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 the laboratory in small groups of 4-6 persons and as self-learning tasks. Each student will answer a quiz in MyCourses on Assignments 1 and 2. The deadline for the quiz is one week after the laboratory assignment. Microbiology exercises form also a part of the Homework 1 and this work will be reported together with the homework exercises.

Assignments 3 and 4 are carried out in the same groups of 4-6 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 13th of April.

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1. Instructions for laboratory analyses

1. Introduction

The purpose of this laboratory project is to strengthen the understanding of the microbiological phenomena and factors affecting the biological 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.

2. Day 1: Analytical methods for monitoring wastewater processes

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 & pilot reactors
2) Analytical methods for nutrients (N&P) (self-learning during Spring 2021)
3) Organic matter analyses (BOD & COD) (self-learning during Spring 2021)
4) Determination of heterotrophic bacteria (to be continued on Thursday)

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 answer a quiz in MyCourses.

2.3 Day 1 - Laboratory work

For this assignment students will be divided into four groups. Each group will carry out the tasks according to the separate schedule. Task 1 will be done using the laboratory pilot reactors. A detailed description of

the two laboratory reactors can be found from Chapter 4. For task 4 synthetic influent and effluent water of the laboratory reactors will be analyzed and also the activated sludge.

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 two 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 reactors influent (MBR) and effluent water flows (MBR, SBR) as well as the waste activated sludge (WAS) flow will be measured.

In our case the WAS will be removed manually once a day. Using sludge retention time of 12 d in sequencing batch reactor (reactor volume 12 l) and sludge retention time of 30 d in membrane bioreactor (reactor volume 15 l), required daily WAS flows would be 730 ml/d (SBR) and 500 ml/d (MBR). NOTE! The amount of sludge removed for analyses has to be taken into account while removing waste activated sludge due to small reactor volumes

Sampling

In order to collect information about the process sampling is needed. From biological processes typically influent and effluent waste is sampled and different sludge samples are taken. In our reactors MLSS, MLVSS and SVI will be analyzed from an activated sludge sample. Sampling can be done as grab samples or as composite sample. In our case, influent and sludge samples will be grab samples and effluent sample will be a composite sample (effluent water collected during about 24 hours).

2.3.2 TASK 2 Analytical methods for nutrients (self-learning during Spring 2021)

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. Ammonium is oxidized to nitrites and nitrates during nitrification. Effluent ammonium, nitrite and nitrate concentrations of the reactors is analyzed in order to assess the nitrification and denitrification in the reactors.

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	Total Organic Carbon Analyzer TOC-L CSH/CSN^{TM} User's Manual, page 286
Total phosphorus	Influent, effluent	SFS 3026
Ortho-phosphate PO_4-P	Influent, effluent	SFS 3025

Nitrate nitrogen NO_3-N	Influent, effluent	Standard Methods for the examination of water and wastewater, 4500-NO3- B. Ultraviolet Spectrophotometric Screening Method
Ammonium nitrogen NH_4-N	Influent, effluent	The measurement is carried out using an ammonia gas electrode.

2.3.3 TASK 3 Organic matter analyses BOD and COD (self-learning during Spring 2021)

Biochemical oxygen demand (BOD) is the amount of dissolved oxygen consumed by aerobic biological organisms to break down organic material present in a given water sample at certain temperature over a specific time period. The BOD value is most commonly expressed in milligrams of oxygen consumed per litre of sample during 5 or 7 days of incubation at 20°C and is often used as a surrogate of the degree of organic pollution of water.

Chemical oxygen demand (COD) is the amount of oxygen consumed to oxidize all of the organic carbon completely to CO_2 and H_2O. COD can be done using either potassium dichromate or potassium permanganate as the chemical oxidant. Historically permanganate has been used, but nowadays dichromate is usually used for wastewater.

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

3.3.2 TASK 4 Bacteria cultivation (to be continued on Thursday March 11th)

In this task cultivation method is used to evaluate the amount of heterotrophic bacteria in the SBR and MBR effluent and 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 result, 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 and effluent from SBR and MBR reactors is used. Results of the CFU/ml calculation taking into account the dilutions will be submitted in HW1.

3. Day 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.

This assignment is divided into four tasks:

1) Suspended solids and volatile suspended solids (self-learning during Spring 2021)
2) Sludge volume index (self-learning during Spring 2021)
3) Microscopic examination of sludge
4) Determination of heterotrophic bacteria (counting of the CFUs)

3.2 Reporting

Microscoping and determination of heterotrophic bacteria will be part of Homework 1 assignments and submitted to MyCourses with HW1. These tasks are also included in the quiz.

3.3 Day 2 – Work description

The work is divided into four tasks. Tasks 1-3 will be carried out in the laboratory during the Thursday laboratory session March 5th. For Task 4 the plating will be done on Tuesday March 3rd.

Different tasks are described in detail below.

3.3.1 TASK1 SUSPENDED SOLIDS AND VOLATILE SUSPENDED SOLIDS (self-learning during Spring 2021)

Mixed liquor suspended solids and mixed liquor volatile suspended solids concentration

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.

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.

3.3.2 TASK2 SLUDGE VOLUME INDEX (self-learning during Spring 2021)

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:

SVI (ml/g) = settled sludge volume (ml/l) x 1000/MLSS(mg/l)

SVI will be measured from both SBR and MBR reactors. Mixed liquor suspended solids (MLSS) concentration will be given.

3.3.1 TASK 3 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 HW1.

3.3.2 TASK 4 Bacteria cultivation (started on Tuesday March 9th)

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 result, 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 and effluent from SBR and MBR reactors is used. Results of the CFU/ml calculation taking into account the dilutions will be submitted in HW1.

4. 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.

4.2 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 14±1°C (8±1°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 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 m^2 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	Temperature 14°C DO: 3-4 mg/l à 1,5 mg/l react: only aerated	Temperature 14°C DO: 6 mg/l
2	Temperature 14°C DO: 3-4 mg/l (aerobic) react: mixed and aerated	Temperature 14°C DO: 6 mg/l
3	Temperature 14°C DO: 3-4 mg/l	Temperature 14°C DO: 6 mg/l

	react: mixed (longer) and aerated	
4	Temperature 8°C DO: 3-4 mg/l react: only aerated(aerobic)	Temperature 8°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). $NaHCO_3$ 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]
$CH_3COONa * 3 H_2O$	130.8	$FeCl_3 * 6 H_2O$	1.5
Yeast extract (Mereck 3753)	209.7	H_3BO_3	0.15
Peptone (Biokar)	184.68	$CuSO_4 * 5 H_2O$	0.03
NH_4Cl	38.2	KI	0.18
KH2PO4	35.1	$MnCl_2*4H_2O$	0.12
$CaCl2 * 2 H_2O$	70	$(NH_4)6Mo_7O_{24} * 4 H_2O$	0.04
$MgSO_4 * 7 H_2O$	60.9	$ZnSO_4 * 7 H_2O$	0.12
$NaHCO_3$ (SBR/MBR)	218.75/328.2	$CoCl_2 * 6 H_2O$	0.15
Nutrient solution*	0.3 ml	EDTA	10

The synthetic wastewater will have quality characteristics approximately as shown in Table 2,

Table 2: Estimated quality characteristics of the influent wastewater.

Influent	mg/l
BOD_7	340
COD_{Cr})	505
N_{tot}	55
NH_4-N	21
P_{tot}	11

4.3.3 Weekly monitoring schedule

Monitoring assignment is preferably carried out in pairs. For nitrification test (Monday) the whole group will attend. 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). For nitrification test about 3 hours for the whole group should be anticipated. Please note that some of the analytical methods require steps that are carried out the next day or days, e.g. suspended solids and volatile suspended solids measurements and BOD.

Day	Basic daily measurements	Sampling and lab analysis	Calculated process values
Tuesday (whole group)	pH, temperature, DO in the reactor, NH_4-N in the effluent, flow rates (influent, effluent, WAS)	BOD (influent,effluent, only SBR) COD (influent,effluent)	SRT, loads, removal %
Wednesday	pH, temperature, DO in the reactor, NH_4-N in the influent and effluent, flow rates (influent, effluent, WAS)	PO_4-P (influent&effluent) NO_3-N (influent&effluent) SS (influent&effluent) Sample (effluent) for Ntot &Ptot	SRT, loads, removal rates %
Thursday	pH, temperature, DO in the reactor, NH_4-N in the effluent, flow rates (influent, effluent, WAS)	MLSS, MLVSS (reactor), settleability evaluation of sludge using a microscope	SRT, loads ,
Friday	pH, temperature, DO in the reactor, NH_4-N in the effluent, flow rates (influent, effluent, WAS)	PO_4-P (influent&effluent) NO_3-N (influent&effluent) SS (influent&effluent)	SRT, loads including sludge loading rate and volumetric loading rate, removal %, SVI, VSS/SS ratio
Monday (whole group)	pH, temperature, DO in the reactor, NH_4-N in the influent and effluent, flow rates (influent, effluent, WAS)	Nitrification rate test (see Ch. 4.3.4)	SRT, loads, removal % nitrification rate BOD/N,P –ratio COD/N,P-ratio

Familiarize 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 (Fri and

Mon). Based on this calculate the amount of sludge that still remains to be removed and remove the amount.

4.3.4 Nitrification rate measurement

Nitrification rate

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 both pilot reactors. It is used to analyze the effect of changes in process conditions to reaction kinetics.

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 ammonium oxidizing bacteria AOB), 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 $\mu_{A,max}$ (1/h) the following equation can be used, where $X_{B,A}$ is the MLVSS (g/l) and Y_A is the yield of AOBs (g of biomass produced or g $COD_{produced}/gN_{consumed}$).

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

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

5. 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 (14 → 8C)

6. 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.