AALTO UNIVERSITY
Department of Built Environment
Water and Environmental Engineering

WAT-E2180

BIOLOGICAL TREATMENT PROCESSES OF WATER AND WASTE

Laboratory Analyses

Contents

Determination of pH value

Determination of pH is one of the most important and frequently used tests in water chemistry. pH is a term to express the intensity of the acid or alkaline condition of a solution. It is a way to express the hydrogen-ion concentration, or more precisely, the hydrogen-ion activity.

Reagents:

1. Commercial pH buffers pH 4, pH 7 and/or pH 9 are used to calibrate the pH-meter depending on the expected (or target) pH of the sample.

Procedure:

Take the pH buffer in to the beaker and calibrate the pH-meter according to the manufacturer's instruction. The pH-meter is calibrated beforehand, so it is ready for the use. If you want, you can check it with one buffer.

Take carefully the cap away from the probe. Rinse the probe well with reverse osmosis water (RO-water) and immerse it in the beaker where the well-shaken sample is. Measure pH. The reading is directly pH of the sample. After all the measurings, rinse probe well with RO-water and put the cap back. There is 3 M KCl inside the cap.

The results are reported to one decimal place.

Literature: SFS-EN ISO 10523, dated 2012. Water quality. Determination of pH

Determination of suspended solids (SS) and volatile suspended solids (VSS)

The measurement of amount of solid material is important in all kinds of liquids and semiliquid materials ranging from potable water through polluted waters, domestic and industrial wastes and sludge produced in treatment processes. Strictly speaking, all matter except the water contained in liquid materials is classified as solid matter.

According to this method suspended solids is determined by filtration through a glass-fiber filter (Whatman GF/A), which is prewashed and dried in temperature 105 °C (SFS-EN 872).

Procedure:

Mark and weigh two prewashed glass-fiber filters per sample. Using a vacuum filtration apparatus the sample is filtered through a glass-fiber filter. Filtrate influent about 100 ml and effluent about 1000 ml so that filter time is not more than 1–2 minutes. Shake samples very well before filtrating. Wash the volumetric glass after each filtration with a small amount (10–20 ml) of distilled water. Rinse also edges of filter apparatus. Remove filter and place it on the edge of aluminum dish. Filters are then dried at 105 °C for at least 1 hour. Transfer filters into desiccator for 30 minutes and weigh them.

VSS is determined so that dried, weighed filter with mass of the residue is placed into the porcelain crucible. Transfer the crucible with the filter in the oven of 550 °C for 1 hour. Place hot crucibles in the glass exicator, where in porcelain plate.

Use always glass exicator with porcelain plate, when you take sample out of the oven at 550 °C.

sample	lab. number	filter mass in the beginning, (g)	volume of sample, ml	filter and residue after 105 °C, (g)	filter and residue after 550 °C, (g)

Calculation:

$$X = 1000 * (a-b)/V$$

X = SS or VSS, mg/l
a = mass of the filter and residue, mg
b = filter mass, mg
V = volume of the filtrated sample, ml

The results are reported in milligram per liter using two expressive numbers. If the result is smaller than 2 mg/l then it is reported “smaller than 2 mg/l”.

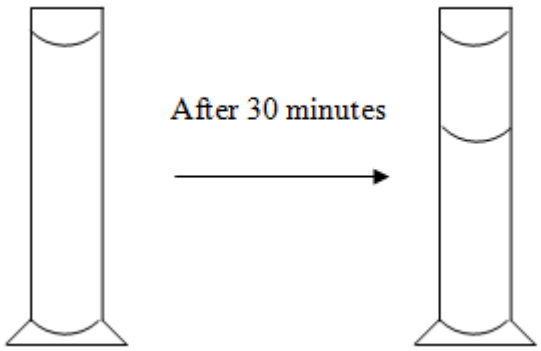
Literature: SFS-EN 872, SFS 3008 (in Finnish)

Sludge Volume Index – SVI

Sludge volume index is considered to be the volume, in milliliters, of 1 gram of suspended solids after 30 minutes of settling. It is the most widely used indicator of sludge settleability, settling characteristics and floc formation.

Procedure:

Collect a sample of activated sludge, mix gently, and pour into the cylinder. After 30 minutes, record the volume to which the sludge settles in mL/L.



Calculation:

$$SVI = \frac{\left(Settled\ volume\ of\ sludge \frac{mL}{L}\right) \times \left(1{,}000 \frac{mg}{g}\right)}{\left(MLSS \frac{mg}{L}\right)} = \frac{mL}{g}$$

Interpreting results:

Typically, SVI of conventional activated sludge should be in the range of 100-200 mL/g for producing clear, high quality effluent. Lower numbers indicate dense and granular sludge while the higher numbers show poor settling properties.

Determination of nitrate nitrogen (NO_3-N)

This method is suitable for samples with low organic matter contents, i.e. uncontaminated natural waters and potable water supplies.

Reagents:

1. 1 M HCl

2. ***Procedure:***

Filter each sample trough 0,45 µm membrane filter. Fill 25 ml volumetric flask with filtered sample up to mark or pipette 25 ml of sample into the 50 ml erlenmeyer flask. Add 0,5 ml 1 M hydrochloride acid and mix. Use wavelength of 220 nm to obtain NO^{3-} -reading, and 275 nm to determine interference due to dissolved organic matter. Use 1 cm UV cell.

Calculation:

$abs_{NO3} = abs_{220nm} - 2 * abs_{275}$ $(2 * abs_{275} < 10\% abs_{220})$.

Use standard curve to obtain sample concentrations in mg/l N.

Literature:

Standard Methods for the examination of water and wastewater, 21st edition 2005, pages 4-120 − 4-121, 4500-NO3- B. Ultraviolet Spectrophotometric Screening Method

Determination of total nitrogen with GanimedeN analyzer

In waters and wastewaters, the forms of nitrogen in greatest interest are nitrate, nitrite, ammonia and organic nitrogen.
Ammonia, nitrite, and many organic nitrogen-containing compounds in the test sample are oxidized to nitrate using peroxide sulfate and by boiling at elevated pressure in closed container. Nitrate is measured spectrophotometrically.
GanimedeN analyzer is used in this analysis. Sufficient amount of oxidizing agent is added automatically. Oxidation happens in a special chamber under 6 bar pressure in temperature 150 °C. Oxidation reaction is very quick in these conditions, only 1,5 minutes. The amount of nitrogen is measured at UV-area using wavelength of 210 nm.

Procedure:
Shake the sample and pour it in the sample cup. Add also a small magnet into the cup (for mixing purpose). Place cups in the sampler. The names of the samples are written into the control unit. With the control unit we start also the measurement. Equipment injects sample, oxidizes it, and measures the concentration automatically.

Results:
When the measurement is ready, results are fetched to the control unit and shown to the user. Results can then also be transferred to a computer.

Literature:
SFS-EN ISO 11905-1 and Standard Methods for the Examination of Water & Wastewater (21st edition 2005), page 4-120 + Manual of the GanimedeN analyzer

Determination of orthophosphate and total phosphorus

Phosphorus is a macronutrient, which is necessary to all living cells. It is limiting nutrient for algal growth in most lakes. Phosphorus determinations are important in assessing the potential biological productivity of surface water. In many areas, there have been established limits for the amounts of phosphorus that may be discharged to receiving bodies of water, particularly lakes and reservoirs. Because of the importance of phosphorus as nutrient in biological methods of wastewater treatment, its' determination is essential with many industrial wastewaters and in the operation of wastewater treatment plants. In wastewaters, phosphorus is present in several forms. That is why total phosphorus is typically determined for wastewaters.

Orthophosphate (PO_4^{3-})

Reagents:

1. Ascorbic acid (SFS 3025, 4.4)
2. Acid molybdate solution (SFS 3025, 4.6)

Procedure:

Filter the sample using 0,45 µm filter. Pipette 25 ml of the sample into a 50 ml erlenmeyer flask. Pipette also 25 ml of RO-water for zero-sample. Add 1 ml of ascorbic acid, mix, add 1 ml of acid molybdate solution and mix again to all samples (also to zero-sample).

Measure the absorbance of solution using the spectrophotometer at wavelength of 880 nm (1 cm OG cell) after a period between 10 and 30 minutes. After measurements, wash the cell carefully with detergent and water (Do not use a brush!), and finally rinse the cell with RO-water.

Calculation:

Subtract the absorbance of zero sample from the absorbance of the samples. Use the calibration curve (i.e. regression line) to get the concentration of the orthophosphate mg/l P. Finally notice the dilution factor, if samples have been diluted.

Literature: SFS 3025 (in Finnish)

Total phosphorus

First inorganic phosphorus compounds are oxidized to orthophosphate with persulfate in acid conditions. Oxidation takes place in an autoclave under the pressure at temperature 120 °C. Orthophosphate forms with antimony and molybdate antimony phosphomolybdate complex, which ascorbic acid reduces to blue colored complex compound.

Reagents:

1. Potassium peroxidesulfate solution II (SFS 3026, 4.4)
2. Ascorbic acid (SFS 3026, 4.5)
3. Acid molybdate solution (SFS 3026, 4.6)

Procedure:

Pipette 25 ml of sample or its' dilution into a 50 ml oxidation flask. Add 5 ml potassium peroxidesulfate solution II and mineralize in an autoclave under the pressure and temperature of 120 °C for 30 minutes. Use RO-water as a zero sample and handle it like every other sample.
Let samples cool down. It takes around 2− 2,5 hours to autoclave and cool samples.

Add to each 50 ml volumetric flask 1 ml of ascorbic acid, mix, and after 30 seconds add 1 ml of acid molybdate solution. Mix well. Measure the absorbance of each solution using the spectrophotometer at wavelength of 880 nm (1cm OG cell) after a period between 10 and 30 minutes.
After measurements, wash the cell carefully with detergent and water (Do not use a brush!), and finally rinse the cell with RO-water.

Calculation:

Subtract the absorbance of zero sample from the absorbance of the samples. Use the calibration curve (i.e. regression line) to get the concentration of the total phosphorus. Finally notice the dilution factor, if samples have been diluted.

Literature: SFS 3026 (in Finnish)

Determination of heterotrofic bacteria

Culture media:
R2A-agar, 22 °C, 68±4h

Growing temperature and time:
22 °C, 48

Procedure:

Melt sterile solid agar medium (R2A- agar) in boiling water. Maintain melted medium in an oven between 44 and 46 °C, preferably no more than 3 hours. Use a sterile pipette tips for transferring samples. Select the dilution so that the total number of colonies on a plate will be between 30 and 100. When discharging sample portions, hold pipette at an angle of about 45° with tip not touching bottom of petri dish. Lift cover of petri dish just high enough to insert pipette. Pipette sample so that it stays on the petri dish by drops. Pour liquefied medium into each dish by gently lifting cover just high enough to pour. Mix melted medium thoroughly with test portions in petri dish, taking care not to splash mixture over the edge, by rotating the dish first in one direction and then in the opposite direction. Let plates solidify on a level surface. After medium solidifies, invert plates and place in incubator. Incubate for 48 hours at 22 °C.

Results:

Express the results as the number of colony-forming units per milliliter (cfu/ml) of the sample.

Literature:
SFS-EN ISO 6222, dated 1999. Water quality enumeration of culturable micro-organisms. Colony count by inoculation in a nutrient agar culture medium.
SFS-EN ISO 8199, dated 2008. Water quality. General guidance on the enumeration of micro-organisms by culture.

Chemical oxygen demand (COD_{Cr}) with closed reflux method. Oxidation with dichromate

Chemical oxygen demand (COD) is defined as the amount of a specified oxidant that reacts with the sample under controlled conditions. The quantity of oxidant consumed is expressed in terms of its equivalence.

Samples are boiled for two hours in the closed tubes with concentrated sulfuric acid, silver catalyst and known amount of dichromate. Portion of dichromate is reduced by the oxidizing matter of the sample. Excess of dichromate is titrated with ferrous ammonium sulphate.

Reagents:

1. Potassium dichromate solution, 0.04 mol/l
2. Sulfuric acid / silver sulphate solution
3. Ferroin indicator solution
4. Potassium hydrogen phthalate 500 mg/l (control solution, standard)
5. Ferrous ammonium sulphate, ≈ 0.07 mol/l

Procedure:

Determination is made in special reacting tubes, which can be plugged. Reaction tubes include 1.0 ml of potassium dichromate solution and 3.0 ml of sulphuric acid / silver sulphate solution.

Duplicate determinations are made. Pipette 2.0 ml well mixed sample into the reaction tubes, close and mix tubes. If sample turns green, it contains too much organic matter and sample has to be diluted.

Check the accuracy of the determination and the reagents by measuring COD_{Cr}-value of the potassium phthalate solution (standard). Theoretical value of this liquid is 500 mg/l. In addition to the standard two blank samples are made using 2.0 ml of RO-water. This means pipette either standard solution or distilled water both into two reaction tubes and handle like samples.
Put all the tubes (8) in the same time in the block heater (150 °C), which is in a fume cupboard. Heat tubes two hours.

Let the tubes cool down to the room temperature. Add 1–2 drops of ferroin indicator and titrate excess of dichromate with ferrous ammonium sulphate solution direct in the reaction tube. Take as the end point of the titration the first sharp colour change from blue-green to reddish brown.

Concentration of used ferrous ammonium sulphate solution: _____ mol/l

During the determination of COD_{Cr} becoming **waste is hazardous waste**. After titration the liquid from the reaction tubes is collected in the beaker located in the fume cupboard and **it is not allowed** to rinse it in the sewer.

Calculation:

$$COD_{Cr} = (8000 \cdot c_{Fe} \cdot (V_1 - V_2) / V_3) \cdot f$$

COD_{Cr} = chemical oxygen demand, mg/l
C_{Fe} = concentration of the ferrous ammonium sulphate solution, mol/l
V_1 = volume of Fe-solution consumed by the blank sample (average of the two determinations), ml
V_2 = volume of Fe-solution consumed by the sample (average of the two determinations), ml
V_3 = sample volume, ml
f = dilution factor
8000 = milliequivalent (= mole mass/2) weight of oxygen · 1000 ml/l

Results are reported as follows:
result $\leq$ 50 COD_{Cr} mg/l, accuracy is 1 mg/l
50 COD_{Cr} mg/l < result $\leq$ 100 COD_{Cr} mg/l, accuracy is 5 mg/l
100 COD_{Cr} mg/l < result $\leq$ 1000 COD_{Cr} mg/l, accuracy is 10 mg/l
result > 1000 COD_{Cr} mg/l , accuracy is 100 mg/l

Literature: Standard Methods for the Examination of Water and Wastewater

Determination of biochemical oxygen demand after *n* days (BOD_n) with OxiTop-system

Biochemical oxygen demand after *n* days (BOD*n*) means the mass concentration of dissolved oxygen consumed under specified conditions by aerobic biochemical oxidation of organic and/or inorganic matter in water. *n* is incubation time, normally 7 days.

Allylthiourea (ATU) is added to prevent the oxygen demand caused by nitrification.

***Procedure**:*

Measure samples and ATU into the OxiTop bottles. The amount of the sample and ATU depend on the expected BOD concentration. They are listed in OxiTop manual. Put one magnet in each bottle. Set the rubber "cap" on top of the bottle, and place six pieces of sodium hydroxide pellets into it. Cork with the pressure-measuring heads, and turn them airtight. Don't tighten too much. Place the bottles on the magnet stirrer, which is located in the incubator preserving stabile temperature. Make sure that the magnets rotate smoothly. Finally the measurement is started with the infrared OxiTop-controller.

Results:

After one week results will be read with the infrared OxiTop-controller and transfer with the special program to the computer.

Draw also a graph of the results.

Literature: OxiTop manual, SFS-EN 1899-1

Ammonium nitrogen from wastewater using ammoniagas electrode

Dissolved ammonium is converted to $NH_{3(aq)}$ by raising pH above 11 with a strong base. $NH_{3(aq)}$ diffuses through the membrane, change the internal solution pH that is sensed by a ion-selective electrode.

Reagents:

1. ISA-solution (strong NaOH+methanol), ***POISONOUS!***
2. Standards: 40 mg/l N and 4 mg/l N

Procedure:

Before measuring the sample, measure standards. Suitable standards for wastewaters are 40 and 4 mg/l N. Measure with volumetric glass 25 ml standard 40 mg/l N into the 50 ml's (narrow and high) beaker. Add 0.5 ml ISA-solution. Put the magnet inside the beaker and mix with magnet stirrer in very slow speed. Immerse NH_3-electrode in the beaker so that the membrane in the bottom of the electrode do not damage. Wait until reading is stabile (ready light appears). Rinse electrode and measure 4 mg/l N standard. The difference between the readings of the standards should be 55 – 60 mV.

Measure samples in the same way: measure 25 ml sample, add 0.5 ml ISA-solution, mix and take the readings. Rinse the electrode carefully between measurements.

Check the standards after samples have been measured. Finally rinse the electrode well and immerse it into the storage container.

Calculation:

The measured potentials are plotted on a semilogarithmic paper against the ammonium ion content of the standard solutions used. There is concentration mg/l N in the Y-axis and mV-readings in the X-axis. Read the nitrogen concentrations directly from the regression line. Results are reported to whole number in terms of mg/l N.

Literature: Instruction of NH_3-electrode

Determination of nitrite nitrogen (NO_2-N)

Because nitrite is unstable compound, determination should take place quickly after sampling at the latest in 5 hours

Reagents:

1. Sulphanilamide
2. NED (N-(1-naphtyl)-ethylene diamine dihydrochloride)

Procedure:

Pipette 25 ml sample or its' dilution into 50 ml erlenmeyer, add 0,5 ml sulphanilamide and mix well. After 4−6 minutes add 0,5 ml NED and mix. For zero sample pipette RO-water 25 ml and treat it like samples. Measure the absorbance of each solution using the spectrophotometer at wavelength of 545 nm (1 cm OG cell) after a period between 20 minutes and 2 hours.

Calculation:

Subtract the absorbance of zero sample from the absorbance of the samples. Use the calibration curve (i.e. regression line) to get the concentration of the nitrite nitrogen. Finally notice the dilution factor, if samples have been diluted.

Literature: SFS 3029

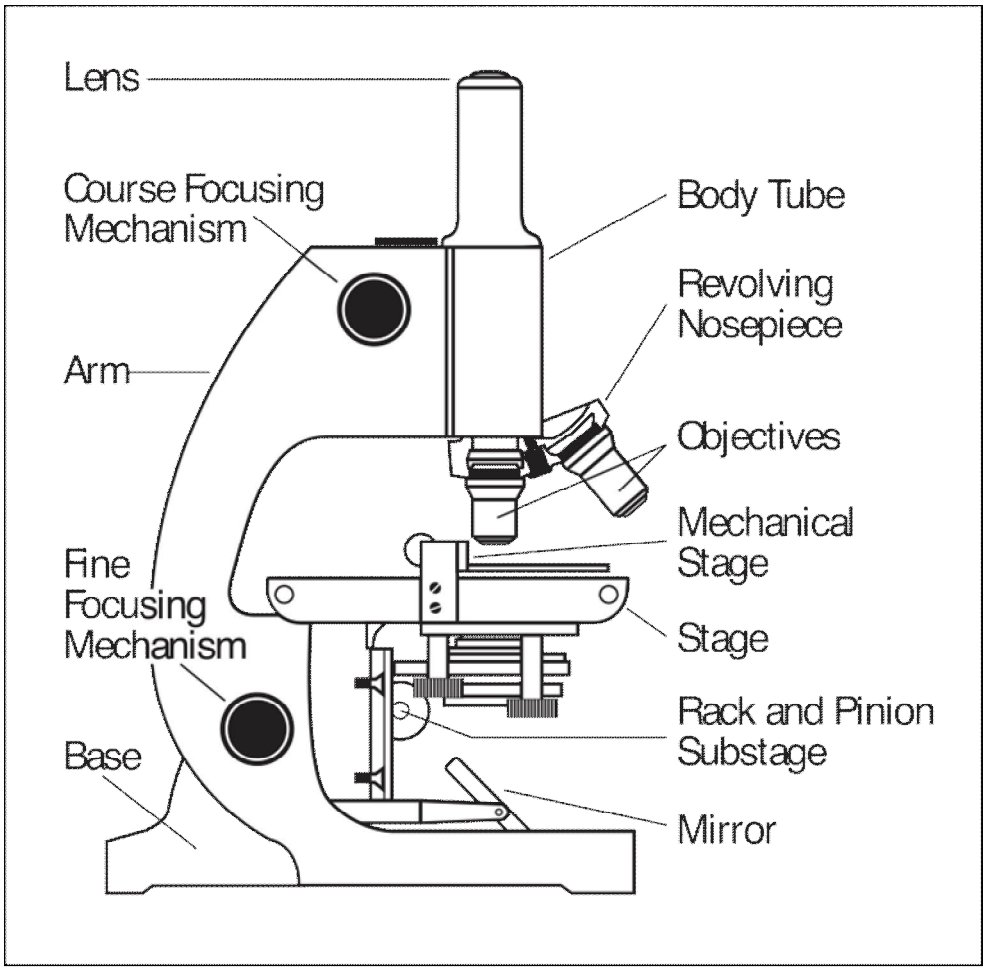
Light Microscopy

Microscopes are tools used to enlarge images of small objects so that they can be studied. The compound light microscope is an instrument containing **two lenses**, which magnifie, and a variety of **knobs to resolve (focus)** the picture. Because it uses more than one lens, it is sometimes called the compound microscope in addition to being referred to as being a light microscope.

Materials

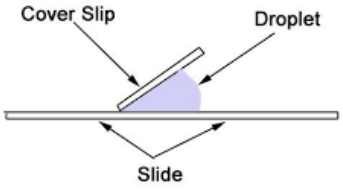
- Microscope
- Glass slides
- Coverslips
- Dropping pipettes
- Fresh activated sludge sample

Microscope parts



Procedure of slides preparation

1. Clean cover slip and slide.
2. Use pipette to grab a sample of activated sludge.
3. Place one drop of sample from the pipette to the middle of the glass slide.
4. Pick up cover slip by two corners.
5. Pull cover slip along glass slide towards drop of the sample
6. As soon as cover slip touches the drop allow cover slip to fall onto glass slide.
7. Pick up glass slide. Place on microscope stage.
8. Move stage up to within approximately 0.5 cm of objective on 10X.
9. Use the coarse then fine adjustments on the microscope to bring the sample into the field of focus.
10. Turn to 40X and repeat #9.
11. Identify organisms in the activated sludge.



Microscopic Examination

When performing a microscopic examination, fill out a worksheet that at a minimum records:
1. Date and time of sample
2. Sample type, location
4. Type of microorganism (free swimming/stalked Ciliates, ratifers etc.)
5. Approximate number within field view
6. size of floc particle (use 10 x to determine)

The WWTP operators typically should examine 3 slides per samples.

General care

1. Keep the body of the microscope clean. Wipe up spills immediately.

2. Do not leave the ocular tubes open for long periods of time or dust may get into the system. Always keep the microscope covered when not in use. Dust leads to dirty optics and a severe decrease in optical resolution. Dirt in the optical system is, without a doubt, the worst enemy of the microscopist.

3. Never use anything but lens tissue to clean any of the optical parts of the microscope. Do not confuse lens tissue with filter paper or wipes; there are profound differences among these types of paper.

4. ***Objective lenses should be used carefully****; they are the most important and most expensive part of the microscope. The following cautions should be routine:*

- *Always begin an observation using the 10X objective. Move from lower power to higher power objectives carefully in order to avoid ramming the objective into the slide, cover slip, or mechanical stage. Since the objectives are approximately parfocal, one should move to a higher power objective only when the specimen is in focus with the lower power objective. Constantly observe the objective front lens from the side while rotating the nosepiece.*
- ***Never use the coarse focus adjustment while observing the sample through the ≥40X***

Determining nitrification rate using activated sludge

Reagents:

- Nutrient solution: phosphate buffer (2 mM) + $(NH_4)_2SO_4$ (500 μM)
- Inhibitor solution: 1M ClO_3^-
- Sulphanileamide (SA)
- 1-naphtyl-ethylene diamine dihydrochloride (NED)

1) Measure 100 ml of nutrient solution in three erlenmeyers.
2) Add 1 ml of inhibitor solution to each erlenmeyer.
3) Add 20 ml of activated sludge to each erlenmeyer.
4) Mix and take 0 sample with syringe from the three erlenmeyers.
5) Place the erlenmeyers in a shaker for 1 hour and start mixing. Take time with stopwatch.
6) With a syringe, take a 6.0 ml sample from each erlenmeyer every 15 minutes (t_{15}, t_{30}, t_{45}, t_{60}), and filter the samples with a 0.2 μm filter to test tubes.

Determining the nitrite concentration of the samples:

1) Pipette 5.0 ml of filtered sample or its dilution to another test tube.
2) Add 125 μl of SA solution to the test tubes and mix.
3) After 4-6 minutes (take time with stopwatch), add 125 μl of NED solution to the test tubes and mix.
4) Place the test tubes in darkness.
5) Measure the absorbance of the samples with spectrophotometer at wavelength 545 nm after a period between 20 minutes and 2 hours. Use 1 cm OG cell.
6) Use standard curve to get concentrations. Remember the dilution factors.

Determining the dry matter in sludge:

1) Weigh the crucibles. Measure about 10 ml of mixed activated sludge to crucibles and weigh them. Make parallel samples.
2) Dry the samples in the oven at 105 °C for 15-20 hours. Let them cool down in desiccator and weigh them.
3) Place the crucibles in the oven (550 °C) for 2 hours. Let them cool down in desiccator and weigh them.

Calculation of the results is done in Homework 3.