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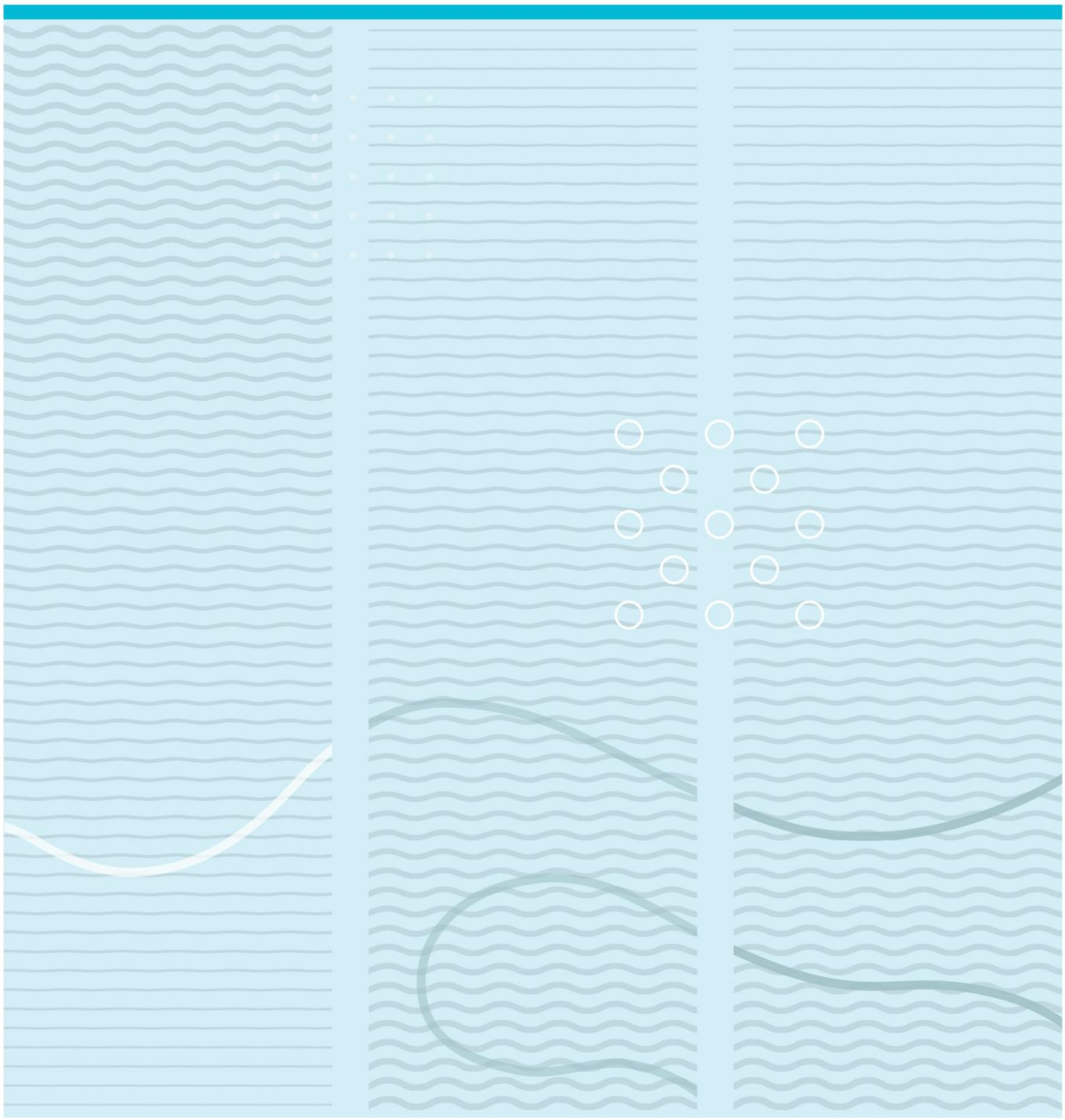
Kinetic study on biological sulphide removal at low temperatures



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Abstract

Sulphides derive as side products in industrial wastewaters or are formed by sulfate-reducing bacteria in anaerobic environments. Sulphides need to be removed, by physico-chemical or biological methods, because of their adverse effect on nature.

During biological sulphide oxidation, nitrate could be used as an electron acceptor, while sulphides serve as the electron donor and oxidizes into harmless elemental sulphur or sulphate. Understanding the kinetics of biological sulphide oxidation is of utmost importance for reactor design. Batch experiments consisting of synthetic media (wastewater) and granular sludge were perform to study the effect of temperature (25 °C, 15 °C and 10 °C) on the kinetics of sulphide removal.

Results showed three distinctively phases: a first phase, which is chemical, where a small fraction of sulphide is immediately oxidized into sulphate. Subsequently the concentration of sulphate decreases. This is followed by a biochemical phase that consists of the initiation of the bacterial denitrification process. During this phase all reduced forms of sulphur are oxidized into sulphate, this phase is clearly dependant on temperature that affects the lag-phase. During the last phase, intracellular elemental sulphur is oxidized into sulphate in the reactor, which creates a higher concentration than the initial concentration of sulphur in the reactor. Concentration changes as a function of time for the different analytes involved, are given in detail in this work.

Keywords: Batch experiment – Anaerobic sulphide oxidation – Temperature - Kinetics

Contents

Preface

I would like to thank my supervisor, Carlos Dinamarca, for guidance and support throughout this study.

I also would like to thank my co-supervisor and flatmate, Michal Sposob, for supporting and guiding me through my experiments and helping me perform successfully them during the night and in the early morning.

Next I want to thank ISU, for making me feel welcome here in Norway and for organizing activities outside of the university.

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1 Introduction

1.1 Research challenge

Industrial wastewaters include high levels of nitrates and sulphides. Nitrate-rich wastewater derives from agriculture and industry (Mahmood et al. 2007). Wastewater that has high concentration of sulphide originates from petrochemical industry, paper mills, sewers, photographic processing and tanneries (Jing et al. 2010) (Vannini et al. 2008).

Hydrogen sulphide (H_2S) is a poisonous, colourless gas, which has a strong odour. The strong odour is similar to the smell of rotten eggs. The recognition of H_2S is only observable by humans at low concentrations (Geertsma 2016). At long exposure or at high concentrations H_2S numbs the olfactory nerve; hence it is no longer possible to smell the presence of H_2S. The smell of rotten eggs is observable because H_2S arises through the conversion of organic substances which contains sulfur (f.e. methionine, cysteine) by sulfate reducing bacteria (SRB) in an anaerobic environment (f.e. at the bottom of a septic tank). Table 1 shows the effects of H_2S on humans (Waterstofsulfide. 2016).

Table 1 Effects of H_2S on the human body by increasing concentration (Geertsma 2014)

Concentration (ppm)	**Effects on the human body**
0,1	Odour is sensible for humans
5,0	Odour of rotten eggs
50	Irritation of respiratory system, losing ability to smell H_2S
100	Coughing and irregular breathing Deadly if exposure is between 8 – 24 hours
200	More coughing and harder breathing, photophobic Deadly if exposure is between 8 – 24 hours
250	Even more coughing and harder breathing, fatigue Deadly if exposure is between 4 – 8 hours
500	Nauseous, serious palpitations, body starts to tremble Deadly if exposure is between 0,5 – 1 hours
800	Unconsciousness Deadly in two minutes
1000	Death

When natural gas containing H_2S is combusted, sulfur dioxide (SO_2) is formed. The SO_2 emission is increasing due to an intensive use of fossil fuels. The importance of having desulfurizers at refineries, natural gas plants, power plants is undeniable. Hence none or a very small amount of SO_2 is formed. SO_2 is a source for acid rain (Feenstra 1982) (Verstraten 1982).

There are many technical solutions for sulphide removal but biological sulphide removal is the most environmental friendly and economical one. There is a variety of solutions/technologies but just a few studies that deals with the kinetics of biological sulphide removal at different temperatures (Kuhn et al. 1983).

There are two methods to remove H_2S: physico-chemical processes and biological processes. Physico-chemical processes are more expensive (because of the use of other chemicals), more complex and have a more negative impact on the environment than biological processes. Biological processes are energy friendly (Gabriel et al. 2003). They can be operated at atmospheric pressure and are not toxic to the environment (Mahmood et al. 2007) (McFarland et al. 1989) (Dinamarca 2014).

1.2 Physico/chemical sulphide removal methods

There are a lot of physico-chemical sulphide removal methods like Claus process, stripping, chemical precipitation, oxidation, H_2S scavenger, scrubbers and membrane technologies.

The Claus process consists in two steps. Firstly, the removal of the acid gases out of the main gas stream and secondly the sulfur components in the acid gas are converted into elemental sulfur.

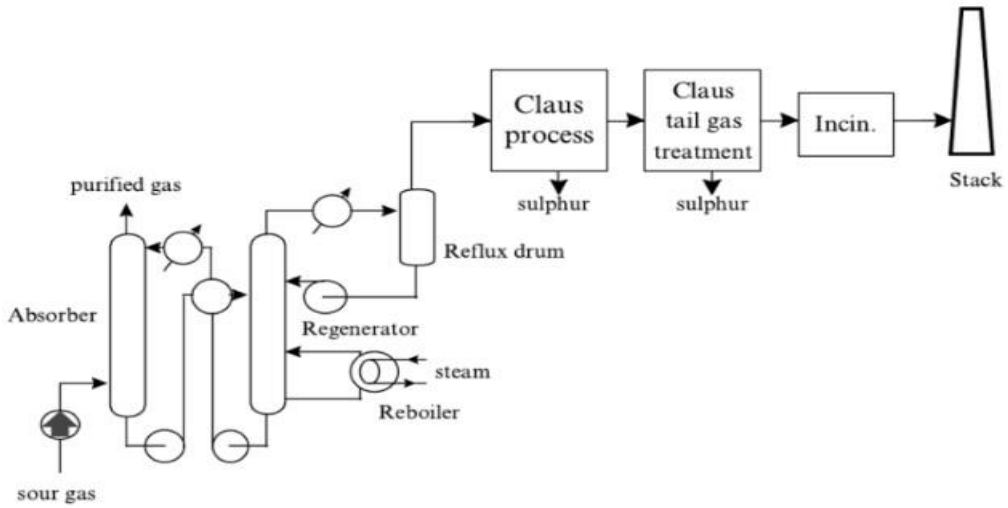

Figure 1 Gas treating and sulfur recovery process (Lens et al. 2005)

The gas containing H_2S passes the absorber (Figure 1), where solvent reacts with the gas flow leading to gas purification. The contaminated solvent is regenerated, where the

solvent is heated and H_2S is desorbed. The leaned solvent is cooled and recycled to the absorber. The gas leaving the top of the regenerator undergoes the Claus process. In this process H_2S is oxidized into elemental sulfur under oxygen presence. One third of H_2S is combusted to SO_2 according to equation 1. While two third of H_2S reacts with SO_2 into elemental sulfur.

$$H_2S + \frac{3}{2}O_2 \rightarrow SO_2 + H_2O \quad (1)$$

$$2H_2S + SO_2 \rightarrow 3S + 2H_2O \quad (2)$$

$$H_2S + \frac{1}{2}\,O_2 \rightarrow S + H_2O \quad (3)$$

The ratio of O_2 to H_2S must be controlled to avoid excessive SO_2 emissions. Later the sulfur needs to be removed from the Claus tail gas. Hence the sulfur recovery in the Claus plants increases. At the end, the remaining H_2S is passing through an incinerator, where is combusted to SO_2 which is emitted to the atmosphere (Nagl et al. 1997) (Lens et al. 2005).

1.3 Biological sulphide removal methods

1.3.1 Principle

Under anaerobic conditions sulphate reduce to sulphide by bacterial activities (Buisman et al. 1990). However nitrate can undergo an autotrophic denitrifcation, by avoiding the reduction of sulphate into sulphide because nitrate has a higher redox potential than sulphate. So chemically, bacteria prefer to reduce nitrate instead of sulfate. Nitrate is not used as an electron acceptor by the sulphate-reducing bacteria SRB, but by the chemolithotrophic or photoautotrophic nitrate reducing, sulfide oxidizing bacteria (NR-SOB) where sulphide is the electron donor. (*Thiobacillus denitrificans* and *Thiomicrospira denitrificans*). Nitrate is reduced into dinitrogen while sulphide is oxidized into sulphate (Krishankumar et al , 1999).

According to following equations, sulphide is oxidized into elemental sulphur or sulfate depending on the ratio between NO_3^- and HS^- (Kleerebezem et al. 2002) (Dinamarca. 2014).

$$14.5HS^- + 5NO_3^- + 0.2NH_4^+ + HCO_3^- + 20.5H^+ \rightarrow CH_{1.8}O_{0.5}N_{0.2} + 2.5N_2 + 14.5S + 17.5H_2O \quad (1)$$

$$3HS^- + 3.9NO_3^- + 0.2NH_4^+ + HCO_3^- + 1.4H^+ \rightarrow CH_{1.8}O_{0.5}N_{0.2} + 1.95N_2 + 3SO_4^- + 2.2H_2O \quad (2)$$

The optimal pH of the autotrophic denitrification is 7-8 (Oh et Al) (Yamamoto-Ikemoto et Al). If the pH is below 7, the denitrification process is not complete and intermediate products like nitrite is present. When pH is below 5, H_2S appears in an ionized form (HS^- and S^{2-}) and non-ionized form (H_2S). The non-ionized form of H_2S is retained into the liquid phase what allows to the penetration of the cell membrane and hinder disulphide bridges between the polypeptide chains, thus obstructing coenzyme activities and sulphur assimilation process (Chen, Y. Et al)(Yongsiri et Al).

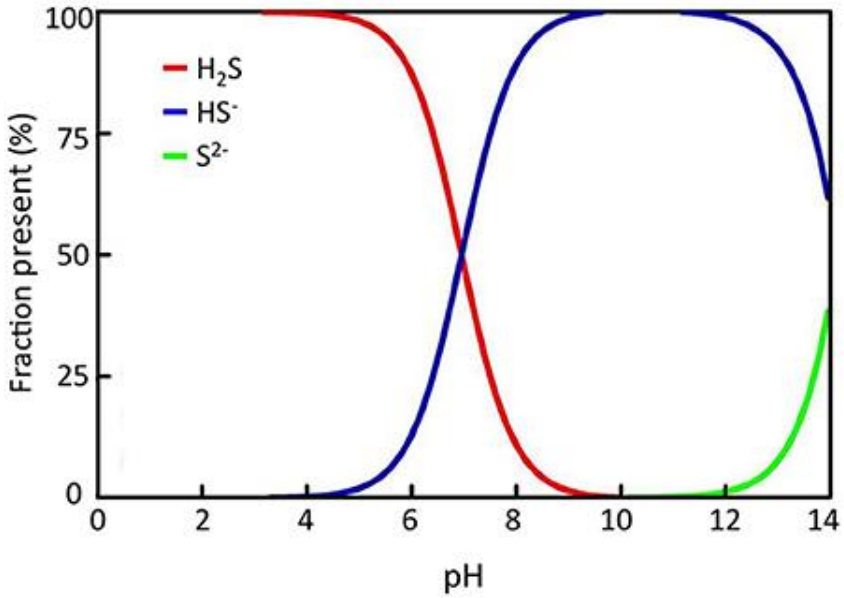

Figure 2 H_2S appearance under different pH values (Holmer et al. 2014)

1.3.2 Reactors

Different reactors for biological sulphide removal are used f.e. biofilters, bioscrubbers and microbial fuel cells.

Many biofilm reactors have been proposed for wastewater treatment. Biofilm up flow sludge blanket (USB) which contains no fixed bed, but a granular sludge. Bubbling

fluidized bed reactor (BFB) applying as fluid bed mineral support to treat a food-processing and paper industry wastewater (Holst et Al, 1997).

An upward flow anaerobic sludge blanket (UASB) and expanded granular sludge bed reactor (EGSB) have integrated sludge granulations concept for the anaerobic treatment of wastewater with high impurities load.

A biofilm airlift suspension reactor (BAS). In a BAS O_2 has been used as the electron acceptor and the airlift suspension is created by pumping air into the process (Moghanloo et Al, 2010).

Another example for the anaerobic treatment of wastewater with high impurities load is the internal circulation reactor (IC). IC consists of two UASB reactors. The two reactors are placed on top of each other. One reactor is heavily loaded, while the other is lightly loaded. In the first stage, gas is gathered and it stimulates internal circulation.

The configuration of mentioned biofilm reactors are shown in Figure 3.

The benefits of particulate biofilm reactors are high surface area of the biofilm, high biomass concentration and high age of the biomass can be obtained. The high age is interesting for anaerobic environments, because in an anaerobic environment bacteria grow slowly. Therefore, they need time to grow.

The disadvantages are long start up times when there is a formation of biofilm on carriers. It is hard to control the thickness of the biofilm (Nicolella et al. 2000).

Remarkably, no study on the kinetics of biological sulphide removal at low temperature was actually performed, while this study benefits all mentioned biochemical technologies.

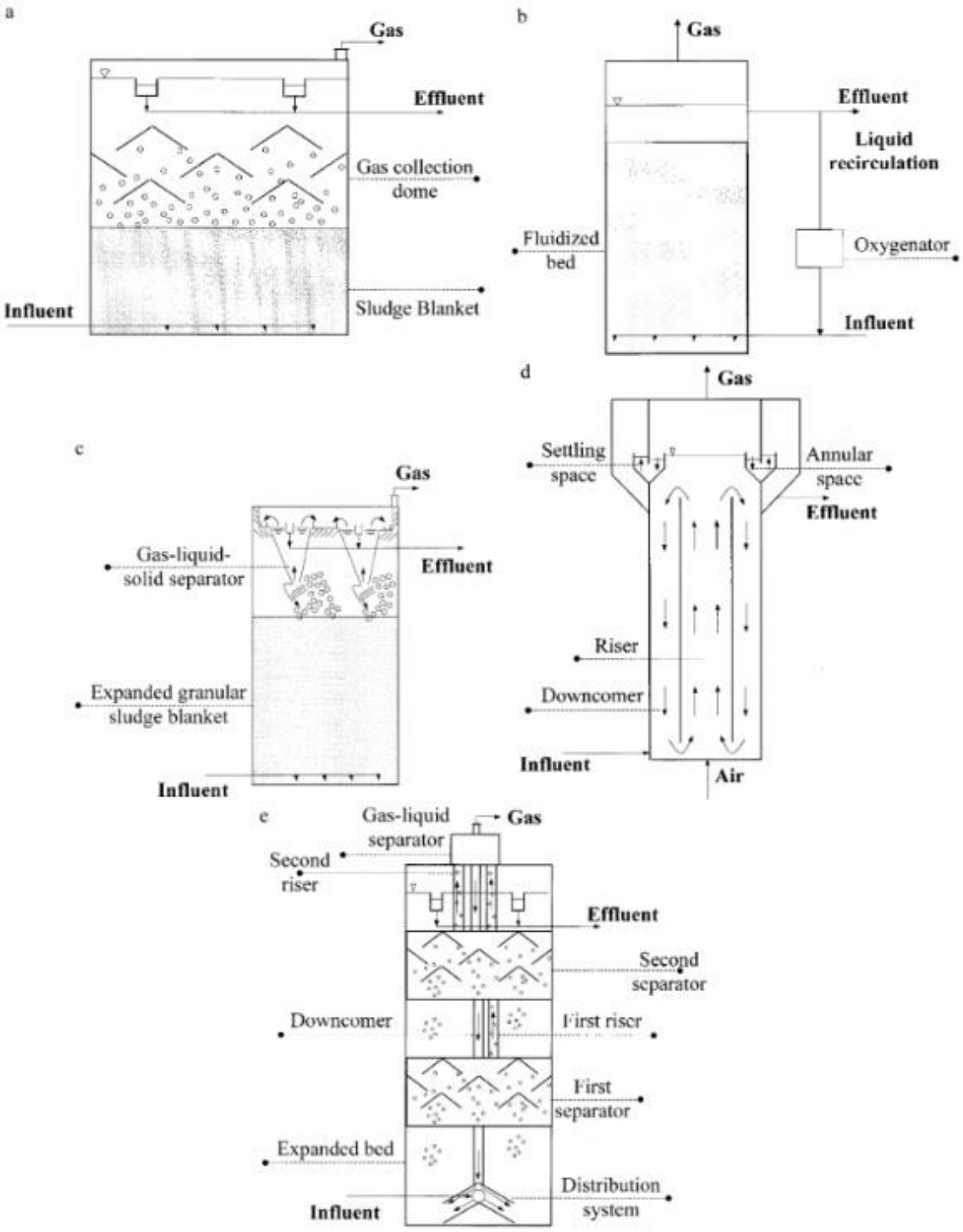

Figure 3 Configuration of biofilm reactors (a) USB, (b) BFB, (c) EGSB, (d) BAS and (e) IC (Nicolella et al. 2000)

2 Methods

2.1 Experimental plan

A series of batch experiments (Table 2) consisting of 500 mL glass reactors were performed to study the kinetics of sulphide removal by a suspended bacteria culture. Firstly, an optimal dosage of biomass (sludge) was evaluated in batch series. Then the optimal dosage of sludge was added to the synthetic wastewater to study the kinetics. Subsequently batches without biomass, nitric acid (HNO_3) and sodium sulphide ($Na_2S{\cdot}9H_2O$) were carried out to assess the effect of those substances on the kinetics of sulphide removal. During the experiment, samples were taken every 4 hours. The samples were analyzed by measuring the concentration of nitrate (NO_3^-), sulphate (SO_4^{2-}), thiosulphate ($S_2O_3^{2-}$), phosphate (PO_4^{3-}) and total sulphur. Due to the presence of phosphates as the buffer, sulphide concentration was calculated by using a mass balance (methylene blue method is not selective in the presence of phosphates).

Table 2 Overview experimental plan

Temperature ^oC	**Objective**	**Total number of reactors**	**Granular sludge[1]**
25	Optimal dosage of the sludge	6	Sample 1
	Kinetics study	3	Sample 1
	Kinetics without biomass	2	Sample 2
	Kinetics without HNO_3	2	Sample 1
	Kinetics without $Na_2S{\cdot}9H_2O$	1	Sample 2
15	Kinetics study	2	Sample 2
	Kinetics without HNO_3	2	Sample 2

[1] Two different samples of sludge were used during the experiments.

	Kinetics without $Na_2S{\cdot}9H_2O$	1	Sample 2
10	Kinetics study	3	Sample 2
	Kinetics without HNO_3	1	Sample 2
	Kinetics without $Na_2S{\cdot}9H_2O$	1	Sample 2

2.2 Materials

2.2.1 Granular Sludge

The sludge originates from a UASB methanogenic reactor, which treats wastewater from the pulp and paper industry at "Norske Skog Saugbrugs" Halden, Norway. The granules size ranged between 1 and 4 mm. The sludge was homogenized 10 minutes by Heidolph homogenizer to achieve a suspended bacteria culture. During the experiments two samples of granular sludge were used. The total (TS) and volatile solids (VS) were measured.

2.2.2 Synthetic media

Synthetic wastewater consists of an equal volume of solution I and solution II (Table 3). Sodium sulphide hydrate ($Na_2S{\cdot}9H_2O$) was used as a source of sulphide (electron donor), while nitric acid (HNO_3) was used as a source for nitrate (electron acceptor). Potassium phosphate was used as pH buffer during the experiments to reach an alkaline conditions (pH = 8.5-9.0). An overall composition of Solution I and II is in Table 2. Solution I was enriched with the mixture of four substrates consisting among others the mineral and vitamin solutions (Table 4). These components are necessary to grow autotrophic denitrifiers.

Table 3 Composition of Solution I and II

Solution	**Substrate**	**Volume** mL	**Mass** g
Solution I	HNO_3[2]	0.028	
	Solution A	4.000	
	Solution B	0.800	
	Solution C	0.800	
	Solution D	0.400	
Solution II	$Na_2S \cdot 9H_2O$		0.300
	K_2HPO_4		0.384
	KH_2PO_4		0.192

Table 4 Composition of solutions A, B, C and D according to Wolin, E.A., et al. (1963).

Vitamin Solution	**Ingredients**	**Concentration** g/L
Solution A	NH_4Cl	10
	NaCl	10
	$MgCl_2 \cdot 6H_2O$	10
	$CaCl_2 \cdot 2H_2O$	5
Buffer solution; B	K_2HPO_4	300

[2] The purity factor of the used nitric acid is 69.65 % solution density is 1.41 kg/L

Mineral solution; C	$MnSO_4 \cdot H_2O$	0.04
	$FeSO_4 \cdot 7H_2O$	2.7
	$CuSO_4 \cdot 5H_2O$	0.055
	$NiCl_2 \cdot 6H_2O$	0.1
	$ZnSO_4 \cdot 7H_2O$	0.088
	$CoCl_2 \cdot 6H_2O$	0.05
	H_3BO_3	0.05
Vitamin solution; D	Biotin	0.02
	Folic acid	0.02
	Pyridoxine hydrochloride	0.1
	Riboflavin	0.05
	Thiamine	0.05
	Nicotinic acid	0.05
	Pantothenic acid	0.05
	Vitamin B_{12}	0.001
	p-aminobenzoic acid	0.05
	Thioctic acid	0.05

2.3 Expermental setup

The batch experiments were performed in a water bath at 25, 15 and 10 °C. The reactors comprise of 500 mL glass with Teflon caps filled with 390 mL synthetic media (specific volume is based on 3.2)**.** The experiment takes place at anoxic conditions. The reactor was sparked by helium gas to remove the oxygen out of the reactor. $Na_2S{\cdot}9H_2O$ solution was added to the reactor just at the beginning because sulphides tend to oxidize very quickly. Hence it is added briefly before the sludge at the beginning of the experiment.

Afterwards the reactor was placed in a water bath for one hour to equilibrate on the selected temperature. The $Na_2S{\cdot}9H_2O$ solution was added first to the reactors followed by sludge, which was added into the reactor with a syringe. Thereafter the sludge is mixed with the solution very carefully until all of the sludge is mixed in the solution. Immediately after the sludge is mixed, the reactor is set for sampling. For each sample that has been taken, 5 mL is taken out of the reactor using an unique syringe for each reactor and poured out in the sink. This is because there is a remaining volume of liquid entrapped in the reactor tube. Then another 5 mL was collected and prepared to analysis. Figure 4 shows the batch experiment set-up.

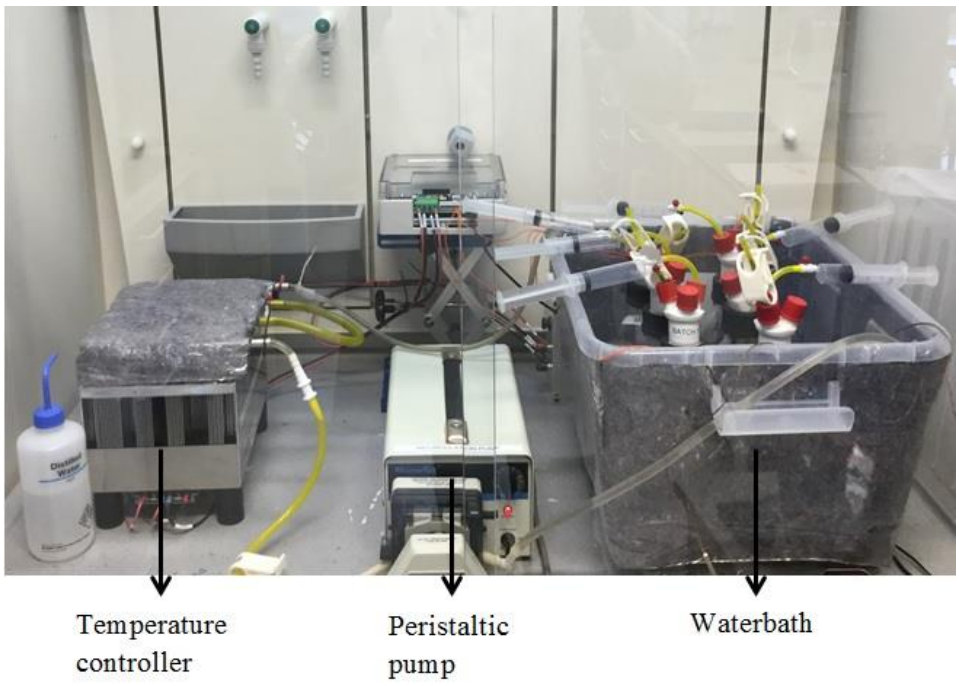

Figure 4 Setup batch reactor process

The peristaltic pump in the setup of the batch reactor process is necessary to pump the water to the waterbath through the temperature controller.

2.4 Sample preparation

The sample was first filtrated through a glass fiber 0.45 μm pore size filter. Next 0.5 mL of the filtrate was pipetted into glass vials. In one glass vial 1 mL of $KMnO_4$ is added to oxidize reduced sulphur species into sulphate. Therefore, this sample is called the oxidized sample. Further deionized water was added to each sample until the total volume of the sample reaches 10 mL. Prepared samples were transferred into 1.5 mL vials and analyzed with the ion chromatography.

2.5 Analytical methods

2.5.1 Ion chromatography

The ion chromatograph (Dionex ICS 5000) (Figure 5) measures wide range of anions and cations. The employed method was developed for NO_3^-, PO_4^{3-}, SO_4^{2-} and $S_2O_3^{2-}$ determination. Sample separation was performed by an IonPac AS11-HC 2 mm analytical column and as eluent KOH at 22 mM is selected. The gradient started at 6 minutes, ramped up in 3 minutes to 45 mM and kept at that concentration for another 4 minutes. The data acquisition time is 13 minutes. The injection volume was 10 μL and the flow rate was 0.3 mL/min. Peak areas of analytes were calculated into concentrations based on the method calibration (mmol/L). The concentration of SO_4^{2-} in the oxidized sample represent the total sulphur concentration in the reactor.

Figure 5 Ion chromatograph (Dionex ICS 5000)

2.5.2 Total solids (2540B US-standard)

Total solids (TS) is the amount of solids that are remained after evaporation of the sample. They are the sum of the total suspended solids and the total dissolved solids. While measuring the total solids of the granular sludge, 5 mL of the sludge was poured into a porcelain dish and placed for 24 hours in an oven at a temperature of 103-105 °C. Afterwards the sample was cooled in a desiccator. The increase of weight towards the dry and empty porcelain dish represent the total solids.

Thus, the total solids were calculated by following formula:

$$TS = \frac{(m_{residue\ +dish} - m_{dish}) \times 1000}{V_{sample}} \quad (1)$$

where:

TS	= Total solids (mg total solids/L)
$m_{residue\ +\ dish}$	= weight of dried residue + dish (mg)
m_{dish}	= weight of dish (mg)
V_{sample}	= Volume of sample (mL)

(Eaton et al., 1995)

2.5.3 Volatile solids (2540E US-standard)

The sample was placed in a muffle oven for one hour at a temperature of 550 °C. Subsequently the sample was placed in a desiccator. The loss in weight during ignition of the sample represent the volatile solids.

The volatile solids were measured by following formula.

$$VS = \frac{(m_{before\ ignition} - m_{after\ ignition}) \times 1000}{V_{sample}} \quad (2)$$

where:

VS	= Volatile solids (mg volatile solids/L)
$m_{before\ ignition}$	= weight of residue + dish before ignition (mg)
$m_{after\ ignition}$	= weight of residue + dish after ignition (mg)
V_{sample}	= Volume of sample (mL)

(Eaton et al., 1995)

3 Results and discussion

3.1 Overall process description

Based on the observations made from the series of batch experiments it is hypothesized that the overall process has three distinctive phases. Following hypothesis has been formulated:

1) The first phase is purely chemical; at the beginning of the process a small fraction of sulphide is immediately oxidized into sulphates. Subsequently the concentration of sulphate decreases. Sulphate is reduced into thiosulphate, while sulphide is oxidizing into elemental sulphur and thiosulphate. The reactions in this phase are purely chemical.

2) The second phase consists of the initiation of the bacterial denitrification process. During this phase all reduced forms of sulphur are oxidized into sulphate, unless the third phase initiates before all forms are oxidized. The oxidation is purely biochemical and is highly dependable on the temperature.

3) During the last phase, the sulphur-containing sludge is releasing sulphates into the process. That is why the amount of total sulphur is increasing after 48 hours.

The overall process is discussed more in detail in the following chapters.

3.2 Optimal dosage of the sludge

The objective of this experiment was to evaluate how much volume of granular sludge is required to perform biological sulphide removal. Six batches were prepared with a different amount of volume for sludge and synthetic media, whereas the total volume was fixed on 400 mL. Table 5 shows the composition of each batch. The experiment ran for 72 hours at 25 °C and during the experiment, 7 samples were taken from each batch.

Table 5 Overview composition reactors during sludge volume optimization experiment

Batch name	**Synthetic media** mL	**Sludge** mL
Batch 1 and 2	300	100
Batch 3 and 4	350	50
Batch 5 and 6	390	10

Standard error of the mean was used to calculate an error bars on the plots. The standard error is calculated by following formula:

$$SE = \frac{s}{\sqrt{n}}$$

Where:

SE = Standard error of the mean

s = Standard deviation on the concentration of the batches

n = number of batches

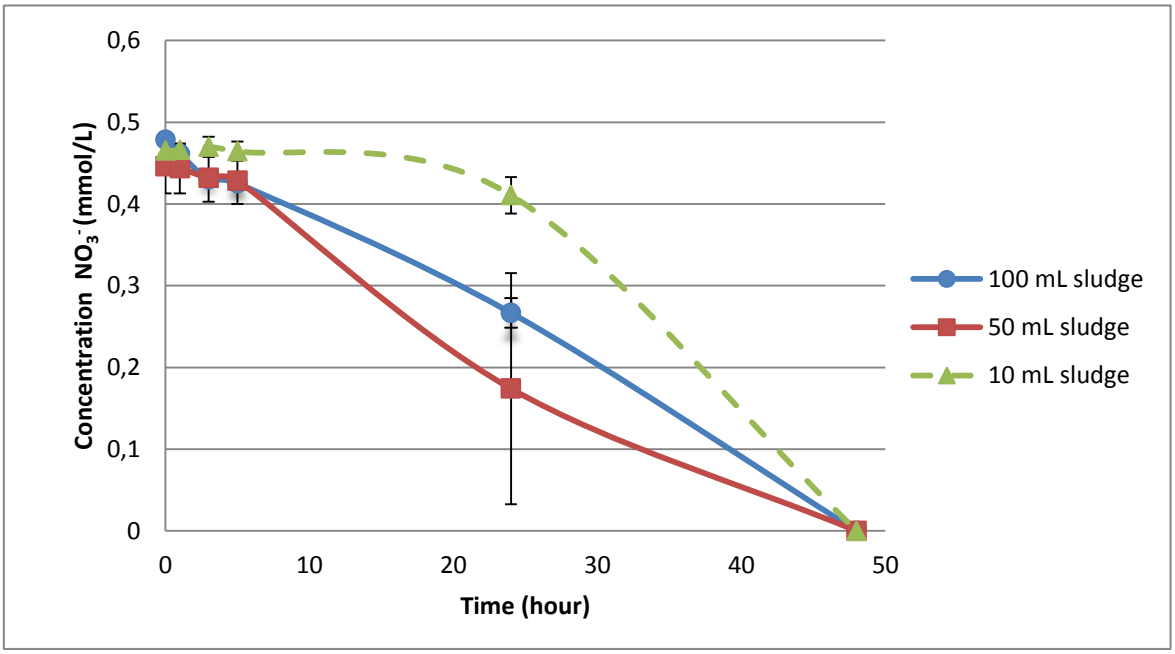

Figure 6 Concentration of nitrate in time at T = 25°C with different volumes of sludge

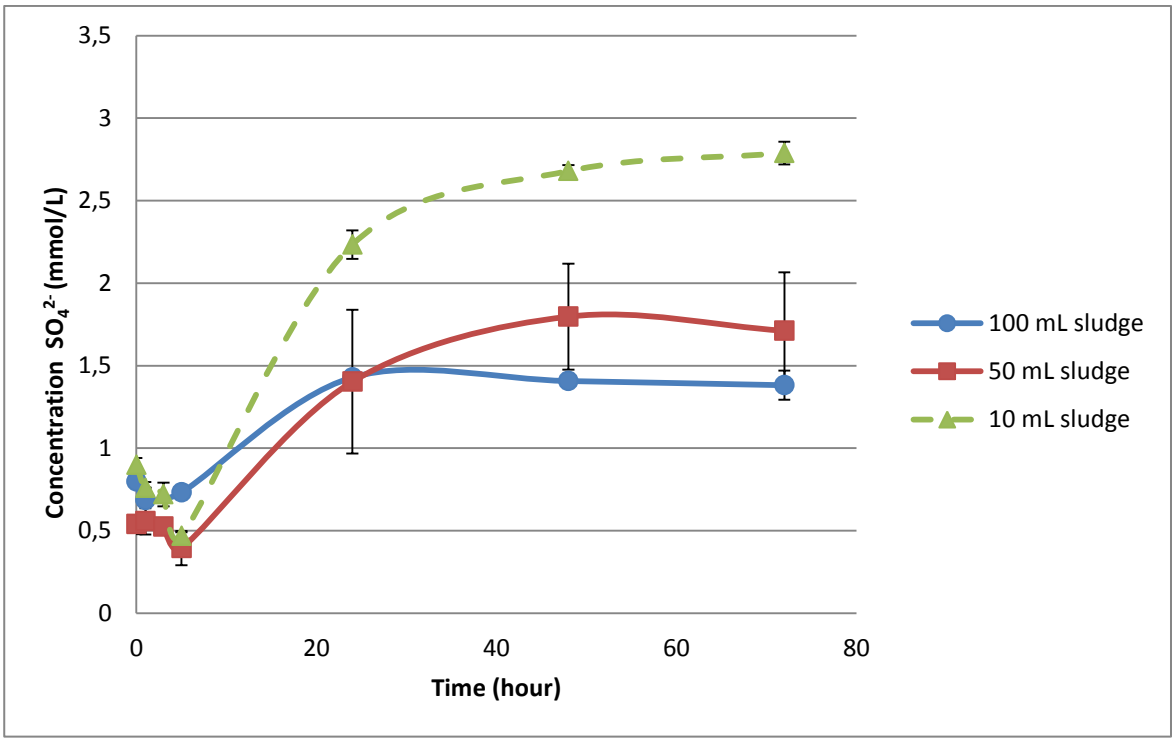

Figure 7 Concentration of sulphate in time at T = 25°C with different volumes of sludge

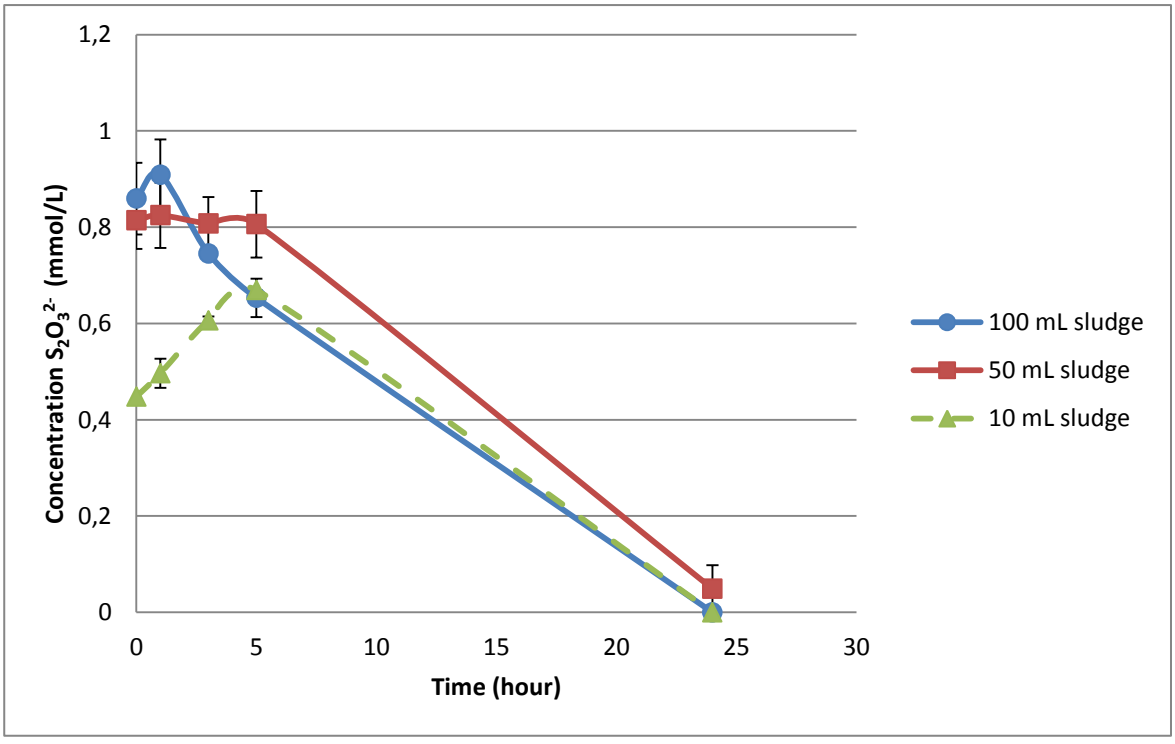

Figure 8 Concentration of thiosulphate in time at T = 25°C with different volumes of sludge

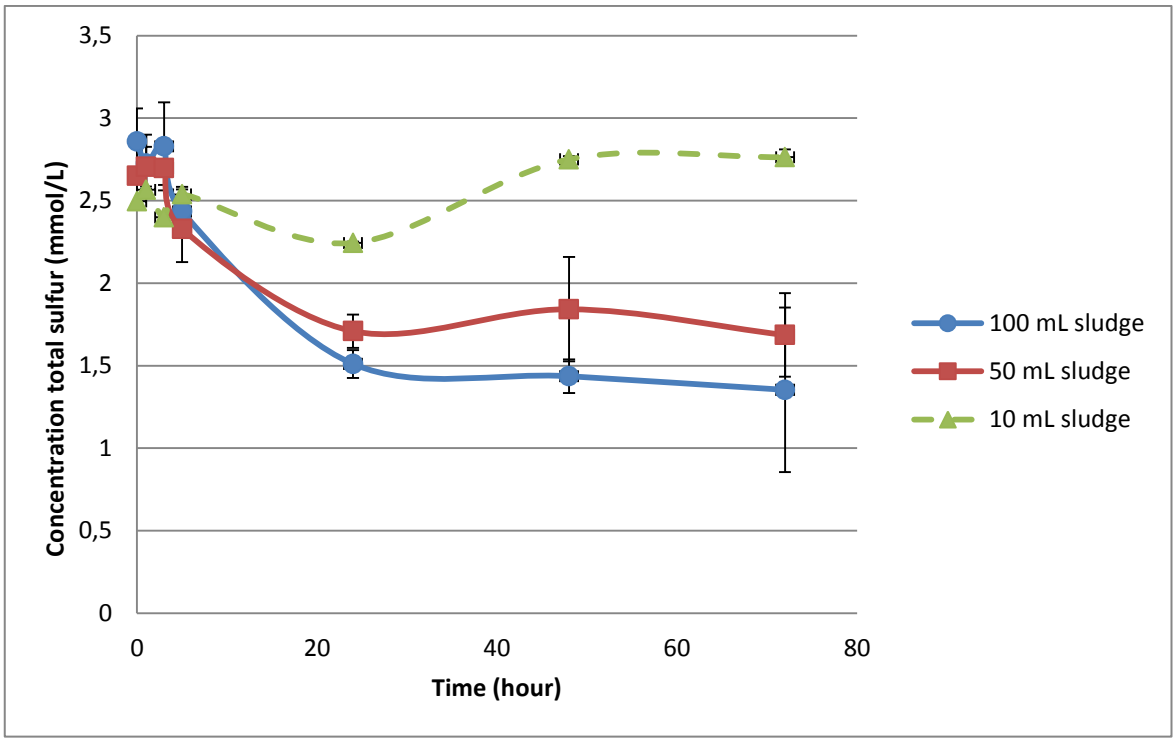

Figure 9 Concentration of total sulphur in time at T = 25°C with different volumes of sludge

According to Figure 6, an autotrophic denitrification occurred in each reactor. The concentration of NO_3^- decreases in time and after 48 hours NO_3^- is fully converted into N_2. Subsequently the conversion of thiosulphate into sulphate is similar in all experimental cases (Figure 7 and 8). The optimal dosage seems to be the one with least amount of sludge (10 mL sludge). When 10 mL sludge is applied into the reactor, it has two benefits compared to 50 and 100 mL of sludge. Obviously there is less usage of elemental sulphur and sample preparation procedure is faster, because filtration time of the sample, which consists the less amount of sludge is shorter.

Thus, 10 mL of granular sludge and 390 mL of synthetic media was applied in all further experiments.

3.3 Analytes concentration at different temperatures

Three experiments have been performed at three different temperatures (25 °C , 15 °C and 10 °C) to study the kinetics of analytes in time in the reactor. The concentration of the analytes (NO_3^-, SO_4^{2-}, $S_2O_3^{2-}$, S^{2-} and total sulphur) are plotted vs. time at different temperatures and are more in detail explained in following chapters.

3.3.1 Nitrate

According to Figure 10, nitrate consumption in the reactor is strongly influenced by the temperature. The higher the temperature, the less time it takes to fully consume NO_3^- due to bacterial denitrification.

The main observation under different temperature stages was the lag phase time. At 25 °C, there is a short period where bacteria need to adjust to new environment before the denitrification process starts. At 15 °C the lag phase takes much longer (24 hours) where at 10 °C it lasted for 40 hours. The rate of denitrification is discussed more in detail in 3.5.2, 3.5.3 and 3.5.4.

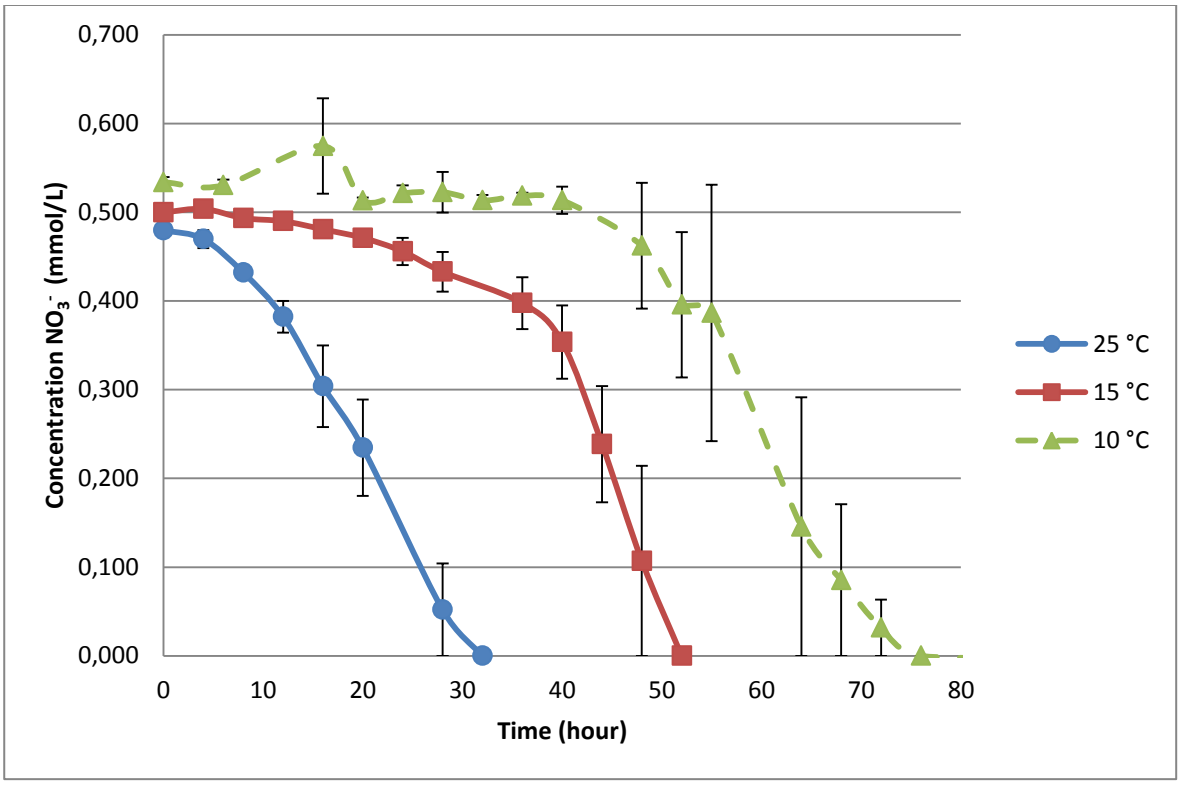

Figure 10 Concentration of nitrate in time at different temperatures

3.3.2 Sulphate and thiosulphate

First, a decrease in SO_4^{2-} concentration and an increase of $S_2O_3^{2-}$ has been observed. This change in concentration wasn't significantly affected by temperature (Figure 11). Afterwards there is a period, which is dependable on the temperature. In this period the

concentration of SO_4^{2-} and $S_2O_3^{2-}$ stay constant (Figure 12). At 10 °C this period lasts from 6 until 40 hours for SO_4^{2-} and from 28 until 48 hours for $S_2O_3^{2-}$. However at 25°C this period lasts only for 4 hours for SO_4^{2-} and 12 hours for $S_2O_3^{2-}$.

There can't be made a significant conclusion for the measurements at 15 °C, because the samples between 20 and 48 hours were measured too late and hence all reduced sulphur species were oxidized into sulphate. Although based on the figure the constant period lasts shorter than at 10 °C and longer than at 25 °C.

After the constant period of SO_4^{2-}, the concentration increases. The concentration of $S_2O_3^{2-}$ doesn't decrease at the same time, but a few hours later. The reason why is that due to the bacterial denitrification, reduced sulphur species like S^{2-}, $S_2O_3^{2-}$, $S_2O_4^{2-}$ and SO_3^{2-} are acting as an electron donor and are oxidized into SO_4^{2-}. Elemental sulphur (S) is oxidized into $S_2O_3^{2-}$. Therefore, there is not immediately a decrease visible in the concentration of $S_2O_3^{2-}$, but is there an increase visible at SO_4^{2-} (Figure 12).

The same phenomena can be observed in Figure 13, the concentration of SO_4^{2-} is increasing immediately after the initiation of the bacterial denitrification, while the concentration of $S_2O_3^{2-}$ stays constant for a while before it is decreasing.

At 10 °C (Figure 14) the concentration of SO_4^{2-} seems to be rising before the denitrification starts. The increase of total sulphur is explained in 3.4.1.

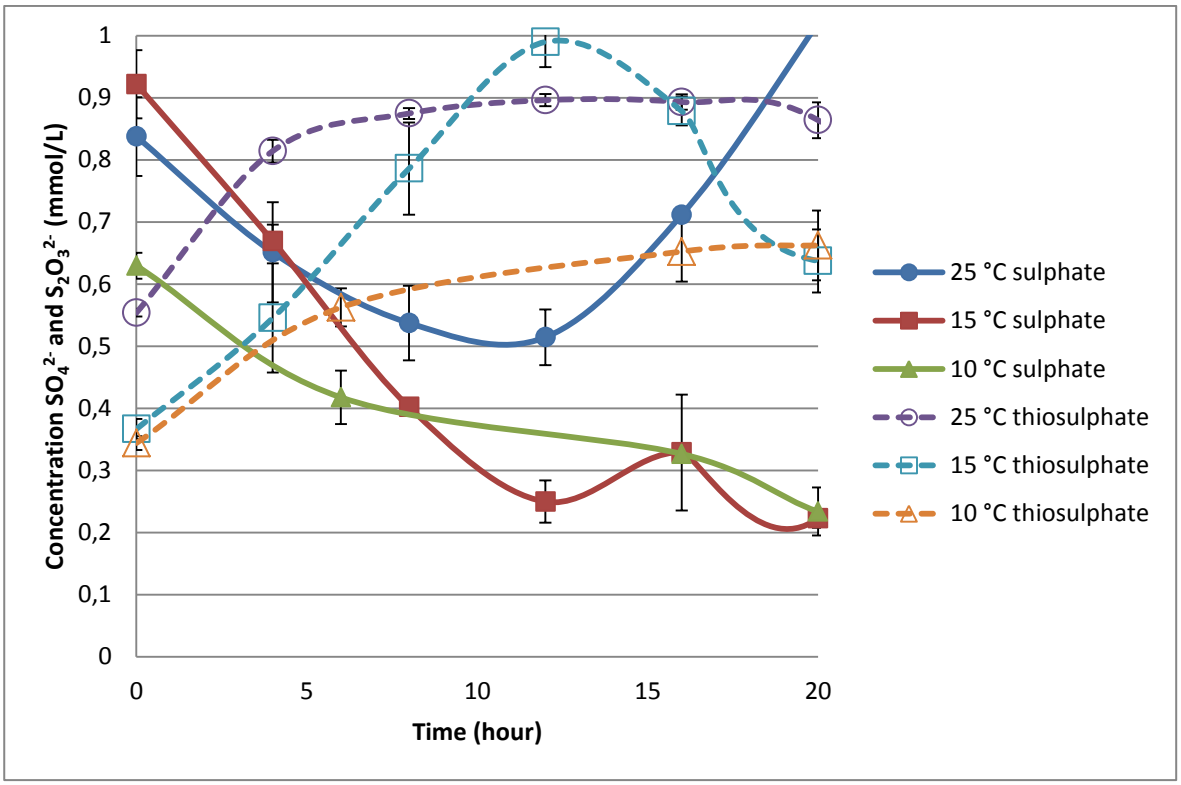

Figure 11 Concentration of sulphate and thiosulphate in time at different temperatures (first 20 hours)

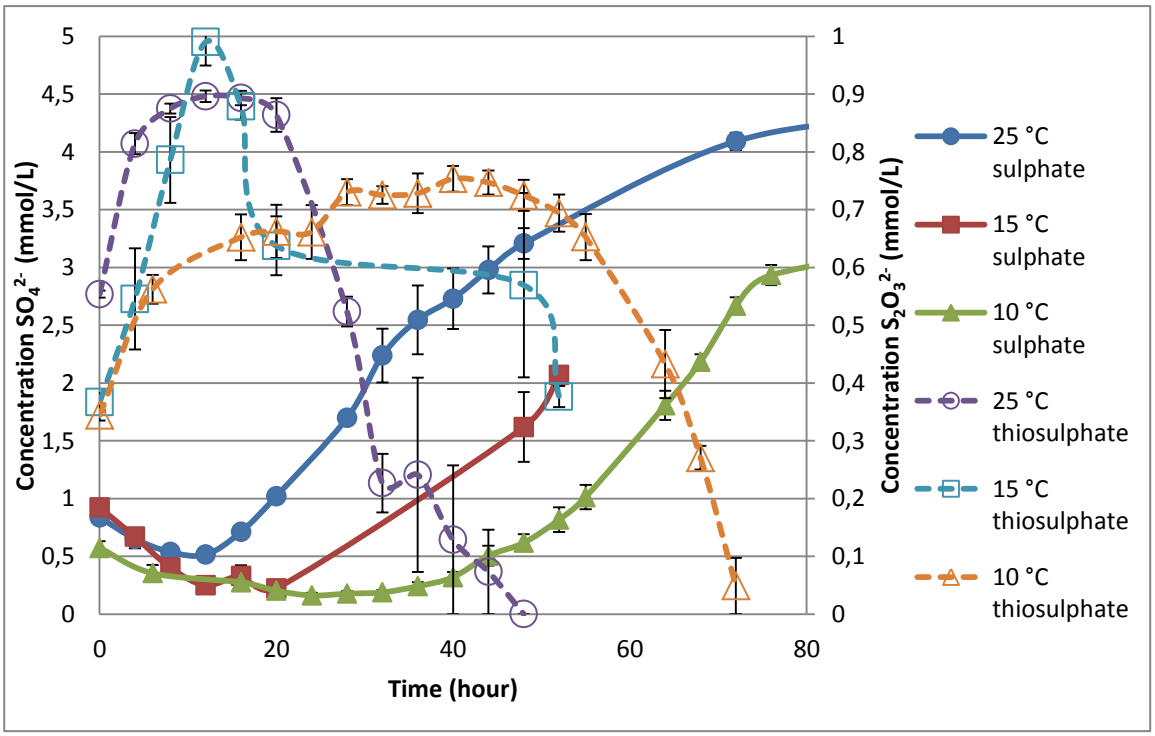

Figure 12 Concentration of sulphate and thiosulphate in time at different temperatures

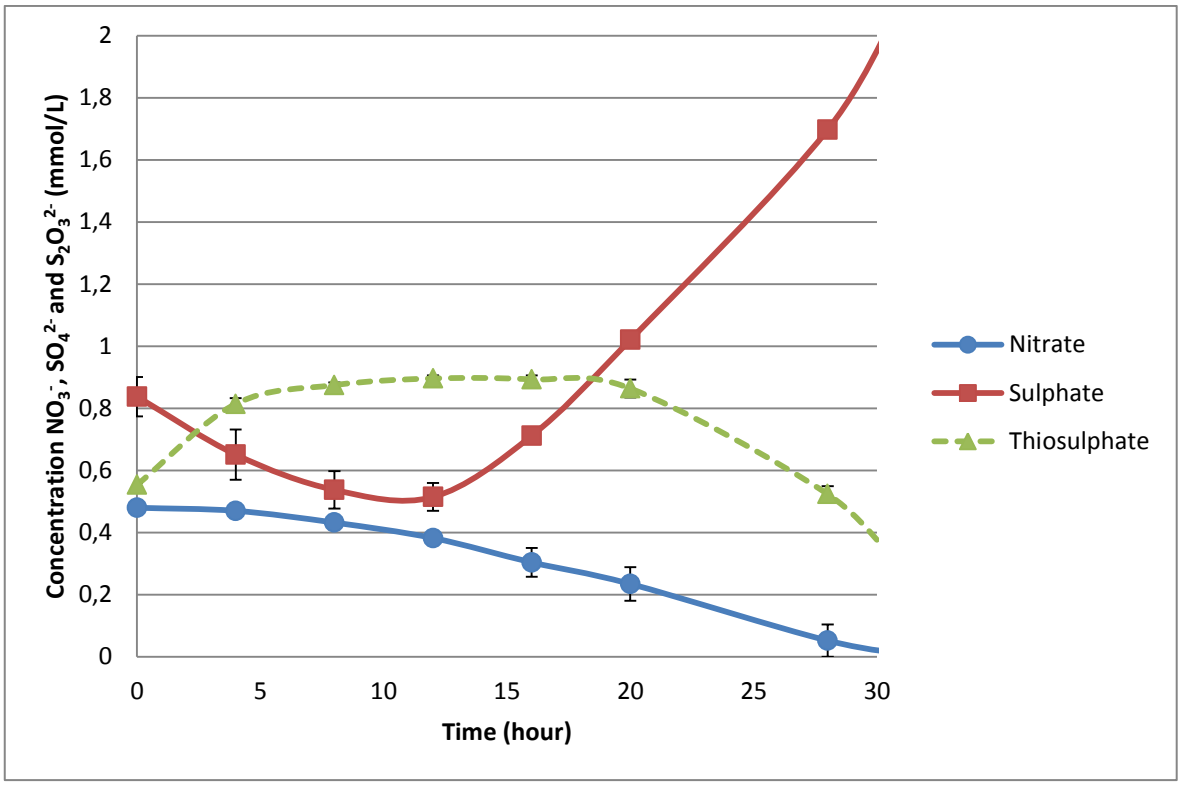

Figure 13 Concentration of nitrate, sulphate and thiosulphate in time at T = 25 °C

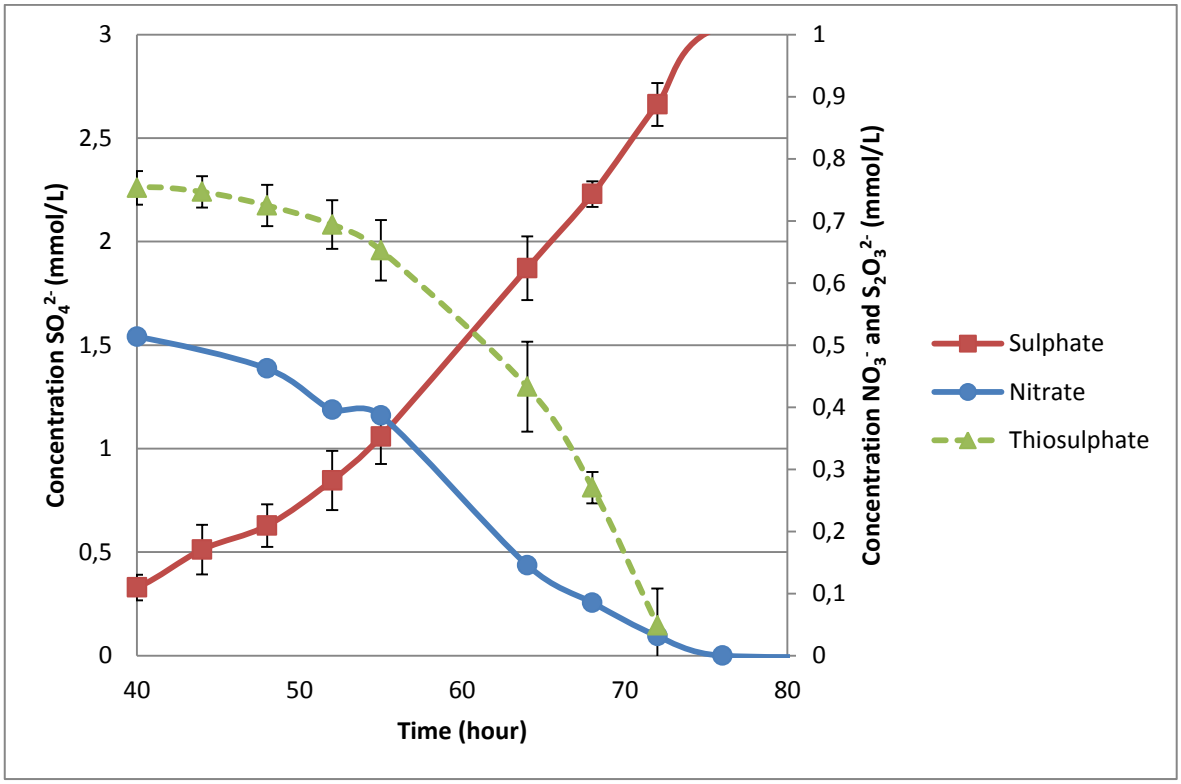

Figure 14 Concentration of nitrate, sulfate and thiosulfate in time at T = 10 °C

3.3.3 Sulphate and total sulphur

Figure 15 shows that the concentration of total sulphur isn't constant during the process. In the beginning the concentration stays constant. The constant period (16 hours) is not significantly influenced by the temperature. Subsequently there is a decrease in total sulphur at 10 and 15 °C and an increase of total sulphur at 25 °C. The decrease of total sulphur is possible due to the formation of elemental sulphur. Elemental sulphur appears as bacterial inclusion bodies/globules. Therefore, during a sample measurement, elemental sulphur stays behind in the reactor (attached to granules) or doesn't pass through the filter and hence a lower concentration of total sulphur is measured.

After 44 hours, all total sulphur is oxidized into sulphate at 25 °C, while it takes 52 hours at 10 °C. Here again the samples at 15 °C are not representative and the measurement were also stopped too early. But based on figure 12 the curve at 15 °C fits between 25 and 10 °C.

The increase of total sulphur is discussed in detail at 3.4.1.

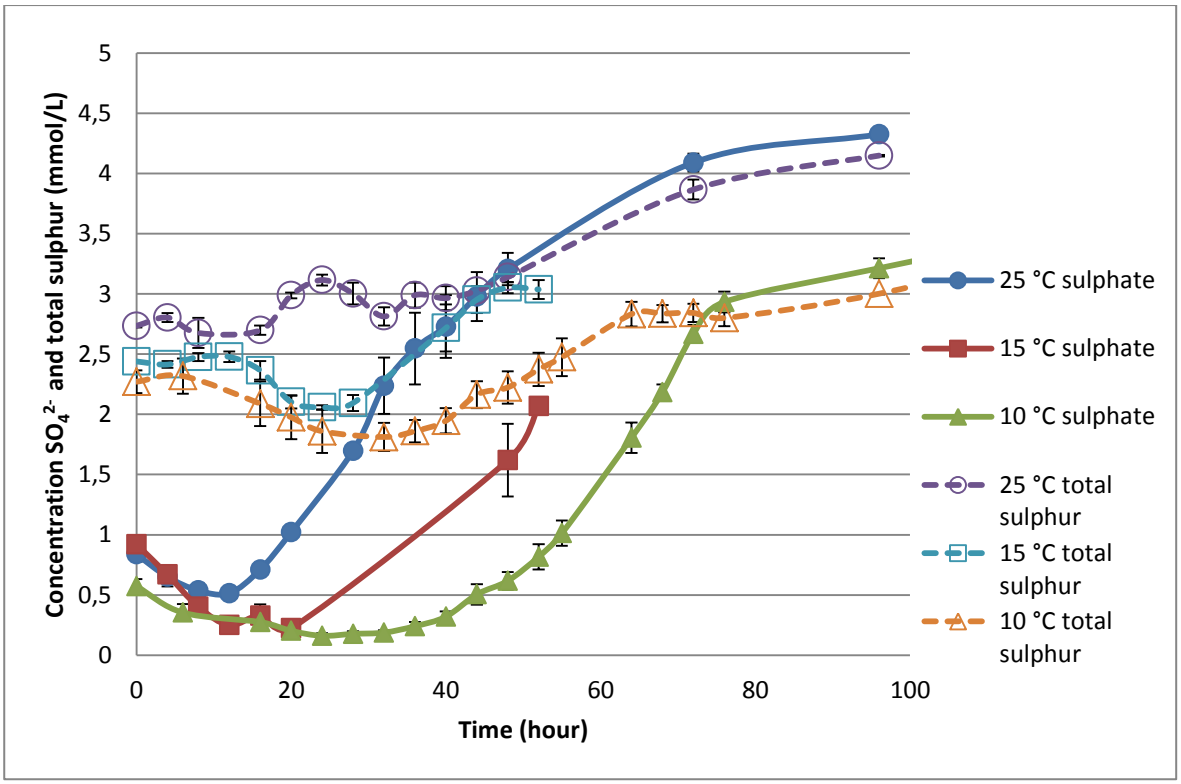

Figure 15 Concentration of sulphate and total sulphur in time at different temperatures

3.3.4 Sulphide

The concentration of sulphide is calculated by following sulphur mass balance :

$$m_{S^{2-}} = m_{S\text{-}SO_{40X}^{2-}} - m_{S\text{-}SO_4^{2-}} - m_{S\text{-}S_2O_3^{2-}} \quad (3)$$

Wherby $m_{S\text{-}SO_{40X}^{2-}}$ is equal to the mass of sulphur that is present in the oxidized sample.

Figure 16 confirms that sulphide removal is dependable on the temperature. At 25 °C the sulphide removal is faster than at 10 °C. Sulphide removal happens in three phases. Firstly, there is a decrease of the concentration of sulphide, due to the chemical oxidation into $S_2O_3^{2-}$ and elemental sulphur (visible as a decrease in total sulphur) (Figure 19). Secondly, there is also a lag phase in the sulphide concentration. The concentration of S^{2-} stays constant from 16 until 28 hours at 25 °C and from 32 until 64 hours at 10 °C. Thirdly, S^{2-} is oxidized into SO_4^{2-} thanks to the bacterial denitrification.

Bacterial denitrification has an impact on sulphide removal at the end of the denitrification process (Figure 17 and 18). Moreover this topic is further discussed in 3.4.1.

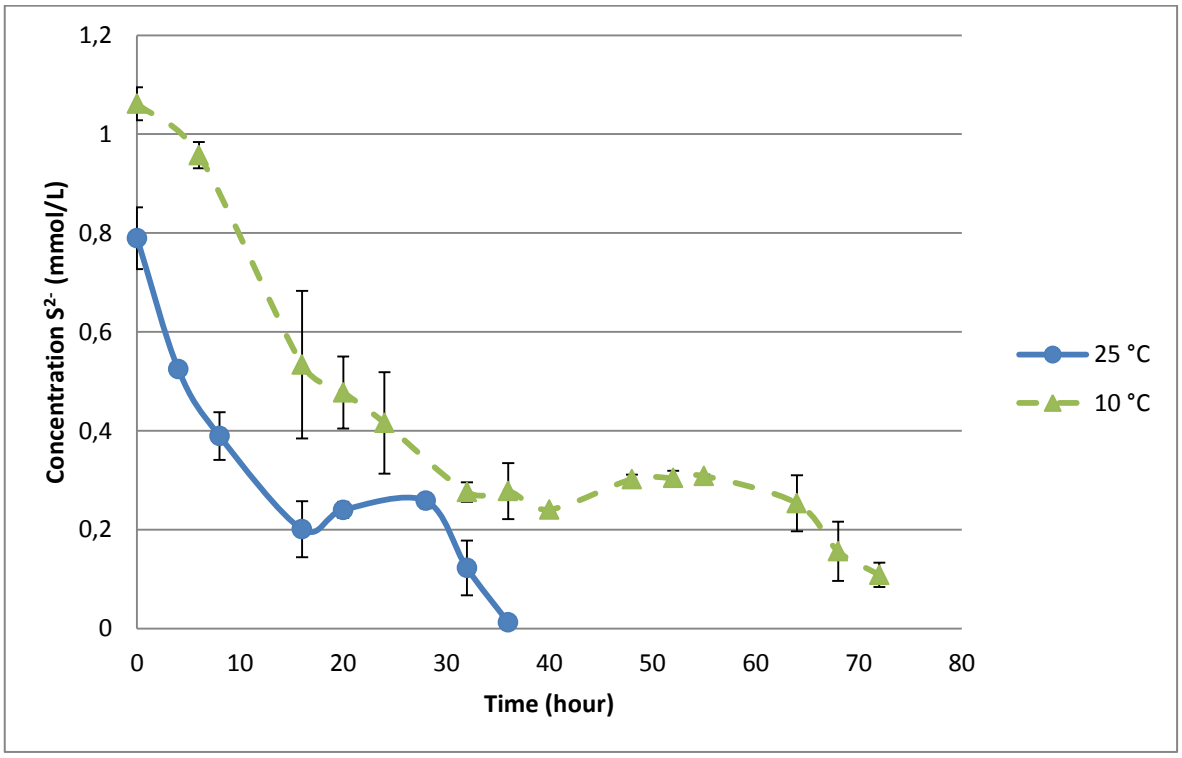

Figure 16 Concentration of sulphide in time at different temperatures

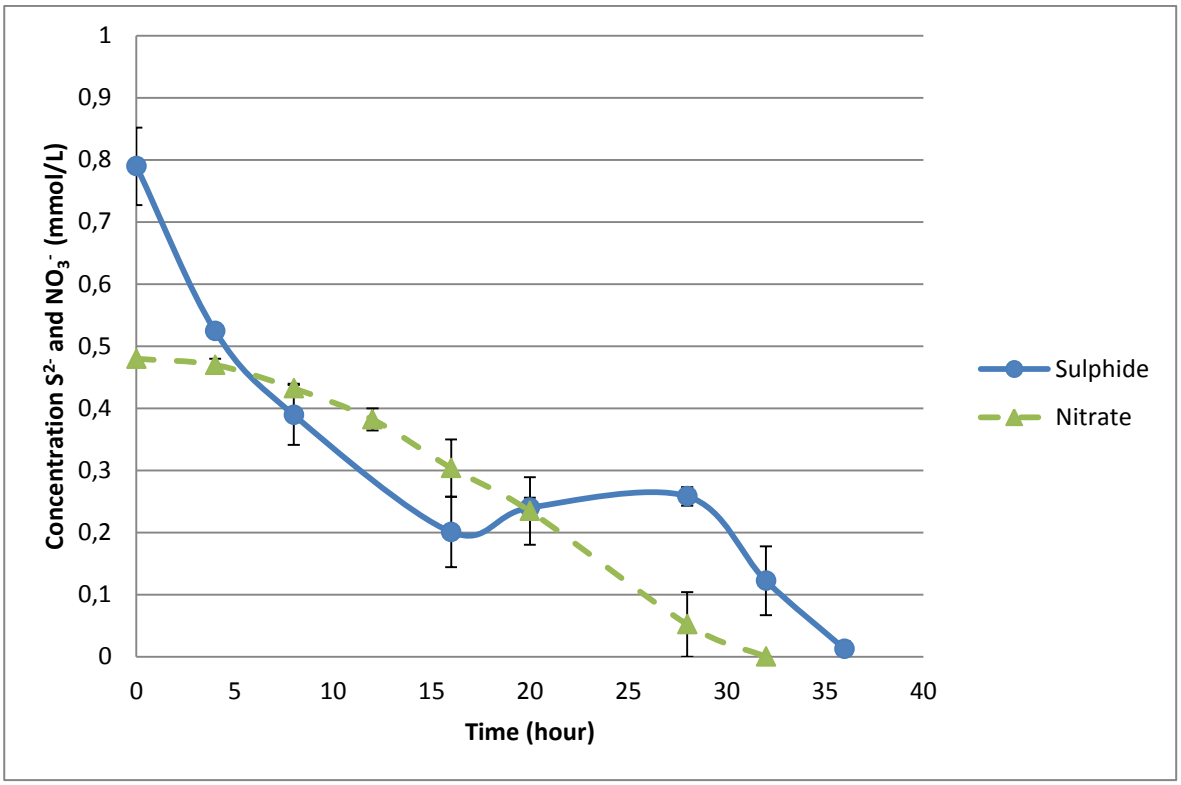

Figure 17 Concentration of sulphide and nitrate in time at T = 25°C

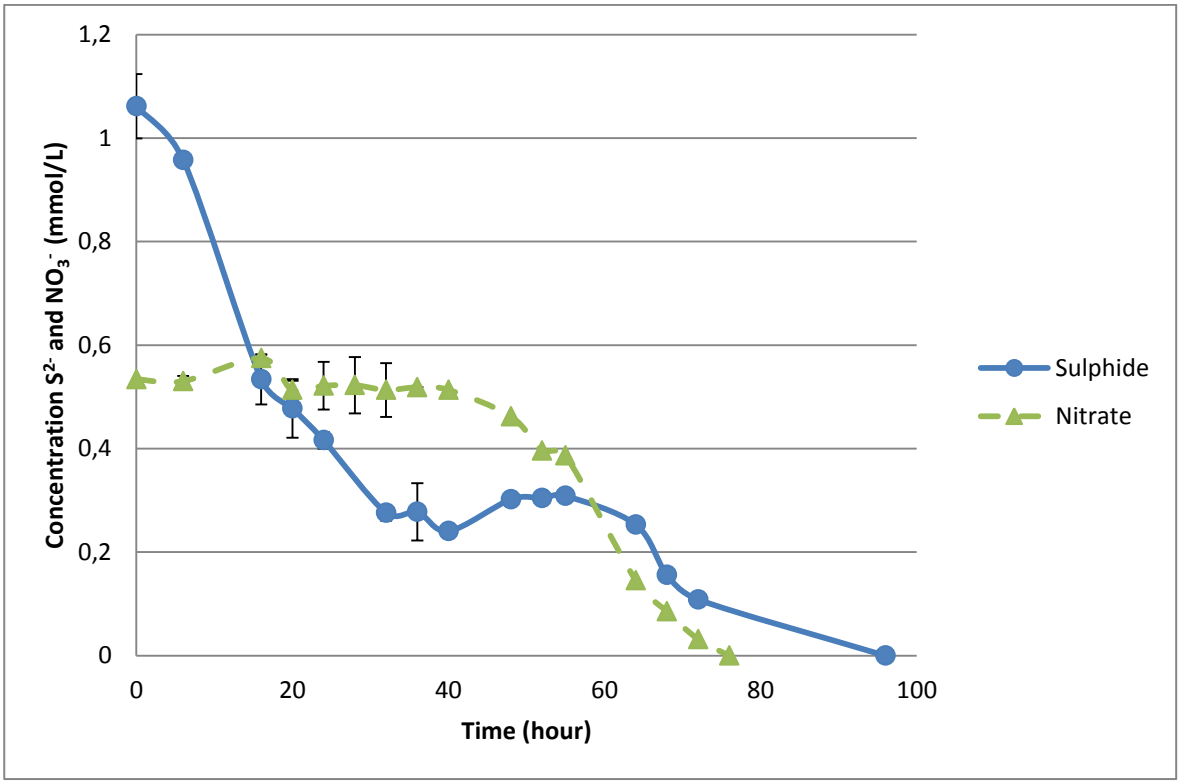

Figure 18 Concentration of sulphide and nitrate in time at T = 10 °C

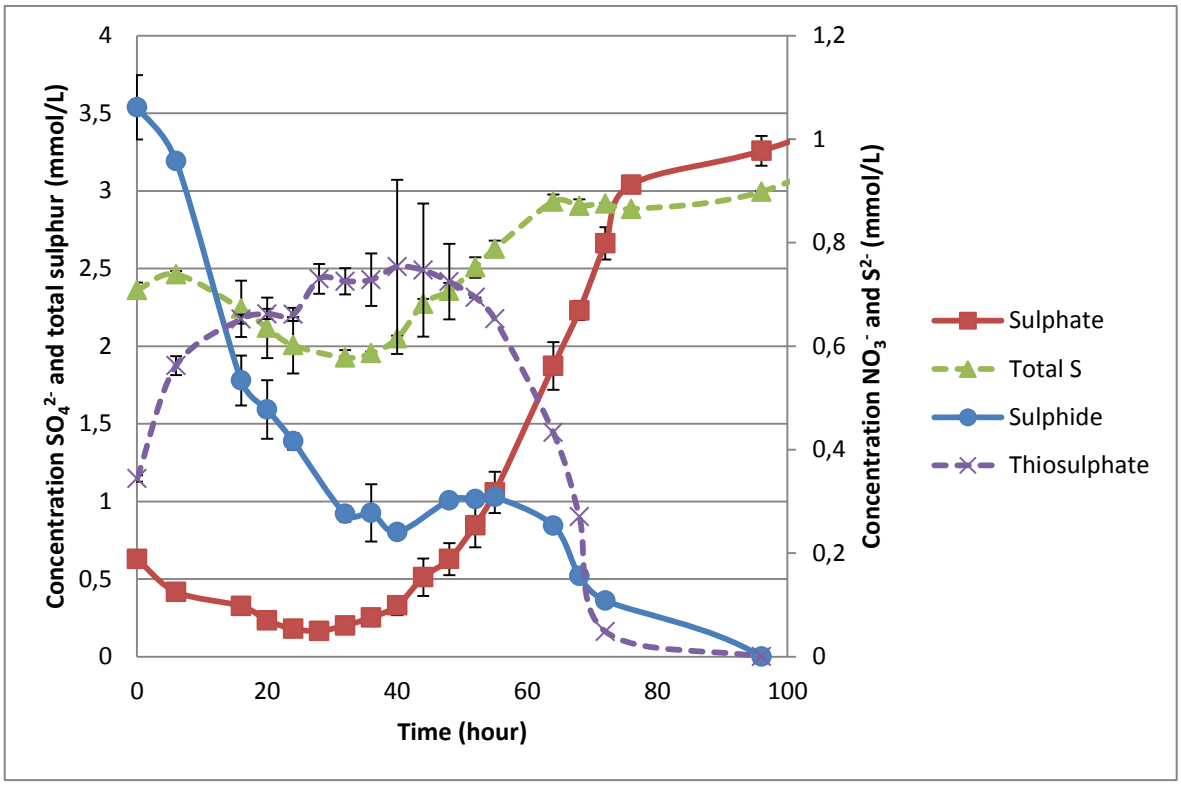

Figure 19 Concentration of sulphide, sulphate, thiosulphate and total sulphur at T =10°C

3.4 The effect of substrates on the concentration of analytes in time

3.4.1 Without Biomass

The analytes study without adding biomass to the reactor was performed at 25 °C to evaluate which reactions are chemical or biochemical in the reactor.

Figure 20 confirms that the consumption of NO_3^- is biochemical. Without biomass in the reactor, the concentration of NO_3^- doesn't change during the experiment.

Figure 21 shows that the decrease of SO_4^{2-} in the beginning of the process is chemical. Because in the beginning the concentration of SO_4^{2-} with biomass and the one without is similar. Subsequently the bacterial denitrification starts and hence the concentration of SO_4^{2-} is increasing.

The concentration of $S_2O_3^{2-}$ without biomass (Figure 22) is increasing higher and longer before the concentration reaches a constant value than for the batch with biomass.

Afterwards the $S_2O_3^{2-}$ in the batch with biomass decreases due to the denitrification, while the batch without biomass stays constant as expected.

Unexpectedly the concentration of total sulphur changes in time (Figure 23). With biomass the total sulphur in the reactor increases and without biomass the total sulphur decreases. Under anoxic conditions and in an *Thiobacillus denitrificans* environment intracellular elemental sulphur is formed out of the biomass. The elemental sulfur is oxidized into SO_4^{2-} (Schedel et Al, 1980). Hence, there is an increase visible in the concentrations of total sulphur and SO_4^{2-}.

Remarkably there is a decrease of total sulphur without biomass. The hypothesis for this decrease could be that the pH of the solution goes down after 48 hours. Hence, S^{2-} and HS^- are converted into gaseous H_2S. H_2S can't be measured in the solution so that's why there is a decrease in the concentration of total sulphur. In the future pH should be measured to confirm hypothesis.

At the end of the process, there is a white precipitation visible on the bottom of the reactor (Figure 25). Therefore, chemical reactions occur between the substances of the synthetic media, which creates an decrease of total sulphur after 48 hours.

Figure 24 shows the impact of biomass on the sulphide removal. Sulphide removal happens in three steps. Without the biomass, is it impossible to have a 100 % sulphide removal, as the third step of the removal process can not be obtained. Because bacterial denitrification is necessary to reach complete removal. From the beginning S^{2-} removal is faster with biomass than without biomass. Hence, bacterial denitrification has already an impact in the first phase. More info about this in 3.5.1.

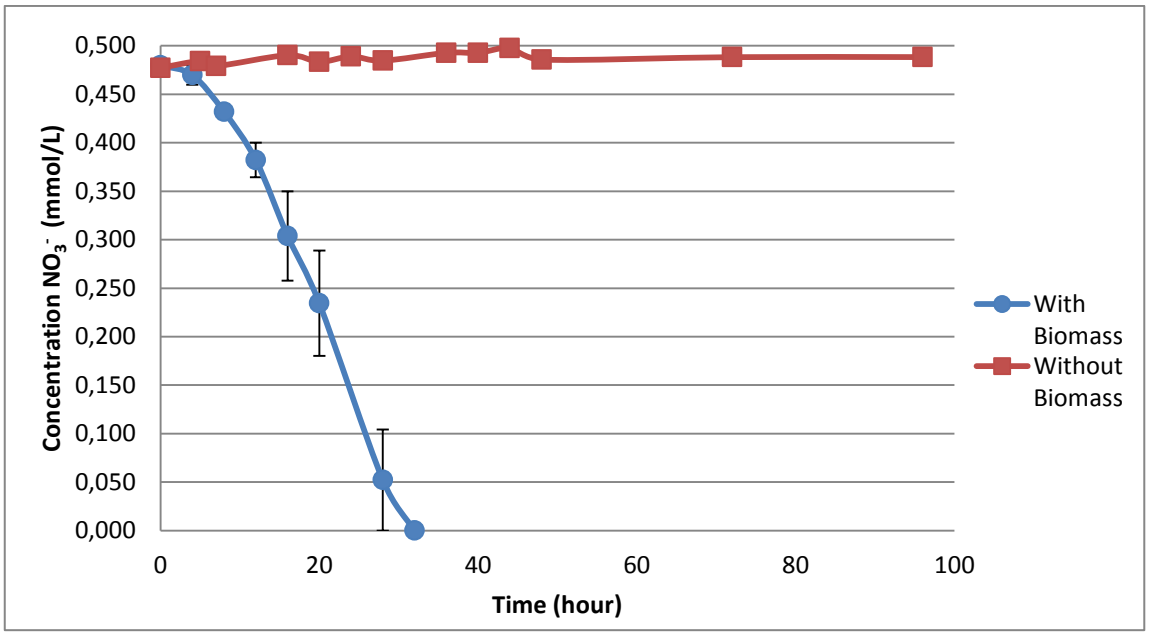

Figure 20 Concentration of nitrate in time with or without biomass at T = 25 °C

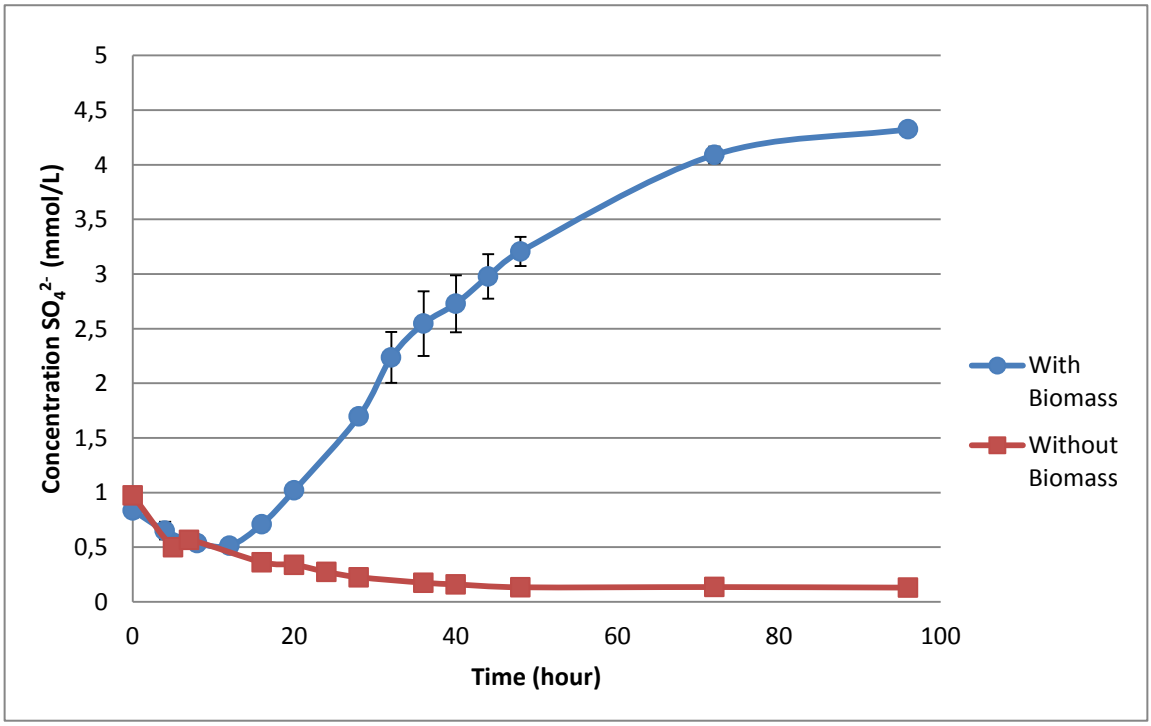

Figure 21 Concentration of sulphate in time with or without biomass at T = 25 °C

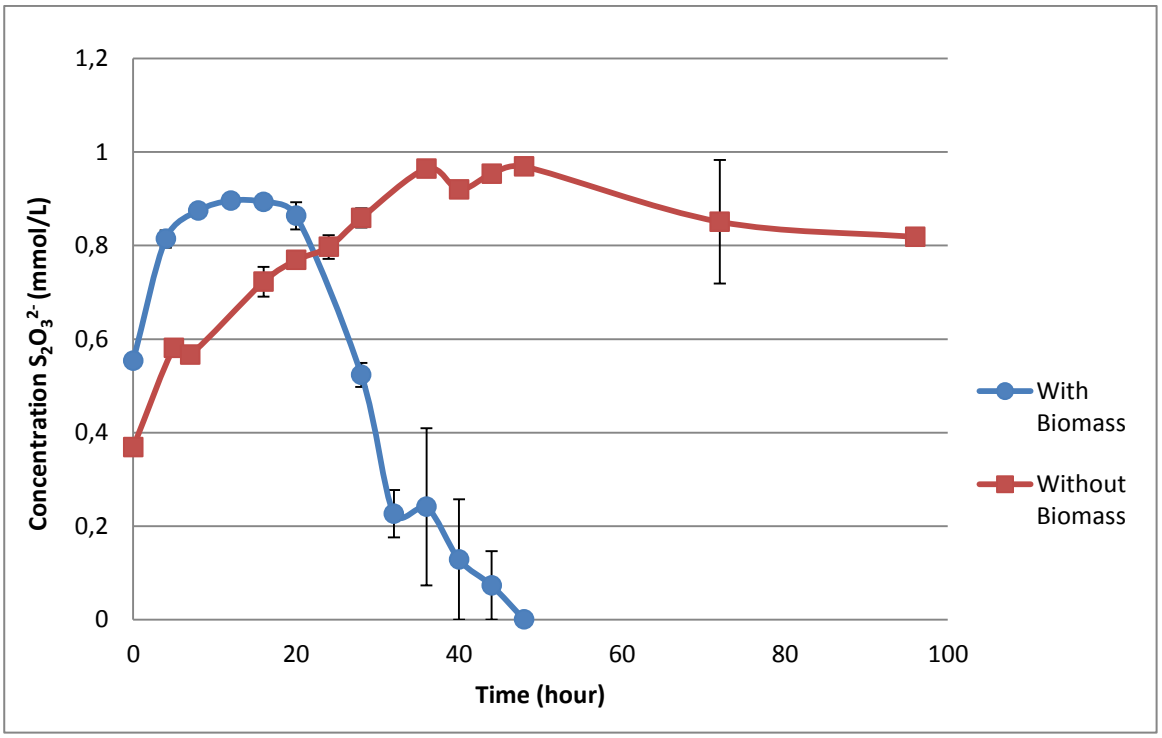

Figure 22 Concentration of thiosulphate in time with or without biomass at T = 25 °C

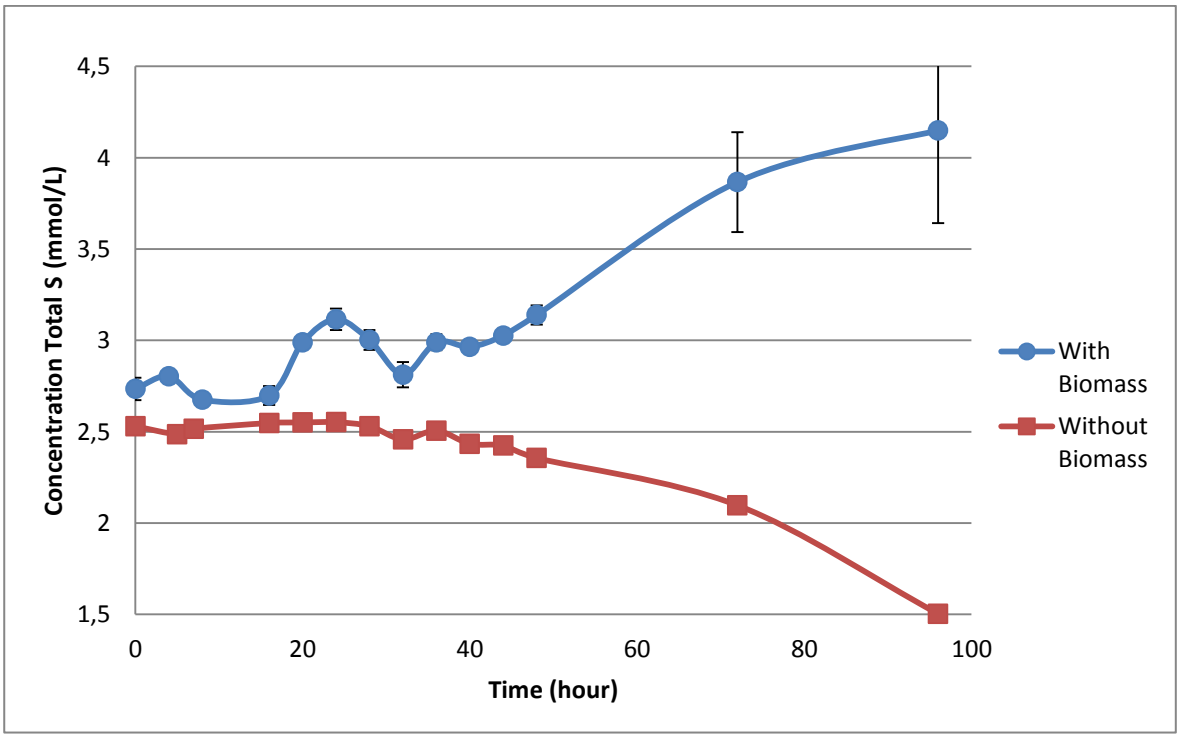

Figure 23 Concentration of total sulphur in time with or without biomass at T = 25 °C

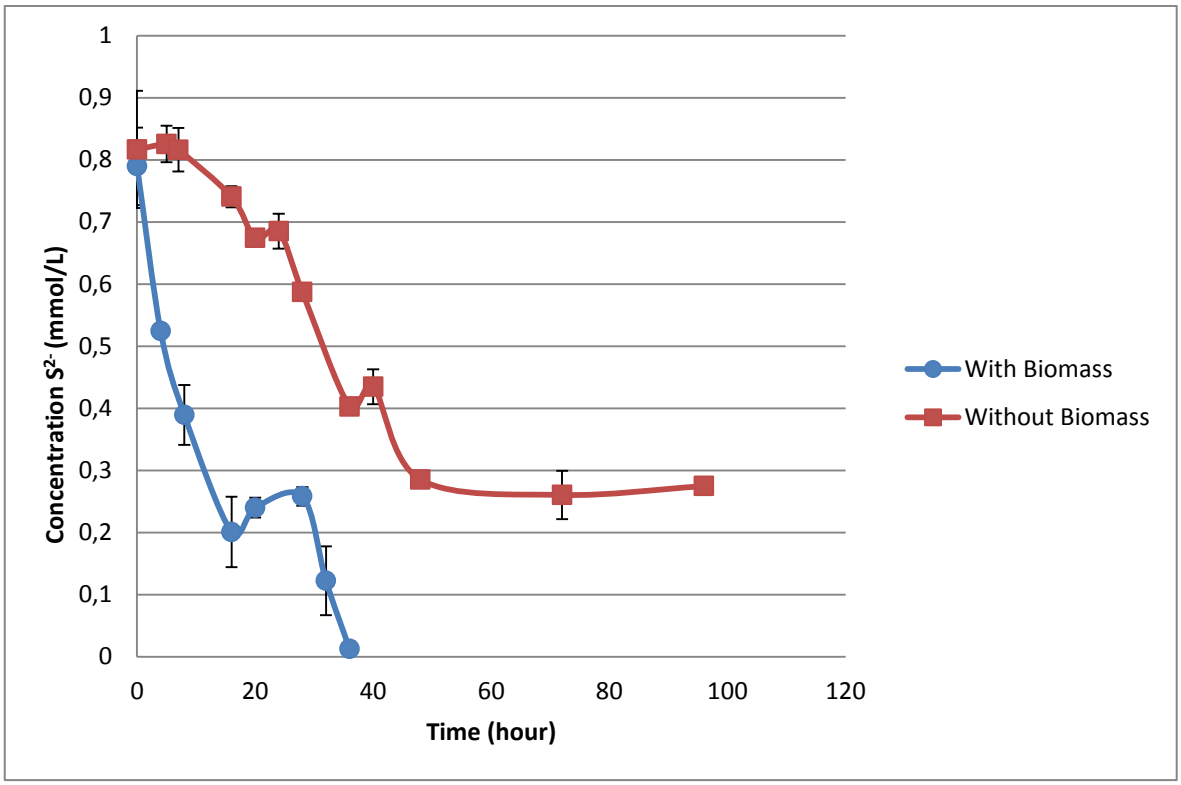

Figure 24 Concentration of sulphide in time with or without biomass at T = 25 °C

Figure 25 Formation of elemental sulphur in bottom reactor without biomass at T = 25 °C

3.4.2 Without HNO_3

A study of the concentration of the analytes without adding nitric acid (HNO_3) to the reactor at 10, 15 and 25 °C was performed to assess the effect of NO_3^- on the sulphide removal.

With or without HNO_3, there was a complete removal of sulphide (Figure 26). With HNO_3, the sulphide removal was faster, but temperature has more influence on the rate of sulphide removal than NO_3^-. Probably there was a small concentration of oxygen (electron acceptor) present in the reactor that oxidized sulphide.

Figure 27 confirms the hypothesis that the increase of SO_4^{2-} is correlated by NO_3^- and therefore by the bacterial denitrification. The growth of the sulphate concentration afterwards is due to the sulphur containing sludge.

Figure 28 shows that the concentration of $S_2O_3^{2-}$ without HNO_3 remains constant in time. Hereby there is another evidence that the second phase of the process, the bacterial denitrification oxidizes all reduced sulphur species into sulphate. Moreover, if there is no denitrification process, $S_2O_3^{2-}$ concentration remains constant.

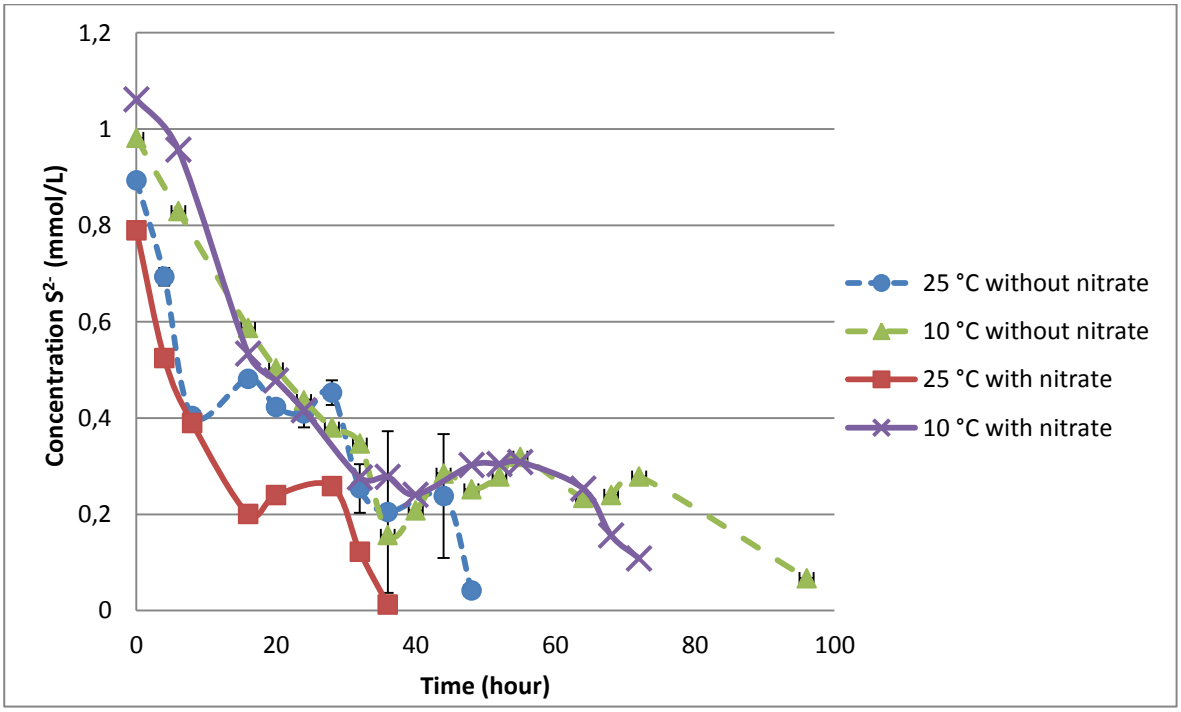

Figure 26 Concentration of sulphide in time with or without HNO_3 at different temperatures

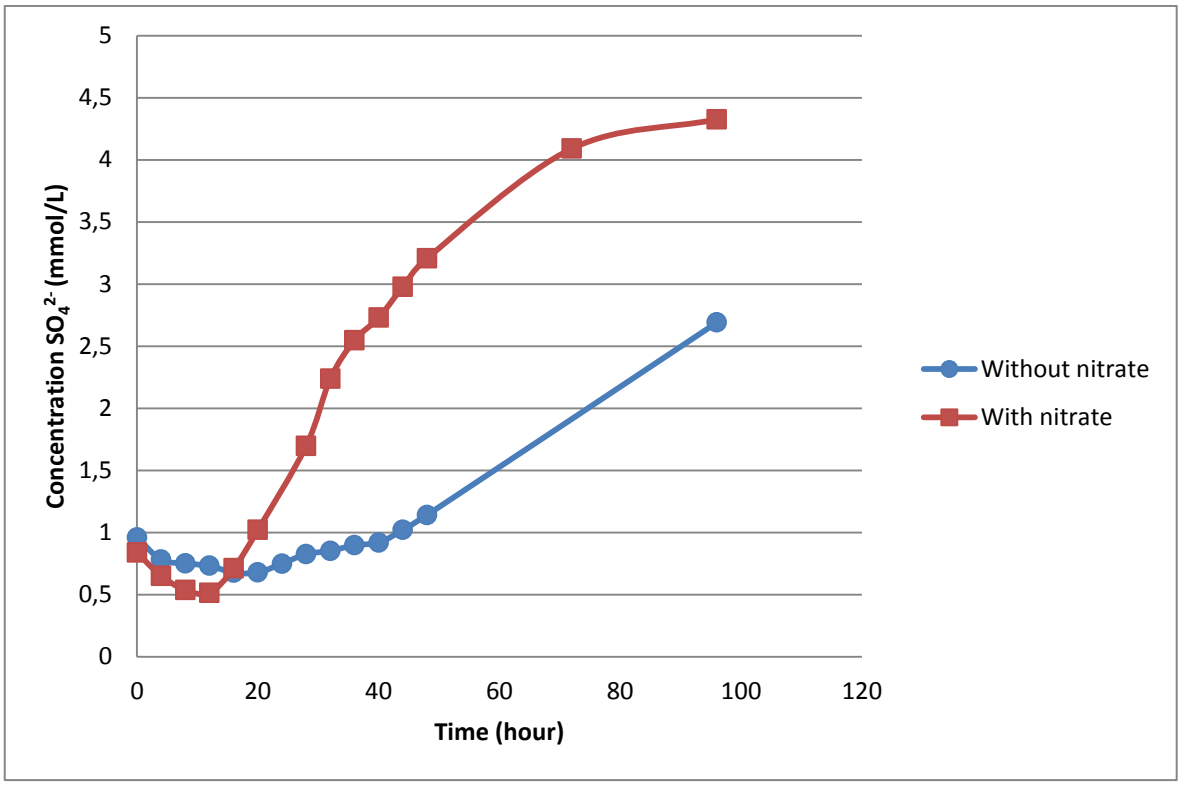

Figure 27 Concentration of sulphate in time with or without HNO_3 at T = 25 °C

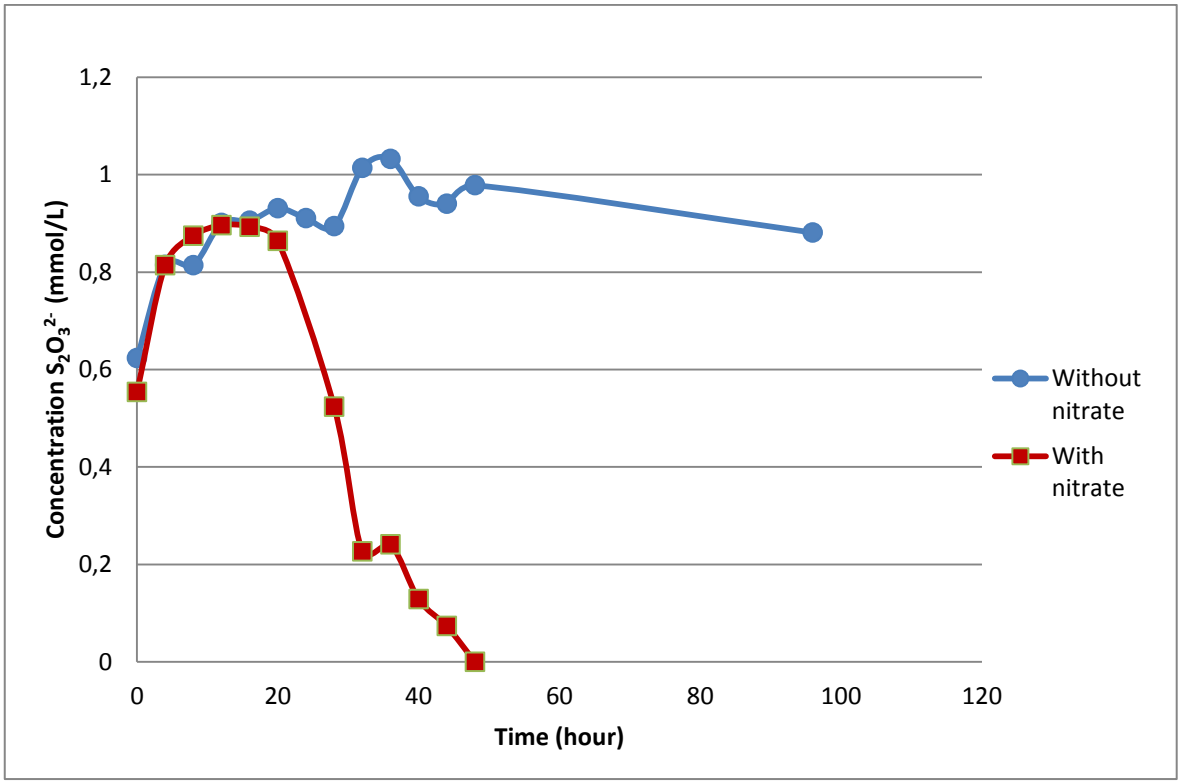

Figure 28 Concentration of thiosulphate in time with or without HNO_3 at T = 25 °C

3.4.3 Without $Na_2S \cdot 9H_2O$

A concentration study of the analytes without adding $Na_2S \cdot 9H_2O$ to the reactor was performed at 10, 15 and 25 °C to assess the impact of sulphide on the process. Figure 29 shows that in the beginning of the process a part of the sulphide is immediately oxidized into sulphate. Subsequently the concentration of SO_4^{2-} is increasing due to the sulphur containing biomass (Figure 31).

S^{2-} has an impact on the bacterial denitrification (Figure 30). Without S^{2-} (electron donor), the rate of the nitrate (electron acceptor) consumption is slower than with sulphide presence.

Figure 32 shows that without sulphide, there only is a bacterial denitrification at 25 °C. Therefore, it can be concluded that kinetics of NO_3^- consumption is strongly influenced by temperature.

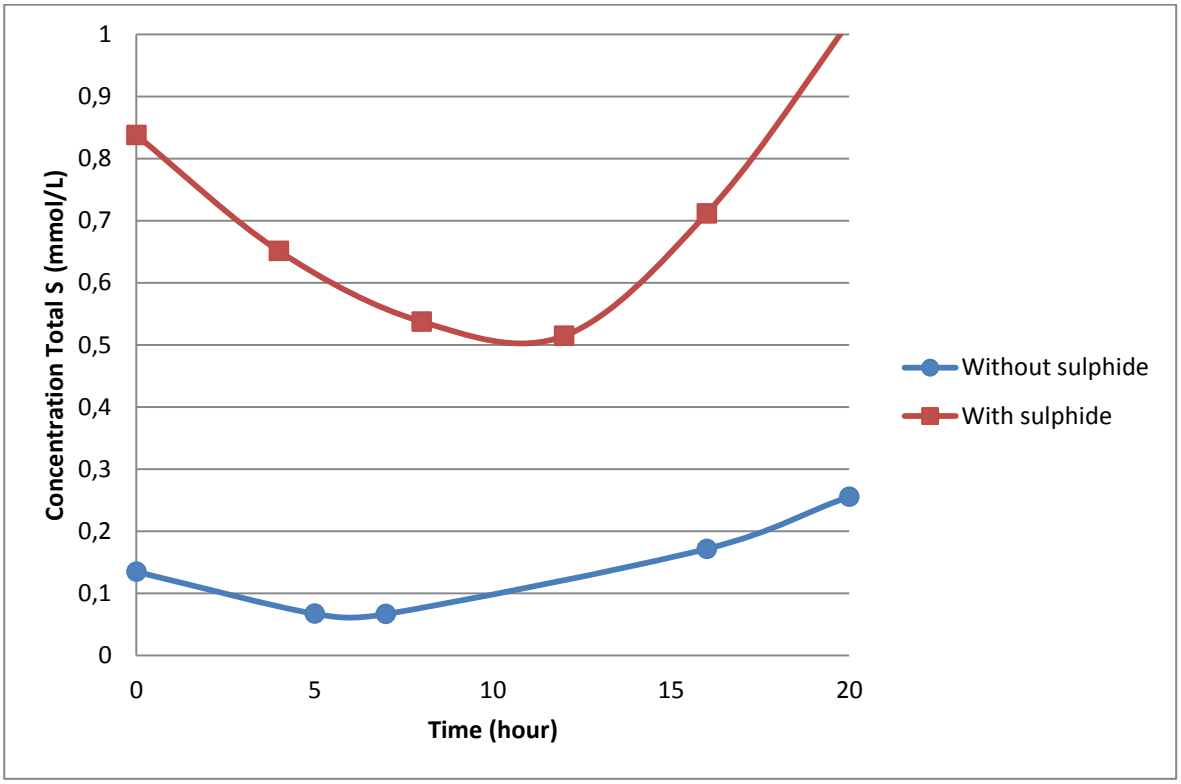

Figure 29 Concentration of sulphate in time with or without Na_2S at T = 25 °C (first 20 hours)

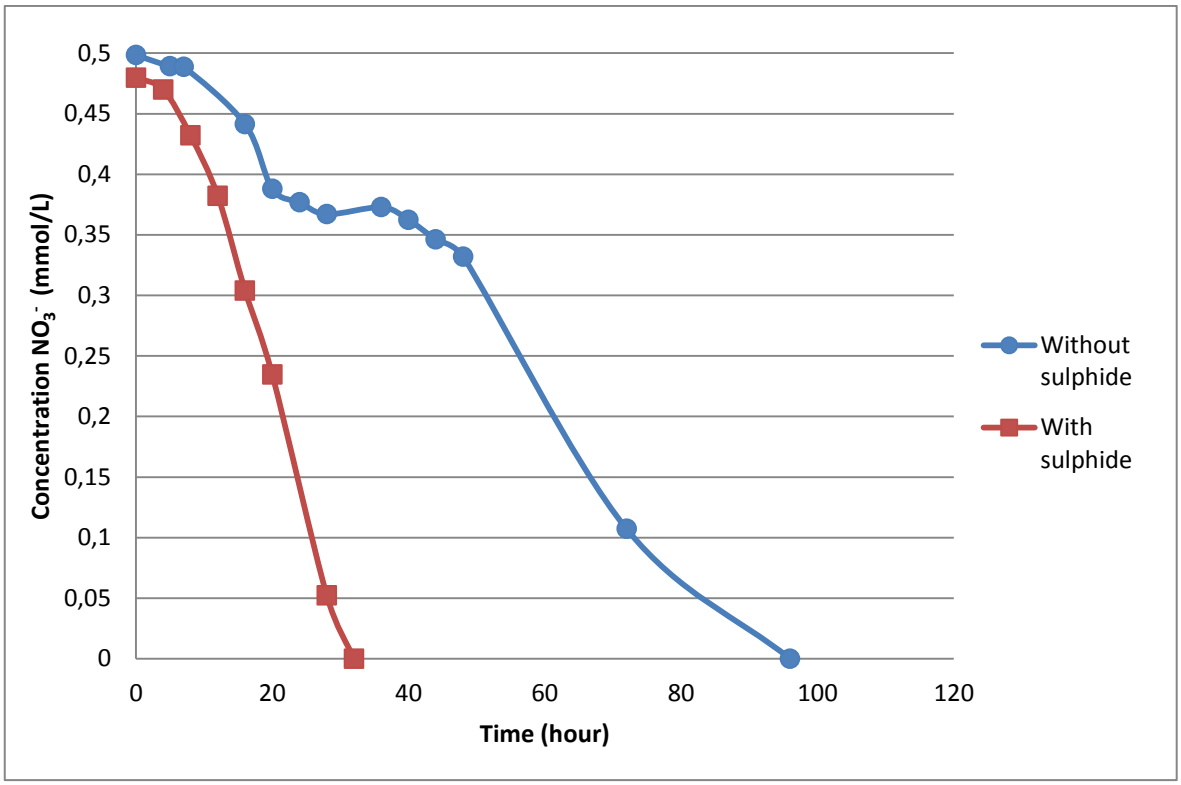

Figure 30 Concentration of nitrate in time with or without Na_2S at T = 25 °C

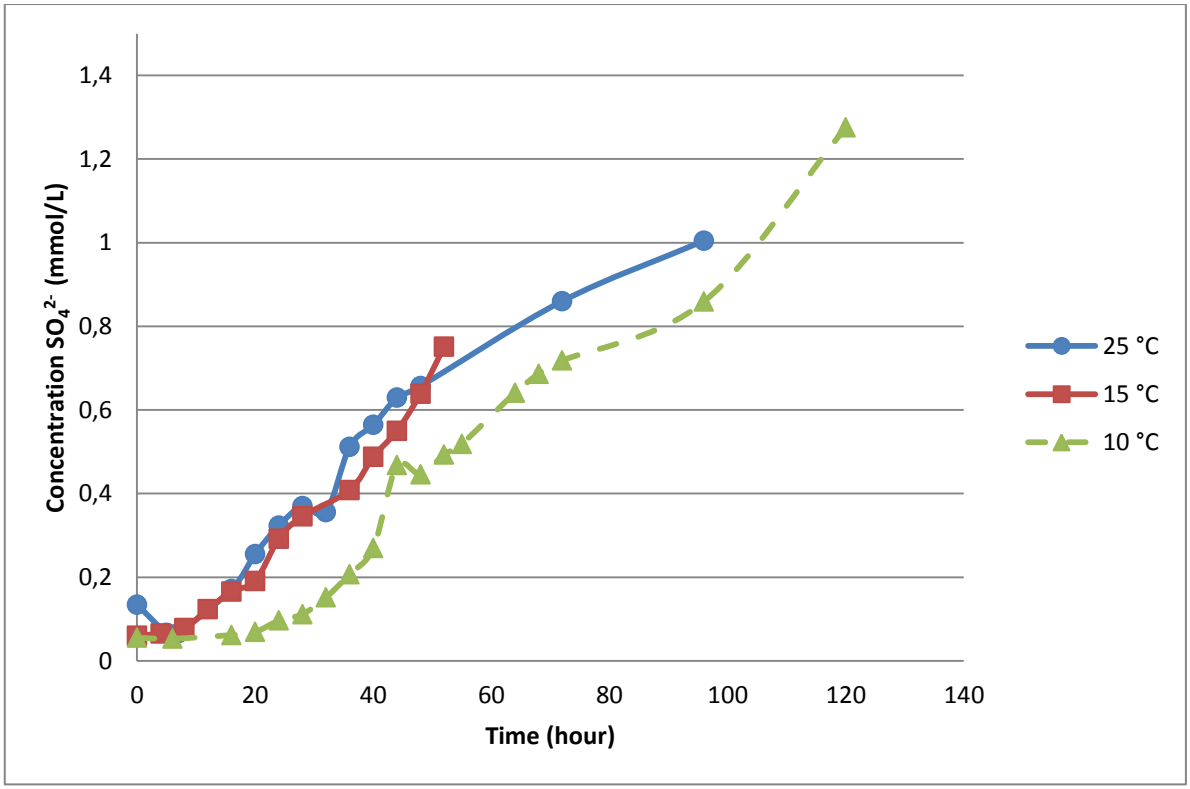

Figure 31 Concentration of sulphate in time at different temperatures (without Na_2S)

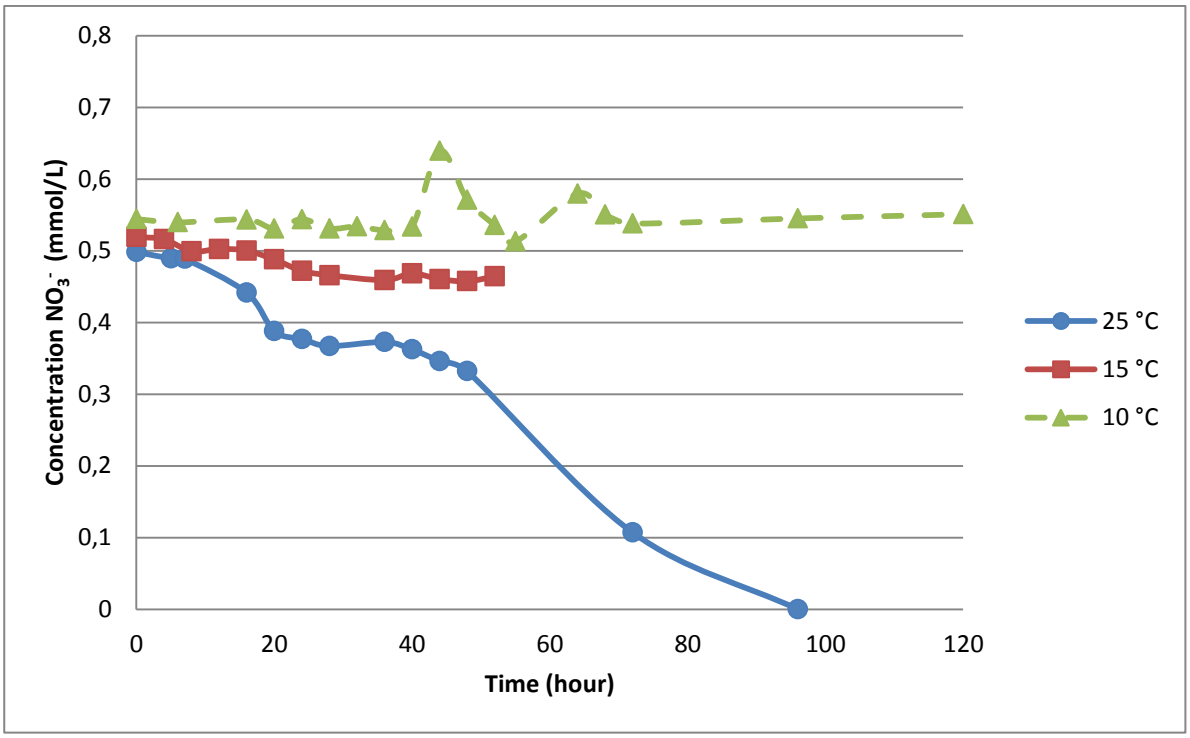

Figure 32 Concentration of nitrate in time at different temperatures (without Na_2S)

3.5 Kinetics of analytes at different temperatures

The rate was calculated according to following formula:

$$\text{Rate} = \frac{C_{n-1} - C_n}{t} \times 1000 \qquad [\frac{\mu mol}{L/h}] \quad (4)$$

Where:

C_{n-1} = Concentration previous sample [mmol/L]

C_n = Concentration sample [mmol/L]

t = time between sample meassurement [hours]

For instance to calculate the rate of the nitrate consumption between 0 and 4 hours at a temperature of 25 °C, the concentration after 4 hours is subtracted from the concentration after 0 hours (Appendix, Table 20) and is divided by the number of hours in the time interval, which is 4 hours, the number of hours between sample measurement.

In following chapters the kinetics of each phase in the process are described.

3.5.1 Kinetics of the first phase

During the first phase of the process, SO_4^{2-} is reduced into $S_2O_3^{2-}$ and S^{2-}. The first phase has ended when the bacterial denitrification initiates. Following tables (Table 6, 7 and 8) show the rate of sulphate reduction at different temperatures. 25 °C (0-4 hour)

Table 6 Kinetics of the first phase at 25 °C for sulphate consumption, thiosulphate and sulphide removal

Time h	**Molar rate SO_4^{2-}** µmol/L·h	**Molar rate $S_2O_3^{2-}$** µmol/L·h	**Molar rate S^{2-}** µmol/L·h
0-4	46.644	65.093	66.320

Table 7 Kinetics of the first phase at 15 °C for sulphate consumption, thiosulphate and sulphide removal

15 °C	**SO_4^{2-}**		**$S_2O_3^{2-}$**		**S^{2-}**	
Time h	**Molar rate** µmol/L·h	**Average molar rate** µmol/L·h	**Molar rate** µmol/L·h	**Average molar rate** µmol/L·h	**Molar rate** µmol/L·h	**Average molar rate** µmol/L·h
0-4	63.087	55.994	44.701	51.940	32.727	44.720
4-8	66.783		60.137		37.954	
8-12	38.114		50.982		63.480	

Table 8 Kinetics of the first phase at 10 °C for sulphate consumption, thiosulphate and sulphide removal

10 °C	SO_4^{2-}		$S_2O_3^{2-}$		S^{2-}	
Time h	**Molar rate** µmol/L·h	**Average molar rate** µmol/L·h	**Molar rate** µmol/L·h	**Average molar rate** µmol/L·h	**Molar rate** µmol/L·h	**Average molar rate** µmol/L·h
0-6	36.388	18.181	36.367	11.901	17.330	22.278
6-16	7.879		8.996		42.358	
16-20	18.199		2.478		14.059	
20-24	10.258		-0.238		15.367	

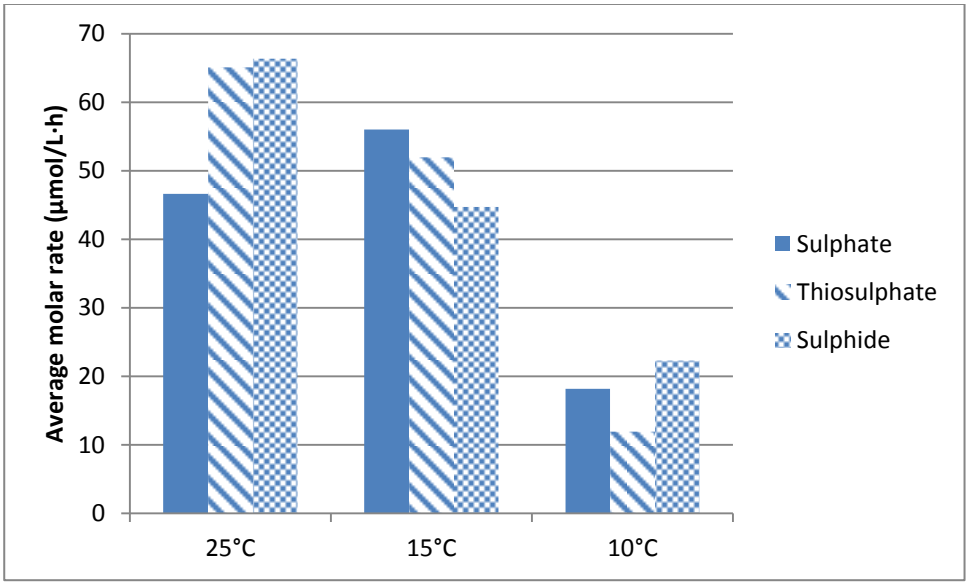

Figure 33 Kinetics of the first phase (time) at different temperatures for $SO4^{2-}$ consumption, $S_2O_3^{2-}$ formation and S^{2-} removal

Figure 33 shows that the average molar rate during the first phase for nitrate and sulphate higher is at 15 °C than at 25 °C. This is contradictory with Arrhenius' Law. The hypothesis is that at 25 °C bacterial denitrification has an impact on the first phase. That's why SO_4^{2-} consumption and $S_2O_3^{2-}$ formation is lower than at 25 °C.

Sulphide removal is dependable on the temperature in the first phase. At 10 °C, the average molar is one third of the S^{2-} removal at 25 °C, while at 15 °C the removal is two third of the S^{2-} removal at 25 °C.

At 10 °C, the first phase lasts longer, because of the very long lag phase of the nitrate consumption. Also the concentration of total sulphur has been diminished significantly after 16 hours. There has been stopped after 24 hours for the calculation of the average molar rate at 10 °C. Even it takes 40 hours when the bacterial denitrification initiates (start 2nd phase). The reason why there has been stopped after 24 hours is because the concentration of SO_4^{2-} started to increase slightly. Therefore, at 10 °C there is an intermediate phase between 24 and 40 hours, which is not a part of the sulfate reduction or the bacterial denitrification.

3.5.2 Kinetics of the second phase

The second phase starts when the bacterial denitrification initiates. During this phase the rate of nitrate consumption, sulphide removal, thiosulphate oxidation and sulphate formation is measured. The second phase ends when the concentration of total sulphur is significantly higher than the initial concentration. This is from 44 hours at a temperature of 25 °C and from 52 hours at 10 °C. The reason why is that the increase of SO_4^{2-} is due to the intracellular releasing of sulphur by the sludge and not by the bacterial denitrification. That is why the rate of the SO_4^{2-} formation is calculated until this time, because afterwards the rate of intracellular sulphur releasing is equal to the SO_4^{2-} formation.

There are only measurements used at 25 and 10 °C, because the measurements at 15 °C were not representative results (3.3.3). Table 9 and 10 show the rates and the average molar rates of the second phase at 25 °C and 10 °C.

Table 9 Kinetics of the second phase at 25 °C for nitrate and thiosulphate consumption, sulphate formation and sulphide removal

25 °C	NO_3^-		SO_4^{2-}		$S_2O_3^{2-}$		S^{2-}	
Time h	**Molar rate** µmol/L·h	**AMR**[3] µmol/L·h	**Molar rate** µmol/L·h	**AMR** µmol/L·h	**Molar rate** µmol/L·h	**AMR** µmol/L·h	**Molar rate** µmol/L·h	**AMR** µmol/L·h
4-8	2.485	16.401	-28.409	38.795	-15.135	8.101	33.757	11.314
8-16	16.012		21.780		-2.299		23.550	
16-20	19.572		77.281		7.310		-9.774	
20-28	17.346		84.529		42.526		-2.276	

Table 10 Kinetics of the second phase at 10 °C for nitrate and thiosulphate consumption, sulphate formation and sulphide removal

10 °C	NO_3^-		SO_4^{2-}		$S_2O_3^{2-}$		S^{2-}	
Time h	**Molar rate** µmol/L·h	**AMR** µmol/L·h	**Molar rate** µmol/L·h	**AMR** µmol/L·h	**Molar rate** µmol/L·h	**AMR** µmol/L·h	**Molar rate** µmol/L·h	**AMR** µmol/L·h
40-48	4.062	8.351	37.161	43.626	3.584	5.613	-7.669	-4.156
48-52	12.639		50.092		7.642		-0.644	

[3] AMR = Average molar rate

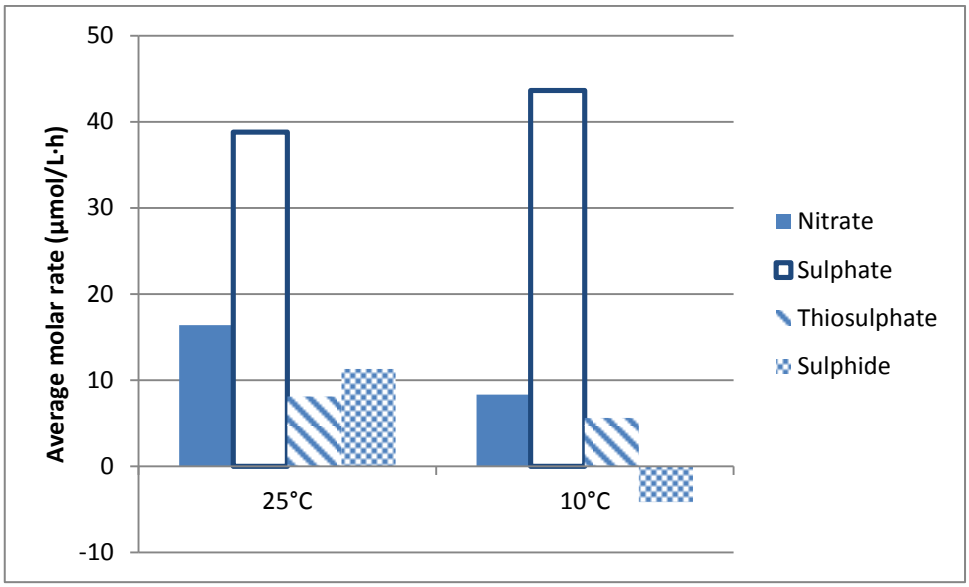

Figure 34 Kinetics of the 2nd phase at different temperatures for SO_4^{2-} formation, $S_2O_3^{2-}$ consumption and S^{2-}removal

According to figure 34, the average molar rate of nitrate at 25 °C is significantly higher than at 10 °C.

The bacterial denitrification lasts from 40 until 76 hours at 10 °C. Bacterial denitrification has an impact on sulphide removal at the end of the denitrification and because the release of elemental sulphur happens already after 52 hours, a negative value for sulphide removal is obtained in this phase. At 25 °C the sulphide removal correlates with the nitrate consumption at 25 °C

Sulphate formation has a higher rate at 10 °C than at 25 °C during the second phase. The reason why is the interference of the denitrification process with the chemical reduction of SO_4^{2-} in the beginning. Hence, the first rate of the SO_4^{2-} formation was negative, because the reduction of SO_4^{2-} into $S_2O_3^{2-}$ was still happening. At 10 °C, the reduction of sulphate had already ended.

3.5.3 Kinetics of the third phase

The third phase of the process starts when the concentration of total sulphur is significantly higher than the initial concentration. Therefore, the sulphate formation is no longer dependable on the bacterial denitrification, but mostly on the release of elemental sulphur by the sludge. Table 10 and 11 show the rates and the average molar rates at 25 °C and 10 °C of the third phase.

Table 11 Kinetics of the third phase at 25 °C for nitrate, thiosulphate consumption, sulphate formation and sulphide removal

25 °C	NO_3^-		SO_4^{2-}		$S_2O_3^{2-}$		S^{2-}	
Time h	**Molar rate** µmol/L·h	**AMR** µmol/L·h	**Molar rate** µmol/L·h	**AMR** µmol/L·h	**Molar rate** µmol/L·h	**AMR** µmol/L·h	**Molar rate** µmol/L·h	**AMR** µmol/L·h
44-48	0	0	57.128	34.574	18.281	6.094	0	0
48-72	0		36.814		0		0	
72-96	0		9.782		0		0	

Table 12 Kinetics of the third phase at 10 °C for nitrate, thiosulphate consumption, sulphate formation and sulphide removal

10 °C	NO_3^-		SO_4^{2-}		$S_2O_3^{2-}$		S^{2-}	
Time h	**Molar rate** µmol/L·h	**AMR** µmol/L·h	**Molar rate** µmol/L·h	**AMR** µmol/L·h	**Molar rate** µmol/L·h	**AMR** µmol/L·h	**Molar rate** µmol/L·h	**AMR** µmol/L·h
52-55	52.411	13.833	64.983	67.449	13.871	27.268	-1.304	9.112
55-64	88.780		88.219		24.400		6.132	
64-68	24.879		94.379		40.567		24.275	
68-72	24.463		121.121		55.475		11.936	
72-96	5.704		22.710		2.029		4.521	
96-120	0		13.279		0		0	

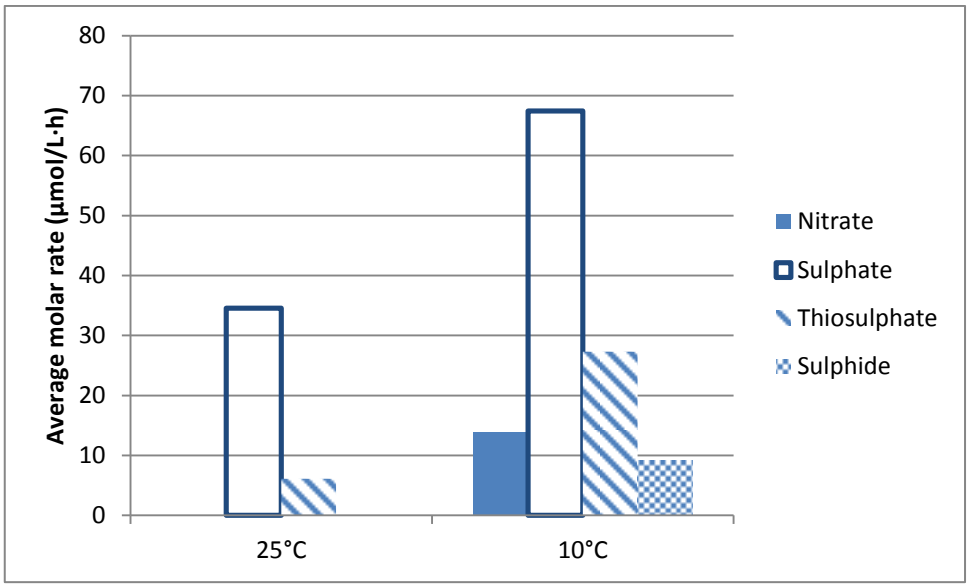

Figure 35 Kinetics of the 3rd phase at different temperatures for, NO_3^- and $S_2O_3^{2-}$ consumption , SO_4^{2-} formation and S^{2-}removal

At 25 °C, there is no sulphide removal anymore (Figure 35), because all sulphide was already removed between the end of the second phase and start of third phase. At 10 °C, bacterial denitrification wasn't completed yet. Hence, there is S^{2-} removal during this phase at this temperature. Because complete sulphide removal is feasible at the end of bacterial denitrification.

During the third phase the average molar rate of SO_4^{2-} is higher at 10 °C, because next to the releasing of sulpur by the sludge, there is still the oxidation of all reduced sulphur species into SO_4^{2-}.

The average molar rate of the SO_4^{2-} formation at 25 °C is equal to the molar rate of intracellular sulphur that has been released by the sludge.

3.5.4 Nitrate consumption

Regardless which kind of phase, the average molar rate of nitrate consumption is calculated to have a better understanding about the kinetics of NO_3^-. The average molar rate of NO_3^- is calculated during the bacterial denitrification. Table 13 shows the molar and average molar rate of nitrate consumption at different temperatures.

Table 13 Kinetics of nitrate consumption at different temperatures

Time h	**Molar rate at 25 °C** µmol/L·h	**Average molar rate at 25 °C** µmol/L·h	**Molar rate at 15 °C** µmol/L·h	**Average molar rate at 15 °C** µmol/L·h	**Molar rate at 10 °C** µmol/L·h	**Average molar rate at 10 °C** µmol/L·h
0-4	2.485		-0.980			
4-8	9.451	16.323	2.587		0.069[4]	
8-12	12.452		0.865			
12-16	19.572		2.284		2.952[5]	
16-20	17.346		2.427		11.754	
20-24			3.804	14.431	-3.098	
24-28	22.795[6]		5.738		1.488	
28-32					-0.832	
32-36			4.421		0.499	
36-			10.987		0.511	

[4] The data is from 0-6 hours
[5] The data is from 6-16 hours
[6] The data is from 20-28 hours

40						
40-44			28.741			13.336
44-48			32.895		4.062[7]	
48-52					11.632	
52-55					12.639	
55-64					5.241	
64-68					8.878	
68-72					24.879	
72-76					24.464	

[7] The data is from 40-48 hours

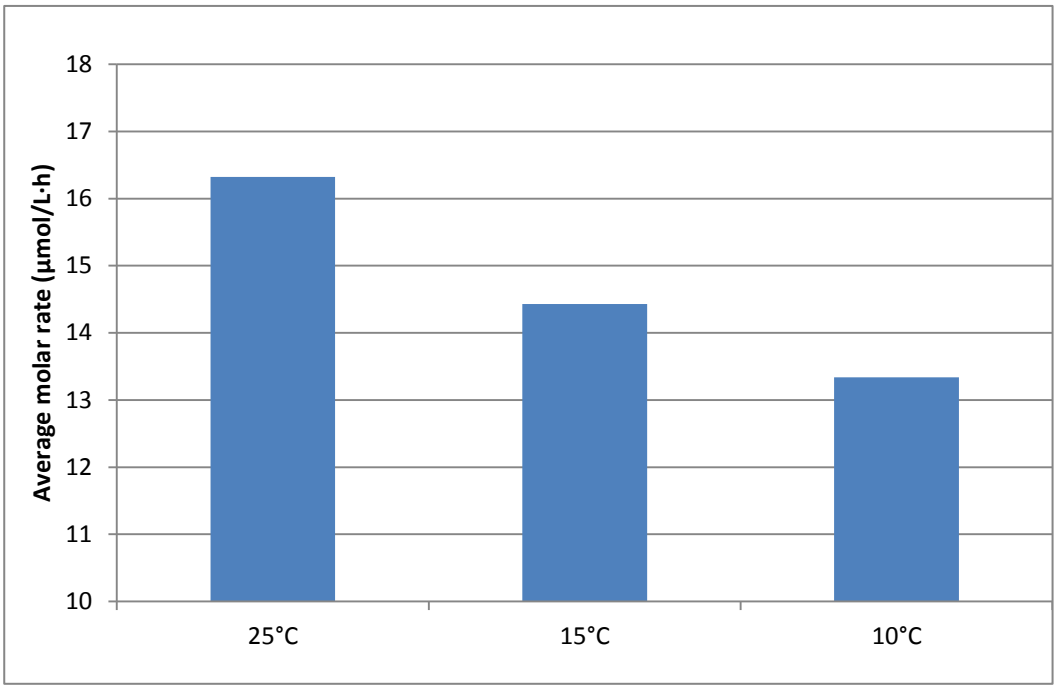

Figure 36 Average molare rate of nitrate consumption at different temperatures

The higher the temperature is, the higher the average molar rate is. Although the difference in molar rate is not ignificant . However the key influence of the temperature on NO_3^- during the process is the length of lag-phase, which is previously discussed in 3.3.1.

3.5.5 Sulphide removal

Like previously performed for nitrate. The average molar rate of sulphide removal is examinded regardless which kind of phase in the process. On the contrary of the average molar rate of nitrate is the average molar rate of sulphide removal rate calculated over the whole area (and not only the bacterial denitrification area). Table 14 shows the molar and average molar rate of sulphide removal at different temperatures.

Table 14 Kinetics of sulphide removal at different temperatures

Time h	**Molar rate at 25 °C** µmol/L·h	**Average molar rate at 25 °C** µmol/L·h	**Molar rate at 10 °C** µmol/L·h	**Average molar rate at 10 °C** µmol/L·h
0-4	66.320		17.33[8]	
4-8	33.757			
8-12	23.550[9]			
12-16			42.358[10]	
16-20	-9.774	24.719	14.059	
20-24	-2.276[11]		15.367	
24-28			17.500[12]	11.400
28-32	33.959			
32-36	27.494		-0.470	
36-40	3.103		9.330	
40-44				
44-48			-7.669[13]	
48-52			-0.644	
52-55			-1.304	

[8] This data is from 0 until 6 hours
[9] This data is from 8 until 16 hours
[10] This data is from 6 until 16 hours
[11] This data is from 20 untiil 28 hours
[12] This data is from 24 until 32 hours
[13] This data is from 40 until 48 hours

55-64			6.132	
64-68			24.275	
68-72			11.936	

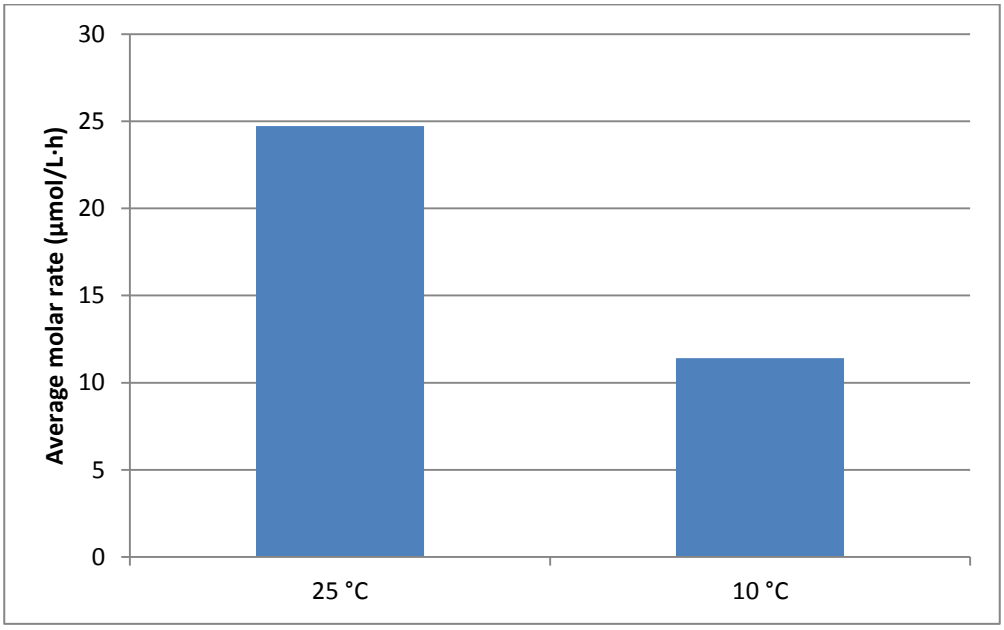

Figure 37 Average molar rate of sulphide removal at different temperatures

At higher temperatures, the average molar rate of sulphide removal is higher than at lower temperatures. The average molar rate at 25 °C is twice as high as at 10 °C. During sulphide removal a lag phase for the concentration of S^{2-} is obtained. At 10 °C the lag phase lasts 32 hours, while at 25 °C the lag phase has a duration of 12 hours. Therefore, the duration of the lag phase has a key influence on the average molar rate of sulphur removal.

3.6 Total and Volatile Solids

The total and volatile solids of the granular sludge from the pulp and paper industry are measured. Two bottles of sludge are used during the experiments. Total and volatile solids are calculated by the formula in 2.5.2 and 2.5.3. Table 15 shows the results of TS and VS.

Table 15 Results Total and Volatile Solids

Sample name	**Total solids** mg TS/L	**Volatile solids** mg VS/L
Sample 1	49.027	41.813
Sample 2	57.040	48.207

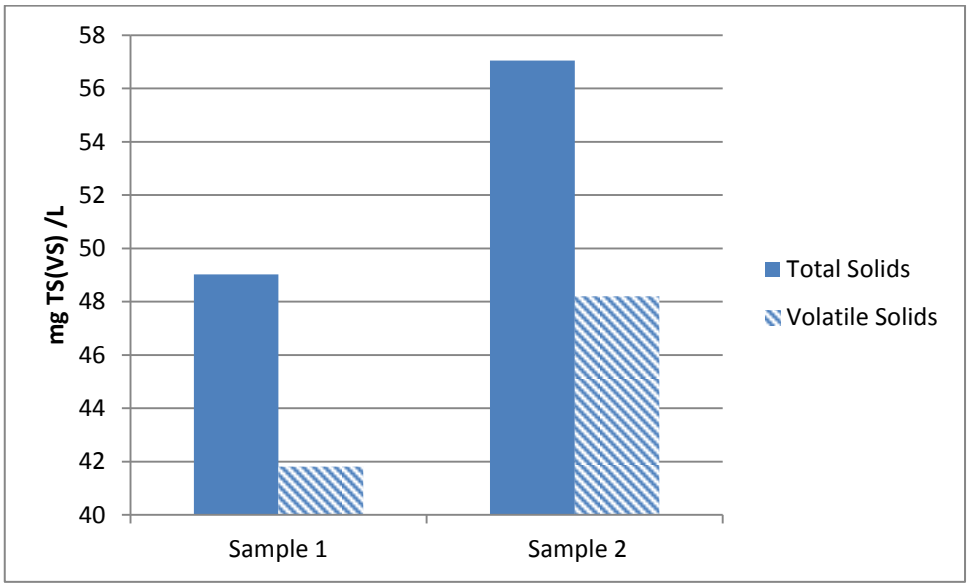

Figure 38 Total and volatile solids for granular sludge

The samples originated from the same lot, although a significant difference between both samples has been observed (Figure 38). Hence, the effect of the two samples is not tested during the experiments. However, in the future it is advisable to use a sludge with a higher reliability in TS and VS during the experiments.

4 Conclusion

The overall process of the batch experiment has three phases. At the start of the first phase, a portion of sulphide is immediately converted into sulphate. Afterwards the concentration of sulphate is diminishing, while it is being reduced into thiosulphate and elemental sulphur. Subsequently the concentration of sulphide is increasing due to oxidation into elemental sulphur. The first phase is mainly chemical. Although at 25 °C, bacterial denitrification is already active at the beginning of the process and therefore, it affects the average molar rates of the analytes. The higher the temperature, the better the sulphide removal is during this phase. During the first phase, the average molar rate of sulphide removal is the highest. At 25 °C the average molar rate is 66.320 µmol/L·h and at at 10 °C 44.720 µmol/L·h.

The second phase starts with the bacterial denitrification. Elemental sulphur and SO_4^{2-} are formed as the result of the oxidation of their reduced forms of sulphur. The oxidation is purely biochemical. The higher the temperature, the less time it takes to fully consume NO_3^- during the bacterial denitrification. Subsequently has temperature an effect on the lag phase time for NO_3^-. At low temperatures the lag phase last longer and therefore, the initiation of the bacterial denitrification is delayed.

The third phase of the process is the phase where intracellular elemental sulphur is being oxidized into sulphate in the reactor, which creates a higher concentration than the initial concentration of sulphur in the reactor. The average molar rate of this phenomena is 34.574 µmol/L·h.

The effects of substrates on sulphide removal were very learn full. Without nitrate there are some sulphide removal which is purely chemical oxidation. Whereas, without biomass this feature was impossible. Furthermore the amount of sulphide influences the rate of the nitrate consumption.

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6 Appendix

Concentration of analytes for different amount of volume for sludge

Table 16 Concentration of nitrate (mmol/L) at different amounts of volume for sludge

Time	Batch 1	Batch 2	100 mL sludge[14]	Batch 3	Batch 4	50 mL sludge[15]	Batch 5	Batch 6	10 mL sludge[16]
0	0.476	0.481	0.479	0.413	0.479	0,446	0.475	0.455	0,465
1	0.458	0.465	0.461	0.413	0.474	0,443	0.472	0.460	0,466
3	/[17]	0.430	0.430	0.403	0.461	0.432	0.482	0.458	0,470
5	0.429	0.422	0.425	0.400	0.457	0.428	0.476	0.453	0,465
24	0.285	0.249	0.267	0.315	0.033	0.174	0.433	0.388	0,411
48	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0

Table 17 Concentration of sulphate (mmol/L) at different amounts of volume for sludge

Time	Batch 1	Batch 2	100 mL sludge	Batch 3	Batch 4	50 mL sludge	Batch 5	Batch 6	10 mL sludge
0	0.748	0.849	0.799	0.478	0.602	0.540	0.941	0.857	0.899
1	0.795	0.577	0.686	0.477	0.637	0.557	0.758	0.761	0.760
3	/	0.960	/	0.489	0.562	0.525	0.792	0.648	0.720

[14] The average concentration of batch 1 and batch 2
[15] The average concentration of batch 3 and batch 4
[16] The average concentration of batch 5 and batch 6
[17] No data was obtained for this point

5	0.735	0.730	0.732	0.292	0.498	0.395	0.446	0.491	0.469
24	1.408	1.450	1.429	0.968	1.838	1.403	2.320	2.148	2.234
48	1.319	1.495	1.407	1.476	2.118	1.797	2.716	2.643	2.679
72	0.895	1.868	1.382	1.356	2.067	1.712	2.858	2.719	2.788

Table 18 Concentration of thiosulphate (mmol/L) at different amounts of volume for sludge

Time	**Batch 1**	**Batch 2**	**100 mL sludge**	**Batch 3**	**Batch 4**	**50 mL sludge**	**Batch 5**	**Batch 6**	**10 mL sludge**
0	0.785	0.934	0.859	0.755	0.873	0.814	0.438	0.458	0.448
1	0.982	0.835	0.908	0.757	0.892	0.825	0.466	0.527	0.496
3	/	0.745	0.745	0.752	0.863	0.808	0.597	0.615	0.606
5	0.693	0.613	0.653	0.737	0.875	0.806	0.669	0.669	0.669
24	0	0	0	0.098	0	0.049	0	0	0
48	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0

Table 19 Concentration of total sulphur (mmol/L) at different amounts of volume for sludge

Time	**Batch 1**	**Batch 2**	**100 mL sludge**	**Batch 3**	**Batch 4**	**50 mL sludge**	**Batch 5**	**Batch 6**	**10 mL sludge**
0	2.657	3.059	2.858	2.495	2.807	2.651	2.533	2.459	2.496
1	2.900	2.548	2.724	2.586	2.826	2.706	2.570	2.561	2.565

3	2.296	2.828	2.828	2.491	2.698	2.698	2.411	2.399	2.399
5	2.285	2.584	2.435	2.128	2.529	2.328	2.507	2.568	2.537
24	1.427	1.595	1.511	1.608	1.810	1.709	2.234	2.252	2.243
48	1.334	1.539	1.436	1.526	2.158	1.842	2.771	2.730	2.750
72	0.855	1.853	1.354	1.433	1.939	1.686	2.811	2.716	2.763

Concentration of nitrate at different temperatures

Table 20 Concentration of nitrate (mmol/L) in time at T = 25°C

Time	Batch 1	Batch 2	Batch 3	Average
0	0.482	0.477	0.480	0.480
4	0.453	0.488	0.469	0.470
8	0.418	0.443	0.435	0.432
12	0.347	0.407	0.392	0.382
16	0.212	0.340	0.359	0.304
20	0.133	0.251	0.319	0.234
28	0	0	0.156	0.052
32	0	0	0	0

Table 21 Concentration of nitrate (mmol/L) in time at T = 15 °C

Time	Batch 1	Batch 2	Average
0	0.499	0.501	0.500
4	0.504	0.503	0.504

8	0.488	0.498	0.493
12	0.485	0.494	0.490
16	0.482	0.480	0.481
20	0.466	0.476	0.471
24	0.441	0.471	0.456
28	0.410	0.455	0.433
36	0.368	0.427	0.398
40	0.312	0.395	0.354
44	0.173	0.304	0.239
48	0	0.214	0.107
52	0	0	0

Table 22 Concentration of nitrate (mmol/L) in time at T = 10 °C

Time	**Batch 1**	**Batch 2**	**Batch 2**	**Average**
0	0.528	0.540	0.508	0.525
6	0.537	0.524	0.514	0.525
16	0.628	0.521	0.513	0.554
20	0.510	0.517	0.495	0.507
24	0.530	0.512	0.516	0.520
28	0.545	0.500	0.496	0.514
32	0.519	0.507	0.524	0.517

36	0.515	0.522	0.508	0.515
40	0.498	0.529	0.512	0.513
48	0.391	0.533	0.517	0.480
52	0.314	0.478	0.498	0.430
55	0.242	0.531	0.469	0.414
64	0	0.291	0.377	0.334
68	0	0.171	0.299	0.235
72	0	0.063	0.210	0.137
76	0	0	0.168	0.084