APPLIED CHEMISTRY LABORATORY PRACTISE

Written for students studying chemistry,

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1. BIOMASS PROCESSING: INVESTIGATION OF BIOMASS WASTE

1.1 Theoretical introduction

The cogeneration and application of the bio-refinery concept requires the wide usage of biomass. The principle of the bio-refinery is – as in the case of oil-refinery - to process every carbon atom of the biomass, including the resultant waste, in order to produce energy or some kind of product (even chemical source materials).

The purpose of the laboratory practice is related to the bio-refinery concept: investigating the composition of renewable waste and producing product from it.

The European Union divides the potential biomass materials to three categories, not including the energy crops[i]:

1. Agricultural residues: agricultural waste materials. For example: corn cob, straw of cereals, sunflower shell, rice shell, (they are mainly used as fertiliser on fields).
2. Forestry residues: forestry waste materials, wood waste. For example: twigs, bark, wood shavings, sawdust.
3. Food waste: waste materials coming from human nutrition, scrapings.

These waste materials mainly consist of water content and plant cell wall components, from that in the point of industry cellulose, hemicellulose and lignin are the most important. Other materials are there to be found in biomass waste (such as salts, oils), that can be usually extracted with the help of a solvent (water or organic solvent), therefore these are called extract materials.

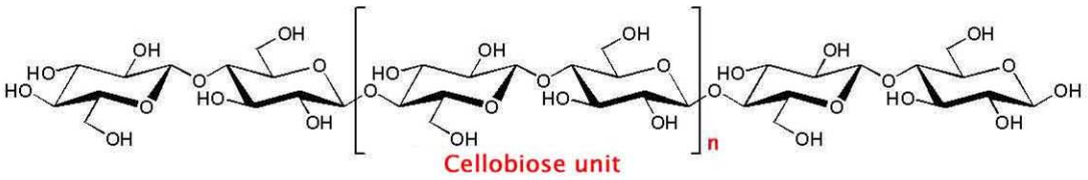
1. 1 Table: The lignocellulose content of agricultural waste materials regarding to the dry matter content [ii]

Agricultural waste materials	Cellulose (m/m %)	Hemicellulose (m/m %)	Lignin (m/m %)
Hardwood trunk (stem)	40–55	24–40	18–25
Softwood trunk (stem)	45–50	25–35	25–35
Seed shell (walnut)	25–30	25–30	30–40
Wheat straw	30	50	15
Beef manure[iii]	17.4	21.9	12.2

The average water content of the air-dry milled trunk is 10-30%[iv], but the moisture level of the herbaceous plants is higher. The average composition of different types of woods per dry matter is the following: 40-50% cellulose, 20-30% lignin, 20-30% hemicellulose, few % resin, tannin, mineral salt.

1.1.1 Cellulose, hemicellulose, lignin

Cellulose is a polysaccharide of highest volume in the flora that is a structural component of the primary cell wall of green plants. Cellulose is derived from D-glucose units, which condensate through β-1,4-glycosidic bonds (Figure 1.1).



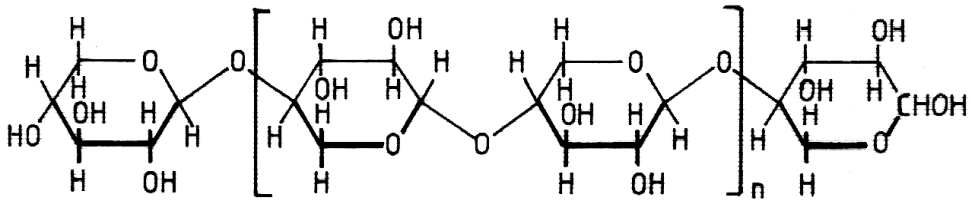
1. 1. Figure: The structural formula of cellulose

Hemicellulose is a collective term including polysaccharides that are involved in structuring cell walls along with cellulose. They bind to cellulose to form a network of cross-linked fibres in order to fix the plant cell wall.

Hemicelluloses contain pentosane (polysaccharide of pentose units bound together) in largest quantities, but they include hexosans as well. Furthermore, we consider cellulose-structured polysaccharides that have significantly smaller molecular mass as cellulose, as hemicellulose.

Practical difference between cellulose and hemicellulose is the fact, that cellulose is insoluble in sodium hydroxide solution, while polysaccharides of a cellulose-like structure of smaller molecular mass, and other polysaccharides of hemicellulose is soluble. The acidic hydrolysis of hemicellulose takes place under milder circumstances, than cellulose's: at room temperature cellulose can be hydrolysed with concentrated hydrochloric acid or 60-80 % sulphuric acid; with 1-2 % sulphuric acid or hydrochloric acid, hemicellulose hydrolyses at 100-130°C, cellulose hydrolyses only at 150-180°C (above 100°C under pressure).

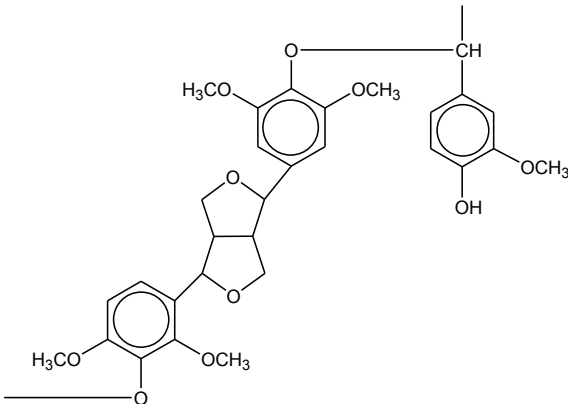
Hemicellulose content of plants includes mainly pentosans, and xylans built of D-xylose units linked by 1-4-glycosidic bonds are the most frequent in pentosanes.



1. 2. Figure Structure of xylan

Xylans contain small amount of D-arabinose, D-galactose and D-glucuronic acid besides D-xylose.

L**ignin** fills the molecular sized spaces in the cell wall between cellulose and hemicellulose components, it strengths the mechanical resistance of the cell wall. The term lignin is a collective term: it means the wall material of coarse plant parts that is built from aromatic constituents. In different wood types the structure of lignin differs, but there are structural similarities: methoxy groups and phenolic hydroxyl groups are often linked to the aromatic ring, in parts uniting the aromatic units is heterocyclic oxygen to be found. The following formula serves as a partial characterisation of the lignin's structure (Figure 3.), that shows one, often repeated, characteristic part of the lignin molecule:



1. 3. Figure: Structure of lignin

1.1.2 Chemical industrial utilization of biomass waste materials

There are two areas of biomass waste material's direct utilization:

A. Cellulose production
B. Chemical processing of waste materials' carbohydrates

A. In cellulose production the most frequently used method is the bisulphite-, acidic-, and alkaline process. Cellulose is used for a wide range of products to be manufactured, its main application is paper production. Amongst cellulose ester derivatives, cellulose acetate, organic esters and mixed esters containing higher number of carbon atoms have industrial importance.

B. The chemical process of the biomass waste materials depends on the target product. Not including the energetic recovery, the conversion methods and their products are shown in the following table:

1. 2 Table: Biomass conversion methods not including the energetic recovery

Category	Method	Product
Thermochemical conversion[v]	Pyrolysis	Pyrolysis oil
	Catalytic liquefying	Bio-oils
	Carbonization	Charcoal
	Gasification	Depending on circumstances CH_4, CO, CO_2 and H_2 different proportions of mixture, so biogas, process gas, wood gas
Physicochemical conversion	Hydrolysis	Monomers, from them chemical-industrial raw materials
	Organosolv process	Aromatic components
	Transesterification	Biodiesel
Biochemical conversion	Enzyme hydrolysis	Monomers, from them ethanol, butanol, acetone, lactic acid
	Anaerobic digestion	Biogas
	Composting	Compost, organic fertilizer

There are different processes of hydrolysis amongst physicochemical methods depending on the temperature and acid concentration:

- We hydrolyse all of the polysaccharides in one step.

- Two-step hydrolysis: firstly the starch and hemicellulose under mild circumstances, then the cellulose under stronger circumstances.

1.1.3 Plant analysis - Methods

Different measurement methods can result in different outcome for the same component. That's the reason why indicatation of the measurement methods is always compulsory alongside with the results.

The water content of the air-dry biomass material can be determined by drying in drying oven.

Hot water extraction is an option for determining extract materials. In this way mineral salts, organic acids, organic bases and their salts, sugars etc. can be extracted from plants. Hot water can partially hydrolyse polysaccharides due to the presence of free acids. So the duration time and temperature of the hot water extraction must be given precisely.

The essence of determining cellulose is to remove extract materials, the mass of the lignin, hemicellulose and the residue is measured. In practice, in order to prepare cellulose we use cc. HNO_3:96 % ethanol in 1:4 ratio. It causes the lignin to nitrate and oxidize, the hemicellulose is hydrolysed, the resultant products and the extract materials can be dissolved in alcohol.

We determine lignin, which is the residue of the biomass material that cannot be hydrolysed with acid. For this purpose the most frequent process is to use 64-72% sulphuric acid or concentrated hydrochloric acid. The optimal quantity of acid can be determined in preliminary examination. It is required that the acid dissolves fully the polysaccharides, however lignin suffers less breakdown. The decrease of methoxide content indicates the breakdown of the lignin. When the smallest quantity material at biggest methoxide content remains, that is the optimal acid concentration. According to the preliminary examinations, the 72% sulphuric acid is the most adequate.

1.2 Practical work

Aim of the laboratory

1. Determining the water content of biomass waste material
2. Determining cellulose content of biomass waste material per dry matter
3. Cellulose acetate foil production from cellulose

1.2.1 Determination of water content

Weight out on analytical scale 3x1 g of biomass waste material in three, preweighed ground-glass weighing pans. Put the pans with open caps in drying oven at 105°C for two hours. Let them cool down in the dessicator (with open top), not to let them absorb any moisture. From the decreasing mass, calculate the average mass percentage water content of the material.

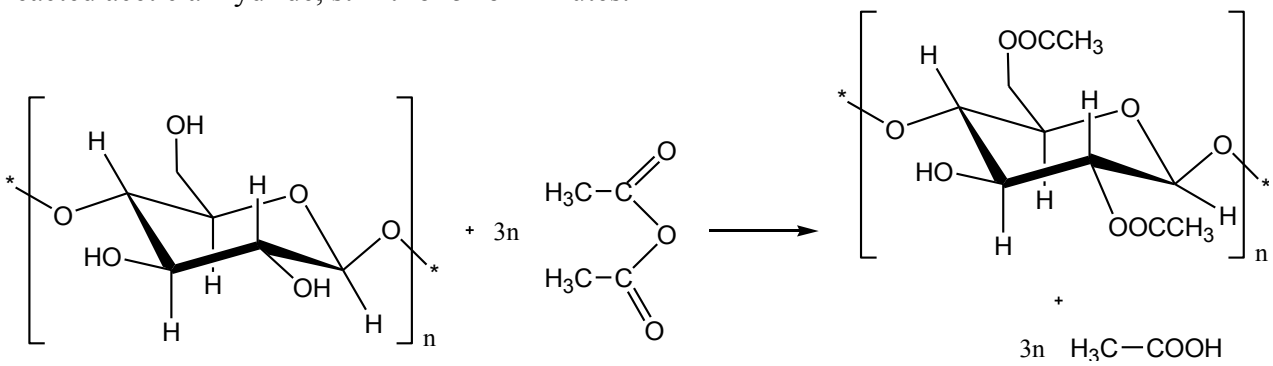
1.2.2 Determination of cellulose content

Pour 20 cm^3 96% ethanol into a 100 or 250 cm^3 Erlenmeyer flask, then during continuous shaking add 5 cm^3 65% nitric acid under the hood. Place 1 g, weight in on analytic scale biomass material into 100 or 250 cm^3 round-bottomed flask, and then add ethanol-nitric acid mixture. Place water-cooled condenser on the flask and put it into waterbath under the hood. Don't forget to open the cooling water of the condenser! Boil the sample for 15 minutes over the waterbath. Pour the solution from the solid material. Prepare new, previously described ethanol-nitric acid mixture, and pour it to the remaining solid material after the pouring. Boil the mixture for 15 minutes. Repeat the previous operations, so manage the material for 3x15 minutes, every time with new ethanol-nitric acid mixture, and carry out three-step extraction.

Measure the mass of the G3 glass-filter! Rinse the residues (cellulose) with a little abs. ethanol into G3 glass-filter, remove the alcohol by suction, and then mixing it with 5x5 cm^3 abs. ethanol wash through the filter, and with removing by suction each of step of alcohol.
The drying of the resultant wet cellulose mixture is carried out in drying oven at 105°C according to chapter 1.2.1, but drying time should be decreased to 20 minutes.
From the decreasing mass, calculate the average, mass percentage water content of the wet cellulose. Determine the percentage of the resultant dry product's mass of the waste material's dry matter.

1.2.3 Preparing cellulose acetate foil

The essence of the reaction is the acetylation with acetic anhydride of the free hydroxyl-groups of cellulose glucose units (Figure 4.). Before adding the acetic anhydride, the cellulose have to be partially hydrolysed with glacial acetic acid containing sulphuric acid, in order to create suspension of short cellulose fragments. The dispersed cellulose molecules are accessible for acetic anhydride. The product is taken into methanol-, dichloromethane-phase, where parallel to the vaporisation of the solvent, the cellulose acetate is separated as a foil.
Weight in 0.5 g of cut cellulose in a form of paper wadding into vessel that can be heated. Add a mixture of 15 cm^3 glacial acetic acid and 2 drops of cc. sulphuric acid. Stir it for 20 minutes and keep at 70-80°C. Then add 5 cm^3 acetic anhydride and stir it for 20 more minutes. Give 1 cm^3 distilled water to the suspension and for hydrolysing the incompletely reacted acetic anhydride, stir it for 5 for minutes.



1. 4. Figure: Preparing cellulose acetate

Pour the mixture in 50 cm^3 distilled water and filter the solid product in details through filters placed on the Büchner funnel. Wash the solid product with small amounts of 20 cm^3 of distilled water, and then leave the vacuum on for 5 minutes. Heat up to the boil the mixture of 18 cm^3 dichloromethane and 2 cm^3 methanol, then give the filtered, vacuum-dried cellulose acetate to it. Be mindful of using watertight tools, because water dissociates cellulose acetate! Drain the resultant solution with 1 g or less of Na_2SO_4. After the draining, it has to be decanted into Petri dish (pour the solution into Petri dish, when the solid material is left over), and let the solvents be vaporised under the hood. Measure the mass of the dried foil.

Task: Submit the processed cellulose acetate foil
The practice was created according to the laboratory practice of ELTE TTK Environmental Technology (Környezettechnológia)

REPORT

<table>
<tr><td colspan="3">Applied Chemistry – I. laboratory</td></tr>
<tr><td>Name of the student</td><td>Biomass processing: Investigation of biomass waste</td><td>Date

Team</td></tr>
<tr><td colspan="3">1. Water content of biomass waste material (measurement data and calculation)

2. Cellulose content of biomass waste material (measurement data and calculation)

3. Cellulose acetate foil (measurement data)

Evaluation</td></tr>
</table>

2. BIODIESEL PRODUCTION FROM VEGETABLE OILS

2.1 Theoretical introduction

Unmodified plant origin/vegetable fats can be used for diesel engine propulsion; this is the straight vegetable oil (SVO). It is more beneficial for the engine if we mix it with kerosene or diesel fuel. Further possibility is transforming fats into biodiesel. Fresh or used fats can also be applied for all these three methods.

By definition biodiesel is the methyl or ethyl esters of fatty acids. Biodiesel is cleaner compared to the SVO and can be used for any diesel-fuelled engines without transforming the engine; moreover, its properties in cold weather are better. Contrary to SVO, biodiesel has been tested for a long time, and is applied in a lot of countries. Biodiesel is clean, safe, and ready for use as alternative fuel, conversely, most of the SVO fuel systems are in pilot phase. Disadvantage of the biodiesel is its higher cost, as it is produced from SVO.

The properties, such as the viscosity and flashpoint of the public-distributed biodiesel must be checked according to the standards. In case of ester-based fuels composition is being also checked, such as acid-, lye- (base), and organic acid residual content. The aim of the investigations is to avoid the corrosion of the engine's parts. The unsaturated content of the ester is being checked by the iodine value: for example, sunflower-based biodiesel can only be mixed with crude oil in limited quantities according to the limiting rules of the standards.

2. 1 Table: Relevant standards for bio- and petroleum-diesel, and few typical values

		European Union, biodiesel	**PETROLEUM DIESEL petroleum**
Characteristics	**Measurement Unit**	**EN 14214:2008**	**EN 590:1999**
Standard		FAME	Diesel
Density 15°C	g/cm³	0.86-0.90	0.82-0.845
Viscosity 40°C	mm²/s	3.5-5.0	2.0-4.5
Distilling character	% @ °C		85%,350°C - 95%,360°C
Flashpoint	°C	101 min	55 min
Sulphur content	mg/kg	10 max	350 max
Water content	mg/kg	500 max	200 max
Cetane number		51 min	51 min
Acid content	mgKOH /g	0.5 max	
Methanol content	%mass	0.20 max	
Ester content	%mass	96.5 min	
Monoglyceride	%mass	0.8 max	
Diglyceride	%mass	0.2 max	
Triglyceride	%mass	0.2 max	
Total glycerine	%mass	0.25 max	
Iodine value		120 max	

In 2003 the European Committee for Standardization created a standard for determining the quality of Fatty Acid Methyl Ester (FAME). In Table 2.1 the density, water content, the pollution content and chemical composition is shown.

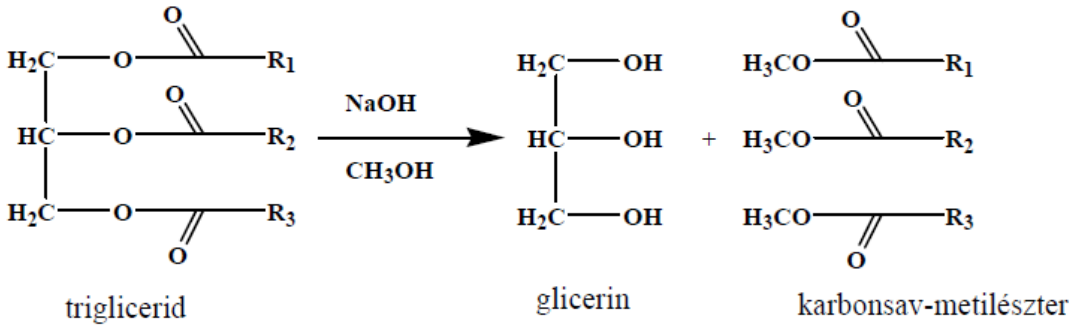
2.1.1 Comparison of biodiesel and petroleum

The gross heat of combustion of biodiesel is 9-13% lower than petroleum. The viscosity of biodiesel can be twice as big as the viscosity of petroleum, and minimal point of its flashpoint is 101°C.
Bio-fuels have lower energy and torque at bigger consumption. The physical and chemical properties of the ethyl and methyl esters, their available performance are similar, their energy content is almost the same. The viscosity of the ethyl esters is bigger, while its cloud point and pour point is smaller than the methyl esters. According to the burning tests, the methyl esters can produce more energy and torque than the ethyl esters. There is no difference between them in consumption point of view.
A few of the advantages of the ethyl esters are the smaller soot production during burning, the lower exhaust temperature and lower pour point. Ethyl esters more likely use up injectors and have bigger glycerine content, as methyl esters[vi].

2.1.2 Reaction

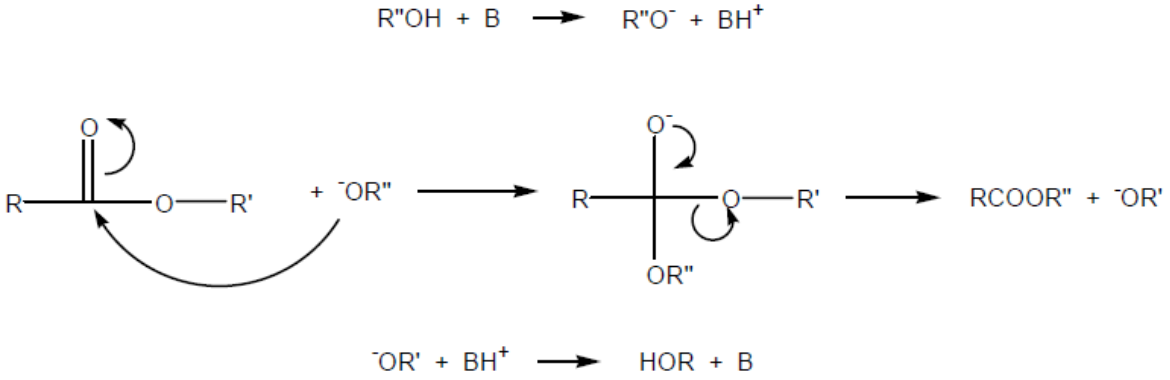
During the production of biodiesel we transform triglycerides (fats and oils) into methyl and ethyl esters, while as a byproduct glycerine is arose. The product and the byproduct create a two-phase system, in which the upper phase is the ester and the lower phase is the glycerine. The process is called transesterification, when the glycerine is changed into monohydric alcohol in the presence of base catalyst. The quantity of the catalyst is usually 0.3-3% NaOH or KOH of the vegetable oil. According to stoichiometry 3:1 = methanol: triglyceride is needed to the reaction, but the methanol is added in excess, typically in 3-6:1 molar ratio[vii].

Chemical equation



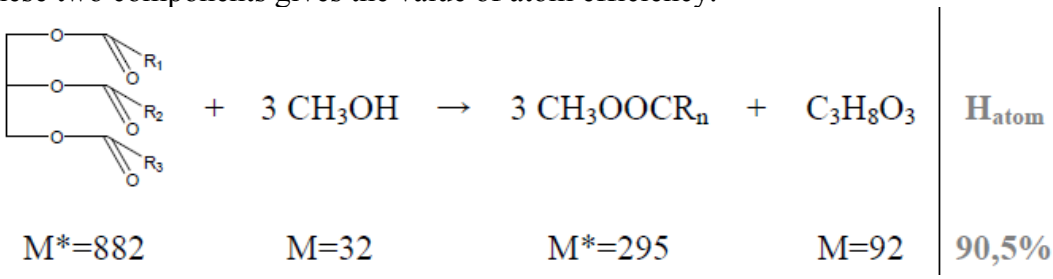
HOOC-R_1, R_2, R_3:
- Palmitinsav [$CH_3(CH_2)_{14}COOH$]: **4.0 - 9.0 %**
- Sztearinsav [$CH_3(CH_2)_{16}COOH$]: **1.0 - 7.0 %**
- Olajsav [$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$]: **14.0 - 40.0 %**
- Linolsav [$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$]: **48.- 74.0 %**

Reaction mechanism



Atom economy

Atom economy is the conversion efficiency of a chemical process, defined as the molecular mass of the desired product compared to the molecular mass of all reactants. The ratio of these two components gives the value of atom efficiency.



H-atom = 100*Mdesired product / Mall reactants= 100*3*295/(882+3*32) = 90.49%
Where M: Average molecular mass (g/mol).

Applied Green Chemistry principles

More efficient energy consumption
Application of renewable raw materials
Usage of catalysts

2.2 Practical work

The aim of the laboratory

2.2.1 Transesterification

Presentation of the transesterification reactions based on the biodiesel production. Introduction/Use of the Höppler viscosity meter. Tasks:

1. Measure the mass of the 100 ml vegetable oil, then heat it up to 40-50°C.
2. Weight out same amount of sodium hydroxide (catalyst) as the 1% of the oil's mass.
3. Give the weighed out solid sodium hydroxide to the 20 ml 99+% methanol and stir it till it is fully dissolved. In the balanced chemical reaction small amount of sodium methoxide arose.
4. Add the already prepared sodium methoxide solution to the preheated vegetable oil, then heat it up to 60±1°C. Keep it on this temperature for 40 minutes, while stirring intensively, using water-cooled condensers.

5. After the reaction time let the reaction mix cool down and be separated. The methyl ester is the upper phase, the glycerine phase is the lower phase. At room temperature the phase-separation takes a long time, but keeping the mix at 40-45°C speeds the process up.
6. Separate carefully the upper layer (phase), then neutralize it with a low quantity of acetic acid. After this, wash it with water a few times. Separating phases takes a long time, but its speed can be increased by heating it up to 40-45°C.
7. Dry the ester phase on watertight magnesium sulphate for a few minutes, then filter it.
8. Measure the mass of the product and calculate the yield (the amount of product obtained in a chemical reaction – around 90%). Measure the viscosity of the product.

2.2.2 Viscosity measurement

Measure the viscosity of the vegetable oil and biodiesel at 20°C, and as a comparison measure the linseed-oil's viscosity as well. The viscosity of the oils is usually measured by the Ostwald kinematic viscosity meter, but in the laboratory Höppler meter is available, which measures the dynamic viscosity. (The kinematic viscosity of sunflower oil on 40°C is 31,1-32,6 cSt, so it is in the range of $3{,}25*10^{-5}$ m^2/s).
In order to start the measurement first of all, switch on the thermostat of the viscosity meter, adjust the given temperature. Fill up the tubes with the sample bubble-freely, then place in the ball nr.3 of known mass and density. Close the tube with its top! Turn the device upside-down and measure the sedimentation time of the ball. Repeat the measurement at least five times! Calculate the dynamic viscosity of the oil with the help of the equation written in the device script, then calculate the kinematic viscosity!
Measure the density of vegetable oil, biodiesel and linseed oil by the piknometer on 20°C!

Practice was created on the basis of „Journey to Forever: Make your own biodiesel" article and „Kovács F, Hancsók J: A repce- és napraforgóolaj átészterezése motorhajtóanyaggá" lecture.

Required chemicals:

Vegetable oil: 100 ml/ 874*
Anhydrous methanol: CH_3OH 20 ml/19.42 g
Sodium hydroxide (solid): NaOH kb. 1 g
Acetic acid: CH_3COOH
Linseed-oil
* The used sunflower oil composition according to the GC-MS measurement: palmitic acid-(9%); linoleic acid- (90%); stearic acid-glycerolester (1%)

Required tools:

Heated magnetic stirrer and magnetic stir bar
Two-necked round-bottomed flask (250 ml)
Separating funnel
Water-cooled condenser
Erlenmeyer flasks, or beakers (25, 50, 100 ml)
Stopper
Höppler viscosity meter
Piknometer
Thermometer

REPORT

<table>
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<tr><td>Name of the student</td><td>Biodiesel production from vegetable oils</td><td>Date

Team</td></tr>
<tr><td colspan="3">1. Density and viscosity of vegetable oils

2. Preparation of the reaction

3. Density and viscosity of the produced biodiesel

4. Evaluation</td></tr>
</table>

3. WATER TREATMENT: INVESTIGATION OF ION EXCHANGE IN PHASED SYSTEM

3.1 Theoretical introduction

Ion exchange processes are getting more and more widely used in different industries (preparing boiler feedwater; extracting valuable or environmentally polluting materials, etc.)
The stationary-bed ion exchange process consists of two cyclical operations. The cyclical factor can be explained by the following: one of the two phases is stationary (resin phase), so the concentration is changing in the function of place and time. Stationary state cannot happen, so the saturation period has to be followed by a regenerating period, one after another cyclically.
During the laboratory practice we investigate a cation exchange on cation exchanger (Amberlite IR-120 type) resin. The Amberlite IR-120 is a strongly acidic cation exchanger synthetic resin, in which liquid-phase cations get bonded to it and H^+ ions are let into the liquid. The theoretical capacity of the resin is 1,8 ekv/dm^3, so on 1 dm^3 of the resin there is 1.8 mol H^+ ion, which cation is interchangeable, so cations with identical charges can be exchanged.
In order to determine the intensity of material exchange between the phases we have to know the balances of ion exchange and the mathematical correlations determining the speed of ion exchange and the characteristics of the ion-exchange resin (for example **capacity**)[viii].

3.1.1 Balance of ion exchange

Considering ion exchange as a reversible process, the exchange-equation in exchanging monovalent Na^+ and H^+ ions is the following:

$$R-H+Na^+ \Leftrightarrow R-Na+H^+ \qquad (1)$$

Where R is the class of compound's sign building up the resin.
According to the (1) equation, the latent equilibrium constants can be expressed for bicomponent system (Equations are valid only if the system is not disturbed i.e. samples are not taken and mass balance is complete):

$$K_H^{Na} = \frac{x_H \cdot y_{Na}}{y_H \cdot x_{Na}} \qquad (2)$$

$$K_{Na}^{H} = \frac{x_{Na} \cdot y_H}{y_{Na} \cdot x_H} \qquad (3)$$

where $y_i = \frac{q_i}{Q}$ dimensionless concentration in the resin phase

$x_i = \frac{c_i}{c_0}$ dimensionless concentration in the liquid phase

q_i concentration of *i* component-to-be changed in the resin phase (mol/dm^3 wet resin)

Q total concentration of cations in the resin (capacity) (mol/dm^3 wet resin)

c_i concentration of *i* component-to-be changed in the liquid phase (mol/dm^3)

c_0 total concentration of cations in the liquid phase (mol/dm^3).

It can be seen, that the bigger difference in the latent equilibrium constant, the easier to separate H^+ and Na^+ components with the help of ion exchange resin. If H^+ ions are to be extracted from the solution, then relation of $K_{Na}^{H}>1$ is the adequate.

3.1.2 Kinetics of ion exchange

Ion exchange is a complex process and is carried out via the following steps[ix]:

1. The ion-to-be-exchanged through the boundary layer diffuses to the surface of the resin (external diffusion).
2. The ion penetrates to the inside of the resin (internal diffusion).
3. The ion-exchange reaction takes place.
4. The exchanged ion diffuses to the surface of the resin (internal diffusion).
5. With diffusion it gets into the solution through the liquid film (external diffusion).

The kinetics of the ion exchange is determined mainly by the diffusion processes. In our case the reaction speed of the ion exchange is bigger than the diffusion speed, so the external diffusion, the internal diffusion or both determine the kinetics. For diluted solutions the external diffusion, for concentrated solutions the internal diffusion plays an active role. Change in hydrodynamic characteristics can affect the boundaries of the speed-determining processes. In this case the thickness of the boundary layer is changed. With this the following phenomena can be explained: having the same c_0 concentration value and D diffusion constant, and a change in hydrodynamic characteristics, the external diffusion inhibition is replaced with internal diffusion inhibition.

In case of phased system liquid is poured onto given quantity of resin. In order to increase the material exchange we stir the system, because in turbulent flow, material exchange is enhanced by turbulent diffusion in the liquid phase. A molecular layer emerges on the solid material, in this case on the surface of the resin in which material exchange is achieved by molecular diffusion. According to the *film model* this molecular layer means all of the resistance during material exchange. Intensive contact is established in case of turbulent stirring, so the thickness of the film layer is reduced to minimum and the ion exchange process can be increased. In phased system the material exchange occurs between the two layers until the resin is exhausted, so the concentration in the liquid phase is constant. The governer of the material exchange is the difference to the balanced concentration.

At the end of material exchange $\frac{dc}{dt}=0$. Then the concentration of the solution can be expressed according to the balance reaction, and equation (4) is given as follows:

$$c = c(0) - \frac{V_{gy}}{V_f} \cdot Q \qquad (4)$$

Where c(0) is the concentration of the liquid at the starting time (t=0).

3.2 Practical work

Aim of the laboratory work:

1. Removing Na^+ ions from solution with the help of H^+-based ion exchange resins.
2. Understanding and use of the flame photometer.

3.2.1 Removing Na+ ions from solution with the help of H+-based ion-exchange resins.

1 cm^3 Amberlite IR-120 ion-exchange resin has to be soaked for 15-20 minutes into five-fold quantity of 0.1 mol/L HCl solution, so the exchange ions of the resin are only H^+ ions. After removing the hydrochloric acid solution and washing with distilled water, we place the resin into 250 or 500 cm^3 beaker on magnetic stirrer. Prepare 250 cm^3 NaCl solution with concentration of 100 ppm Na^+, then take 200 cm^3 of it and place into a beaker. Check the Na^+ concentration of the starting sample: investigate with flame photometric method. Place a pH-meter into the solution and at the same time start measuring the time with a stop watch and start stirring. Follow the pH-changing of the sample. Take samples from the solution in 2, 5, 10, 30 and 60 minutes and determine the Na^+ concentration of the sample with the help of flame photometer. (Suggestion: take 1 cm^3 sample and dilute it 25-times, later 10-times dilution is enough).

3. 1 Table: Measurement table

Elapsed time from the beginning of measurement [min]	Quantity of sample taken [cm^3]	Na^+ concentration of sample [ppm]	Na^+ concentration of sample [mol/dm^3]	pH of the solution	H^+ concentration of sample according to pH [mol/dm^3]
0					
2					
5					
10					
30					
60					

3.2.2 Understanding of the flame photometer

Flame photometer uses sodium and potassium colour filters. The information needed for operating the photometer can be found in the manual placed next to the equipment. During operation you need to pay attention to wash the intake manifold with twice-distilled water between each sample. Calibration is needed before operation. The equipment has linear calibration until 10 ppm concentration, this has to be taken into account.

Required chemicals:
NaCl
HCl of 0.1 mol/L concentration
Standard solutions of flame photometer
Ultra-pure water, or twice distilled water, 1000 mL.

Required tools:
Amberlite IR-120 ion-exchange resin
Magnetic stirrer
Magnetic stir bar
pH-meter
Flame photometer
5 and 10 cm^3 pipette for sampling
1 piece of 250 ml volumetric flask
5 pieces of 50 ml volumetric flask for dilution of solution

1 piece of 250 or 500 ml beaker
double distilled water (normally ultrapure water) for making the calibration solutions for the flame photometer

REPORT

Applied Chemistry – III. Laboratory		
Name of the student	**Water treatment: investigation of ion exchange in phased system**	Date
		Team

1. Measurement table.
2. Represent the change of sodium ion's concentration in the function of time!
3. Calculate the total concentration of cations in the liquid phase (c_0)!
4. Calculate the value of q_{H^+}! (exchanging H+ component concentration in the resin phase (mol/dm^3 wet resin))
5. Calculate the latent equilibrium constants!
6. Calculate the volume of Amberlite IR-120 ion exchange resin needed for 1 m^3 100 mg/L Ca^{2+} concentration water in order to be fully decontaminated of calcium! (The full capacity of ion exchange resin can be calculated by the $M_T = V \cdot Q$ relation, and we assume that the system is perfectly contacted, where all the capacity can be used.)
7. Evaluation

4. WASTEWATER CLARIFICATION

4.1. Theoretical introduction

Significant part of environmental pollution is caused by untreated wastewater (for example in India). However in Hungary, sewage treatment is adequate as a result of approaching environmental issues in a complex way, applying Integrated Pollution Prevention and Control (IPPC). The aim of the directive is to realize integrated prevention and control for pollution derived from activities having environmental impact. Wastewater can be divided into two main categories:

- Public sewage water
- Industrial wastewater, more acceptably process water term.

Wastewater treatment is carried out in three or four stages. In the first step mechanical treatment is applied (bar screen and sand channel); while in the second step chemical treatment is implemented: coagulants and flocculants are being fed depending on the quantity of suspended matter and phosphorus content in wastewater. During this, the formed precipitate is removed by sedimentation in the pre-sedimentation tank. In the following third stages organic materials are being decomposed with the help of microorganisms in the aerated biological tank. The biological tank consists of more compartments, where nitrification and denitrification take place, in this way not only the organic materials, but the nitrogen content can be removed from wastewater. In the fourth treatment stage during the final sedimentation, the conditions are being created for the possibility of releasing the treated, cleaned water into natural waters. From the biological tank the residual sludge is being removed, and a known part of it is being recycled.

4.1.1 Removal of suspended matter content

Wastewater always contains more or less suspended matter that (before or after usage) has to be removed before releasing the water back into natural waters. Coarse granules as a part of suspended solids can be removed by the means of mechanical treatment (sedimentation, filtration).

Significant part of the suspended matter content is colloidal sized. Colloidal particles do not settle, they float even though their density is bigger than water's. In municipal wastewater these small particles have negative charge so they repel each other. The spontaneous attachment of these particles, so the flaking requires long time (months). In order to remove the colloidal particles, the stabilising forces must be inhibited and bigger particles (aggregates) have to be created, that can be eliminated from water with mechanical phase separation. For creating aggregates the widely spread method is the coagulation-flocculation process, that can be implemented by chemical-dosing. A few of the practically used methods and fundamentals are defined:

Clarification: it includes the micro- and macro flaking by chemical-dosing and the phase separation (most often sedimentation).

Coagulation: during water treatment, it means the destabilization of colloidal particles; it is induced because of the limiting or ceasing repulsive forces between the particles. The destabilization can be achieved by:

— Charge neutralization, for example: electrolytes,
— Specially sorbing chemical compounds.

Flocculation: flaking; the destabilized (coagulated) particles are attached to each other in order to create bigger aggregations.

In water treatment the charges of suspended matters are being neutralized by Al(III)- and Fe(III) chemical compounds, occasionally compounds of bivalent metals (for example the economical use of calcium hydroxide). From metal salts (after dosing them into water) positive intermediate products (poly hydroxide compounds) are generated. These can neutralize the negative charge of colloidal particles. In the proceeding part of hydrolysis the temporary compounds lose their charge and with absorbing colloids they create poorly soluble hydroxy flakes that can be separated from water in a form of macroscopic precipitates.
Hydrolysis is completed with the carbonate hardness in a way of the following gross process:

$$Al_2(SO_4)_3 + 3\ Ca(HCO_3)_2 = 3\ CaSO_4 + 2\ Al(OH)_3 + 6\ CO_2 \qquad (5)$$

(Clarification using aluminium sulphate is only efficient when the wastewater contains at least 0.5 mval hydrogen carbonate ion per litre (proton acceptor), and its total hardness is above 5 nKº.)

4.1.2 Operation methods

In order to ensure rapid and homogenous distribution, chemicals are added to the wastewater in a form of solution or suspension. The quantity of chemicals depends on the water quality, generally in a range of 5150 mg $Al_2(SO_4)_3/dm^3$ water and 0.1—1.0 mg polymer/dm^3 water. Addition of water-soluble polymers results in the formation of flakes that evolve via bridging. The first step of the process is the sorption of polymers on the surface of solid particles, this is the micro-flaking. Then comes the attachment of micro-flakes into well-settling flakes. The structure of polymers allows the development of macro-flaking. On the surface of colloidal particles one part of the polymer molecules adsorbs, the other part moves freely in the solution and is capable of attaching to other particles. In this way polymers create a bridge between flake units so enable the webbing of micro-flakes. The formed macro-flakes are bigger, their structures are more solid than the metal hydroxide flakes, so they enable more efficient solid-liquid separation. (The application of cation polyelectrolyte method is not used in clarification of water, only applicable in sludge treatment due to its economic disadvantage.)

Flygtol-method

Bentonite has granules with great expanding ability, big relative surface and good sorption (ion-exchange) characteristics. Sodium bentonite establishes chemical interaction with the polymer, its application helps in increasing the size of the flakes and the clarification efficiency as well. During clarification water suspension is used. The method, when we apply a rock of sodium-ion form (Sodium bentonite) as an auxiliary flocculant, is called Flygtol-method.

Feeding metal ions in the form of polymer

Nowadays trivalent metal ion containing flocculants are widely used in a form of inorganic polymer. Such basic poly(aluminium chloride)-type flocculant having a chemical formula of $Al_n(OH)_mCl_{3n-m}$ is efficient in a wider pH range than aluminium sulphate, its hydrolysis is less dependent on temperature, does not require auxiliary flocculant and its application in treated water results in smaller aluminium concentration. The process of coagulation, flocculation depends significantly in most cases on temperature and pH.

The charges of colloidal particles in water and the ion form of flocculants are pH dependant (for example the optimal pH range for the hydrolysis of Al(III) compounds is 6-6.5.)

Mechanical circumstances play an important role during applying flocculants. When adding chemicals, the wastewater and the chemical must be mixed at a quick pace. On one hand this ensures the homogenous distribution of chemical, while on the other hand it allows the chemicals to react in a highly reactive way with the suspended matter particulates.

Hereinafter in order to let the primary aggregates to impact, so to improve the flake-size, slow stirring has to be ensured. The blending velocities (the evolving velocity gradients in liquid) and blending times have to be optimized, as well. When blending time is improved, not only the formation of aggregates is happening, but the aggregates are being cut as well.

4.1.3 Determining the turbidity of waste water sample

Turbidity consists of light absorbing and light scattering discrete particles present in water. Particles causing turbidity are finely suspended inorganic and/or organic materials. Besides concentration of particles in solution, turbidity depends on the properties (refraction features) of particles, their shape and size, therefore the rate of turbidity cannot define directly the quantity of suspended matter. It is an adequate and fast method for comparative analysis. Turbidity can be measured by determining absorption of light: in this way, turbidity can be expressed in FTU units (Formazin Turbidity Unit). Measuring scattered light from the discrete particles present in sample at a 90° scattering angle from the incident light gives the intensity, which can be a measurement method as well. In this way turbidity is given in NTU units (Nephelometric Turbidity Unit). For comparative (calibration) goals, standards are used in both cases, inducing known turbidity (given absorption and scatter), having particle-size distribution that is easily reproducible, applying organic suspension with given concentration.

4.2 Practical work

Aim of the laboratory work:

Durng this laboratory practise we are going to investigate the chemical treatment of wastewater, together with the processes of the pre-sedimentation, primarily the determination of suspended matter and their removal will be carried out. The efficiency of flocculants used for clarification of model wastewater samples will be determined.
On the laboratory a rock named Sodium bentonite will be used as an auxiliary flocculant.

Applied methods for quality analysis of wastewater:
The following properties of wastewater samples will be determined on the laboratory practice:

- pH
- turbidity (FTU)
- sediment volume (cm^3/dm^3), in 30 minutes depositing volume in Imhoff cone (slurry volume)

The quality analysis has to be done with homogenised wastewater samples. The pH-meter has to be calibrated previously. Before measuring turbidity dilution series has to be prepared from known FTU values and calibration curve must be determined. The origin point of calibration refers to the value of distilled water.

Determination of depositing sediment volume of the wastewater sample:
Pour 1 litre of homogenised wastewater into the Imhoff cone placed on the holder, and let it settle for 30 minutes. Note the deposited sludge volume after 30 minutes of settling.

Used flocculants:

- 20 mg/ml $Al_2(SO_4)_3$ solution or Al(III)-content polyaluminum chloride equivalent to $Al_2(SO_4)_3$ solution
- 0.1%-os Magnafloc-27 (MLT-27) cationic flocculant (added dropwise)
- 0.1%-os Magnafloc-340 (MLT-340) anionic flocculant (added dropwise)

Clarification
The clarification of the wastewater sample has to be carried out by the "jar test" used in practice. Into 3 pieces of 500 mL beakers dispense 250 mL sample and 3 different flocculants. The batching and the fast mixing of flocculants must be achieved in 30 seconds, then the solution has to be stirred slowly for 3 minutes. After stopping the stirring we investigate the sedimentation of flake by gauging the sedimentation time on known sedimentation path length. After the sedimentation, get a sample from the cleared-out upper phase (upper 1/3 of the sample) and determine its turbidity (this is possible 5 minutes after stopping the stirring).

Chemical volume needed for clarification depends on the quality of wastewater sample. Define the applicable volume of chemicals according to the pH value determined for the samples.

4. 1 Table: Volume of chemicals needed for clarification in the function of pH

pH	$Al_2(SO_4)_3$ volume (mg/L *wastewater*)
6.0 – 6.6	0
6.7 – 7.0	10
7.1 – 7.4	15
7.5 – 8.0	25

APPENDIX
In accordance with Regulation 204/2001 (X.26.), the Government determined the following emission thresholds for sewer:
pH 6.5—10
Sedimentation matter* suspended matter; 150 mg/L *
*should be measured if the sediment volume is above 5 cm^3/dm^3 in 10 minutes of sedimentation. Above this value, a fine has to be paid.

Preparing calibration series from 4000 NTU stock, measuring absorbance on 300 nm.
In case of appropriate sedimentation, values under 100 NTU can be achieved.
Dilution scale suggested for calibration: 25, 50, 100, 200, 400 NTU.

Required chemicals:
$Al_2(SO_4)_3$ or BOPAC
MLT-27 cationic and MLT-340 anionic flocculant of 0.1% solution

Required tools:
Magnetic stirrer
Magnetic stir bar
pH-meter
Spectrophotometer
3 pieces of 5 or 10 cm^3 pipette for sampling
5 pieces of 50 ml volumetric flask for turbidity dilution scale
3 pieces of 500 ml beaker
Imhoff cone
Stop watch
Model wastewater is prepared by the technician.

The practice was created according to the laboratory practice of ELTE TTK Environmental Technology (Környezettechnológia)

Report

Applied Chemistry – III. Laboratory		
Name of the student	**Wastewater clarification**	Date Team

1. Measurement table.
2. Calculate the calibration curve of turbidity measurement!
3. Compare the turbidity of the samples carried out by the three different flocculants and determine which one is the most efficient!
4. Compare the settling times measured during clarification!
5. Evaluation

5. ONE OF THE BASIC PROCESSES OF BIOTECHNOLOGY: YEAST PROPAGATION

5.1 Theoretical introduction

Biotechnology: integrated application of biochemistry, microbiology and technical sciences in microbiological processes, when micro-organisms or their certain constituents (for example enzymes) are used for industrial production purposes.

Biotechnology can be found in several industrial areas, but its food- and pharmaceutical industrial application is of utmost importance. The key process of biotechnology is the fermentation. Fermentation is the process, where microorganisms due to their normal way of life and propagation produce compounds that compose a product of consumer society. Beers, wines, B12 vitamins and penicillin derivatives are produced by fermentation. Lactic acid monomer creating the biodegradable polylactic acid polymer is made by fermentation with the help of Lactobacillus strains.

One of the families of producing microorganisms is the yeast-fungus, from which the most well-known is the common baker's yeast (*Saccharomyces cerevisiae* as formal name). *Saccharomyces* strain is used in several fermentation technologies and the yeast extract is propagated, in this way the cost of the whole technology can be decreased.

It fits to the biorefinery concept, if byproducts generated in biomass conversion process, the unused raw materials and wastes serve as raw materials in auxiliary technology, so the integrated consumption is achievable. This proves the fact as well, that the sugar content of sweet sorghum was used for lactic acid fermentation, additionally yeast was propagated on the plant's starch content (originally byproduct) what ensured the yeast extract, which is necessary to lactic acid fermentation[x].

The quantity of the desired sample can be reached by ensuring the right conditions for the key players of fermentation processes, the microbes. These are the followings:

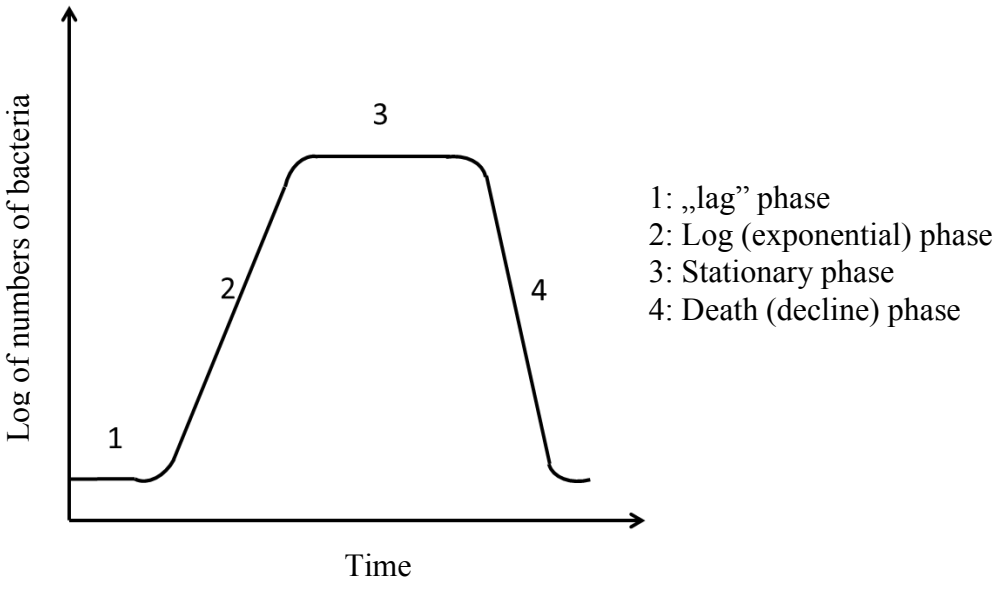
- Optimal temperature
- Optimal nutrient content
    - Carbon-
    - Nitrogen-
    - Phosphorus source
- Ideal oxygen supply

Saccharomyces cerevisiae results in diverse processes, when carbon source and oxygen supply differs.

- In the presence of oxygen, cells are enabled to develop on sugar or starch substratum. The adequate oxygen supply can be achieved by stirring, by the so called shaker flask method, where the surface of material exchange increases due to the thin film layer created on the wall of the flask, so the diffusion of oxygen from air to the liquid is enhanced.
- Without air supply alcoholic fermentation develops, so yeast transforms carbohydrate nutrient into ethanol.

5.1.1 Microbial growth

Reproduction forms of microbes are cell-cleavage and budding. (In view of the fact that the laboratory was made for chemistry students, only the concepts needed for the laboratory are explained here.) Characteristic reproductive way of yeast-fungus is the budding that means an asymmetric reproduction. The portion of the cell body is thrust out and then becomes separated forming a new individual cell. The daughter cells behave in different ways in the next cycle.



5. 1. Figure: Bacterial growth phases in mixed liquid phase sample[xi]

1. „**lag" phase**: Resting-cells are grafted in sterile medium: bacteria adapt themselves to growth conditions, they are not yet able to divide.
2. **Log (exponential) phase:** The bacterial population undergoes exponential growth. $N(t)=N(0)\cdot\exp(\mu\cdot t)$, where μ is the specific growth rate (the quality of the microbe and the environment define the value of it, it is reciprocally proportional to the culture's generation time)
3. **Stationary phase**: There is no change in number of cells, growth stops (caused by lack of nutrients or enrichment of metabolites).
4. **Death (decline) phase**: Cells are maturing, the number of them is decreasing according to exponential kinetics.

5.1.2 Optical density

Bacterial growth is monitored by Optical Density (OD) measurement. The method is based on the following: we place given amount of sample into a cuvette, we light it at a given wavelength. The absorbance will differ when samples with different cell numbers are measured, so the density of sample solution changes along with the cell number. The correlation between absorbance and cell number can be explained by the Lambert-Beer law, but the disadvantage of the method is that it cannot distinguish between living and not living cells, because dead cells contribute to the turbidity of the sample and absorb light as well.
In the early phases of yeast propagation examination, it is an appropriate method together with the Bürker chamber cell counting (practical part). According to previous investigations

Optical density of yeast cells is determined at 300 nm wavelength[xii]. (Suggestion: use a scanning to determine the wavelength showing the optimal absorption.)

5.2 Practical work

Propagation of traded Budafoki yeast on sugar content model wastewater medium.

Preparation:

1. Tools used during measurement have to be sterilized in exsiccator for 120 minutes at 160°C in order to avoid their contamination.
2. In order to shorten the incubation time, yeasts are heated to room temperature.

5.2.1 Preparation of sugar-content medium

Prepare growth medium of 100 ml 5 g/l sucrose, 1 g/l glucose, 0.5 g/l fructose concentration.

Perform propagation experiments in 100 mL thermostable Erlenmeyer flask at 36°C.
For appropriate propagation of *Saccharomyces cerevisiae* yeast-fungus, measure in 40 mL wastewater of given sugar concentration and implement it with the following compounds:

- 0.04 g KH_2PO_4
- 0.012 g $MgSO_4$
- 0.08 g NH_4Cl

After solving these, add the yeast to it!

- 0.0048 g Budafoki yeast

Perform the experiment for 90 minutes. From the initial sample, then in every 30 minutes take 1 mL of sample and measure their absorbance at 300 nm (Optical Density). By the advancement of budding, the absorbance of samples increases as well, and this gives indirect information on the cell numbers. After measuring the absorbance, accomplish the cell counting in Bürker chamber. With micropipette take 0.17 μL from the 1 mL sample and drip the cell-suspension in the pit created on the chamber, cover it with coverslip. Set the microscope to 40' magnification, then calculate the cell on the selected areas.

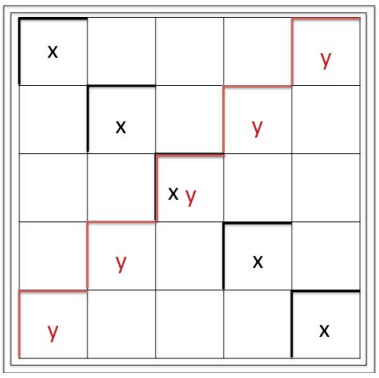
5.2.2 Procedure of Bürker chamber cell counting

Setting the microscope:
Set the 1,4x magnification on the lense. At 10-times magnification on the microscope, the human hair is visible). The gridlines of Bürker- chamber can be seen at 40x magnification.

This method is used primarily for counting of yeasts and mould spores. Bürker chamber contains two rectangular areas. The area of the small squares is 1/400 mm^2, the big ones' are 1/25 mm^2. Their depths are 0.1 mm when covered with coverslip. The cell number of 1 mm can be calculated as follows: the average cell number of the small squares has to be multiplied by 4×10^6, the big squares by 2.5×10^5. Bürker chamber contains two rectangular scales separated by a hollow, in this way it allows the examination of two suspensions. The original thought is to choose the squares that has to be counted randomly from the whole surface, but because of transparency reasons it is easier to count the cells starting from a square bordered with double (or triple) lines, going in a diagonal way. After this, the squares on the other diagonal have to be handled the same way, so the square in the intersection of diagonals is counted twice. For practical reasons the cells located on the line of the left, upper square are included in the square, and the cells located on the line of the right, lower square are not included in the counting. In order to determine the cell number of one suspension, at least 500

cells have to be counted. The cell groups and gemmiferous yeast cells are counted as one, and in this case cell mass number is given. If the culture is too dense, the sample has to be diluted before counting. The lower limit of sensitivity is 10^6 cell/ml. So we count the average cell number of one small square and multiply it by the right factor (4×10^6).

How to count cell in the Bürker-chamber



Task:

1. Calculate cell number in 1 mL of sample, then plot it in the function of time.
2. Determine in which propagation phase the yeasts are.
3. Define the correlation between absorbance and cell number.

Required chemicals:

0.04 g KH_2PO_4
0.012 g $MgSO_4$
0.08 g NH_4Cl
0.0048 g Budafoki yeast
Sucrose
Glucose
Fructose

Required tools:

Heated Erlenmeyer flask (If there is no flask, we apply water bath.)
Thermostat
Microscope + Bürker chamber
Spectrophotometer + cuvette
1 cm^3 pipette for sampling
Micro pipette
4 pieces of 10 mL volumetric flask for dilution of yeast suspension, if needed

REPORT

<table>
<tr><td colspan="3">Applied Chemistry – V. Laboratory</td></tr>
<tr><td rowspan="2">Name of the student</td><td rowspan="2">One of the basic processes of biotechnology: yeast propagation</td><td>Date</td></tr>
<tr><td>Team</td></tr>
<tr><td colspan="3">1. Measurement table: for the absorbance and Bürker chamber measurements
2. Plot the cell number in 1 mL of sample in the function of measurement time.
3. Allocate the reproductive phase of the yeast.
4. Note the correlation between absorbance and cell count
5. Evaluation</td></tr>
</table>

6. PRODUCTION OF ADIPIC ACID

6.1 Theoretical introduction

Under natural conditions adipic acid can be found in beet sugar juice and treacle or molasses, but it arises in rancid fats, as well. Its soft sour-salty flavour is persistent, that is why it is likely to be used in coatings, for example in fruit chewing gums. Adipic acid does not bind the humidity of air, so it is widely used as flavour enhancer in powder-like foodstuff that have long storage time, for example in drink powder (its maximal concentration is 10 g/dm^3). Adipic acid is used as the substitute of table salt, it is applied in the production of cosmetics and medicine. It is labelled in food-related applications as E 355[xiii]. The daily input quantity is 5 mg/bodyweight kg. There is no known side effect, it decomposes in the human body and leaves with urine.

Its application in the plastic industry is more significant, adipic acid serves as building block of nylon, so it is one of the parent compounds of „Nylon 66" polymer. „Nylon 66" is a polymer consisting of adipic acid and 1,6-diaminohexane units bonding with amide linkages.

The production of adipic acid is carried out from cyclohexane as starting compound. Cyclohexane is a colourless, transparent liquid that peroxidases due to sunlight and air, so it is recommended to distill it before use or store it in stabilized form.

The carbon-carbon double bond in cyclohexene participates mainly in addition reactions, but depending on the reaction conditions, it can oxidise as well. Under mild conditions only the π-bond breaks and vicinal diol appears. Under forceful circumstances complete chain disruption is carried out, and depending on the conditions formation of oxo-compounds (aldehydes) or carboxylic acid takes place.

In traditional industrial procedure nitric acid is applied as strong oxidiser for oxidative cleavage of alkenes. Under laboratory conditions the reaction is carried out with hot potassium permanganate ($KMnO_4$), while big amount of manganese dioxide (MnO_2) precipitate appears as byproduct.

6.1.1 Outlook: Green chemistry production of adipic acid

For the production of adipic acid there is a "green" method that is an alternative to the known reactions. Oxidation of cyclohexene with hydrogen peroxide is catalysed by sodium wolframate (Na_2WO_4)[xiv]. This method is environmentally friendlier than the reaction of nitric acid or potassium permanganate. Despite the fact that the mechanism of the reaction is not fully known we can assume that the wolframate ion plays a similar role as the similar-structured permanganate ion. But with the help of hydrogen peroxide the transiently reduced wolfram compound is oxidised to wolframate, so on the contrary, wolframate ion works as the catalyst. In order to ensure the wolframate to exert its effect, phase transfer catalyst has to be applied. Aliquat 336 – a quaternary ammonium salt – takes the water soluble anion into organic phase.

6.1.2 Chemical equation

$$3C_6H_{10} + 8KMnO_4 + 4H_2O \rightarrow 3HOOC-(CH_2)_4-COOH + 8KOH + 8MnO_2$$

Cyclohexene + potassium permanganate + water → adipic acid + potassium hydroxide + manganese dioxide

Atom efficiency

$3\ C_6H_{10} + 8\ KMnO_4 + 4\ H_2O \rightarrow 3\ C_6H_{10}O_4 + 8\ KOH + 8\ MnO_2$

M=82 M=157 M=18 M=146 M=56 M=87 g/mol

$$atom\ efficiency = \frac{n_{product} \cdot M_{product}}{\sum_{i-1}^{x} n_{reactant,i} \cdot M_{reactant,i}} = \frac{3 \cdot 146}{3 \cdot 82 + 8 \cdot 157 + 4 \cdot 18} \cdot 100 = 28\%$$

6.2 Practical work

Aim of the laboratory practice:

During this laboratory practice the oxidative cleavage of cyclohexene is carried out with the help of potassium permanganate, and the outcome is 1, 6-hexanediodic acid, adipic acid. The aim is to produce an organic chemical industrial raw material, adipic acid, which is the monomer of „Nylon 66" polymer. Oxidative chainwidth according to organic chemistry is shown *via* the catalytic oxidation of cyclohexene.

Steps of practice:

1. Measure 100 ml of water, 1.6 g of cyclohexene and 4.9 g of potassium permanganate into a 250 ml Erlenmeyer flask. And add 0.8 ml of 10% sodium hydroxide solution.
2. Let the reaction go for 30 minutes on heated magnetic stirrer. Make sure that the reaction mixture is heated up to around 100°C in 10 minutes, then keep this temperature for 20 more minutes while stirring it moderately. For better thermal transmittance, cover the flask with aluminium foil.
3. (If the magnetic stirrer cannot be heated, stir the reaction mixture for 10 minutes on magnetic stirrer, then place it to hot water bath for 20 minutes. Rate of material exchange highly depends on stirring, so yield depends on it as well.)
4. When reaction time is over, place one drop of the mixture on filtering paper. If purple colour is observed, potassium permanganate is not fully reacted. The potassium permanganate residue has to be destroyed by little quantity of sodium hydrogen sulphite solution.

   $2MnO_4^- + 5SO_3^{2-} + 6H^+ \longrightarrow 2Mn^{2+} + 5SO_4^{2-} + 3H_2O$
5. Filter the reaction mixture with the help of filtering paper in order to remove the manganese dioxide produced as byproduct. Wash the precipitate left on the filter with water.
6. Pour the mother-liquor into 150 ml beaker, add few silica particles and distill it above gas jet until it is 30 ml. If the solution is colourful, clarify it with a little of activated carbon. Adjust the pH to 2 in hot state with concentrated hydrochloric acid, in this way the product is separated in crystal state. (pH-paper should be used.)
7. Filter and dry the separated crystals on room temperature.
8. Weight the mass of the product and calculate the yield. (Expected yield is 46%)
9. Yield: mol number of the desired product proportionally to initial materials' total mol number

Required chemicals:
Stabilized cyclohexane (few g)
Potassium permanganate (few g)
Sodium hydroxide solution (10%)
Sodium hydrogen sulphite (few g)
Concentrated hydrochloric acid
Carborundum

Required tools:
Heated magnetic stirrer and magnetic stir bar
Erlenmeyer flask (250 ml)
Stopper
Filtering paper
Funnel
Beaker (150 ml)
pH-paper

REPORT

<table>
<tr><td colspan="3">Applied Chemistry – VI. Laboratory</td></tr>
<tr><td rowspan="2">Name of the student</td><td rowspan="2">Production of adipic acid</td><td>Date</td></tr>
<tr><td>Team</td></tr>
<tr><td colspan="3">1. Describe the implementation of reaction
2. Calculation of yield
3. Evaluation</td></tr>
</table>

7. ISOLATION OF STARCH FROM AGRICULTURAL/FOOD INDUSTRIAL PRODUCTS

7.1 Theoretical introduction

The wide range of consumption of biomass has a significant role in energy production, as well as in the application of biorefinery concept. The principle of the bio-refinery is – as in the case of petroleum-refinery - to process every carbon atom of the biomass, including the resultant waste, in order to produce energy or some kind of product (even chemical source materials).

7.1.1 Starch and its production

Starch is built from α-D-glucose molecules, and it can be regarded as the mix of polysaccharides of different molecular mass formed from their polycondensation. Its chemical formula is $(C_6H_{10}O_5)_n$, where *n* is a big number. Starch consists of two different structural and "average molecular mass" element, the spiral-shaped and not-branching amylose (20-28%) and the branched amylopectin (72-80%). The average molecular mass of amylose is 50-200, while the molecular mass of amylopectin is 300-2000 kDa. The degree of polycondensation shown by the "*n*" value is in the range of 300-1200, but decisively around 1000 for amylose, and in the case of amylopectin it is around 5000, moving between 1500 and 12000 depending on the origin of starch.

The most common raw materials of starch production are cereals of high starch-content: wheat, corn, barley, rice, rye or other plant storage organs (modified stems, root, etc.), for example potato. Independently of the materials, isolation of starch has to be started by cell disruption. With the help of mechanical, chemical methods, or with the combination of these, the cell wall has to be broken, and the starch particles become free has to be separated from other constituents.

If required, raw materials are swollen in order to stir up constituents. During swelling, the increase of osmotic pressure in the cells results in partial break-up of the cell wall. Then by means of mechanical methods (grinding, milling) cell walls are smashed, the starch-particles are washed from the builders, and are separated from the wash water by sedimentation. The raw starch is separated from the contaminations (proteins, colouring materials, dissolved amino acids, organic and inorganic salts, etc.) by repeated washing combined with sedimentation. After drying and packaging, the cleaned product will be used in air-dried condition. The above-mentioned processes can be carried out by batch or continuous operation methods. The procedure applying flowed and circulated washing water became prevalent, but on the practice the repeated batch washing will be implemented.

7.1.2 Characteristics of starch

Starch has a plant-specific shape and structure, it deposits in a form of more or less linear dimension and white particle (see: appendix). The size of the particles are likely between 150 and 200 μm. Starch molecules are longitudinally stretched, they are placed between the spherical glycogen molecule and fibre-shaped cellulose molecule.

Starch does not dissolve in cold water or ethanol. Shaking it together with water creates a faster or slower settling suspension. With iodine it provides a high-sensitivity colour

reaction. This reaction is suitable for the determination of starch and iodine (iodometric indicator!). By heating carefully the water-starch suspension, starch particles swell at first, then they create a colloid solution. By cooling down the colloid, the solution solidifies into a dense gel (starch paste). The gel-forming ability of starch is due to the presence of amylopectin component. The temperature range providing the gel-forming is relates to the origin of starch, so for example for potato starch it is 60-65°C and for wheat starch it is 70-80°C.

7.1.3 Utilisation of byproducts

A lot of valuable mineral salt, compounds supporting condition for life of microorganisms (amino acids, proteins, sugar, and other organic active agents) dissolve into the washing water, and accumulate in it. Materials dissolved in these technological waters, being generated as byproducts during starch production and then being concentrated via evaporation. Concentrated byproduct for example corn jam can be produced, that can be utilized as valuable material in microbiological industry (for example in production technology of antibiotics) for making growth medium.

After the washing process the remaining reinforcing filler still contains some of the starch, other valuable organic and inorganic materials, and digestible components. These are primely used for animal nutrition.

Valuable byproduct of the wheat starch production is gluten. Gluten is applied for production of amino acids, as raw material for adhesive and for producing infant formula. A lot of industry, including pharmaceuticals (formulation), textile industry, cosmetic industry, explosive industry (nitrate starch), plastics industry (compostable plastic foils), food industry (condenser, starch syrup) and alcohol industry consumes starch.

7.2 Practical work

Aim of the laboratory:

The aim of the laboratory belongs to the biorefinery concept: product manufacture from biomass material. In this case the isolation of starch and its quantification and microscopic investigation is implemented.

Tasks to be carried out:

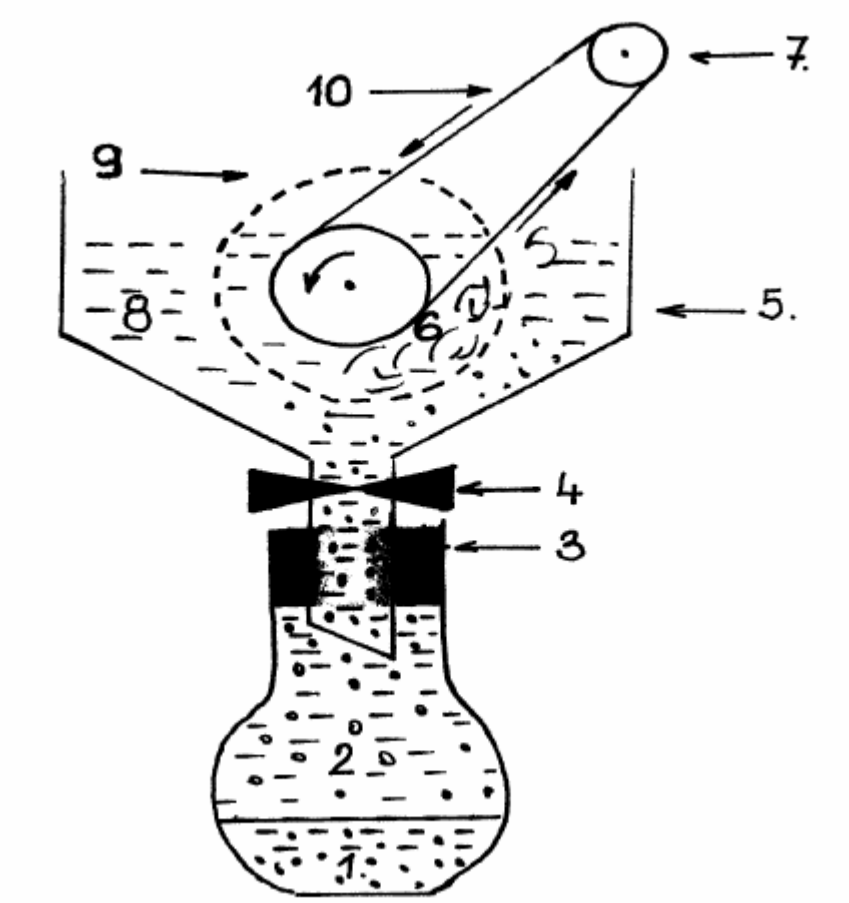
1. Lysis of potato and isolation of starch, note the mass balance and the diagram of leaching speed-time.
2. The microscopic investigation of the isolated starch (linear size and shape). Then compare potato-starch with and known-origin starches, the identification of the unknown starch sample.

7.2.1 Extraction of potato-starch

Weigh circa half kg of potato. The contaminations on the tubers has to be washed, then rasp them until mash. From the potato mash, the starch can be isolated via using washing drum.

Starch particles can be isolated from potato mash via using washing drum. First of all the drum has to be prepared. Open the tap under the tank. Attach a 750 (maximum 2000) ml wide-mouthed round-bottomed flask in a liquid tight way to the snag of the tap. The flask has to be fixed to a scaffolding. Disassemble the washing drum covered with bolting cloth out of the washing drum and place it next to the device on the table with filler upwards in order not to let the cloth get injured (to secure it against displacement). Fill up the tank with tap water till the sign (around 5 cm below the edge of the tank). Then open the drum and place it with the filler upwards above the tank in a way that the shaft of the drum is supported on the edge

of the tank. Quantitatively put the potato mash into the washing drum. Suspend the potato mash remaining in the bowl in little water, then decant it into the drum at one sweep. Close the drum and assemble it back to the tank. Check carefully the fixations, and start the device. Adjust the drum's revolution with the controller to 15-20 RPM. We do the rpm adjustment as the following: fixate a characteristic state of the spinning drum (for example when the filler gets to the liquid's surface), then using a stopper, count the revolutions under a time unit (for example: one minute). Note the adjusted idle speed. The washed starch particles will be collected in the flask attached to the bottom of the water tank. Continue the spinning of the drum and the washing for 20 minutes. At the end of the washing stop the spinning. Close the tap at the bottom of the water tank. Disassemble the flask with the collected starch and attach to its place a new flask filled with tap water.
The schematic structure of the washing device is shown on the next figure.



(1./ starch, 2./ sedimentation flask, 3./ gasket (drilled plastic stopper), 4./ one-hole closing tap, 5./ washing tank, 6./ grated potato, 7./ engine of controllable idle speed, 8./ wash water, 9./ washing drum coated with bolting cloth, 10./ power transmission)

The previous set of operations has to be repeated three times. If there is a loss of water in the washing tank because of changing the flasks, refill it with tap water.

Filtering the suspension of starch-content

With the help of suction flask, Büchner funnel and precisely cut filtering paper remove the water-content of starch by applying water-jet vacuum. **Practical advice**: the filtering paper should not reach the side of the funnel (or a part of the particles will get into the filtrate). Weigh the mass of the filtering paper before wetting it. In order to reach great grip, wet the

paper and then dry-suck it. At first, the supernatant liquid has to be decanted onto the filter. With the help of the last part of the liquid, the settled starch is suspended, and at one sweep we get the suspension onto the filter. The remaining starch has to be decanted with tap water onto filter. After the last decant, the filter cake should be sucked for more 10 minutes. Then remove the filter cake along with the filter paper, place it on an appropriate sized watch glass and measure its wet mass.
Stir up the filter cake on the watch glass used for weighing, then determine its moisture content. This moisture content is considered valid for the washed starch in the following 3 steps.

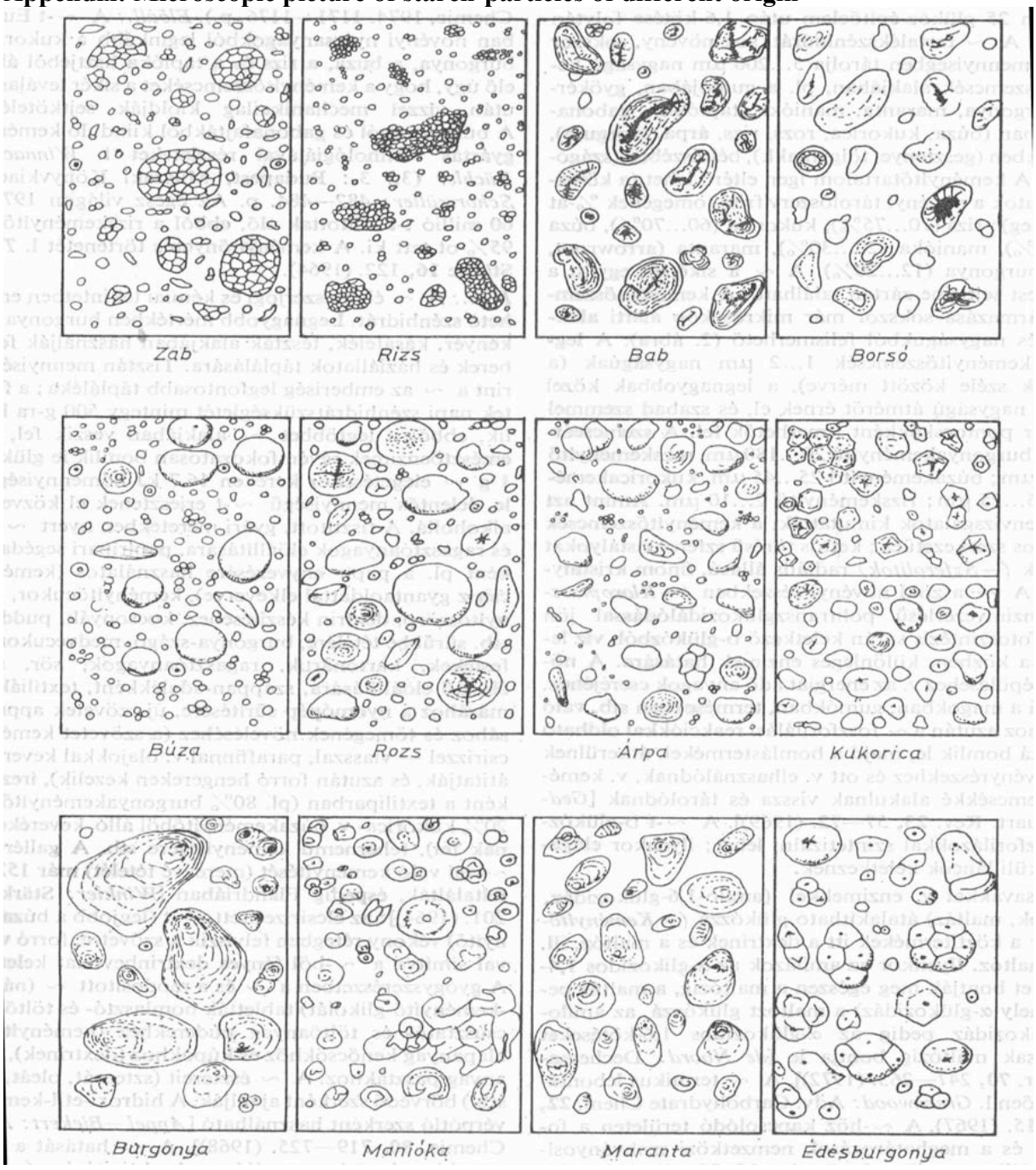
Determining the dry matter content of starch
Measure known quantity of wet starch into a previously weighed measuring utensil, and dry it at 105°C for 2 hours (with removing the cover!). At the end of the second hour, place the cover onto the utensil with forceps. Let the bowl cool down in desiccator for 20 minutes, then by weighing it again, determine the loss of mass of the starch sample (removed water). After the process of washing out, the potato mash has to be thrown out to trash and the washer drum has to be cleaned. Get a rubber tubing of appropriate diameter onto the tap of the water tank and lead the water into the laboratory sink by opening the tap. At open tap position, the inside of the drum has to be washed out with water-jet. After the cleaning process, leaving the draining-tap at open position, the drum has to be placed back into the washing tank.

7.2.2 Microscopic investigation

Setting the microscope:
Use 1.4x magnification on the lense. Human hair is visible if 10-times magnification is set on the microscope.

The isolated potato-starch and an unknown-origin starch is investigated by suspending it in water, in the form of so-called aqueous preparation. After practicing the use of the microscope in empty status, and if the preparation is well-done, adjust the microscope to 600-800 times magnification. In this magnification, scan the object-slide and choose a characteristic, well-articulated part of the microscopic picture. The following observations are to be done on this part. Draw the picture of the field of vision on grid paper. The criteria of a good drawing can be studied on the sample pictures in the appendix. Make an effort to draw the field of vision in scale. Identify the characteristic linear size of few particles. Provide scale.
Then investigate the unknown-origin grist, identify its origin. For the identification use the microscopic pictures of the appendix. The drawing has to be annexed to the report, and the applied magnification has to be represented.

Appendix: Microscopic picture of starch-particles of different origin



Starch-particles of different plants in 200-times magnification according to Gassner

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Required tools:

Washer drum

Potato grater

Light microscope with equipage

Tools needed for the microscopic investigations (coverslip, forceps)
Mixing engine with scaffolding and equipage (flexible shaft, churn-dasher)
Desiccator
Tools needed for assembly (flask tongs, scaffoldings)
Chemical spoon, rubber tubing, rubber cone, rubber plug, vacuum rubber tubing
Büchner funnel
Filtering paper
Scissors
1000, 500, 200 mL suction flask
2000, 750 mL short-necked, wide-mouthed round-bottom flasks
1 and 3 L pot, 3 L pan
1 or 2 L measuring cylinder
25 mL beakers for the microscopic investigation
Porcelain bowl
Textile for the filtration and washing

Required materials:
Potato (half a kg/team)

REPORT

Applied Chemistry – VII. Laboratory		
Name of the student	**Isolation of starch from agricultural/food industrial products**	Date
		Team

1. Calculate the mass balance: Sketch in tabular form the extracted wet starch mass from the half kg of potato during the 4 x 20 minutes washing time, the moisture content, the dry matter content and the extracted dry starch mass. Calculate the potato's starch-content in mass-%, as well.
2. Leaching speed - time diagram: Plot the total mass of extracted starch in the function of leaching time. Fit a function on it! (Plotted points: t_1m_1; $t_2(m_1+m_2)$; t_3-$(m_1+m_2+m_3)$; $t_4(m_1+m_2+m_3+m_4)$.
3. Microscopic investigation of the isolated starch (linear size and shape): field of vision
4. Microscopic investigation of the starch sample of unknown origin: field of vision
5. Evaluation

8. HYDROLYSIS OF STARCH

8.1 Theoretical introduction

The chemical characteristics of starch are given by the molecules' polyalcohol character and the molecules' polysaccharide character. The free hydroxyl groups of the molecules are responsible for the reactions that the aliphatic- and poly-alcohols give:

- they can be easily esterified with inorganic acids and carboxylic acids,
- ether linkages can be built up,
- formation of acetal ring happens due to the reaction with oxo compounds.

A lot of derivate is of particular importance, for example:

- nitric acid ester of starch, the starch nitrate as an industrial explosive material,
- acetic acid ester of starch, used as a material in pharmaceuticals' formulation, is a component of starch-based adhesives,
- its copolymer containing ether- or ester-linkages is compostable, and can provide environmentally friendly agricultural foil and packaging material in sales.

8.1.1 Main products of starch

1. Expandable starch: used as adhesive.
2. Soluble (or modified) starch: starch is not soluble in cold water, gel-forming does not happen. Hydrolysing under moderate conditions the starch results in water-soluble starch that does not expand during solution, the viscosity of its aqueous solution is smaller than the viscosity of the colloid solution generated by the original starch. Starch is being suspended in volatile, dilute mineral acids (0.1-0.2 N), then it is hydrolysed at a temperature smaller than the gel-forming temperature. The generated product is water-soluble, does not form a gel, and when cooling down the solution, it does not become gelled. It still provides the iodine-starch reaction that is used as a determination method in analytics.
3. Products produced by hydrolyses: C_6-fractions.

Starch, as most of the polysaccharides is degradable, i.e. can be hydrolysed. The hydrolytic degradation can be carried out by the following chemical equation in both enzymatic way or by cooking with hot, dilute mineral acids.

$$(C_6H_{10}O_5)_n + n\text{-}1\ H_2O \rightarrow \text{catalyst} \rightarrow n\ C_6H_{12}O_6$$

Parameters of the acidic, enzymatic and combined hydrolysis in Table 1.

Table 1 Parameters of the acidic, enzymatic and combined hydrolysis

Parameters	Acidic	Acidic/enzymatic	Enzymatic
Rate of dissociation	75 - 85	92 - 95	97 - 98
Glucose-content (%)	80 - 85	90 - 91	97 - 98
Reversion (%)	10 - 12	5 - 8	~ 1

During dissociation with hot dilute mineral acids, the H^+ - ions act as catalysts, while enzymatic hydrolysis, the amylase enzymes catalyse the hydrolysis providing bio-catalysis.

Starch hydrolysed in the enzymatic way: mainly wort of fermentable sugar (maltose) is produced for purposes of alcoholic fermentation methods. Usually there is no need to use isolated starch, because in alcohol production the product is isolated by distillation (see: brewing), in other cases the different components of the material are built into the product.

Table 2 Summary of products created by hydrolysing starch

Products	**Dextrose equivalent (DE)**	**Application**
Maltodextrin	3-20	flour improver
		fermentation industrial adhesive
Starch-syrup	15-30	flour improver, filler
	30-50	sweeteners, conserver
	50-75	beverage technology, confectionery industry
Starch-sugar	60-80	food and beverage industry
		confectionery
Glucose	96-98	crystalline glucose, dextrose syrup

The abbreviation DE stands for the dextrose equivalent, which is a value between 0 and 100. The dextrose equivalent means the rate of hydrolysis. For starch DE=0, while for glucose DE=100.

Dextrin: intermediate product of the starch degradation. It can be produced by spraying starch with mineral acid (0.2% hydrochloric acid or nitric acid) and then applying heat treatment at 120-150°C. The other way is without using acid, generating by applying higher temperature heat treatment (170-190°C). Dextrin is utilised as adhesive-, binder- and thickener material in fabric industry, applied in ink- and paper production.

Starch syrup: In Hungary the primarily used syrup material is potato starch. When making starch syrup, the starch is suspended in water of 2.5-3 times the starch quantity, 0.2% of sulphuric acid is added, then it is being hydrolysed in closed autoclave at 120°C (1-2.5 atm) until the sample taken shows red-brown colour with the iodine probe. It takes usually one hour. The hydrolysate contains oligosaccharides, glucose (ca. 40%) and dextrines (ca.

40%). The hydrolysate is being neutralised with calcium carbonate, then clarification with active carbon and filtration is applied. The hydrolysate depending on the purpose of consumption is adjusted to 45-80% dry matter content. The syrup is utilised in food and beverage industry (candy production, making sweets and fondant) and canning industry (as fruit flavour). The original, esthetical form of food industrial decorations is preserved for a long time, because dextrin content of the syrup prevents the sugar from crystallisation.

Glucose: at its production complete hydrolysis is carried out. In order to achieve more and more complete hydrolysis, diluter starch-suspension is implemented and the conditions are stronger (time, pressure, temperature). The hydrolysate showing negative alcohol-probe is being cleaned, evaporated, inoculated and crystallised. The crystallised product can be separated from the supernatant by centrifugation. The crystallised glucose is being washed and dried on the centrifuge. Glucose is utilised in food industry for making caramel, formulation of medicinal products, making infant formula and production of sorbitol (material of synthetic L-ascorbic acid production).

8.2 Practical work

Tasks to be carried out:

1. Investigation the starch' rate of hydrolysis with 0.1 M hydrochloric acid in the function of time.
2. Investigation the starch' rate of hydrolysis with 0.1 M sulphuric acid in the function of time.
3. Confirming the presence of reducing sugar.
4. Iodine probe.

Prepare 2 pieces of 250 ml beakers and fill them halfway with water. Using them as water bath, place them onto a tripod and heat them with Bunsen burner. (4-4 pieces of tubes go to both beakers.) As the boiling temperature is reached, turn down the flame, so the water does not boil too much. During this, prepare 8 pieces of tubes and label them (1-8). Give 5 ml of 3% starch solution to all of the tubes.
As a blank sample, by the means of graduated pipette add 3 ml of Benedict solution into the 1. and 5. tube, and place them into water bath for minimum 5 minutes (5-10 minutes).
Give to the 2., 3., and 4. tube 1 cm^3 0.1 M hydrochloric acid and place them into water bath. Note the time. Continue the boiling for further 5, 10 and 15 minutes consecutively.
Give the 6., 7., and 8. tube 1 cm^3 0.1 M sulphuric acid and place them into water bath. Note the time. Continue the boiling for further 5, 10 and 15 minutes consecutively.
After finishing the process of boiling, remove the tubes from water bath, cool them down under tap water, then neutralise them with the help of solid $NaHCO_3$ and place them on the tube rack. Using drop-glass take a sample from each tube and get the iodine probe done.
Add 3 ml of Benedict solution to the tubes labelled as 2, 3, 4 and 6, 7, 8 and place them back to the water bath for 5 minutes. Then cool down the samples again, check their colour and measure the quantity of produced precipitation.

Analytics

1. Iodine probe

Preparation of potassium iodate iodine (Lugol's) solution: 5% I_2 solved in 10% aqueous KI solution.
The samples are investigated with iodine probe hereinafter. By the means of a glass rod take one drop of the actually analysed tube and add it to a clean tube containing 2-3 ml water. Mix

the content of each tube with the rod and add 1 drop of Lugol's solution. Observe the colour of the samples reacting with the Lugol's solution. If the determination of the colour proves difficult because of the dark colour, gradually dilute the sample with water as long as the colour can be evaluated for sure.
An iodine molecule fits perfectly into the amylose helix of the starch. In this new environment the molecules are bound by the weak van der Waals bond. This has an effect to the electron structure and the wavelength of the absorbed light, so the result is a blue coloured mixture. At higher temperature the blue colour disappears. This reaction is suitable for determination of small amount of starch (or iodine), as well.

2. Benedict probe

The group prepares the Benedict reagent as follows:
In one litre of water dissolve: 173 g of sodium citrate, 100 g of watertight sodium carbonate and 17.3 g of copper(II)-sulphate-5-hydrate. The reaction goes down similarly to the Fehling probe: the aldehyde group reduces the copper(II) ion into copper(I) ion, during which the aldehyde group is oxidised. The copper(I) ion comes off as brown Cu_2O precipitation. This method is suitable to determine the presence of reducing sugars.

3. Quantitative determination of the precipitation content by centrifugal separation

Decant the contents of the tubes cooled down to room temperature into centrifuge tubes (made of plastic or glass) and centrifuge them. Adjust the centrifuge to 2000 1/s or to an idle speed ensuring bigger acceleration than this and set the operation time to 10 minutes minimally. (In the instrument book of the centrifuge, look at the maximal idle speed of the actually assembled rotor (type plate), and the acceleration due to the distribution of mass belonging to the maximal idle speed.)
Only even number of tubes can be placed into the rotor of the centrifuge, locating them in couples on an imaginary line going through the axis. Weighing on Tara scale, the masses of the tubes in pairs (including their contents) have to be identical. In order to balance the masses always use Tara scale during filling the tubes. Only 75-80% of the volume of tubes can be filled up with liquid. As the rotor accelerates, at higher filling rate one part of the liquid can be splashed into the inner side of the centrifuge, contaminating it.
Attention:
1./ Operating the centrifuge with open cover is of high risk! By-passing, bracing the latch of the cover is forbidden!
2./ Stopping the moving rotor (even at switched off engine) by the means of hand or any other hand-held object is forbidden!
3./ The centrifuge can only be started and operated with a rotor of balanced mass.

REPORT

				Benedict probe			
Tube	*Acid*	*Content*	*Iodine probe*	*Colour of the solution*	*Colour of the precipitation*	*Mass of the precipitation*	*Hydrolysis %*
1	blank sample	3% starch solution					
2	by adding 1 ml 0.1 M HCl	3% starch solution after 5 minutes of acidic boiling					
3		3% starch solution after 10 minutes of acidic boiling					
4		3% starch solution after 15 minutes of acidic boiling					
5	blank sample	3% starch solution					
6	by adding 1 ml 0.1 M H_2SO_4	3% starch solution after 5 minutes of acidic boiling					
7		3% starch solution after 10 minutes of acidic boiling					
8		3% starch solution after 15 minutes of acidic boiling					

The quantity of seceded Cu_2O is not equivalent to the quantity of reducing sugar present, but the reduced copper ions' quantity is in empirical correlation with the reducing sugar content of the solution. (The exact reducing sugar content can be determined by the Bertrand I, II, III solutions, but the process is too long for the time frame of this course, so we implement the Benedict probe.) We assume that the quantity of formed Cu_2O precipitation is proportional to the glucose content and based on this, we calculate the rate of hydrolysis in %.
Evaluation: Compare the effect of the two acids and boiling time to the rate of hydrolysis!

Required tools:
Bunsen burner, 2 pieces
8 pieces of tubes
Tube rack
2 pieces of 250 ml beakers
5 ml graduated pipette
1 ml pipette, 2 pieces
Drop-glass
Centrifuge, centrifuge tubes (made of plastic or glass)

Stopper, 2 pieces

Required chemicals:
Potassium iodide
Iodine
Copper sulphate-5-hydrate
Sodium citrate
Anhydrous sodium carbonate
3m% starch solution
0.1 M HCl
0.1 M H_2SO_4

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