



AALTO UNIVERSITY

Department of Built Environment

Water and Environmental Engineering

WAT-E2180

BIOLOGICAL TREATMENT PROCESSES OF WATER AND WASTE

Laboratory Work Instructions

Assignments 1 and 2 are carried out in large group (all the course's students). Each student should submit a short individual report from Assignments 1 and 2.

Assignments 3 and 4 are carried out in groups of 4-8 persons. Each group will be responsible for the laboratory reactors during one week. The group's data and notes should be collected in the lab notebook and submitted to MyCourses at the end of the week of pilot work. Groups will also analyze and present their results to the rest of the students in a session on 9th of April.

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1. Content for the report of Assignment 1 (Personal lab notebook)

2. Introduction

The purpose of this laboratory project is to strengthen the understanding of the microbiological phenomena and factors affecting the processes. The laboratory work also introduces the most relevant analytical methods and tools – chemical, physical and microbiological - used for monitoring biological processes.

Experimental work is carried out using real wastewater as well as synthetic wastewater simulating typical municipal wastewater. The focus of this work is on municipal wastewater but the same methods and processes are widely applied in different fields of environmental engineering.

The work is divided into four assignments. Assignments 1 & 2 will introduce the monitoring tools and work as training for the following reactor monitoring tasks. During assignment 3 the students will operate two different bioreactors - sequencing batch reactor and membrane bioreactor - during one week and should understand the operation of the two reactors. Each week the reactors will be operated in different conditions. Assignment 4 is about analyzing the observed results and explaining the factors affecting the process. The results will be also compared to other scientific studies on these factors.

3. Assignment 1: Analytical methods for monitoring processes and organic fractions of wastewater

2.1 General description

In this laboratory assignment, students will familiarize themselves with the common analytical methods used to monitor wastewater quality or wastewater treatment process. This assignment is divided into four tasks:

- 1) Basic process measurements
- 2) Analytical methods for nutrients (N&P)
- 3) Activated sludge monitoring
- 4) Organic matter in wastewater

The objective of this assignment is to learn how the basic analytical methods and on-line measurements are carried out. These methods will be used during assignment 3, where the pilot reactors are operated.

2.2 Reporting

To support the following laboratory tasks, all the students will independently prepare a simplified description of the methods used in this assignment.

2.3 Laboratory work

For this assignment students will be divided into four groups. Each group will carry out all four tasks one after another. Tasks 1 and 3 will be done using the laboratory reactors. A detailed description of the two laboratory reactors can be found from Chapter 4. For tasks 2 and 4 synthetic influent and effluent water of the laboratory reactors or Viikinmäki WWTP influent and effluent will be analyzed.

2.3.1 TASK 1 Basic on-line and process measurements:

Monitoring the pH in the biological reactor

For many biological processes optimal pH range is between 6.5 and 9. Optimal pH for nitrification reported in literature ranges between 7.5 and 9. Reaction rates slow down significantly when pH decreases under 6.8 and nitrifying bacteria stop working when pH drops under 5. pH also affects the equilibrium of different ions in water and it is important to understand which is the ion taking part in the reaction. For example in water solution, ammonium ions are in equilibrium with ammonia. If pH rises, the equilibrium moves towards ammonia, which leads to less efficient nitrification as bacteria oxidize ammonium ions instead of ammonia. In addition, ammonia is poisonous to nitrifying bacteria.

The pH in the reactors is monitored using SFS-EN ISO 10523, dated 2012.

Monitoring the temperature in the biological reactor

Temperature is a significant factor affecting nitrification rate and efficiency. The effect of temperature on the reaction rate of a biological process can be expressed with the following equation):

$$k_T = k_{20} \Theta^{(T-20)}$$

where

k_T = reaction-rate coefficient

k_{20} = reaction-rate coefficient at 20 °C

Θ = temperature-activity coefficient

T = temperature, °C

The temperature in the reactors is monitored with the thermometer.

Monitoring the dissolved oxygen concentration in the biological reactor

Dissolved oxygen (DO) is important in biological processes because only oxygen in dissolved form can be accessed by the microbes. Biological processes have different optimal DO concentrations. For example optimal nitrification requires sufficient soluble oxygen concentration in the biological process, usually ca 2-3 mg/l. Oxygen should also be evenly distributed to prevent the formation of anoxic zones in the aeration tank by ensuring efficient mixing and sufficient oxygen transfer to water.

The DO in the reactors is monitored with the oxygen probe.

Monitoring flow rates of water and sludge flows

It is important to monitor and control all the flow rates in the process. In biological processes we typically have to output flows – clean effluent water and sludge containing the microbial biomass together with solids in the influent. Furthermore, there might be several recycle flows, such as return activated sludge flow or internal nitrate recycle flows that need to be measured.

From our two reactors influent and effluent water flows as well as the waste activated sludge (WAS) flow will be measured. In our case the WAS will be removed manually once a day.

Monitoring ammonia concentration

Wastewater mostly contains nitrogen in the form of ammonium. Ammonium is oxidized to nitrites and nitrates during nitrification. Effluent ammonium concentration of the reactors is analyzed in order to assess the nitrification in the reactors.

The measurement is carried out using an ammonia gas electrode.

2.3.2 TASK 2 Analytical methods for nutrients

Nitrogen and phosphorus are nutrients that are needed for the growth of biomass and other biological organisms. However, the excessive amount of nitrogen or phosphorus causes harm in the receiving water, such as eutrophication and oxygen depletion.

Nitrogen in wastewater is mostly derived from urea and excreta and is found in nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+) and amino acids in proteins. In ammonification, bacteria oxidize organic nitrogen compounds releasing nitrogen to wastewater as ammonia, which is the most common form of nitrogen in influent

Phosphorus in wastewater is mostly derived from urea, excreta and different detergents. Phosphorus compounds can be divided into dissolved and solid fractions which are both further divided into reactive, chemically bound, organic and acid-hydrolysable fractions. In municipal wastewater 2/3 of the phosphorus is typically reactive ortho-phosphates and the rest is different polyphosphates. Organic phosphorus is typically less than 1 mg/l. Reactive phosphorus fractions can be analyzed with colorimetric methods.

Following nitrogen and phosphorus fractions will be analyzed from synthetic influent and effluent:

Compound	Samples	Method
Total nitrogen	Influent, effluent	SFS-EN ISO 11905-1; GanimedN analyzer
Total phosphorus	Influent, effluent	SFS 3026
Ortho-phosphate $\text{PO}_4\text{-P}$	Influent, effluent	SFS 3025
Nitrate nitrogen $\text{NO}_3\text{-N}$	Influent, effluent	Standard Methods for the examination of water and wastewater, 4500-NO3- B. Ultraviolet Spectrophotometric Screening Method

2.3.3 TASK 3 Activated sludge monitoring and influent and effluent solids

Sludge monitoring

Mixed liquor suspended solids (MLSS) is the concentration of suspended solids in the biological process based on suspended growth. MLSS is an important part of the activated sludge process to ensure that there is a sufficient quantity of active biomass available to consume the applied quantity of organic pollutant at any time. MLSS varies between 2 – 8 g/l in conventional activated sludge processes and 7 – 15 g/l in membrane bioreactors.

The portion of the MLSS that is actively contributing to the process is referred to as the Mixed Liquor Volatile Suspended Solids (MLVSS). The volatile solids concentration in a sample of mixed liquor will consist mostly of microorganisms and organic matter. As a result, the volatile solids concentration of mixed liquor is approximately equal to the amount of microorganisms in the water and can be used to determine whether there are enough microorganisms present to purify the water.

Sludge Volume Index (SVI) is used to describe the settling characteristics of sludge in the biological process. It has become the standard measure of the physical characteristics, such as settleability of activated sludge. It is defined as 'the volume (in ml) occupied by 1 gram of activated sludge after settling for 30 minutes' and it is calculated using equation:

$$\text{SVI (ml/g)} = \text{settled sludge volume (ml/l)} \times 1000 / \text{MLSS(mg/l)}$$

MLSS, MLVSS and SVI will be measured from both SBR and MBR reactors. MLSS will be measured using SFS-EN 872 and MLVSS using SFS 3008.

Suspended solids in reactor effluents

SS concentration in the reactor effluents will be measured using SFS-EN 872.

2.3.4 TASK 4 Organic matter in wastewater

Due to the time required for these analyses the BOD and COD analyses will be partly only demonstrated during Assignment 1.

BOD and COD analyses will be carried out using following methods:

Compound	Samples	Method
Biological oxygen demand BOD	Influent, effluent	OxiTop manual, SFS-EN 1899-1
Chemical oxygen demand COD	Influent, effluent	SFS 5504

4. Assignment 2: Microbiological methods

3.1 General description

Practical assignments are designed to give the student the basic understanding of microbiological lab techniques and brief introduction to bioinformatics. Students will acquire basic skills on microbial identification from conventional light microscopy and bacterial growth on agar plates to interpretation of genome sequences using open databases and drawing phylogenetic trees. In addition, they should have developed an understanding of microbial ecology and biotechnological functions for microorganisms.

3.2 Reporting

Students have to provide activated sludge investigation report for the process engineer of WWTP on the state of microbial composition. Both SBR and MBR sludge will be included.

From the Task 4 students have to submit identification results and detailed characterization of one unknown microorganism.

These tasks will be part of Homework 3 assignments and submitted to MyCourses with HW3.

3.3 Work description

The work is divided into four tasks. Tasks 1-3 will be carried out in the laboratory during the Tuesday laboratory session March 19th. For Task 2 the plating will be done on Tuesday March 12th. Task 4 is carried out with the whole group during Thursday lecture on March 14th in lecture room 286/287 of the Water building.

Different tasks are described in detail below.

3.3.1 TASK1 Microscopic examination of activated sludge

Microscopic examination is an important tool for evaluation the sludge characteristics and process conditions. E.g. floc size and structure can be evaluated. The amount, type and activity of protozoa in the sludge will also indicate what kind of process conditions are occurring. The microscopic examination will be done with three different activated sludge samples – laboratory SBR, laboratory MBR and Viikinmäki WWTP sludge. The observations comparing SBR and MBR sludge will be reported in HW3.

3.3.2 TASK 2 Bacteria cultivation (started on Tuesday March 12th)

In this task cultivation method is used to evaluate the amount of heterotrophic bacteria in the sludge. Living bacteria cells (and certain yeasts and fungi) will grow on agar-support and resulting colonies can be calculated by visual inspection. It is assumed that one cell forms one colony but in reality bacteria cells might be in water as pairs or short chains. Also, many bacteria can't be cultured in laboratory conditions. As a results, the number of colonies is always smaller than the actual amount in the sample. Heterotrophic plate count is mainly used for water quality monitoring. In this assignment sludge from

SBR and MBR reactors is used. Results of the CFU/ml calculation taking into account the dilutions will be submitted in HW3.

3.3.3 TASK 3 Nitrification rate measurement

Respirometry tests, substrate uptake rate tests and e.g. nitrification rate test can be used to study reaction kinetics of a given biomass. In this task, nitrification rate is measured and calculated for sludge taken from Viikinmäki WWTP.

Normally, nitrification proceeds in two steps, with ammonium being oxidized to nitrite by one group of autotrophic nitrifiers and nitrite to nitrate by another. Since nitrite is being oxidized as it is being produced, the rate at which ammonium is oxidized is equal to that at which nitrite plus nitrate accumulates. Addition of chlorate to the samples prevents nitrite from being oxidized to nitrate. When nitrite oxidation is completely and specifically blocked, the rate at which nitrite alone accumulates is equal the rate of ammonium oxidation. This gives an advantage, since methods for nitrite plus nitrate are less convenient and possibly less sensitive than for nitrite alone.

In the experiment nitrite concentration is measured during one hour in a mixed reactor where it can be assumed that ammonium (substrate of AOB bacteria), oxygen nor pH are limiting the process. The measured increase in nitrite concentration per unit of time or the slope of nitrite build-up i.e. the nitrite production rate (NPR) can be thus considered to be the maximum nitrification rate (gN/Lh) for AOBs in the sludge. To link the measurement to the maximum specific growth rate of AOBs $\mu_{A,max}$ the following equation can be used, where $X_{B,A}$ is the MLVSS (g/l) and Y_A is the yield of AOBs (g COD_{produced}/gN_{consumed}).

$$\Gamma_{v,max\ nit} = NPR = \frac{d[NO_2 - N]}{dt} = \frac{\mu_{A,max} \cdot X_{B,A}}{24 \cdot Y_A}$$

In the assignment the nitrification rate is calculated per unit of volume (gN/Lh) and per active biomass (gN/gMLVSSh). Calculations are submitted in HW3.

3.3.4 TASK 4 Introduction to bioinformatics and online databases for microbial community analyses.

Single sequence identification demo and exercise. Bacterial characterization principles. If possible, bring your computer to this exercise. One computer per 2-3 students is acceptable.

5. Assignment 3: Daily monitoring of the pilots

4.1 General description

This assignment will be carried out in four groups. Each group will operate and monitor two laboratory pilot reactors during one week. One reactor is a sequencing batch reactor (SBR) where all the steps of the activated sludge process take place in the same volume. The reactor is first filled, then left to react and settle and finally emptied. The other reactor is a membrane bioreactor (MBR) which is a suspended growth reactor where solid separation is carried out using membrane filtration. Reactors are operated with different sludge retention times. Most of the operation is automated. More detailed information about the reactors is presented in Chapter 4.3. Both reactors have a back-up reactor operated by the course assistant. The objective of this assignment is to monitor both reactors using methods from Assignments 1 and 2. The monitoring results will be used as a basis for the analysis of the process performance in Assignment 4.

5.2 Reporting

The results from this assignment will be reported in the lab notebook and in the shared excel file (MyCourses).

4.3 Laboratory work

4.3.1 Pilot reactors

SBR pilot



Sequencing Batch Reactors (SBRs) with operating volumes of 12 litres are operated in temperature-controlled chamber at $17\pm 1^\circ\text{C}$ ($10\pm 1^\circ\text{C}$ for Group 4). Feeding with synthetic wastewater during feeding sequence is done by peristaltic pumps with flow rate of 6 l/d. Fine bubble aerator is placed in the bottom of the reactors for constant aeration $2-3 \text{ l min}^{-1}$. The SBR reactor is operated with an SRT of 12 days.

The SBR is operated automatically two cycles per day. The cycles are different for different groups:

- Group 1&4: (720 min for a cycle) composed of Fill (10 min), React (590 min), Settle (75 min), Draw (8 min) and Idle (37 min) stages.
- Group 2: (720 min for a cycle) composed of Fill (10 min), AnoxReact/Mix (190 min), React (400 min), Settle (75 min), Draw (8 min) and Idle (37 min) stages

- Group 3: (720 min for a cycle) composed of Fill (10 min), AnoxReact/Mix (290 min), React (300 min), Settle (75 min), Draw (8 min) and Idle (37 min) stages

MBR pilot



An MBR with 15 l operational volume is operated. The MBR contains one flat-sheet submerged membrane with 0.11 m² of membrane surface made of chlorinated polyethylene with pore size 0.4 μm. Feeding with synthetic wastewater and permeate control were done by peristaltic pumps with flow rate of 15 l/d (operational flux is 0.14 m d⁻¹). Fine bubble aerators are placed in the bottom of the reactors for constant aeration (5.0 l min⁻¹) for keeping the membrane surface clean during filtering as well as providing AS with oxygen. The MBR reactor is operated with an SRT of 30 days.

Operational conditions for groups 1-4

The pilots will be operated according to the following table:

Group	SBR	MBR
1	DO: 3-4 mg/l → 0,5 mg/l react: only aerated (aerobic)	DO: 6 mg/l
2	Temperature 17C DO: 3-4 mg/l react: anoxic + aerobic	Temperature 17C DO: 6 mg/l
3	Temperature 17C DO: 3-4 mg/l react: anoxic + anaerobic + aerobic	Temperature 17C DO: 6 mg/l
4	Temperature 17C → 10 C DO: 3-4 mg/l react: only aerated(aerobic)	Temperature 20C → 10 C DO: 6 mg/l

4.3.2 Synthetic wastewater

The reactors are operated using synthetic wastewater which is prepared by the course assistant. Synthetic wastewater is based on average wastewater compositions of the Suomenoja and Viikinmäki WWTPs (Kuronen, 2005). NaHCO₃ was added in order to control the alkalinity in the reactors. Complete mixture of the synthetic wastewater shown in Table 1.

Table 1 Composition of synthetic wastewater

Substance	Conc. [mg/l]	*Nutrient solution compound	Conc. [mg/l]
CH ₃ COONa * 3 H ₂ O	130.8	FeCl ₃ * 6 H ₂ O	1.5
Yeast extract (Mereck 3753)	209.7	H ₃ BO ₃	0.15
Peptone (Biokar)	184.68	CuSO ₄ * 5 H ₂ O	0.03
NH ₄ Cl	38.2	KI	0.18
KH ₂ PO ₄	35.1	MnCl ₂ *4H ₂ O	0.12
CaCl ₂ * 2 H ₂ O	70	(NH ₄) ₆ Mo ₇ O ₂₄ * 4 H ₂ O	0.04
MgSO ₄ * 7 H ₂ O	60.9	ZnSO ₄ * 7 H ₂ O	0.12
NaHCO ₃ (SBR/MBR)	218.75/328.2	CoCl ₂ * 6 H ₂ O	0.15
Nutrient solution*	0.3 ml	EDTA	10

4.3.3 Weekly monitoring schedule

Monitoring assignment is carried out in pairs. Within the group, decide who is monitoring the process and when. Fill in a student and an approximate time to the monitoring plan in MyCourses. Monitoring should take place during working hours of the lab (8 am – 3 pm), and will likely take at least 2 hours for two persons to run simple analyses and check pilot operations (longer for additional tests).

Day	On-line measurements	Sampling and lab analysis	Calculated process values
Wednesday	pH, temperature, DO in the reactor, NH ₄ -N in the effluent, flow rates (influent, effluent, WAS)	PO ₄ -P (effluent) NO ₃ -N (effluent) BOD (effluent) COD (effluent)	SRT, loads, removal % BOD/N,P –ratio COD/N,P-ratio
Thursday	pH, temperature, DO in the reactor, NH ₄ -N in the effluent, flow rates (influent, effluent, WAS)	PO ₄ -P (effluent) NO ₃ -N (effluent) SS (effluent) MLSS, MLVSS (reactor) evaluation of sludge using a microscope	SRT, loads, removal %
Friday	pH, temperature, DO in the reactor, NH ₄ -N in the effluent, flow rates (influent, effluent, WAS)	PO ₄ -P (effluent) NO ₃ -N (effluent) Sample (effluent) for N _{tot} & P _{tot} SVI (reactor)	SRT, loads, nitrification removal %

		evaluation of sludge using a microscope	
Monday	pH, temperature, DO in the reactor, NH ₄ -N in the effluent, flow rates (influent, effluent, WAS)	PO ₄ -P (effluent) NO ₃ -N (effluent) BOD (effluent) COD (effluent)	SRT, loads, nitrification removal % BOD/N,P -ratio COD/N,P-ratio
Tuesday	pH, temperature, DO in the reactor, NH ₄ -N in the effluent, flow rates (influent, effluent, WAS)	PO ₄ -P (effluent) NO ₃ -N (effluent) SS (effluent) MLSS, MLVSS (reactor) evaluation of sludge using a microscope	SRT, loads, nitrification removal %

Familiarise yourself with the operation of the reactors and their equipment by testing and adjusting the process. Perform at least the following steps every day:

- Verify the influent and effluent flow rates by measuring the yield of the pump per impulse with e.g. a graduated cylinder.
- Verify the functioning of aerators in both reactors and the mixer in SBR (groups 3 and 4) by visual inspection.
- Calculate the amount of waste activated sludge that should be removed daily from the reactors (SBR and MBR). Calculate how much sludge has been removed for sludge analyses (Thu, Fri and Tue). Based on this calculate the amount of sludge that still remains to be removed and remove the amount.

6. Assignment 4: Analyzing the performance of the pilot processes in different process conditions

Each group will analyze the performance of the reactors during their week. Some scientific papers will be given and they should be used to deepen the analysis of the processes. Special attention should be paid to the differences in conditions between the SBR and MBR reactor. Were the two reactors operated similarly during the week? What were the differences and how did they affect the results?

The groups will operate the reactors as follows:

1. DO changed in SBR (0,5 mg/l and 3-4 mg/l)
2. Anoxic conditions in SBR → denitrification
3. Anaerobic conditions in SBR → biological P removal
4. Temperature effect (17 → 10C)

7. Reporting the monitoring work

The monitoring work and the analysis of the results will be reported as presentation. The presentation will have three parts:

- Observations of the lab reactors, analysis of the performance (about 10 min)
- Presentation of another experimental study (based on the scientific paper studied) (about 10 min)
- Planned experimental study with the lab reactors and expected results (about 10 min)

For this assignment you can divide the group into three sub-groups having different roles:

- presenters
- competitors
- planners.

The expected length of the presentation is 30 minutes (10 min for each part) and 20 – 25 slides. More detailed instructions of the content will be given later during the course.

The presentation should be submitted to MyCourses after the final seminar.

Appendices

1 Content of the personal lab notebook (Example)

- 1) Basic process measurements
pH – for each measurement steps shortly explained with some special hints (e.g. locations, deviations from instructions etc)
Temperature
Dissolved oxygen
Flow rates
Ammonia

- 2) Sampling
Influent
Effluent
Sludge (SBR&MBR)

- 3) Analytical methods for influent and effluent
BOD
COD
Nutrients N&P
SS

- 4) Activated sludge monitoring
MLSS
MLVSS
SVI