)

$$\text{HCHO} + \text{I}_2 + 3 \text{ OH}^- \rightarrow \text{HCOO}^- + 2 \text{ } \Gamma + 2 \text{ H}_2\text{O}$$

b) CH₃COCH₃ (acetone)

$$CH_3COCH_3 + 3~I_2 + 4~OH^- \rightarrow CHI_3 + CH_3COO^- + 3~I^- + 3~H_2O$$

c) L-ascorbic acid

L-ascorbic acid + $I_2 \rightarrow$ L-dehydroascorbic acid + 2 HI

- B.III.2What is characteristic for the structure of glucose molecule? Which physicochemical property results from the structure of glucose molecule and how is the instrumental method for the determination of such substances called? Circle one of the following answers.
- A Conjugated system of bonds; absorption of UV light; UV spectrometry
- B Asymmetric (chiral) carbon; optical rotation; polarimetry
- C Charged centre; electrical conductivity, voltammetry
- D Ester groups; volatility; gas chromatography
- B.III.3 How many millilitres of 0.0923 M $Na_2S_2O_3$ are consumed in the titration of iodine released from a chromic acid solution and potassium iodide, when the same volume of chromic acid solution for alkalimetric titration was consumed 18.92 mL of 0.1006 M NaOH?

The determinations are described by following reactions:

2
$$\text{CrO}_4^{2-}$$
 + 6 Γ + 16 H^+ \rightarrow 2 Cr^{3+} + 3 I_2 + 8 H_2O
 I_2 + 2 $\text{S}_2\text{O}_3^{2-}$ \rightarrow $\text{S}_4\text{O}_6^{2-}$ + 2 Γ

$$H_2CrO_4 + 2 NaOH \rightarrow Na_2CrO_4 + 2 H_2O$$

18.92 ml of 0.1006 M NaOH = 1.903 mmol NaOH

 $1.903 \ mmol \ NaOH \sim 0.9517 \ mmol \ H_2CrO_4 \sim 1.428 \ mmol \ I_2 \sim 2.855 \ mmol \ Na_2S_2O_3$

 $2.855 \text{ mmol of } 0.0923 \text{ M Na}_2\text{S}_2\text{O}_3 = 30.93 \text{ mL}$



C. Density of beer, CO₂ production

TASK C.I: MEASURE DENSITY AND DETERMINE DEGREE OF BEER

| C.I.1 Rec | Record your measurements to the following table 5 pts. | | | | | |
|-------------------------|--|--|--|---|--|--|
| Number of measur. | m ₀ (g) mass of empty pycnometer | m _{wp} (g) mass of the pycnometer filled with water | m _{Ip} (g) mass of the pycnometer with light beer | m _{2p} (g) mass of the pycnometer with dark beer | | |
| 1 | 152 | 347 | 349 | 350 | | |
| 2 | 153 | 347 | 349 | 350 | | |
| 3 | 153 | 347 | 349 | 350 | | |
| 4 | 153 | 347 | 349 | 350 | | |
| 5 | 152 | 347 | 349 | 350 | | |
| Mean | 152,6 | 347 | 349 | 350 | | |

C.I.2 Calculate the mass of distilled water in the pycnometer (denote it by m_w), the mass of the light beer sample in the pycnometer (m_1) and the mass of the dark beer sample in the pycnometer (m_2) . Write the expressions in terms of m_0 , m_{wp} , m_{Ip} and m_{Ip} and the value. Do not forget to write unit!

$$m_w = m_{wp} - m_p = 194.4 \text{ g}$$

 $m_I = m_{Ip} - m_p = 196.4 \text{ g}$
 $m_2 = m_{2p} - m_p = 197.4 \text{ g}$

C.I.3 Copy the lab temperature (t) from the white-board to the Answer sheet. Use Graph 1 to determine the density (ρ_w) of distilled water at the temperature t. Calculate the volume (V_w) of the distilled water in the pycnometer, write the formula for the volume and the value with units.

$$t = 22^{\circ}\text{C}$$

$$\rho_{w} = 998 \text{ kg m}^{-3} \qquad 1 \text{ pt.}$$

$$V_{w} = m_{w} / \rho_{w} = 0,1948 \text{ m}^{3} \qquad 2 \text{ pts.}$$

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| | n terms of the mass of the | |
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| sample and the volume V_w . Calculate the density of the | | |
| the dark beer sample (ρ_2). Do not forget to state correct | | 1. 44/4/4 00 54 |
| | | b 11/4/11 00:54 |
| $\rho = \underline{m/V}_{\underline{W_{\mathbf{A}}}}$ | kleu | opmaak: Tekstkleur: Aangepaste r(RGB(0;112;192)) |
| $\rho_1 = 1008 \mathrm{kg/m^3}$ | | b 10/4/11 23:54 |
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| | | b 10/4/11 23:55 |
| $\rho_2 = \underline{1013 \text{ kg/m}^3}$ | | opmaak: Niet onderstrepen, stkleur: Rood |
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| C.I.5 Calculate the volume fraction of the alcohol and the | | |
| sample and the degree of the dark beer sample. The d | | |
| the light beer is $\rho_{1hw} = 1040 \text{ kg/m}^3$ and for the dark beer | | |
| | 3 pts. | ib 11/4/11 00:55 |
| Alcohol volume fraction of the light beer: 4.13% | | b 11/4/11 00:55 opmaak: Lettertype:12 pt, Vet, |
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| Degree of the light beer: 10 | | b 10/4/11 23:56 |
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| Alcohol volume fraction of the dark beer: 8.99% | ikgh | ib 10/4/11 23:56 |
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TASK C.II: QUANTITATIVE ESTIMATION OF CO₂ PRODUCTION BY YEAST

C.II.1 Weigh all sugar cubes together.

Total mass of the sugar is: 9 g 1 pt

C.II.2 Calculate the lab thermodynamic temperature.

T = 295 K 1 pt

| | C.II.3 Fil | .II.3 Fill the following table | | | | | | 5 pts. | | |
|---|------------------|--------------------------------|-------------|------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| 1 | Time (min) | <u>4</u> 0 | <u>5</u> ,0 | <u>6</u> 0 | <u>7</u> ,0 | <u>8</u> 0 | <u>9</u> 0 | 100 | 110 | 120 |
| ĺ | d (cm) | <u>3</u> | 3.3 | 3.6 | 3.8 | 4.0 | 4.3 | 4.6 | 4.8 | 5.0 |
| ĺ | $V(\text{cm}^3)$ | 14.1 | 18.8 | 24.4 | 28.7 | 33.5 | 41.6 | 50.9 | 57.9 | 65.4 |
| | p (kPa) | 109.3 | 108.6 | 108.0 | 107.6 | 107.3 | 106.9 | 106.5 | 106.3 | 106.1 |
| 1 | n (mol) | 6.10-4 | 8.10-4 | 1.0.10-3 | 1.2·10 ⁻³ | 1.5·10 ⁻³ | 1.8·10 ⁻³ | 2.2·10 ⁻³ | 2.5·10 ⁻³ | 2.8·10 ⁻³ |

C.II.4 Derive the formula for the carbon dioxide amount of substance by terms of the balloon diameter, lab thermodynamic temperature and constants. 2 pts.

Calculations:

Atmospheric pressure $p_a = 101.325 \text{ kPa}$

Formula for the gas volume: $V = \pi d^3/6$

Formula for the gas pressure: $p = p_a + 2C/d$

Formula for the amount of substance: $n = (p_a + 2C/D)\pi d^3/(6RT)$

| , | |
|---|------|
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DO NOT FORGET TO INCLUDE YOUR GRAPHS TO ANSWER SHEET! 6 pts,

C.II.5 What is the maximal possible amount of substance of carbon dioxide, if you suppose that all sucrose was converted to alcohol and carbon dioxide? Use the results of the TASK A.II.

3 pts,

Calculations:

9 g of sucrose correspond to 0,027 mol. From each mol of sucrose yeast produces 4 mol of CO₂. Thus we could get at most 0,1 mol of carbon dioxide.

Maximal possible amount of substance is: 0.1 mol

THAT'S ALL!

CONGRATULATIONS!

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ikahih 11/4/11 01:00

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