CIE4485 Laboratory Experiment:

N Removal

Course:	CIE4485 Wastewater Treatment
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Lab

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1 Introduction

In the nitrification process Kjeldahl-nitrogen (NKj, the sum of organic fixed nitrogen and ammonium), is transformed to nitrate. The organic nitrogen is first transformed to ammonium (NH_4^+). The oxidation of ammonium goes in two steps and is accomplished by two different types of autotrophic bacteria.

Nitrosomonas
$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2\ H^+ + H20$
Nitrobacter
$NO_2^- + 0.5\ O_2 \rightarrow NO_3^-$

Summed reaction
$NH_4^+ + 2\ O_2 \rightarrow NO_3^- + 2H^+ + H_2O$

Nitrification only happens if the relevant organisms are present in the sludge. The sludge age must at least be equal to the reciprocal value of the growth rate of the organisms. At moderate temperatures (<20°C), the growth rate of Nitrosomonas is the lowest and hence determines the minimal sludge age to be maintained for nitrification. Because the growth rate of the bacteria decreases with lower temperatures, higher sludge ages are necessary during wintertime for nitrification.

If the oxygen concentration in an activated sludge mixture becomes very low most heterotrophic organisms can change from oxygen to nitrate as electron acceptor for their energy demand. In this process nitrate is converted to elementary nitrogen gas in some intermediate steps. The nitrogen gas escapes to the atmosphere. This process is called denitrification. As long as nitrate is present in an anaerobic environment it is called anoxic. Almost all kinds of activated sludge will denitrify with sufficient low oxygen concentration. The rate of denitrification also depends on the presence of organic material (carbon source) necessary for energy supply (electron donor) and cell synthesis.

The kinetics of these processes are described by the Monod equations:

Nitrification: $$r_N = -\frac{dC_A}{dt} = \mu_N \frac{f_N X}{Y_N} \frac{C_A}{K_A + C_A} \frac{C_O}{K_{ON} + C_O} \quad [1]$$

Denitrification: $$r_D = -\frac{dC_N}{dt} = \mu_D \frac{f_D X}{Y_D} \frac{C_N}{K_N + C_N} \frac{C_C}{K_C + C_C} \frac{K_{OD}}{K_{OD} + C_O} \quad [2]$$

Symbols are explained in table 1

Tabel 1 List of symbols

Symbol	Description	Unit
r_N	Nitrification rate	kg N/(m^3.d)
r_D	denitrification rate	kg N/(m^3.d)
X	suspended solids concentration	kg SS/m^3
f_N	fraction nitrifiers in sludge	-
f_D	fraction denitrifiers in sludge	-
μ_N	maximum growth rate nitrifiers $0.47 \cdot (1.103)^{(T-15)}$	d^{-1}
μ_D	maximum growth rate denitrifiers $4 \cdot (1.103)^{(T-15)}$	d^{-1}
Y_N	yield factor nitrifiers	0.15 kg SS/kg oxidized N
Y_D	yield factor denitrifiers	5.8 kg SS/kg reduced NO_3^--N
C_A	NH_4^+-N concentration	mg N/L
C_N	NO_3^--N concentration	mg N/L
C_O	oxygen concentration	mg O_2/L
C_C	concentration carbon source	mg/L
K_{ON}	half-velocity degradation constant during nitrification	1.0 mg O_2/L
K_{OD}	half-velocity degradation constant[1] during denitrification	0.1 mg O_2/L
K_A	half-velocity degradation constant[1] of NH_4^+-N	1.0 mg NH_4^+-N /L
K_N	half-velocity degradation constant[1] of NO_3^--N	0.2 mg NO_3^--N /L
K_C	half-velocity degradation constant[1] of carbon source	10 mg COD/L
[1] The half-velocity degradation constant is the concentration at one-half of the maximum specific substrate utilization rate without any limitation		

If the concentration of all reagents is sufficiently high there is no limitation of the transformation rates and all terms with the half-velocity concentration in the denominator become 1. The denitrification process contains an inhibition factor for oxygen ($K_{OD}/(K_{OD}+C_o)$); that process stops with high oxygen concentrations, because the inhibition term for oxygen becomes zero.
Under stationary conditions the kinetics can be simplified to a zero-order process where the reaction rates are independent of the concentrations of the reagents involved. The reaction equations simplify to the general form:

$$r = -\frac{dC}{dt} = k_0 \cdot X \qquad [3]$$

All constants are brought together in a new constant k_0, the zero-order rate constant, expressed in kg substrate per kg SS per unit of time. This value k_0 will be determined in the experiment for the temperature applied in the test. Equation [1] shows that the actual oxygen concentration is influencing the k_0-value.

With the help of the reaction rate and measured concentrations the average fraction nitrifiers during the experiment can be calculated with [1]. Simply recalculating k_0 to other temperatures and oxygen concentrations is not allowable because the fraction nitrifiers will change as well. Moreover the growth rate of the all organisms will change, making things really complicated.

The fraction of denitrifying organisms in presence of the extra carbon source is calculated with equation [2] when we assume that $C_C >> K_C$. With this known value it is possible to calculate the denitrification rate for the condition with a low concentration of carbon source. Such a condition exists for example during simultaneous nitrogen removal in an aeration tank of a low-loaded activated sludge plant with an assumed degradable COD (BOD) of about 5 mg/l. Difficult or non-degradable compounds, causing the higher COD-concentration in effluents of such treatment plants, cannot serve as carbon source.

The objective of the denitrification experiment is an acquaintance with the nitrification and denitrification processes. The kinetic constants of nitrifying and denitrifying sludge from a full-scale treatment plant will be determined. The determined kinetic constants can be used for the design of a treatment plant. For an optimum design the relations between the kinetic constants and the temperature and pH should be investigated too. However, due to the duration of this experiment these relations will not be studied during this practical.

2 Experiment

The experimental set-up consists of two buckets, one with an aeration element, the other with a stirrer. In the first bucket 10 liter of activated sludge is aerated; the conditions are suitable for nitrification. In the other bucket, 10 liter of activated sludge is stirred under anaerobic conditions, suitable for denitrification. Through the analysis of samples, which are sampled with regular time intervals, the changes in concentration of ammonium-N and nitrate-N can be measured.

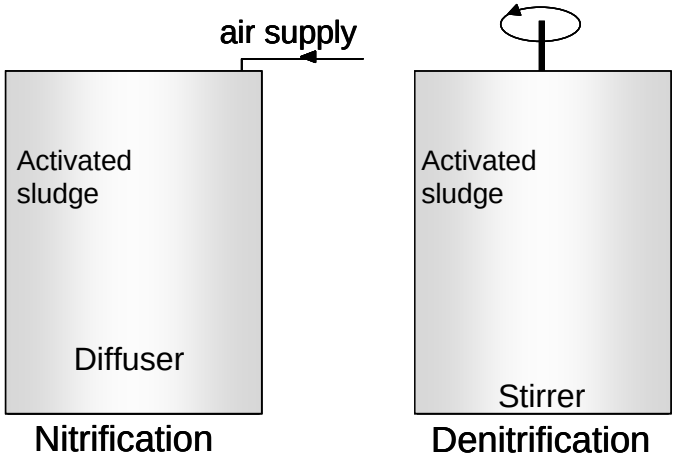


Figure 1 Experimental set-up nitrogen removal

3. Procedure

The procedures of sections 3.2 and 3.3 are carried out simultaneously. The sludge is taken from the storage vessel in which the sludge is maintained. Don't forget to return the sludge to this storage after the experiment.

3.1 General

- The oxygen concentration in both buckets has to be measured with the oxygen electrode every 30 minutes during the experiment.
- The measured suspended solid (SS) concentrations are considered as average values for both series. The sludge growth is too low to measure an increase of the SS-concentration.
- Begin and end temperatures are averaged for both series.
- Samples are filtered directly after sampling. *Why?*
  Filter paper can be used without drying.
- Mark the samples to make clear to which part of the experiment every sample belongs.
- Write down the time of sampling and the corresponding number of each sample.
- Clean up after finishing the experiment according to the given procedure.

3.2 Nitrification

- Add 10 L of activated sludge to the aeration bucket and start aerating. The oxygen concentration must be at least 2 mg/L, adjust the aeration if this value is not reached.
- Add 25 mL ammonium-chloride solution (60 g NH_4Cl/L).
  How much is the NH_4^+-N concentration increased by performing this step? (Molair weight N=14 g/mol, H = 1 g/mol; Cl=35.5 g/mol). Calculate this while you are waiting to sample.
- Measure the temperature and take a sample for SS-analysis[1].
- Measure the pH and bring it to a value between 7.0 and 7.5 by adding 1 M *HCl* or 1 M *NaOH*; write down the new pH after correction. The measuring period starts at that moment.
- Take the next 9 samples of activated sludge (50 mL) with intervals of 15 minutes.
- After sampling filter the samples immediately.
- Measure the ammonium concentration of the filtrate (§ 6.2).
- After the last sample measure again the temperature and pH.

3.3 Denitrification

- Fill the stirred bucket with 10 L activated sludge and switch on the stirrer.
- Add 10 mL sodium nitrate solution (100 g $NaNO_3$/L),
  How much is the NO_3^--N concentration increased by performing this step? (Molair weight N=14 g/mol, O = 16 g/mol; Na=23 g/mol). Calculate this while you are waiting to sample.
- Add 10 grams sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$) as carbon source.
  How much is the COD concentration increased by performing this step? (COD value acetate: 1.067 gCOD/gAc$^-$; Molair weight: Acetate ($C_2H_3O_2^-$) =59 g/mol, Na = 23 g/mol). Calculate this while you are waiting to sample.
- Measure the temperature and take a sample for SS-analysis[1].
- Measure the pH and bring it to a value between 7.0 and 7.5 by adding 1 M *HCl* or 1 M *NaOH*; write down the new pH after correction.
- Measure the oxygen concentration and wait until it is decreased to about 0.1 mg O_2/L; the sludge is anoxic. The measuring period starts at that moment.
- Take the next 8 samples of the activated sludge (50 mL) with intervals of 15 minutes.
- After sampling filter them immediately.
- Measure the concentration of nitrate-N in the filtrate (§ 6.3).
- After the last sample measure again the temperature and pH.

[1] During sampling, let one group member perform the SS analysis, since this sample needs to dry for 2 hours!

4 Analyses

4.1 Suspended solids concentration

1. Weigh the beaker together with the pre-dried filter in it (accuracy: 4 decimals): M_{dry}.
2. Take an activated sludge sample of about 100 ml and place it on the stirring device.
3. Rinse the filtration device with demineralized water.
4. Place the filter in the filtration device and wet it with some demineralized water.
5. Create a vacuum underneath the filter.
6. Bring 50 mL (V) of the activated sludge sample on the filter, using a sludge pipette.
7. Rinse the filtration device with demineralized water and filter the sample.
8. Return to atmospheric pressure.
9. Fold the filter and sweep residual sludge adhered to the filtration device.
10. Put the filter back in the corresponding beaker. Dry the beaker in an oven at 105 °C for 2 hours.
11. Place the beaker in a desiccator to cool down for 15 minutes.
12. Determine the weight of the beaker + filter (in 4 decimals): M_{total}.
13. The suspended solids concentration G_a is: $G_a = \frac{M_{total} - M_{dry}}{V}$ g/l

4.2 Ammonium-nitrogen concentration

This is a standardized procedure with of the Merck Company, based on the measurement of colour intensity (photo spectrometry).

The analysis is according to the Merck instructions. Put the tubes the right way into the spectrophotometer. Look after the indicated concentration, expressed in mg N/L, and the dilution factor.

4.3 Nitrate-N concentration

This is a standardized procedure with of the Merck Company, based on photo spectrometry.
Dilution of the sample is required because the expected concentrations exceed the measuring range.
The dilution factor is 4:

1. Bring with a pipette 25 mL filtrate in a volumetric flask of 100 mL.
2. Fill with demineralized water to the grade mark.
3. Put the stopper on the flask and mix gently by rotating the flask upside-down.
4. Encode the flasks.

The analysis is according to the Merck instruction. Put the tubes the right way into the spectrophotometer. Look after the indicated concentration, expressed in mg N/L, and the dilution factor.

5. Shut-down procedure

After the experiment is finished, you need to clean the set-up and leave everything nnice and tidy (at the set-up upstairs as well as in the laboratory at the ground level). To finish, the following steps need to be made:

- close the air valve (if applicable) ;
- switch off the stirrer (if applicable);
- empty the buckets with sludge in the sludge storage vessel ;
- clean the buckets ;
- clean the air diffuser (if applicable) ;
- clean the stirrer blade (if applicable) .

Rinse the pH electrode with demi water and fill the rubber cap with fresh 3 M KCl solution. Put back the electrode in the cap and close the slide.

Rinse the DO electrode with demi water and put it back in the white calibration vessel. Tighten the black ring so the vessels sticks to the electrode.

Clean all sample vessels and glassware that has been used (in the laboratory at the ground level as well as at the set-up) as well and put it in the cabinet under the lab table.

6. Elaboration

The results are strongly dependent of the sludge quality. Although much effort has been put in maintaining a good sludge quality, success is not guaranteed.

- Plot the measured concentration of the nitrogen component as a function of time for both buckets. Give your comments on the position of the points on these lines.

- Calculate with a linear regression procedure the slopes of the lines (if necessary by omitting outliers), which are equal to the growth rate constants for nitrification and denitrification respectively, expressed in kg N per kg SS per day. Give your comments on the calculated rate constants.

- Calculate the fraction of nitrifying and denitrifying organisms in the sludge mixtures, averaged over the whole experiment.

With the use of the rate constants from the experiment a full-scale aeration/denitrification tank for a treatment plant with advanced biological nitrogen removal has to be designed. Keep in mind that limitation of the denitrification process might happen with low concentrations of the carbon source, which on its turn is dependent of the design.

(If no adequate growth constants are found experimentally use the following values that are applicable for 20 ºC: Nitrification 0.09 kg NH_4^+-N per kg SS per day (without limitation!!) and denitrification 0.15 kg NO_3--N per kg SS per day (without limitation!!)).

1. Find the total tank volume and the percentage necessary for denitrification with the use of mass balances.

2. Make a control calculation on the eventual limitation of the carbon source, using the calculated reaction rates and bacteria fractions from the experiment.

Design data:

- Influent flow: 10,000 m^3/day
- Influent BOD: 250 mg/L
- Influent NKj-concentration: 50 mg/L
- Required effluent NH_4^+-N: 5 mg/L
- Required effluent NO_3^- -N: 5 mg/L

7. Further reading

Metcalf & Eddy Inc., 4th Edition 2003: Wastewater Engineering – Treatment and Reuse Chapters 7-10 and 8-5

Appendix I - Ammonium-N Concentration

The ammonium concentration is determined with a standardized reagent test from Merck Company (# 00683). It is based on the measurement of colour intensity i.e. photo spectrometry.

The analysis is according to instructions below. It is a user defined method (adjusted to this experiment), based on the Merck test kits. Put the tubes the right way into the spectrophotometer. Look after the indicated concentration, expressed in mg N/L, and the dilution factor.

Ammonium Analyses

Chemicals: Spectroquant Reagent Test# 00683
Spectrophotometer: Merck Nova 60

Procedure: User defined method #383
Range: 2.0-50.0 mg/l NH_4^+-N

Remark:

- For addition of NH4-1 (concentrated Sodium Hydroxide) use a dosing pump on the bottle.

Appendix II - Nitrate-N Concentration

The nitrate concentration is determined with a standardized reagent test from Merck Company (# 14773). It is based on the measurement of colour intensity i.e. photo spectrometry.

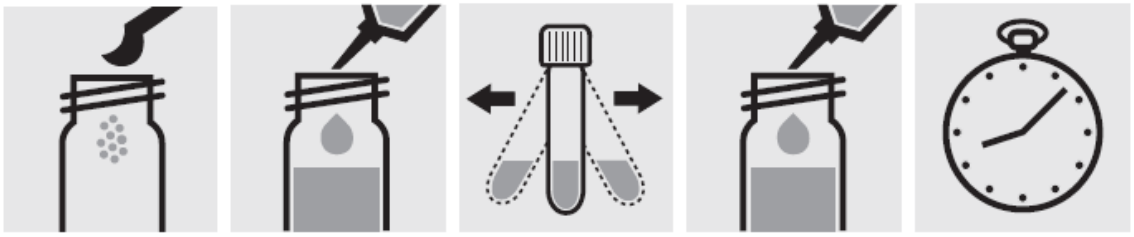
Dilution of the sample is required because the expected concentrations exceed the measuring range. The dilution factor is 4:
1. Bring with a pipette 25 mL filtrate in a volumetric flask of 100 mL.
2. Fill with demineralized water to the grade mark.
3. Put the stopper on the flask and mix gently by rotating the flask upside-down.
4. Encode the flasks.

The analysis is according to instructions below. It is a user defined method (adjusted to this experiment), based on the Merck test kits. Put the tubes the right way into the spectrophotometer. Look after the indicated concentration, expressed in mg N/L, and the dilution factor.

Nitrate Analyses

Chemicals: Spectroquant Reagent Test# 14773
Spectrophotometer: Merck Nova 60

Procedure: User defined method #373
Range: 1.0-20.0 mg/l NO_3^--N



Place 1 blue microspoon of **NO_3-1A** into an empty round cell (Empty cells, Cat.No. 14724).

Add 5.0 ml of **NO_3-2A** using a pipette into the cell.

Shake **vigorously for 1 minute** to dissolve the solid substance.

Add 1.5 ml of the sample using a pipette and mix.
Caution, cell becomes very hot!

Reaction time: 10 minutes

Remark:

- For addition of NO3-2A (concentrated Sulphuric acid) use a dosing pump on the bottle.

Appendix IV - Suspended Solids Concentration

- Weigh the beaker together with the pre-dried filter in it (accuracy: 4 decimals): M_{dry}.
- Take an activated sludge sample of about 100 mL and place it on the stirring device.
- Rinse the filtration device with demineralized water.
- Place the filter in the filtration device and wet it with some demineralized water.
- Create a vacuum underneath the filter.
- Bring 50 mL (= V) of the activated sludge sample on the filter, using a sludge pipette.
- Rinse the filtration device with demineralized water and filter the sample.
- Return to atmospheric pressure.
- Fold the filter and sweep residual sludge adhered to the filtration device.
- Put the filter back in the corresponding beaker. Dry the beaker in an oven at 105 °C for 2 hours.
- Place the beaker in an exsiccator to cool down for 15 minutes.
- Determine the weight of the beaker + filter (in 4 decimals): M_{total}.

The suspended solids concentration G_a is: $G_a = \frac{M_{total} - M_{dry}}{V}$ g/L.