European Union Science Olympiad

Jahresbericht 2015/16

Mag. Peter Holub
Regionales Netzwerk für Naturwissenschaften und Mathematik Kärnten

Vom

Bundesministerium für Unterricht, Kunst und Kultur

gefördert

Inhalt

European Union Science Olympiad

Wissenschaft für Morgen|Training auf höchstem Niveau

Die EUSO ist ein naturwissenschaftlicher Teamwettbewerb der Europäischen Union für Biologie, Chemie und Physik. Österreich war 2016 zum schon neunten Mal mit zwei Teams bei der EUSO, die heuer in Tartu stattfand, vertreten.

2015 fand die EUSO vom 26. April bis 3. Mai 2015 in Klagenfurt statt.
Rund 350 Personen (MentorInnen, teilnehmende Jugendliche, EUSO 2015 Team) hatten die Gelegenheit, an sieben am Gelingen dieser naturwissenschaftlichen Großveranstaltung mitzuwirken. Das Organisationsteam des Nawizentrums der Pädagogischen Hochschule Kärnten, geleitet von Mag. Sigrid Holub, leistete hervorragende Arbeit. Die mehrjährige Entwicklung der Aufgabenstellungen durch ein Team des Nawizentrums und der Karl-Franzens-Universität Graz, unterstützt von zwei Physikern der Pädagogischen Hochschule Steiermark zahlte sich aus. Die Rückmeldungen aus den 25 teilnehmenden Ländern der Europäischen Union waren hervorragend. Das Österreichische A-Team errang im Rahmen des Wettbewerbs eine Goldmedaille, das junge B-Team Bronze.

Das Credo der EUSO

- begabten SchülerInnen die Möglichkeit geben, ihre Talente zu entfalten
- Das Interesse an Wissenschaft und des forschenden Lernens zu wecken bzw. zu fördern
- Durch die Eindrücke und Erfahrungen der EUSO auf eine mögliche Teilnahme an weiteren Internationalen Olympiaden vorzubereiten

Ziel des Wettbewerbs

- Öffentliche Interesse auf die naturwissenschaftliche Ausbildung lenken
- Ermittlung der besten SchülerInnen der Europäischen Union im naturwissenschaftlichen Bereich

- Wertschätzung der Wissenschaft in der Allgemeinheit
- Intensivierung der Zusammenarbeit zwischen europäischen Bildungssystemen
- Individuelle Ideen und Konzepte innerhalb der gesamten Europäischen Union zu verbreiten
- Vorbereitung europäischer SchülerInnen auf die Internationalen Olympiaden

Mehr dazu unter: **www.euso.eu und www.euso.at**

Vorbereitungswoche Klagenfurt

25 SchülerInnen aus sechs Bundesländern wurden von insgesamt acht TrainerInnen eine Woche lang, vom 22.02. - 26.02. 2016 am Europagymnasium Klagenfurt und im Nawizentrum der Pädagogischen Hochschule Kärnten auf den Teamwettbewerb in Estland vorbereitet.
Neben den fachspezifischen Trainingseinheiten wurden auch Teamfähigkeit und Englisch als Arbeitssprache angeboten.

Trainingstage in Potsdam

Sechs Jugendliche schafften es in die Qualifikation und somit zum Intensivtraining, das heuer wieder in Kooperation mit dem deutschen EUSO-Nationalteam in Potsdam stattfand (14. - 17. März 2016).

Diese Trainingstage mit den deutschen EUSO KandidatInnen waren für die zwei österreichischen Nationalteams eine tolle Herausforderung und dementsprechend lehrreich, da dieses Intensivtraining schon starken Wettbewerbscharakter hatte.

TeilnehmerInnen 2016

Farbcodierung (bei den Namen): Gelb: Trainingsfokus Chemie, Grün: Trainingsfokus Biologie,Blau : Trainingsfokus Physik

Familienname	Vorname	Bundesland
Ademi	Zarije	Oberösterreich
Balaka	Pia	Wien
Büttner	Victoria	Vorarlberg
Cavaliere	Flavia	Steiermark
Gradauer	Stefan	Wien
Gritzky	Othmar	OÖ
Gruber	Regina	Wien
Hasenbichler	Manuel	Steiermark
Kaufmann	Helene	Steiermark
Klaus	Elisabeth	Kärnten
Koegler	Florian	Wien
Kölbl	Sebastian	Steiermark
Kuchling	Zorah	Steiermark
Kutleša	Kevin	Steiermark
Likawetz	Matthias	Steiermark
Perner	Wanda Melina	Wien
Prettenthaler	Jakob	Steiermark
Rehbein	Viktoria	Salzburg
Reichmayr	Anna	Steiermark
Rupp	Markus	Kärnten
Schantl	Michael	Steiermark
Schmidt	Sara Catherine	Steiermark
Stölzl	Theo	Steiermark
Winkler	Lucia	Steiermark
Wlattnig	Hannah	Steiermark

Trainingsteam

Verantwortliche	Stamminstitution	Fach
Mag. Dieter Winkler	*Bischöfliches Gymnasium Graz*	*Physik*
Matthias Diez	*Karl Franzens Universität Graz*	*Physik*
Mag. Karl Brachtl	*Regionales Netzwerk Kärnten*	*Chemie*
Mag. Sabine Seidl	*Nawizentrum der Pädagogischen Hochschule Kärnten*	*Chemie*
Mag. Christine Ottowitz	*BG/BRG Villach St. Martin*	*Biologie*
Dr. Christina Morgenstern	*Nawizentrum der Pädagogischen Hochschule Kärnten*	*Biologie*
Mag. Sigrid Holub	*Regionales Netzwerk Kärnten*	*Biologie*
Mag. Peter Holub	*Regionales Netzwerk Kärnten*	*Biologie*
Dr. Peter Willitsch	*Regionales Netzwerk Kärnten*	*Mathematik*

EUSO 2016 in Estland

Die Olympiade fand vom 7.bis 14. Mai 2016 in Tartu und Tallinn statt.

Die SchülerInnen und BetreuerInnen waren im unterschiedlichen Hotels untergebracht und trafen einander nur nach den jeweiligen Wettbewerbstagen.

Die Aufgabenstellungen wurden von mehreren Teams der Universität Tartu vorbereitet und waren gut ausgearbeitet.

Die österreichische Delegation in Tartu

Delegationsleitung:	Mag. Peter Holub
Mentorin Biologie:	Dr. Christina Morgenstern
Mentorin Chemie:	Mag. Sabine Seidl
Mentor Physik:	Mag. Dieter Winkler

Team A	**Team B**
Kevin Kutleša	Anna Reichmayr
Regina Gruber	Elisabeth Klaus
Sebastian Kölbl	Jakob Prettenthaler

Von links: Alina Gruber(Guide), Dieter Winkler, Sebastian Kölbl, Regina Gruber, Kevin Kutleša, Elisabeth Klaus, Jakob Prettenthaler, Anna Reichmayr, Christina Morgenstern, Sabine Seidl, Peter Holub

Die Delegation wurde durch die österreichischen Guides, Alina Gruber, Anna Fister, Matthias Sommeregger und Gabriella Orsolya verstärkt, die schon bei der EUSO 2015 in Klagenfurt im Einsatz waren.

Resultate 2016

Einmal Silber, einmal Bronze für Österreich!

Die Österreichischen Teams schlugen sich sehr gut. Team A errang eine Silbermedaille, das junge B-Team Bronze.

Team A Sebastian Kölbl, Regina Gruber, Kevin Kutleša

Team B Anna Reichmayr, Jakob Prettenthaler, Elisabeth Klaus,

Medaillenspiegel

GOLD MEDALS AND TROPHY OF THE EUSO:

Germany, Team A

Isabel Berenice Richter

Konstantin Schwark

Leo Pavlovio Gitin

GOLD MEDALS:

Portugal, Team A - Guilherme Vilela Alves, Luís Miguel Martins Costa e Silva, Raul Pombo Monteiro

Slovakia, Team B - Juraj Michalec, Martin Orságh, Matúš Kopunec

Slovenia, Team B - Aleksej Jurca, Luka Govedič, Urban Duh

Estonia I, Team A - Kaarel Hänni, Karl Paul Parmakson, Richard Luhtaru

SILVER MEDALS

Czech Republic, Team A - Lukáš Fiedler, Miroslava Novoveská, Šimon Karch

Hungary, Team A - Mátyás Sajgó, Péter Ótott Péter, Tamás Kovács

Hungary, Team B - Ákos Harangozó, Bence Béla Botlik, Gergely Illés

Lithuania, Team A - Elena Everatt, Ignas Šakuro, Justas Terentjevas

Estonia I, Team B - Airon Johannes Oravas, Joosep Kaimre, Loona Volke

Netherlands, Team A - Ezra Thomas Bekkering, Marouscha Puister, Stan Pieter Jan Koenis

Germany, Team B - Johannes Jan Günzl, Kai Lukas Gipp, Luis Quentin Langfeld

Romania, Team A - Alina-Alexandra Gudei, Petru Cotrut, Tudor-Cristian Cozma

Romania, Team B - Elena Diana Gabroveanu, Irina - Malina Strugaru, Razvan -Mihai Ursu

Ireland, Team A - Darragh Glynn, Emily Rose McCarthy, Yan Li

Croatia, Team A - Mirko Duvnjak, Paula Vidas, Vilim Lendvaj

Estonia II, Team A - Hannes Kuslap, Kirke Joamets, Uku Hannes Arismaa

Ireland, Team B - Ryan James Bell, Thomas Cornally, Thomas Elliot McCarthy

Sweden, Team B Carl Arvid Lunnemark, Nils Evert Hammar, Per Ludvig Göte Forslund

Czech Republic, Team B - Adam Babák, Jakub Kára, Jan Preiss

Austria, Team A - Kevin Kutleša, Regina Gruber Sebastian Kölbl

Greece, Team A Anthony Constantine Kriezis, Nickolas Stathas, Efstratios Tsakalidis

Lithuania, Team B - Justas Janickas, Sintija Raudonytė, Veronika Everatt

Slovenia, Team A - Gregor Igličar, Martin Rihtaršič, Vid Kermelj

Italy, Team A - Gregorio Rebecchi, Stefano Cavana, Stefano Pigozzi

BRONZE MEDALS (in alphabetical order)

Austria, Team B - Anna Reichmayr, Elisabeth Klaus, Jakob Prettenthaler

Belgium, Team B - Lionel Philippe Mozin Nicolas Francis Rotheudt Tom François Winandy

Belgium, Team A - Alex Joseph Willems, Daniël Denis Withoeck, Gust Michel Popelier

Bulgaria, Team A - Dimitar Tomov, Vanya Milanova, Vasil Ivanov

Bulgaria, Team B - Mina Tsakovska, Silvi Koycheva, Viktor Georgiev

Croatia, Team B - Luka Bulić Bračulj, Noa Jelić Matošević, Tea Arvaj

Cyprus, Team A Anastasia Polyviou, Ioannis Kestoras, Panayiota Artymata

Cyprus, Team B - Crystalleny Kossiva, Maria Pieri, Maria Petrou

Denmark, Team A - Caja Lilhav Christoffersen, Hannah Ravn Bingley, Liv Brandhøj

Truelsen

Denmark, Team B - Anna Olivia Jeppesen, Josefine Neerup-Lundh, Rune Sjørslev Blom

Estonia II, Team B - Juri Volodin, Mihhail Lebedev, Roman Oleinik

Finland, Team B - Konsta Akseli Keijonen, Mike Anton Sebastian Bäck, Rasmus Joonatan Sarkanen

Finland, Team A Eka Verneri Luhtanen, Joonas Akseli Lahikainen, Kaisa Helinä Antikainen

Greece, Team B - Dimitrios Apostolidis, Apostolos Argyros, Dimitrios Gavridis

Italy, Team A - Andrea Pasotti, Emanuele Manzi, Marco Piacentini

Latvia, Team B - Austris Mazurs, Rūta Ozoliņa, Sindija Kezika

Latvia, Team A - Amanda Zvaigzne, Kršjānis Kucins, Vladislava Kurtukova

Luxembourg, Team A - Loris Picco, Max Wenner, Pit Bermes

Luxembourg, Team B - Anna Hellers, Lavinia Kadar, Raffaël Marth

Netherlands, Team B - Hendrik Holwerda Koen, Joris van der Heijden, Kornelius Jan Doedens

Portugal, Team B - Constança Albuquerque, Maria Matilde Soares da Silva, Rodrigo Rosmaninho

Slovakia, Team A - Andrej Tekel, Filip Farkas, Martin Marek

Sweden, Team A - Alicia Sara Thorborg van Hees, John Ivar Oliver Lindström, Julia Susanna Salwén

Aufgabenstellungen 2016

Task 1

General instructions

Wear the lab coat at all times in the lab.

Eating and drinking in the lab is strictly prohibited. Ask a lab assistant if you want to drink or go to the toilet.

It is highly advisable to wear disposable gloves and protective goggles when handling chemicals.

Defective and broken equipment will be replaced by a lab assistant, if you ask for it. Nevertheless you are expected to clean spilled liquids etc yourself.

Behave during experiments environmentally friendly! Please find for produced waste suitable bin for disposal– paper, plastic, metal, glass or wet waste!

All paper used, including rough work paper, must be left on the desk at the end of the experiment.

All results must eventually be entered into your *yellow* Answer Sheet (or the Excel files).

Please save all files with your experimental data on the desktop of your team's laptop!

Only the *yellow* Answer Sheet and the Excel files will be marked.

The experiment consists of three Tasks and can be completed either individually or as a team.

Milk day

TASK A.1 Fat in milk

Milk is a natural system that can be described as a colloid containing proteins, lipids, and carbohydrates (mainly lactose). Today, your task is to investigate those components of milk in a dairy lab using physical, chemical, and biological methods. Your work is financed by a dairy company called cowBOOM. cowBOOM intends to launch a series of specialised milk products. Your studies will enable determining different properties of the milk samples and their marketing suitability.

General materials:

- laptop
- pens
- 2 waterproof markers
- 2 pencils (the mechanical pencil is for Task A.1)
- ruler
- scissors
- long and short forceps
- Post-it papers
- clock
- calculator
- distilled water (500 mL bottle)
- safety goggles
- paper towels
- paper bin (blue label)
- plastic bin (yellow label)
- glass bin (green label)
- yellow wet waste jug

TASK A.1 Fat in milk

Milk is a natural colloidal emulsion of fat globules, as well as a hydrocolloid suspension of casein micelles, dispersed in a water-based solution (Figure 1.1). Each fat globule is surrounded by a membrane, which keeps the individual globules from joining together. The volume fraction, content, and size of those particles significantly influence the properties of dairy products.

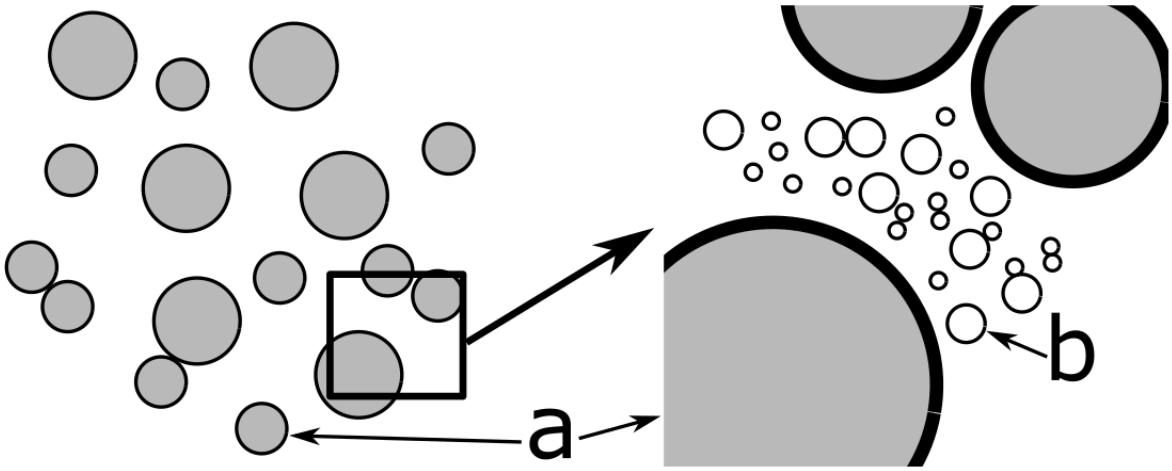


Figure 1.1. Fat globules (a) and casein micelles (b) in milk.

The size of milk fat globules (the tiny fat droplets) ranges from 1–15 μm depending on the cow breed and season. Commercial milk is usually homogenised – the fat globules of natural milk are mechanically broken down to smaller droplets, so that they would not be able to float to the top to form a layer of cream.
When light passes through milk, it is scattered by the milk fat globules and casein micelles via reflection and diffraction. Scattering decreases the transparency of milk and weakens the light passing through a milk layer.
In this task different light scattering effects are used for studying the size and the concentration of microscopic particles.

Samples

- standard glass microspheres in a tube, marked “Glass”
- milk samples in tubes marked “K”, “L”, “M” and “N”.

  Tubes contain milk of different properties (in random order):

  natural (non-homogenised) milk with a fat content of 3.7 %,
  homogenized milk with a fat content of 3.7 %
  natural (non-homogenised) milk with a fat content of 2.0 %
  homogenized milk with a fat content of 2.0 %

List of necessary equipment:

- optical stand with a green laser (wavelength λ = 532 nm), a sample holder, and a screen
- 14 microscope slides
- automatic pipette and pipette tips
- 4 paper clips
- paper spacers (a paper sheet for cutting spacers)
- test object (a paper sheet with printed text)

Task A.1.1 Estimating particle size using light diffraction

A.1.1.1 Estimating size of microscopic glass spheres

In this task you will estimate the size of standard glass spheres by observing their diffraction pattern on a screen. When laser light passes through a substance containing small particles and falls on a screen, an image of a bright central dot surrounded by concentric circular rings is formed (Figure 1.2).

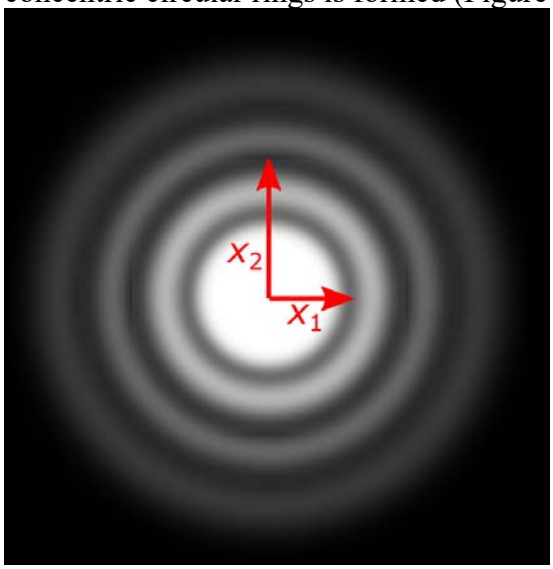


Figure 1.2. Diffraction of light from monodispersed (equally sized) spheres. Distances from the centre to the first and second minima are marked as x_1 and x_2, respectively.

The angular distribution of the scattered light on screen depends on the size of scattering particles in sample. For small diffraction angles, the diameter D of spherical particles can be estimated from their diffraction pattern using the formula

$$D = k\,\lambda\,L\,/\,x, \qquad \text{(Formula 1.1)}$$

where λ is the wavelength of the laser, L is the distance from the object to the diffraction image, and x is the distance (on the screen) from the center of the diffraction

pattern to the diffraction minimum. The coefficient k is different for each diffraction minimum: k equals 1.22 for the first diffraction minimum (first dark circle from the center), and k equals 2.23 for the second diffraction minimum.

Experimental procedure:

1. Align the green laser, sample holder, and screen on the optical stand. According to the stand ruler, the approximate initial position of the aperture of the laser (where the light emerges from the laser) should be at 40 cm, the sample at 48 cm, and the screen at 80 cm.
2. Prepare a sample by placing the glass spheres from the tube labelled "**Glass"** to the centre of a microscope slide. Place another slide on top of the first slide, and press the slides together with two paper clips.
3. Insert the prepared sample into the sample holder.
4. Turn on the green laser. Make sure that the light passes through the sample and falls on the screen.
5. Move the laser and the screen in relation to each other to get as clear diffraction maxima and minima as possible. If necessary, use a sheet of paper as a shade to improve the observation conditions!
6. **If the laser light is too weak to observe any pattern on the screen, a lab assistant can provide a new battery or change the laser (no points will be deducted)**.

   A.1.1.1.1 Observe the diffraction pattern on screen. Sketch the approximate graph of the light intensity I depending on the displacement x from the center of pattern. In addition, mark the positions of the observed diffraction minima x_1 and x_2 on the graph.

   A.1.1.1.2 Measure (3 times) the distance from the laser beam centre to the first and to the second diffraction minima, and the distance L from the sample to the screen. (Each time you may change the positions of the laser, sample and screen to get the best diffraction pattern. You can choose yourself on which side of the screen you'll measure the positions of the diffraction maxima and minima). Write the results in the table!

   TURN OFF THE LASER whenever you are not using it!

   A.1.1.1.3 Calculate the diameters of the glass spheres (using Formula 1.1) corresponding to the three measurements of the first and second diffraction minima.

   A.1.1.1.4 Calculate the average diameter of the glass spheres and estimate its uncertainty (measurement error).

   A.1.1.2 Estimating approximate size of milk particles

   Unlike glass spheres, a thin layer of milk does not scatter light in a manner that creates clear diffraction rings, because all milk fat globules are not of equal size. The intensity of the scattered light on the screen will smoothly fade with the distance from the laser beam centre.

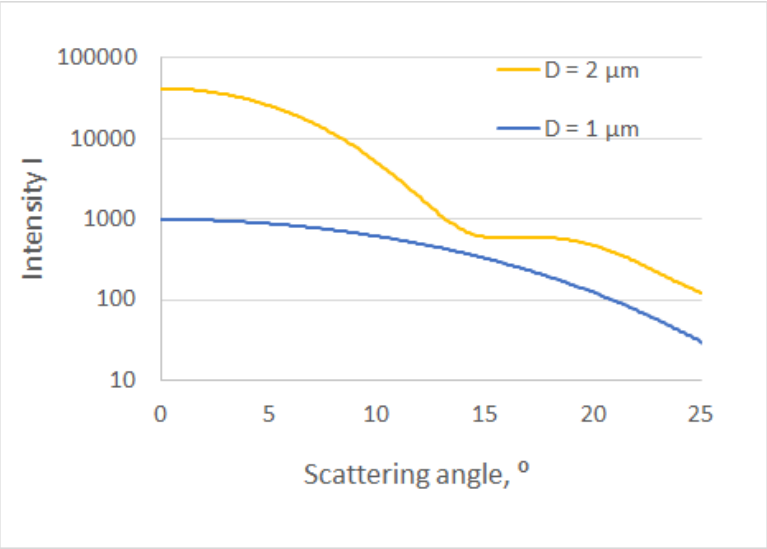


Figure 1.3. Example of the angular distribution of the intensity of scattered light for particles of different diameter D.

Still, the width of the illuminated area, and the angular change of the light intensity depend on the diameter of the fat globules in milk (see Figure 1.3 for a simulated distribution).

Experimental procedure:

1. Prepare a sample from milk in tube "K", which is a natural non-homogenised milk with a 3.7 % fat content.
2. Use the automatic pipette to drop 10 µl of milk from tube "K" onto centre of a slide marked with "K". Flip the tube gently for mixing milk before taking it into pipette.
3. Use paper spacers to fix the thickness of the milk layer between the slides. Cut appropriate spacers from a paper sheet, and put them on both sides of the milk droplet.
4. Cover the slide with another microscope slide marked "K".
5. Carefully press the slides together using two paperclips.
6. Rearrange the experimental setup: on optical stand put the sample holder and the screen as close to each other as possible.
7. Insert the prepared milk sample into the sample holder.
8. Turn the green laser on. Make sure that light passes through the sample and falls on the screen. Move the laser and the sample holder to get a well observable illuminated area of scattered light on screen. (You can choose yourself on which side of the screen you'll observe and measure the illuminated area. Use a paper sheet as a shade, if necessary.)

A.1.1.2.1 Observe the illuminated area on the screen. Sketch the approximate graph of the light intensity I depending on the displacement x from the beam axis. Mark the distance x_{milk} (measured in A.1.1.2.2) on the graph.

A.1.1.2.2 Measure the approximate distance x_{milk} from the beam axis to the edge of the illuminated area (half-width of the illuminated area), and the distance L_{milk} from the sample to the screen. **TURN THE LASER OFF!**

A.1.1.2.3 Estimate the approximate size of the milk fat globules. Use the parameters measured in task A.1.1.2.2 to calculate the diameter of the milk fat globules D_{milk} from the Formula 1.1. Use $k = 2.23$.

A.1.2 Characterising milk by transparency observations

In this task you'll compare four milk samples (**K, L, M,** and **N** described in the beginning of Task A.1) in order to identify their specific properties.

When looking at an object (*e.g.* typed text) through a thin layer of milk, the object seems less sharp and less visible. The light reflected from the object scatters on milk fat globules on its path through milk. The scattered light appears as haze, obscuring the observed object, *e.g.* reducing its visibility. The transparency of the milk layer decreases, when scattering increases.

A light quantum is scattered whenever it hits a fat globule of cross section $S = \pi D^2/4$, where D is the diameter of the globule. The degree of scattering in a thin layer of milk of thickness z depends both on the milk fat content γ (volume fraction of fat globules) and the average size (diameter D) of fat globules. When a thin milk layer is illuminated by light of intensity I_0, then the intensity of scattered light I can be described by the formula

$$I \sim I_0 \gamma z / D \qquad \text{(Formula 1.2)}$$

A.1.2.1 Find out theoretically, which of the four milk samples described in Table 1.1 would exhibit the strongest, and which the weakest scattering. The samples include homogenised and natural (non-homogenised) milk (whereas $D_{hom} < D_{nat}$) of two different fat contents (whereas $\gamma_1 < \gamma_2$).

Table 1.1. Characteristics of milk samples 1–4.

Sample	Globule size	Fat content
1	D_{hom}	γ_1
2	D_{hom}	γ_2
3	D_{nat}	γ_1
4	D_{nat}	γ_2

Experimental procedure:

1. Take 8 microscope slides, and label pairs of slides with letters **K, L, M,** and **N**.
2. Put four slides close to each other on the paper sheet with printed text (test object).
3. Prepare milk samples **K, L, M,** and **N** as described for tube **K** in steps 1–4 in the experimental procedure of Task A.1.1.2.
4. Compare visibility of the printed text through samples **K, L, M,** and **N.**

A.1.2.2 Observe the text through the milk layers and determine the two samples exhibiting the strongest and the weakest scattering (the worst and the best visibility, respectively). Identify the properties of the two selected samples**,** taking into account the description of the samples in the beginning of Task A.1, and the results of Task A.1.2.1.

A.1.2.3 Summarize the identification of the milk samples in the table. Decide how each of the samples **K, L, M,** and **N** have been processed and what is their fat content. Take into account your previous answers and helpful information given in the text.

Task A.1.3 Estimating size of milk fat globules by measuring light attenuation

In task A.1.2 you investigated the scattered light from a thin layer of milk. Here you will measure the fraction of light that can pass through a thick layer of water, containing a small amount of milk. In pure water, light rays can freely move along a straight path. If milk is added to the water, fat globules get in the way of light rays and obstruct their movement along their original straight path. The longer the path is that light has to travel through a medium containing particles, the higher the probability is that the light ray will hit a particle. The fraction of freely transmitted light can be described by the Beer-Lambert law:

$$I = I_0 e^{-NSz}, \qquad \text{(Formula 1.3)}$$

where I is the intensity of transmitted light, I_0 is the intensity of incident light, N is the number of particles per unit volume, z is the thickness of the layer, and S is the cross-section area of a single particle. Assuming that all the fat globules in milk are of equal diameters D, each of them acts as a spherical obstacle with a cross-sectional area

$$S = \pi D^2/4 \qquad \text{(Formula 1.4)}$$

in front of the light's path.

In this experiment, you will measure light attenuation by adding milk to a container of fixed thickness, initially filled with pure water. Adding milk leads to the increase of the number of particles (fat globules), which leads to the decrease of transmitted light intensity. The aim of the experiment is to measure the dependence of the light attenuation coefficient on the concentration of milk in water in order to find out the parameters necessary for calculating the size of fat globules.

You will be using a Lux meter to quantify the intensity of a laser beam that has travelled through the medium containing fat globules. However, some light from the background environment (such as sunlight entering from the windows and the room lighting) influences the reading of the Lux meter. In order to get the intensity corresponding to the laser beam only, you need to subtract the reading corresponding to the background light. Detailed instructions of the procedure are given to you below.

Equipment and materials:

- red laser on a stand
- water container (50 mL graduated bottle with blue cap)
- Lux meter on a stand
- automatic pipette and pipette tips
- milk sample in a tube labelled **K**
- Excel file “Milk A.1.3 Country Team A B.xslx” on the desktop of your team’s laptop

Experimental procedure:

1. Place the red laser, the water container, and the Lux meter on your table so that the laser beam would pass through the water container on its way to the sensor of the Lux meter.

Important: Place the water container so that the laser beam enters the container perpendicularly to its side. Place your setup so that the laser beam is directed towards the window.

2. Place the water container about 20–40 cm away from the Lux meter.
3. Plug the red laser in a USB port of the computer. Turn on the computer. Adjust the position of the laser, so that the light beam would be roughly horizontal.
4. Make sure that the laser beam is focused (the laser spot is as small as possible) on the Lux meter sensor, and that it enters the sensor through the hole in front of the sensor. If needed, adjust the lens in front of the laser by turning the cap.
5. Turn on the Lux meter. Take measurements in the lowest range marked "2000".
6. Fill the water container with distilled water up to the 50 mL mark. Note that the thickness of the water layer is z = 25 mm.
7. **Important:** It is crucial that after positioning the light sensor and the laser, their **positions remain fixed** for the rest of the experiment. If you accidentally move the laser or sensor, it is advised to start over with the measurements (starting with clean water).

   A.1.3.1 Measure and write down **in the Excel file** the light intensities and the total volume of milk in water in each step of your experiment as described in steps 8–12. Also, write down the volume of water V_0.
8. Take the reading I_0 of the Lux meter corresponding to the intensity of transmitted laser light through pure water. (This reading also includes contribution from the background environment.) Write the measured value I_0 **in your Excel file** table. **In case of computer problems (troubles with software, files not found, etc), ask a lab assistant for help (no points will be deducted).**
9. Measure the reading B_0, corresponding to the light intensity coming from the background environment. To do so, block the laser beam with your hand, take the reading of the Lux meter and write down the B_0 value **in your Excel file.**
10. Using an automatic pipette with a tip, take a portion of milk of volume $V_1 = 20\,\mu L$ from the tube labelled **K**. Add milk into the water container and mix the solution by closing the container and turning it over a few times.
11. Put the container back into its former position. Measure and write down (**in the Excel file**) the reading I_1 of the Lux meter as the laser beam passes through the container. Block the laser beam and write down (**in the Excel file**) the reading B_1 corresponding to the background light.
12. Repeat the procedure by each time adding 20 µL of milk into the container. After adding each portion, mix the solution, write the total volume of added milk V_i, the reading I_i of the Lux meter, and the reading B_i of the Lux meter **in the Excel file**. Repeat the procedure until 10 droplets of milk have been added, or until reading I_i will be close to the reading B_i corresponding to the background light.

    A.1.3.2 For each data point, calculate the volume concentration C_i of milk in water and the relative transmission coefficient $\alpha_i = (I_i - B_i)/(I_0 - B_0)$. Do the calculations **in the Excel file**.

A.1.3.3 Plot a graph of the natural logarithm of the relative transmission coefficient $\ln \alpha_t$ versus the milk concentration in water C_i (**in the Excel file**).
A.1.3.4 Plot the trendline fitting your data to a linear relation $\ln \alpha = -aC$ **in the Excel file.** Find out the numerical value of parameter a. Write the absolute value of a **in the Answer sheet**.
A.1.3.5 The milk sample labelled **K** contains a volume fraction of fat globules (total fat volume in the unit volume of milk) $\gamma = 0.037$. Derive a formula for the number density N_0 of the fat globules in milk (number of fat particles in the unit volume of milk, in SI units $1/m^3$) in terms of the globule diameter D, and γ.
A.1.3.6 Derive a formula for the number density N of the fat globules in the mixture of milk and water in terms of C(milk concentration in water), D (diameter of a single fat globule), and γ.
A.1.3.7 Derive a formula for calculating the diameter D of the fat globules in your milk sample and calculate the numerical value of D. ***Hint*:** note that by taking the logarithm of the Beer-Lambert law $I = I_0 e^{-NSz}$ (discussed in the introduction of task A.1.3), we get a linear relation $\ln \alpha = -NSz$ between the logarithm of transmission coefficient and the number density of particles (fat globules). Use your experimental results for calculating the numerical value of the diameter of the fat globules.

Milk day

TASK A.2 Cheese manufacturing and protein content

Milk is a natural system that can be described as a colloid containing proteins, lipids, and carbohydrates (mainly lactose). Today, your task is to investigate those components of milk in a dairy lab using physical, chemical, and biological methods. Your work is financed by a dairy company called cowBOOM. cowBOOM intends to launch a series of specialised milk products. Your studies will enable determining different properties of the milk samples and their marketing suitability.

General materials:

- laptop
- pens
- 2 waterproof markers
- 2 pencils (the mechanical pencil is for Task A.1)
- ruler
- scissors
- long and short forceps
- Post-it papers
- clock
- calculator
- distilled water (500 mL bottle)
- safety goggles
- paper towels
- paper bin (blue label)
- plastic bin (yellow label)
- glass bin (green label)
- yellow wet waste jug

TASK A.2 Cheese manufacturing and protein content

Cheese is produced from milk by treatment with special bacteria and rennet enzymes in the presence of Ca^{2+}. During cheese manufacturing, several stages of milk processing are carried out, including acidification, coagulation, dehydration, and maturation steps. Today, you will learn the basic principles of cheese manufacturing and get an insight into its underlying biochemical and biophysical aspects.
In this task, you will prepare cheese. In addition to milk, three other main ingredients are necessary for cheese manufacturing: $CaCl_2$, a mixture of proteolytic enzymes called rennet, and a specific culture of bacteria called starter bacteria. The bacteria produce lactic acid, which decreases pH to around 5.0–6.0. At a lower pH, certain milk proteins called caseins start to aggregate. In addition, such pH is optimal for the initial stages of the reactions carried out by rennet, which performs hydrolysis of casein and leads to enhanced coagulation and curd formation. The calcium ions added to the raw milk further facilitate coagulation by neutralizing the electrostatic repulsion of the negatively charged coagulating micelles, and thus leading to the formation of a thicker curd. You have been provided with all of the ingredients necessary for making cheese. $CaCl_2$, rennet and bacteria are labelled **substance A, B,** and **C** (in random order) and your task is to identify which is which.

Task A.2.1 Cheese manufacturing

List of necessary equipment:

- milk in a 300 mL plastic bottle (labelled **MILK**)
- substance A (20 mg in microcentrifuge tube; 3 tubes)
- substance B (3 mg in microcentrifuge tube; 3 tubes)
- substance C (20 µL in microcentrifuge tube; 3 tubes)
- 8 centrifuge tubes (50 mL)
- tube rack
- 12 Pasteur pipettes
- 4 plastic Petri dishes
- 250 mL beaker
- plastic funnel (white)
- 4 filtering tissues (in mini-grip)
- jug with warm water (water bath), to be asked from lab assistant after performing steps 1–4

Experimental procedure:

1. Shake the milk bottle moderately to mix the different layers well. Be aware not to shake too intensely to avoid foam formation!
2. Pour approximately 50 mL of milk into 4 centrifuge tubes (50 mL). Mark the tubes **I, II, III,** and **IV**.
3. Dissolve the pre-weighed amount of substance A (20 mg) in 1 mL of distilled water (measure using a Pasteur pipette), add it to the 50 mL centrifuge tube marked **I.**

a. Repeat the same for tubes **II** and **III** (take new aliquots of substance A)

4. Using a new Pasteur pipette, take about 1 mL of the mixture from the centrifuge tube labelled **I** and transfer to the microcentrifuge tube containing substance B (3 mg). Suspend it in the mixture and then transfer back to the centrifuge tube marked **I.**
    a. Repeat the same for tubes **II** and **IV** (take new aliquots of substance B)
5. Mix the contents by inverting the closed tubes and let all the mixtures incubate for 30 minutes inside the water bath (**ask for the warm water bath from a lab assistant**). Meanwhile, answer the theoretical questions.

A.2.1.1 Which fraction of milk has lower density – cream, or skimmed milk? Why?

1. cream, because of higher fat content
2. skimmed milk, because of lower fat content
3. cream, because of higher protein content
4. skimmed milk, because of lower protein content
5. cream, because of higher sugar concentration
6. skimmed milk, because of lower sugar concentration

A.2.1.2 In cheese-making facilities, raw milk is separated into fractions of different densities (cream and skimmed milk) by applying centrifugal force. The lipid content of these fractions and of standardized milk can be measured using near-infrared spectroscopy or other methods.
A cowBOOM facility received 200.0 L of raw milk with an established fat content of 4.1%. For cheese-making, standardized milk with a fat content of 2.9% is required. The facility has a separator, which can prepare cream (with a fat content of 20%) and standardized milk from the raw milk. How many litres of cream and standardized milk can be prepared out of 200.0 L of raw milk? In calculations, ignore the minor differences in densities of milk and its separation products!

Continue cheese-making!

6. Using a new Pasteur pipette, add substance C (20 μL aliquot in the microcentrifuge tube; analogous to addition of substance A) to the 50 mL centrifuge tube marked **I.**
    a. Repeat the same for tubes **III** and **IV** (take new aliquots of substance C)
7. Mix the contents by inverting the closed tubes and incubate all tubes for 30 minutes inside the same water bath.
8. Separate the liquid and solid fractions (whey and curd, respectively) in tubes **I–IV** by filtering. Put the filtering tissue into the provided plastic funnel and place the funnel into a clean 50 mL centrifuge tube and pour the contents of the incubated tube onto the filtering tissue. To facilitate filtering, you can mix the contents of tubes **I–IV** using a Pasteur pipette. For each tube, use new filtering tissue and new centrifuge tube, but the same plastic funnel. Wash and dry the funnel and the spatula before each filtering. Mark the 50 mL centrifuge tubes **WHEY I, WHEY II, WHEY III,** and **WHEY IV**, respectively, and save them for Task A.2.2. After each filtering, transfer the curd left in the tissue onto a new Petri dish. Mark these **CURD I, CURD II, CURD III,** and **CURD IV**, respectively, and save them for Task A.2.2.

A.2.1.3.1 Fill in the table concerning curd formation in different samples **in the Answer sheet.**

A.2.1.3.2 Which one Petri dish has the softest precipitate? Do not take into account the dish(es) with no precipitate. Write the correct Roman numeral **in the Answer sheet.**

A.2.1.4 Based on your observations and the results of Task A.3 (performed by your teammate), determine the function of each substance used for cheese manufacturing **in the Answer sheet**. Choose from the following options:

1. causes the formation of curd
2. enhances the formation of curd
3. consumes lactose

A.2.1.5 Based on the introductory text and answers 1–3 of Task A.2.1.4, determine the biological/chemical identity of substances A, B and C.

1. $CaCl_2$
2. rennet
3. starter bacteria

Task A.2.2. Bradford Assay for Determination of Total Protein Content

During the process of making cheese from milk, you will notice the emergence of two phases: cheese curd and whey. The sedimentation of cheese curd from milk occurs due to the coagulation of the milk protein called casein, which is achieved by adding proteolytic enzymes (rennet) that perform specific biochemical modifications of casein (discussed below). Alternatively, coagulation can be triggered by strong acidification of milk, which results in the precipitation of casein at a low pH.

Coagulation of milk by acidification

List of necessary equipment:

- milk, **WHEY I** and **CURD I** (used or produced in Task A.2.1)
- 1 M HCl (0.5 mL in microcentrifuge tube)
- 2 Pasteur pipettes
- 3 centrifuge tubes (15 mL)
- filter paper disc (in mini-grip)
- plastic Petri dish
- Excel file “Milk A.2.2 country Team A/B.xlsx” on the desktop of your team’s laptop

Experimental procedure:

1. Make sure to have your lab coat, gloves and goggles on.
2. Using a Pasteur pipette, transfer 10 mL of milk to a new 15 mL centrifuge tube. Label the tube **Acid coagulation.**
3. Using a clean Pasteur pipette, carefully transfer 0.5 mL of 1 M HCl from the microcentrifuge tube to the centrifuge tube with milk. Close the tube and invert it several times.
4. Separate the liquid and solid fractions by filtering into a new 15 mL centrifuge tube (use the filter paper!). While the filtering takes place, you can solve theoretical tasks. Once you have separated the largest portion of the liquid (you do not have to wait until all liquid has come through the filter), label the collecting tube **LIQ**.

5. Transfer the pellet to a new Petri dish and label the dish **SOL**.

The Bradford protein assay is an analytical spectroscopic method for determination of the total protein content of a sample. The assay is based on measuring absorbance of ultraviolet (UV) and visible (VIS) light in a sample solution mixed with the so-called Bradford reagent (Coomassie Brilliant Blue G-250 dye). Depending on the pH of the solution, the free dye can exist in three states: cationic (red), neutral (green), and anionic (blue). At a fixed low pH, the dye is predominantly in the protonated red form (λ_{max} = 470 nm). However, when the dye binds to proteins (due to hydrophobic and ionic interactions), it is converted to a stable unprotonated blue form (λ_{max} = 595 nm). This blue protein-dye complex can be detected at a wavelength of 595 nm in an assay using a spectrophotometer or microplate reader.

In order to improve the accuracy of the assay, a calibration curve is usually made using known amounts of a relatively cheap and pure protein (such as bovine serum albumine, BSA). Subsequently, the concentration of the total protein in a sample of interest can be established by comparing the absorbance values on the BSA calibration curve and the absorbance value measured for the sample of interest.

A.2.2.1 In the Figure 2.1 below, absorbance of a blank sample without protein, a less concentrated protein sample, and a more concentrated protein sample is presented. Assign to each sample the corresponding data series on Figure 2.1.

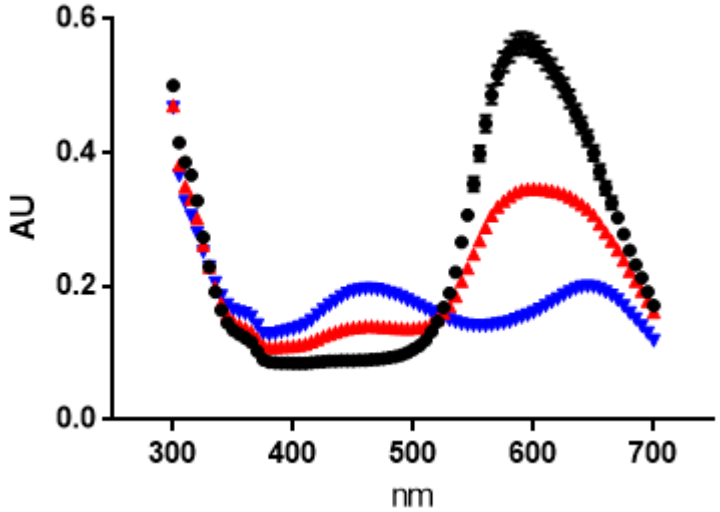


Figure 2.1. The absorbance of the samples (in AU, arbitrary units) at the different UV-VIS wavelengths (in nanometers, nm). (●) data series 1, (▲) data series 2, (▼) data series 3.

Preparation of the dilution series. Spectrophotometry.

List of necessary equipment:

- milk (same as used in Task A.2.1)
- Bradford reagent (5 mL in centrifuge tube)
- Bovine serum albumine, BSA (1.5 mg in microcentrifuge tube)
- Buffer (phosphate buffered saline, PBS; 20 mL in centrifuge tube)
- 1% solution of Triton X (3 mL in centrifuge tube)
- 15 Pasteur pipettes
- 10 microcentrifuge tubes (2 mL volume)
- microcentrifuge tube rack

- microspatula
- tabletop vortex
- automatic pipette and tips
- microtiter plate (transparent 96-well with transparent flat bottom)

Experimental procedure:

1. Using a Pasteur pipette or an automatic pipette, add as precisely as possible 1.5 mL of phosphate buffer saline (PBS) to the 1.5 mg Bovine serum albumin (BSA) in the microcentrifuge tube. Invert and vortex the tube until all of the BSA has dissolved (3–5 minutes).

   NB! You can use the same Pasteur pipette for adding PBS in all of the steps of the given experimental procedure, if you are sure that the pipette has not been contaminated. If you are not sure about it, take a clean Pasteur pipette every time.
2. Prepare a 1% milk solution into a microcentrifuge tube: using a Pasteur pipette, transfer 1 mL of PBS to the tube and then, using the automatic pipette, add 10 μL of milk to the same tube. Vortex the mixture and label the tube **MILK#1**.

   NB! Discard the pipette tip from the automatic pipette after every pipetting!
3. Prepare a 0.02% milk solution into a new microcentrifuge tube: using a Pasteur pipette, transfer 1 mL of PBS, and then using an automatic pipette, add 20 μL of mixture **MILK#1** to the same tube. Vortex the mixture and label the tube **MILK#2**.
4. Prepare a 1% solution of the liquid fraction separated from the acidified milk. Using a Pasteur pipette, transfer 1 mL of PBS into a new clean microcentrifuge tube; then, using the automatic pipette, add 10 μL of the mixture **LIQ** to the same tube. Vortex the mixture and label the tube **LIQ#1.**
5. Prepare a 0.2% solution of the liquid fraction separated from the acidified milk. Using the automatic pipette, first add 160 μL of PBS to a clean microcentrifuge tube and then add 40 μL of mixture **LIQ#1** to the same tube. Vortex the mixture and label the tube **LIQ#2.**
6. Transfer a small visible amount of the solid fraction separated from the acidified milk (from the Petri dish **SOL)** into a clean microcentrifuge tube using the spatula provided. Label the tube **SOL#1**. Using a Pasteur pipette, transfer 1 mL of the 1% solution of Triton X to this microcentrifuge tube (Triton X is a detergent that should facilitate the solvation of proteins contained in the pellet). Vortex the microcentrifuge tube thoroughly for 3–5 minutes until the pellet is broken down to smaller pieces.

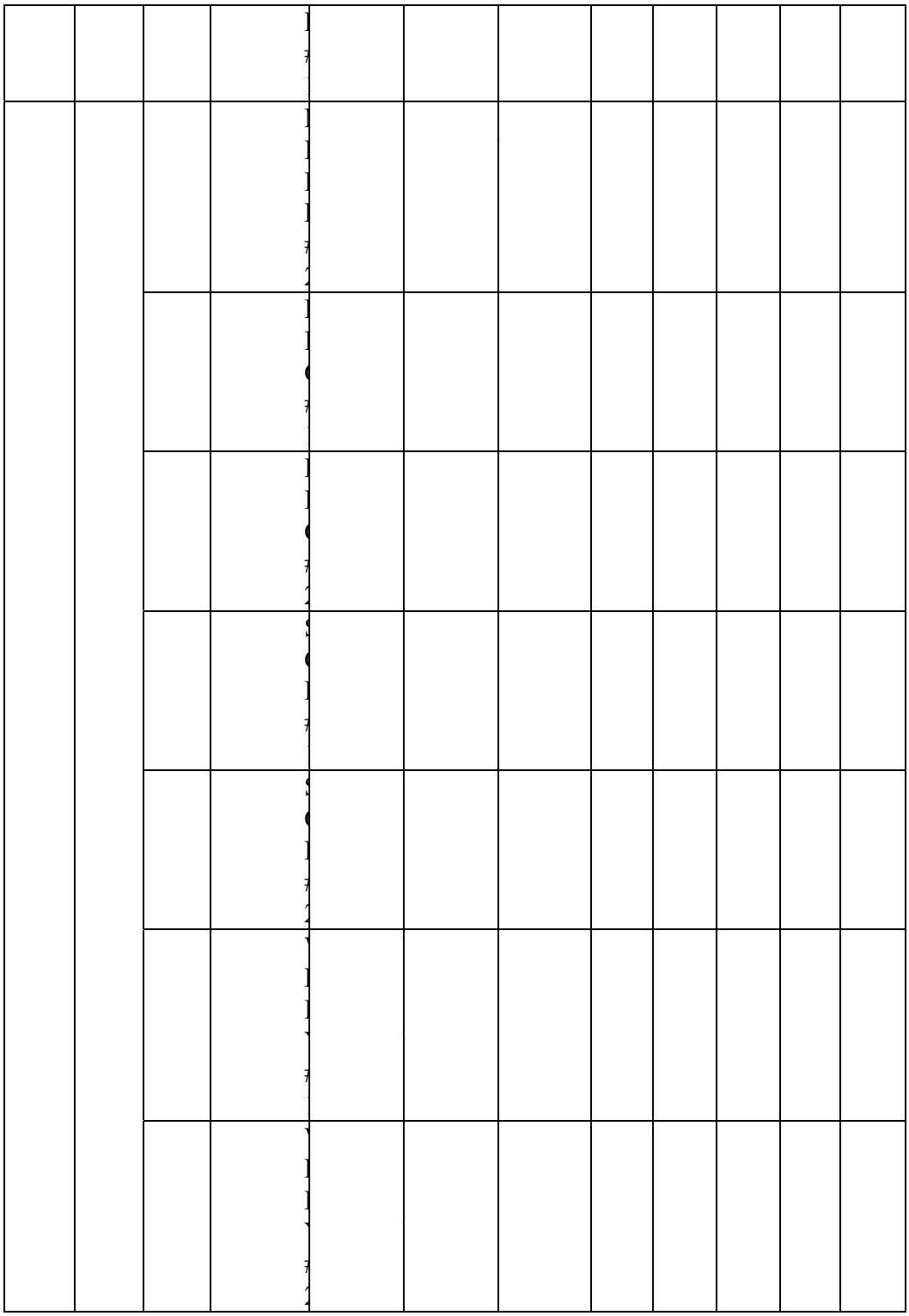
   In case you obtained no solid fraction, ask a lab assistant for help (you will be given a ready-to-use sample, but 2 points will be deducted for that).
7. Prepare a 2% solution of the supernatant from the previous step. Using a Pasteur pipette, first add 1 mL of PBS to a clean microcentrifuge tube and then, using the automatic pipette, add 20 μL of the liquid fraction of **SOL#1** into the same tube. Make sure to transfer only the liquid fraction without solid pieces. Vortex the mixture and label the tube **SOL#2**.
8. Prepare a 1% solution of **WHEY I.** Using a Pasteur pipette, transfer 1 mL of PBS into a clean microcentrifuge tube; then, using the automatic pipette, add 10 μL of whey to the same tube. Vortex the mixture and label the tube **WHEY#1**.

9. Prepare a 0.2% solution of whey. Using the automatic pipette, first add 160 µL of PBS into a clean microcentrifuge tube and then add 40 µL of mixture **WHEY#1** to the same tube. Vortex the mixture and label the tube **WHEY#2**.
10. Using the spatula, transfer a small visible amount of cheese **CURD I** into a clean microcentrifuge tube and label it **CURD#1**. Using a Pasteur pipette, transfer 1 mL of the 1% solution of Triton X to this microcentrifuge tube; vortex thoroughly for 3–5 minutes until the pellet is broken into smaller pieces.

In case you obtained no curd, ask a lab assistant for help (you will be given a ready-to-use sample, but 2 points will be deducted for that).

11. Prepare a 2% solution of the liquid fraction of the contents of tube **CURD#1**. Using the Pasteur pipette, first add 1 mL of PBS to a clean microcentrifuge tube and then add 20 µL of the liquid fraction of **CURD#1** to the same tube with the automatic pipette. Make sure to transfer only the liquid fraction without solid pieces. Vortex the mixture and label the tube **CURD#2**.
12. Now you should have, in total, 11 samples in microcentrifuge tubes and you can start pipetting those onto the microtiter plate. Be careful with pipetting, as protein-containing solutions tend to form bubbles, which may compromise the measurement! If bubbles are formed, you can try to break those with the sharp end of a pipette tip.
13. Check that your team name is written on the lid of the plate (**otherwise consult with a lab assistant**). Remove the lid of the plate and keep an eye on it so that none of the plates accidentally get swapped between the teams.
14. First prepare the BSA dilution series on the microtiter plate using an automatic pipette.
    a. Pipette 140 µL of PBS to well H1. Then pipette PBS to the wells A1–G1 (75 µL to each well).
    b. Add 10 µL of the BSA solution to well H1 and mix it thoroughly with the pipette by pipetting up and down.
    c. Take 75 µL of the solution from well H1 and transfer it to well G1; mix thoroughly as before, and transfer 75 µL of the resulting solution to well F1. Repeat these steps until you reach well B1.
    d. From B1, do not transfer the solution to A1, but discard together with the pipette tip. Now you should have 75 µL of liquid in all wells of column 1.
    e. Repeat the same procedure with column 2.
15. Now pipette your other samples into the wells of the microtiter plate (75 µL of each sample) as shown in the Table 2.1. In case of **SOL#1** and **CURD#1**, make sure to transfer only the liquid fraction without any solid pieces. Each time, mix contents of the well thoroughly with the pipette by pipetting up and down.

Table 2.1. Layout of the microtiter plate for Bradford assay



16. Finally, add 75 µL of diluted Bradford reagent to all of the wells that contain liquid (when adding the Bradford reagent, mix the contents of the well a couple of times by pipetting up and down). The wells containing proteins should turn blue. Put the lid on the plate, incubate the plate for 5 minutes at room temperature. Then **give the plate to a lab assistant,** who will measure the absorbance values at 590 nm using a microplate reader.

A.2.2.2 Calculate the final total concentration (in mg/mL) of BSA in the wells A1–H1 of the microtiter plate. Using absorbance values measured by the assistant, calculate the

average absorbance values for each of the BSA concentrations (*e.g.*, wells H1 and H2) and the PBS controls. Fill in the table **in the Excel file**.

A.2.2.3 Plot the data from A.2.2.2 **in the Excel file** (BSA concentration on the x-axis and the average absorbance values on the y-axis).

A.2.2.4 Using the absorbance values measured by the assistant, calculate the average absorbance values for each of the samples in the microtiter plate columns 4–7 (*e.g.*, wells A4 and A5). Fill in the table **in the Excel file**.

A.2.2.5 Using the calibration curve made in Task A.2.2.3 and the calculated average absorbance values, estimate the approximate total protein concentration (in mg/mL) for all of the samples in the microtiter plate columns 4–7. If the absorbance value measured for the samples is out of the range of the calibration curve, use sign **<** if the total protein concentration is less than the minimum, or **>** if it is more than the maximum. **If you cannot obtain a calibration curve with your data, ask an assistant for help** (you will be given a ready-to-use set of data, but 4 points will be deducted for that). Fill in the table **in the Excel file**.

A.2.2.6 Using the most precise averaged results, calculate the total protein concentration (in mg/mL) in the samples. Fill in the table **in the Excel file**.

Task A.2.3 SDS-PAGE for Monitoring Changes in Protein Composition

The approximate protein composition of raw milk is given in Table 2.2.

Table 2.2. Relative protein content of raw milk and the molecular weight values of corresponding proteins as established by gel electrophoresis.

Protein Family	Content in Raw Milk, %	Observed Molecular Weight, g/mol (Da)
α-caseins	45–55	32 000
β-casein	25–35	29 000
κ-casein	8–15	25 000
β-lactoglobuline	7–12	17 000
α-lactalbumine	2–4	12 000

During heat treatment, milk proteins may interact and form chemical complexes with high molecular weight (over 50 000 Da).

In cow milk, about 90% of casein exists as macromolecular aggregates termed micelles. In raw milk, aggregation of these micelles is prevented by the water-soluble tail (glycomacropeptide) attached to casein. When rennet is introduced, its enzymes hydrolyse specifically the stabilizing outer layer of micelles resulting in the formation of para-casein with lower molecular weight and higher hydrophobicity. In this way, aggregation of hydrophobic particles is initiated and followed by the further extensive growth of the casein clusters until curd is formed. Curd traps water, fat and bacteria.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a technique that is used to separate proteins according to their molecular weight. The proteins in the sample are denaturated using a detergent (sodium dodecyl sulfate, SDS) and heat, whereas SDS also binds to the amino acid residues of the protein and gives them negative charges. The sample is then applied onto a porous gel, and electric current is passed through the system, causing migration of the negatively charged molecules

towards the positive electrode. As polyacrylamide gel restrains larger molecules from migrating as fast as smaller molecules, separation of proteins according to their molecular weight occurs. Usually, a mixture of proteins – with the individual components' molecular weights known – is applied as a standard on a separate lane of the same gel to simplify the analysis of the results. After electrophoresis, the proteins in the gel can be visualized using protein-binding dyes such as Coomassie Brilliant Blue.
A.2.3.1 Figure 2.2 below is an example of an SDS-PAGE gel stained with Coomassie Brilliant Blue dye. Each lane on the gel represents one sample. Indicate on the figure **in the Answer sheet**, at which height on the gel you can see a band corresponding to the (*) α-casein, (**) β-casein, (***) κ-casein, (#) β-lactoglobuline, (‡) α-lactalbumine.

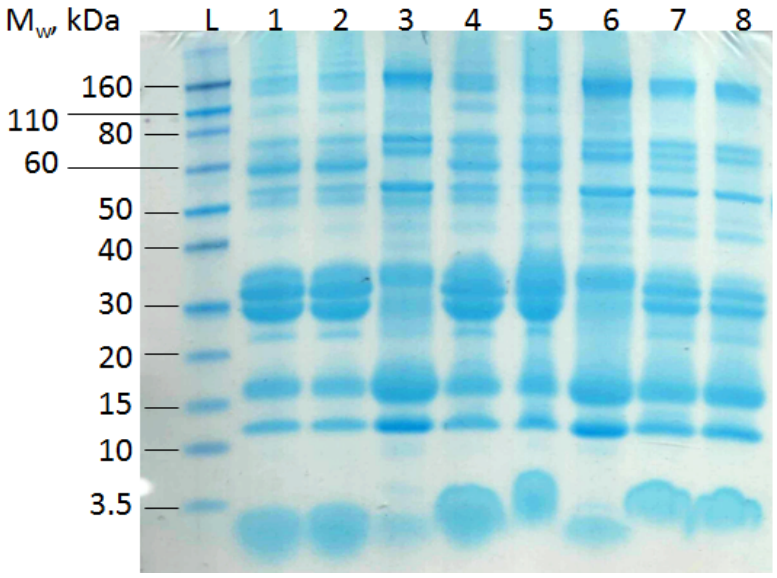


Figure 2.2. An SDS-PAGE gel stained with Coomassie Brilliant Blue dye. The leftmost lane (marked L) contains a mixture of proteins with known molecular weights (M_w) shown on the left in kilodaltons (kDa). Lanes 1 and 2 contain milk; lane 3 contains the liquid fraction separated from the acidified milk; lanes 4 and 5 contain the supernatant of solid fraction separated from the acidified milk; lane 6 contains whey; lanes 7 and 8 contain the supernatant of cheese curd I.
A.2.3.2 Using gel quantification software, the total band intensities in the regions of 35–25 kDa, 17 kDa and 12 kDa were calculated for different lanes. The results are shown as percentages of total intensity (relative content, %) of the corresponding lanes (see Figure 2.3). Provide an explanation for the observed trends **in the Answer sheet** by marking each of the following statements as either true (write +) or false (write 0).

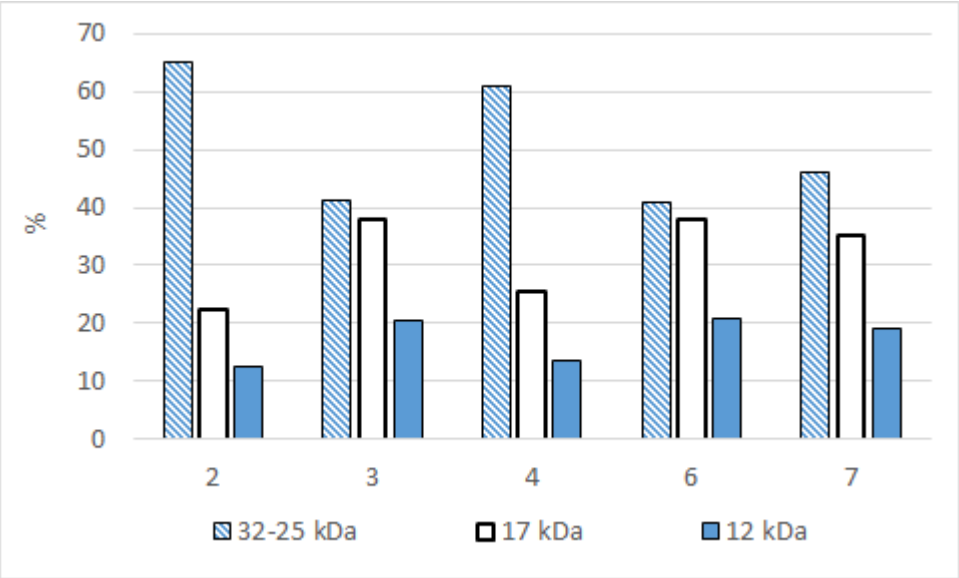


Figure 2.3. Relative content (%) of proteins with different molecular weights in the indicated lanes of the gel. The numbering of the lanes corresponds to that of Figure 2.2.

number	statement
1	The relative content of proteins with molecular weight values of 32–25 kDa, presented on the graph, does not reflect the physico-chemical processes occurring during milk acidification or cheese production.
2	The relative content of caseins is reduced in the liquid fraction separated from the acidified milk and in whey because caseins become poorly soluble during cheese production and milk acidification.
3	The relative content of caseins is reduced in the liquid fraction separated from the acidified milk because caseins are degraded during acidification.
4	The relative content of caseins is reduced in the supernatant of the cheese curd because caseins are degraded during cheese production.
5	The relative content of β-lactoglobuline and α-lactalbumine is increased in whey because these proteins are produced by bacteria during cheese making.
6	The relative content of β-lactoglobuline and α-lactalbumine is increased in the liquid fraction separated from the acidified milk and in whey because the relative content of caseins in supernatants is decreased.
7	The relative content of proteins with molecular weight of 17 kDa and 12 kDa is increased in the supernatant of the cheese curd because those represent the products of enzymatic degradation of casein.

8	The protein composition of milk and the supernatant of the solid fraction of acidified milk is dramatically different, as acid degrades proteins.

Milk day

TASK A.3 Iodometric Titration of Lactose

Milk is a natural system that can be described as a colloid containing proteins, lipids, and carbohydrates (mainly lactose). Today, your task is to investigate those components of milk in a dairy lab using physical, chemical, and biological methods. Your work is financed by a dairy company called cowBOOM. cowBOOM intends to launch a series of specialised milk products. Your studies will enable determining different properties of the milk samples and their marketing suitability.

General materials:

- laptop
- pens
- 2 waterproof markers
- 2 pencils (the mechanical pencil is for Task A.1)
- ruler
- scissors
- long and short forceps
- Post-it papers
- clock
- calculator
- distilled water (500 mL bottle)
- safety goggles
- paper towels
- paper bin (blue label)
- plastic bin (yellow label)
- glass bin (green label)
- yellow wet waste jug

TASK A.3 Iodometric Titration of Lactose

Lactose is a disaccharide composed of one galactose and one glucose moiety. The lactose content of a solution can be measured by iodometric titration, where iodine acts as the oxidizing agent and lactose acts as the reducing agent. A known and excessive amount of iodine is added to a sample of milk with an unknown lactose content. Some of the iodine reacts with the lactose in the sample. As the next step, the titration of the excess I_2 is carried out with a $Na_2S_2O_3$ solution of known concentration. "Blank" sample (distilled water) is also titrated the same way as the milk, to assess and correct for the loss of iodine by the process (not by the analyte). From the difference between the added ("blank") and titrated iodine content, it is possible to calculate how much iodine reacted with lactose. Using this data, the lactose content of the milk sample can be calculated.

You will be given a fermented milk sample as well. It is made from the same milk as you titrate, but substance B (same as in Task A.2.1) was added to it a day earlier. You have to determine how the lactose content changes during that time (the same, higher, or lower concentration).

A.3.1 In the Answer sheet you will find the molecular structure of lactose. Write the equilibrium equation of the reaction through which lactose turns into an open chain compound. Encircle the structural feature that endows lactose its reducing properties.

A.3.2 Write your answers in the Answer sheet. Below you will find equations depicting the reactions taking place during the titration described above. Balance these equations – write a number in front of each compound, except when the number should be 1.

$$C_{12}H_{22}O_{11}\text{(lactose)} + \quad I_2 + \quad NaOH \rightarrow \quad C_{12}H_{21}O_{12}Na + \quad NaI + \quad H_2O$$

$$Na_2S_2O_3 + \quad I_2 \rightarrow \quad NaI + \quad Na_2S_4O_6$$

In the practical task the lactose mass percentage of milk and fermented milk will be measured. For this iodometric titration will be used.

List of necessary equipment:

- milk (in 300 mL plastic bottle)
- fermented milk (in 50 mL plastic container)
- solution of $CuSO_4$ (in 50 mL plastic container)
- 0.5 M NaOH (in 50 mL plastic container)
- 1 M HCl (in 50 mL plastic container)
- 0.5% starch (in dropping bottle)
- 0.05 M I_2 (in 100 mL Erlenmeyer flask covered with aluminium foil)
- 2 volumetric flasks with plastic stoppers (250 mL)

- 4 Erlenmeyer flasks with glass stoppers (200 mL)
- 2 beakers (250 mL)
- 100 mL beaker (for distilled water)
- 10 mL volumetric pipette
- 25 mL volumetric pipette
- glass rod
- burette on a retort stand (filled with 0.1 M $Na_2S_2O_3$)
- beaker under the burette (100 mL)
- filter paper discs (in mini-grip)
- plastic funnel (blue)
- 7 Pasteur pipettes
- 3 plastic centrifuge tubes (15 mL)
- scales
- 3 sheets of aluminium foil
- 2 pipette fillers

Ask a lab assistant if you need more chemicals ($Na_2S_2O_3$ solution will be added to the burette by the lab assistant), filter paper discs or paper towels. No points will be deducted.

Experimental procedure:

NB! Besides blank, you need to prepare and titrate two samples – milk and fermented milk. Please pay attention to step 13 for better time management!

1. **Before each time you take a sample**, shake the milk bottle and fermented milk container thoroughly but moderately to mix the different layers well. Do not shake the sample too intensely in order to avoid foam formation!
2. Weigh about 10 g of milk into a 250 mL **volumetric flask** using a Pasteur pipette. Write down the exact weight in Table 3.1 **in the Answer sheet**. Add distilled water until the flask is about half full and stir thoroughly.
3. To precipitate the milk proteins, add approximately 5 mL of the $CuSO_4$ solution, using Pasteur pipettes or 15 mL centrifuge tubes for the volume measurements, and stir the mixture. Next, add approx. 4 mL of the 0.5 M NaOH solution to the mixture, using Pasteur pipettes or 15 mL centrifuge tubes for the volume measurements and stir the mixture again!
4. Fill the flask with distilled water to the mark, put the plastic stopper on, shake thoroughly and allow 20 minutes for the reactions to take place. Apply Post-it paper with the initial time on the flask for convenience!

5. Once 20 minutes have passed, use a dry paper filter and glass rod (if necessary) to filtrate your solution into the 250 mL beaker.
6. Measure 25.00 mL of the filtrate into a 200 mL Erlenmeyer flask.
7. Add 10.00 mL of the 0.05 M I_2 solution to the mixture and stir moderately.
8. Add approximately 7.5 mL of 0.5 M NaOH solution using a Pasteur pipette or 15 mL centrifuge tube to the mixture and stir it.
9. Close the Erlenmeyer flask with a glass stopper and protect the solution from light by wrapping it into aluminium foil for 20 minutes.
10. Next, add approx. 4 mL of 1 M HCl solution, using a Pasteur pipette or 15 mL centrifuge tube, and **write down the initial reading** of the burette. Then start to titrate the free iodine with the 0.1 M $Na_2S_2O_3$ solution. When your solution has turned light yellow, add a few drops of starch solution, until the starch solution will turn your solution dark blue. Titrate until the blue color disappears and the solution doesn't turn blue for the next 30 seconds. Record your results in Table 3.2 **in the Answer sheet**.
11. Repeat steps 6–10 as many times as you think is necessary to be able to calculate the lactose content of the sample accurately.
12. Repeat the experiment with a "blank" sample (distilled water). For the blank sample, only carry out steps 6–11 and use distilled water instead of the filtrate in step 6. Record the results in Table 3.2 **in the Answer sheet**. The volumetric pipette, 250 mL beaker, and funnel should be rinsed with distilled water and the funnel wiped with paper towels, if necessary. You may use the sink at the end of your lab table.
13. Using fermented milk instead of milk, repeat steps 1–11. This titration does not have to be very precise (two parallel titrations are more than enough). It is important to assess whether the lactose content in fermented milk is greater, equal, or smaller than in the milk sample. Record the results in Tables 3.1 and 3.3 **in the Answer sheet.** The 25 mL volumetric pipette should be rinsed with fermented milk filtrate and 250 mL volumetric flask should be rinsed with distilled water, if necessary.

A.3.3 Check that you have filled in the tables 3.1, 3.2 and 3.3 **in the Answer sheet** as required.

A.3.4 Calculate the concentration of lactose in milk and fermented milk (mass %). From Tables 3.2 and 3.3 **in the Answer sheet**, use only the accepted volume. Molar mass of lactose is 342 g/mol.

TASK A.3.5 Milk production – Integration of Results

In this task you will have to decide whether the milk you have been analysing in the previous Tasks (Tasks A.1–A.3) is suitable for various marketing ideas of a start-up company called cowBOOM.

A.3.5.1 cowBOOM would like to sell beneficial milk that has vitamin D added to it. Figure 3.1 shows the dependence of the solubility of vitamin D in milk relative to the fat

content of milk. Based on the results of Task A.1, which milk would you choose for this purpose (**K, L, M**, or **N**)? For standardization and quality reasons, it is also important that cream does not form on top of the milk.

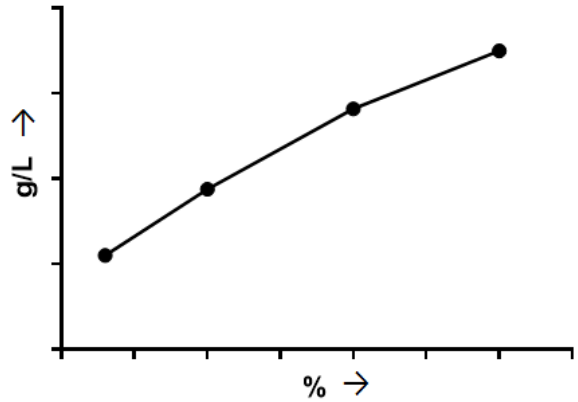


Figure 3.1. Solubility (g/L) of vitamin D relative to the fat content of milk (%). The arrows indicate the direction of increase.

A.3.5.2 cowBOOM intends to produce allergy-free milk for people who are allergic to a certain type of antibiotic – gentamicin. Figure 3.2 shows the dependence of lactose content of milk treated with substance B (as in Task A.2.1) on the incubation time in the presence or absence of the antibiotic. Do you think that the milk you used in Tasks A.2 and A.3 would be suitable to market as gentamicin allergy free milk?

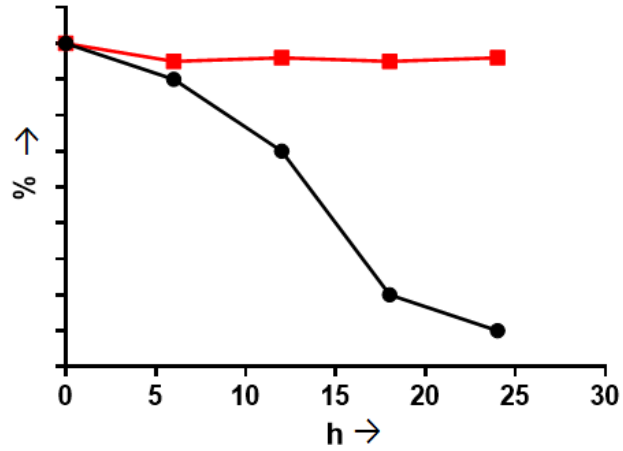


Figure 3.2. Relative content (%) of lactose dependent on the incubation time (h) of milk with substance B in the absence of gentamicin (●) , or in the presence of traces of gentamicin (■). The arrows indicate the direction of increase.

A.3.5.3 Approximately 65% of people in the world are lactose intolerant. cowBOOM would like to capitalize on this and sell milk aimed at persons who have lactose intolerance. Such milk should contain less than 0.01% of lactose by mass. Do you think that the milk you used in Tasks A.2 and A.3 would be suitable to label as lactose-free?

A.3.5.4 Finally, cowBOOM intends to sell allergy-free milk to people who are allergic to casein. Such milk should contain less than 0.005% of caseins (total of α, β and κ) by mass. Do you think that the milk you used in Tasks A.2 and A.3 would be suitable for this marketing purpose?

Task 2

General instructions

Wear the lab coat at all times in the lab.

Eating and drinking in the lab is strictly prohibited. Ask a lab assistant if you want to drink or go to the toilet.

It is highly advisable to wear disposable gloves and protective goggles when handling chemicals.

Defective and broken equipment will be replaced by a lab assistant, if you ask for it.

Nevertheless you are expected to clean spilled liquids etc yourself.

Behave during experiments environmentally friendly! Please find a suitable bin for disposal of produced waste paper, plastic, metal, glass or wet waste!

All paper used, including rough work paper, must be left on the desk at the end of the experiment.

All results must eventually be entered into your *yellow* Answer Sheet (or the Excel files).

Please save all files with your experimental data on the desktop of your team's laptop!

Only the *yellow* Answer Sheet and the Excel files will be marked.

The experiment consists of three Tasks and can be completed either individually or as a team.

Battery day

TASK B.1 Aluminium-air battery

It is important to start preparing the longest task B.1 immediately and later measure parameters of the batteries quickly with two team members. Task B.2 may need help from your physicist in the beginning, otherwise it is microbiology. Task B.3 is much shorter and is intended to be started by your chemist after helping to prepare task B.1 electrodes.

As the Earth's oil and gas reserves are moving closer to depletion with every day, we need to find new and more effective ways to harvest renewable energy and to store it. Electrical energy can be stored in batteries and in fuel cells, both of which may have a very complex design.

In batteries and fuel cells the energy from the chemical reaction is directly transformed into electrical energy. The oxidation and reduction half reactions are separated to achieve it. The oxidation takes place at the anode and reduction at the cathode. Electrodes can be inert or dissolvable. The electrodes are immersed in the electrolyte. Usually a membrane is used to separate the anode and cathode compartment, whereas ions can pass through the membrane. The membrane avoids short-circuit and/or mixing of the electrolytes. The electric current in the outer circuit could be used to power the electric motors and lights.

Here we challenge you to build a few battery types using only some simple materials. Your physicist´s task is to develop a cheaper and more energy dense battery for an electric car (task B.1). Your chemist´s task is to develop a new superbattery formula to replace ordinary 1.5 V battery cells (task B.3). Your biologist´s task is to study a battery using the cheapest “fuel” possible (task B.2).
Notice that the pages 2, 11 and 25 are identical.

***General materials*:**

- laptop
- 3 pens, 2 pencils
- 2 waterproof markers
- ruler
- scissors
- thread
- short forceps
- Post-it papers
- toothpicks
- clock
- calculator
- periodic table of elements
- A3 list of blue paper

- distilled water bottle (500 mL bottle)
- safety goggles
- paper towels
- paper bin (blue label)
- plastic bin (yellow label)
- glass bin (green label)
- metal bin (red label)
- yellow wet waste jug

TASK B.1 Aluminium-air battery

Your first task today is to assemble two aluminium-air battery cells. You will then characterize one cell's power output and use the other cell to power a model car in a race. The car track will be open during the competition: from beginning of the 3rd competition hour until to the end of competition. Come to the race as soon as your battery is ready! If you come in the end of competition time you'll lose time in the live queue.

Chemicals:

- 23 g (40 mL) of active carbon
- 10% NaOH solution (in a 125 mL container; same as drainage cleaning liquid) BE CAREFUL! Handle with gloves! Do not drop NaOH onto your skin or clothes! Wear safety glasses!
- two-component epoxy glue in two syringes (labelled as "glue" and "hardener" in a mini-grip)

Materials:

- 2 empty Tic-Tac containers
- a model car on a piece of wood
- stainless steel bowl
- knife and spoon
- stainless steel mesh (17 x 8.8 cm)
- sandpaper
- aluminium foil (70 μm thick)
- plastic foil (a base for gluing)
- 2 syringes (5 mL) with plastic needles (labelled as NaCl and NaOH in a mini-grip)
- thick and thin metal plates (3 +2)
- thick white metal base (for cooling)
- bolts, nuts and wrench
- 3 multimeters, 10 wires with banana plugs and 8 crocodile clips (to be shared by team members for different tasks)
- 11 resistors (566 kΩ, 10 kΩ, 1 kΩ, 470 Ω, 100 Ω, 47 Ω, 18 Ω, 10 Ω, 4.7 Ω, 2.2 Ω, 1 Ω) (shared)
- Excel file "Battery B.1.5 [Country Team A/B].xslx" on the computer's desktop
- video "Al-air battery – car race challenge" on the computer's desktop

Materials placed in the lab separately from your working place:

- board stove, stainless steel pot, pot holders
- handy press made from 12 t hydraulic jack (one or two in the lab)

Task B.1.1 Preparation of carbon cathodes and other components

Aluminium–air batteries produce electricity from the reaction of the air oxygen with aluminium. Such batteries have one of the highest energy densities, because a classical oxidizing compound at a cathode (such as MnO_2 in common batteries) is replaced by air oxygen. The aluminium–air batteries working in aqueous solution are electrically non-

rechargeable. Once the aluminium anode is consumed by its reaction with atmospheric oxygen to form hydrated aluminium oxide, the battery will no longer produce electricity. However, it is possible to mechanically recharge the battery with new aluminium anodes.

In order to use air oxygen as an oxidizer in a battery (or a fuel cell), you need a chemically inert, electrically conductive electrode having large surface area. A suitable material is porous carbon, for example active carbon from a water cleaning filter.

Instructions for preparation of aluminium-air batteries are shown in a demonstration video ("Al-air battery - car race challenge") on the desktop of your laptop.

You may need assistance from your chemist for completing steps 1-9.

1. Go to your stove! Turn on the stove by turning the knob to the red line (aiming to heat the stove up to 100 ºC). Place the empty pot on the stove. Watch the video.
2. Use scissors to cut out four current collectors from the given stainless steel mesh. **Think how!** The size and shape of the current collectors is depicted in Figure 1.1. Don't waste mesh material, you have been given only the minimum amount needed. Take care when cutting the mesh to avoid injury from the sharp edges of the mesh.

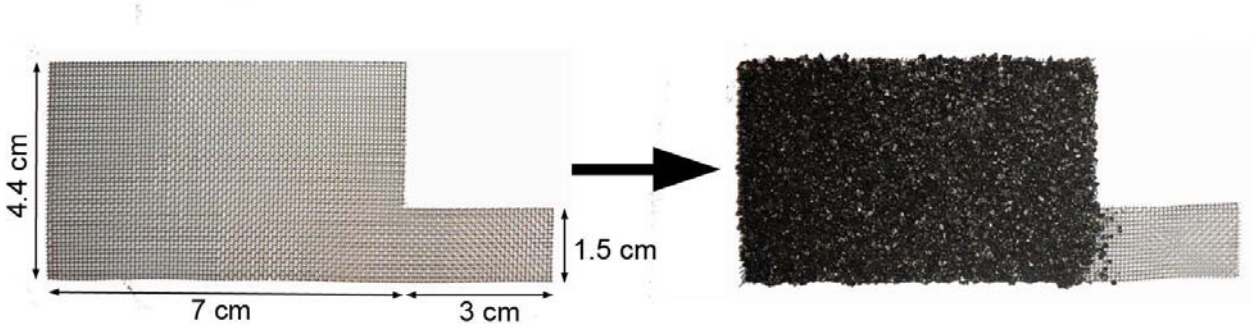


Figure 1.1. A metal mesh is needed as a current collector for a poorly conducting porous carbon electrode.

3. Prepare a total of 0.6 mL epoxy glue. Take 5 parts of glue and 1 part of hardener and mix them together (~1 min) with a toothpick on the plastic foil. Be sure to wear gloves when handling the glue. **Do not touch anything else with these gloves**, except the current collectors; after gluing discard these gloves.
4. Cover both sides of the current collector, except for the protruding part (see figure 1.1), with a thin layer of epoxy glue layer on both sides by rubbing it with gluey gloved fingers. Repeat it with all four current collectors. Gluey electrodes can be placed on the plastic foil only. Use as little glue as possible but enough to cover the whole area thoroughly. See video for the gluing method. **Take off the gloves after you finish gluing** (you may put on a new pair)! **Avoid getting glue anywhere else!**
5. Active carbon (from a water filter) has already been crushed and filtered for you.
6. Take the thick press plate with bolts as the base plate and place a thinner metal plate onto it. Cover the thin metal plate sparsely, but uniformly with a ~1 mm thick carbon particle layer. Cover ~0.5 cm larger area than two electrodes would take.

7. Above the stainless steel bowl, shake the carbon uniformly onto both sides of a glue-coated current collector (except for the protruding part). The carbon should stick on the mesh evenly as shown in the demonstration video. Do the same with another current collector.
8. Place two cathodes (now with carbon we may call these so) on the carbon layer on the metal plate as shown in the video and shake uniformly additional carbon on top of the electrodes so that current collectors are completely covered.
9. Add a second thin metal plate on top of the cathodes. Repeat the procedure described in steps 7 and 8 similarly with two other current collectors.
10. Finally, add another thin metal plate and then the thick steel plate with holes. Tighten bolts with nuts one by one using fingers. In the following procedures try holding the press plates only horizontally to avoid spilling carbon out.
11. Take your press plates to a hydraulic press (one or two per lab). Apply 12 t pressure using the hydraulic jack from your grandpa's car. You have to pump the press quite strongly. Tighten the bolts again when under pressure. **You may ask for assistance.**
12. Relieve pressure by turning the hydraulic jack tap one full turn counter-clockwise and remove the press plates.
13. Place the press plates on the preheated stove and put the stainless steel pot upside down over the press. Heat it for 35 minutes to harden the glue faster. Meanwhile you may solve theoretical problems (Tasks B.1.2. and B.1.6.).
14. Using scissors cut out two aluminium foil pieces with the same shape and dimensions as the current collectors shown in Figure 1.1. These will later function as the anodes. Clean the foil with sandpaper!
15. Use double folded tissue paper as the membrane. Cut out paper membranes out of the folded paper tissue with the dimensions a few mm larger at each side compared to the aluminium anode.
16. Turn off the oven (35 min later). Use potholders to take the press plates to your working table and place onto a white metal base to cool for at least 10 minutes.

Task B.1.2 The chemistry and physics of the aluminium-air battery

The aluminium-air battery cell has an air cathode and an aluminium anode. The following reactions take place at the electrodes during discharge:

- anode: $Al + 3OH^- \rightarrow Al(OH)_3 + 3e^-$ $E^0(Al(OH)_3/Al) = -2.3$ V,
- cathode: $O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$ $E^0(O_2/OH^-) = +0.4$ V,

where E^0 is the standard reduction potential (This is the voltage for the half-reaction when the concentration of the reactants is 1 mol/L and the pressures of the gasses are 1 bar). Reduction potential is a measure of the tendency of chemical species to acquire electrons and thereby to be reduced.

B.1.2.1 Write a balanced chemical reaction equation for discharging of the battery by combining two half-reactions. What is the number of electrons z transferred in the reaction?

B.1.2.2 Calculate the standard cell potential E^0_{cell} for the reaction obtained in B.1.2.1 by combining two half-reaction potentials.

B.1.2.3 Calculate the theoretical energy density of Al-air battery, which is Gibbs energy per mass of aluminium in units MJ/kg. The standard Gibbs energy ΔG^0 (in units J/mol) of the cell can be calculated from the equation

$$\Delta G^0 = -zFE^0_{cell},$$

where z is the number of electrons, and F is the Faraday constant (96485 $\frac{A \cdot s}{mol}$).

B.1.2.4 Derive a formula for the circuit (Figure 2.1) relating the voltage U with the current I and the battery resistance r, and the electromotive force E *(which exhibits the electrochemical potential of the battery)*.

Task B.1.3 Battery assembly

17. Remove the cathodes from the press plates after they have cooled (about 10 min). Take care when removing the nuts - use pot holders not to get burnt as the plates probably haven't cooled down completely. Tap the electrodes gently to remove excess carbon.

B.1.3.1 Show your air cathodes to a lab assistant who will take photos of them. If you have failed to produce the cathodes, the lab assistant will provide you with replacements with a penalty point and 1.5 second additional time in the car race for each replaced cathode (if the replaced cathodes will be used in the car race).

A battery needs a cathode (where reduction reaction takes place) and an anode (where oxidation reaction takes place). You have already made them both for your battery. These two must be electrically disconnected. This can be achieved with a non-conductive porous membrane e.g. paper. Carbon electrodes are placed outside to get contact with air oxygen.

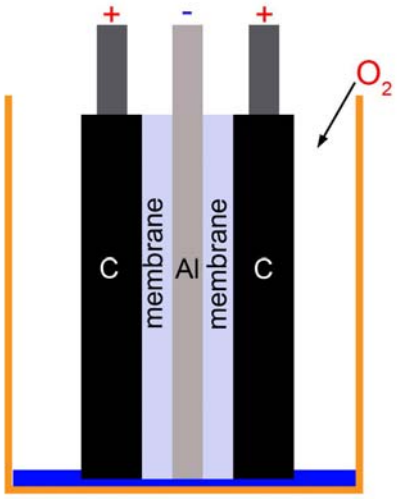


Figure 1.2. Al-air battery cell contains two carbon cathodes ("C"), two membranes ("membrane") and one aluminium foil ("Al").

18. Assemble one battery cell {cathode | membrane | anode | membrane | cathode } as shown in figure 1.2 and in the video. Wrap thread

tightly around the assembled battery. Check that the electrodes are tightly pressed against each other.

19. Assemble the second battery cell in a likewise manner.
20. Place both battery cells into Tic-Tac containers.
21. Check for short-circuit (there must be infinite resistance between the electrodes). If necessary, repack the electrodes.

B.1.3.2 Demonstrate to a lab assistant that you have no short-circuit in either cell. The lab assistant will also take photos of the assembled cells. If there is short-circuit in either of the cells or the cells are too loosely packed, the lab assistant may ask you to repack the cells. If there is still short-circuit in the second attempt, you will be provided with replacement cell(s) for penalty points (5 penalty points for each replaced cell) and 5 second penalty time in the car race (if the replaced cell will be used in the car race).

Task B.1.4 Model car race

You will use one battery cell in the car race and the other cell in the following voltage and power measurements.
A battery also needs an electrolyte - an ionically conductive solution to carry the charge from one electrode to another. Here the electrolyte is the 10% NaOH solution (in video sewage cleaning liquid).

22. Attach the first battery to the model car on a piece of wood. Use two crocodile clips to connect motor wires to the electrode protruding connectors.
23. Take 6 mL of 10% NaOH solution in a syringe. Do not add the electrolyte to the Tic-Tac container before you are told to do so. Let a lab assistant know that you are ready for the race, he or she will take you to the race track.
24. With one hand hold the car in the air. The lab assistant will tell you when to add 5 mL electrolyte to the battery cell.
25. When 5 mL of the electrolyte is added, check which way the car moves. Let it drive only a very short distance, ~10 cm. Now your will be transferred to the start line. Hold your car 1-2 cm above the start line, wheels freely spinning in the air. When start command ("ready" and starting pistol bang) is given, place the wheels on the ground at the start line and let it go.
26. The race assistant will measure the time your car covers the given distance. If the car is not able to cross the finish line, points will be awarded based on the distance covered.
27. You may try racing once more. You can add ~1 mL electrolyte to boost the battery before the second start. The best result will be recorded on your Answer Sheet.

Task B.1.5 Voltage and power measurements

Make the measurement of voltage and power with the other cell. Place the car on the wooden plate and remove carefully the first Tic-Tac box. Insert the second Tic-Tac box with new cell inside. Use the car model (placed on the wooden plate) as the stand for the Tic-Tac box.

B.1.5.1 Set up the electric circuit in accordance with the Figure 2.1 for current and voltage measurements with different resistors (similarly to Task B.2.1). Leave one of

the aluminum battery terminals unconnected! Once you've set the circuit up ask for a lab assistant's approval. During the discharge the aluminum may become coated with a thin film of different reaction products. Therefore, start using the resistor with the highest resistance and end with the one with the lowest value.
If you fail to set up a correct circuit, you will get a second chance to do it yourself. If you fail to do so for the second time, the lab assistant will provide you with a correct circuit but you lose points.

B.1.5.2 Electric current and voltage measurements with NaOH as the electrolyte.

28. Take 5 mL of 10% NaOH solution into the syringe. Add 5 mL of 10% NaOH solution into the battery cell with a syringe aiming at the top of the membrane, to ensure that the membrane is imbued with NaOH.
29. Work quickly, because part of aluminium dissolves in NaOH due to corrosion and it reduces power in time.
30. Measure the current and voltage values for all resistances starting with resistor with the highest resistance. Record the values in the Excel file Battery B.1.5 Country Team A B.xsls. Add 3 drops of NaOH solution to the top of the membrane before each measurement to maintain the constant working conditions of the cell. For the four lowest resistors change the current setting to 10 A. Do not forget to change multimeter wire position for the 10 A setting!

B.1.5.3 Calculate the power for each data point from task B.1.5.2 in the Excel file. The graph appears when you fill the table with the experimental data.

B.1.5.4 Read the maximum power measured from the graph in the Excel file. Write the answer in the Answer Sheet.

B.1.5.5 Use the voltage (y) versus current (x) graph in the Excel file to plot a linear trendline and the trendline equation. The graph appears when you fill the table with the experimental data. Estimate from the graph the internal resistance r of the electrolyte. Write the answer in the Answer Sheet.

B.1.5.6 Why basic electrolyte (NaOH solution) is better compared to neutral solution (NaCl solution) in an Al-air battery?

Task B.1.6 Real life applications

B.1.6.1 What is the maximal energetic efficiency of the assembled aluminium-air battery, based on the highest voltage achieved with the 10 % NaOH solution? Efficiency = maximum measured voltage / theoretical voltage calculated in Task B.1.2.

B.1.6.2 Which of your observations prove that the battery efficiency is lower than 100%?

B.1.6.3 My green car drives up to 700 km, but I have a feeling that it is not environmentally green. It has an internal combustion engine, which has 20% fuel-to-wheel efficiency, and it accommodates 40 kg gasoline, which has energy density of 44 MJ kg^{-1}. I have an idea to take the engine out and replace it with an electric motor and replace the gasoline tank with the Al-air battery. How far could such electric car drive if

it would have 40 kg of aluminium in the aluminium-air battery (the energy density of aluminium-air battery was calculated in B.1.2.3)? Assume that the efficiency of the battery is as high as calculated in task B.1.6.1 and electric motor has 90% efficiency.

B.1.6.4 The aluminium-air battery has higher power than the microbial fuel cell, because the aluminium-air battery has ... (Choices are in the Answer Sheet).

Battery day

TASK B.2 Microbial fuel cell and its microbes

It is important to start preparing the longest task B.1 immediately and later measure parameters of the batteries quickly with two team members. Task B.2 may need help from your physicist in the beginning, otherwise it is microbiology. Task B.3 is much shorter and is intended to be started by your chemist after helping to prepare task B.1 electrodes.

As the Earth's oil and gas reserves are moving closer to depletion with every day, we need to find new and more effective ways to harvest renewable energy and to store it. Electrical energy can be stored in batteries and in fuel cells, both of which may have a very complex design.

In batteries and fuel cells the energy from the chemical reaction is directly transformed into electrical energy. The oxidation and reduction half reactions are separated to achieve it. The oxidation takes place at the anode and reduction at the cathode. Electrodes can be inert or dissolvable. The electrodes are immersed in the electrolyte. Usually a membrane is used to separate the anode and cathode compartment, whereas ions can pass through the membrane. The membrane avoids short-circuit and/or mixing of electrolyte. The electric current in the outer circuit could be used to power the electric motors and lights.

Here we challenge you to build a few battery types using only some simple materials. Your physicist´s task is to develop a cheaper and more energy dense battery for an electric car (task B.1). Your chemist´s task is to develop a new superbattery formula to replace ordinary 1.5 V battery cells (task B.3). Your biologist´s task is to study a battery using the cheapest “fuel” possible (task B.2).

Notice that the pages 2, 12 and 25 are identical.

***General materials*:**

- laptop
- 3 pens, 2 pencils
- 2 waterproof markers
- ruler
- scissors
- thread
- short forceps
- Post-it papers
- toothpicks
- clock
- calculator
- periodic table of elements

- A3 list of blue paper
- distilled water bottle (500 mL bottle)
- safety goggles
- paper towels
- paper bin (blue label)
- plastic bin (yellow label)
- glass bin (green label)
- metal bin (red label)
- yellow wet waste jug

TASK B.2 Microbial fuel cell and its microbes

Your task is to study a battery that uses the cheapest "fuel" possible, and identify the bacteria isolated from the microbial fuel cell.

List of necessary equipment:

- microbial fuel cell
- 11 resistors (566 kΩ, 10 kΩ, 1 kΩ, 470 Ω, 100 Ω, 47 Ω, 18 Ω, 10 Ω, 4.7 Ω, 2.2 Ω, 1 Ω) (shared with other tasks)
- 3 multimeters (shared with other tasks)
- 10 wires (shared with other tasks)
- 8 crocodile clips (shared with other tasks)
- Excel file "Battery B.2.1 [Country Team A/B].xslx" on the computer's desktop
- video "Microbial fuel cell – DIY Elbonian style" on the computer's desktop

Task B.2.1. Microbial fuel cell

Commercial fuel cells need expensive platinum as their catalyst, but it is also possible to use bacteria as living catalysts. The fuel can be anything bacteria eat – sugar, vegetables, meat; but also cheaper substances, such as spoiled food, mud, wastewater etc. Some bacteria are able to release organic or inorganic compounds, which will oxidize at the anode. Building a bacterial fuel cell is simple and you can do it yourself at home using only household materials.

One cell has been assembled for you few weeks ago, because the bacterial community in the fuel cell needs time to grow. By now, the cell has reached some intermediate power level but not maximum. A video about the preparation of the cell (Microbial fuel cell – DIY Elbonian style) can be seen on the computer desktop. The cell consists of two porous active carbon electrodes in mud/wastewater mixture. One electrode is in the bottom (the anode), the other is on the top (the cathode) and has contact with air oxygen.

Your task is to study this cell – measure its voltage, current, and analyze why and how it works.

Your task is to investigate the electrical power output and the internal resistance of the cell. For that you have to set up an electric circuit (Figure 2.1) and measure voltage and current values using different resistors.

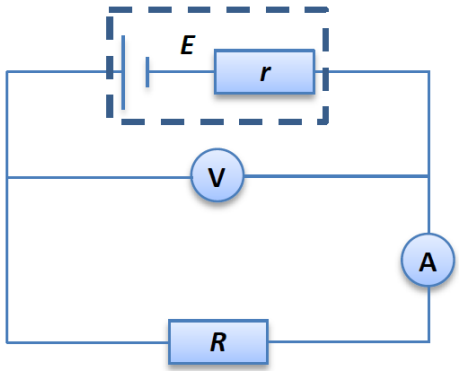


Figure 2.1. An electric circuit consisting of a battery (with an internal resistance (designated *r*) and electromotive force *E*), a resistor (designated *R*), an ammeter (designated A), and a voltmeter (designated V).

Experimental procedure:

1. Set up the circuit (Figure 2.1), but **leave one battery wire unconnected**. Use one multimeter to measure voltage and the other to measure current. Use the highest valued resistor first.
2. Once you've set the circuit up, **ask for a lab assistant's approval**. If you fail to set up a correct circuit, you will get a second chance to do it yourself. If you fail to do so for the second time, the lab assistant will provide you with a correct circuit for but you lose points.
3. Be sure **not to** short-circuit the microbial fuel-cell before the measurements.
4. Measure the voltage and the current in the circuit with all provided resistors. Use the 2 V DC measurement range for voltage measurements. Use the DC microampere range for the first current measurement with the highest resistance, and change the range with decreasing resistance, when necessary.

B.2.1.1 Fill in the resistance-voltage-current-power table **in the Excel file *Battery B.2.1 Country Team A B.xsls*** by making necessary measurements and calculations.

B.2.1.2 What is the maximum voltage achieved? What is the maximum current achieved? What is the maximal power achieved? Write your answers **in the Answer sheet.**

B.2.1.3 Use a voltage (*y*) *versus* current graph (*x*) in **the Excel file** to plot a linear trendline and the trendline equation. The graph appears then you fill the table with the experimental data. The internal resistances of the cell is $r = -$slope. Write the internal resistance *r* **in the Answer sheet.**

B.2.1.4 What is the total mass of microbial fuel cells if these would be used as a current source for a 75 kW electric car? The mass of a single microbial fuel cell is ∼300 g. Take the fuel cell power from task B.2.1.2**.**

B.2.1.5 Where could the microbial fuel cell in practice be used?

B.2.1.6 Look at how the cell has been constructed and decide, **in the Answer sheet,** which bacterial community forms at the bottom of the mud cell.

1. aerobic
2. anaerobic

B.2.1.7 Propose a simple chemical equation for discharging of the bacterial cell. Give the anode and cathode reactions separately. Assume that the food that the bacteria consume is some hydrocarbon $(CH_2O)_n$. Use protons and water to balance the equation.

TASK B.2.2 Identification of a bacterial strain isolated from a microbial fuel cell

An unknown bacterial strain has been isolated from a microbial fuel cell and its purified culture (marked as **X**) has been plated onto a Petri dish containing LB+lactose agar medium (LB is a nutrient rich medium used for growing bacteria). Your task is to identify the bacterial strain by performing or studying the results of eight microbiological tests. An identification table (Table 2.1) and a table of transition ranges of various pH indicators (Table 2.2) are given below. For your convenience, you may mark the results of your tests for the unknown strain in the bottom row of Table 2.1, but only the final results **in the Answer sheet** will be graded.

List of necessary equipment:

- microscope
- automatic pipette and tips
- 4 microscope slides (on Petri dish)
- 10 toothpicks (on Petri dish)
- bacterial strains on Petri dish with LB+lactose agar
- distilled water in 2 mL centrifuge tube (on rack)
- long forceps (on rack for centrifuge tube)

Table 2.1. Macromorphological, cellular, biochemical and physiological parameters of different bacterial genera

Genus		**Shape**			**Pigmentation**	**Motility**				
Bacillus		bacillus			none	+				
Enterobacter		coccobacillus			none	+				
Erwinia		coccobacillus			none	+				

Escherichia		coccobacillus			none	+				
Klebsiella		coccobacillus			none	-				
Micrococcus		coccus			yellow	-				
Pseudomonas		bacillus			none	+				
Raoultella		coccobacillus			none	-				
Salmonella		coccobacillus			none	+				
Serratia		coccobacillus			red/none	+				
Shigella		coccobacillus			none	-				
Staphylococcus		coccus			none	-				
Unknown strain										

CAUTION! All materials that have been in contact with bacteria must be placed in the proper container (**plastic bin**)!
Please use rubber gloves while handling bacteria and colour solutions. Avoid touching your mouth, face, or eyes with your fingers during the whole duration of this experiment! Wash your hands thoroughly with warm water and soap after finishing the task.

B.2.2.1 Gram staining

Gram staining is a method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative). The name comes from the Danish bacteriologist Hans Christian Gram, who developed the technique.

Gram staining differentiates bacteria by the chemical and physical properties of their cell walls – in Gram-positive bacteria, the cell walls consist of a thick layer of peptidoglycan, while in Gram-negative bacteria, it is thin. In a Gram stain test, Gram-positive bacteria retain the crystal violet dye and therefore look violet after the staining, Gram-negative bacteria retain the counterstain (commonly safranin) and look red or pink after staining.

List of necessary equipment:

- crystal violet solution (in dropping bottle)
- Lugol's solution (in dropping bottle)
- safranin solution (in dropping bottle)
- ethanol (in dropping bottle)
- immersion oil (in glass dropping bottle)
- unknown bacterial strain culture grown on LB+lactose agar (marked as **X**)
- Gram-positive and Gram-negative bacterial strains grown on LB+lactose agar (marked as **G+** and **G-**, respectively)
- spirit lamp (**ask from a lab assistant, when necessary**)

Experimental procedure:

1. Take the microscope slide and pipet two separate water drops on it, side by side, up to 100 µL each, from the 2 mL centrifuge tube as shown in Figure 2.2. Suspend a very small amount of bacterial mass from Gram-positive and Gram-negative bacterial strains (marked as **G+** and **G-**, respectively) from LB+lactose agar plates (use a toothpick, be careful not to bring any agar along!) in the separate drops of distilled water and spread out the drops to facilitate faster drying. The suspensions should be only very slightly hazy. Do not let the drops get in contact with each other! Due to subsequent washes, all marks should be made onto the matte edge of the microscope slide using a pencil.

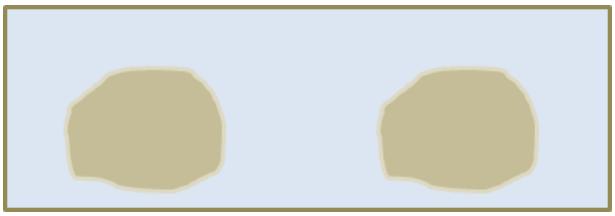


Figure 2.2. The layout of a microscope slide with two bacterial suspensions.

2. Suspend a small amount of the unknown bacterial strain from the plain LB+lactose agar plate (marked as **X**) into a single drop of distilled water on a separate microscope slide.
3. Let the slides dry (drying may take up to 20 minutes). You may carefully remove last droplets with a paper tissue.
4. **Ask a lab assistant to fix the bacteria** onto the microscope slide.

5. Put the microscope slides on a paper tissue and cover all the dried suspensions with crystal violet (Figure 2.3 shows the procedure for controls, same applies for the unknown sample). After 1 minute, rinse the slides with distilled water for a few seconds above a wet waste jug. Shake off the excess water from the surface.

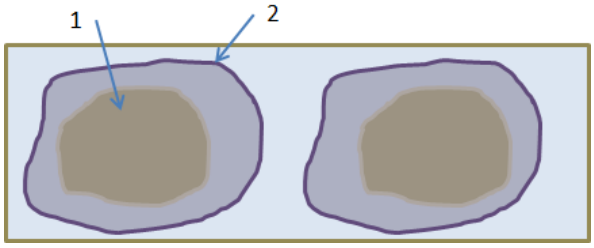


Figure 2.3. Heat fixed suspensions must be completely covered with colour (1 – heat fixed suspension, 2 – colour solution).

6. Cover all the samples with Lugol's solution. After 1 minute, rinse the slides with distilled water.
7. Cover all the samples on one slide with ethanol for 30 seconds and rinse with distilled water immediately after that. Repeat with other slide.
8. Cover all the samples with safranin. After 30 seconds, rinse with distilled water. Shake off the excess water from the surface.
9. Let the slides dry (about 10 minutes). Before microscopy, make sure that the slides have fully dried!
10. Examine the stained samples under the microscope, using the 100x oil immersion objective lens.

B.2.2.1.1 Determine the Gram reactivity of the unknown bacterial strain **in the Answer sheet**. Use **G+** and **G-** as references.
B.2.2.1.2 Determine the shape (Figure 2.4) of the unknown bacterial strain **in the Answer sheet** based on what you see in the microscope.

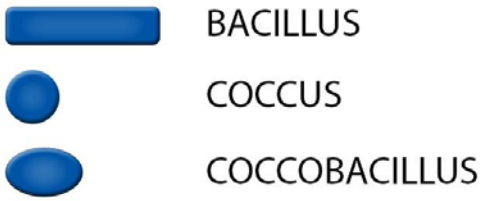


Figure 2.4. Shape types of bacteria (a coccobacillus is a shape in between a coccus and a bacillus).

B.2.2.1.3 If you want to repeat the staining, then ask the lab assistant for a new slide. Once you've determined the shape and Gram reactivity of the unknown strain and you are sure that this will be your final result, **show the prepared and in focus microscope slide to a lab assistant and ask for their approval.** The lab assistant will indicate the true Gram reactivity and shape for you **in the Answer sheet.**

B.2.2.2 Hanging drop method for determining motility

List of necessary equipment:

- special thick microscope slide with a shallow concavity
- microscope slide
- coverslip
- vaseline (in 2 mL centrifuge tube)
- unknown bacterial strain grown on LB+lactose agar (marked as **X**)

Experimental procedure:

1. Pipet 30 µL of distilled water on the unknown bacterium **X** biomass on the Petri dish with LB+lactose agar, and wait for 3 minutes. In the meantime, smear some vaseline around the shallow concavity with a toothpick (NB! Insure not to put the vaseline within the concave surface".)on a special thick microscope slide (Figure 2.5). Vaseline will be used to “glue” the cover slip and the microscope slide together. Use a small amount of vaseline to create a gentle ring, it is not necessary to make the ring continuous. Alternatively, only four marks/dots of vaseline can be made around the shallow concavity.

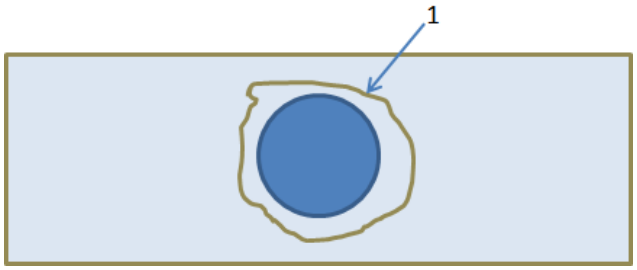


Figure 2.5.1 – vaseline ring.

2. Pipet 10 µL of the bacterial suspension from the LB+lactose agar from the culture dish onto the clean microscope slide. Take care to only take the liquid and not aggregated bacterial biomass! If the suspension is too hazy, add 10 µL of water to dilute the suspension. Pipet a small drop (the size should not be bigger than 2 mm x 2 mm - you can achieve it by taking in 10 microlitres and letting out only half of it) of suspension onto the center of a cover slip (Figure 2.6).

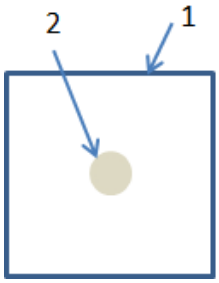


Figure 2.6. 1 – coverslip, 2 – drop of the bacterial suspension.

3. Put the special microscope slide on top of the coverslip, concavity down, and press slightly to create an airtight chamber (Figure 2.7).

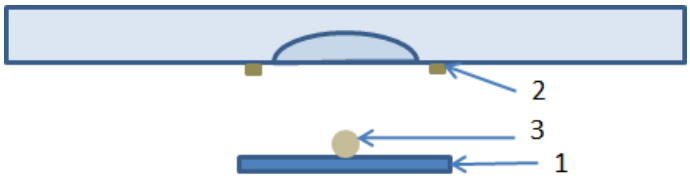


Figure 2.7.1 – cover slip, 2 – vaseline ring, 3 – drop of the bacterial suspension.

4. Flip the slide over. The droplet of bacterial suspension should be hanging down from the coverslip (Figure 2.8).

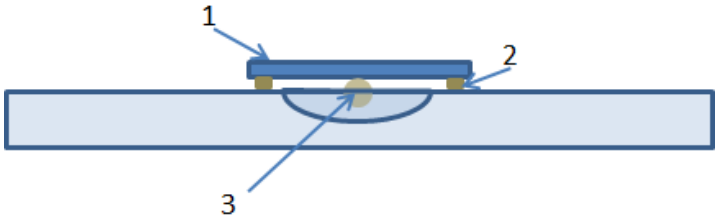


Figure 2.8. Cross-section of the finished hanging drop microscope slide (1 – cover slip, 2 – vaseline ring, 3 – drop of the bacterial suspension).

5. Examine the edge of the drop of the bacterial suspension under the microscope (40x objective).

B.2.2.2.1 Observe the bacteria and determine **in the Answer sheet**, if the unknown bacterial strain is motile or not.

B.2.2.2.2 Once you've determined the motility of the unknown strain, **show the prepared and in focus microscope slide to a lab assistant and ask for their approval.** The lab assistant will indicate the true motility for you.

B.2.2.2.3 Is it possible to see bacterial flagella with the microscope you have used? Mark your answer **in the Answer sheet.**

B.2.2.3 Oxidase test

This test will determine whether the studied strain possesses an enzyme called cytochrome c oxidase. The colorless test reagent acts as a substrate for the enzyme. The oxidized reagent forms the colored compound Wurster blue. This cytochrome system is usually only present in aerobic organisms that are capable of using oxygen as the terminal electron acceptor. The end-product of these reactions is either water or hydrogen peroxide.

List of necessary equipment:

- unknown bacterial strain grown on LB+lactose agar (marked as **X**)
- bacterial strains known to possess or lack cytochrome c oxidase (marked **OX+** and **OX-**, respectively) grown on LB+lactose agar

- 3 oxidase test strips (on Petri dish)

Experimental procedure

1. Touch the bacterial biomass with an oxidase test strip. Repeat with separate test strips for all three bacterial strains.
2. Observe the inoculated area for up to three minutes. If the area turns dark-blue, then the result is positive. If a color change does not occur within three minutes, the result is negative (Figure 2.9).

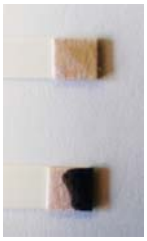


Figure 2.9. Examples of the negative (upper) and positive (lower) oxidase test.

B.2.2.3.1 Determine the presence of the cytochrome c oxidase in the unknown bacterial strain **in the Answer sheet**.

B.2.2.4 Catalase test

This test will determine, if the strain of interest possesses catalase – an enzyme that catalyzes the decomposition of hydrogen peroxide. Catalase is an important enzyme protecting the cells from oxidative damage by reactive oxygen species.

List of necessary equipment:

- unknown bacterial strain grown on LB+lactose agar (marked as **X)**
- hydrogen peroxide solution (in 2 mL centrifuge tube, 3%)
- microscope slide (on Petri dish)

Experimental procedure:

1. Pipette 20 μL of 3% hydrogen peroxide solution on the microscope slide.
2. Use a toothpick to transfer biomass of the studied bacterial strain to the drop of hydrogen peroxide. Observe whether gas bubbles form.

B.2.2.4.1 Determine the presence of catalase in the unknown bacterial strain **in the Answer sheet**.

B.2.2.4.2 Write and balance the equation for the decomposition of hydrogen peroxide in the appropriate box **in the Answer sheet**.

B.2.2.5 β-galactosidase (ONPG) test

The β-galactosidase test is used for differentiation of bacterial genera. β-galactosidase is an enzyme that catalyzes the breakdown of disaccharides (for example lactose) thus

releasing monosaccharides. The test is based on the use of a chemical calledONPG. It is an artificial substrate structurally similar to lactose, with the exception that glucose is substituted with an o-nitrophenyl group. If the organism possesses β-galactosidase, the enzyme will split ONPG , releasing o-nitrophenol, which is a yellow-colored compound.

List of necessary equipment:

- unknown bacterial strain grown overnight on LB+lactose agar (marked as **X**)
- β-galactosidase-positive and β-galactosidase-negative bacterial strains grown on LB+lactose agar (marked as **ONPG+** and **ONPG-**, respectively)
- ONPG (in 2 mL centrifuge tube, 4 mg/mL)

Experimental procedure:

1. Using a pipette, transfer 10 μL of the ONPG solution directly onto three bacterial biomasses growing on the medium (LB+lactose) and observe them, when 10 minutes have passed (for better observation, place the dish on white paper).

B.2.2.5.1 Determine the presence of the β-galactosidase in the unknown bacterial strain **in the Answer sheet**.

B.2.2.5.2 Which of the chemical substances listed in the the table **in the Answer sheet**, are products of a reaction catalyzed by β-galactosidase when lactose is a substrate?

B.2.2.6 Urease test

Urea [$CO(NH_2)_2$] has an important role in the metabolism of nitrogen-containing compounds by animals. Some bacteria can produce an enzyme called urease for creating more favourable conditions for their growth. Bacteria that possess urease, hydrolyze urea and use its decomposition products as a primary source of nitrogen. The urease test identifies the organisms that are capable of hydrolyzing urea.
For the test, bacteria are grown overnight on Christensen's agar (peptone – 1.0 g; glucose – 1.0 g; NaCl – 5.0 g; Na_2HPO_4 – 1,2 g; KH_2PO_4 – 0.8 g; yeast extract – 0.1 g; Phenol Red – 0.012 g; agar – 15 g are all dissolved in 1 L of distilled water, autoclaved and filter sterilized urea (5 mL 40%) is added to the medium). The pH of the medium is 6.8–6.9 and salmon pink in colour.

Table 2.2. Transition ranges of pH indicators (Table used also for Tasks B.2.2.7. and B.2.2.8.)

pH indicator	Transition range	Colour in acidic environment	Colour in alkaline environment
Bromocresol Green	3.8–5.4	yellow	bluish green
Bromophenol Blue	3.0–4.6	yellow	blue
Bromothymol Blue	6.0–7.6	yellow	blue
Methyl Red	4.4–6.4	red	yellow

Phenol Red	6.8–8.4	yellow	red

List of necessary equipment:

- rack with test tubes containing Christensen's agar marked as **U1** (not inoculated), **U2** (inoculated with *Proteus vulgaris*), and **U3** (inoculated with unknown bacterial strain)

B.2.2.6.1 Write and balance the equation for the hydrolysis of urea by urease in the appropriate box **in the Answer sheet.**

B.2.2.6.2. In the tube rack, locate test tubes marked **U1, U2** and **U3. U1** is a control sample not inoculated with bacteria. Tube **U2** has been inoculated with bacterium *Proteus vulgaris,* and tube **U3** with your unidentified strain. The transition ranges of various pH indicators are given in the Table 2.2. Study the test tubes and decide, whether the following statements are true (+) or false (0) **in the Answer sheet.**

B.2.2.6.3 In the Answer sheet, determine the ability of the unknown bacterial strain to hydrolyze urea.

B.2.2.6.4 Which of the statements listed, **in the Answer sheet,** are true (+) and which are false (0) regarding urease negative bacteria growing on Christensen's agar?

B.2.2.7 Citrate test

The citrate test identifies the ability of the bacterial strains to utilize citrate as a carbon and energy source. Strains are grown on Simmons' citrate agar (containing Na-citrate as a single carbon source and Bromothymol blue as a pH indicator). The pH of the prepared medium is 6.9 and its color is green. Since only very few bacteria are able to use agar as a carbon source, we can say that this medium selects for bacteria that are capable of using citrate as a sole carbon source. When Na-citrate is used as a carbon and energy source in this environment, sodium carbonate is released as a by-product.

List of necessary equipment:

- rack with test tubes containing Simmons' agar marked as **C1** (not inoculated), **C2** (inoculated with *Proteus vulgaris*), and **C3** (inoculated with unknown bacterial strain)

B.2.2.7.1 Write **in the Answer sheet**, what impact will the sodium carbonate, produced during the utilization of the Na-citrate, have on the pH of the growth medium? The transition ranges of various pH indicators are given in the Table 2.2.

B.2.2.7.2 In the tube rack, locate the test tubes marked **C1, C2** and **C3. C1** is a control sample not inoculated with bacteria. Tube **C2** has been inoculated with bacterium *Proteus vulgaris* and tube **C3** with your unidentified strain. Study them and determine

the ability of the unknown bacterial strain to use Na-citrate as carbon and energy source **in the Answer sheet.**

B.2.2.8 Oxidative-fermentative (OF) test

The oxidative-fermentative test is used to determine whether bacteria metabolize carbohydrates oxidatively (O), by fermentation (F), or are nonsaccharolytic, and therefore have no ability to use the carbohydrate in the medium. During fermentation, sugars are converted to acids, gases, or alcohol. According to the type of fermentation, following acids are produced: formic acid, lactic acid, butyric acid, etc.

The OF medium, developed by Hugh and Leifson, is used to differentiate between these types of bacteria. The medium consists of the following: peptone – 2.0 g; NaCl – 5.0 g; K_2HPO_4 – 0.3 g; glucose – 10.0 g; Bromothymol Blue – 0.03 g; agar – 3.0 g dissolved in 1 L of distilled water. The pH of the prepared medium is 7.1 and its color is green.

The test is performed simultaneously in two test tubes containing Hugh-Leifson's agar. After inoculation of both tubes, one of them will be covered with an agar cap to prevent the diffusion of oxygen into the medium and to create anaerobic growth conditions. The agar cap covering the medium can also be used to detect the formation of gaseous byproducts that will push it up during the incubation.

Based on the results of the OF test, bacteria are divided into one of three main groups:

1. Bacteria that ferment glucose. During the anaerobic process of fermentation, pyruvate is converted to a variety of mixed acids depending on the type of fermentation.
2. Bacteria that oxidize glucose. Nonfermenting bacteria metabolize glucose using only aerobic respiration and therefore only produce a small amount of weak acids during the Krebs cycle and Entner-Doudoroff (glycolytic) pathway.
3. Bacteria that neither ferment, nor oxidize glucose. These bacteria oxidize peptone instead, as their source of carbon and energy, releasing ammonia into the medium.

List of necessary equipment:

- rack with test tubes containing OF medium, marked as **OF1** (without agar cap) and **OF2** (with agar cap), inoculated with unknown bacterial strain

B.2.2.8.1 Write in the Answer sheet, which gases are produced during the fermentation of glucose. Keep in mind the acids, produced during the fermentation, and their end products!

B.2.2.8.2 How would you expect the test tubes, inoculated with four different types of bacteria, to look like? Use Figure 2.10 below to fill the table **in the Answer sheet.** The transition ranges of various pH indicators are given in Table 2.2.

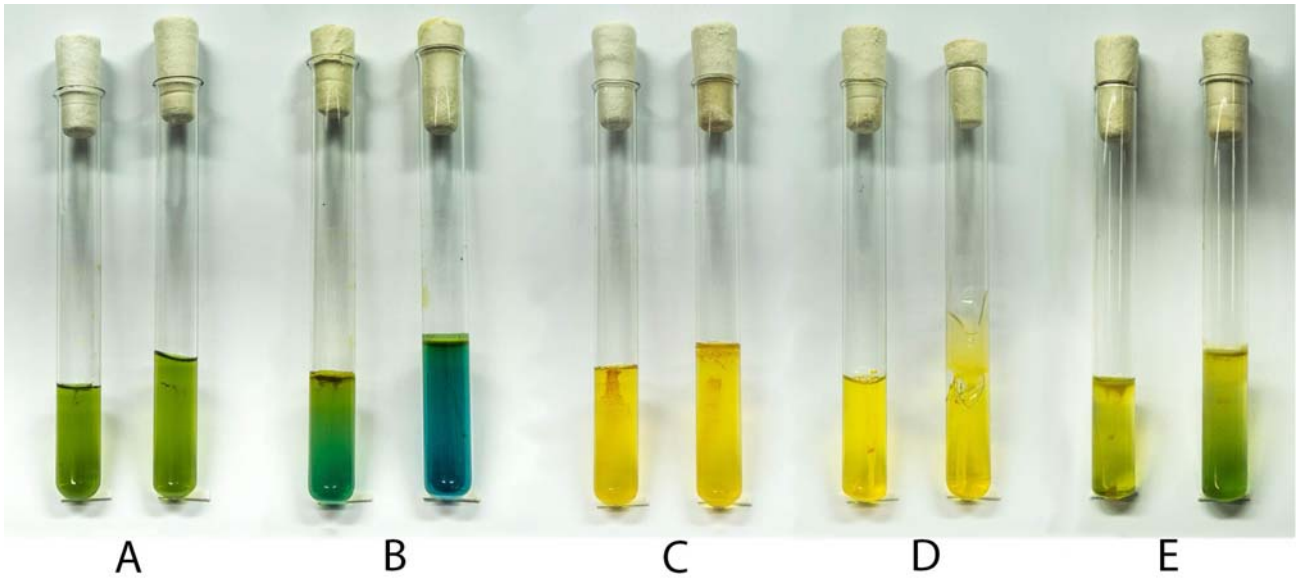


Figure 2.10. Pairs of test tubes with Hugh-Leifson's agar (in each pair, the right tube is covered with agar cap) inoculated with bacteria having different metabolism types (B, C, D, E) and test tubes not inoculated with bacteria (A).

B.2.2.8.3 In the tube rack, locate the test tubes marked **OF1** and **OF2**. Study them and determine **in the Answer sheet,** how the unknown bacterial strain uses glucose as a carbon and energy source – i.e. which type of metabolism does it have and whether it produces gas or not?

B.2.2.9 Identification

Having completed eight tests on the unknown bacterial strain, use the Table 2.1 and determine, which bacterial genus this strain belongs to. Write the correct answer **in the Answer sheet.**

Battery day

TASK B.3 Constructing an aqueous battery with highest possible voltage

It is important to start preparing the longest Task B.1 immediately, and later measure the parameters of the batteries quickly with two team members. Task B.2 may need help from your physicist in the beginning, otherwise it is microbiology. Task B.3 is much shorter and is intended to be started by your chemist after helping to prepare electrodes for Task B.1.

As the Earth's oil and gas reserves are moving closer to depletion with every day, we need to find new and more effective ways to harvest renewable energy and to store it. Electrical energy can be stored in batteries and in fuel cells, both of which may have a very complex design.

In batteries and fuel cells, the energy from the chemical reaction is directly transformed into electrical energy. The oxidation and reduction half reactions are separated in order to achieve it. The oxidation takes place at the anode and reduction at the cathode. Electrodes can be inert or dissolvable. The electrodes are immersed in the electrolyte. Usually a membrane is used to separate the anode and cathode compartment, whereas ions can pass through the membrane. The membrane avoids short-circuit and/or the mixing of electrolyte(s). The electric current in the outer circuit could be used to power electric motors and lights.

Here, we challenge you to build a few battery types using only simple materials. Your physicist´s task is to develop a cheaper and more energy dense battery for an electric car (Task B.1). Your chemist´s task is to develop a new superbattery formula to replace ordinary 1.5 V battery cells (Task B.3). Your biologist´s task is to study a battery using the cheapest “fuel” possible (Task B.2).

Notice that pages 2, 12 and 29 are identical.

***General materials*:**

- laptop
- 3 pens, 2 pencils
- 2 waterproof markers
- ruler
- scissors
- thread
- short forceps
- Post-it papers
- toothpicks
- clock

- calculator
- periodic table of elements
- A3 list of blue paper
- distilled water (500 mL bottle)
- safety goggles
- paper towels
- paper bin (blue label)
- plastic bin (yellow label)
- glass bin (green label)
- metal bin (red label)
- yellow wet waste jug

NB! Proceed to this task only after your team has reached the protocol point 11 in Task B.1.1 (i.e., after the electrodes are ready to be taken to a hydraulic press).

In this task, you have to construct a battery cell with the maximum possible voltage.

You are given a glass cell, which has two chambers, separated by a porous membrane. The aim is to light two light-emitting diodes (LEDs) with your battery. Can you do it?

Chemicals:

- 4 chemicals to be used in a battery:
  - solid $K_2Cr_2O_7$ (in 5 mL tube) (NB! handle with gloves, do not breathe the dust)
  - solid $CuSO_4 \cdot 5H2O$ (in 5 mL tube)
  - solid $KMnO_4$ (in glass bottle)
  - 10% NaCl solution (in 50 mL plastic container)
- 10% H_2SO_4 solution (in 100 mL glass bottle) (NB! Handle with gloves, do not drop onto your clothes or skin.)
- 10% NaOH solution (in 125 mL plastic container) (shared with other tasks) (NB! Handle with gloves, do not drop onto your clothes or skin.)
- Five 10 cm long wires (in mini-grip labelled **B.3**, marked as **XA (standing for Fe)**, **XB (standing for Pb)**, **XC (standing for Al)**, **XD (standing for Zn)**, **XE (standing for Cu)**).
- 10 cm long carbon rod with increased surface area on one end (in mini-grip labelled **B.3**, a piece of carbon fibre tube from a toy helicopter tail)

Materials:

- U-shaped glass tube with a porous glass membrane between the sections, on a retort stand
- 2 plastic tubes with green cap (10 mL)
- microspatula
- a white and a red LED

electronic scales

- multimeters with wires and crocodile clips (shared with other tasks)

Examine the standard reduction potentials (Table 3.1) and decide which of the available components (electrode materials, solutions and solids) to use in order to put together an aqueous battery with the highest possible voltage. Note that if the electrode itself is not mentioned in a reaction formula, then it is wise to take an inert electrode, such as carbon.

Table 3.1. Standard reduction potentials in aqueous solution

$[Al(OH)_4]^- + 3\,e^- \rightleftarrows Al + 4OH^-$	$E^0 = -2.33$ V
$Al^{3+} + 3\,e^- \rightleftarrows Al$	$E^0 = -1.66$ V
$Li^+ + e^- \rightleftarrows Li$	$E^0 = -3.04$ V

$Zn^{2+} + 2e^- \rightleftarrows Zn$ $E^0 = -0.76$ V
$Zn(OH)_4^{2-} + 2e^- \rightleftarrows Zn + 4OH^-$ $E^0 = -1.20$ V
$Fe(OH)_2 + 2e^- \rightleftarrows Fe + 2OH^-$ $E^0 = -0.89$ V
$Fe^{2+} + 2e^- \rightleftarrows Fe$ $E^0 = -0.44$ V
$PbO + H_2O + 2e^- \rightleftarrows Pb + 2OH^-$ $E^0 = -0.58$ V
$PbSO_4 + 2e- \rightleftarrows Pb + SO_4^{2-}$ $E^0 = -0.36$ V
$Pb^{2+} + 2e^- \rightleftarrows Pb$ $E^0 = -0.13$ V
$Cu^{2+} + 2e^- \rightleftarrows Cu$ $E^0 = +0.34$ V
$O_2 + 2H_2O + 4e^- \rightleftarrows 4OH^-$ $E^0 = +0.40$ V
$MnO_4^- + 2H_2O + 3e^- \rightleftarrows MnO_2 + 4OH^-$ $E^0 = +0.59$ V
$MnO_4^- + 8H^+ + 5e^- \rightleftarrows Mn^{2+} + 4H_2O$ $E^0 = +1.51$ V
$NiO_2 + 4H^+ + 2e^- \rightleftarrows Ni^{2+} + 2OH^-$ $E^0 = +1.59$ V
$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightleftarrows 2Cr^{3+} + 7H_2O$ $E^0 = +1.33$ V
$PbO_2 + 4H^+ + 2e^- \rightleftarrows Pb^{2+} + 2H_2O$ $E^0 = +1.47$ V

Experimental procedure:

1. Select the best possible oxidizing system – choose an electrode, a chemical, and a solution of acid or base. Dissolve about 0.5 g of the chosen chemical in either 5 mL of the acid or base solution in a 10 mL plastic tube. Exercise caution when handling acids and bases.
2. Select the best possible reducing system – choose an electrode and a solution of acid or base. Pour 5 mL of acid or base solution to a second 10 mL plastic tube. Exercise caution when handling acids and bases.
3. Pour the 5 mL of the reducing solution to the U-tube's left compartment, and at the same time, the same amount of the oxidizing solution to the right compartment. It is important that both liquid levels are equal, rotate the U-tube holder for equalization of the levels of the solutions, if necessary.
4. Scrape the electrodes with sand paper, before inserting them into the U-shaped tube.

B.3.1 Measure voltage and short-circuit current of your battery.

B.3.2 Try to light a red LED (minimum voltage 1.6 V) and a white LED (minimum voltage 2.3 V). Note that a LED works only, if it is connected the right way. **Call a lab assistant, so that he/she could check that you have managed to light a red and a white LED**. If the voltage of the battery is higher than the minimum voltage of a white LED, but the LED does not light, call a lab assistant anyway. If you are not able to produce sufficiently high voltage, you may try other solutions/electrodes and call the assistant one more time for this purpose (no penalty).

B.3.3 Write the anode reaction, the cathode reaction, and the total balanced reaction that takes place in your battery during discharge.

B.3.4 Are the metals, provided for you, stronger reducers in acidic or basic solutions?

B.3.5 Are the oxidizers, given in Table 3.1, stronger oxidizers in acidic or basic solutions?

Unterstützung durch

Regionales Netzwerk für Naturwissenschaften und Mathematik Kärnten

Regionales Netzwerk für Naturwissenschaften und Mathematik Steiermark

IMST- Innovationen machen Schulen Top