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DEEPCLEANTECH-PROJECT: OPTIMIZATION AND MANAGEMENT OF CHEMICAL AND MICROBIOLOGICAL PROCESSES (2018-2020) – RESEARCH REPORT

THE PROJECT TEAM: **Maarit Janhunen, Eero Antikainen, Tero Reijonen, Olli Torvinen, Tero Kuhmonen** and **Jari Sonninen**

RESEARCH REPORT

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PREFACE

This report has been written during the spring 2020 and contains the results from DeepCleanTech project taken place between year 2018 and 2020. The focus of the project's research was on optimizing microbiological processes to remove nitrogen and sulfate from mining water. The technological approach and operation of the processes is based on the long-term experience of the Savonia Water Laboratory. The report includes 3 separate subreports, covering entity A on the microbiological nitrogen removal process, entity B on the sulfate reduction process, and entity C on calcium precipitation experiments. In addition, the published Theses are linked to entities B and C. The experimental operation of the sulfate reduction process has been based entirely on collaboration with Sulfator Oy (entity B). The digital models implemented by VTT are the basis for calcium precipitation experiments (entity C).

The authors acknowledge Business Finland – the Finnish Funding Agency for Innovation, Savonia University of applied sciences, Finnish Minerals Group and Sulfator Oy for funding this DeepCleanTech project. In addition, authors would like to acknowledge the support within the steering group from Valmet, Outotec and Langis.

Kuopio 14.9.2020

Authors

1. INTRODUCTION

Environmental permits are tightening for the mining industry and, at worst, new investment projects will be left without funding. Improving the efficiency of wastewater treatment requires further research from both a nitrogen and a sulfur removal perspective and a metal removal perspective. In the DeepCleanTech -project, the work package of the Savonia subproject "*Experimental validation of selected cases*" investigated the validation of selected process solutions with laboratory-scale experimental arrangements. The purpose of the experimental arrangements was to ensure the functionality of both chemical precipitation processes and microbiological reduction processes in batch and continuous processes. The main focus of the pilot work is on the development and verification of the following processes:

- Optimization of the microbiological nitrification-denitrification process (ND -process): for the nitrogen removal process, the aim is to optimize both the nitrification and denitrification phase, the process parameters affecting the microbiological process so as to maximize the conversion of nitrogen compounds to nitrogen gas.
- Optimization of the microbiological sulfate reduction process (SRB -process); for the sulfate reduction process, the aim is to optimize the process parameters affecting the microbiological process so that the conversion from sulfate to sulfide and hydrogen sulfide can be maximized in the process. Particular attention is paid to the optimal control of the feed solution as well as the process conditions and running parameters and to the rapid correction of possible change situations in a direction favorable to microbial function.
- Use of biosolution in calcium precipitation experiments. Calcium has been enriched from neutralization processes in mine effluents. At high concentrations, calcium is detrimental to the technique of the microbiological reduction method of sulfate, for example, and should naturally be reduced to an appropriate level prior to the microbiological process.

The report consist of three subreport: ND process entity A, SRB process entity B and calcium precipitation tests entity C. Each subreport includes a brief theoretical review of the topic, verification of experimental activities, and the reached results and conclusions. The ND process has been verified with process equipment developed by Savonia for nitrogen-concentrated waters in the mining industry. The pilot operation based on the SRB process has been based entirely on a valuable collaboration with the Sulfator Oy. Similarly, the specifications of the calcium precipitation experiments were based on digital modeling.

1.1 Microbiological processes - industrial applicability

Microbiological applications as part of water treatment technology have increased significantly in Finland and especially in cold water treatment. Utilizing microbiological applications as part of water purification technology is the advantageous availability of microbes. Microbes can themselves be grown from ditch sediments or wastewater, and utilizing them in the process costs virtually nothing, with microbes doing the work as part of water purification. When utilizing microbiological processes, good examples of these are the utilization of MBBR technology at the Ruka wastewater treatment plant and Stora

Enso's Mill of Oulu. The mining industry will have stricter limit values for both nitrogen and sulfate in the near future. Valuable groundwater areas must be effectively protected. The real need for nitrogen removal by the mining industry is typically a few tens of milligrams and the amount of water to be treated varies at the level of 3.5-5 million m^3/a. In the popularity of microbiological applications, cost-effectiveness is a crucial factor. The waters can be purified with relatively short residence times. Solutions for commercial suppliers of MBBR technology to the customer are still a relatively expensive investment, for example for carriers where recyclability or the recoverability of recycled materials should be taken into account.

For sulfate, the industry needs solutions to remove it. Sulfate should therefore be seen as a raw material, not as a waste or a problem. High sulfate concentrations can be further processed to new products for industrial needs. In the cold conditions of the North, the removal of sulfate microbiologically poses its own challenges. If the sulfate removal process is not controlled, it will, at worst, prevent industrial investment and environmental permits. Further research is still needed to manage and ensure stable conditions for the process.

1.2 Benefits to Finnish process industry

During the DeepCleanTech -project there was ongoing discussion about the project benefits to Finnish process industry. This chapter sums up the results of these discussions with emphasis on modelling and considering the whole project consortium.

Chemical models are increasingly utilized in the industry for deep technology development, customer service and marketing. Digitalization is included in the strategy of the most of Finnish industrial companies. Technology providers nowadays regularly use expert systems such as digital twins and extended databases for both marketing, process design, operator training and web-based services e.g. as maintenance support. All these solutions are based on process models in different detail level.

Valmet has practiced long-term development work in which the know-how and utilization of chemistry through multi-phase programs, particularly VTT's ChemSheet, has created a unique and internationally recognized competitive advantage to the company. In the future, special attention will be paid on the control of contaminant behavior in forest industry by innovative means to control of the chemistry of non-process elements and help the customers to make their processes greener. The research of both topics has been the key focus in DeepCleanTech.

Modelling also creates new know-how on corrosion control and sustainable material selection for process equipment. Outotec actively utilizes the models in the development of new process concepts and their further marketing to customers. Digital twin models are also increasingly used for dynamic process control as well as in practical process commissioning and operator training. Physical and chemical models describing the process phenomena combined with black-box models is an interesting development tendency that has an important role in digitalization and has been also an interest in DeepCleanTech.

Modeling reduces the risks in new process solutions. Finnish Minerals Group focusses on developing new holistic methods for metals recovery, using the multicomponent models to support development of optional process concepts. The usage of modelling is often costeffective way to evaluate the viability of new process options. Certainly, the models need to be validated through experimental work.

SME companies will benefit on the computational root cause analysis of new monitoring techniques, in developing novel remote sensors for environmental analysis and when designing new innovative bioactive processes. Good understanding of phenomena helps avoiding the problems and fastens the troubleshooting. The computational results can be further connected to experimental knowledge with the help of new expert systems, softsensor solutions and artificial intelligence algorithms utilizing machine learning.

Research institutes VTT and Savonia, industrial companies Valmet, Outotec and Finnish Minerals Group and SMEs Langis and Sulfator were involved in the DeepCleanTech consortium.

The examples of new modelling methods developed in the project include:

- In the project theory has been developed for time-dependent redox process modelling. The new method describe the oxidation-reduction pairs as constraint in thermodynamic modelling and widens the applicability of models. The method can be applied e.g. for process control in hydrometallurgical processes or corrosionmanagement.
- The chemical models describing the aqueous and brine solution are commonly applicable in diluted or medium concentrated solutions. The new model/theory, which connects Pitzer activity model to adsorption models, developed in the project, enables the modelling of aqueous multiphase multicomponent systems from extremely dilute solutions to dry product. The method can be applied in industry were large concentration range is needed.
- Machine learning (two-layer feed-forward neural network model) was applied for predicting mean activity coefficients for ion pairs in concentrated aqueous solutions. The presented methodology might be used when developing new processes related to circular economy, where fundamental properties of used metals are not know. It might provide a rapid tool for screening concepts prior to conducting experiments or measuring material properties for previously unknown cation-anion-pairs.
- The Constraint Gibbs free energy (CFE) -method has been applied successfully in many processes, however the systematic method to add slow reactions to thermodynamic system has been missing. In bioprocesses there are typically multiple reaction to be constrained. Selecting these constraints in CFE by intuition has been challenging. The work performed enables systematic and automated addition of constraints and thus widens the applicability of CFE. The developed methodology can be applied also to the addition of equilibrium reactions to kinetic reaction systems. Another new development of CFE is its usage to produce phase diagrams for timedependent reactive systems using extents of reaction as diagram axis. Use of such computational diagrams has been commonplace for static systems for several decades. With the new extension of CFE dynamic reaction conditions can now be analysed respectively without doing kinetic experiments which often are elaborate and timeconsuming.

In addition, the biological processes, which use for example in hydrometallurgy is increasing, were researched in the DeepCleanTech -project. The chemical state of the process affects biology e.g. pH and in addition, bacterial activity has an effect on the chemical properties of the system. The project has researched the microbiological treatment of sulfate-containing mining water by the means of experiments and modeling, utilizing the industry's natural sulfate reducing bacteria (SRB) strain. Sulfate should not be seen as a waste but as a raw material for industry. Recycling sulfur compounds back into the raw material results in cost savings for production. In the project the computational dynamic phenomena description has been created for the microbial sulfate reduction process. In the future, the model can be used to give guidance for process management.

The importance of the usage of digitalization tools while aiming on greener processes is emphasized in Valmet's parallel project. In the project the NPE accumulation have been modelled in order to obtain knowledge on NPE behavior in recovery cycle and further on move away from fossil fuels. Valmet's customers, i.e process industry have already shown a lot of interest in the project and are interested in its progress. Utilization of the results of the project will be even more important in the future as the emission limits become even stricter and the knowledge of what impacts NPE accumulation has on the process, becomes more significant.

The main benefit of DeepCleanTech to Outotec is the new much more effective process modeling platform to Outotec process designers and R&D experts. This makes possible to create more competitive technology offers to mineral processing and metallurgical industry, and increase technology sales. The new communication interfaces enables the new process advisors, optimizers, training simulators and digital twins, which creates new continuous digital service based business. Outotec new products and services gives lot of benefits to the process industry: For example: more stable and safe process operation; better recoveries, grades, energy efficiencies and less environmental emissions; better availability and performance; easier management of raw material changes; operation forecasting and scenario analysis; and continuous improvement of the operation.

The DeepCleanTech -project have studied the digitalization and modelling in process industry from many different angles. In addition, the project has researched the microbiological treatment of mining waters. All developed methods and models are steps toward better process design, efficiency and control and thus steps towards lower virgin raw material and energy consumption, better product quality, and environmental friendly processes. The models deepens the understanding of chemical phenomena behind the processes, which in such is a competitive edge. The key in research has been thermodynamic modelling and especially the applying the constraint equilibrium calculations in industrial processes. The project has also provided the opportunity for international networking by organizing the "*Latest in modelling symposium - In Honour of Professor Pertti Koukkari's 65th Birthday*" 12.9.2019, which gathered almost 100 process modelers from academy and process industry.

REPORT PART A:

MICROBIOLOGICAL WATER TREATMENT OF NITROGEN RICH MINE WATER

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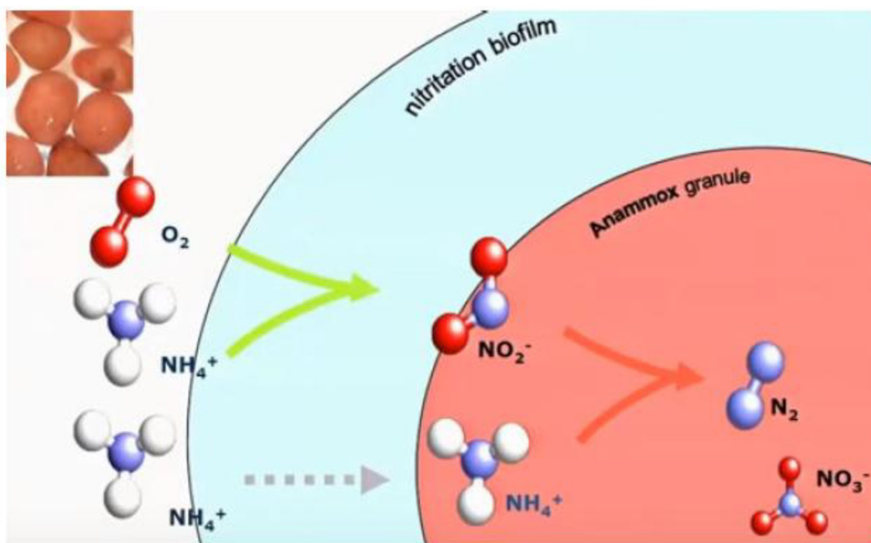


2 MICROBIOLOGICAL NITROGEN REMOVAL

Mining waters are nitrogen concentrated, which comes from the industrial production process. In recent years, the community has begun to impose nitrogen removal obligations on wastewater treatment plants based on the EU Water Framework Directive and the Government Decree derived from it (888/2006). The nitrogen removal obligation of these wastewater treatment plants is assessed on a plant-by-plant basis.

One of the goals of the DeepCleanTech -project was to test and pilot nitrogen removal microbiologically with laboratory-scale pilot equipment. The nitrification-denitrification process (ND-process) is based on the operation of autotrophic nitrifiers and heterotrophic denitrifiers. Nitrifying bacteria operate in the aerobic part of the nitrogen removal process, where ammonium (NH_4^+) is oxidizes to nitrite (NO_2^-) and nitrate (NO_3^-), using carbon dioxide produced mainly by heterotrophic bacteria as their carbon source. Denitrifying bacteria operate in the anoxic part of the denitrification process, where they use oxygen of the nitrite and nitrate molecules and finally nitrogen is reduced to nitrogen gas (N_2).

The DeepCleanTech -project carried out a 43-week long microbiological nitrogen removal test run on mining water in ND-process, with the aim of achieving the most efficient nitrogen reduction possible.

Nitrogen is a colorless, tasteless, non-combustible and non-toxic gas that occurs as a diatomic molecule. About 78 % of the earth's atmosphere is nitrogen and it also occurs in soil and natural waters as various salts. Nitrogen is a vital substance because it is needed for the formation of amino acids and nucleic acids. Table 1 shows the different forms of nitrogen (Sohlo, 2011).

Table 1. Nitrogen in various forms (Sohlo, 2011).

Nitrogen gas (N_2)	Pure nitrogen, 78 % of the earth's atmosphere.
Ammonium (NH_4^+)	Decomposition product of organically bound nitrogen, plant food source.
Ammonia (NH_3)	Toxic gas, soluble in water. Formed from ammonium at high pH.
Nitrate (NO_3^-)	Oxidation product, plant food source.
Nitrite (NO_2^-)	Intermediate in the oxidation of ammonium to nitrate.
Proteins	A building block of cells, consists of an amino acid chain.

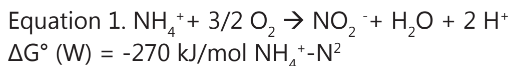
In wastewater, nitrogen is present organically and inorganically bound to nitrite-, nitrate- and ammonium nitrogen. Bacteria oxidize organic nitrogen compounds, releasing nitrogen into the water as ammonia. This process is called ammonification, which occurs before nitrification. In water, ammonia and ammonium ions are in equilibrium. In wastewater, most of the nitrogen is in the form of ammonium and nitrogen comes from e.g. proteins and urea. Because nitrogen is a plant nutrient and can cause eutrophication, it must be removed from wastewater before it is discharged into water. In addition, the nitrogen compounds are in a soluble form, allowing them to accumulate in water bodies. Nitrogen in water can increase the growth of algae, which, when decomposed, consumes a large amount of oxygen. In addition, ammonium nitrogen consumes oxygen in the body of water as bacteria oxidize ammonium to nitrate. In nature, nitrogen is removed from water through nitrification and denitrification, whereupon ammonium nitrogen eventually ends up in atmospheric nitrogen.

In general, the most commonly used processes for nitrogen removal in addition to biological processes are ion exchange and catalytic oxidation. Reverse osmosis has been studied for the removal of total nitrogen from water. Due to the huge amount of wastewater generated in the mines, the reduction step before biological treatment may be economically feasible. For this purpose, membrane filtration is a promising option, as it simultaneously removes other impurities in addition to nitrogen-containing compounds and requires less energy than many other concentration processes. Ammonium and nitrate have a molecular weight of less than 70 g/mol, and therefore the reverse osmosis process is viable for their removal. The membrane-based separation process is quite simple, the devices are compact and modular, and can be operated continuously (Mattila, 2007).

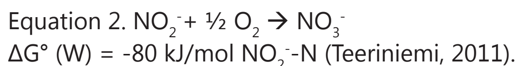
Biological nitrogen removal is based on the activity of two types of bacteria: nitrifying autotrophs and denitrifying heterotrophs. Heterotrophic bacteria use an organic carbon as source of nutrients and function in the anoxic part of the nitrogen removal process. Autotrophic bacteria use carbon dioxide (CO_2) as their carbon source and act in the aerobic part of the nitrogen removal process (Teeriniemi, 2011).

The traditional nitrification process is practically based on the metabolism of a few autotrophic micro-organisms. The process takes place in two simultaneous reactions. In the first, ammonium (NH_4^+) is oxidized to nitrite (NO_2^-) by ammoniumoxidizing bacteria (AOB).These bacteria are included to be bacteria of the genus *Nitrosomonas*. In the second stage, nitrite oxidizing bacteria (NOB) oxidize nitrite to nitrate (NO_3^-). In general, this is considered to becaused by nitrobacteria. Other bacteria such as *Nitrospiira*, *Nitrococcus* and *Nitrocystis* are also involved in the process. Since the potency of other nitrifying-capable bacteria does not differ significantly from that of *nitrosomonas* or *nitrobacteria*, nitrification is generally considered to be a clearly two-step process controlled by two groups of bacteria and well-known stoichiometry and kinetics (Teeriniemi,2011).

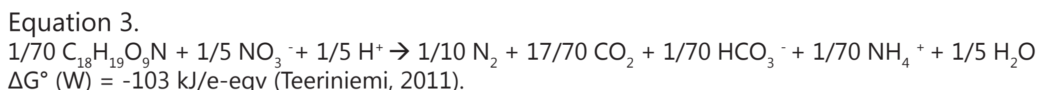
The reaction performed by AOB is according to the following equation:


Equation 1. $NH_4^+ + 3/2\ O_2 \rightarrow NO_2^- + H_2O + 2\ H^+$
$\Delta G°\ (W) = -270$ kJ/mol NH_4^+-N^2

The reaction performed by the NOB is as follows:


Equation 2. $NO_2^- + ½\ O_2 \rightarrow NO_3^-$
$\Delta G°\ (W) = -80$ kJ/mol NO_2^--N (Teeriniemi, 2011).

It should be noted that nitrogen removal does not occur during the nitrification step but during denitrification. Denitrification occurs by heterotrophic bacteria. Denitrification can be simplified by the following reaction equation

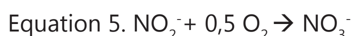
Equation 3.

$1/70\ C_{18}H_{19}O_9N + 1/5\ NO_3^- + 1/5\ H^+ \rightarrow 1/10\ N_2 + 17/70\ CO_2 + 1/70\ HCO_3^- + 1/70\ NH_4^+ + 1/5\ H_2O$
$\Delta G°\ (W) = -103$ kJ/e-eqv (Teeriniemi, 2011).

2.1 Nitrification

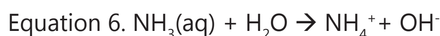
In nitrification, autotrophic or self-sufficient bacteria use the soluble oxygen in the wastewater to oxidize ammonium nitrogen to nitrite and further to nitrate in a two-step process. This is how the bacteria get the energy they need. However, the energy from oxidation is small, so the bacteria grow slowly. Nitrification reactions take place inside bacteria in the cytomembrane, i.e. they are biochemical reactions. Nitrite is only an intermediate in nitrification that does not accumulate in the system, so its formation is a limiting factor for total nitrification. There are also other intermediates in nitrification, such as hydroxylamine, but they are so short-lived that they are not usually shown in the reaction equations. A two-step reaction to oxidize ammonium nitrogen to nitrate requires oxygen about 4.6 g of O_2/g N (Sohlo,2011).

Ammonium nitrogen is oxidized to nitrate nitrogen, which is reduced to nitrogen gas during denitrification. The micro-organisms assimilate the organic nitrogen from the ammonium nitrogen they use for nutrient into their cell mass, whereby when they die, part of the assimilated nitrogen returns to the use of other micro-organisms. In nature, nitrogen removal takes place in two stages, first ammonium nitrogen is oxidized to nitrite and then to nitrate by bacteria according to the following equations (Nieminen, 2011):

Equation 4. $NH_4^+ + 1,5\ O_2 \rightarrow NO_2^- + H_2O + 2\ H^+$


Equation 5. $NO_2^- + 0,5\ O_2 \rightarrow NO_3^-$

Ammonium nitrogen is present in water as either ammonia (NH_3) or ammonium ion. The reaction of ammonium nitrogen with water is as follows (Mattila, 2007):


Equation 6. $NH_3(aq) + H_2O \rightarrow NH_4^+ + OH^-$

The net reaction:

Equation 7. $NH_4^+ + 2\ O_2 \rightarrow NO_3^- + 2\ H^+ + H_2O$.

Nitrification produces an acid which reacts with the carbonate in the water, so that the pH can drop sharply if the alkalinity of the water, i.e. the buffering capacity, is low (Sohlo, 2011).

Temperature and pH can influence the form in which ammonium nitrogen is present. Above pH 9.25, 50 % of the ammonium nitrogen is in NH_3form. If the temperature remains constant, a decrease in the pH unit reduces the proportion of ammonia in the water to one tenth. The proportion of ammonia, on the other hand, triples as the temperature rises to ten degrees celsius, whatever the pH. Nitrification requires a lot of oxygen to work. The oxygen content should not be less than 1 mg/l, but the best results are obtained with concentrations above 2 mg/l. A really high oxygen content has no detrimental effect on the operation of the process, in which case this only affects the costs of the process (Nieminen, 2011). The efficiency of nitrification doubles as the temperature rises by 10 ° C, denitrification also depends on temperature, but the efficiency of the process is already doubled to 4 °C with increasing temperature. Typical nitrogen removal efficiencies at municipal wastewater treatment plants are 1 kg/N/m^3/d (Mattila, 2007).

The dissolved oxygen concentration in the reactor must be at least 2 mg/l. With a smaller amount of oxygen, the removal efficiency decreases. At an oxygen level of 6 mg/l, the same removal efficiency can be achieved with a retention time of 6 hours. The most efficient nitrogen removal is achieved when the amount of soluble oxygen is kept above 2 mg/l (average 89.1 %), at lower oxygen concentrations, anaerobic conditions occurred and the ammonia concentration in the water increases. As the biofilm increases, the concentration of soluble oxygen decreases rapidly due to the higher bacterial activity (Barwal, 2014).

Nitrification also requires a sufficient amount of nutrients. The ratio of nitrogen to phosphorus (N:P) should be 100:1. If the phosphorus content of the influent water is low, it is advisable to add phosphorus to the process, which will increase the degree of nitrification and achieve good purification results. According to studies, especially in biofilm reactors, the proportion of phosphorus has emphasized the efficiency of reduction (Nieminen, 2011).

Nitrifying bacteria are fairly sensitive organisms, so changing conditions prevent them from growing. Too much ammonium nitrogen can inhibit the growth of these organisms. The optimal pH is between 7.5 and 8.6. At lower pH conditions, nitrification can be successful. In addition, temperature affects the growth rate of bacteria (Nieminen, 2011).

Nitrification bacteria are common in soil, freshwater, seawater, wastewater, and household water. Nitrification bacteria are aerobic autotrophs, meaning they produce the energy they need under oxygenated conditions. The bacterial group that oxidizes ammonium nitrogen to nitrite is different from the bacterial group that oxidizes nitrite to nitrate. Bacterial genera that oxidize ammonium nitrogen to nitrite are abundant, the most important of which are *Nitrosomonas* and *Nitrobacter*. Bacteria that oxidize ammonium nitrogen receive more energy than those that oxidize nitrite, so that under unfavorable conditions, ammonium-oxidizing bacteria grow faster and nitrite begins to accumulate in the system. Even relatively low nitrite concentrations can be toxic to the process (Sohlo, 2011).

Nitrification bacteria need a carbon source (carbon dioxide or other inorganic carbon compound), phosphorus, and trace elements for cell growth. These bacteria mostly use carbon dioxide as their carbon source, which is sufficiently available in water. They also utilize ammonium as a nutrient source and thus do not nitrify everything. Nitrification bacteria are very sensitive to inhibitors that prevent the oxidation of ammonium to nitrate (Sohlo, 2011).

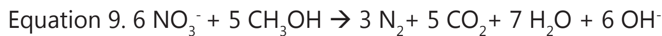
2.2 Denitrification

Nitrification alone does not remove nitrogen from wastewater, but the final nitrogen removal takes place in denitrification. Many chemo-organotrophic, lithototrophic and phototrophic bacteria and some fungi reduce nitrite and nitrate to nitric oxide, nitrous oxide and finally nitrogen gas while oxidizing the organic material according to Equation 8. Nitrogen gas is thus released back into the atmosphere. In addition to nitrogen, denitrification releases carbon dioxide. Denitrification takes place under anoxic conditions, i.e. there is no dissolved oxygen in the water, in which case the bacteria use oxygen of

nitrite and nitrate molecules to breathe (Mattila, 2007) (Sohlo, 2011):

Equation 8. $2\ NO_3^- \rightarrow 2\ NO_2^- \rightarrow 2\ NO \rightarrow N_2O \rightarrow N_2$

Denitrification bacteria are mainly heterotrophic, i.e. they need an external carbon source. The carbon source can be an internal and wastewater-containing organic matter or an external carbon source that is fed to the denitrification process. The external carbon source should be readily degradable, such as methanol, ethanol, glycol or acetate. Methanol is often used in a ratio of 3 kg methanol/kg nitrate nitrogen (Equation 9). It is also economical and does not cause a large increase in biomass in the process (Mattila, 2007) (Sohlo, 2011).



Equation 9. $6\ NO_3^- + 5\ CH_3OH \rightarrow 3\ N_2 + 5\ CO_2 + 7\ H_2O + 6\ OH^-$

Denitrification operates in an anaerobic circumtanses, and the optimal conditions for the growth of organisms are also important. The ramp-up time of an anaerobic process is usually quite long as it can take 2-4 months. This is because denitrifying microbes are very slow growing. A typical biomass also grows in the reactor, where microbes thrive. Biomass growth is also slow and needs time to adapt to the new environment (Mattila, 2007).

2.3 The effect of temperature on nitrogen removal efficiency

Nitrification and denitrification processes are temperature dependent. Temperature affects not only the metabolic activity of bacteria, but also the transport of gases in water. Roughly, it can be seen that the activity increases with increasing temperature. At some point, however, a point is reached where the activity collapses due to too high a temperature. Correspondingly, at too low a temperature, the activity is zero or the bacterial activity is very slow. The effect of temperature on the reaction rate of biological processes can be described by the following equation:

Equation 10. $\mu_T = \mu_{15} \times e^{\theta \times (T-15)}$

where,

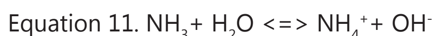
μ_T = Growth rate at T °C
μ_{15} = Growth rate at 15 °C
Θ = temperature-activity factor
T = temperature (°C) (Sohlo, 2011).

Temperature affects nitrification the most of all environmental factors. The growth rate and activity of nitrification bacteria increase as the temperature rises up to 30 °C. Above 45 °C and below 4 °C nitrification is inhibited. The optimum temperature for nitrification is 28-32 °C. When the temperature drops below 10 °C, the nitrification rate is only about 20 % of the nitrification at 30 °C. In this case, nitrite can start to accumulate in the water, as the cold affects *Nitrobacteria*, which oxidize nitrite more than *Nitrosomonas*, which oxidize ammonium. The rate of denitrification also increases with increasing temperature. Denitrification is also aided by the fact that oxygen is less soluble in warmer water as the temperature rises (Sohlo, 2011).

2.4 The effect of pH on nitrogen removal efficiency

Nitrification is a pH sensitive process due to the pH sensitivity of the enzyme that catalyzes the reaction. Reaction rates decrease significantly when the pH drops below 6.8. When the pH is below 5, nitrifying bacteria cease to function, but nitrification can occur by organotrophic bacteria and fungi that decompose organic carbon. The optimum pH for nitrification is between 7.5 and 8.6. Nitrification consumes the alkalinity of the water, i.e. the pH decreases unless a base is added to the water to raise the pH. Hydrated lime ($Ca(OH)_2$) or sodium hydrogen carbonate ($NaHCO_3$) is generally used to raise the pH and maintain a suitable level. The amount of chemical added is affected by the initial alkalinity of the wastewater and the amount of oxidized ammonium. After nitrification, the alkalinity should be at least 50 mg $CaCO_3$/l (Sohlo, 2011).

In aqueous solution, ammonium ions are in equilibrium with ammonia according to Equation 11. As the pH rises high, the proportion of ammonia is higher. In this case, nitrification is less efficient because the bacteria oxidize ammonium ions and not ammonia. In addition, ammonia is toxic to bacteria (Sohlo, 2011).



Equation 11. $NH_3 + H_2O <=> NH_4^+ + OH^-$

where,

NH_3 = ammonia
H_2O = water
NH_4^+ = ammonium-ion
OH^- = hydroxide-ion (Sohlo, 2011).

Denitrification bacteria are not as pH sensitive as nitrification bacteria, so denitrification occurs over a wider pH range. The optimum pH range for denitrification is from 6.5 up to 9 and as the pH drops below 6.5, the denitrification rates decrease. On the contrary as in nitrification, in denitrification the alkalinity increases, i.e. the pH increases due to the OH^- ions formed (Sohlo, 2011).

2.5 Inhibition of nitrogen removal process

In addition to temperature, oxygen concentration, and pH, various inorganic and organic toxins and metals also affect nitrification and denitrification. Nitrification bacteria are more sensitive to contaminants than organotrophic bacteria because they do not get enough energy to repair the damage. In general, reaction rates decrease significantly under the influence of toxins and contaminants, allowing bacteria to continue to grow and oxidize more slowly. Bacterial inhibition can be temporary, short-term (acute) or long-term (chronic). Sometimes toxins can kill bacteria completely. Because nitrogen removal bacteria are sensitive to toxins at low concentrations, they are good indicators of toxins. Compounds that are toxic to bacteria include e.g. solvents, phenols, alcohols, ethers and benzene. Ammonia formed at high pH and nitric acid formed at nitrite at low pH are also toxic to bacteria. However, these can be prevented by maintaining the pH and alkalinity of the aeration step at an appropriate level. The effects of toxic or harmful emissions can affect nitrogen removal bacteria. According to the literature, the limit values for inhibition are as follows: Sulfate 500 mg/l, Zinc 0.3 mg/l, Copper 0.35 mg/l, Nickel 0.25 mg / l, Chrome (VII) 0.25 mg/l and Chrome (III) 0.05 mg/l (Gerardi, 2002) (Sohlo, 2011).

3 CARRIER REACTOR TECHNOLOGY

3.1 Moving bed biofilm reactor (MBBR)

In recent years, carrier-containing bioreactors have become more common as a wastewater treatment technology. These particles are called carriers because they are kept in constant motion by aeration. The main purpose of the carrier particles is to increase the surface area in the reactor where the active biomass grows as a biofilm (Nieminen, 2011).

The advantages of MBBR technology are high-end wastewater treatment technology with high treatment efficiency: low operation, maintenance and replacement costs, as well as reliable and robust operation. In addition, this particular technique can be applied to a wide variety of wastewater streams ranging from 10,000 to 100,000 up to 150,000 m^3 for daily flows. MBBR has been shown to be effective in removing up to 90 % of chemical oxygen and 95 % of biochemical oxygen demand provided the retention time is sufficiently long. The performance of MBBRs depends directly on the carrier, the surface area they provide, the dissolved oxygen and the amount of organic load (Barwal, 2014).

A biofilm or membrane is a phenomenon in which micro-organisms grow on the surface of a carrier. The biofilm grows on the protected inner surface of the carrier, making it an effective biofilm.This also serves as an important design parameter in the selection of cleaning technology. The biofilm grows internally due to the biocarriers and the dissolved contaminants decompose in the wastewater stream. The carrier increases productivity by providing an active surface area that maintains microbial function and metabolism. Different types of carriers are available in different shapes and preparations from different materials. The most typical are carriers made of polyethylene (HDPE) or polypropylene (PP). Carriers are generally cylindrical in shape with a cross inside the cylinder and longitudinal 'fins' outside (Barwal,2014).

In general, the service life of carriers varies between 10 and 30 years. The plastic material has a proven long service life without material deterioration. If necessary, the material can be replaced or supplemented in the reactor (Barwal, 2014).

MBBR technology is based, like all other carrier processes, on a non-clogged biofilm reactor in which biofilm is formed on the surface of the carrier. In addition, the reactors have a very low energy loss. The biofilm grows on the surface of the carrier and is in constant motion in the reactor. The movement in the aerobic reactor is caused by aeration, whereby air is fed to the reactor from small openings, whereby bubbles are formed. In anaerobic and anoxic reactors, the movement of the support is caused by a mechanical stirrer (Nieminen, 2011).

Particles of different sizes and shapes can be used as a carrier for the biofilm. In MBBR technology, the reactor can be filled with 70 % support, giving a potential biofilm growth area of up to 500 m^2/m^3. However, the biofilm is mainly formed inside the cylindrical carrier, whereby the amount of biofilm formed outside the cylinder is small. In this case, a better efficient biofilm growth area can be determined to be 350 m^2/m^3 on average. Therefore, the effective surface area of the carriers is taken into account in the surface area, in which case the surface area does not include the outer surface of the particle (Nieminen, 2011).

There are many different carriers available. Their specific surface areas range from 100 m^2/m^3 to 5,000 m^2/m^3. However, the surface area of the carrier is not the most decisive factor in cleaning efficiency. The most important thing in the development of a biofilm is the transfer of sufficient oxygen and nutrients from the liquid to the biofilm. This allows for good conditions for the biofilm to grow. Other important factors in selecting a carriers are also the openness of the body, the turbulence of the flow on the surface of the biofilm, and the amount of carrier to be filled. As a result, the specific surface area of the carrier itself has a relatively small effect on how well the process works. The inner part of a body with a large surface area can be filled with sludge, so that oxygen and nutrients can no longer enter the surface of the biofilm. As a result, the choice of carrier type is influenced by the required oxygen content and energy demand (Nieminen, 2011).

Size, transparency and durability are important in the choice of carriers to maximize benefits. It is also good to pay attention to the durability of the carriers, as the carriers wear out in the process. The carriers rub against each other, the walls of the basins and other structures. Some pieces may also collapse when the support ring on their edge wears out. The mixer may cause breakages in the pieces. Broken carriers and pieces leaving them can clog partitions and travel to piping and pump trees. In particular, very small carriers can become clogged, which also reduces their effectiveness. Clogged carrier particles can be washed with certain washers, but it is quite cumbersome in the middle of the process. The transport of carriers and broken pieces is prevented by means of mesh and strainer structures. This also places demands on these structures, because the smaller the solid particles, the denser the network structures must also be. Very dense mesh structures can in turn become clogged with sludge and small pieces of carrier (Nieminen, 2011).

One of the most important factors in the formation of a biofilm is the movement of the various components into and out of the carrier. The formation of an effective biofilm is really important in order to achieve good cleaning results. The most ideal biofilm in the carrier process is really thin and distributed over the entire surface of the carrier. In addition, the turbulence generated in the reactors is important, which is what MBBR technology is trying to achieve. Good vorticity helps to transport the nutrients needed for biofilm formation while keeping the biofilm thin by friction (Nieminen,2011).

Reactors containing carrier achieve really good biological purification results. These reactors have been developed from the traditional activated sludge process and the fluidized bed reactor. The carrier reactors consist of an aerobic and an anaerobic reactor, giving the purest possible result. The aerobic reactor is important for the removal of nitrogen and organic carbon. Hydraulic residence time (HRT), i.e. the time it takes for the water to be purified to pass through the reactor, is particularly important in nitrogen removal. Ammonium nitrogen is removed by 97 % when the retention time of the aerobic reactor is more than 1.25 days. The temperature and the retention time have a direct effect on the degree of nitrification, so that a good cleaning result can be achieved with a sufficient retention time. At higher temperatures, a shorter retention time is sufficient, and at low temperatures, changes of a few degrees immediately affect the retention time (Nieminen, 2011).

Important factors influencing nitrification are dissolved oxygen, ammonium nitrogen content above 3 mg/l, and temperature. All of these factors must be considered to maximize the rate of nitrification. The following equation can be used to determine the rate of nitrification:

Equation 12. $r_N = 213,6 * (DO-1,15) * 1,047^{(T-20)}$

where,

r_N= nitrification rate (mg N/m^2)
DO = dissolved oxygen (mg/l)
T = temperature (°C) (Nieminen, 2011).

A carrier-based bioreactor has many useful properties compared to others. The microbes growing inside the reactor are efficient and require less space because the carriers have a lot of surface area to which they can grow. There is a high sludge content inside the reactor, leaving a smaller amount of sludge out of the reactor. Sludge deposition has a long removal time, allowing microbes to develop well. The organisms used for wastewater treatment can be processed very easily. Microbes living under aerobic as well as anoxic conditions are able to coexist. The reactors are also smaller in size, so they take up less space than other cleaning methods. The reactor also does not require sludge recovery because the necessary microbes grow on the surface of the support as biofilm (Nieminen, 2011).

3.2 Anaerobic fixed bed reactor

Anaerobic fixed bed reactor technology can be used for microbiological nitrogen removal. The reactor consists of two parts, both a cylindrical or rectangular column and a three-stage gas-liquid-solid separator. The water to be examined is pumped from the bottom of the reactor. Biological reactions take place all the way through very active sludge beds and cover areas. The gas formed in the process and the sludge trapped inside the gas bubbles rise upwards. A gas is separated from the effluent, which continues its journey to the gas collection unit. The effluent liquid exits the upper end of the reactor (Ang, 2012).

In an anaerobic reactor, mixing can be accomplished by mechanically or by internal circulation. In addition, the movement of the gas bubbles that form and flow upwards brings natural turbulence and floats the sludge, which enhances the contact between the wastewater and the biomass. In addition to the gas bubbles, the internal circulation of the reactor mixes the wastewater under study and recirculates it through the sludge bed. In connection with the circulation, it is also possible to heat the reactors by connecting the circulation pipes to the heater, through which the wastewater is heated. Heating can also be implemented with separate heaters on the outside of the reactors (Ang, 2012).

In an anaerobic reactor, biofilm formation allows for a short retention time of the wastewater and a high retention time of solids. Under the right conditions, the equipment can be shut down for extended periods of time without significantly impairing the ability of the microbes to function. Sludge, water and biofilm from one reactor can also be transferred

and used as a ramp-up inoculum for another reactor. The reactor also has disadvantages, such as its long ramp-up phase. The ramp-up phase has a strong impact on the efficiency and stability of the reactor and is influenced by several physical, chemical and biological factors. Influencing factors include the type of wastewater and its properties, the prevailing operating conditions and the amount of inoculum available, and the activity of the biomass in it. During the ramp-up phase, the reactor must not be overloaded and its temperature must be kept constant. In addition, the inoculated microbes must be given a sufficient stabilization phase to allow them to adapt to the new conditions in the reactor before the test effluent is fed to the reactor (2-8 month) (Ang, 2012).

3.3 Granulation to carrier surface

The biofilm formed in the microbiological process has a constant interaction with microorganisms. The viability, size and density of the resulting biofilm in the sludge particles control the efficiency of the reactors. The formation of sludge granules is emphasized because the granules not only support biofilms but create buoyancy and descent in the granule-liquid contact reactor (Abbasi, 2011).

Anaerobic granules are the envelope for the biofilm formed spontaneously in the metabolism of anaerobic bacteria. In other words, the granules are in dense particles in symbiosis with anaerobic micro-organisms. A typical granule is a real microecosystem hiding millions of organisms per gram of biomass. However, no single species can completely decompose waste. Once the reactor is started and the influent flows in the column and is in contact with the microbes attached to the carriers, the formation of granules can begin slowly. Figure 1 verifies this phenomenon. The conditions must be optimal for the process, for example in terms of pH, nutrient availability and water upflow rate. Rapid changes in temperature can cause decomposition, so for this reason the temperature in the reactor must be constant. Gradually, different mutually beneficial groups join together to form roughly spherical groupings. These clusters are thus called granulations, ranging in size from 0.1 millimeters to 5 millimeters, and have better shear forces than the flake-like sludge. The pellet sludge has better settling properties than normal sludge, so it can withstand higher loading rates, which in turn leads to better reactor efficiency. Granulations can also withstand high shear stress of gas and liquid without disintegration. In addition, they offer better resistance to, for example, shock loads and toxins than dispersed micro-organisms (Abbasi,2011).

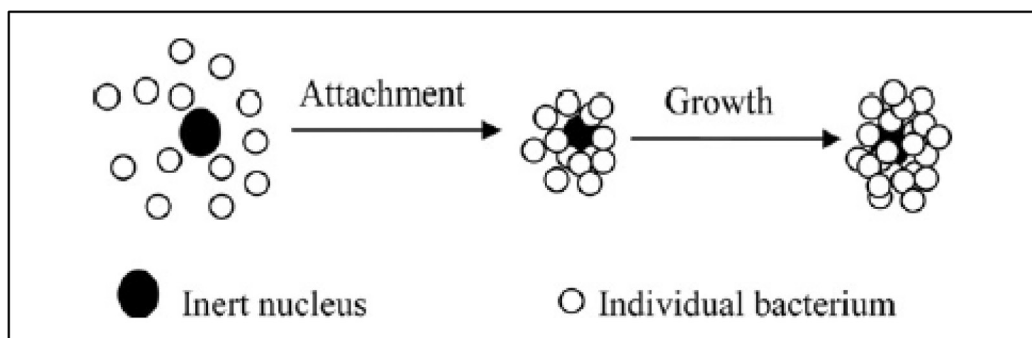


Figure 1. Formation of granulation as the microflora adheres around the nuclear (Abbasi, 2011).

Ideally, the granulation should contain concentric layers of near-spherical biofilms with different groups of bacterial trophies (producers, consumers and degraders). Each of these groups of bacteria is expected to play its own role in the decomposition of wastewater and the production of biomass and extracellular polymeric substances (ECPs) in its immediate environment. Extracellular polymeric substances are large molecular compounds secreted by micro-organisms into their environment, which strengthen the functional and structural integrity of biofilms (Abbasi, 2011).

4 OPERATIONAL FACTORS AFFECTING DENITRIFICATION

During the microbiolocal nitrogen removal test run, there are several operational factors that strongly influence the denitrification. These include the amount of substrate, oxygen-free conditions (free molecular oxygen), sufficiently strong and active denitrifying population, pH, temperature, nutrients, and redox potential. The most critical factor is the presence of a substrate and an adequate supply of carbon, as well as completely anaerobic circumtances (Gerardi, 2002).

About 80 % of the bacteria in the activated sludge of the municipal wastewater treatment plant are facultatively anaerobic and capable of denitrification. Bacteria in the activated sludge process are present in very large quantities in bulk as well as in solids (MLVSS). Unless there are ramp-up conditions, leaching, toxicity, or recovery from toxicity in the treatment process, the population of denitrifying bacteria should be sufficient and active to ensure denitrification under favorable operating conditions (Gerardi, 2002).

Denitrification can take place over a very wide pH range. Denitrification is relatively insensitive to acidic conditions and can be slowed down at low pH. Optionally, anaerobes are capable of floc formation in the pH range of 6.5-8.5 while being capable of denitrification. To optionally ensure the activity of anaerobic bacteria and nitrifying bacteria, the pH in the aeration tank should be above 7. The most optimal range for denitrification is 7-7.5 (Gerardi, 2002).

Denitrification is a biologically significant reaction, it occurs more rapidly at warmer, and correspondingly denitrification occurs more slowly as the temperature decreases. Denitrification is inhibited when the temperature drops below 5. To compensate for the activity of denitrifying bacteria at colder temperatures, the number of denitrifying bacteria can be increased by increasing the solids (MLVSS) (Gerardi, 2002).

Denitrification is completely dependent on nitrification, and thus nitrification is also a biologically significant reaction. The warmer temperature favors faster nitrate ion formation. In addition, warm wastewater has a lower affinity (ability to bind) to dissolved oxygen than cold wastewater. Thus, dissolved oxygen is more easily consumed by the warmer conditions, and denitrification occurs more easily under warmer conditions (Gerardi, 2002).

The main nutrients for facultatively anaerobic bacteria are nitrogen and phosphorus. Due to the higher energy intake and production of cells in aerobic respiration of facultative anaerobic bacteria compared to anaerobic, nutrient guidelines for anaerobic respiration can be used. In aerobic respiration, bacteria need half a milligram of ortho-phosphate for every one ammonium ion and every three nitrate ions (Gerardi, 2002).

Nitrate and nitrite ions are present as part of the bacterial degradation of BOD with the redox potential of the operation ranging from +50 to -50 mV. Redox potential measures oxidizing ions such as NO_2^- and NO_3^- from wastewater, as well as reducing agents such as NH_4^+. In the millivolt range mentioned above, oxygen is either completely absent or at very low concentrations. While nitrate and nitrite ions are at relatively high concentrations (Table 2) (Gerardi, 2002).

Table 2. Redox potentials according to conditions (Gerardi, 2002).

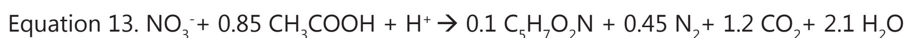
Redox potential, mV	Electron acceptor	Circumtances
> + 50	O_2	aerobic
+50 → -50	NO_2^-, NO_3^-	anaerobic

4.1 Carbon source of nitrogen removal process

The amount of substrate or BOD is the most significant factor in denitrification, not so much the amount of nitrite or nitrate. The higher the amount of BOD - especially the amount of soluble BOD in simplified terms - the greater the need for electron acceptors such as free molecule oxygen, nitrite and nitrate ions. The greater the need for electron acceptors, the greater the potential for denitrification to occur even under aerobic conditions. If the demand for electron acceptors exceeds the oxygen supply of free molecules, facultative anaerobes or denitrifying bacteria exchange their enzymatic metabolism from free molecular oxygen to nitrite or nitrate ions as electron acceptors (Gerardi, 2002).

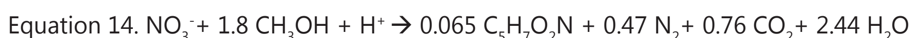
Denitrifying bacteria use many common organic compounds and unusual organic compounds as a source of carbon and energy. In general, they are able to utilize municipal wastewater compounds efficiently. Several organic compounds can be used as a carbon source, such as acetic acid, ethanol, glucose, methanol, and molasses. The reaction equations with acetic acid and methanol are shown below:

Denitrification of acetic acid as a carbon source:



Equation 13. $NO_3^- + 0.85\ CH_3COOH + H^+ \rightarrow 0.1\ C_5H_7O_2N + 0.45\ N_2 + 1.2\ CO_2 + 2.1\ H_2O$

Denitrification of methanol as a carbon source:



Equation 14. $NO_3^- + 1.8\ CH_3OH + H^+ \rightarrow 0.065\ C_5H_7O_2N + 0.47\ N_2 + 0.76\ CO_2 + 2.44\ H_2O$

Methanol is the most widely used source of carbon when it needs to be added to the process. This is because methanol is the simplest organic compound that is in a soluble form and is rapidly absorbed into the bacterial cell wall and readily degraded (Gerardi, 2002).

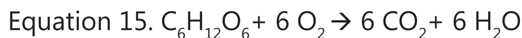
Complete denitrification or removal of nitrite and nitrate ions generally occurs when the ratio of BOD to those ions is approximately 3:1. By reducing this ratio to 3:2, nitrite and nitrate ions decompose more easily. Generally, a ratio of 3:1.21 is used to achieve complete denitrification (Gerardi, 2002).

Complete denitrification of nitrate ions requires 2.5 mg/l methanol per milligram of nitrate. When microbes use 2.5 mg/l methanol, a new biofilm (cell wall) of 0.5 mg per liter is formed. Of this 0.5 mg by mass, 0.06 mg is bound to the cell wall as organic nitrogen. Nitrogen that does not adhere to the cell wall or sludge is released as molecular nitrogen into the atmosphere (Gerardi, 2002).

That same can also be expressed per nitrite. 1.5 mg/l of methanol is needed for every mg of nitrite. When this amount of methanol decomposes, 0.3 mg of new biofilm (cell wall) is formed, which contains 0.04 mg of organic nitrogen when trapped in the cell wall and the rest is released into the atmosphere (Gerardi, 2002).

4.2 Free oxygen

Free elemental oxygen inhibits denitrification by competing with nitrite and nitrate ions as electron acceptors to decompose BOD. If the free elemental oxygen is in the vicinity of the bacterial cell and ends up in the cell wall, the cells use the elemental oxygen according to the following reaction equation:


Equation 15. $C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$

The use of free elemental oxygen is more advantageous than the use of nitrite ions and nitrate ions because its use provides more energy to the cells and allows them to grow faster. The amount of oxygen that is toxic to denitrification is relatively small. A concentration of dissolved oxygen at a concentration of <1.0 mg/l inhibits denitrification. However, if dissolved oxygen surrounds the floc particle, denitrification occurs in the nucleus of the particle. The particle must be at least more than 100 µm in diameter for this to happen. In such a situation, the bacteria breathe using both dissolved oxygen and oxygen molecules of nitrite and nitrate ions at the same time. Bacterial cells around the floc particle use dissolved oxygen to breathe, while in the floc nucleus they breathe with nitrite and nitrate (Gerardi, 2002).

5 DESCRIPTION OF LABORATORY EQUIPMENT

Microbiological nitrogen removal consists of aerobic nitrification and anaerobic denitrification. Figure 3 presents the laboratory-scale pilot equipment used with its various process units where MBBR technique was simulated. The three-stage microbiological test equipment consists of a 100 liter influent tank with automatic pH adjustment (to 8.5 with 1 % NaOH solution). In influent tank the correction of the ammonium nitrogen and nitrite nitrogen balance was adjusted to 1:1 and carbon source (sodium acetate) to level 1.2 times the amount of nitrate. Between the influent tank and the nitrification step was the supply of nutrients. The nutrients were initially fed directly into the influent tank, which is why microbiology began to function and remove nitrogen already in the influent tank (water is process industry water and naturally contains microbes). After the influent tank is the nitrification reactor, R1, where the carriers were free in the reactor and move by means of aeration. Aeration was carried out with a tube aerator (Figure 2) so that the oxygen was in the nitrification step at all times at least 2 mg/l. In practice, the amount of oxygen varied between 5-8 mg/l. This was also visually evident in the floating motion of

the carriers with a diameter of 10 mm. The nitrification reactor was heated with heating mats and the optimum operating temperature was 28 °C. By these measures, ammonium nitrogen was efficiently oxidized via nitrite to nitrate.

After the nitrification water went to a control tank where the pH was adjusted and carbon nutrient was added (raising to 1.2 times the amount of nitrate). There was constant paddle mixing in the control tank. With efficient nitrification, the pH dropped to level 5 in control tank. The addition of sodium acetate in the control tank itself raised the pH. The automation adjusted the pH to an optimal level (pH about 7.00) using 4 % nitric acid (HNO_3) or 1 % lye (NaOH).

Figure 2. Nitrification tube aerator (Figure: Maarit Janhunen).

This was followed by two-stage anaerobic reactors (R2 and R3) in which nitrate is reduced to nitrogen gas. Mixing in anaerobic reactors takes place by an internal circulation which ensures contact between the biofilm and the water to be purified. The carriers in the anaerobic reactors were 16 mm and 25 mm in diameter (half and half) and the carriers was not bound in any way. Figure 4 shows a model of the carrier. The advantage of a smaller carrier is a larger surface area for biofilm formation. The anaerobic reactors were covered and the first anaerobic reactor also had heating with an external heating mat to an optimum level of 35 ° C. Table 3 shows the calculated values for the reactor surface area according to the degree of filling of the carrier for the reactor volume used in nitriofication with a surface area of 4.5 $m^2$and in denitrification 3.6 m^2.

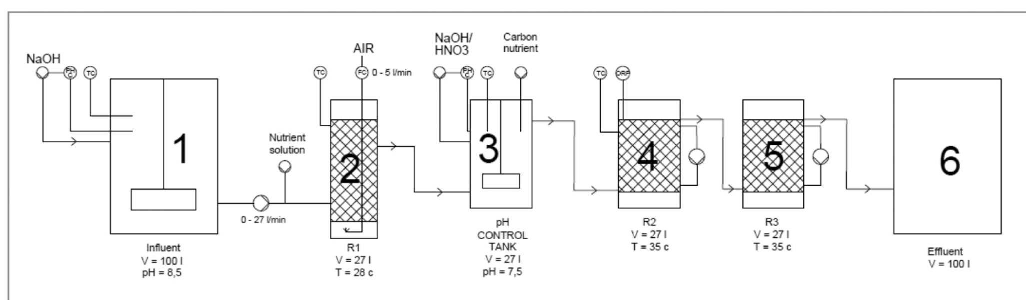


Figure 3. Microbiological nitrogen removal equipment based on MBBR technology (Figure: Tero Reijonen).

Microbiological nitrogen removal based on MBBR technology includes the following units:

1. Influent tank 100 L, balancing ammonium nitrogen and nitrite nitrogen (continuous mixing). Automated pH adjustment with lye to 8.5.

2. Reactor 1 (R1). Aerobic reactor, with volume of 27 l (1 l air/h/R per liter) with 10 mm diameter carriers, oxidation of ammonium nitrogen to nitrate.
3. Control tank, with volume of 27 l with constant mixing. Addition of carbon and nutrients to the anaerobic phase.
4. Reactor 2 (R2). Anaerobic 27 l reactor with 16/25 mm diameter carriers and internal circulation. Reduction of nitrate to nitrogen gas.
5. Reactor 3 (R3). Anaerobic 27 l reactor, certifies the operation of the previous phase. Internal circulation, no heating. Reduction of nitrate to nitrogen gas.
6. Effluent tank, 100l.

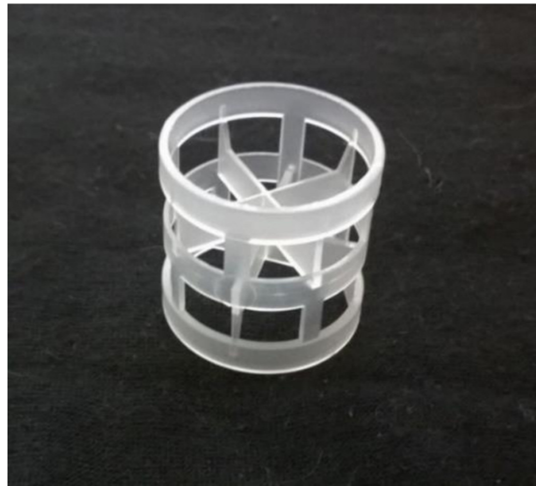


Figure 4. Plastic polypropene carrier used in reactors (Figure: Maarit Janhunen).

Table 3. Reactor area load capacity calculated according to the degree of filling of the carriers.

High, mm	Carrier, m^2/m^3	Nitrification, m^2/R	Denitrification, m^2/R
7,2	500	**4,5**	
16	320		2,2
25	210		1,4
		Total:	**3,6**

6 MINING WEATER

The test run used wastewater from the Finnish mining industry with a nitrogen concentration (total nitrogen) ranging from 700 to 1,200 mg/l. Typically, mining waters have less than 100 mg/l of nitrogen, so this water was strongly concentrated. Table 4 shows the chemical composition of the mine water from the first water batch delivered to the laboratory. Water was supplied for research use in a cube container in approximately 700 liter water batches.

Table 4. Chemical composition of mining water used in the test run (sample analyzed by ICP-MS at the University of Eastern Finland), results expressed in mg/l. Inhibitors marked on red and boosters at purple colour.

B	Na	Mg	Al	Si	P	K
2,4	4,7	44,6	3,6	25,4	7	36,9
Cr	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**
0,005	2	< 0,0	0,03	0,07	0,02	7,8
Ca	**V**	**Ba**	**Pb**	**TOC**	**NO_3^--N**	**NH_4^--N**
91,6	0,02	0,009	0,02	1,2	520	535
As	**Rb**	**Sr**	**Cd**	**Cs**	**NO_2^--N**	**SO_4^{2-}**
0,02	0,05	2,3	0,005	0,003	6	100

As can be seen from Table 4 the proportion of nitrogen is fairly evenly distributed between nitrate and ammonium nitrogen (green). In addition, there were some milligrams of nitrite nitrogen in the water. Sulfate in water contained only about one hundred milligrams, so it was not a disturbance to the microbiological process. In addition, the most typical inhibitory metals are highlighted in red in the table. Of these, zinc exceeds the limit of inhibition in the literature, however, this was not removed from the process water prior to the microbiological process. In addition, "boosters", that accelerate granulation on the surface of the carriers, are marked in purple in the table. These accelerators should be about 20-30 mg/l in the water. Calcium was naturally present in the water, but iron and aluminum were added as the nutrient solution combined with the process.

7 RESULTS OF MICROBIOLOGICAL NITROGEN REMOVAL

7.1 Adaption and inoculation

Inoculum were grown in the laboratory into the denitrification phase for two months. It is known that anaerobic bacteria are slow to grow and may require up to two to six months of adaptation or growth. The aim of the test run was to use an inoculum grown naturally from nitrogen-containing water in the mining industry. The stock thus grown would be stronger and would also be more resistant to changes in the process condition. The rearing was carried out in large container (started on 28 June 2018), where about 30 % of the volume of carriers (16 mm in diameter) was placed. The packings were selected according to the diameter of the column to obtain an optimal surface area with respect to flow. "Nitrogen water" was added to the products, which contained 9 l of nitrogenous mining water (TN = 1,000 mg/l), 21 l of tap water and 10 l of anaerobic primary sludge from municipal wastewater treatmentplant. In addition, sufficient main nutrients, phosphorus and carbon, were added to the recipients (Total-N:Total-P 100:1 and carbon:nitrite $C:NO_3$ 1.2:1), as well as trace elements. Potassium dihydrogen phosphate was used as the phosphorus source and sodium acetate as the carbon source. All ingredients were mixed well and the pH was finally adjusted to 7.5 with lye. Container was covered with a lid and left to stabilize in the laboratory at 25 degrees. During the adaptation phase, nothing was added but, the pH, oxygen and Redox potential were occasionally examined. Measurements showed that the reduction potential was negative, the pH was in the optimal range (6.5- 9), and oxygen-free conditions were prevalent.

After an adaptation phase of about two months, on August 21 2018, the inoculated cultured recipients were transferred to two anaerobic reactors of the equipment (Figure 3). The available carriers were evenly divided into two reactors. The first of the reactors, R2, was completely filled with inoculum. The second reactor R3 was charged with dilute mining water to make it completely filled. Both reactors were turned on for internal circulation at such a rate that water circulates through the process once an hour. Reactor R2 was heated with external heating mats to 35 degrees.

On 27 August 2018, the ramp-up was started in accordance with the table below (Table 5), the table also describes the preliminary plan made for the entire ramp-up. In the ramp-up phase, the aerobic reactor was filled by adding carriers (10 mm in diameter) to 30 % of the reactor volume, 3 l of sludge from the municipal sewage treatment plant activated sludge plant and filling it with influent containing 30 % of the mining nitrogen

water and rest was a tap water. The influent was diluted because as such it was too concentrated for the nitrogen concentration to enter the process. Thus, the entire test run was performed with water with a TN of about 300 mg/l. The influent tank had a pH adjustment to 8.5 as automation. The nutrients were fed immediately after the influent tank before the first reactor and were fed under the same equilibrium conditions as already described in the theoretical part.

Table 5. Preliminary test plan for the ramp-up of the wastewater process under study.

Test week	Feed, l/d	HRT, pv	NH_4-N, mg/l	NO_3-N, mg/l	TN, mg/l
1	2,25	12,00	150	150	300
2	4,5	6,00	150	150	300
3	6,75	4,00	150	150	300
4	9,0	3,00	150	150	300
5	11,25	2,40	150	150	300
6	13,5	2,00	150	150	300
7	15,75	1,71	150	150	300
8	18,0	1,50	150	150	300
9	20,25	1,33	150	150	300
10	22,5	1,20	150	150	300
11	24,8	1,09	150	150	300
12	27,0	1,00	150	150	300

For the first two weeks, the ramp-up of the test run was kept at the same feed, 2.25 l/d. When the nitrification worked properly, the pH drops due to the formation of hydrogen and acids, and the pH was too low for the anaerobic step. The situation was rectified by installing an automatic pH adjustment in the control tank. During the third test week, the feed was increased to 4.5 l/d. At the same time, it was found that nitrification is unable to oxidize ammonium nitrogen at the target level. A review of the literature found that bacteria work more efficiently if ammonium nitrogen and nitrate nitrogen are in a 1:1 ratio in influent. This balance was taken into account from test week 5. Similarly, a separate carbon feed was added to the control tank, with nitrification using all the carbon added to the beginning of the process. In addition, the nutrient and carbon supply to the influent tank was converted between the influent tank and the aerobic reactor. After these changes, the test run could be normally continued as planned, lasting a total of 21 test weeks (during the Christmas break feeding was 16 l/d for two weeks).

Process analysis were performed on both the ramp-up phase and the actual test run, and Table 6 shows the used measurement equipment and methods. Of the measurements, pH, ORP, conductivity, dissolved oxygen and temperature, and TN and TOC were measured on average three times a week. The various forms of nitrogen, ammonium, nitrate and nitrite, and phosphorus were measured once or twice per week.

Table 6. Measurement methods and equipment used in process analysis.

Analysis	Unit	Equipment	Method
pH		WTYW pH 3110, Elektrode Sentix 41	SFS3021 (1979)
Redox potential (ORP)	mV	Hach Multi HQ40d, Intellical™ MTC101 ORP	Internal method
Conductivity	mS/cm	Hach Multi HQ40d	SFS-EN 27888 (1994)
Dissolved oxygen	mg/l	Hach Multi HQ40d	Internal method
Temperature	°C	Hach Multi HQ40d,	SFS3021 (1979)
Total Nitrogen, TN	mg/l	Analytic Jena Multi N/C2100S	Internal method
Total Organic Carbon, TOC	mg/l	Analytic Jena Multi N/C2100S	SFS-EN 1484 (1997)
Ammoniumnitrogen	mg/l	Hach Lange DR6000	LCK303/304 ISO 7150-1, DIN 38406 E5-1
Nitratenitrogen	mg/l	Hach Lange DR6000	LCK339 ISO 7890-1-2-1986, DIN 38405 D9-2
Nitritenitrogen	mg/l	Hach Lange DR6000	LCK342 EN ISO 26777, DIN 38405 D10
Phosphate phosphorus and total phosphorus	mg/l	Hach Lange DR6000, HACH Lange HT 200 S	LCK349 ISO 6878-1-1986, DIN 38405 D11-4
Alkalinity	mg $CaCO_3$/l	Automatic titrator TitraLab AT1000 seriers, centrifuge Thermo Scientific SL16R	SFS3005
Volatile Fatty Acids, VFA	mg CH_3COOH/l	Automatic titrator TitraLab AT1000 seriers,	Internal method

7.2 Load capacity of reactor technology

When the test run was at full capacity, the feed was 27 l/d and the retention time was 24 h per process unit, the loadability of the process technology could be calculated. Table 7 shows the amounts of nitrogen fractions fed to the nitrification. The first column shows the amount of total nitrogen per hour and per reactor cube. The next three columns calculate the amount of total nitrogen, ammonium nitrogen and nitrate nitrogen per reactor packing area and per hour. The values have been calculated as an example for 7 test weeks from the actual measurement results. Accordingly, Table 8 shows the corresponding values fordenitrification.

Table 7. Feeding amount of nitrogen to nitrification phase.

TN, $g/h/Rm^3$	TN, $mg/h/Rm^2$	NH_4-N, $mg/h/Rm^2$	NO_3-N, $mg/h/Rm^2$
11,90	72,10	30,81	33,84
7,88	47,73	37,60	42,17
8,96	54,29	35,86	38,01
11,31	68,54	31,31	34,60
13,75	83,34	30,56	43,52
14,82	89,82	29,92	53,62
14,20	86,09	36,04	46,21

Table 8. Feeding amount of nitrogen to denitrification phase.

TN, $g/h/Rm^3$	TN, $mg/h/Rm^2$	NH_4-N, $mg/h/Rm^2$	NO_3-N, $mg/h/Rm^2$
9,88	74,53	4,75	53,14
8,29	62,58	0,03	55,35
9,67	72,96	6,57	48,11
10,67	80,52	14,28	48,43
12,76	96,32	17,64	36,79
13,06	98,55	26,79	62,26
13,65	103,03	36,16	51,89

7.3 Results of adaptation

An examination of the results can be done by estimating the ramp-up phase, however, these cannot be considered as exantadic results because the process equipment is not run at full capacity. The reductions have been calculated from the stage of the test run when they can be reliably determined and the water to be purified has passed through the process equipment in its entirety.

Figure 5 shows the feed rate of the nitrification ramp-up phase and the reactor surface area with respect to ammonium nitrogen loading. Figure 7 corresponds to the values for the denitrification phase. The feed has been carefully started at the beginning, and during the first five weeks there were problems with the functioning of the microbiology. From week 6 onwards, the feed was steadily increased until week 17. Weeks 18-19 represent the Christmas break, when the feed has been such that the influent tank has had enough water over the holidays.

After this, the feeding has started to increase again and starting from week 22 it is the target 27 l/d. The maximum reactor load per square meter has been about 30 mg NH_4-N/h/Rm^2 in nitrification and about 60 mg NO_3-N/h/Rm^2 in denitrification. Values are calculated per hour and per influentflow.

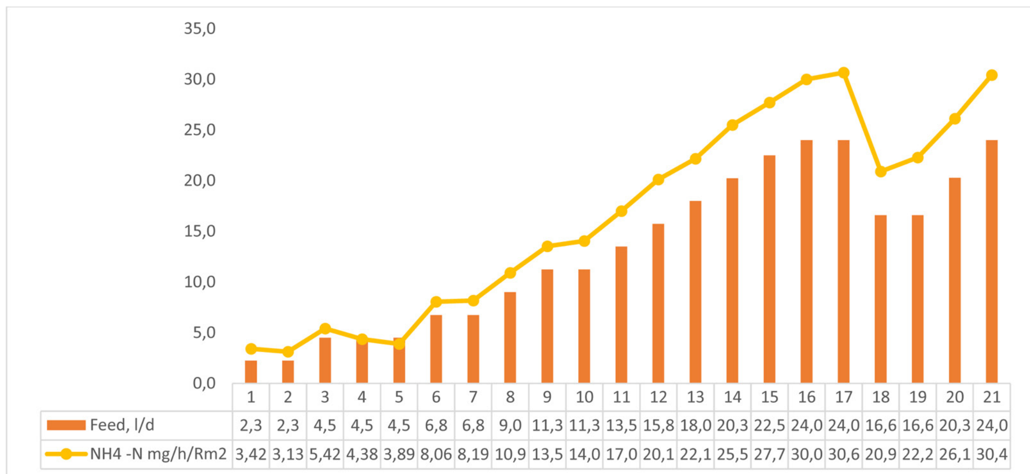


Figure 5. Load capacity and feed rate of the nitrification in ramp-up phase.

Ammonium nitrogen has been fed to the nitrification in a relatively steady stream at a concentration of about 150 mg/l (Figure 6, blue line). Nitrification should oxidize ammonium nitrogen as efficiently as possible through nitrite to nitrate. This is observed from the results from week 5 onwards. During the first weeks of the experiment, no optimization of the amount of carbon and no ammonium nitrogen-nitrite nitrogen equilibration were taken into account in the nitrification phase. When these were repaired in week 4, oxidation began to proceed as desired. Of course, not all of the ammonium nitrogen could be removed and some went through the process with reductions, currently over 90 % (NH_4-N).

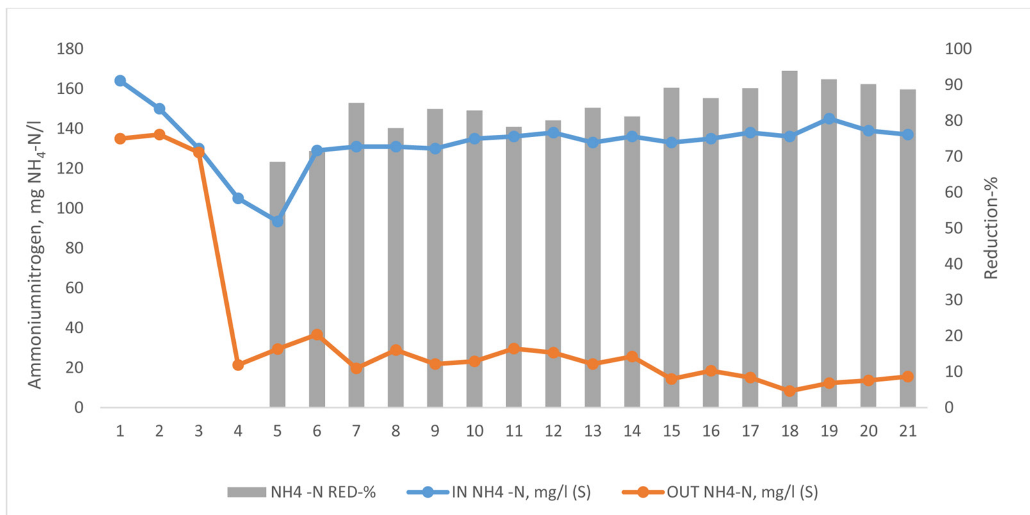


Figure 6. Amount of ammonium nitrogen in influent and effluent in the nitrification stage of the ramp-up phase and the reduction.

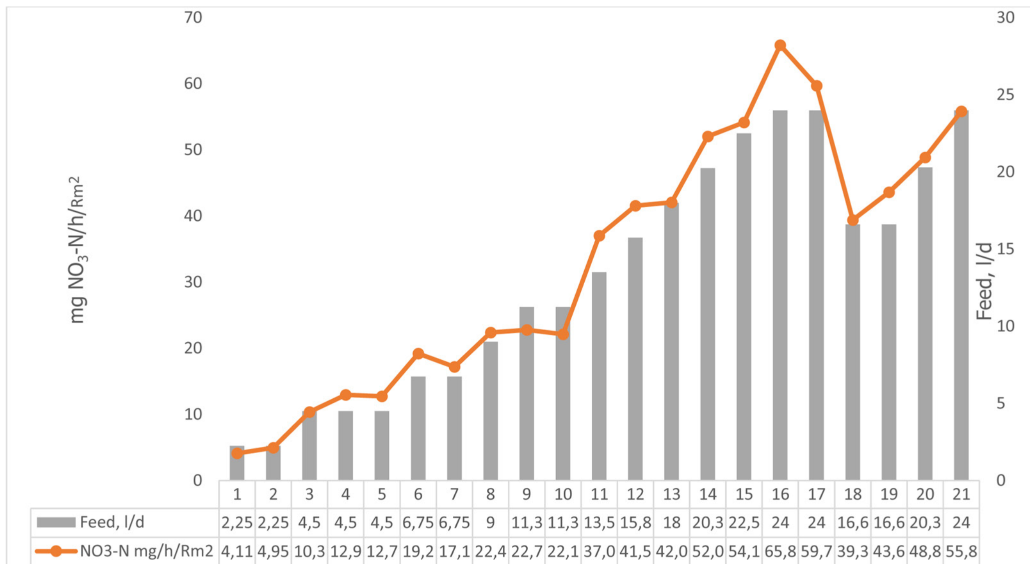


Figure 7. Load capacity and feed rate of the denitrification in ramp-up phase.

The amount of nitrate nitrogen in the denitrification phase has ranged from 150 to 250 mg/l (Figure 8, blue line). The range is caused by the reliability of the nitrification phase and the oxidation of ammonium nitrogen to nitrate. At week 5, a separate carbon feed to the denitrification phase (feed to the control tank) is introduced. From the results of the graph, it can be seen that from week 10 onwards, the reduction is almost complete.

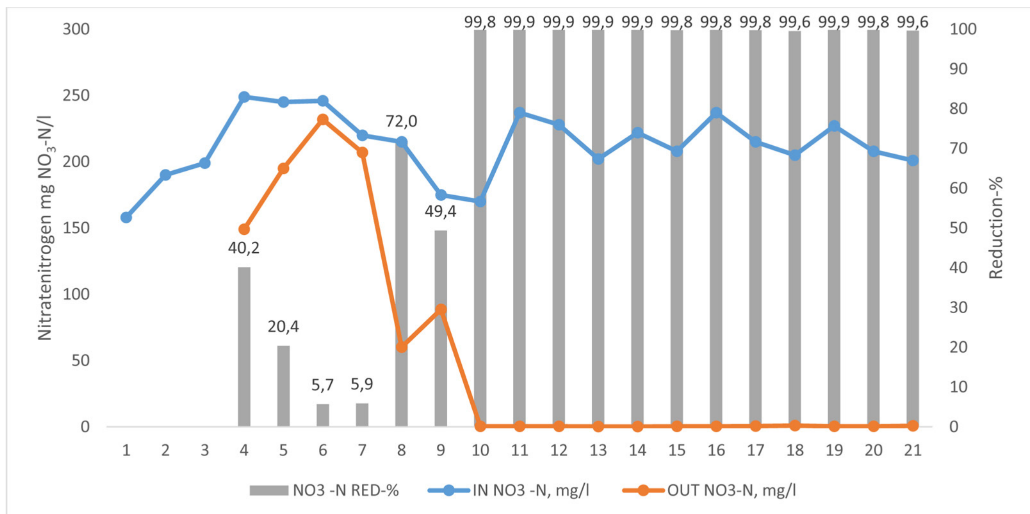


Figure 8. Amount of nitrate nitrogen in influent and effluent in the denitrification stage of the ramp-up phase and the reduction.

7.4 Results of laboratory-scale piloting

A test run after the ramp-up phase was performed for 22 weeks. The aim of the test run was to remove nitrogen as efficiently as possible and to have a retention time of 24 hours per process unit. In practice, this meant that nitrogen-concentrated mining water to be purified was fed to the process equipment per reactor volume, i.e. 27 l per day. Thus, the delay of one process unit became one day. The first eight weeks (weeks 1-8) test run was carried out with a target retention time, after which nitrification collapsed and a new ramp-up was initiated. The second ramp-up phase was carried out much faster than before (weeks 9-14), the denitrification still had a strong population, so only the nitrification had to be taken into account in the ramp-up phase. At week 15, the process equipment was run again at full efficiency. From week 16 onwards to week 20, the temperature dependence of the denitrification was tested by dropping every 5 °C at a time (35 °C → 20 °C) and at 20 °C the process was run for three weeks (weeks 18-20). This was followed by a two-week run (weeks 21-22) at full capacity at in optimal temperature range (35 °C).

This chapter first reviews the implementation of nitrification and the results obtained (7.4.1 Results of nitrification). The results of denitrification are then dealt with separately in a separate chapter (7.4.3 Results of denitrification). Examining the results also seeks to find possible correlations between different measurements. The composition of alkalinity and fatty acids from both microbiological stages has been examined (7.4.2 Fatty acids and alkalinity innitrification stage & 7.4.4 Fatty acids and alkalinity in denitrification stage). Finally, the elemental analysis of the sludge and water fractions formed in the test run is completed (end of the test) (7.4.5 Elemental analysis of the pilot and sludge composition).

7.4.1 Results of nitrification

4.5 m^2 was used as the surface area of the carriers in the nitrification reactor. Figure 9 shows the amount of nitrogen-concentrated water fed to the nitrification step per day (gray bar) and, for ammonium nitrogen, the reactor surface area loading per hour. The variation in the amount of feed shows the challenges posed by the nitrification step and the restarting of the process. The restart of the nitrification was carried out with the activated sludge of the municipal wastewater treatment plant filling about 10 % of the reactor volume and the rest was the water of the mining industry (diluted) to be examined. The reactor was aerated for 24 hours and then the feed was started with a flow of 9 l/d (week 8). The ramp-up has proceeded cautiously as shown in the figure, the dripping of the week 13 feed is due to the Easter break. The lower flow at weeks 17 & 18 is due to denitrification temperature testing and thereby impaired total nitrogen reduction, resulting in reduced feed. However, the nitrification stage has had a fairly uniform loading of the reactor surface area as ammonium nitrogen throughout the test run, about 30 mg NH_4-N/h/Rm^2.

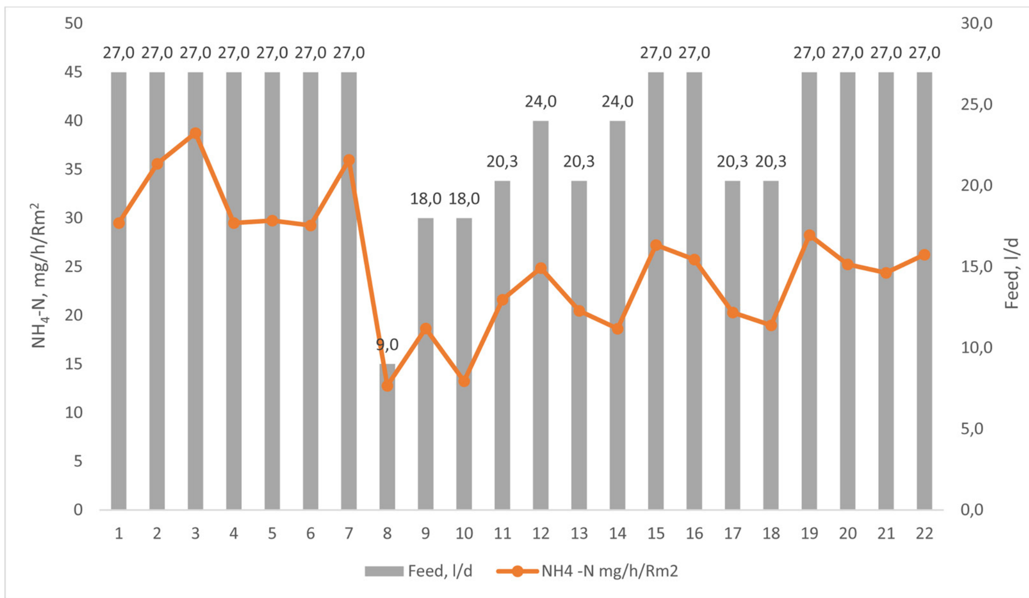


Figure 9. Laboratory pilot nitrification reactor surface load capacity and feed rate.

Correspondingly, Figure 10 shows the ammonium content fed in and out of the nitrification step and the calculated reduction percentage. The graph shows a relatively steady amount of ammonium nitrogen in the influent and a correspondingly decreasing percentage reduction from test week 2 to week 8. Looking more closely at the oxidation of ammonium nitrogen in nitrification at weeks 1-8 (Figure 11) a clear linear increase in ammonium nitrogen concentration in nitrification is observed from week 4 onwards. After the ramp-up phase, the operator had changed and the ammonium nitrogen - nitrite balance had not been taken into account with sufficient accuracy in the nitrification. In waters with a high nitrogen concentration, oxidation during nitrification does not occur without chemical equilibrium correction. The proportion of nitrate in nitrification should increase with the effective oxidation of ammonium nitrogen, the influent itself already contained 100-150 mg/l nitrate. As Figure 11 verifies, the nitrate nitrogen concentration in nitrification (R1) is above the influent level only at individual weeks.

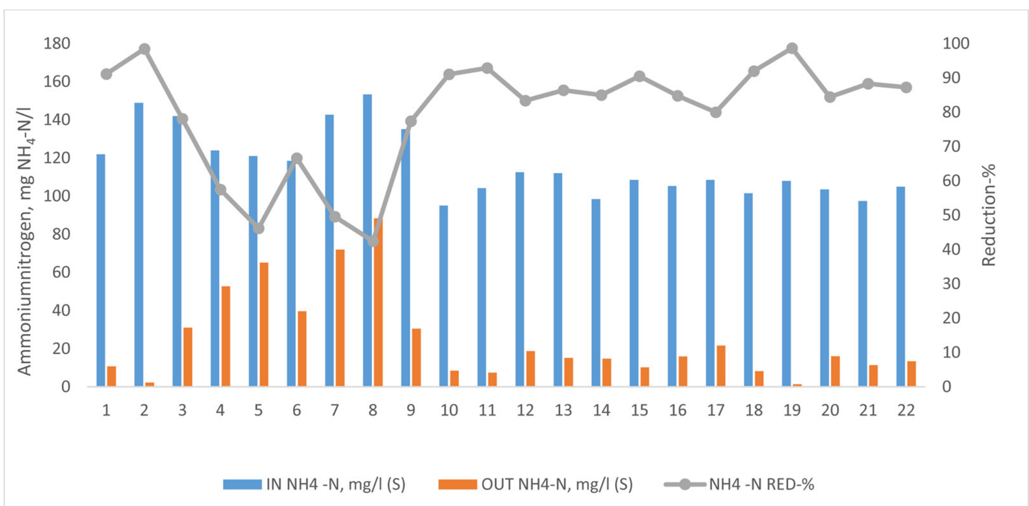


Figure 10. The amount of ammonium nitrogen in and out of the nitrification stage of the laboratory piloting and the calculated reduction percentage.

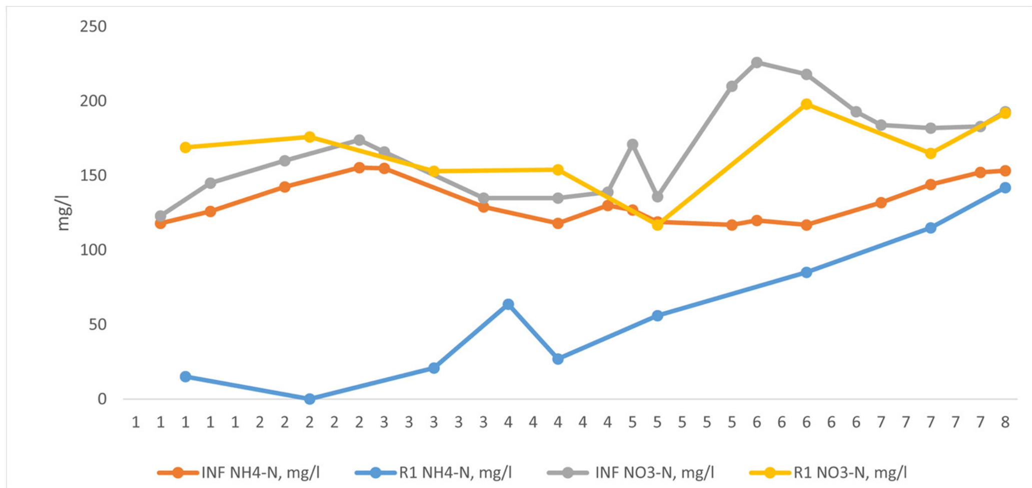


Figure 11. Ammonium and nitrate nitrogen concentrations for influent and nitrification for the first 8 weeks.

Similarly, looking at the progress of nitrification after week eight (Figure 12), it has been able to operate relatively smoothly (compare Figure 10 reduction uniformity). In nitrification, oxidation takes place efficiently: nitrate nitrogen is higher in nitrification than it is in influent, and ammonium nitrogen is oxidized from 100-150 mg/l to 0-50 mg/l.

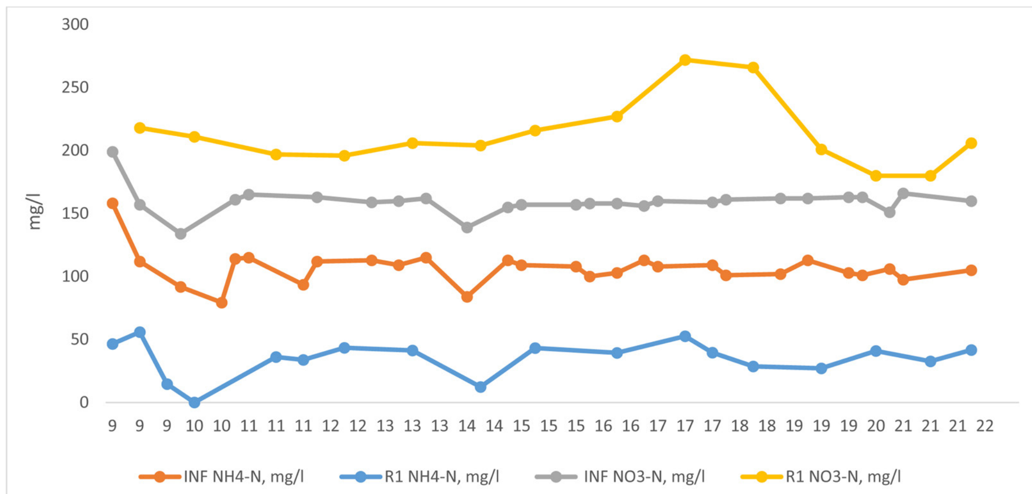


Figure 12. Ammonium and nitrate nitrogen concentrations for influent and nitrification for weeks 9-22.

When looking at the dependence of two measurements using calculus of probability, it is stated that when the correlation coefficient is 1, there is a complete linear dependence between the variables. Similarly, -1 means a complete negative dependence. The more the correlation coefficient deviates from zero, the stronger the dependence between the variables. The correlation can be found to have a dependence between two variables when it is at least 0.65. Dependence should be considered in the long term so that it can be considered reliable. The review has been performed for both the ramp-up and the laboratory pilot test weeks (43 weeks).

The following figures (Figure 13 - Figure 16) show the dependence of the oxidation of ammonium nitrogen in the nitrification step on other determined parameters, i.e. pH, temperature, conductivity and redox potential. The only clear relationship can be verified between ammonium nitrogen and temperature (Figure 16).

According to the literature review, it would be possible to indicate the efficiency of nitrification and the oxidation of ammonium by measuring the conductivity. The conductivity measurement was more advantageous to implement and it would be possible to obtain a correlation curve between these two variables and a corrective formula to be derived from it. However, this requires that the correlation must first be considered over a longer period of time and must be kept constant in order for comparability to be established. Based on the measurements (Figure 14), it has not been possible to analyze a clear cause-and-effect relationship as to why a dependency relationship between the two measurements is not found. On the other hand, the test run has not progressed steadily, which may have contributed to the issue.

The redox potential is another important low-cost measurement that should be above +50 mV in the aerobic process. When measuring the redox potential, the electrical potential required to transfer electrons from one substance to another (oxidant to reductant) is measured. Redox reactions are equilibrium reactions that do not go spontaneously and completely in either direction. By influencing the reaction conditions (concentrations of substances, temperature and pH), oxidation-reduction reactions can be controlled. In nitrification, this means an increase in the redox potential and a shift in the equilibrium of the reaction towards the oxidizing components (conversion of ammonium via nitrite to nitrate). Similarly, once the redox potential has risen to a high level, biodegradation activity no longer occurs because biodegradable, reducing compounds no longer exist. With respect to the redox potential, the oxidation of ammonium nitrogen remains at a really low level of dependence at only 0.2 (Figure 15).

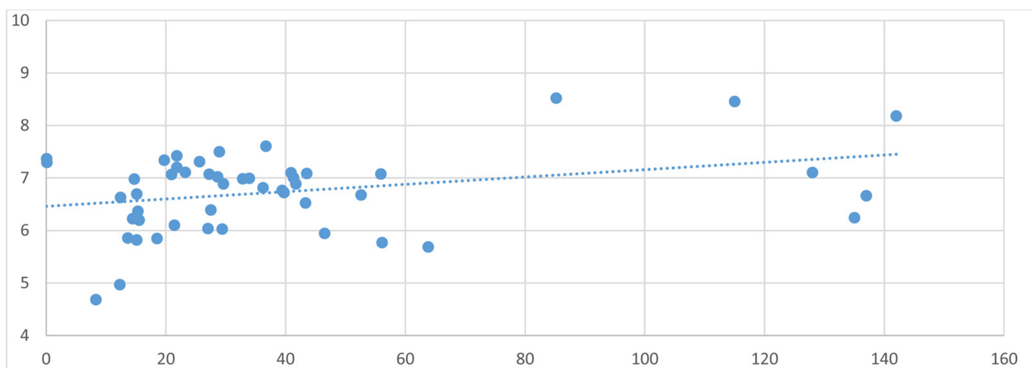


Figure 13. Nitrification ammonium nitrogen & pH, correlation coefficient 0.32.

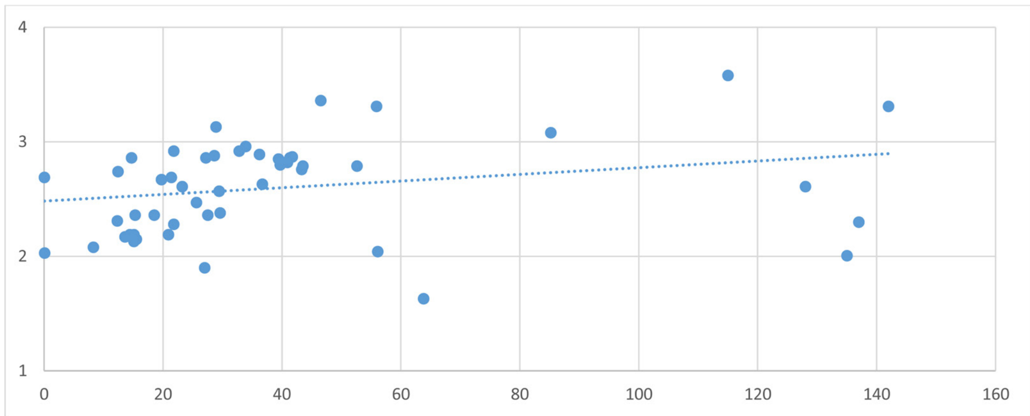


Figure 14. Nitrification ammonium nitrogen & conductivity, correlation coefficient 0.25.

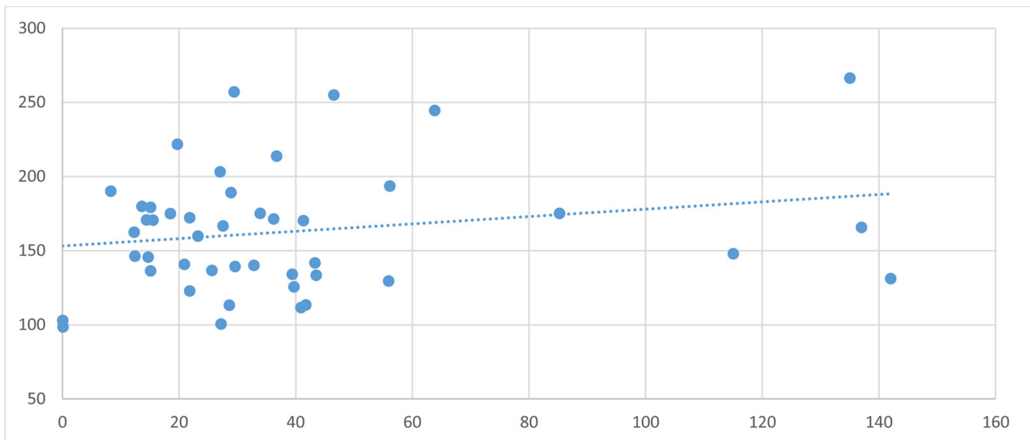


Figure 15. Nitrification ammonium nitrogen & redox potential, correlation coefficient 0.20.

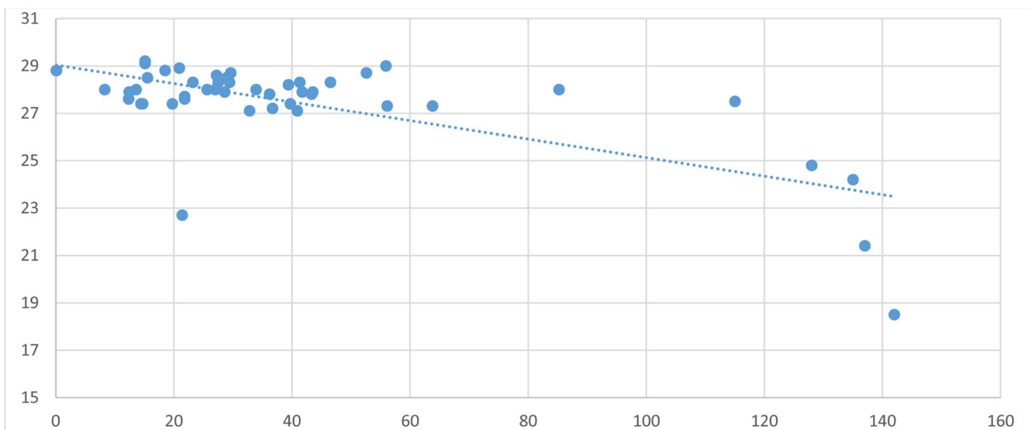


Figure 16. Nitrification ammonium nitrogen & temperature, correlation coefficient 0.69.

7.4.2 Fatty acids and alkalinity in nitrification stage

Alkalinity describes the buffer capacity of a process, ie its ability to resist a change in pH, and is expressed as the equivalent amount of calcium carbonate (mg $CaCO_3$/l). In addition to hydroxides, carbonates and bicarbonates, alkalinity is caused by silicates, phosphates, borates, arsenates and aluminates.

Similarly, volatile fatty acids (Volatile Fatty Acid, VFA) are produced by acid-forming bacteria. The phenomenon can be verified as a rapid change in pH to a detrimental low, resulting in impaired bacterial function and even the cessation of the process altogether. The assay method determines the amount of all volatile fatty acids present at a given point in time, expressed as the equivalent amount of acetic acid.

Alkalinity and fatty acids were monitored from the nitrification step in the test run during weeks 15-22. From the point of view of the optimal operation of the process, the alkalinity after nitrification should be at least 50 mg/l. No corresponding limit value has been defined for fatty acids. Figure 17 shows the results of nitrification alkalinity and fatty acid determinations and their ratios. Nitrification is divided into two reactions, the first (Equation 16) being ineffective and the latter (Equation 17) even less efficient.

Equation 16. 34 mol $NH_4^+ \rightarrow$ 1 mol CO_2 $\Delta G° = -267{,}5$ kJ/mol

Equation 17. 100 mol $NO_2^- \rightarrow$ 1 mol CO_2 $\Delta G° = -87$ kJ/mol

According to the Figure 17, the alkalinity is fairly uniform, generally above 50 mg/l from test week 18 onwards. In weeks 15-16 and 19-22, the retention time was 24 h. For weeks 17 and 18, the retention time was 20.3 h, ie the feed was slightly lower.

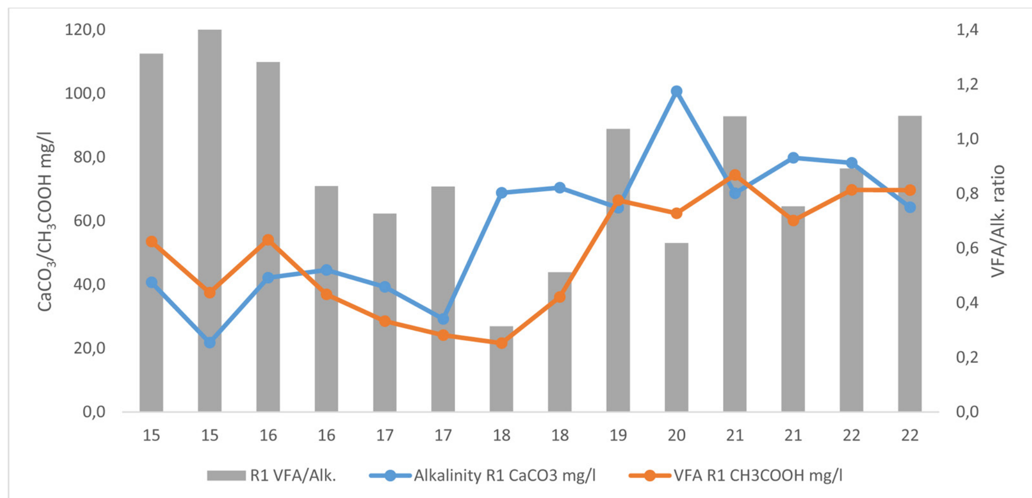


Figure 17. Concentrations of alkalinity and volatile fatty acids after the nitrification step and their ratio.

7.4.3 Results of denitrification

In denitrification, the reduction of nitrate took place efficiently already in the first anaerobic stage, so that no more nitrogen remained in the second reactor to be purified. The results of the latter denitrification step are thus not discussed in this report. The used surface area of the carriers was 3.6 m^2 in the denitrification stage. Figure 18 shows the amount of water from the nitrification fed to the denitrification step per day (gray bar) and, for nitrate nitrogen, the reactor surface loading area per hour. The variability in the amount of feed comes directly from the challenges posed by the nitrification step and the restart of that process step at week 8. The denitrification step did not need to be restarted at any point during the test run. In the graph, the drip of the week 13 feed is due to the Easter season and the lower flow of the weeks 17 & 18 is due to the temperature testing of the denitrification step and thereby the reduced reduction of total nitrogen, whereby the feed was reduced. However, for the denitrification step, the loading of the reactor surface area as nitrate nitrogen has been fairly even throughout the test run, about 60 mg NO_3-N/h/Rm^2.

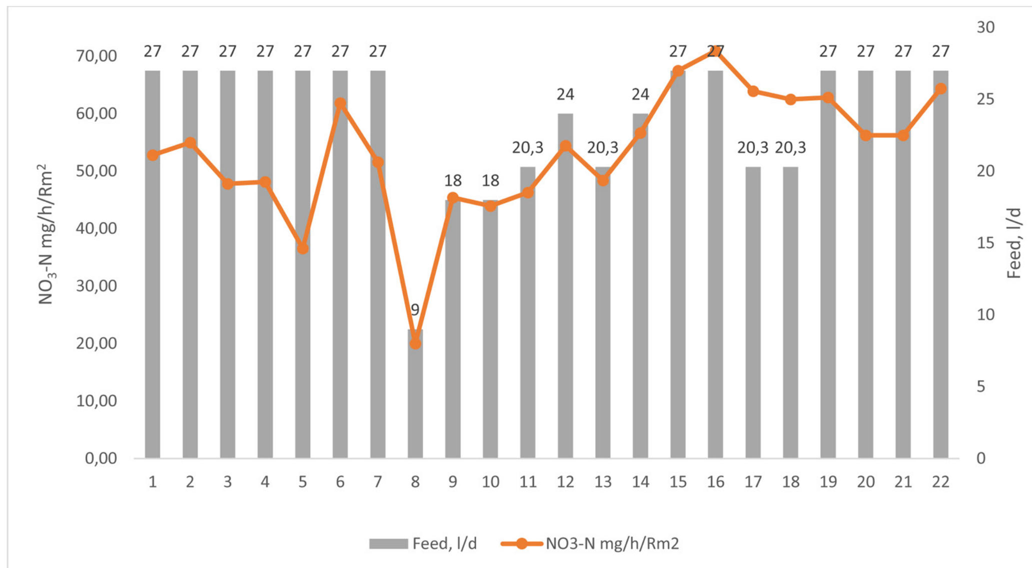


Figure 18. Laboratory pilot denitrification reactor surface load capacity and feed rate.

Correspondingly, Figure 19 shows the nitrate content of the water fed into and out of the denitrification step and the calculated reduction percentage. The graph shows the relatively uniform amount of nitrate nitrogen in the water after nitrification (denitrification influent, marked in the graph IN NO_3-N) as well as the really efficient operation of the process. At weeks 9 and 10, nitrate reduction has not been fully effective. This reflects the failure and restart of nitrification at week 8, when there has been less feed to the denitrification step. In this case, the microbes have fallen into a dormant state and awakened and reactivated as the feed increases from week 9 onwards.

In week 21, there is a momentary collapse in denitrification, from which the process phase recovers very quickly. During weeks 15-20, an attempt was made to drip the temperature in denitrification, Table 9 shows the changes in temperature for those weeks. At week 16, a very small change in nitrate reduction was observed. As a result, the feeds for

weeks 17 and 18 were slightly reduced, but in week 19 the feed was again at the normal target level. At the beginning of week 21, the temperature was raised back to 35 degrees from 20 degrees, which is seen in that week as a momentary decrease in the reduction. After this, the microbes are again accustomed to the new temperature range.

Table 9. Denitrification phase temperature tests for weeks 15-22.

Week	Days	Feed, l/d	HRT, h	Temperature, °C
15	29.4.-5.5.2019	27,0	24,0	35,0
16	6.-12.5.2019	27,0	24,0	30,0
17	13.-19.5.2019	20,3	32,0	25,0
18	20.-26.5.2019	20,3	32,0	20,0
19	27.-2.6.2019	27,0	24,0	20,0
20	3.-9.6.2019	27,0	24,0	20,0
21	10.-16.6.2019	27,0	24,0	35,0
22	17.-23.6.2019	27,0	24,0	35,0

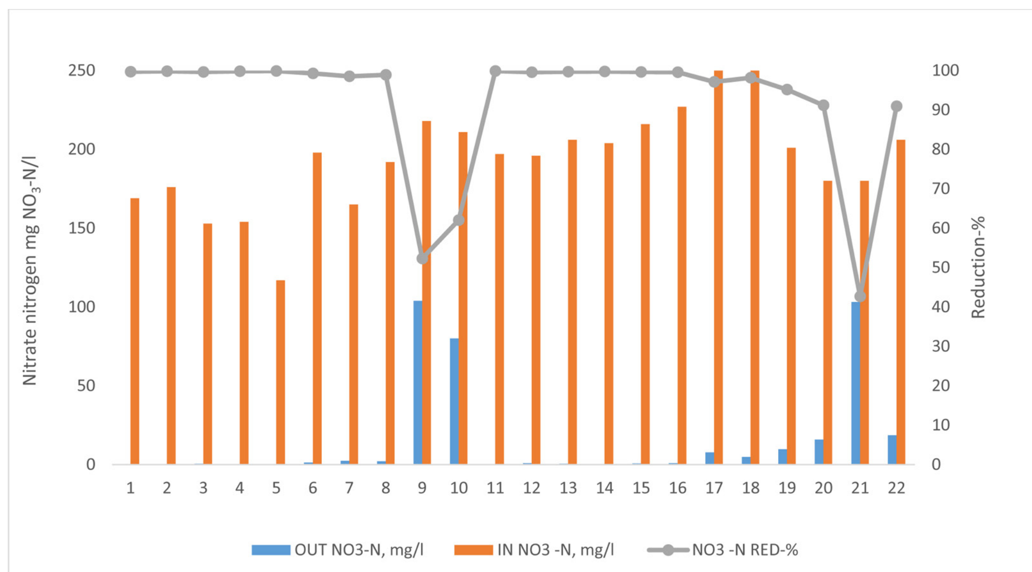


Figure 19. The amount of nitrate nitrogen in and out of the denitrification step of the laboratory pilot and the calculated reduction.

For denitrification, calculations of probability were similarly performed between two measurements for the entire test run, i.e., ramp-up and laboratory piloting, for a total of 43 weeks. The following figures (Figure 20 - Figure 23) show the dependence of the nitrate nitrogen reduction of the denitrification step on the Redox potential, conductivity and pH. A very clear relationship between nitrate nitrogen reduction and redox potential and a weak relationship between pH and conductivity can be verified. According to the literature, the redox potential in denitrification should be between +50 mV and -50 mV, however, in the implemented test run it was evenly at the level of - 400 mV.

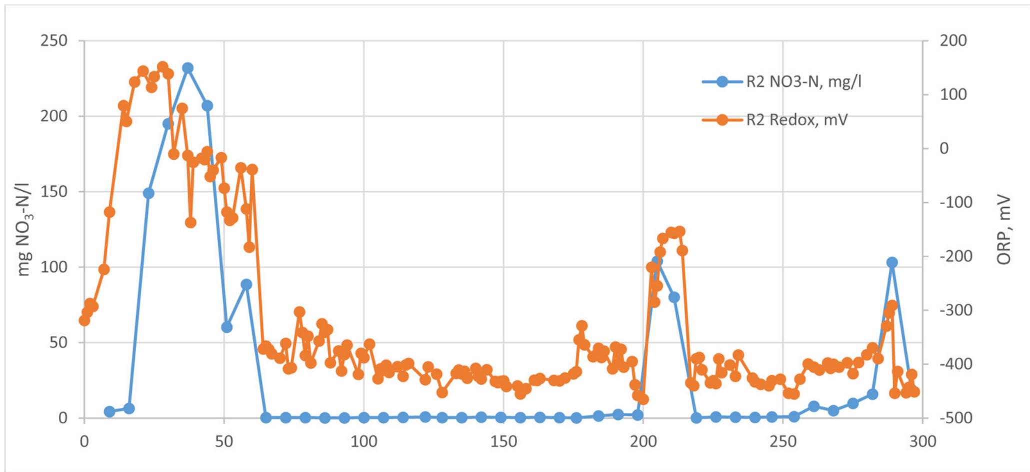


Figure 20. Measurements of denitrification nitrate nitrogen and redox potential for 297 measurements day.

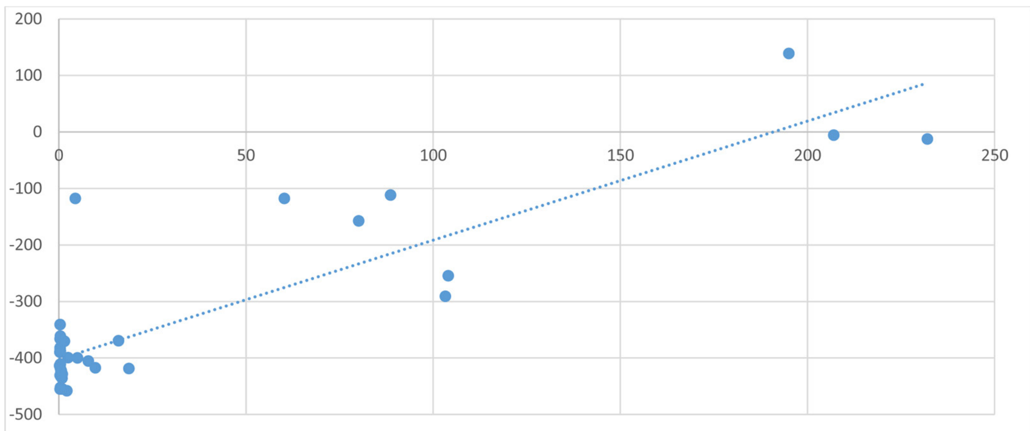


Figure 21. Linear dependence of nitrate nitrogen and redox potential for denitrification, correlation coefficient 0.87.

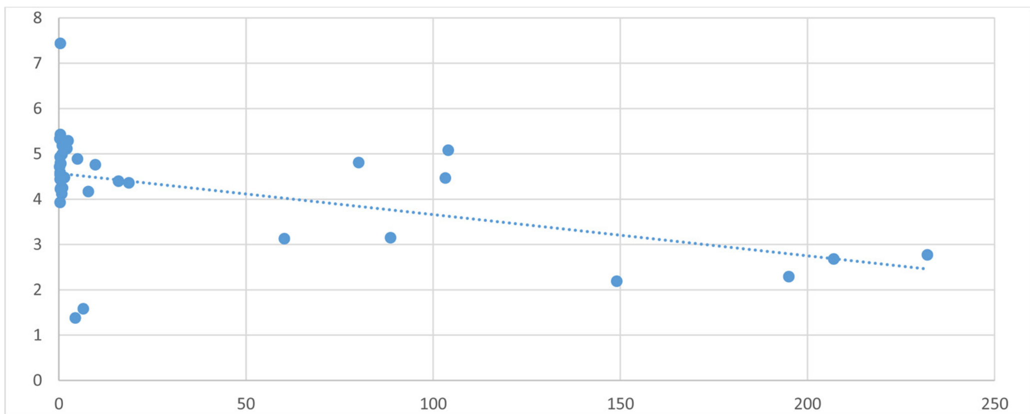


Figure 22. Linear dependence of nitrate nitrogen and conductivity for denitrification, correlation coefficient 0.51.

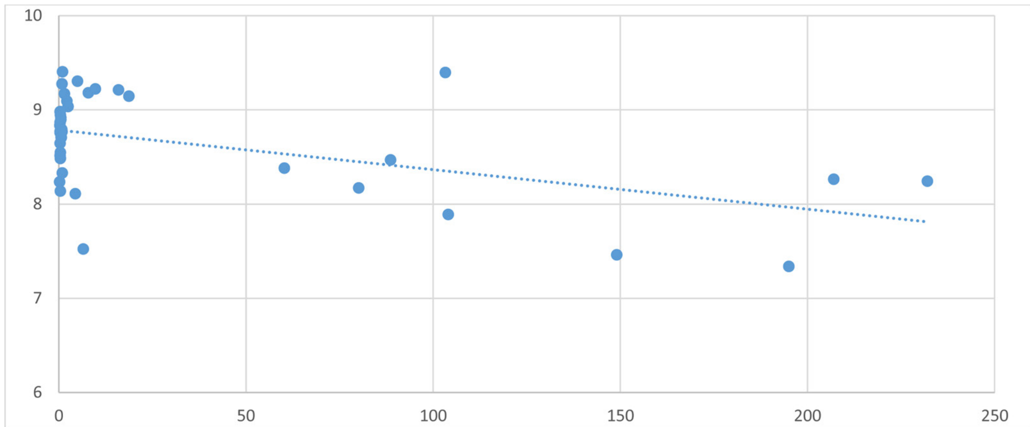


Figure 23. Linear dependence of nitrate nitrogen and pH on denitrification, correlation coefficient 0.51.

7.4.4 Fatty acids and alkalinity in denitrification stage

Fatty acids and alkalinity were similarly determined for the denitrification step during weeks 15-22, the results of the assay can be seen in the following figure (Figure 24). When first looking at the alkalinity in the denitrification step, it is found to be very uniform in the order of about 2.500 mg/l, i.e. very high. It is known that denitrification microbes are able to operate in a very wide pH range of 6.5-9. As one of the products of microbial metabolism in the denitrification step, bicarbonate and carbon dioxide are released into the process while the microbiology is operating efficiently. These react with the carbon source sodium acetate to form carbonate in the process. This explains the high value of the buffer capacity of the process on the one hand and the constant value on the other hand. Correspondingly, the concentration of fatty acids varies on average between 200-300 mg/l and is very constant throughout the series of measurements. When microbiology works effectively, microbes can take advantage of the fatty acid released while releasing soluble phosphate into the liquid phase. Looking at the ratio of these two parameters, it is found to be below 0.2 at all times. This, too, favors microbiological activity.

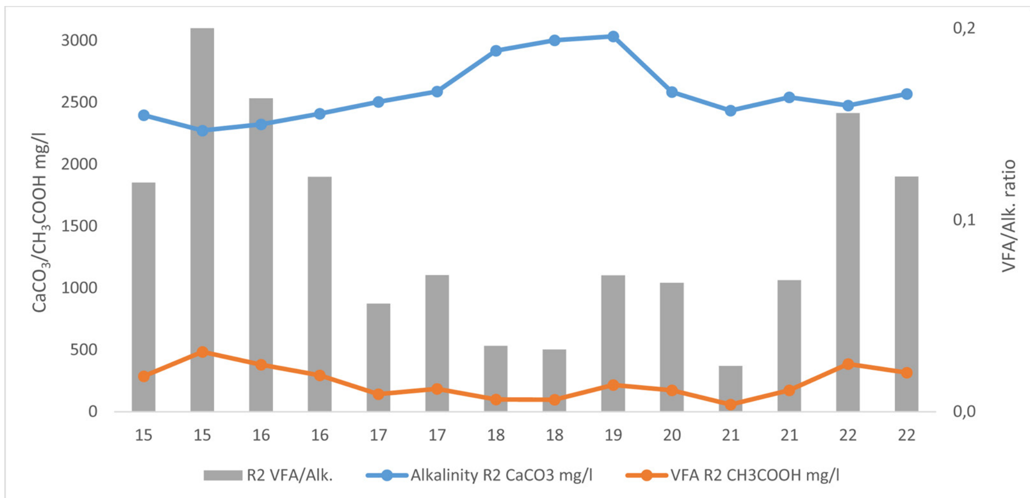


Figure 24. Concentrations of alkalinity and volatile fatty acids of the denitrification step and their ratio.

7.4.5 Elemental analysis of the pilot and sludge composition

The chemical composition of the feeding water was previously shown in Table 4. From research point of view is important to understand which trace elements and nutrients are essential for bacteria and what should be added to the process. Table 10 shows the chemical composition of liquid phase and sludge of the control tank and denitrification at the end of the 43 weeks long test run. In table added nutrients is marked in blue, identified inhibitors in red and boosters in purple. Sodium comes from carbon source (Sodium acetate) and potassium from phosphate source (Potassium hydrogenphosphate).

From the booster's aluminium and iron level is increased to 30 mg/l in influent. It can be seen from the results that bacteria can take trace elements advantages of its growth and small pieces of those metals end up to sludges. Calcium was not added to influent, its level was naturally adequate in the water. The sodium content increases strongly from the carbon source (up to 250 times) and is also deposited in the sludge to some extent. Similarly, bacteria are able to utilize the amount of added phosphorus. Potassium also comes with this chemical, but its level does not change much during the process, ie it can be utilized by bacteria.

Influent water consist little amounts of inhibitors chrome, cupper, nickel and zinc. Only the zinc can be identified in very small quantitys. In the influent zinc is level 8 mg/l, at the end of the process it is 0.3 mg/l. The level of inhibition is 0.3 mg/l according to the literature. However, the inhibition condition was not met in this testrun.

Table10. Liquid phase and sludge chemical composition of control tank and denitrification phase (sample analyzed by ICP-MS at the University of Eastern). Liquid results expressed in mg/l and for sludge per dry matter. Inhibitors marked on red, boosters at purple and added substances at blue.

Liquid phase	Na 23 (g/L)	Mg 24 (mg/L)	P 31 (mg/L)	K 39 (mg/L)	Ca 44 (mg/L)	Al 27 (µg/L)
Control tank	1,2	10,6	0,4	25,2	8,0	329,7
Denitrification, R2	1,3	11,4	3,4	21,7	3,8	61,7
Liquid phase	Mn 55 (µg/L)	Fe 56 (µg/L)	Co 59 (µg/L)	Zn 66 (µg/L)	Se 82 (µg/L)	Mo 98 (µg/L)
Control tank	6,7	13,6	0,9	27,5	4,9	2,9
Denitrification, R2	82,0	10,7	1,6	17,1	7,6	6,2

Sludge	Na 23 (g/kg)	Mg 24 (g/kg)	P 31 (g/kg)	K 39 (g/kg)	Ca 44 (g/kg)	Al 27 (g/kg)
Control tank	31,6	10,4	6,2	<1	332,6	1,6
Denitrification, R2	35,2	3,3	4,2	<1	333,9	1,8
Sludge	Mn 55 (g/kg)	Fe 56 (g/kg)	Co 59 (g/kg)	Zn 66 (g/kg)	Se 82 (g/kg)	Mo 98 (g/kg)
Control tank	2,8	1,9	0,1	3,9	0,0	0,0
Denitrification, R2	2,4	8,0	0,1	5,4	0,1	0,0

Metal concentrations of the water samples were analysed by inductively coupled plasma mass-spectrometry (ICP-MS) using a NeXION 350D ICP-MS instrument (PerkinElmer Inc., Waltham, MA, USA) equipped with ESI PrepFAST autosampler (Elemental Scientific, Omaha, NE, USA). Elements were determined against certified multi-element calibration standard (TraceCERT Periodic Table Mix 1, Sigma Aldrich) in acid conditions (1.7 % HNO3, TraceMetal™grade, Fisher Chemical). Calibration range used for Na, Mg, K, Ca, Fe and Al was 4-400 µg L^{-1} and 1-100 µg L^{-1} for other elements. Three replicates were obtained

for each sample. The data was processed using PerkinElmer Syngistix Data Analysis Software™. Before ICP-MS analysis water samples were diluted into calibration range with conc.

8 CONCLUSION OF MICROBIOLOGICAL NITROGEN REMOVAL

The aim of the research project was to gain extensive experience in the microbiology, chemistry and technical functionality of the ND process laboratory scale piloting. In the test run, nitrogen-concentrated wastewater from the Finnish mining industry was tested in a long-term test run lasting 43 weeks, simulating the MBBR technology. The aim of the processes was to reach a retention time of 24 hours per process unit. The main goal was to operate in the optimal temperature range and to test the temperature dependence for denitrification.

The test run was started with the inoculum growth phase in the denitrification phase, after which the actual ramp- up phase was started. A viable and efficient bacterial strain is the lifeblood of a microbiological application. The strongest population is obtained by growing from an original source, such as mining water or ditch sediment. Denitrification was successful in increasing the population when it was done slowly over two months and the population functioned effectively throughout the experimental run. Similarly, the nitrification population was taken from the municipal wastewater plant (activated sludge).

In the ramp-up phase, the process was carefully run up, lasting 21 weeks. In the case of nitrogen-concentrated waters, the proportion of ammonium nitrogen in the water was quite high concentration that the bacteria could not completely oxidize it. When nitrite-ammonium nitrogen equilibrium 1: 1 was introduced in ramp-up week 5, the oxidation worked efficiently. From the ramp-up, it can be seen that the nitrification is the neck of the flask and the reliability of the denitrification can be ensured by a sufficiently long adaptation step. During the ramp-up phase, the importance of monitoring analytics is also emphasized to ensure the desired reactions. In addition, the redox potential alongside the pH measurement acts as a guiding parameter/measurement.

Based on the results, it can be observed that the nitrification of the population is significantly more challenging to manage. The microbiology of nitrification collapsed a couple of times during the test run, but on the other hand, we also learned how nitrification can be ramped-up in a few weeks. When evaluating the results of the nitrification step, it is found that the load is of a relatively high order per carrier area (30 mg NH_4-N/h/Rm^2) when the reactor area was used as a capacity of 4.5 m^2. The results of Figure 10 show the reduction of ammonium nitrogen. Immediately after the first week of the experiment, the reduction was found to gradually weaken and eventually completely collapse at week 8. This is purely due to the fact that the chemical nitrogen balance was not taken into account and the bacteria were unable to oxidize all the ammonium nitrogen (Figure 11). In addition, carbon dosing was not optimal. Correspondingly, from test week 10 onwards, the process reduction has been relatively steady and always at least 60 percent. Figure 12 illustrates the uniformity of nitrification and the efficiency of operation. The figure shows all measurements from those weeks, measurements 2-3 times a week. When oxidation works effectively, ammonium nitrogen is oxidized to nitrate and thus the nitrate level of nitrification is higher in nitrification than in influent.

The results of the nitrification were statistically examined by means of probability calculations. No clear cause-and-effect relationship was obtained between measurements, the only clear relationship could be found between nitrification and temperature. The review was performed for all 43 weeks of all measurements. The results were partly influenced by the implementation of the measurements (some of the measurements were performed on-line and some a few times a week) and the measurement was not concurrent.

The amount of alkalinity and volatile fatty acids was estimated for experimental weeks 15-22. The alkalinity in the nitrification section should be at the level of 50 mg/l to ensure sufficient buffer capacity. Looking at the entire assay interval, it is observed that during test weeks 15-22, the influent ammonium nitrogen load has been very even at 30 mg NH_4-N/h/$Rm^2$and there are no large differences in the reductions between the weeks. The small difference between alkalinity and fatty acids is supported by the fact that some microbes can accumulate in the process, which reduces their movement and slows down the respiration of the cells. Nitrification energies are part of aerobic conversion.

The reliability of denitrification throughout the test run was really high and the importance of the long adaptation phase is emphasized when interpreting the results. Denitrification was loaded with about 60 mg NO_3-N/h/$Rm^2$and the surface area of the carriers was 3.6 m^2. The denitrification was two-stage comprising two successive reactors, but in practice the first reactor reduced the nitrate so efficiently that no nitrogen remained on the second one. In the reduction of denitrification (Figure 19), a collapse is observed at test weeks 9 and 10, which is directly due to the restart of nitrification at week 8. The second drop is in week 21, which is explained by raising the temperature back from 20 degrees to 35 degrees.

Temperature testing (Table 9) was tested over weeks 15-21 by dropping the temperature 5 degrees per week. A very small reduction was observed at week 17, when the feed was reduced and the retention time was increased to 32 hours from 24 hours. Thereafter, at week 18, the temperature was dropped to 20 degrees and the feed was raised back to the target daily retention time. At week 21, the next change was made and the temperature was raised back to 35 degrees and this large temperature change is directly reflected in the reduction. As a whole, it can be stated that the microbes adapt well to the change once a week, and the increase in the retention time compensates for the fluctuation of the reduction. The rate of denitrification always increases above 5 ° C and oxygen is less soluble in warmer than cold water.

For denitrification, a cause-and-effect relationship was similarly sought by examining probability calculus. A very clear relationship could be observed between nitrate reduction and redox-potential. Correspondingly, a weak dependence was seen with conductivity and pH as reference values. In the denitrification of the test run, the Redox potential was in the order of steady -400 mV. The nitrites and nitrates produced in the nitrification diffuse and migrate with the water column wave movements into oxygen-free water layers, where they act as substrates for the denitrification process to remove nitrogen. The occurrence of heterotrophic denitrification is regulated by the availability of organic carbon and nitrate. Although denitrification is an anaerobic process, it is dependent on the substrates produced by the aerobic nitrification process. The efficiency of denitrification is regulated, inter alia, by the ratio of organic carbon to nitrate in the environment. When the ratio of organic carbon to nitrate in the environment is low, denitrification dominates

the reduction processes, which can be seen from the reduction potential measurements. This measurement also indicates population activity.

The alkalinity in the denitrification step was in the uniform order of 2,500 mg/l, i.e. a very high level. As one of the products of microbial metabolism in the denitrification step, bicarbonate and carbon dioxide are released into the process while the microbiology is operating efficiently. These react with the sodium acetate, the carbon source, to form carbonate in the process. This explains the high value and the constant value of the buffer capacity in the process. Correspondingly, the proportion of fatty acids was in the order of a uniform 200 mg/l. As a result, this means that the microbes have utilized the acids formed as food and have not deposited in the reactor.

Element concentrations were evaluated for the aqueous phase control tank and denitrification as well as sludges. Iron and aluminum were fed into the microbiological process as "boosters" to accelerate granulation on the surface of the carriers. Calcium is considered such a booster too, but it was naturally abundant in water and there was no need to add it. As can be seen from the results in Table 10, the boosters have been well utilized and there is almost no residue in the aqueous phase. In the control tank, the concentrations are slightly higher, but naturally the population of denitrification has taken advantage of them while still operating. Similarly, when examining the same values for sludge, it is found that calcium is more abundant in sludges (a few hundred grams), but aluminum and iron are only in the order of a gram.

Phosphorus was also fed to the process as potassium hydrogen phosphorus, so potassium also was added into the process. The results in Table 10 show that phosphorus has been used as a nutrient and potassium has also been able to be utilized as microbes grow and replicate themselves. Concentrations in sludge are also very moderate, with potassium remaining below one gram and phosphorus even a few grams.

Of the inhibitors, only zinc is naturally present in mining water in the order of about 8 mg/l, which exceeds the limit of 0.3 mg/l in the literature. Based on the results, it is observed that microbes have also used zinc in part and its share in the aqueous phase of the end of the experiment remains smaller than in the initial situation. There are a few grams of zinc in the sludge. Zinc had not beed inhibit the microbiological process metabolism at any time of the testrun.

Of the trace elements, manganese, cobalt, selenium and molybdenum were added to the process, the concentrations of which were also evaluated at the end of the test run. The concentrations in the water fed to the process were raised to 30 mg/l. The results in Table 10 show that the concentration in the aqueous phase is at the microgram level, so trace elements are necessary to treat nitrogen-concentrated waters and to ensure process efficiency. There is almost no trace elements in the sludge.

In conclusion, the results of the test run, the reduction for total nitrogen was more than 90 % throughout the test run, as shown in Figure 25. Especially from test week 11 onwards, a very stable result is seen in nitrogen reduction. For these weeks, the nitrogen in the feedwater has also been in a more even order than at the beginning of the experiment.

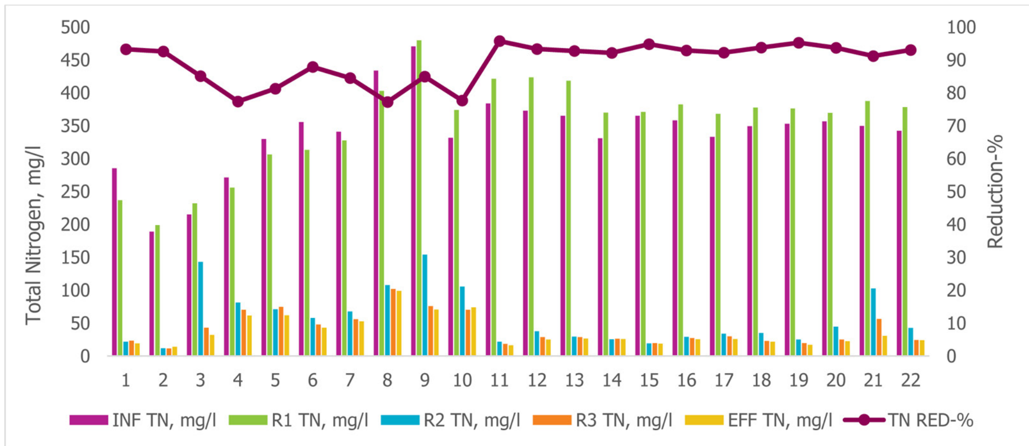


Figure 25. Total nitrogen concentration levels at different process stages for the total test run period.

In nitrogen-concentrated waters, nitrification requires a chemical balance of nitrite-ammonium nitrogen. Maintaining microbiological activity also requires consideration of an external carbon source as well as the TN:TP:C balance. These can ensure the natural growth of microbial mass. However, care must be taken with regard to the carbon source, as autotrophs can easily displace heterotrophs. Nitrification is dramatically more sensitive to microbiological changes, so it could be two phases for the future. The mixing of the reactors is an important part of the controllability of the whole process. If the microbes are allowed to accumulate and concentrate in the process, their movement is reduced and the respiration of the cells is slowed down.

REPORT PART B:

MICROBIAL REDUCTION OF SULFATE FROM MINING WATER

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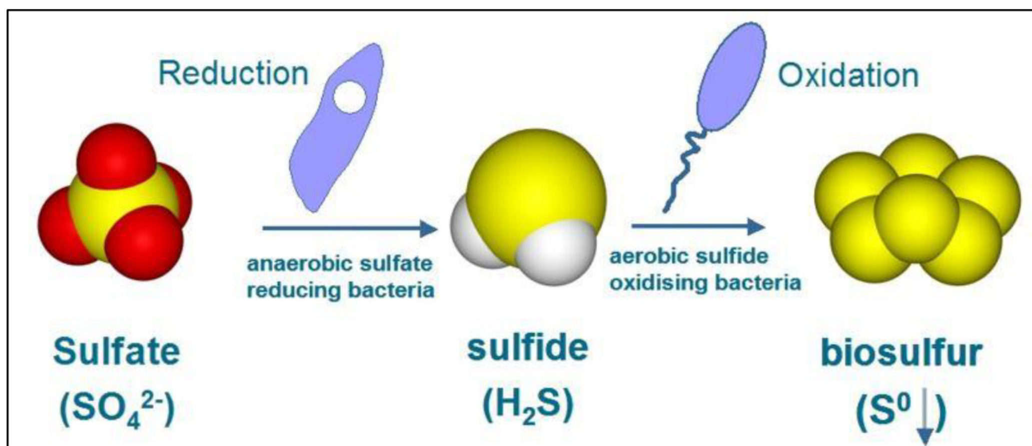


9 SULFATE RICH WASTEWATERS AND TREATMENT TECHNOLOGIES

One of the biggest problems in mining industry is acid runoff. These are released when soluble sulfur-containing compounds and dissolved metal sulfate salts are released from the soil together with either the water used in the process or the rainwater from the open pit, mine and side rock or tailings treatment plants. When sulfur compounds are oxidized and with the water used for the operation, sulfuric acid is formed, which in turn dissolves metals in the rock and causes the pH of the runoff to drop. Acidic runoff contaminates both waterways and soil without treatment. The soil, which has lost its buffering capacity as a result of acidification, also lets in metal sulfides, which accumulate in sediments and pollute groundwater reserves (Cole, 2011).

In the DeepCleanTech -project, a laboratory-scale test run based on the sulfate reduction process, duration 9.10.2018-29.11.2019, has been implemented in cooperation with Sulfator Oy. The aim of the project was to verify the microbiological sulfate reduction process with laboratory-scale continuous equipment. The aim of the test run was to optimize the process parameters affecting the process so that the reduction of sulfate can be maximized. The test run has been technically carried out in the water laboratory of Savonia University of Applied Sciences and Sulfator Oy has acted as a test run expert.

This report first theoretically reviews the conditions of challenging microbiological process, the original sources of water formation and the microbial metabolism. In support of the theoretical study, the project has included the thesis “Microbiological sulfate reduction process from industrial waters” (Bachelor Thesis). The test run is divided into two separate tests, the implementations and results of which are presented in the experimental section of this report. The test run has been carried out with synthetic wastewater.

Sulfate reduction is a bacterially catalyzed process that occurs naturally in anaerobic aquatic environments, resulting in the reduction of aqueous sulfate and the precipitation of metals as sparingly soluble compounds. Sulfate reduction can be utilized in the treatment of mine water in a variety of applications. The mining industry generates large amounts of sulfate-containing wastewater, which is very harmful to all water bodies, accelerates the amount of acidic water in the mine dewatering and greatly retains water reuse and recycling. Mine effluents and mine production contain large amounts of sulfate ions, but acidic waters in the mining area are generally the largest source of sulfate. The transport of sulfate-containing water is a natural process that accelerates considerably as a result of mining (Lakso, 2005 & Mroueh, 2008).

Globally, sulfate concentrations in mine effluents range from 250 mg/l to 1 000 mg/l, of which the 250 mg/l limit is set in the United States, while concentrations of 1 000 mg/l are approved in Brazil and Chili. In Finland, no general limit has been set for the amount of sulfate separated, as separate limit values are set for each mine and new regulations are expected in the future. These mine-specific limit values depend on e.g. the mining area, the mining products, the quality of the ore and the size and shape of the discharge waters surrounding the mine. If the discharge point is located, for example, in a catchment area (Figure 26), the lower water areas and the shapes of their bays and bottoms must be taken into account in terms of water turnover. As an example, one of the most important factors influencing the state of the lake is e.g. the average time taken for the

water to change, i.e. the water retention. This largely affects the general quality requirements of mine discharge water (Lakso,2005).

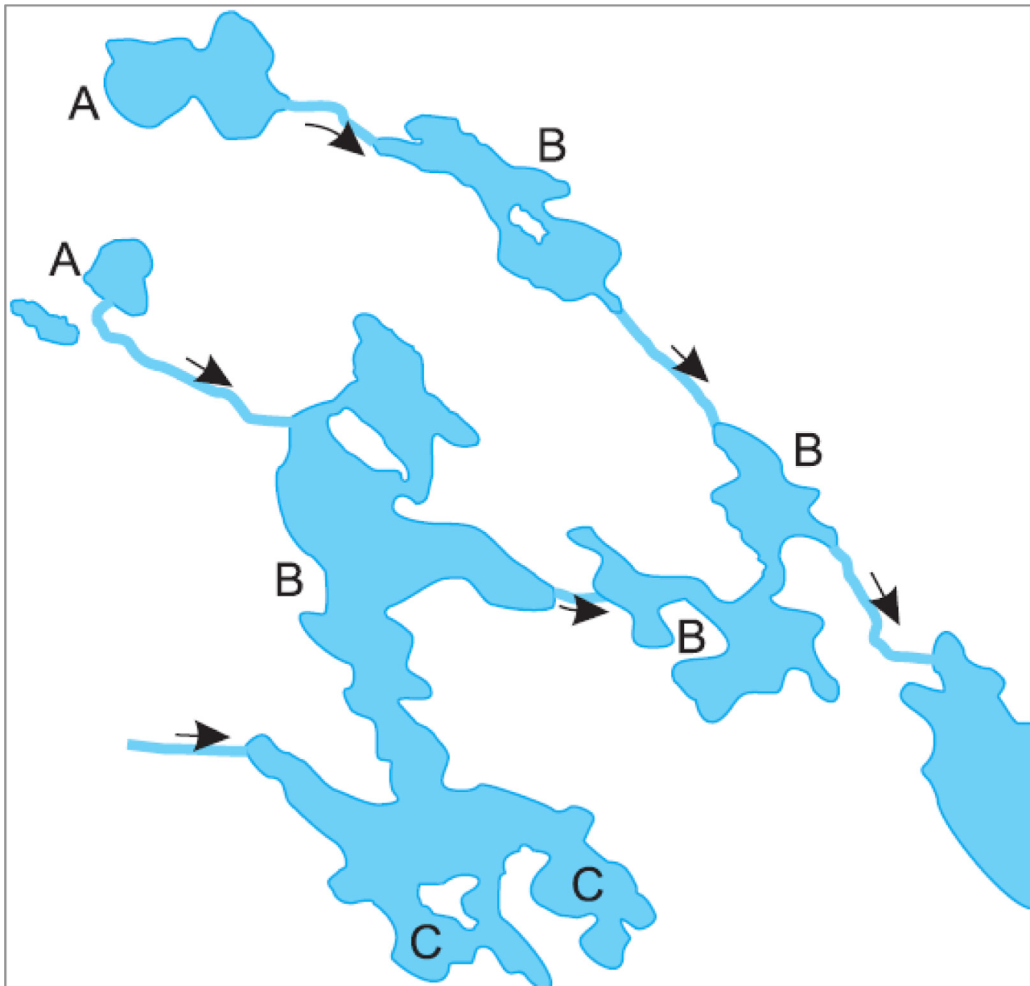


Figure 26. Structure of a small Finnish watershed with top lakes (A), flow-through lakes (B) and bays protected from water turnover (C) (Lakso,2005).

The most common metals present in mining waters are e.g. Cu, Zn, Pb, Cd, Ni, Cr, Hg. Most of these also act as trace elements and substrates for microbial growth. At high concentrations, however, they are either toxins or antimicrobial agents. However, metal-tolerant strains that are continuously exposed to combinations of metals such as nickel and zinc or copper can produce synergistic or cumulative toxic effects from these combinations more than when exposed to a single metal alone. Toxic concentration limits for sulfate reducing bacteria (SRB = Sulfate Reducing Bacteria) are copper (2-50 mg/l), zinc (13-40 mg/l), lead (75-125 mg/l), cadmium (4-54 mg/l), nickel (10-20 mg/l), chromium (60 mg/l) and mercury (74 mg/l). Also, metal sulfides in insoluble form are either toxic to SRB strains or act as inhibitors by binding to the surface membrane structures of the micro-organism, preventing normal cellular metabolism. Sulfur compounds themselves also inhibit SRB function: sulfate <thiosulfate <sulfite <total sulfide (S^{2-}) <H_2S (Cole,2011).

Passive desulfurization methods include oxidation, reduction, adsorption and precipitation. Artificial wetlands can be set up in so-called PMDTS (Passive Mine Drainage Treatment Systems) to treat runoff. In this method, all the above mentioned desulfurization methods are carried out. PMDTS pools require a relatively large area of land. When the artificial wetland is well constructed and functioning properly, the removal of sulfur and metals (e.g., Cd, Cu, Pb, and Ni) works by using fungal symbiotics formed in the aerobic and anaerobic zone of the wetland plants and their roots. Typically, species that thrive in swamps and acidic environments include sedges, hornbills and osmanthus. As a basis and basis in the PMDTS basin, runoff water is biogeochemically treated from metallic organic matter, such as peat. Fungal antibiotics have been found to work e.g. in composts and especially in an anaerobic environment as a source of sulfur-reducing microbes (including *desulfovibrio* and *desulfotomaculum*) and as a store of their substrates. Fungal symbiotics reduce runoff water sulfate to sulfide in the anaerobic root zone formed in the wetland, which in turn precipitates the metals (Me) in the water to metal sulfides (MeS). Once the acid-causing sulfate and its acids have been removed, the pH of the wetland water begins to rise. When the pH rises close to neutral, the conditions are also optimal in the oxygenated layer of the wetland, e.g. for iron and manganese oxidizing microbes. Iron and manganese generally precipitate as hydroxides or alternatively form colloids and complexes with the organic matter of the pool (Cohen, 2006).

Active methods for the treatment of acid runoff, in particular for the removal of sulfur compounds, include limestone neutralization, ion exchange, membrane filtration, reverse osmosis, solution extraction and biological treatment. The problem for the efficiency and implementation of the methods has been e.g. toxic metal concentrations in the water passing through the treatment, environmentally toxic secondary compounds formed during the treatment and the costs required for the treatment. The main goal in both passive and active acidic wastewater treatment methods is that after the treatment, the water is sufficiently pure to be either discharged into natural waters or reused in an industrial process (Cohen, 2006;Pepper;Gerba;& Gentry, 2015; Boelius, 2017).

In limestone neutralization, the principle is to use a filter matrix containing porous limestone to neutralize acidic runoff water. The filtrate matrix can be constructed in almost any shape, from a columnar cylinder to a limestone wall built in the water catchment area (Schumann, ym., 2015; Gidert, ym., 2011).

In ion exchange, the principle is to bind and release the charged ions, compounds and/or molecules in a sample to an ion of opposite charge, which is usually bound to a stationary separation matrix. A solution (eluent) with the same charge as the sample is used for the separation, but the ion concentration of the eluent is considerably lower. The ion exchange capacity is affected by the pH and ionic strength of both the sample and the eluent (An, 2014).

Membrane filtration and reverse osmosis are inversely based on a natural physical phenomenon (osmosis) that is also used by plant roots to obtain water. In osmosis, the so-called a semipermeable membrane with liquids of different ionic concentrations on different sides. In other words, the less saline liquid tends to equilibrate with the saltier liquid, so that eventually the liquids on both sides of the membranes have the same ion concentration. Membrane filtration generally utilizes a vacuum to force the liquid to be cleaned through a membrane of a certain pore size. Impurities in the liquid remain on the

membrane and clean liquid penetrates the membrane. Membrane filtration is commonly used to remove microbial size contaminants. In reverse osmosis, on the other hand, the goal is to remove smaller impurities (e.g., salts) than in membrane filtration. In reverse osmosis, the saline liquid is forced under pressure through a semipermeable membrane of a certain pore size, whereby the salinity of the membrane-permeable liquid is lower than that of the semipermeable liquid (Fu, 2018; Schmidt, ym., 2016).

In bioleaching, microbes form acids with soluble metals from ores through oxidation/ reduction reactions. The acids formed must be further processed to separate the metals. Bioleaching uses a variety of built environments from reactor models to earth embankments or combinations thereof. The problem with the method is e.g. the end product is sulfate remaining in the run-off water, which forms sulfuric acid in aqueous solution. As further processing methods for bioleaching, e.g. ion exchange, membrane and reverse osmosis filtration, or combinations thereof (Ma,2018).

One form of biological treatment is a so-called immobilized microbial matrix attached to an organic filtration material. The method uses either a single microbial strain, such as sulfate-reducing microbes (SRBs), or a mixture of multiple microbial strains, such as SRBs, methanogens, nitrifying and denitrifying microbes, exploiting each other's ability to use each other's end products as their own energy source or other substrate (Li, 2017; Pepper;ym., 2015).

10 MICROBIOLOGY OF SULFATE REDUCTION PROCESS

The microbiological sulfate reduction process is based on the reduction of sulfate and sulfite to sulfide and hydrogen sulfide under anaerobic conditions, which are subsequently partially oxidised to sulfur by sulfate-reducing bacteria. When the sulfate is reduced e.g. sulfide as well as other sulfur compounds, they are separated from the feed water before final discharge back into the water body. Many sulfate reduction methods have been tested, such as electrochemical, chemical and microbiological sulfate reduction methods (Wejima, 2020).

10.1 pH and temperature

The majority of sulfate reduction processes (SRBs) have been studied near neutral pH due to faster bacterial growth, but low pH tolerant reducing bacteria have also been used in sulfate reduction, even at pH 3. In general, sulfate reduction works best at pH 7-8. Most of the SRBs studied are neutrophilic or mildly acid-resistant. Reports of SRBs that withstand acidic conditions have been published to a very limited extent. For optimal growth, the pH is recommended to be > 7.5 and is also proportional to potential hydrogen sulfide toxicity at lower pH levels. Low pH is toxic to the process as it acidifies the cytoplasm and generally impairs microbiological metabolism (Papirio, 2012, p.18-27).

The growth of anaerobic bacteria slows down considerably when the temperature drops below 14–16 ˚C. The optimum temperature for the sulfate reduction process is 25-35 ˚C, operating temperatures can range from room temperature (25 ˚C) to mesophilic temperature (45 ˚C). In general, the growth and conversion rate of sulfate reducing bacteria ac-

celerates at higher temperatures, but the energy required to heat a bioreactor is directly proportional to its maintenance cost. Thus, it is most convenient to operate the bioreactor as close as possible to the temperature of the water fed to the sulfate-containing reactor. At low temperatures, however, chemical and biological kinetic activities are clearly slowed down. Many species have been found to have reacted to and adapted to rapid changes in temperature immediately (Papirio, 2012 & Coaltech, 2020).

When the temperature drops below 14–16 °C, the growth of anaerobic bacteria slows down considerably. At the highest temperatures, sulfate reducing agents can take over power over methane producers and the temperature should be in the range where the sulfate reducing agent strain used in the process works best. Even momentary changes in temperature can limit the sulfate reduction efficiency of the process and, at worst, give power to other micro-organisms. The reactor is naturally and self-sufficient to neutralize acidification with bicarbonate, which is one of the products of the sulfate reduction process. The amount of acidic substances in the feedwater affects the pH of the process and if the process does not naturally have time to produce the necessary alkalinity enhancers, the amount of acidic substances in the feedwater should be controlled before being fed to the process. The buffering capacity can also be improved by adding alkaline compounds such as calcium carbonate to the process (Greben,2000).

10.2 Redox potential

The oxidation reduction potential is the electrical potential required to transfer electrons from a compound or element, i.e., an oxidant, to another compound or element, i.e., a reducing agent, used to qualitatively measure the oxidation state of water in water purification processes. Under reduced conditions, such as in deep stratified lakes or lake sediments, the redox potential is low, less than 100 mV, or even a completely negative value. In addition, the redox potential characterizes important carbon and nutrient cycling reactions, such as rivers and swamps. Sulfate reducing bacteria need special environmental conditions for sulfate reduction such as low redox potential (Rumbaugh, 2018 & Søndergaard, 2009).

In wastewater treatment biology, the importance of redox potential affects electron donors (often organic compounds) and electron acceptors. These electron motions produce energy that is used by biological organisms operating in the waters to be purified. The redox potential thus has a direct relationship to the electron acceptors available in the water to be purified. Table 11 shows the most common electron acceptors in the water to be purified in order from best to weakest compared to the energy produced in the microbial metabolism and the reduction of the redox potential (Rumbaugh, 2018).

Table 11. The role of different electron acceptors in microbial metabolism (Rumbaugh, 2018).

Oxygen	Under aerobic conditions, oxygen acts as an electron acceptor. At neutral pH, it represents an ORP ≥ 0. In aerobic metabolism, it provides more energy for microbiological growth and regeneration.
Nitrate/nitrite	Many microbiological organisms are able to use nitrate as well as nitrite as an alternative electron acceptor when dissolved oxygen is not available (however, some continue to use nitrate/nitrite under aerobic conditions). Nitrate/nitrite as the final electron acceptor produces more energy than the electron acceptors as shown in the figure below (Figure 27)
Sulfate	As the redox potential drops below -125 mV at neutral pH, sulfate using microbes begin to appear. Sulfate is converted to reduced sulfur ion and increasingly to hydrogen sulfide as the pH decreases. The energy from sulfate reduction is lower than that of oxygen, nitrate and iron.
Organic compounds	When the electron is received by an organic compound, fermentation occurs. This is the first step in the microbiological process of anaerobic digesters and also occurs in anaerobic functions at low redox values. This process produces short chain fatty acids such as acetic, butyric and propionic acids.
Methane	Methane production occurs when organic acids and hydrogen are used to produce methane by archaeological organisms such as methanogenic bacteria. In this case, the low redox potential (<-400 mV) enables energy production by combining carbon dioxide, hydrogen, and organic compounds to form methane as the final product.

When the redox potential is > 200 mV, iron begins to have a high phosphorus absorption capacity, while at a low redox value, iron is reduced and phosphorus begins to be released from the compounds formed with the iron. If this activity is related to the sulfate reducing bacteria in the sulfate reduction process and to the methanogenic bacteria and the competition between them, the redox potential in the water should always be as close as possible to the value of the reducing agents used by the sulfate reducing agents. However, there must be a limit to the value so that organic compounds do not start to degrade, which in turn favors methanogenic bacteria (Søndergaard,2009).

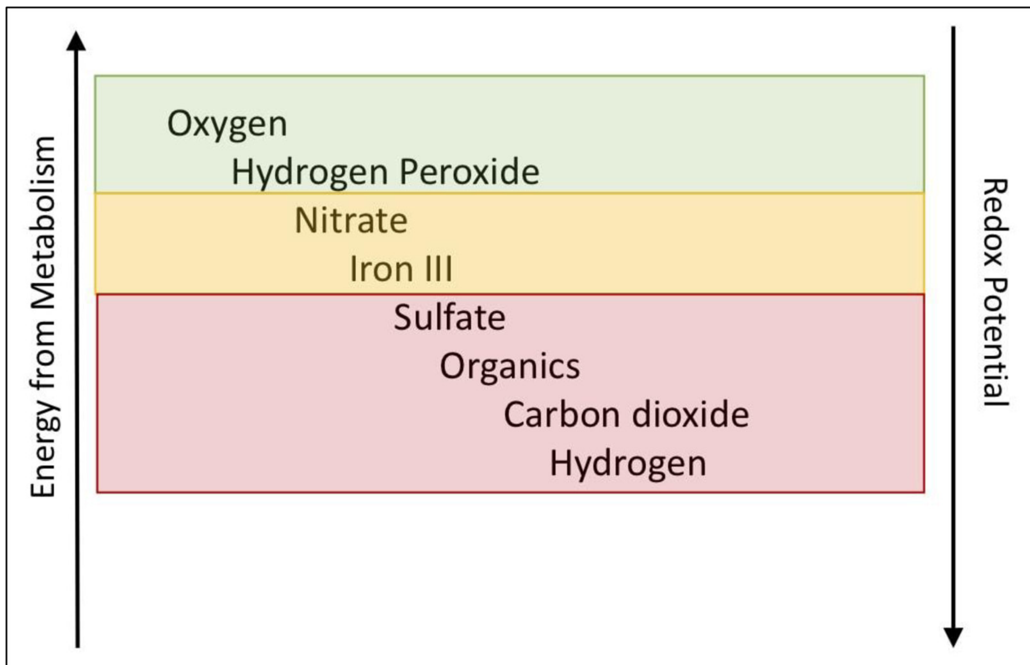


Figure 27. The amount of energy produced in bacterial metabolism and the ratio of the value of the redox potential (Rumbaugh, 2018).

Nitrate has been found to inhibit all or part of sulfate reduction, even in small amounts, when the *desulfovibrio* strain has acted as a reducing bacteria. This is possibly due to the fact that nitrite is reduced directly to ammonia by the nitrate reduction process but more likely the nitrite is oxidized in the mine water to nitrate during storage where the nitrate formed has been used as an electron acceptor in the oxidation process. Nitrate can potentially be used as an electron acceptor in the sulfide oxidation process by sulfite oxidizing bacteria. Figure 27 states that the use of this nitrate is due to the redox potential at the time of operation. The amount of nitrate and sulfate available may also be relevant. Sulfate-reducing bacteria require a redox potential of at least -150 to -200 mV for them to function properly. If the redox potential is higher than this, in the presence of higher oxygen, sulfate values remain constant and no sulfides are formed. Sulfate reduction efficiency was not observed to change when redox values were up to -400 mV (Rumbaugh,2018).

10.3 Carbon source in sulfate reduction process

Sulfate-reducing bacteria needs carbon as a nutrient source to keep sulfate reducing metabolism functional. A good carbon source is e.g. ethanol, methanol, acetic acid, acetate and lactate. All sulfate reducing bacteria are generally capable of utilizing hydrogen gas (H_2), lactate, and pyruvate. In addition, most incompletely oxidizing sulfate reducing agents utilize malate, sulfonate, and primary alcohols such as methanol, ethanol, propanol, and butanol, while fatty acids, lactate, succinate, and benzoate are used as electron donors by organisms capable of complete oxidation (Mroueh,2008).

10.3.1 Ethanol

Ethanol (C_2H_5OH) is used as an antiseptic drug, solvent, and product of the metabolism of humans, animals, and many microbes. Ethanol also acts as a good carbon source and electron donor for microbes in the microbiological process. The use of ethanol is considerably more expensive compared to methanol. In the microbiological process the use of ethanol could be more favorable than methanol, due to the strong growth of methanol in methane-producing bacteria (Greben, 2000).

Ethanol is the most commonly used substance due to its relatively low cost, easy of transport, and good suitability for many sulfate-reducing bacteria. Biological oxidation of ethanol catalyzes the enzyme dehydrogenase of alcohol to form acetaldehyde. This reaction is very energy intensive. Acetaldehyde is further oxidized to acetic acid and hydrogen ion or can be condensed with ammonia to form ethanolamine. Ethanolamine acts as an important ingredient in bacterial cell membranes and also acts as a storehouse of carbon and nitrogen that bacteria use during starvation (Arnold, 2017).

Studies have shown that the reduction efficiency of sulfate is more than 80 % when ethanol is used as a carbon source. Strains of *Desulfovibrio desulfuricans* and *Desulfobacter postgatei* have been found to oxidize ethanol completely. Sulfate-reducing strains using ethanol form a weaker biomass and in many cases ethanol is partially reduced to acetate. Equation 18 shows the reduction of sulfate from ethanol to acetate, hydrogen sulfide and water. The reaction does not produce compounds that increase buffer capacity. Weaker biomass production may be due to the fact that other competing micro-organisms use the formed acetate in their activities and the increase in their activity impairs the growth status of sulfate-reducing bacteria as well as the general activity (Papirio, 2012, p. 18- 27).

Equation 18. $2\ CH_3CH_2OH + SO_4^{2-} \leftrightharpoons 2\ CH_3COO^- + H_2S + 2\ H_2O$.

10.3.2 Methanol

Methanol (CH_3OH) acts as an amphiprotic solvent, fuel, product of metabolism in humans, animals and microbes, and acts as a good carbon source, especially for methane-producing bacteria. Sulfate reducing agents are able to use methanol as a carbon source, but methane producers have a dominant position over sulfate-reducing agents. Methanol is used because of its availability and low cost and has been widely used in sulfate reduction applications. The use of methanol is not desirable for the sulfate reduction process because methanol is capable of inducing the growth of methanogenic microbes, taking away growth potential from sulfate reducing strains. At mesophilic temperatures (20–45 °C), the growth of sulfate reducing agents is slow, with a doubling time of more than a day, with methanol being used as the carbon source. Correspondingly, under thermophilic (above 55 °C) conditions, sulfate reduction activities are more intense than methanogenesis (Pubchem, 2020 & Papirio,2012).

Methanol has been studied to be a weak alternative as a carbon source in the sulfate reducing process. The results showed a low level in terms of chemical oxygen demand and sulfate reduction. It has been found that methanogenic bacteria displace sulfate-reducing bacteria if methanol is used as the carbon source. If hydrogen or decomposed

organic matter is available, sulfate-reducing bacteria will be able to displace methanogenic bacteria and gain dominance. If neither substance is available, methanol is used for methanogenesis instead of sulfate reduction. Under thermophilic conditions, the use of methanol as an energy source has been up to 50 times better than under mesophilic conditions. Differences in sulfate reduction at these temperatures may be due to the preference of sulfate-reducing bacterial strains at different temperatures for methanol as an energy source (Greben,2000).

10.3.3 Asetic acid and acetate

Acetic acid (CH_3COOH) is a synthetic carboxylic acid with antibacterial and antifungal properties. Acetic acid can improve the solubility of fatty substances, allowing increased fatty acid accumulation on cell membranes or other cell walls. It is a weak acid that can inhibit carbohydrate metabolism, causing death to living organisms (Pubchem, 2020).

Acetate (CH_3COO^-) is a monocarboxylic acid anion and is important as a product of human and yeast metabolism. Acetate is the salt of acetic acid. Microbiological growth with acetate alone occurs if the carbon content (C_2) of the acetate is oxidized and the reaction produces equivalent reducing agents. The electrons transferred in the process are further converted into cellular material. This reaction pathway also produces large amounts of carbon dioxide from acetate. Alternatively, acetate fermentation can be a significant source of energy, but other substances such as hydrogen and organic compounds are needed to provide similar reducing agents or precursors for cellular carbon synthesis. Propionate and butyrate are common fermentation products in anaerobic processes, such as the sulfate reduction process, and various sulfate-reducing bacteria have been found to consume all or part of these substances as acetate. However, the use of hydrogen as a source of further energy is necessary for the sulfate-reducing bacteria to be able to oxidize propionate and butyrate (Papirio, 2012, p. 18-27 & Zeikus, 1997, p. 514- 541).

Very few sulfate reducing agents are able to use only acetate as their source of energy. For example, *Desulfotomaculum acetoxidans* and a few bacteria capable of impure oxidation are able to use acetate as their carbon source if hydrogen gas is used as an electron donor (Papirio, 2012, p. 18-27).

Hydrogen respiration (hydrogen gas transferred through the cell membrane to hydrogen ion) produces energy by the motive force of the proton. In methanogenesis, the use of hydrogen requires four times the availability of energy compared to acetate fermentation. The use of acetate as the sole energy source can be very stressful for some bacterial strains such as in this case methanogenic as well as sulfate reducing agents. Their growth may therefore be inherently slow, and in the long run, the use of acetate alone may be completely inadequate to maintain the normal growth and function of the bacteria. However, any alternative electron donor present in the acetate solution is capable of acting as a source of energy for microbial growth. Also, the persistent inability to isolate and grow acetate-fermenting methanogenesis, where acetate acts as the sole electron donor, may be the result of unsuitable growth conditions (Zeikus, 1997, p.514-541).

10.3.4 Lactate

Lactate ($CH_3CHOHCOOH$) is a lactic acid anion that is an active variant in human biology. Lactate acts as a very good carbon source for the bacteria in the sulfate reduction process and is used in many laboratory experiments and is the most optimal carbon source for microbiological processes. The disadvantages of using lactate are in its production. Lactate is very difficult to produce chemically and due to its complexity its price is very high for use on an industrial scale. Lactate is a superior electron donor compared to ethanol, acetate or propionate. When comparing energy and biomass production, the use of lactate produces more bicarbonate, thus neutralizing the acidity of the effluent in a better way (Papirio, 2012, p. 18-27).

Only certain sulfate-reducing bacteria, such as the *Desulfotomaculum* -strain, are able to oxidize lactate to carbon dioxide, while the *Desulfovibrio desulfuricans* -strain oxidizes lactate to acetate. If lactate does not degrade in the sulfate reduction process, it can be an inhibitory factor or even lethal to sulfate-reducing bacteria (Papirio, 2012, p. 18-27).

10.4 Toxicity factors of the sulfate reduction process

Various organic compounds can have toxic effects on the function of sulfate reducing bacteria, especially in low pH ranges, because organic acids are mainly in their non-ionized form. The acids can migrate to the bacterial cell and acidify their plasm. At high concentrations, this leads to bacterial death. Too low pH is toxic to bacteria because it acidifies their plasm and thus prevents them from getting energy (Papirio, 2012, p. 18-27).

Heavy metals such as zinc and copper can have stimulating effects at low concentrations and toxic and inhibitory effects at the higher concentrations. In addition, metal sulfides can limit the activity of sulfate reducing bacteria. Studies have shown that metal sulfides act as a shield and prevent a reactive substance, such as sulfate or organic matter, from entering the necessary enzymes or bacteria. However, sulfate reducing bacteria retained their sulfate removal functionality, so metal sulfides are not lethal to them. Thus, all metal sulfides formed in the process must be removed immediately in order for the efficient sulfate removal operation to continue. However, the metals must be in free form in order for their toxic and antimicrobial effects to occur. A typical hierarchy for inhibiting the microbiological functions of metals that increase in the process, from the most toxic to the less toxic, is as follows: Copper (Cu)> Cadmium (Cd)> Nickel (Ni)> Zinc (Zn)> Chromium (Cr)> Lead (Pb) (Coaltech, 2020).

Excessive accumulation of acetate due to bacterial metabolism can acidify the process, impairing its sulfate reduction efficiency. The amount of excess sulfate in the process can also affect its efficient operation, as it can cause an increase in redox potential and acidification. These changes in condition may be beneficial to other bacteria involved in the process, weakening the dominance of sulfate reducing agents. In addition, large amounts of bacteria that collect sulfate into biomass, such as L-cysteine, cause acetate accumulation, which in turn increases the acidity of the process. The fungi in the process can also increase the production of acetate, organic acids and hydrogen by fermentation and thus its total acidity using organic compounds in the absence of oxygen (Arnold, 2017).

One of the factors hindering the operation of the process is considered to be sulfide, which is assumed to be present in the process as free hydrogen sulfide. Inhibitors are known to correlate with free hydrogen sulfide and total sulfide concentrations. Microorganisms may have two different inhibition thresholds, with the lower threshold correlating with free hydrogen sulfide and the upper threshold correlating with total sulfides. In addition, sulfide toxicity is affected by differences in bacterial growth patterns, delayed growth, or granular growth. The toxicity concentration of sulfide in the form of free hydrogen sulfide was found to be between 100 and 180 mg/l at pH 7.6. This depends on which substrate the bacteria use in metabolism (Coaltech, 2020).

The toxicity of the process can be acute (short-term and immediate) or chronic (long-term and recurrent). Acute process toxicity may not destroy the process but may slow or stop the process altogether. Some bacteria have the ability to adapt to long-term changes in the process. These changes may be related to the acidity, alkalinity, conductivity of the process, and the substances, compounds, and metals circulating in the process. All chemical and microbiological functions have a substrate that is used in bacterial metabolism. As this substrate is trans formed by bacteria, its pH changes at the same time, affecting the overall pH of the process. For example, methane-producing bacteria are particularly sensitive to hydrogen sulfide from sulfate reduction because it directly inhibits bacterial metabolic activity and free hydrogen sulfide gas can be removed from the process by carbon dioxide, hydrogen, or methane production (Karlsson, 2011).

11 SULFUR AND SULFATE REDUCING BACTERIA

Sulfate-reducing bacteria comprise a wide variety of different bacterial species that have adapted to different environmental conditions as well as substrates. Bacteria are able to use a variety of carbon sources as energy sources from complex cellulose compounds to simple carbon compounds and are even able to use hydrogen in their metabolism (Coaltech, 2020).

The element sulfur reducing prokaryotes are *Desulfuromonas acetooxidans*, which uses small organic hydrocarbons such as acetate, ethanol and propanol as a carbon source. The reaction with acetate is according to the following reactionequation.

Equation 19. $CH_3COO^- + 2\ H_2O \rightarrow 2\ CO_2 + 4\ S^{2-} + 8\ H^+$.

Sulfate reduction reactions are generally anaerobic. The SRB strain is known to reduce sulfate and use hydrogen as an electron donor according to the following formula equation:

Equation 20. $4\ H_2 + SO_4^{2-} \rightarrow S^{2-} + 4\ H_2O$.

There are many strain, such as *Desulfobacter*, *Desulfobulbus*, *Desulfococcus*, *Desulfonema*, *Desulfosarcina* and *Desulfotomaculum*. Sulfate-reducing microbes use sulfate as an electron acceptor. Gram (-) *Desulfovibro* DMSS-1 acts as a reducing agent in marine sediments and bog oxygen-free deposits. It also acts as a nitrogen reducing agent. Typically, iron serves as a substrate for accelerating SRB cell mass growth and sulfur reduction. If the iron intake is less than 1 μM and the ammonium intake is less than 0.1 mM, the

reduction of sulfate is slowed down and the end is prevented. If there is too much iron, it forms a complex with hydrogen sulfide, which in turn can slow down the reduction of sulfur and prevent the growth of SRB cell mass (Pepper;ym., 2015; Sim;ym.,2012).

Sulfate-reducing anaerobic prokaryotes can be divided into three taxonomic classes according to their growth environment temperature and membrane structure properties:

1. Mesophilic proteobacteria (more than 25 genera) that are gram negative a thin peptidoglycan layer inside the cell membrane and a thick lipid layer outside the cell membrane. These genera include e.g. *Desulfivibrio, Desulfomicrobium, Desulfobulbus, Desulfobacter, Desulfobacterium, Desulfococcus, Desulfosarcina, Desulfomonile, Desulfonema, Desulfobotulus* and *Desulfoarculus*.
2. Gram-positive bacteria characterized by a thick multilayered peptidoglycan cell membrane. These bacteria are able to form spores and can also withstand higher ambient temperatures. These genera include e.g. *Desulfotomaculum* and *Desulfosporosinus.*
3. Thermophilic sulfate-reducing bacteria (Pepper;ym., 2015; Sim;ym.,2012).

Classes 1 and 2 range from psychophilic microbes to thermophilic genera. In addition, anaerobic sulfate reducing archaea can be mentioned as a separate group. Archaea differs from bacteria according to their different cell membrane structures and mode of reproduction. What is common to archaea and bacteria, in turn, is that neither has a separate nucleus, nor separate intracellular membrane structures. Archaea has been found e.g. from hot springs and submarine volcanoes formed at the seams of continental seabeds. According to metabolism, sulfate-reducing bacteria can be divided into two categories:

1. Completely oxidizing sulfate-reducing bacteria that use formate, benzoate, butyrate, acetate or pyruvate as a carbon source. The final product is hydrogen carbonate (HCO_3^-), hydrogen sulphide (HS^-) and hydrogen (H^+).
2. Partially oxidizing sulfate-reducing bacteria that use either carbon dioxide or organic compounds as a source of carbon and energy, such as lactate, ethanol, propionate or pyruvate. The final product is acetate (CH_3COO^-), bicarbonate (HCO^-), hydrogen sulfide (HS^-) and hydrogen (H^+). In addition, there are bacteria that reduce sulfur to sulfide, such as *desulfuromonas*, *desulfurella*, *sulfurospirrilium* and *campylobacter*. These are unable to reduce sulfate to sulfide. Electron acceptors can also be sulfur-free compounds nitrite, nitrate, ferric iron, arsenate, chromate, uranium and oxygen molecules (Pepper;ym., 2015; Sim;ym., 2012; Berg, 2019; Madsen, 2016).

The optimal pH for SRB strains is 6.9-8.5 (at a rough estimate between 5-9). There are also strains that reduce sulfur even at pH 10. Acid ore mines also have acidophilic/tolerant strains with a characteristic growth medium pH of 2.9 (*desulfotomaculum*). Mix of SRB strains is much more resistant to environmental pH fluctuations than pure strains. The pH of the environment also affects the dissociation of the sulfide when the pH is ≤ 6, the sulfide is mainly undissociated in the form of H_2S. As the pH rises, H_2S dissociates into the form HS^- and further as the pH rises ≤ 8.5 HS^- dissociates into S^{2-}. Finally, if the pH rises to ≤ 10, S^{2-} is the only form of sulfide (Pepper;ym., 2015; Sim;ym., 2012).

When the temperature of the growth environment rises to 25-30 ˚C, the activity of SRB accelerates considerably and when the temperature rises above 40 ˚C, the activity slows

down (Table 12). Stocks have been separated from the mining environment and processed, which operate efficiently in sulfate reduction even in the temperature range of 2-16 ˚C. Cultivation of psychophilic strains is a slow and sensitive process. Once the strain has been adapted to low temperatures, the effect of the temperature limit values disappears and in the future SRB occurs from the lowest accustomed temperature to the initial optimum upper limit for the strain (Pepper;ym., 2015; Sim;ym., 2012; Berg, 2019; Madsen, 2016).

Table 12. The sulfate reducing agent bacteria genera and their commonly used carbon sources. I: partially oxidizing, C: completely oxidizing, NR: no oxidizing properties known (Boelius, 2017).

Genus	Oxidizing	Source of carbon	Temperature (˚C)
Mesofilic: Gram (-)			
Desulfivibrio	I	Lactate	25-35
Desulfomicrobium	I	Lactate, Malate/H_2	25-40
Desulfobulbus	I	Propionate	28-29
Desulfobacter	C	Acetate	28-32
Desulfobacterium	C	Butyrate; Formate/H_2	0-31
Desulfococcus	C	Pyruvate	28-35
Desulfosarcina	C	Benzoate	33-38
Desulfomonile	NR		30 (optimum)
Desulfonema	C	Benzoate	30 (optimum)
Desulfobotulus	I	Propionate, Lactate	28-39
Desulfoarculus	C	Butyrate	20-39
Meso- and thermofilic: gram (+)			
Desulfotomaculum	C / I	Butyrate/Lactate	35-60
Desulfosporosinus	C / I	Malate, glycerol	28 (optimum)

12 METABOLISM OF SULFATE REDUCING BACTERIA

Sulfate reduction is dependent on several microbiological subprocesses. By adjusting the ratios of nutrients and trace elements to the organic carbon in the material to be treated, the desired biological processes can be facilitated or slowed down. Sulfate reduction is directly dependent on the parallel nitrogen and phosphorus modification processes. Depending on the carbon and oxygen, the above processes are also in competitors with each other. In anaerobic wastewater treatment, for example, sulfate can be used to remove nitrogen. In this reaction, the sulfate acts as an electron acceptor, reducing the sulfate to sulfide and oxidizing the nitrogen to nitrate. In the further reaction, the sulfide acts as an electron donor for autotrophic denitrifying microbes, whereby the sulfide is reoxidized to sulfate and the nitrate is reduced to nitrogen gas (N_2). In this process, a high concentration of sulfate inhibits methanogenesis. Methanogenesis is the most common competing microbiological process that impairs and inhibits sulfate removal. That is, if the chemical oxygen demand increases (COD > S) as the sulfate is reduced, the methane-producing microbes begin to activate and eventually the reduction of the sulfate is inhibited. When it is desired to know which subprocess is dominant, one of the useful methods is to measure the oxidation-reduction potential (ORP) of the solution.

When the ORP of the solution is above + 50 mV, aerobic microbes predominate, using molecular oxygen and organic matter as a carbon source to increase their cell mass. For anaerobic SRB, the optimal ORP is less than -50 mV and free molecular oxygen is not available and bicrobes use sulfate as a source of oxygen. In the measuring range from

+50 to -50 mV, both facultative and obligatory microbes predominate. When the ORP drops below -100 mV in the decomposition of organic matter, the power takes on the fermentation of mixed acids, forming ions of carboxylic acids and alcohols. When the ORP drops to -300 mV methanogenesis is predominat process, which uses acids and alcohols from mixed acid fermentation as a source of both oxygen and carbon. The predominance of SRB and methanogenesis can also be adjusted by the ratio of the concentration of sulfate to be reduced and the carbon source. If the carbon source is acetate, the ratio of acetate to sulfate should be less than two for the sulfate reduction to reach its optimum efficiency. When the ratio of acetate to sulfate is between two and three, the "battle" between sulfate reduction and methanogenesis is most intense. When the ratio of acetate to sulfate is greater than three, conditions favor methanogenesis (Wu, 2013; Gerardi, 2003).

In the normal, biochemical cycle of nature, sulfur is either reduced from organic sulfur or sulfate compounds to sulfide or the element is oxidized from sulfur or from various forms of sulfur oxidation by soil chemolithotrophic bacteria to sulfate. Oxidation is carried out by either sulfur oxygenase or sulfite oxygenase. Sulfate is transported into the cell by specific carrier proteins. Thus, it can be said that autotrophs convert sulfur from soil water to in organic sulfate, and heterotrophs, in turn, can utilize and modify both organic and inorganic sulfur in their metabolism (Wu, 2013; Gerardi, 2003).

The sulfur containing biomolecules, and their variants are responsible for cellular respiration, cell membrane for the transport of compounds, molecules, and metals, curettage, the tissue protection against radicals and toxic compounds, and the activities of the enzymes. In addition, sulfur containing biomolecules methylate DNA, are involved in the synthesis of proteins, of which, for example, methionine (a hydrophobic thioether compound) is also involved in interpeptide reactions, inhibiting intracellular unwanted oxidation reactions and cysteine (a thiol compound). In addition, sulfur biomolecules affect the regulation of the extracellular matrix, the metabolism of fats, the non-toxic modification of contaminants, and the production/activation of signaling enzymes specific to them (Wu, 2013; Gerardi,2003).

In prokaryotes, cellular respiration occurs in the cytoplasm. In cellular respiration, several oxidation-reduction reactions take place, as a result of which the organic starting material e.g. lactate and gaseous hydrogen are oxidized, the sulfate is reduced, the ATP needed by the cell for metabolism is formed and the cell mass increases. More specifically, the following occurs: lactate is oxidized through a number of enzymatically controlled reactions to acetate, which in turn is used in the synthesis of ATP (by the enzyme acetyl-CoA). While substrate-directed ATP synthesis occurs, a second ATP synthesis also takes place, the purpose of which is both to catalyze the abowe mentioned ATP synthesis and to create channels for proton and electron exchange through the cell membrane, the so-called for chemiosmotic proton flow and electron transport through the heme network. A chemiosmotic proton flow is formed when protons released from lactate degradation react with the cytoplasmic hydrogenase enzyme to reduce hydrogen. Hydrogen diffuses through the cell membrane into the periplasm, where it is reoxidized by the periplasmic hydrogenase enzyme. Using a chemiosmotic proton current, the sulfate is reduced to sulfite in assimilation (Figure 28). In this reduction, the inorganic sulfate acts as an electron acceptor and is reduced to a sulfide within the cell. Intracellular sulfate reduction is a tightly controlled reaction in which the resulting sulfide is immediately bound to an organic compound, serine (O-acetylserine), from which the cell in turn synthesizes cysteine.

With the nucleophilic -SH group of cysteine, sulfur bridges (S-S bridges) are also formed in proteins. These bridges allow proteins and enzymes to be either active or dormant. The proper circulation of proteins also affects the function of cellular antibodies and their activity. In the synthesis of cysteine, the Ferredoxin (Fe-S-) cluster required for the functioning of the dissimilar ion channel is also formed. Sulfur mineralization is the removal of sulfur from an organic compound. Serine is also needed for this. The enzyme serine sulfhydrolase releases sulfur in cysteine in response to the above. Another important enzyme, cysteine sulfhydrolase, acts in the cell to remove both sulfide and ammonium. Removal reactions occur in both aerobic and anaerobic environments (Wu, 2013; Gerardi, 2003).

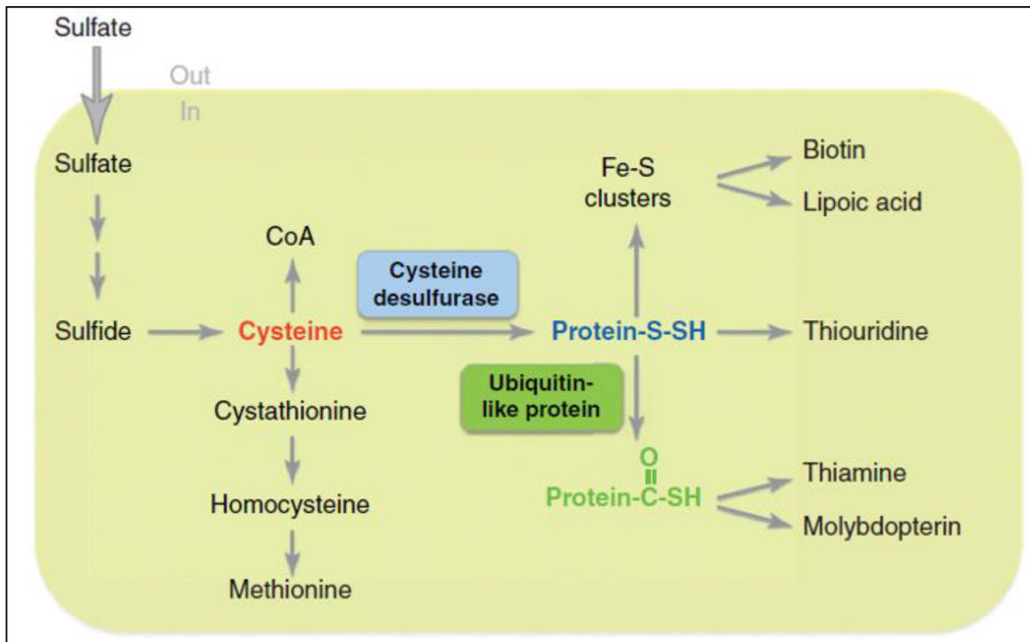


Figure 28. Sulfate assimilation in a bacterial cell (Liu, 2012).

The electrons released during lactate oxidation, in turn, are transported to the dissimilative reduction of sulfate through specific cell membrane channels formed by the c-type cytochrome (inside the cell membrane) and the Fe-S cluster (outside the cell membrane, Figure 29). This so-called group of hemiproteins also forms cell membrane channels for several other ion-carrying complexes. The channel formed by the C-type Fe-S cluster and the cytochrome is sensitive to hydrogen sulfide formed in the periplastic state, as hydrogen sulfide can seize the iron acting as the central atom of the heme itself, whereby the entire channel ceases to function. Dissimilative sulfur reduction is more common for SRB bacteria because the energy source commonly used in assimilative reduction is hydrogen, while in dissimilative reduction the source of both carbon and energy is an organic molecule. In both dissimilative and assimilative sulfate reduction, the end product is hydrogen sulfide (Wu, 2013; Gerardi, 2003).

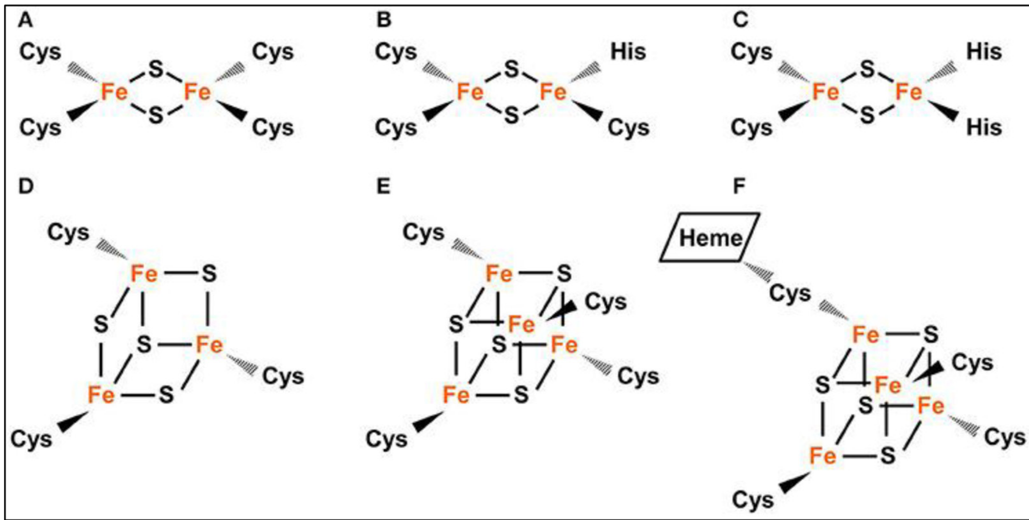


Figure 29. Six known structures of the Ferredox cluster (Lu, 2018).

Sulfur-reducing prokaryotes are a diverse group of anaerobic bacteria found in every air, weather, and soil condition on Earth. They have a significant impact on the Earth's sulfur and carbon cycles. When the surrounding material does not contain sulfur to be reduced for SRB use, they can take the energy and nutrients they need, e.g. by using organic acids and alcohols. At the same time, they produce hydrogen, acetate and carbon dioxide. They are also able to produce methane from organic matter through methanogenesis. In other words, SRBs can be classified as either sulfidogenic or acetogenic in their metabolism. This property is useful for the survival of a species in an environment with a limited number of species-specific electron acceptors (SO_4^{2-}). When sulfate is released (e.g., from soil sediment) in the anaerobic zone of soil, where methanogenic archaea are generally the predominant species of microbes, SRBs win the struggle for survival by removing nutrients (hydrogen and carbon) and substrates (propionate) and butyrate from the archaea. In general, SRBs and methanogens do not fight each other if there is enough organic matter in the environment for food and if there is enough sulfur in the environment to reduce to SRBs, SRBs and methanogens live side by side fighting mainly for the substrate hydrogen (H_2). When there is no usable sulfur in the environment, SRBs break down organic matter into food. This produces hydrogen, which is used by methanogens to produce methane. SRBs, in turn, continue to use methane in their metabolism as the final "electron well" instead of sulfate. This symbiotic way of life of the two species is called syntrophy. Methane-producing SRB strains with a similar morphology to methanogens include e.g. *Desulfuromonas*, *Desulfovibro* and *Desulfomonas* (Plugge, 2011; Gerardi,2003).

13 IMPLEMENTATION OF THE LABORATORY SCALE TEST RUN

In the mining industry, water is used in mining and enrichment, which results in sulfate, among other things. Sulfate is a common emission from mines and, although it is quite harmful to health, it is a good idea to calculate the sulfate concentrations in mining waters before discharging them into natural waters. In nature, sulfate can pose a risk of acidification and eutrophication, among other things.

One part of the DeepCleanTech -project was to pilot on a laboratory scale microbiological sulfate removal in the form of a longer test run. At the same time, further efforts were made to optimize the sulfate reduction process with practical experience. In practice, the pilot was divided into two different test runs about a year long test run period, because the sulfate-reducing bacteria proved to be challenging to control. In both experimental runs, a strain of unwanted methanogenic microbes formed. This messed up the first test run so that it ended up being stopped. In the second test run, based on the experience of the first run, it was possible to prepare better and be able to operate the sulfate reduction process reasonably despite the formation of methane. Due to methanogenic microbes and technical problems, neither test run could be driven beyond the run-up phase, which is why there is no data on the test runs at full flow (hydraulic retention time of one day).

13.1 Description of laboratory equipment

Microbiological sulfate reduction was carried out in one reactor based on UASB- (Upflow Anaerobic Sludge Blanket) technology. The treated wastewater is fed to the bottom of the reactor and an upward flow is generated in the reactor by an internal circulation. This allows efficient contact with water as well as the sulfate reducing bacteria contained in the sludge blanket and carriers. The upward flow even helps gases the rising of the process from the reactor.

Figure 30 shows the laboratory-scale pilot equipment used in test run II with its various process steps. The equipment for the first test run did not included influent tank and a separate carbon supply. The reactor was run in a batch type, adding the necessary feed water during the working day.

The equipment included an influent tank with a continuous mixer with a capacity of 100 liters. This involved adjusting the nutrients and sulfate content of the process. The pH of the process (reactor) was also adjusted via influent, by adjusting the pH of the influent relative to that measured from the reactor. 10 % sodium hydroxide (NaOH) or 4 % sulfuric acid (H_2SO_4) was used as pH adjusting chemicals.

The second stage was a 27-liter reactor in which the actual sulfate reduction took place by means of sulfate reducing bacteria (SRB). SRBs use a sulfur compound, sulfate, instead of oxygen, for cellular respiration, which is reduced in the process to sulfides (S^{2-}), hydrogen sulfides (HS^-), and hydrogen sulfide (H_2S). For the second stage, influent was pumped to the bottom of the reactor. Carbon nutrient was also pumped to the same line with its own pump (Figure 30). The reactor contained carriers (enclosed in a cage) on the surface of which sulphate reducing bacteria were able to adhere and form flocs. The

carriers used were made of polypropylene (Figure 4), 16 mm (320 m^2/m^3) in height and diameter. There were about 6 liters of carriers in the cage, so their calculated area brought to the reactor was a total of about 1.92 m^2. The reactor was also equipped with internal circulationand heating to bring better contact between the bacteria and the treated effluent and to keep the operating temperature under control at 28 °C. The reactor was also prepared for the supply of nitrogen gas, which, if necessary, sought to increase the efficiency of hydrogen sulfide removal as a gas from the process. The reactor lid also had a bushing with a shut-off valve to which a gas collection bag could be connected or the gas concentrations in the reactor could be measured with a gas analyzer. The final step in the equipment was an effluent tank into which the liquid leaving the reactor was collected so that it could be analyzed in the form of an aggregate sample, if necessary.

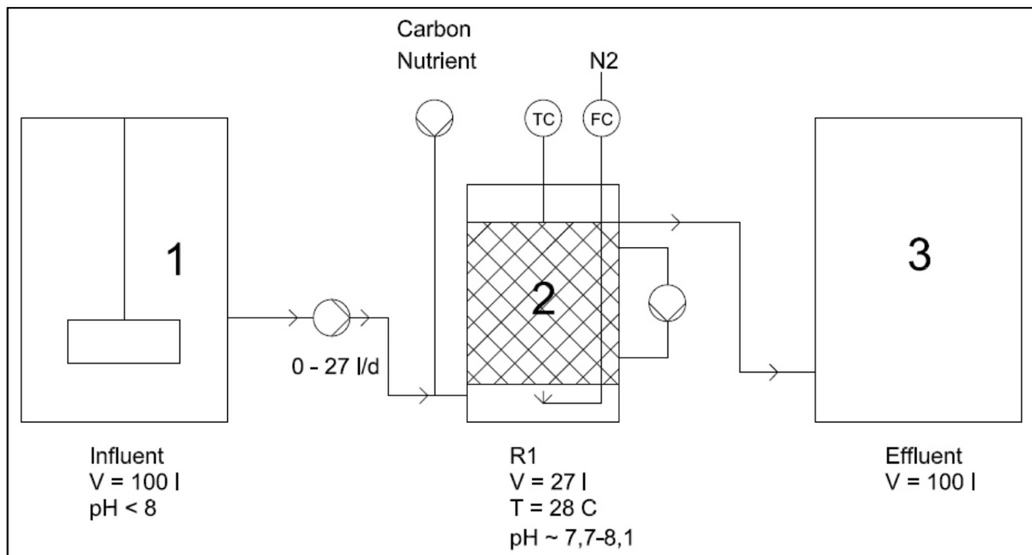


Figure 30. UASB technology microbiological sulfate reduction equipment (Figure: Olli Torvinen).

The equipment includes the following units with their functions:

1) Influet tank with a capacity of 100 liters, where nutrients as well as sulfate content and pH were adjusted (continuous mixing). Manual pH adjustment relative to the pH of the reactor was practically keptbetween 6.5 and 7.5.
2) A 27-liter reactor based on UASB technology with carriers in the screen area. The reactor was equipped with heating, internal circulation and a separate carbon feed. In addition, there was readiness to feed nitrogen gas to the bottom of thereactor.
3) Effluent tank with a capacity of 100 liters.

13.2 Inoculation and adaptation to the reactor

Anaerobic bacteria, including sulfate reducing bacteria, are slow growing. Sulfate reducing bacteria usually require about 2 to 6 months for the growth and adaptation phase. Inoculations grown from the bottom sediment of a ditch saturated with sulphate-containing water from the mining industry were used in the test runs. In this way, the aim was to obtain a functional and efficient strain accustomed to sulfate under natural conditions. In both of the two experimental runs, inoculum growth and adaptation in the

reactor took place in almost the same way, mainly with different volumes differing. The ramp-up phases of test runs I and II, on the other hand, were carried out in different ways. Initially, sulfated water from the mining industry was to be used during test runs, but due to practical supply difficulties, it was decided to use synthetically produced wastewater in the pilot. Deionized water and prescription chemicals were used to make the synthetic wastewater. The advantage here was that it was possible to more precisely control what was fed to the reactor. Correspondingly, the disadvantage was that the experimental times did not show the significance of the variation of the constituents contained in the correct process water and their concentrations for the activity of sulfate-reducing bacteria.

In both experimental runs, inoculum were grown in advance in a pre-covered container in a heating cabinet. In the first test run, a larger 45 liter container was used (start on 7 September 2018) and in the second a smaller 15 liter container (start on 15 April 2019). In the first run, the amount was larger because it was desired to prepare an inoculum as well in case the adaptation in the reactor had not taken place as desired. The bottom sediment, carriers and synthetic nutrient solution of the ditch were placed in the container. The nutrient solution contained carbon, sulfate, nutrients, and some calcium according to the desired initial concentrations. The carbon source at the beginning of the first test run was sodium acetate (CH_3COONa), which was changed during it (19.12.2018) to acetic acid (CH_3COOH), which was also used during the second test run. Sodium sulfate (Na_2SO_4) was used as the sulfate source and ammonium sulfate ($(NH_4)_2SO_4$) and potassium dihydrogen phosphate (KH_2PO_4) were used as nutrients. The source of calcium was calcium chloride ($CaCl_2$). In addition to these, sodium bicarbonate ($NaHCO_3$) was initially added to enhance the formation of alkalinity and sodium sulfide (Na_2S) to raise the sulfides to the desired initial concentration. All ingredients were mixed well and the pH was finally adjusted to 7.7 with sulfuric acid. Container was covered with a lid and left in the oven to stabilize at about 28 degrees. During the inoculation, the container were mixed and analyzed 1-2 times a week. The measurements monitored the pH, soluble oxygen, and redox potential, as well as sulfate, sulfide, ammonium, phosphate, and carbon concentrations. Based on the analyzes, conditions were adjusted or nutrients and carbon were added when needed. The inoculum was grown under anaerobic conditions and the pH was maintained at 7.7 to 8.0 throughout the growth step.

After about a month of inoculation (I: 9.10.2018 and II: 6.5.2019), the inoculum was transferred to the reactor to adapt in the cases of both test runs. A little bottom sediment from the inoculum was placed in the bottom of the reactor as a sludge bed, about one-third of the biosolution from the heating cabinet container was poured to the reactor, and the carriers were placed inside the reactor. In addition to these, a synthetic nutrient solution was added to the reactor to reach the desired initial concentrations. The synthetic nutrient solution contained the same components as in the growth step, but in addition to these, it contained trace elements. These included magnesium, iron, aluminum, manganese, nickel, cobalt, copper, and zinc. The heating of the reactor was set to 28 degrees by means of a thermostat and the internal circulation was determined so that the biological solution circulated about the volume of the reactor once an hour. Conditions and nutrient consumption were monitored in similar ways as for inoculum and were adjusted directly to the reactor as needed.

The purpose of the adaptation was to ensure, before the start of the ramp-ups, that the action of the sulfate-reducing bacteria had started. This was verified based on sulfate and sulfide analyzes. When the SRB operation starts, the sulfate concentrations in the reactor start to decrease rapidly and the sulfide concentrations increase accordingly. It was possible to analyze the calculation of the sulfate content because during the adaptation no new sulfate was added to the reactor after the start.

In the ramp-up of test run I, pH, redox potential, soluble oxygen, conductivity, and temperature were monitored (Table 13). Sulfate and sulfide concentrations were analyzed 2 to 5 times per week and ammonium, nitrate, phosphate, and COD concentrations 1 to 3 times per week by LCK tests based on Hach spectrophotometric measurements. The amount of acetate was titrated 3-5 times a week and in addition the amount of organic carbon was monitored 1-2 times a week with a TOC (Total Organic Carbon) analyzer. In addition, from test week five onwards, alkalinity was analyzed to ensure process well-being and adequate buffer capacity.

In the ramp-up of test run II, the follow-up analyzes (Table 13) were performed as described in the previous paragraph with the following exceptions. The COD content was no longer determined at all because acetate titration and TOC determination proved to be sufficient. TOC determinations were performed less frequently than in test run I, 0 to 2 times per week. In addition, a titration method was taken from week 5 of the test run in parallel with the sulfide assay. Alkalinity was monitored from the beginning of inoculation weekly until ramp-up week 7, after which at random. Gas concentrations were monitored from the beginning of the ramp-up, as a rule, daily to the end. In addition to these, influet pH, redox potential, soluble oxygen, conductivity and temperature, and sulfate content were monitored weekly from the start of the ramp-up. Similar measurements were also made of effluent, but less frequently than weekly.

Table 13. Measurement methods and equipment used in process analysis.

Analysis	Unit	Measuring equipment	Method
pH		WTYW pH 3110, Elektrod Sentix 41	SFS3021 (1979)
Redox potential (ORP)	mV	Hach Multi HQ40d, IntellicalTM MTC101 ORP	Internal method
Conductivity	mS/cm	Hach Multi HQ40d	SFS-EN 27888 (1994)
Soluble oxygen	mg/l	Hach Multi HQ40d	Internal method
Temperature	°C	Hach Multi HQ40d	SFS3021 (1979)
Total Organic Carbon, TOC	mg/l	Analytic Jena Multi N/ C2100S	SFS-EN 1484 (1997)
Ammoniumnitrogen	mg/l	Hach Lange DR6000	LCK303/304 ISO 7150-1, DIN 38406 E5-1
Nitratenitrogen	mg/l	Hach Lange DR6000	LCK339 ISO 7890-1-2-1986, DIN 38405 D9-2
Phosfate- and total phosphorus	mg/l	Hach Lange DR6000, HACH Lange HT 200 S	LCK349 ISO 6878-1-1986, DIN 38405 D11-4
Chemical Oxygen Demand, COD	mg/l	Hach Lange DR6000, HACH Lange HT 200 S	LCK514 ISO 6060-1989, DIN 38409-H41, -H44
Sulfate	mg/l	Hach Lange DR6000	LCK353, Barium Sulphate
Sulfide	mg/l	Automatic titrator TitraLab AT1000 seriers	Internal method
Acetate	mg/l	Automatic titrator TitraLab AT1000 seriers	Internal method
Alkalinity	mg/l	Automatic titrator TitraLab AT1000 seriers, Centrifuge Thermo Scientific SL16R	SFS3005
Gases: CH4, CO2, O2	%	Biogas5000	Internal method
Gases: H2S, NH3	ppm	Biogas5000	Internal method

13.3 First testrun

The start-up of the first test run was started (19 February 2019, approximately 5.5 months after the start of inoculation), when the activity of sulfate-reducing bacteria was determined on the basis of the analyzes. In other words, the sulfate concentration had decreased from 5,000 mg/l to less than 900 mg/l and at the same time the sulfide concentrations had increased to approximately 560 mg/l (Table 19). The sulfate concentration was below 500 mg/l at the lowest and the sulfides were above 700 mg/l at the highest. The ramp-up followed the plan in the following table (Table 14), according to which the process was to be run up within 30 working days. The aim was to increase the sulfate load in the reactor on a daily basis, either by increasing the daily influet feed rate or the sulfate concentration, in order to finally reach a full flow of 27 l/d at the reactor load with an influet sulfate concentration of 7,000 mg/l. The required amount of influet for each run-up day (Table 14) was prepared in the mornings and pumped through the bottom of the reactor during the day. For these reasons, the configuration of the test equipment was not yet at this stage similar to the process diagram, but included only the reactor and effluent tank. The reactor also did not yet have a separate carbon feed and no nitrogen gas dosing at thisstage.

Table 14. Plan for test run I.

Day	Feed, l/d	HRT, d	SO_4-level, mg/l	SO_4 load, g/d
1	0,7	40,0	1 400	0,9
2	0,8	33,3	1 400	1,1
3	0,8	33,3	1 680	1,4
4	1,0	27,8	1 680	1,6
5	1,2	23,1	1 680	2,0
6	1,2	23,1	2 030	2,4
7	1,4	19,4	2 030	2,8
8	1,7	16,2	2 030	3,4
9	1,7	16,2	2 450	4,1
10	2,0	13,6	2 450	4,9
11	2,4	11,3	2 450	5,9
12	2,4	11,3	2 870	7,0
13	2,9	9,2	2 870	8,4
14	3,5	7,7	2 870	10,1
15	3,5	7,7	3 500	12,1
16	4,2	6,5	3 500	14,6
17	5,0	5,4	3 500	17,5
18	5,0	5,4	4 200	21,0
19	6,0	4,5	4 200	25,2
20	7,2	3,8	4 200	30,2
21	7,2	3,8	5 040	36,2
22	8,6	3,1	5 040	43,5
23	10,4	2,6	5 040	52,2
24	10,4	2,6	6 020	62,6
25	12,5	2,2	6 020	75,1
26	15,0	1,8	6 020	90,1
27	15,0	1,8	7 000	108,2
28	18,5	1,5	7 000	129,8
29	22,3	1,2	7 000	155,8
30	26,7	1,0	7 000	186,9

The influet contained all the necessary ingredients. The chemicals were dosed according to the desired concentrations in the following table (Table 15) in proportion to the feed-in feed rate and sulfate concentration on the day of start-up. In the table, the carbon content is given only for the sodium acetate used beginning of the test run. In December 2018, the carbon source was switched to acetic acid (80 %), which was also dosed based on the results of the analysis. The acetic acid was made into a 30 % aqueous solution, the pH of which was adjusted to 5.5 to 6.0 with 50 % sodium hydroxide.

Table 15. Concentrations of chemicals of influent in test run I.

Component	Needed amount,g/l	Chemical	Dosing, g/L INF
C	0,4	CH_3COONa	1,39
SO_4	1	Na_2SO_4	1,48
NH_4	0,15	$(NH_4)_2SO_4$	0,55
PO_4	0,05	KH_2PO_4	0,21
Ca	0,10	$CaSO_4$*2 H_2O	0,43
Mg	0,03	$MgSO_4$ * 7 H_2O	0,30
Al	0,001	$Al_2(SO_4)_3$ * 14 H_2O	0,01
Cu	0,001	$CuSO_4$ * 5 H_2O	0,004
Co	0,002	$CoSO_4$ * 7 H_2O	0,01
Mn	0,002	$MnSO_4$ * H_2O	0,01
Fe	0,001	$FeSO_4$ * 7 H_2O	0,004
Ni	0,002	$NiSO_4$ * 6 H_2O	0,01
Zn	0,002	$ZnSO_4$ * 7 H_2O	0,01

13.4 Second test run

The start-up of the second test run was started (13 June 2019, approximately 2 months after the start of inoculation), when analyzes showed that the sulfate content had decreased from 3,000 mg/l to about 1,200 mg/l and at the same time sulfide level has increased to about 400 mg/l (Table 20). The ramp-up followed the plan in the following table (Table 16), according to which the process was to be ramp-up in 12 weeks. As a learned from test run I, it was found that it makes more sense to run the process up more calmly and thus give the sulfate reducing bacteria more time to get used to the growing sulfate load. The aim was thus to increase the influet feed rate and sulfate content once a week, after which we drove with the same daily load every week.

Table 16. Plan for test run II.

Ramp-up week	Feed, l/d	HRT, d	SO_4-level, mg/l	SO_4 load, g/d
1	2,25	12,0	2 000	5
2	4,5	6,0	2 400	11
3	6,8	4,0	2 800	19
4	9,0	3,0	3 200	29
5	11,3	2,4	3 600	41
6	13,5	2,0	4 000	54
7	15,8	1,7	4 400	69
8	18,0	1,5	4 800	86
9	20,3	1,3	5 200	105
10	22,5	1,2	5 600	126
11	24,8	1,1	6 000	149
12	27,0	1,0	6 400	173

The influent contained all the ingredients required for the process and was dispensed with chemicals according to the concentrations in the following table (Table 17) in proportion to the feed-in week and the sulfate content. No carbon concentration has been given in the table because it was dosed in the required amount based on the analyzes. The carbon source was acetic acid, which was added directly to the influent tank at the beginning of the ramp-up, but already during the second week of ramp-up it was found that a precipitate formed in the influent tank and the pH tended to rise. From this it was

concluded that under the nutrients and suitable conditions, some kind of functional microbial had formed in the influet tank, which caused the above, mentioned problems with its actions. For this reason, it was decided to separate the carbon supply into its own line and replace the influent tank with a new and clean one. At the same time, nitrogen supply readiness was added to the reactor, whereby the equipment was operated with the configuration shown in the previously shown figure (Figure 30). The carbon solution was made in a separate 20 liter canister by diluting acetic acid (80 %) and pumped with its own dosing pump. The pH of the solution was adjusted to 5.5-6.0 with 50 % sodiumhydroxide.

Table 18 shows the dose levels of the trace elements used in test run II, where the concentration of this trace element was 1,000 mg/l and the dosage was given in g/L influet. This made it easier to administer them to the influent. The selenium supplement was included in the trace elements on 30 August 2019. In the second test run, sodium bicarbonate was also added into the influent during November because of the desire to enhance the alkalinity formation of the process in the reactor.

Table 17. Concentrations of chemicals of influent in test run II.

Component	Needed amount,g/l	Chemical	Dosing, g/L INF
SO4	1	Na_2SO_4	1,48
NH_4	0,15	$(NH_4)_2SO_4$	0,55
PO_4	0,05	KH_2PO_4	0,21
Ca	0,10	$CaSO_4*2H_2O$	0,43

Table 18. Trace elements of the influent.

Element	Recepi, g/L Deionized water	Chemical	Dosing, g/L INF
Mg	10,14	$MgSO_4*7\ H_2O$	10
Al	11,02	$Al_2(SO_4)_3*1\ 4H_2O$	5
Cu	3,93	$CuSO_4*5\ H_2O$	1
Co	4,77	$CoSO_4*7\ H_2O$	1
Mn	3,08	$MnSO_4*\ H_2O$	2
Fe	4,98	$FeSO_4*7\ H_2O$	5
Ni	4,48	$NiSO_4*6\ H_2O$	2
Zn	4,40	$ZnSO_4*7\ H_2O$	1
Se	1,41	SeO_2	1,5

14 RESULTS OF MICROBIOLOGICAL SULFATE REMOVAL

The following chapters represent the results of test runs I and II. The sulfate reductions were not calculated in the results because the test runs did not go beyond the ramp-up and due to the partial reciprocating adjustment of the feed rates, this could not be done very reliably either.

14.1 Result from the first test run

The ramp-up of test run I was carried out according to plan (Table 14) until the run-up day 20. After that, an elevated methane concentration was observed in the gas measurement in test week 6. By the next ramp-up week, the concentration had already risen to an average of 15.9 %. During ramp-up week 7, the amount of carbon going to the reactor began to be limited while feeding small amounts of sulfate-containing water to maintain sulfate levels. According to the literature, in this way conditions are provided in which sulfate-reducing bacteria should be in a more favorable position than methanogenic microbes. The higher carbon content, combined with the low sulfate content, in turn favors methane producers. In a couple of weeks, these measures did not achieve the desired result, in which case it was stated that the project still has time to start a new test run. The new inoculum was placed in growth (April 15, 2019) and the first test run was terminated during run-up week 10. The reactor equipment was emptied and disinfected for the next testrun.

Table 19 shows the main results of the first test run. Based on the experience and results of the experimental run, it was concluded that the formation of the methanogen strain was facilitated by the factor that impaired the function of the sulfate-reduced-bacterial strain. In reality, the event is more likely the sum of several things, but the reasons identified were at least the use of a selected inoculum, the accumulation of sulfides in the reactor, i.e., sulfide toxicity, and the accumulation of acetate (carbon) in the reactor.

Table 19. The main results of the first test run.

Test week	pH	Redox, mV	Conductivity, ms/cm	SO_4^{2-}, mg/l	S^{2-}, mg/l	TOC, mg/l	H_2S, ppm
Inoculation	7,96	-486	12,0	4 280		615	
Inoculation	7,70	-409	12,2	5 030		542	
Inoculation	7,85	-394	12,0	4 833		137	
Inoculation	7,91	-438	12,4	4 375		236	
Inoculation	7,97	-463	13,2	4 957		259	
Inoculation	7,76	-435	14,5	3 950		356	
Inoculation	7,72	-431	14,6	3 870		163	
Inoculation	7,90	-441	15,1	3 950		337	
Inoculation	7,89	-430	15,1	3 970		171	
Inoculation	7,81	-421	15,3	4 305		256	
Inoculation	7,87	-419	16,0	4 655		258	
Inoculation	7,84	-439	15,6	3 950		0	
Inoculation	7,54	-382	15,8	3 545		0	
Inoculation	7,80	-437	15,7	4 016		313	
Inoculation	7,85	-419	14,4	3 784		327	
Inoculation	7,86	-430	13,3	3 368		212	
Inoculation	7,68	-445	12,7	3 220		268	
Inoculation	7,73	-465	13,5	1 054		354	
Inoculation	7,86	-455	14,3	877		360	
1	7,90	-454	14,8	1 177		345	2 129
2	7,90	-464	13,5	997		322	425
3	7,80	-464	12,2	1 052		430	468
4	7,84	-454	11,2	938		515	
5	7,74	-457	10,5	1 412	791	277	
6	7,83	-458	10,8	1 668	986	215	547
7	7,97	-452	11,7	1 889	1 094	233	8 109
8	7,81	-452	12,4	2 230	1 091	85	5 965
9	7,72	-447	13,8	3 220	800	53	8 385
10	7,58	-449	14,2	2 920	833	0	10 000

The choice of inoculum had the effect that when it was grown from the bottom sediment of a natural ditch, it presumably already contained methanogenic microbes. Therefore, methanogens were already present in the reactor before they rose. Based on the results (Figure 31 and Figure 32), it can be stated that although the sulfate was reduced evenly during the first 4 weeks of operation (the sulfate content of the reactor did not vary much), carbon has accumulated in the process during that time. Figure 31 shows the results of acetate titration and TOC determinations at different concentration levels, from which the graphs are well followed. The amount of carbon was adjusted on the basis of residual carbon (titration) and the aim was to keep it below 15 milliliters. Figure 31 shows that the first 4 weeks of ramp-up have been over this limit. Carbon was not fed to the reactor in excessive amounts during that time, so the reason for the accumulation is more likely to be that the sulfate-reducing bacterial strain was not as active as expected. In this case, carbon remained on the residues despite the controlled amount of feed. The decreasing carbon concentrations towards the end of the ramp-up are due to the fact that the amount of carbon was limited in an attempt to control the stock of methane producers.

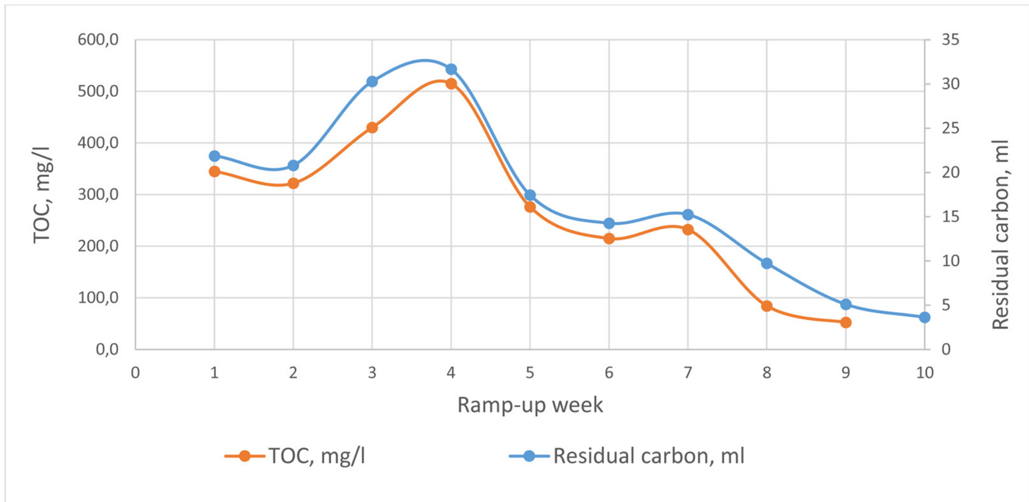


Figure 31. Test run I: amounts of residual carbon and organic carbon during the ramp-up phase.

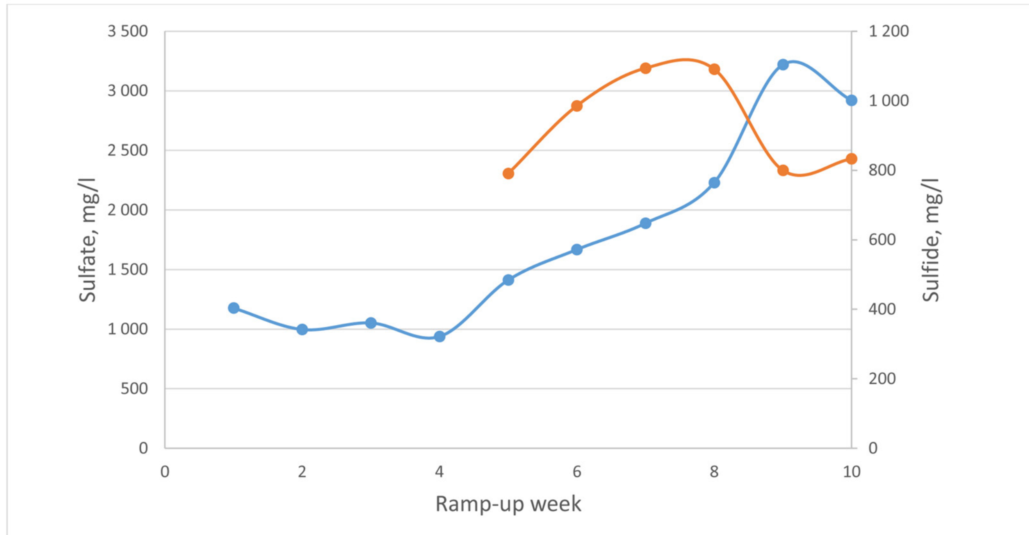


Figure 32. Test run I: sulfate and sulfide concentrations during the ramp-up phase.

The sulfide content (Figure 32) has started to increase after that same week, which also indicates that the efficiency of sulfate-reducing bacteria has started to decrease clearly at that time. Sulfide was measured first 5 rump-up weeks by the Hach LCK method (indicative result), but then the sulfide titration method was included. According to various sources, the toxicity of total sulfides is in the range of 1,000 to 1,200 mg/l, but, for example, the toxicity limit of soluble hydrogen sulfide is already at the level of 100 to 200 mg/l. The proportion of different sulfide compounds in the solution is pH dependent, as shown in Figure 33. The average pH increase during the ramp-up was about 7.8 (Table 9), so that the proportions of sulfide compounds in different pH ranges can be seen from Figure 33 (the proportion of hydrogen sulfide in liquid form is 12 %). In this case, for example, the proportion of soluble hydrogen sulfide in the ramp-up week 6 was about

120 mg/l. Although the toxicity limits of sulfides are high, even sulfide concentrations below the toxicity limits can already slow down the activity of sulfate-reducing bacteria. High sulfide concentrations can also be seen in the gas measurements in the scoreboard (Table 19), which show a clear increase in hydrogen sulfide concentrations from ramp-up week 7 onwards. This indicates that the reactor solution is already so saturated with sulfide (Figure 32) that the sulfide must escape from the liquid phase to the gas phase. The daily increased sulfate load should not have a disruptive effect on the sulfate reduction process, so the decrease in SRB efficiency can be said to be very much due to sulfide toxicity and thus to excessive carbon accumulation in the process.

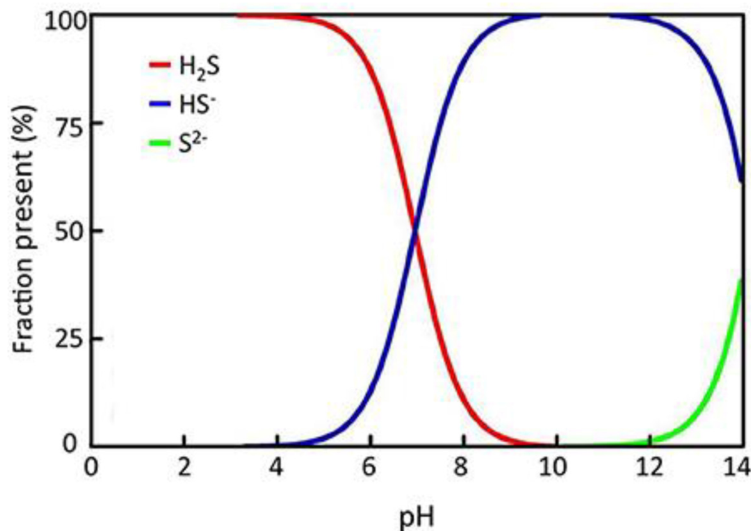


Figure 33. Proportions of sulfide compounds in different pH ranges (Hasler-Sheetal, 2014).

14.2 Result from the second test run

The start-up of test run II was started according to plan (Table 16). The load of sulfate-reducing bacteria was increased on a weekly basis by slightly increasing the flow and sulfate concentration in influent. The ramp-up of test run II was the same as the first run, ie methane was formed during it. The increase in methane concentration was first seen in ramp-up week 5. At that time, the concentration increased by only 0.1 % from the initial concentrations, but in ramp-up week 7 it was found that the methane concentration had already risen to 0.6 %. Based on the experience of the previous test run, it was possible to immediately reduce the carbon supply in order to keep the effect of methanogens to a minimum. By reacting quickly to the situation, the methane content was managed so well that the concentrations peaked at about 9 % in one day. Thereafter, the concentrations generally remained below 0.4 % during test run II.

Table 20. The main results of the second test run.

Test week	pH	Redox, mV	Conductivity, ms/cm	SO_4^{2-}, mg/l	S^{2-}, mg/l	TOC, mg/l	H_2S, ppm
Inoculation	7,97	-340	7,8	2 103	49		
Inoculation	8,03	-398	8,7	2 575	127		
Inoculation	8,03	-394	9,4	2 834	32	216	10
Inoculation	7,92	-420	10,1	3 027	48	140	13
Inoculation	7,78	-438	9,9	2 513	231	160	
Inoculation	7,90	-443	10,8	1 164	688	53	
1	7,99	-453	9,7	1 363	642	150	382
2	7,84	-445	7,5	1 354	563	85	75
3	7,96	-456	6,9	768	754	120	82
4	8,07	-449	7,0	1 159	599		579
5	7,85	-452	7,0	1 970	492		62
6	7,99	-460	7,3	1 121	459		155
7	8,10	-459	7,7	1 163	604	240	303
8	7,49	0	6,4	1 798	395	28	3 459
9	7,09	-420	7,2	2 524		320	22
10	7,21	-424	7,6	2 415	325	110	106
11	7,72	-450	8,1	1 457	510	129	211
12	7,88	-454	8,4	1 769	438	140	435
13	7,98	-466	8,7	1 767	334	70	1 435
14	7,94	-456	8,7	1 916	372	156	122
15	7,85	-456	9,4	1 980	607		576
16	7,92	-450	10,0	2 406	698		2 156
17	8,04	-452	10,0	2 483	664	230	1 785
18	8,14	-450	9,1	1 966	725	250	2 004
19	7,79	-439	9,6	2 492	863	210	1 448
20	8,20	-425	8,9	2 973	176		76
21	7,64	-416	6,4	1 626	110		36
22	7,80	-452	7,0	1 568	419		23
23	7,82	-449	9,5	2 151	591		30
24	7,78	-455	11,2	2 156	665		47

Figure 34 shows the measurement results of sulfate and sulfide for the test run. As previously stated, sulfate reductions could not be reasonably calculated because the feed varied somewhat back and forth due to ramp-up problems. To that extent, it can be very roughly estimated that about half of the added sulfate load was reduced during the ramp-up. This is based on the fact that the sulfate measurements of the reactor and the effluent looked pretty much the same and that the concentration of the inflow was generally about twice that of the previous ones. Unfortunately, in the test run, it was not possible to reach the run with a steady flow after the ramp-up, which would have made more sense in calculating the reductions. At its highest, the influent feed (22 l/d) was from the end of the ramp-up week 22 to the end of the ramp-up week 23. Which means a circulation time of 1.2 days for industrial wastewater treatment (test run target was 1 day). The sulfide concentrations at the bioreactor were at an almost optimal level > 500 mg/l. Sulfate has been reduced based on microbiology and can be used, for example, in metalprecipitation.

The increase in the methane concentration was possible again this time for largely the same reasons as in the previous test run. The inoculum used was the same starting material because nothing else was available. The limits of sulfide toxicity were not exceeded

on the basis of the measurements of the test run, but as the figure below shows, the sulfide concentrations were already high at the beginning of the ramp-up phase. The reason for this was that their concentration already rose to this level at the end of the adaptation phase (Table 20). This has probably had the effect of slowing down SRB activity. In addition, carbon was initially accumulated in the process on the 3rd weekend of the rump-up week, after a fault had occurred in the influent feed pump (no feeding during weekend).At the same time, however, the carbon feed operated normally, which caused a large amount of carbon to accumulate in the reactor (Figure 35). Together, these factors allowed favoriable conditions to methanogens.

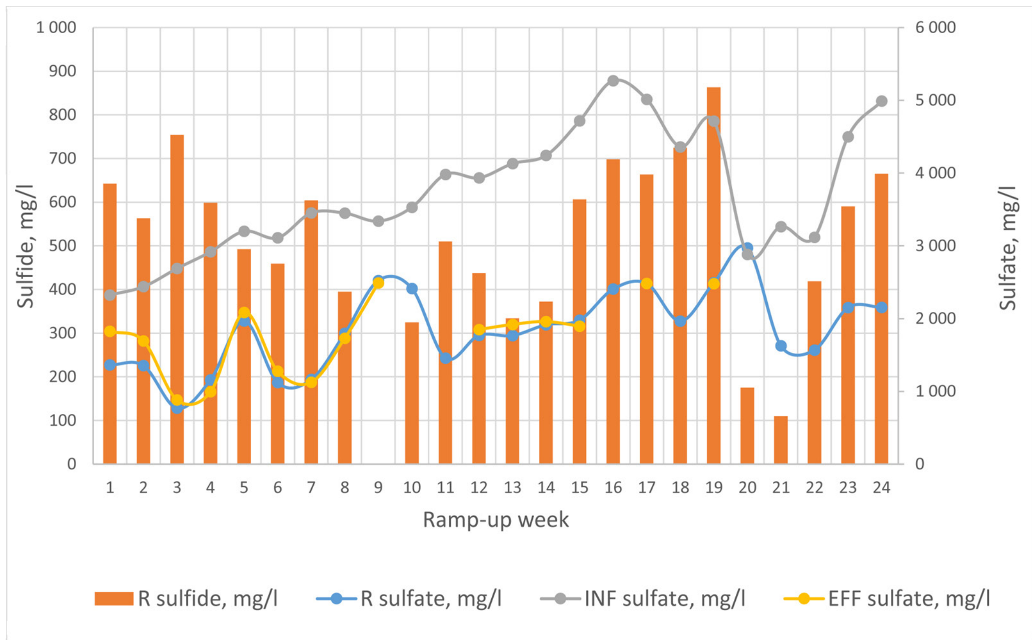


Figure 34. Test run II: sulfate and sulfide concentrations during the ramp-up phase.

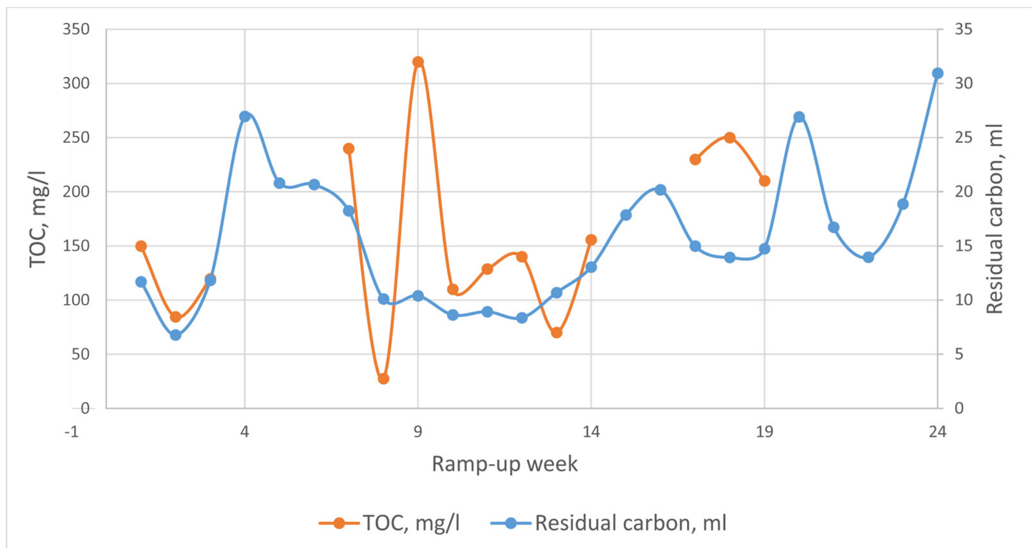


Figure 35. Test run II: residual carbon and organic carbon during ramp-up phase.

During ramp-up weeks 8-10, an attempt was made to remove methanogens from the process by adjusting the pH of the reactor in different directions. This experiment was based on the fact that sulfate-reducing bacteria are able to function better in different pH ranges than methanogens, which are more sensitive to pH fluctuations. The pH of the reactor was adjusted by influent and the influent pH was at a low of 3.3 and at a high of about 9. The pH variation did not appear to have an effect on the decrease in methane concentration, but at week 8 an increase in hydrogen sulfide concentration in the gas phase was seen. This was because as the pH of the reactor solution dropped to about six, the proportion of sulfides changed more in the direction of hydrogen sulfide (Figure 33), which also released more into the gas phase as the solution became saturated with hydrogen sulfide. The most positive effect of the experiment on sulfate-reducing bacteria was that the sulfide concentration dropped briskly momentarily, making the solution less toxic and disruptive to the process. The effect can also be seen in the figure (Figure 34), where during weeks 8 to 10 the sulfate content first increases because the pH variation also affected the activity of sulfate reducing bacteria, but when the sulfide content was lower and the pH stabilized, the sulfate content started to decrease again.

At the beginning of ramp-up week 20, the pH of the reactor had risen to just over nine. This was due to an increase in pH during the pH adjustment of the carbon solution. The pH of the carbon solution was adjusted during the test runs with sodium hydroxide (50 %) to raise its pH to 5.5-6.0. The pH of the solution was measured before weekend to be about 6, but apparently the lye had still not reacted at this stage. Thus, the pH of the solution had continued to rise and was about 11 when measured after the weekend. This has, of course, substantially affected the reactor and the activity of the sulfate-reducing bacteria. This damage has had a positive effect on the sulfide concentration in the reactor. It can be seen from figure (Figure 34) that before the change in pH, sulfide concentrations have been increasing week by week and their levels have already approached threatening toxicity limits. The increase in pH has probably precipitated the sulfides in the compounds in the reactor and thus their concentrations have fallen considerably in the following weeks. The reduction of sulfate has also slowed down with the case and a much more dilute sulfate solution was fed to the reactor for a while, which can be seen in the decreased sulfate concentrations (Figure 34). The same picture of the increase in sulfide levels from week 22 onwards also shows that the activity of sulfate reducing agents has resumed in less than two weeks. This was probably due to the fact that the sulfide content in the reactor was well below the limit at which point they interfered with the process. The rate could also be affected by the addition of sodium bicarbonate from week 21 onwards, which increased the alkalinity of the reactor and thus its well-being.

15 CONCLUSIONS ON MICROBIOLOGICAL SULFATE REMOVAL

Methane was formed in both test runs, but the test run II process method was more reasonable. By increasing the sulfate content and flow in the treated wastewater once a week, the sulfate reducing bacteria have more time to adapt to the increasing load. In both test runs, the ramp-up was not completed, which may be due in part to the aim of too high a sulfate content to be treated. It might make more sense to run the process up to full flow first, even with influent with a maximum sulfate content of 3,000 mg/l, after which the sulfate content of the influent could then be gradually increased to a higher level. During the test runs, the effect of the sulfate content adjusted at the beginning of

the growth phase on the ramp-up rate of the process was also observed. In the first test run, the sulfate concentration in the container of the growth phase was about 5,000 mg/l, while in the second it was only about 3,000 mg/l. This difference was the largest factor, contributing to the fact that in the first test run, it took about 5.5 months from the start of inoculation to the start of the inoculation, while in the second, the equivalent lasted only about 2 months.

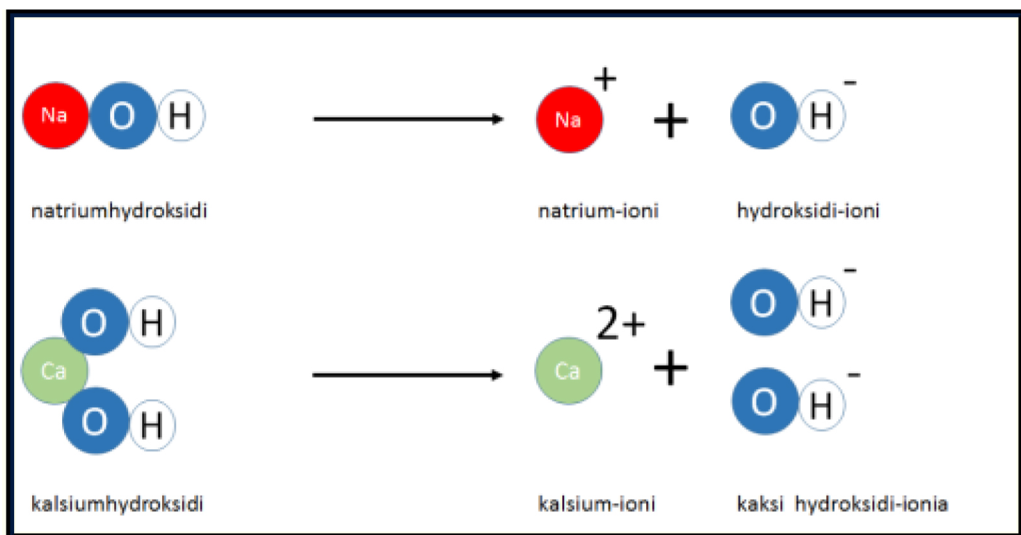
It should pay attention to the choice of inoculum. The natural strain used proved to be effective in sulfate reduction, but it always also contains other microbes which are not necessarily desirable when operating the process. It is always possible to buy clean strains for use in processes, but they are quite expensive and wastewater containing them should not be discharged into the sewer, so it is also a bit complicated operation.

The biggest factor complicating the test runs was sulfide toxicity and the difficulty of controlling it. Based on the results, the sulfate reducing bacteria can be said to have worked quite well, because the sulfide concentrations sometimes rose rapidly too high. Sulfide concentrations approaching the toxicity limit slow down SRB activity and this was occasionally reflected in the decreasing efficiency of the process. The problem with the equipment was that the sulfides were not removed as fast as what was formed. Sulfide levels could be lowered by diluting the reactor solution or by driving sulfides out of solution as hydrogen sulfide gas with nitrogen gas. The first of these occurred to the extent that new influent was fed to the reactor, but no additional dilution was desired because it always washes out bacteria essential to the process. Intermittent feeding of nitrogen gas resulted in more efficient dilution of the sulfide concentrations than the calculated dilution, but feeding the gas to the reactor caused even strong bubbling of the solution, which in turn was feared to detach and disintegrate bacterial blocks formed on the filler surfaces and the sludge bed. Therefore, it could not be used very often to avoid its side effects. The equipment must be developed so that it has, for example, a functioning separate reactor through which the solution of the actual reactor could be recycled. This second reactor would then have, for example, the removal of hydrogen sulfide by a continuous supply of nitrogen gas. Nitrogen gas is inert and does not affect the process and when used in a separate reactor there would be no fear of rinsing the bacteria off the surface of thefillers.

REPORT PART C:

CHEMICAL PRECIPITATION OF CALCIUM FROM MINING WASTE WATER

Authors: Maarit Janhunen and Olli Torvinen



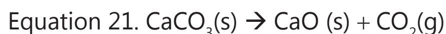
16 CALCIUM REMOVAL FROM INDUSTRIAL WATER

16.1 The presence of calcium and the aims of the study

Calcium is part of second major group in the periodic table, alkaline earth metals. Calcium is most common alkaline earth metal and the fifth most abundant element in the earth's crust (4.1 %). Calcium is a silvery, soft metal that quickly darkens in the air and reacts with water. Pure calcium does not occur naturally, but is present in compounds such as limestone (calcium carbonate $CaCO_3$, which is the most important source of calcium in the industry), gypsum (calcium sulfate), fluorite (calcium fluorite) and apatite (calcium chlorophosphate or calcium fluorophosphate) (Hannola-Teitto, 2006).

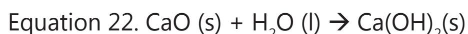
Calcium metal is used as a reducing agent in the manufacture of other metals such as thorium and uranium. It is also used as alloying agent for aluminium, beryllium, copper, lead and magnesium alloys. Calcium is also used in the manufacture of steel to remove impurities from molten iron ore. Slaked lime or calcium hydroxide is used in cement production, soil improvement and water treatment to reduce acidity and in the chemical industry (Royal society of chemistry, 2019).

Limestone, chalk and marble are calcium carbonate. Calcium carbonate is an important building material and a paper additive. Calcium carbonate or hydroxide made from it are used to neutralize acidic waters and reduce acidity. Calcium carbonate is soluble in acids while producing carbon dioxide. For this reason acid rain are harmful for buildings made of limestone and marble. Gypsum is another calcium compound used as a building material, as a concrete additive and in paper coating. When burning limestone in high temperature, the carbonate decomposes to oxide according to the following reaction Equation 21, the reaction also produces burnt lime (CaO) (Hannola- Teitto,2006).



Equation 21. $CaCO_3(s) \rightarrow CaO\ (s) + CO_2(g)$

Calcium oxide reacts violently and exothermically with water to swell and form calcium hydroxide powder, i.e. slaked lime ($Ca(OH)_2$) (Hannola-Teitto,2006).



Equation 22. $CaO\ (s) + H_2O\ (l) \rightarrow Ca(OH)_2(s)$

Calcium hydroxide reacts with carbon dioxide in the air back to carbonate. This reaction occurs, for example, when concrete and mortar harden

Equation 23. $Ca(OH)_2(s) + CO_2(g) \rightarrow CaCO_3(s) + H_2O\ (l)$

Calcium is essential for all living beings and it is especially important for healthy and strong teeth and bone growth. Calcium phosphate is a main component of bone. On average, a person contains about 1 kg of calcium (Royal society of chemistry, 2019).

In this project calcium precipitation was tested in the laboratory by JAR experiments, of which the implementation and results of the tests are presented in this part C of the final report. A separate thesis has been published on the implementation and theory of test series (Bachelor Thesis). The test series were carried out with process water supplied by the industrial plant, using various precipitation chemicals. In addition there was tested a

biological solution of the sulfate reduction process. In the RO reject (water after reverese osmosis) formed in the process industry typically has a high calcium and sulfate content. The purpose of these test series were to remove some of the calcium by precipitation tests, after which the water can be directed to a microbiological sulfate reduction process. The removal of sulfate (SO_4^{2-}) is difficult due to the solubility and stability of the sulfate salts. A common method for removing sulfate to precipitate it into gypsym. The sulfate content can be decreased by lime precipitation to form gypsum or calcium sulfate. More effective sulfate removal methods are precipitating the sulfate with a barium salt or raising pH and precipitating the sulfate with aluminum oxide.

The starting points for the implementation of the test series were the precipitation conditions identified and numerically optimized through digital modelling. One of the most important competitive advantages for the process industry is a thorough understanding of the chemical phenomena observed and exploited in industrial processes. Digitized process understanding and computation based on it accelerate process design, problem solving and inservice diagnostics.

Finland has been a pioneer in process modeling for years. In Finland has been developed for example HCS, Apros, Balas, ChemSheet, Flowbat Kilnsimu and Napcon products to solve problems in the process industry by describing the most important natural laws affecting processes into simulation software. One of the most successful areas is digitalized computational thermodynamics, which has been widely used to solve various problems in the process industry.

16.2 Calcium removal as carbonate

Usually the alkalization of water is accomplished by adding an alkalizing chemical to the water, whereby the carbon dioxide in the water begins to be neutralized to the bicarbonate. At hte same time the pH in the water increases. In lime-based processes, at the same time, the calcium content and water hardness increase (Figure 36). With a water pH of 7, the total carbon dioxide content in the water is about 75 % bicarbonate and 25 % carbon dioxide. When the pH of the water rises to 9, the total carbon dioxide content of the water is 95 % bicarbonate and 5 % carbonate. Calcium bicarbonate is highly water soluble and calcium carbonate is sparingly soluble which means in practice that the pH must be above 9 to precipitate calcium (Vesi- ja viemäriyhdistys, 2002).

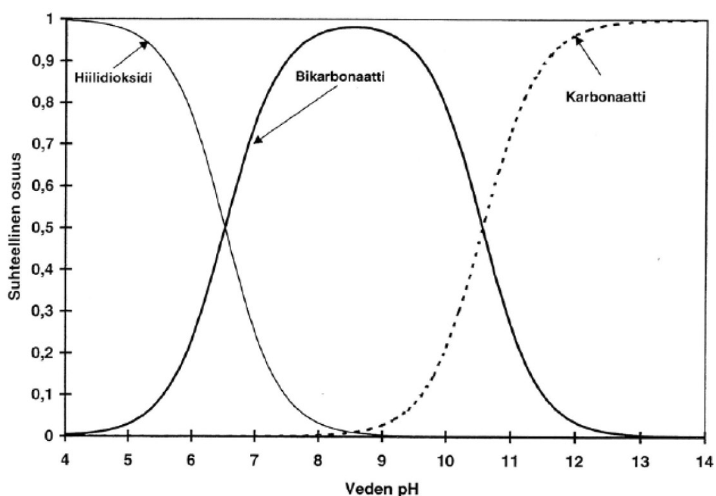


Figure 36. Effect of water pH on relative amounts of carbon dioxide, bicarbonate and carbonate (Vesi- ja viemäriyhdistys, 2002).

In his study, Chen looked at the effect of carbon dioxide on calcium removal (Figure 37 & Figure 38). Carbon dioxide (CO_2) is easily soluble under basic conditions. When carbonate CO_3^{2-}compund is mixed with Ca^{2+}, a precipitate of calcium carbonate ($CaCO_3$) is formed to remove calcium ions. For better absorption of carbon dioxide in water, the pH must be adjusted >10.5. At the same time carbon dioxide gas is fed continuously until saturation. By adjusting reaction time, pH, stirring rate and other conditions, the best result is obtained for the removal of calcium ions (Chen, 2018).

When dissolved, carbon dioxide increases the alkalinity i.e. water buffering resistance against pH change (Vesi- ja viemäriyhdistys, 2002).

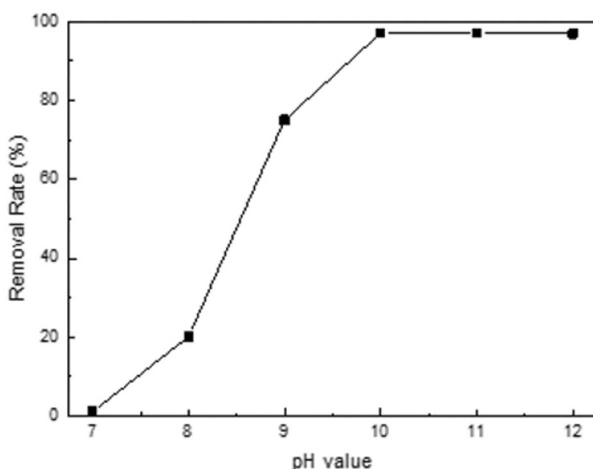


Figure 37. Effect of pH on the removal of calcium ions (Chen, 2018).

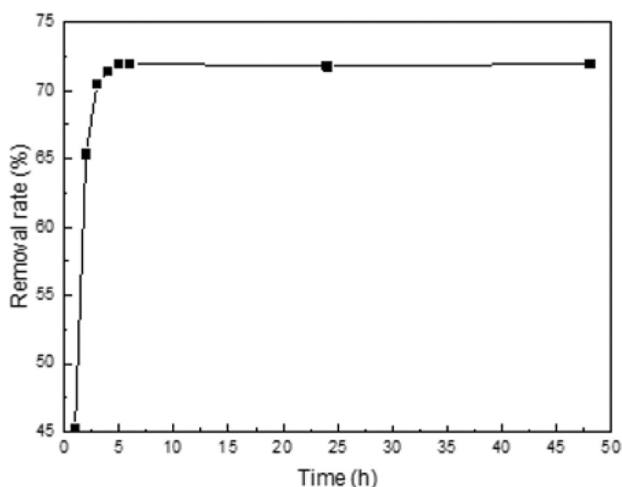


Figure 38. Effect of time on the removal of calcium ions (Chen, 2018).

16.3 Calcium removal by filtration

Calcium salts can be removed from water by reverse osmosis and nanofiltration. Karppanen, 2017 writes that the equipment needed for reverse osmosis and the method of running the process should always be designed according to what kind of raw material i.e. feed water is used. The amount and quality of the impurities affect, among other things, pressure applied, choice of pretreatment and film contamination. When treating process waters, impurities can be almost anything and their concentrations vary depending on the process (Karppanen, 2017).

Optimal pH for reverse osmosis is achieved by feeding acid or base to water prior to feeding to RO membranes. The purpose of decreasing the pH is mainly to prevent crystallization of calcium carbonate. Accordingly, raising the pH mainly aims to reduce the water solubility of metals such as calcium carbonate. Raising the pH also prevents organic and chemical contamination (Karppanen, 2017).

The most common salts that precipitate on the membrane surface are calcium carbonate ($CaCO_3$), calcium sulfate ($CaSO_4$), barium sulfate ($BaSO_4$) ja strontium sulfate ($SrSO_4$), calcium fluoride (CaF_2), calcium phosphate ($CaPO_4$) and magnesium hydroxide ($Mg(OH)_2$). Sulfates and carbonates are sparingly soluble in water and therefore easily crystallized by concentration polarization (Karppanen, 2017).

The action of osmosis is based on separating the two compartments (A) containing pure water and (B) containing salt water with a semipermeable membrane, whereby the pure water tends to pass naturally through the membrane to equalize the salt content of the compartments. Figure 39 represents the working principle of osmosis (Suomen Galvanotekninen Yhdistys, 2003).

The salt content in the compartment (B) decreases until the hydrostatic pressure in the compartment (B) rises above the osmotic pressure causing the passage of pure water. This level difference is characteristic of osmotic pressure and is directly proportional to the salinity and temperature of the water (Suomen Galvanotekninen Yhdistys, 2003).

In reverse osmosis, due to the osmotic equilibrium, it is sufficient to apply a pressure higher than the osmotic pressure to the saline water (B) to reverse the natural osmosis. The direction of water flow is reversed, and since the membrane only passes through pure water, the saline solution cannot pass back through it (Suomen Galvanotekninen Yhdistys, 2003).

Nanofiltration is very close to reverse osmosis as a process. The difference is the higher permeability of ions in membranes used in nanofiltration compared to reverse osmosis (Suomen Galvanotekninen Yhdistys, 2003).

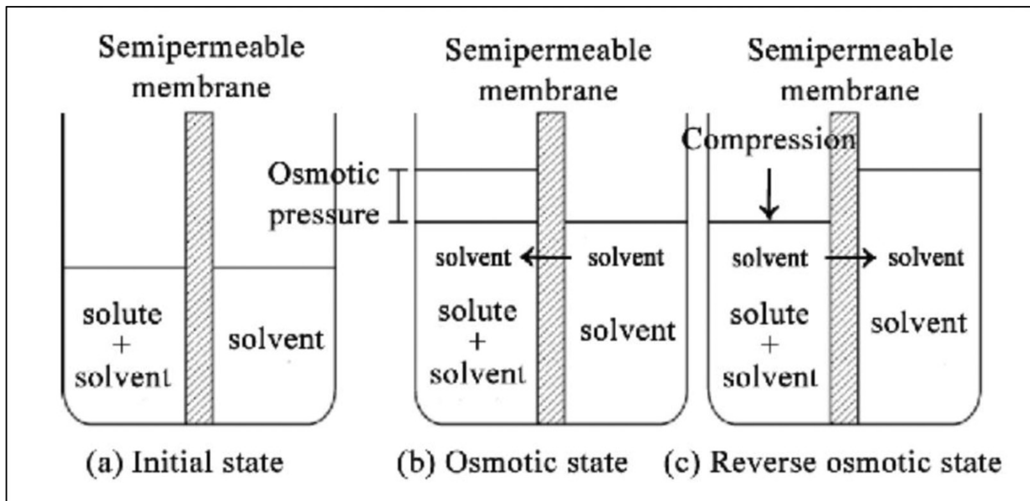


Figure 39. The principle of osmosis (Suomen Galvanotekninen Yhdistys, 2003; Lei Jiang, 2018).

17 CALCIUM PRECIPITATION TESTS IN PROCESS WATER – TEST APPLICATION

Calcium precipitation test were carried out in the laboratory using process water supplied by the industrial plant. Analytical grade chemicals such as bicarbonate calcium hydroxide and sodium hydroxide were used as precipitating chemicals. From biosolution, both the rejection of the sulfate reduction process and the synthetic biosolution were tested. The process water used contained almost one and a half grams of calcium per liter. Table 21 indicates the concentrations of process water and the concentrations of the chemicals used.

Table 21. Rawmaterials and used chemicals.

Material	Compund/Ion	Concentration, m (g/l)
Process water	Ca	1.439
Biosolution	HCO_3	10
Sodium hydroxide (10 w-%)	NaOH	100
		Concentration, m (g/kg)
Lime (dry)	$Ca(OH)_2$	1 000

Through the modeling, it was planned to carry out the test series so that in the first experiment all chemicals are dosed at once and the residual calcium is measured only at the end. In the second experiment, the chemicals were dosed in three steps, each of which was individually measured for residual calcium. Table 22 shows the dosages of chemicals shown in the model for the experiments.

Table 22. Modeling feed rates, measurement only at end of test (lines 1-3) and addition of chemicals in three steps and measurements after each step (lines 4-6).

	Chemical	Process water, ml	HCO_3, ml	NaOH, ml	$Ca(OH)_2$, g
Case 1	HCO_3 + NaOH	500	109.5	7.2	
Case 2	$Ca(OH)_2$ + HCO_3	500	219		1.33
Case 3	HCO_3	500	328.5		
Case 1	HCO_3 + NaOH	500	3*36.5	3*2.4	
Case 2	$Ca(OH)_2$ + HCO_3	500	3*73		3*0.45
Case 3	HCO_3	500	3*109.5		

Precipitation tests were carried out with four different chemical combinations:

1. **Precipitation tests using sodium bicarbonate and sodium hydroxide.** Sodium bicarbonate dissolved in deionized water and 10 % lye as the base was used in the precipitation test. The chemicals were mixed with 500 ml of process water. In two tests, the core precipitate of the corresponding precipitation test was added to the solution and its effect on calcium precipitatioin was investigated.
2. **Precipitation tests using sodium bicarbonate and calcium hydroxide.** In the precipitation test, dissolved sodium bicarbonate mixed with deionized water and dry lime as the base were used. The chemicals were mixed with 500 ml of process-water.
3. **Precipitation tests using sodium bicarbonate.** Sodium bicarbonate mixed with deionized water and mixed with 500 ml of waste water was used in the precipitationtest.
4. **Precipitation tests with a biological solution.** In the precipitation test, real reject water of the sulfate reduction process was used, which was mixed with 500 ml of waste water.

JAR equipment was used to mix the reagents (Kemira Flocculator 2000) (Figure 40). Temperature and pH were measured during tests with a WTW pH 3110 meter. In the tests, the calcium concentrations of the solutions were determined with an EDXRF –analyzer (Energy Dispersive X-ray Fluorescence) at the University of Eastern Finland in Kuopio. X-ray Fluorescence or XRF is a non-destructive analytical technique used to determine the chemical composition of materials. In XRF analysis, the sample is irradiated with X-rays

and the energy bound to the substance is discharged at the fluorescence characteristic of each element. Each element produces a unique combination of fluorescence radiation spesific to that element, enabling the analyzer to identify the element.

Figure 40. Kemira Flocculator 2000 (Ficure: Maarit Janhunen).

Calcium precipitation tests were carried out in the Savonia laboratory during February-March 2019. The pH of the process waste water used was variable, as were the calcium levels. For the precipitation tests, 2-3 replicate 1 000 ml beakers were used to which 500 ml of process waste water was added. After the addition of process waste water, the chemicals used in the tests were add and mixing startd with a JAR flocculator. The mixing was carried out for 30 seconds with fast mixing (100 rpm) and for 10 minutes with slow mixing (40 rpm). After mixing, clarification and precipitation were performed using different precipitation times. Finally, the pH was measured and a sample was taken for calcium EDXRF analysis.

18 RESULTS OF CALCIUM PRECIPITATION TESTS

The water used for the calcium precipitation tests was real industrial process water containing high concentrations of sulfate and calcium. The purpose of the tests was to remove some of the calcium, after which the water can be subjected to a microbiological sulfate reduction process. The aim of the calcium precipitation tests was to reduce calcium to about 400 mg/l, not all calcium was supposed to be removed because microbes also need it for growth. In addition, the goal was to have the lowest post-test pH as the

sulfate reduction process is operated at about pH 7.5. In this way, after the precipitation tests, the pH adjustment is minimized and chemical cost savings are achieved. In all tests, a fast mixing of 30 seconds (100 rpm) and a slow mixing (40 rpm) were used. The precipitation time was slightly varied between different test series.

The first was to test the chemical inputs by dosing all chemicals (Table 22, amounts of chemicals in rows 1-3) at one time. Precipitation time was 60 minutes. Table 23 presents the results for the first set of tests with two replicate tests for each case (cases 1-3). The results show that cases 1 and 2 work and the reductions are almost all over 90 %, but in the case of synthetic biosolution, calcium increases in the process.

Table 23. Calcium precipitation test results for two replicates, measurements only at end.

		Ca, mg/l	Red-%	End pH	Ca, mg/l	Red-%	End pH
Case 1	Process water Ca-level: 1 191 mg/l, pH-level 7.21	49	95.9	11.9	127	89.3	11.9
Case 2		34	97.1	10	29	97.6	9.9
Case 3		7 884	-562	7.6	7 833	-558	7.7

As Table 22 and Table 23 prove cases 1 and 2 worked effectively when chemicals were dosed all at once. The same amounts of chemicals were tested by adding them to the process water in three steps, using 10 minutes precipitation time and in addition performing a fourth measurement 60 minutes after the addition of all chemicals. Table 24 presents the results achieved. In case 1, i.e. the combination of bicarbonate and lye, optimum reduction is achieved 60 minutes after the precipitation time, but unfortunately the pH rises. In case 2, i.e. the combination of dry lime and bicarbonate, sufficient reduction is achieved already after the first dose of chemical has been added. In fact, adding another batch of chemicals does not increase the reduction and with the final bacth and longer precipitation time, the calcium content only increases. In both cases, there are some differences in the parallel tests.

Table 24. Calcium precipitation test results for two replicate tests, three-step chemical addition and three-step measurement plus one additional measurement after 60 minutes of precipitation.

		Ca, mg/l	Red-%	End pH	Ca, mg/l	Red-%	End pH
Case 1	Process water Ca-level: 1 217 mg/l, pH-level 7.6	668	44.7	11.0	667	44.7	10.4
		1 217	-0.8	11.4	1 697	-40.6	10.9
		1 775	-47.1	11.9	1 346	-11.5	10.6
		402	66.7	12.1	442	63.4	11.0
Case 2	Process water Ca-level: 1 217 mg/l, pH-level 7.6	606	50.2	9.3	577	52.6	10.4
		604	50.4	9.3	581	52.3	10.9
		2 392	-96.4	10.6	1 017	16.5	10.6
		1 090	10.5	10.8	571	53.1	11.0

Next, the precipitation enhancement using core precipitate was tested for cases 1 and 2. The dosages of the chemicals were still the same (Table 22). Four varitations at 30, 60, 120 and 240 minutes were used as precipitation times. The amounts of core precipitate in tests were 100 and 200 ml. The following tables (Table 25 and Table 26) show the results of calcium precipitation tests. The results on the left side are for the 100 ml core precipitate and on the right for the 200 ml core precipitate. The results show that core precipitate contributes up to 98 % to calcium precipitation. However, extending the precipitation time does not yield any corresponding benefits. In case 1, the end pH rises to about 12, but in case 2 the end pH remains at 9.

Table 25. Calcium precipitation test results for case 1. Addition of chemicals is done in one step. The results for the 100 ml core precipitate are on the left and for the 200 ml core precipitate on the right.

	Precipitation time, min	Ca, mg/l	Red-%	End pH	Ca, mg/l	Red-%	End pH
Process water	30	236	80.1	11.8	13	98.9	11.8
Ca-level: 1 195 mg/l	60	23	98.1	11.9	137	88.5	12.0
pH-level 7.5	120	37	96.9	11.9	94	92.1	12.0
	240	30	97.5	12.0	32	97.3	11.8

Table 26. Calcium precipitation test results for case 2. Addition of chemicals is done in one step. The results for the 100 ml core precipitate are on the left and for the 200 ml core precipitate on the right.

	Precipitation time, min	Ca, mg/l	Red-%	End pH	Ca, mg/l	Red-%	End pH
Process water	30	19	98.4	9.1	232	80.6	9.8
Ca-level: 1 205 mg/l	60	117	90.3	9.7	750	37.3	9.4
pH-level 7.5	120	26	97.8	9.8	25	97.9	9.8
	240	33	97.3	10.0	33	97.2	9.5

Next the alkaline and lime dose optimization was tested using a constant amount of synthetic biosolution for cases 1 and 2. Table 27 shows the results for case 1 where both vessels contained a constant amount of synthetic biosolution (84 ml →500 ml process water). In the first series of six measurements, starting with 4.9 ml (up to 5.4 ml) of lye and adding 0.1 ml more at the end of precipitation time. The precipitation time was 10 minutes. In another vessel, the same test was repeated starting with 5.5 ml of lye and adding up to 6.0 ml.

Although the synthetic biosolution alone was not capable of precipitating calcium, the results show that even a small amount of lye enhances precipitation in almost all dose amounts to more than 90 %. However, the final pH is quite high considering the next processstep.

Table 27. Optimization of the calcium precipitation tests by adding lye for case 1. A standard amount of synthetic biosolution (84 ml/500 ml process water) was used in the tests.

	NaOH, ml	Ca, mg/l	Red-%	End pH	NaOH, ml	Ca, mg/l	Red-%	End pH
	4.9	156	87.1	11.3	5.5	192	84.1	11.7
Process water	5.0	38	96.9	11.4	5.6	100	91.2	11.7
Ca-level: 1 210 mg/l.	5.1	86	92.9	11.4	5.7	55	95.5	11.7
pH-level 7.9	5.2	18	98.5	11.4	5.8	28	97.7	11.7
	5.3	27	97.8	11.4	5.9	45	96.3	11.8
	5.4	19	98.4	11.6	6	30	97.5	11.8

Dry lime optimization was tested similarly using 73 ml of synthetic biosolution in 500 ml process water. Table 28 shows the results of tests in which the dosage of dry lime started from 0.2 g and continued up to 0.45 g. In the second vessel, testing was performed from 0.5 g to 0.75 g. The results show that the nearest target of 400 g of residual calcium was achieved only by the addition of 0.5 g of dry lime, the final pH then reached 10.7. As a whole, dry lime did not achieve effective precipitation results at the dosage of the biosolution in question.

Table 28. Optimization of calcium precipitation tests by adding dry lime for case 2. A standard volume of synthetic biosolution (73 ml/500 ml process water) was used in the tests.

	$Ca(OH)_2$, g	Ca, mg/l	Red-%	End pH	$Ca(OH)_2$, g	Ca, mg/l	Red-%	End pH
	0.2	898	25.9	8.0	0.5	586	51.6	10.7
Process water	0.25	843	30.1	8.0	0.55	614	49.3	10.9
Ca-level: 1 210 mg/l.	0.3	784	35.3	8.2	0.6	709	41.4	11.0
pH-level 7.9	0.35	775	36.1	8.4	0.65	825	31.8	11.1
	0.4	736	39.3	8.6	0.7	809	33.1	11.1
	0.45	714	41.1	9.1	0.75	1 066	11.9	11.4

Next, the target levels of residual calcium were set at 300 mg/l and 500 mg/l by varying the concentration and amount of the bicarbonate i.e. biosolution to be added to the reaction. For selected solution concentrations the modelling set 12.5 g/l, 14.5 g/l and 16.5 g/l, dosed as indicated in the table below (Table 29). In addition, the amount of lye indicated in the model was dispensed to each volume of biosolution. The precipitation time was 60 minutes after each addition of the lye.

Table 29. Testing of calcium precipitation by varying the volume of the biosolution and the lye, with target levels of 300 mg/l and 500 mg/l. The process water had a starting calcium content of 1 392 mg/l and a pH of 7.5.

HCO_3, g/l	Biosolution, ml	NaOH, ml	Ca, mg/l	Red-%	End pH
		1.8	550	55.2	8.9
		1.9	478	61.1	9.1
12.5	60.9	2.0	449	63.4	9.1
		2.1	438	64.3	9.2
		2.2	392	68.1	9.2
		2.0	668	45.7	9.3
		2.1	830	32.5	9.1
14.5	52.5	2.2	1 870	-52.0	9.3
		2.3	2 478	-101.5	9.1
		2.4	2 030	-65.0	10.5
		2.5	3 403	-176.7	10.7
		1.8	522	57.5	8.9
		1.9	481	60.8	9.0
16.5	46.1	2.0	514	58.1	9.1
		2.1	436	64.5	9.2
		2.2	401	67.3	9.5

From the results of the table it is observed that no residual calcium was reached at 300 mg/l at any dose. A solution concentration of 14.5 g/l does not appear to work with lye in this context, on the contrary, lye increases the calcium concentration. Correspondingly, many variations were obtained at the 500 mg/l level at both a solution concentration of 12.5 g/l and 16.5 g/l. The end pH does not rise very high. Also, the level of 400 mg/l as residual calcium was well achieved with the amount of lye 2.2 ml.

Next, it was decided to seek the optimum precipitation time (Table 30) for the dosages of chemicals optimized in the previous tests, i.e. a biosolution concentrations of 12.5 g/l and 16.5 g/l of lye in an amount of 2.2 ml to 500 ml process water.

Table 30. Optimization of the precipitation time for the combination of the biosolution and the lye. The process water had an initial calcium content of 1 392 mg/l and a pH of 7.5.

Biosolution, g/l	Chemicals	Precipitation time, min	Ca, mg/l	Red-%	End pH
		30	755	45.8	9.3
	60.9 ml HCO_3	60	636	54.3	9.3
12.5	+2.2 ml NaOH	120	468	66.4	9.3
		180	426	69.4	9.3
		240	422	69.7	9.2
		300	411	70.5	9.2
		360	411	70.5	9.1
		30	1 721	-23.6	9.4
		60	575	58.7	9.4
16.5	46.1 ml HCO_3	120	501	64.0	9.4
	+ 2.2 ml NaOH	180	4 490	-222.6	9.3
		240	426	69.4	9.3
		300	416	70.1	9.2
		360	432	69.0	9.2

From the results of the table, a precipitation time of 120 minutes for a concentration of 12.5 g/l in the biosolution is considered sufficient for a residual calcium of about 400 mg/l. With a longer precipitation time no more efficient precipitation is achieved.The end pH is at a level which is also good for further use of process water. Correspondingly, at a concentration of 16.5 g/l in the biosolution, the result cannot be accepted at a precipitation time of 180 minutes. Otherwise, at this concentration, optimum results are obtained after 240 minutes of precipitation time, after which it is no longer enhanced by increasing the time. The end pH remains at the same level with another test, 9.3.

19 CONCLUSIONS ON CHEMICAL PRECIPITATION OF CALCIUM

The purpose of the tests was to investigate the possibilities of calcium removal from the wastewaters of industrial processes, focusing on testing different precipitation methods under laboratory conditions. The target for precipitation tests was a residual calcium level of 400 mg/l and the pH should be less than 9.5. Based on the initial values obtained from VTT, the precipitation tests used parameters identified through digital modeling and calculated to be optimal, which determined the dosages of the precipitation chemicals. The aim of the study was to reduce the calcium content of wastewater to be suitable for microbiological treatment by using coagulant chemicals. The challenge of finding the desired concentration was to find the optimum amount of coagulant chemicals so that the pH would not rise too high or too low, affecting the calcium reduction. In all of the verified tests precipitation started to appear visibly almost immediately after the addition of the precipitating chemicals and the precipitate formed was rapidly settling.

All tests were carried out in liter vessels with a blade stirrer using 500 ml process water. In all tests a fast mixing of 30 seconds (100 rpm) and a slow mixing (40 rpm) were used. By varying the precipitation time, differences were detected between different test series.

The calcium precipitation tests were successful, and chemical precipitation makes it possible to remove almost all of the calcium from the waste water by raising the pH to a sufficiently high level with lye or dry lime (Table 23) together with the biosolution. When all chemicals were dosed at once using a precipitation time of 120 minutes, about 93 % reduction was achieved with lye (case 1) and about 97 % with dry lime (case 2). The biosolution alone (case 3) was unable to precipitate calcium. The final pH for both cases was higher than optimal, 12 for case 1 and 10 for case2.

Cases 1 and 2 were subsequently tested by adding chemicals in three batches and using a 10-minute precipitation time after each addition, plus an additional hour of precipitation after the final step (Table 24). Close to optimal was reached already with the addition of the first batch of chemicals, with calcium falling to 570-670 mg/l in both cases. In case 1 optimal calcium reduction (residual calcium level 400-440 mg/l) is achieved after all chemicals have been added and used a one hour precipitation time. The end pH is at the same level as in the previous test where the chemicals were all added at once. From the results it can be stated that the dosing of chemicals in several batches and with a slightly shorter precipitation time does not enhance the calcium precipitation.

When testing the use of core precipitate for calcium precipitation, the precipitate was found to work well cases 1 and 2 with an amount of 100 ml of core precipitate (and Table

26). In both cases, the optimum residual calcium content is achieved with a 100 ml core precipitate and a precipitation time of 30 minutes. In case 1 the pH of the residue rises to 12, but in case 2 the pH of the residue remains to 9.1. Higher amount of core precipitate and longer precipitation time have no effect on calcium precipitation efficiency.

Lye and dry lime were found to work well in the first dosages of chemical shown by the model. The optimization of these chemicals was further tested separately by changing their dosage and using a short precipitation time of 10 minutes. In case 1 (Table 27) i.e. when adjusting the dose of the lye, no significant change is achieved by increasing the dose. With all dosages the end pH is above 11. In both test vessels, more than 90 % of the calcium is precipitated. Dry lime optimization (Table 28) does not produce as good results, with reductions on average below 50 %.

As the lye worked more efficiently with the biosolution in the calcium precipitation, further precipitation tests were carried out to optimize the concentration of the biosolution and the amount of the lye (Table 29). At concentrations of 12.5 g/l and 16.5 g/l of the biosolution, optimal reductions were found in 2.2 ml of lye with a residual calcium of 400 mg/l. Also the end pH was slightly above 9. In this test was used an hour precipitation time. The tests were repeated using the optimum concentrations of the biosolution already found and the amount of lye was 2.2 ml. The precipitation time was tested from half an hour to six hours (Table 30). Based on the results, a sufficiently long precipitation time of two hours is observed, with a residual calcium level of 400 mg/l and a pH of 9.3.

In the calcium precipitation tests the target was set to about 70 % reduction (400 mg/l residual calcium level) and the end pH shoukd be less than 9.5. The real biosolution of the microbiological sulfate reduction process, i.e. reject, did not achieve the desired results. However, the results were achieved in two cases, using a core precipitate and optimizing a biosolution of sufficient concentration. When using core precipitate with lye, stops the end pH too high. With dry lime the set optimum levels are achieved. Similarly, the synthetic biosolution worked at two concentrations (12.5 g/l and 16.5 g/l) together with the lye to achieve the set goals. With the use of core precipitate the precipitation time is not more than half an hour. In contrast, the biosolution-lye combination with a precipitation time of two hours. By extending the precipitation to a larger scale, the treatment facilitates shorter contact times. In this case smaller processing volumes are also sufficient. However, the widespread use of the process in industry for precipitation of larger water masses is fully verifiable.

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SAVONIA

University of Applied Sciences

DEEPCLEANTECH-PROJECT: OPTIMIZATION AND MANAGEMENT OF CHEMICAL AND MICROBIOLOGICAL PROCESSES (2018-2020) – RESEARCH REPORT

The main goal of the DeepCleanTech -project was to facilitate the definition of process solutions through integrated modelling expertise and experimental testing activities. The measures will also make it possible to identify risks and increase the effectiveness of microbiological applications. The subject of the research was removing nitrogen and sulfate from the mining wastewater. The project has received funding from Business Finland's Co-innovation funding program. VTT has acted as the lead organization for the project and the Savonia Environmental Technology Unit has acted as a co-implementer. The following companies participated in the project consortium: Suomen Malmijalostus Oy, Outotec Oyj, Valmet Oyj, Langis Oy and Sulfator Oy. The operating period of the project was 1.5.2018–30.6.2020.

The mining industry generates a large amount of nitrogen and sulphate-containing wastewater annually. In order to treat this wastewater, environmental permits are tightening in the near future for the mining industry and, at worst, new investment projects will be left without funding. The masses of treated wastewater are huge, typically 3-5 million. m^3 per year. Nitrogen removal was tested by a conventional ND process on nitrogen-concentrated mining waters, where more than 90 % of the nitrogen was removed to nitrogen gas. Similarly, sulfate removal was tested using the SRB strain, which was found to be a very challenging process to control. SRB testing activities were based on co-operation with Sulfator Oy, and VTT also modelled the results so that the microbiological well-being of microbes could be assessed more efficiently. Unfortunately, the test run remained at the level of the ramp-up phase. In addition, calcium precipitation from mining water was tested chemically as well as utilizing the bio solution of the SRB process. The experiments were based entirely on a digital model with the goal of dropping calcium to a level of 400 mg/l. The target was reached by more than 90 % with sodium hydroxide and dry lime.

During the experimental research, nitrogen removal was piloted on a laboratory scale by simulating MBBR technology efficiently and reliably in a test run that lasted almost a year. Nitrification bacteria was found to be more challenging to manage, but on the other hand, work steps were learned to allow nitrification to be re-established very quickly. Correspondingly, the SRB process encountered innumerable challenges in mastering microbiology. After implementation of two test runs, the process was still at ramp-up phase. For the SRB process, much more research is still needed to control the microbiological state of the process.

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