April 12, 2011

PROBLEM 1

All about beer

General instructions

4 Hours

Be aware, practical part should take more time than theoretical!

Wear a laboratory coat and safety glasses at all times within the laboratory.

Eating and drinking is prohibited in the laboratory.

Disposable gloves are provided and must be worn when handling chemicals.

Use just the pen, pencil and calculator provided.

All paper used, including rough work paper, must be handed in at the end of the experiment.

All results must be entered into your answer sheet.

Your calculations must be handed in along with the answer sheet.

Only the final answer book, and attached sheets, will be marked.

The tasks may be carried out in whatever order you wish.

When you finish your tasks, leave everything on the desk. You are not allowed to take anything from the laboratory.

Introduction

Every nation has its own history. Each nation also has its traditional food and drink. While the classical Czech dumplings and sauerkraut, pork, slowly begin to retreat into the background, dominating drink beer does not lose popularity at all. Czech beer is not only drink. It is also a source of national pride. In Bohemia, you can criticize everything, except the Czech beer!

Beer is not a new invention. It was for sure produced at least 10 000 years ago - we can trace beginnings of its fame to the ancient Egypt. It was not a beer in the modern sense. One most famous beer ingredient was missing these days – the hops. It is not entirely clear where and when the hops-supplemented beer first emerged, but this type of production was immediately used by the Slavic tribes.

In the dark middle-Ages beer was brewed in Bohemia mainly in monasteries, or in their vicinity. Breweries have already been documented from the 10th century. We have to stress however, that medieval beer was not identical with the modern one. The taste difference for experienced beer-drinkers, compared to the modern beer disappeared in the 18th century.

The Czech beer is brewed today by 38 companies. The most beer is produced by consortium Pilsner Urquell, just behind are companies Staropramen and Budweiser. Czechs are not just good at brewing beer, but exceptionally successful in drinking it. In the beer drinking Czechs keep for the long time the first place in the world. Consumers in the Czech Republic have a selection of 470 beer brands! So the Czechs are a nation of pub-culture. In the Czech pub most of the social strata meets in equal and friendly terms. Czech Inn is the universal meeting place, people date there and even trade partners sign important contracts during the informal pub appointments…

Here our story begins.

(Adapted by Michael A. Cotter from the story "Small beer on the Bummel" by Petra Kněžíková)

Once upon a time, a long time ago, in a far off country, Mr. & Mrs. Beerbellow lived happily in a small box with their ten children. One day Smallbeer, the youngest son said, "I don't want to live in this crammed little small box anymore. I'm leaving now for the big city of Pardubice" He packed his six hops into his bag and set off alone to seek his fortune.

Many weeks later he arrived at the kingdom of Euscoly2011. He was surprised to find that all the Kind's subjects were in mourning. Smallbeer asked a servant girl he met in the street why everyone was so sad and gloomy. She told him that Her Royal Highness, Princess Clear-Plum-Brandy, the only child of the King and Queen, was in very great danger and no-one knew how to save her. The King had offered his only daughter's hand in marriage to the brave bottle who saved the Princess from Seven-headed Charles Drunkard who lived in a cave outside the town and had vowed to drink her. The King's subjects gave Seven-headed Charles Drunkard many different kinds of alcohol to save the princess, but Seven-headed Charles Drunkard simply drank them down and asked for more.

Smallbeer went to the hotel Hurkabice, which was full of bright young bottles from many different countries all eager to save the Princess from being drunk up. After dinner he went for a walk to the castle, where, to his surprise he saw the Princess at a window. She was beautiful, pure, six x distilled and her bottle glistened in the evening sunlight. They looked at each other and it was love at first sight. Smallbeer decided that he had to save the Princess without sacrificing himself.

He walked up to the castle door and asked the see the King. He said he had a plan to save the Princess. The King was desperate and agreed to see him. He told the King his plan.
"If you succeed you will be a Prince, marry my only daughter and be the heir to my throne; But if you fail, I will toss you into the raging, fast flowing river below and you will be swept over the waterfall, smashed into smithereens on the rocks below, never to be seen again," said the King.

Smallbeer was so much in love with the Princess that he was willing to risk his life for her. That evening, after the pubs closed, as Seven-headed Charles Drunkard was returning, very drunk, from the Hurkabice, Smallbeer called to him at the other side of the river, "Hola, hola, Smallbeer calling, please drink me, please drink me"

Thirsty Seven-headed Charles Drunkard jumped into a river and tried to swim to the other side. But the current was too strong and the water too high and he was carried down stream and over the waterfall, never to be seen again. People say, to this day, that the sound of the waterfall is the roar of Seven-headed Charles Drunkard falling to his certain death on the rocks below.

With Seven-headed Charles Drunkard gone forever and the Princess safe once more, the ungrateful King changed his mind. But the princess pleased with him to let her marry Smallbeer. The King agreed if Smallbeer completed one final task. "You are a stranger in my Kingdom" he said, "Nobody here knows anything about you. But if you teach us how to make beer I will agree to your marriage to the Princess."
Will you assist Smallbeer overcome his final obstacle and help Prince Smallbeer and Princess Clear-Plum-Brandy get married and live happily ever after?

A. Help Smallbeer to study yeast and fermentation

TASK A.I: YEASTS

Yeasts are eukaryotic microorganisms classified in the kingdom Fungi, with the 1,500 species currently described estimated to be only small fraction of all yeast species. Yeasts do not form a single taxonomic or phylogenetic grouping. The term "yeast" is often taken as a synonym for *Saccharomyces cerevisiae*, but the phylogenetic diversity of yeasts is shown by their placement in two separate phyla, the *Ascomycota* and the *Basidiomycota*. The yeast species *Saccharomyces cerevisiae* has been used in baking and in fermenting alcoholic beverages for thousands of years. It is also extremely important as a model organism in modern cell biology research, and is one of the most thoroughly researched eukaryotic microorganisms. Other species of yeast, such as *Candida albicans*, are opportunistic pathogens and can cause infections in humans.

Yeast belongs to the earliest domesticated organisms. Archaeologists digging in Egyptian ruins found early grinding stones and baking chambers for yeasted bread, as well as drawings of 4,000-year-old bakeries and breweries. In 1857, French microbiologist Louis Pasteur proved in the paper "*Mémoire sur la fermentation alcoolique*" that alcoholic fermentation was conducted by living yeasts and not by a chemical catalyst. Pasteur showed that by bubbling oxygen into the yeast broth, cell growth could be increased, but fermentation was inhibited – an observation later called the "Pasteur effect".

Yeasts are chemoorganotrophs, as they use organic compounds as a source of energy and do not require sunlight to grow. Carbon is obtained mostly from hexose sugars, such as glucose and fructose, or disaccharides such as sucrose and maltose. Yeast species either require oxygen for aerobic cellular respiration (obligate aerobes), or are anaerobic, but also have aerobic methods of energy production (facultative anaerobes).

A.I.1 What is a prerequisite for *S. cerevisiae* to become an important model organism? Circle the correct statements in the Answer sheet.

A	[YES]	[NO]	*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	[YES]	[NO]	*S. cerevisiae* can be transformed allowing for either the addition of new genes or deletion through homologous recombination.
C	[YES]	[NO]	As a eukaryote, *S. cerevisiae* shares the complex internal cell structure of plants and animals.
D	[YES]	[NO]	*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 Ethanol fermentation, also referred to as alcoholic fermentation, is a biological process in which sugars such as glucose, fructose, and sucrose are converted into cellular energy and thereby produces ethanol and carbon dioxide as metabolic waste products. Essential set of reactions involved in ethanol fermentation is glycolysis. Which from the following statements is true about glycolysis? Circle correct statements in the Answer sheet.

A	[YES]	[NO]	One of the products is water

B [YES] [NO] Ten ADP molecules (per one glucose molecule) are converted to ten ATP molecules
C [YES] [NO] Glycolysis is typical for eukaryotes
D [YES] [NO] 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 in the Answer sheet.

A [YES] [NO] During fermentation process baker yeast produces heat, water gets evaporated and forms bubbles in the dough
B [YES] [NO] Baker yeast produce carbon dioxide as waste product, which forms bubbles in the dough
C [YES] [NO] Baker yeast produce ethanol and heat as waste product. Evaporated ethanol forms bubbles in the dough.
D [YES] [NO] 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 Most alcoholic beverages are produced by ethanol fermentation by yeast. Wine and brandy are produced by fermentation of the natural sugars present in fruits, especially grapes. Rum is produced by fermentation of cane sugar. Beer and whiskey are produced by fermentation of starches. Yeast is not able to ferment starch directly. Starch needs to be cleaved to simple fermentable sugars. Which technology is used for transformation of starch to simple sugars in brewery? Circle correct statements in the Answer sheet.

A [YES] [NO] Grain kernels that have been germinated are rich source of enzyme amylase which does the job.
B [YES] [NO] Grain kernels are heated and high temperature treatment cleaves the starch to fermentable sugars
C [YES] [NO] Enzyme amylase is produced in bacteria, isolated and are used for starch treatment
D [YES] [NO] Grain kernels are pre-treated with starch splitting bacteria or yeast and then inoculated with ethanol producing yeast strain

The ability of yeast to convert sugar into ethanol has been harnessed by the biotechnology industry to produce ethanol fuel. The crop with highest energy content/m^2 grown in Czech Republic and easily fermentable into ethanol is sugar-beet (*Beta vulgaris)*. Sugar-beet can be grown commercially in a wide variety of temperate climates. During its first growing season, it produces a large (1–2 kg) storage root whose mass is up to 20% sucrose by weight. Sugar-beet yields up to 60 tonnes of tubers in the local soil and climate conditions per hectare (10 000m^2). On the Åland Islands, a rum-like spirit is made under the brand name *Kobba Libre*. In some European countries, especially in the Czech Republic and Germany, sugar beet is also used to make rectified spirit and vodka – (cited from Wikipedia).

A.II.4 Write to the Answer sheet two chemical equations when sucrose is transformed into an ethanol via glucose and fructose first (than to ethanol and CO_2).

A.II.5 Write to the Answer sheet the summary chemical equation of sucrose transformation into an ethanol.

A.II.6 How much ethanol [kg] can be theoretically produced from 1 kg of sugar-beet storage roots? Write your calculation to the answer sheet.

A.II.7 Why is not possible to reach the theoretical maximal sucrose transformation efficiency using fermenting microorganisms? Circle the correct answers in the Answer sheet.

A [YES] [NO] some carbon is released in a form of CO_2

B [YES] [NO] some sucrose will stay in the solution unfermented, because ethanol will block fermentation process

C [YES] [NO] some carbon will end up in the macromolecules allowing the microorganism to grow and divide

D [YES] [NO] reactants are never 100% transformed into products

A.II.8 A litre of ethanol contains circa 21.5 MJ of energy (one litre of ethanol solution in reality contains 96% of ethanol, density of 96% ethanol solution is 800 g/l !!!). What percentage of the total land (area of CR is approx. 78 866 km^2 – CR is 116th largest country in the world) should be used as sugar-beet field, if Czech Republic, with energy consumption of 496 TWh (1Wh = 3600J) 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 to the answer sheet.

A.II.9 What percentage of total land (area of CR is approx. 78 866 km^2) should be used as sugar-beet field, if Czech Republic decide to fuel all the cars with ethanol produced from sugar-beet sucrose? Czech Republic imports each year about 6 million tons of crude oil and needs 5 million tons of diesel and petroleum diesel for the car and train traffic. A litre of petrol/diesel contains in average circa 32 MJ of energy, it´s average density is 0,785 kg/l. Please, do not include into your calculation about extra energy you have to spend for sugar-beet production. Write your calculations to the Answer sheet.

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 to the answer sheet.

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. Draw an image of the optimal shape in the box and describe the sizes in km units.

TASK A.III: ETHANOL TOLLERANCE

Construction of *Saccharomyces cerevisiae* strains with enhanced ethanol tolerance by mutagenesis of the TATA-binding protein gene and identification of novel genes associated with ethanol tolerance. Jungwoo Yang[1] et al. Biotechnol. Bioeng. Article first published online: 3 APR 2011

Since elevated ethanol is a major stress during ethanol fermentation, yeast strains tolerant to ethanol are highly desirable for the industrial scale ethanol production. Comparing global transcriptional profiles of two selected strains ETS2 and ETS3 with that of the control identified 42 genes that were commonly regulated with twofold change. Out of 34 deletion mutants available from a gene knockout library, 18 were ethanol sensitive, suggesting that these genes were closely associated with ethanol tolerance. Eight of them were novel with most being functionally unknown. To establish a basis for future industrial applications, strains iETS2 and iETS2 were created by integrating the SPT15 mutant alleles of ETS2 and ETS3 into the chromosomes, which also exhibited enhanced ethanol tolerance and survival upon ethanol shock on a rich medium. Fermentation with 20% glucose for 24 h in a bioreactor revealed that iETS2 and iETS2 grew better and produced approximately 25% more ethanol than a control strain.

From the above abstract is obvious, that search for yeast strains tolerant to high concentrations of ethanol is important task. Your job will be characterization of the ethanol tolerance (ability to grow in media containing ethanol) of common baker´s yeast.

Material needed:

3 Erlenmeyer flasks
3 plastic inflatable balloons
4.5g sugar cubes
3 x 8 g of baking yeast
96 % pure ethanol
tap water (slightly warm)
glass pipettes and automatic pipettes

Prepare growth media for the yeast culture:

-
- prepare 3 different growth media (100 ml) with final concentration of sucrose 4.5% in 3 Erlenmeyer flasks provided

  A. containing 0% ethanol

  B. containing 10% ethanol

  C. containing 20% ethanol

- inoculate with the yeasts provided (each Erlenmeyer flasks with 8g mass)

Design and perform the experiment indicating efficiency of yeast catabolic metabolism in an environment containing ethanol.

A.III.1	**Observe and draw the results in the appropriate box. Mark the 0 concentration of ethanol 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).**

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.III.3 Which metabolites of sucrose catabolism will be generated in three different growth conditions A-C (use chemical formulae)

A.III.4 Which type/types of metabolism (if any) you expect at the end of your experiment in three different growth conditions A-C? Use abbreviations AE for aerobic and AN for anaerobic.

A.III.5 What factor(s) is/are the major limitation(s) for the active metabolism of yeast in growth conditions A and C? Use abbreviation O for O_2, S for sucrose, C for CO_2, E for ethanol, T for temperature and N for no limitation.

TASK A.IV: YEAST DOMESTICATION

The natural environment rarely provides microorganisms with conditions that enable their growth and reproduction at a maximal rate; microorganisms in the nature need to be able to cope with, for example, nutrient starvation, temperature changes, and lack of moisture and should be able to survive for extended periods without reproducing. One of the ways how to survive adverse conditions is the formation of colonies in which the cells cooperate. It is not surprising that wild-type yeast strains form different colonies from the domesticated ones, which were selected for special purposes under the optimal growth conditions minimizing the need for cooperation.

Your task will be to identify wild-type and domesticated strain colonies, compare their morphology and adaptations.

A.IV.1 You are provided with a Petri dish in which the colonies were grown on a rich medium. The colonies have different morphologies. Identify the wild-type and domesticated strains and draw to the Answer sheet typical examples of selected morphologies. Use the lowest magnification of the microscope and/or magnifying glass.

A.IV.2 Take a tiny amount of yeast cells from a colony (using the pipette tip), resuspend the cells in a small droplet of water (10 microliters) and observe the cells under microscope. Compare observed cell morphologies, draw a representative illustration in the Answer sheet – with asterisk mark the major differences among the strains.

A.IV.3 Characterization of the wild-type and domesticated colonies using Coomassie Brilliant Blue R dye

- at the bottom surface of the Petri dish encircle location of several wild type and domesticated colonies (at least 5 from each – mark with different colours)
- wash away the yeast colonies with tap water
- remove the remnants of the colonies with the provided cotton-stick (cotton-bud) as much as possible, (without damaging the agar surface)
- stain the agar with the Coomassie Brilliant Blue R staining solution for circa 10 minutes (add with Pasteur pipette, cca 5ml per Petri dish)

- wash out the staining solution with tap water, pour the water out from the Petri dish
- observe patterns of blue signals. Identify the patterns corresponding to the wild colonies and domesticated ones and draw typical examples in the Answer sheet. Use the lowest magnification of the microscope and/or magnifying glass

A.IV.4 Which statement/statements about domesticated vs. wild-type yeast cells and corresponding colonies is/are true? Circle correct statements in the Answer sheet

A [YES] [NO] Domesticated colonies are more complex, because selection pressure in energy rich conditions drives cooperative behaviour between individual cells.

B [YES] [NO] 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 [YES] [NO] 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 [YES] [NO] Domesticated colonies are smooth and lack structural complexity and are formed from cells without ability to differentiate into specialized subsets

B. Help Smallbeer to study residual sugars

Yeast alcohol tolerance is prerequisite for quantitative sugar transformation into ethanol. In reality in each beer or wine preparations remains residual sugar, which is important for particular taste characteristics. Residual sugar is usually measured in grams of sugar per litre of wine, often abbreviated to g/l or g/L. Even among the driest wines, it is rare to find wines with a level of less than 1 g/L, due to the unfermentability of certain types of sugars. By contrast, any wine with over 45 g/L would be considered sweet, though many of the great sweet wines have levels much higher than this. For example, the great vintages of Château d'Yquem contain between 100 and 150 g/L of residual sugar. The sweetest form of the Tokaji, the Eszencia - contains over 450 g/L, with exceptional vintages registering 900 g/L. How sweet a wine will taste is also controlled by factors such as the acidity and alcohol levels, the amount of tannin present, and whether the wine is sparkling or not.

There is a special need to produce low sugar beer – especially for diabetics who can´t properly control entry of glucose into cells. It is obvious that any residual unfermented amount of glucose in beer would constitute an increased risk for diabetics. Diätbier is a German specialty beer brewed for diabetics; it uses an unusually complete conversion and fermentation to create a beer with the same alcohol content as an ordinary beer, but almost no residual sugars or carbohydrates. The result is something similar in flavour to Dry Beer.

Measurement of residual sugar in particular steps of beer and wine fermentation is therefore important job for brewer or wine maker. You will perform their duty using one of the most precise techniques.

Iodometric determination of reducing sugars

Reducing sugars are determined by reaction of a water soluble portion of the sample with an excess of standard cupric sulfate ($CuSO_4$) in alkaline tartrate (Fehling's) solution under controlled conditions of time, temperature, reagent concentration and composition, so that the amount of copper reduced is proportional to the amount of reducing sugars in the sample analysed. In this adaptation of Schoorl's method the reducing sugar concentration expressed as glucose, is estimated by iodometric determination of the unreduced copper remaining after reaction.

In this case the residual cupric salt is completely converted into cuprous iodide, with the liberation of an equivalent amount of iodine:

$$2\ Cu^{2+} + 4\ I^{-} \rightarrow 2\ CuI + I_2$$

The iodine formed from iodide is determined by titration with standard thiosulfate, starch being used as indicator. The difference between the blank and the titration of a determination represents reduced copper, and the corresponding amount of sugar (glucose) is learned by inspection of the supplementary table 1 because of the non-stechiometric reaction ratio between glucose and cupric salt.

This method was designed specifically for water soluble dextrins and maltodextrins and is applicable to other carbohydrates, such as low DE (dextrin equivalent) glucose syrups and solids. Dextrins are modified starches prepared from starch by heat treatment in the dry state with or without the addition of small quantities of reagents. The method is not recommended for samples above 30 DE, since it tends to give higher results as the relationship of reduced copper with respect to reducing sugars becomes less linear at the higher DE's.

Apparatus and reagents:

- *Sample:* Glucose (in plastic vial)
- *Tools*: Volumetric flask 100 mL
  2× Erlenmeyer flask (250 mL)
  2× titration flask (250 mL)
  2× pipette 10 mL
  1× Burette 25 mL
  2× Funnel
  1× (Powder) funnel
  2× beaker 150 mL
  1× Graduated cylinder 10 mL
  1× Plastic wash bottle (with distilled water)
- *Chemicals*: Fehling´s solution A (69.28 g CuSO4·5H_2O in 1 litre of water)
  Fehling´s solution B (346.0 g $C_4H_4O_6NaK\cdot 4H_2O$ and 100 g NaOH in 1 litre)
  Standard potassium dichromate solution ($K_2Cr_2O_7$) - real concentration declared by organisers
  0.1 M Sodium thiosulfate ($Na_2S_2O_3$) solution
  Potassium iodide (KI) - solid
  2 M Sulfuric acid H_2SO_4
  Starch indicator

TASK B.I: STANDARDISATION OF 0.1 M $Na_2S_2O_3$ SOLUTION

- Add appropriate volume of distilled water (approx. 50 mL) into a 250 mL titration flask containing 5 mL standard potassium dichromate ($K_2Cr_2O_7$) solution – added by organisers
- Add 5 mL of 2 M H_2SO_4 by the graduated cylinder
- Add approx. 1 g of solid KI and swirl the contents thoroughly.
- Titrate with standardized 0.1 M sodium thiosulfate ($Na_2S_2O_3$) to a light yellow colour.
- Add 5 mL of starch indicator from a graduated cylinder and continue the titration to the disappearance of the blue indicator colour (note: at the end of titration the solution is light blue-green colour according to the Cr^{3+} ions presence).
- In the Answer sheet, record the volume of standardized 0.1 M sodium thiosulfate used in the start position, end position and the difference.
- Perform the analysis at least twice (three times if necessary).
- Calculate the concentration of $Na_2S_2O_3$ solution. Write your calculations and result to the answer sheet.
- Standardisation is characterised by following reactions:

$$Cr_2O_7^{2-} + 6\ I^- + 14\ H^+ \rightarrow 2\ Cr^{3+} + 3\ I_2 + 7\ H_2O$$

$$I_2 + 2\ S_2O_3^{2-} \rightarrow 2\ I^- + S_4O_6^{2-}$$

TASK B.II: ANALYSIS OF GLUCOSE SAMPLE

- Transfer sample in plastic vial quantitatively into volumetric flask (100 mL) and fill this flask up to the mark with distilled water.
- Pipette 10.0 ml of Fehling´s A solution and then 10.0 ml of Fehling´s B solution into 250 mL Erlenmeyer flask.
- Add 10.0 mL of sample (glucose solution) and 20 mL of distilled water to bring total volume to 50 mL of reaction mixture.
- Mix contents of flask by gentle swirling.
- Add 2 small glass beads to prevent bumping while boiling and cover the mouth of the flask with a small glass funnel.
- Please preheat the heater
- Place the flask (**make sure it is dry outside !**) on a hot plate adjusted to bring the solution to a boil in approx. (not more than) 3 minutes
- Continue boiling for exactly 2 minutes (total heating time of 5 minutes).
- Cool quickly to room temperature in a cold water bath.
- Add approx. 2 g of solid KI and swirl the contents thoroughly.
- Add 15 mL of 2 M H_2SO_4 by the graduated cylinder (solution must get brown according to the iodine creation; otherwise the next portion of acid is required).
- Titrate immediately with standardized 0.1 M sodium thiosulfate ($Na_2S_2O_3$) to a light yellow colour.
- Add 5 mL of starch indicator from a graduated cylinder and continue the titration to the disappearance of the blue colour.

- Record to the answer sheet the volume of standardized 0.1 M sodium thiosulfate used in the start position, end position and the difference.

- Perform the analysis at least twice (three times if necessary).
- For the blank sample analysis use 10 mL of distilled water instead of 10 mL of sample.
- Determine the difference between the blank and the mean titration value. Recalculate this volume in terms of precisely 0.1 M $Na_2S_2O_3$ and determine the equivalent glucose mass from Table 1. You can assume that there is a linear relationship between any two consecutive points in Table 1. Result should be expressed in milligrams (mg) of glucose in a glucose solution.

- **Fill your calculations and result to the answer sheet!**

Table 1: Dependency of the glucose amount (mg) on the 0.1 M $Na_2S_2O_3$ (mL) volume equivalent to the reduced copper

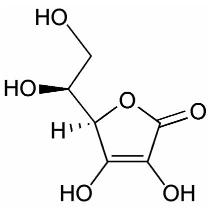
0.1 M $Na_2S_2O_3$ volume (mL)	**Glucose amount (mg)**	**0.1 M $Na_2S_2O_3$ volume (mL)**	**Glucose amount (mg)**	**0.1 M $Na_2S_2O_3$ volume (mL)**	**Glucose amount (mg)**
1.0	3.2	9.0	28.9	17.0	56.3
2.0	6.3	10.0	32.3	18.0	59.8
3.0	9.4	11.0	35.7	19.0	63.3
4.0	12.6	12.0	39.0	20.0	66.9
5.0	15.9	13.0	42.4	21.0	70.7
6.0	19.2	14.0	45.8	22.0	74.5
7.0	22.4	15.0	49.3	23.0	78.5
8.0	25.6	16.0	52.8	24.0	82.6

TASK B.III: SUPPLEMENTARY QUESTIONS

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

a) HCHO (formaldehyde) +OH^-

b) CH_3COCH_3 (acetone) +OH^-

c) 

(L-ascorbic acid)

B.III.2 What 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 in each column.

Characteristic of the Structure	Physicochemical property	Instrumental method
Conjugated system of bonds	absorption of UV light	UV spectrometry
Asymmetric (chiral) carbon	optical rotation	polarimetry
groups	volatility	gas chromatography
Ester groups	volatility	gas chromatography

C. Help Smallbeer to study beer itself

In Bohemia, the Czech beer has been traditionally marked by its "degree", for example 10° beer or 12° beer. The degree determined the concentration of fermentable sugar before fermentation. The beer alcohol content then depends on the amount of fermentable sugars transformed to the alcohol. Nowadays, the beer degree is given by mass fraction of fermentable sugars in hopped wort.

The first intermediate product in the process of beer brewing is called malt wort. It is a sweet solution of sugars and other substances without hops. The malt wort is then boiled together with hops. The product is called hopped wort. By adding yeast, the sugar is transformed to alcohol and carbon dioxide. The alcohol content depends on the density of hopped wort and on the final density of beer. The volume fraction of the alcohol is approximately given by the formula

$$\textit{Alcohol volume fraction}\ (\%) = (\rho_{hw} - \rho)/k$$

where ρ_{hw} is a density of hopped wort (in kg/m^3), ρ is a final density of beer (in kg/m^3) and k is a constant with a value of 7.45 kg/m^3.

The beer degree can be consequently determined by multiplying the alcohol volume fraction by a constant 2.5:

$$\textit{Beer degree} = 2.5 \cdot \textit{Alcohol volume fraction}\ (\%).$$

Due to the different concentration of residual fermentable sugar the beers of the different degree have also different density. The higher is the degree, the higher is the final beer density.

The density can be determined by pycnometer, see Fig. 1. A pycnometer is usually made of a glass bottle with a close-fitting stopper with a capillary tube through it. The bottle can be filled with the big precision by the same volume of different liquids.

Figure 1 - Pycnometer (Source: Wikimedia Commons)

If we know the density of some comparison liquid, we can determine the volume of pycnometer by weighting the empty pycnometer and the pycnometer filled by comparison liquid. Weighting then the pycnometer filled with the liquid of unknown density, we can calculate its density.

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

Apparatus and material: two bottles of beer, distilled water (ask the lab assistant for it), scales (shared with one more team), glass rod, three beakers, small glass bottle, stopper with a tube through it, swab, ruler with scale

Procedure

1. Pour the samples of both beers to the beakers and swirl them by the glass rod so that air bubbles escape from the beer. Allow both beer samples to stand for several minutes at the lab temperature with occasional swirling. Pour the distilled water to the third beaker and allow it also to stand to reach the lab temperature.
2. Meanwhile, weigh the empty dry pycnometer (glass bottle) together with the stopper five times.

   Record your measurements to the Answer sheet (**second column in the table C.I.1**) and calculate the mean.
3. Fill the pycnometer by distilled water to the rim. Insert the stopper. Some amount of water will flow out through the tube. Dry the pycnometer carefully and weigh it. Perform the measurement five times. Do not empty the pycnometer in between the particular measurements. It is enough to add the distilled water to the rim and insert the stopper again.

   Record your measurements to the Answer sheet (**third column in the table C.I.1**) and calculate the mean.
4. Empty and rinse the pycnometer. Fill it with the sample of the light beer. Insert the stopper, dry carefully the pycnometer. Make sure that there are no air bubbles under the stopper and weigh it. Perform the measurement five times. Again, do not empty the pycnometer in between measurements. It is enough just to add a beer sample so as the pycnometer is full to the rim and insert the stopper again.

   Record your measurements to the Answer sheet (**fourth column in the table C.I.1**) and calculate the mean.
5. Empty and rinse the pycnometer. Perform the same procedure as for the light beer for the sample of dark beer. Again, make sure that there are no air bubbles under the stopper.

   Record your measurements to the Answer sheet (**fifth column in the table C.I.1**) and calculate the mean.

Figure 2 – Pycnometer with a dark beer sample on the scales

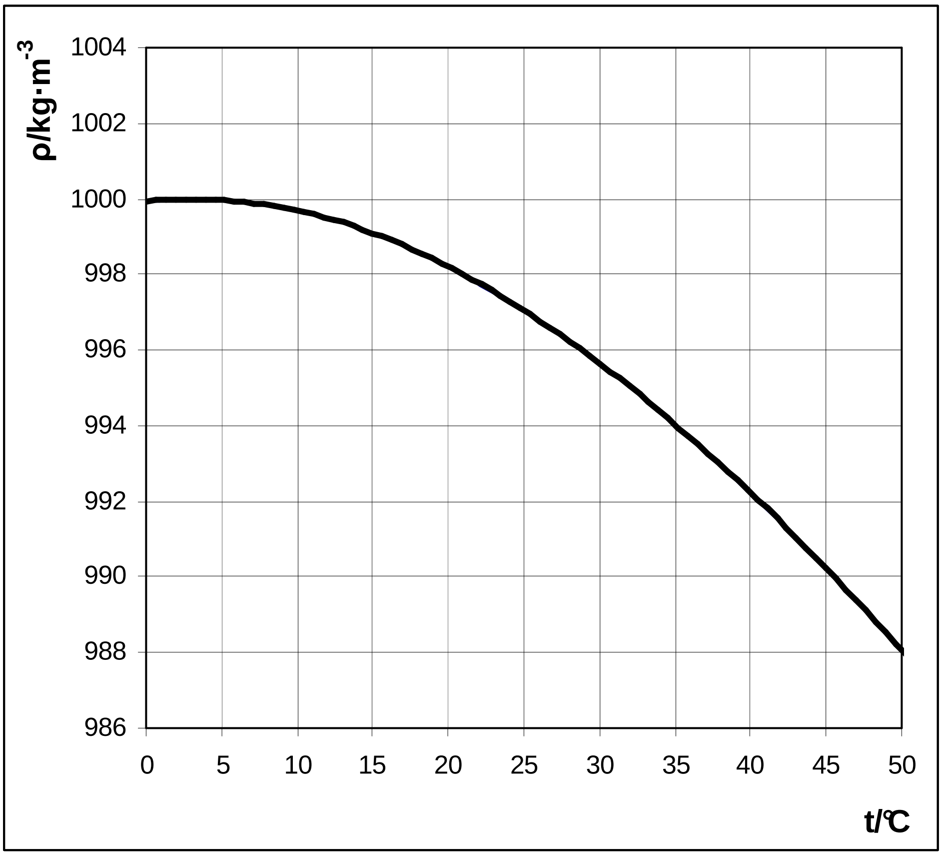
We strongly recommend to start TASK C.II now. You will return back to analyse your data during the measurement in the TASK C.II!

6. Calculate the mass of distilled water in the pycnometer (denote it by $\boldsymbol{m_w}$), the mass of the light beer sample in the pycnometer ($\boldsymbol{m_1}$) and the mass of the dark beer sample in the pycnometer ($\boldsymbol{m_2}$). Write the masses to the Answer sheet (**C.I.2**)

7. Copy the lab temperature from the white-board to the Answer sheet. Use Graph 1 below to determine the density of distilled water. Calculate the volume of the distilled water in the pycnometer. Write your results to the Answer sheet (**C.I.3)**

8. Write to the answer sheet (**C.I.4**) the formula for the density of the beer sample in terms of the mass of the sample and the volume V_w. Calculate the density of the light beer sample (ρ_1) and the dark beer sample (ρ_2). Write your results to the answer sheet.

9. Calculate the volume fraction of the alcohol and the degree of the light beer sample and the degree of the dark beer sample. The density of hopped wort for the light beer is ρ_{1hw} = 1040 kg/m^3 and for the dark beer sample ρ_{2hw} = 1080 kg/m^3. Write your results to the answer sheet (**C.I.5**).



Graph 1 – The dependence of the density of distilled water on temperature

Another way how to measure the density of liquids is to use a hydrometer, see Fig 3. A hydrometer is usually made of glass and consists of a cylindrical stem and a bulb weighted with mercury or lead shot to make it float upright. The liquid to be tested is poured into a tall container, often a graduated cylinder, and the hydrometer is gently lowered into the liquid until it floats freely. The point at which the surface of the liquid touches the stem of the hydrometer is noted. Operation of the hydrometer is based on Archimedes' principle.

C.I.6	For simplicity, consider a hydrometer made of the test tube (of constant cross sections) with the weight inside of the length of 20 cm. Such a test tube is immersed in the distilled water to one half of its length. Calculate the length of immersion of the tube in both of your beer samples. **Write your calculations and results to the Answer sheet.**

Figure 3 - Hydrometer *(Source: Wikimedia Commons)*

TASK C.II: QUANTITATIVE ESTIMATION OF CO_2 PRODUCTION BY YEAST

As you have seen in the part A.II, when yeast consumes and metabolizes sugar, it produces ethanol and carbon dioxide. By measuring the volume and the pressure of produced CO_2 we can determine its amount of substance (compare with TASK A.III1) by means of ideal gas law

$$pV = nRT$$

where p denotes the pressure of the gas, V stands for the volume of the gas, n is required amount of substance, R denotes the molar gas constant (R = 8.314 J $mol^{-1}K^{-1}$) and T is thermodynamic temperature (that can be easily determined form the Celsius temperature written on the white-board).

If we suppose that the shape of inflatable balloon is an ideal ball then for inflated balloon holds approximately true the following equation

$$p = p_a + \frac{C}{r},$$

where p_a denotes the atmospheric pressure, r is a radius of the balloon and C is a constant describing the material properties and wall thickness of the balloon

Apparatus and material: scales (shared with one more team), glass rod, beaker, small glass bottle (pycnometer), ruler with scale, two set squares, inflatable balloon, 2 sugar cubes, baker yeast (approx. 20g).

Procedure

1. Weigh both sugar cubes together. Record your result to the Answer sheet (**C.II.1**)

2. Calculate the lab thermodynamic temperature. Record your result to the Answer sheet (**C.II.2**)

3. Fill the pycnometer up to the rim by the tap water of the lab temperature. Pour the water from the pycnometer to the beaker. Add baker yeast to the beaker and dissolve also all sugar cubes. Mash the yeast by glass rod and properly swirl the content of the beaker.
4. Pour the obtained solution to the rinsed pycnometer up to the rim. Finally set carefully the empty balloon to the pycnometer neck, see Fig 4. Do not stretch the balloon unnecessarily much, it may change its elastic properties near the neck and spoil its shape. If you break the balloon, ask the lab assistant for additional.

Figure 4 – Inflatable balloon on the pycknometer neck

5. Observe carefully inflation of the balloon. After 40 minutes, record every 10 minutes the diameter *d* of the balloon to the Answer sheet (**second row in the table C.II.3**) up to 120 minutes. To measure the diameter use the ruler and both set squares.

6. Derive the formula for the carbon dioxide amount of substance by terms of the balloon diameter, lab thermodynamic temperature and constants. Write your calculations to the Answer sheet (**C.II.4).**

7. For each value of the diameter calculate consequently the volume of the gas inside balloon (*V*), the pressure of the gas (*p*) and the amount of substance of the gas. Write your results to the Answer sheet (**table C.II.3).** Suppose that the shape of the balloon is an ideal ball, neglect the volume of the balloon stem. The current atmospheric pressure is written on the white-board. The value of the constant C = 240 Pa m. Neglect also the original amount of substance of the air in the balloon.

8. Draw graphs of the dependence of the gas pressure on time (**denote the graph by Graph C1**) and the produced amount of substance on time (**denote the graph by Graph C2**)

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. The molar mass of sucrose is 342.2 g/mol. Write your calculations to the Answer sheet.

GOOD LUCK !!!