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# **Are the energy pathways diverted by temperature in biological sulphide oxidation?**

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**Summary:**

Many wastewater streams contain sulphides. Their corrosiveness, toxicity and unpleasant odor makes necessary the application of removal techniques. In the present study the temperature effect (25-35 °C) and N/S ratio (<0.35) impact on biological sulphide oxidation with nitrate as an electron acceptor impact was studied. The experimental trial was conducted in expanded granular sludge bed reactor (EGSB). Nitrate and sulfur components were analyzed using ion chromatography. Additionally, the microscopy analysis was conducted for sludge samples. The almost complete removal of  $\text{NO}_3^-$  was achieved at all tested temperatures and ratios. Average  $\text{HS}^-$ -S removal efficiency over the whole temperature study was 92% and slightly varied with temperature. Electron donor removal tended to decrease with  $\text{NO}_3^-/\text{HS}^-$  ratio decrease from 92% (0.35) to 88% (0.245). Temperature increase caused metabolic shift manifested by increased  $\text{SO}_4^{2-}$  production. The free Gibbs energy analyses were performed, however, no significant changes in overall reaction free Gibbs energy were observed. Microscopy analysis showed the possible presence of sulphur particles in sludge samples. It was suggested that high temperature (35 °C) and/or low ratios (<0.35) could be a reason of low granular sulphur accumulation.

*The University College of Southeast Norway takes no responsibility for the results and conclusions in this student report.*

# Preface

The background of this thesis is related with sulphide removal technologies, especially the technologies based on biological removal by autotrophic denitrification (with nitrate). Different factors can have an impact on the biological removal process of sulphide, two of the most influential are temperature and molar ratio ( $\text{NO}_3^-/\text{HS}^-$ ) which are investigated in this work. Results of this this work show how high temperatures (29-35 °C) and lower than stoichiometric molar ratios ( $\text{NO}_3^-/\text{HS}^- < 0.35$ ) may influence the biological process and the overall sulphide removal efficiency.

The author would like to thank the supervisors: Carlos Dinamarca and Michal Sposob for cooperation and help during the master project work.

Porsgrunn, 15.05.2017

Valerii Vyshniakov



# Nomenclature

ASO - anoxic sulphide-oxidizing

BSR – biological sulphide removal

CSTR - continuous stirred tank reactor

EGSB - expanded granular sludge bed

FeS - iron sulphide

GAC - granular activated carbon

HRT - hydraulic retention time

HS<sup>-</sup> - hydrogen sulphide

L - liter

mg – milligram ( $10^{-3}$  gram)

N – nitrogen

n.d. – not detected

NH<sub>3</sub> – free ammonia

NO<sub>2</sub><sup>-</sup> - nitrite

NO<sub>3</sub><sup>-</sup> - nitrate

°C – degree Celsius

ORP – oxidation reduction potential

PAC - powdered activated carbon

pK<sub>a</sub> – acid dissociation constant

S – sulphur

S<sup>0</sup><sub>acc</sub> – elemental sulphur accumulated inside the reactor

S<sup>0</sup><sub>ss</sub> – elemental sulphur suspended

S<sub>2</sub>O<sub>3</sub><sup>2-</sup> - thiosulphate

SO<sub>4</sub><sup>2-</sup> – sulphate

SOB – sulphide oxidizing bacteria

SRT - solids retention time

UASB - upflow anaerobic sludge blanket

ΔG<sup>0</sup> - standard free Gibbs energy

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# 1 Introduction

Sulphides are present in many kinds of wastewaters i.e. from petrochemical industry, electricity generation, coal gasification and mining industry. The wastewater pipelines are usually made of metal (i.e. stainless steel, steel, copper), sulphur species such as sulphate ( $\text{SO}_4^{2-}$ ) or hydrogen sulphide ( $\text{H}_2\text{S}$ ) behave aggressively towards them causing pipes corrosion (Geldenhuis et al., 2003; Krishnakumar and Manilal, 1999; Ravichandra et al., 2007).  $\text{H}_2\text{S}$  and its ionic forms  $\text{HS}^-/\text{S}^{2-}$  are toxic to humans, environment and has characteristic harsh unpleasant (rotten eggs) odor (Ravichandra et al., 2007; Sposob et al., 2016; Yavuz et al., 2007). Due to these adverse properties it is crucial to remove them from wastewater streams (Cai et al., 2008).

Numerous methods for  $\text{H}_2\text{S}$  removal exist and can be split into two main categories – biological and physicochemical. The non-biological methods have a high efficiency (Li et al., 2009), however, they require more complex technologies and addition of other chemicals what increases the process maintenance costs. Additionally, the great amount of generated side products (i.e. sediments) needs to be removed, treated and/or disposed. Due to that the physicochemical treatment is not environmentally favorable (An et al., 2010; Dinamarca, 2014; Krishnakumar and Manilal, 1999; Ravichandra et al., 2007; Show et al., 2013).

Alternatively, biological sulphide removal (BSR) processes give a high removal efficiency being a more economically and environmentally attractive. BSR can be used for different volumetric flows at low and high  $\text{HS}^-$  load (An et al., 2010; Mahmood et al., 2007a). Biological sulphide oxidation may lead to the production of elemental sulphur ( $\text{S}^0$ ), thiosulphate ( $\text{S}_2\text{O}_3^{2-}$ ) and sulphate ( $\text{SO}_4^{2-}$ ). Such compounds can be recovered and recycled during biological sulphide oxidation (Yavuz et al., 2007).

Nitrogen and sulphur cycles are tightly connected to each other. The biological removal of  $\text{HS}^-$  can be conducted in the presence of nitrate ( $\text{NO}_3^-$ ) or nitrite ( $\text{NO}_2^-$ ) as an electron acceptor source. For denitrification process (nitrate removal) reduced sulphur compound (i.e.  $\text{S}^0$ ,  $\text{HS}^-$ ) can serve as electron donor (Li et al., 2009; Yavuz et al., 2007).

In recent years, simultaneous biological removal of  $\text{HS}^-$  and  $\text{NO}_3^-$  is gaining high attention. The process is economically beneficial and environmentally-friendly (Chen et al., 2017; Show et al., 2013; Yavuz et al., 2007).

Process stability is a critical factor to fulfill for the process to be used in full scale (Chen et al. 2008). Different factors can have an impact on the process stability i.e. hydraulic retention time (HRT), ratios between  $\text{H}_2\text{S}$  and  $\text{NO}_2^-/\text{NO}_3^-$  and pH impact was investigated (Cai et al., 2010; Chen et al., 2009; Mahmood et al., 2007a, 2007b). Hence, efficiency and stability of the process can be improved simultaneously leading to economic benefits.

Microorganisms in BSR processes are very sensitive to changing conditions (i.e. pH). Level of pH may increase as a result of denitrification process which can affect bacterial metabolism hence decrease process efficiency (Mahmood et al., 2007b). Controlling of the reactor pH is one of the challenges for process stability.

Proper ratio between  $\text{HS}^-$  and  $\text{NO}_3^-$  is important parameter for their simultaneous removal. Changing N/S ratio may lead to both positive and negative effects. Under  $\text{NO}_3^-$  presence concentration of  $\text{H}_2\text{S}$  will decrease due to autotrophic denitrification activity. Alternatively, excessive presence of  $\text{HS}^-$  or/and diluted oxygen inhibit denitrifies (Show et al., 2013).

Temperature has also an impact on bacteria involved in BSR process. Thus, it is recommended that during biological treatment processes (i.e. anaerobic digestion) temperature variations should be less than 1 °C (Grady Jr et al., 2011). So far, not many studies considering temperature effect on a simultaneous  $\text{HS}^-$  and  $\text{NO}_3^-$  removal have been performed. Yavuz et al., (2007) observed that with increasing temperature (25 to 35 °C), specific  $\text{HS}^-$  oxidation rate increases. Chen et al., (2008) reported that increasing temperatures has a positive effect on microorganisms metabolic rate during anaerobic digestion process. However, increasing temperature results in higher concentrations of process inhibitors (e.g. free ammonia ( $\text{NH}_3$ )). Previously published research considering low temperatures (<25°C) impact on simultaneous  $\text{HS}^-$  and  $\text{NO}_3^-$  removal process in EGSB reactor showed that it is possible to treat wastewater with low  $\text{HS}^-$  load under different temperature conditions. Minor influence of temperatures in a range from 25 to 20 °C on  $\text{SO}_4^{2-}$  and  $\text{S}_2\text{O}_3^{2-}$  formation was observed. Further temperature decrease to 15-10 °C led to increasing concentrations of  $\text{SO}_4^{2-}$  and  $\text{S}_2\text{O}_3^{2-}$  (Sposob et al., 2016). Temperature changes affect sulphur based denitrifying systems and sulphide removal efficiency drop dramatically when temperature decreasing from 20-25 °C to 5-10 °C (Zhou et al., 2011). However, it has been reported that highly efficient denitrification process can be conducted at 3 °C using  $\text{S}_2\text{O}_3^{2-}$  as electron donor source (Di Capua et al., 2017).

The objective of the present study was to investigate how different factors such as high temperatures (>25°C) and different stoichiometric N/S molar ratios (<0.35) influence the biological  $\text{HS}^-$  oxidation. Experimental trial was performed using the expanded granular sludge bed (EGSB) reactor based on the approach developed at USN.

## 2 Literature review

### 2.1 Sulphide removal methods

#### 2.1.1 Physicochemical sulphide removal

Different physicochemical methods for  $\text{HS}^-$  removal are described in literature i.e. Claus process, Lo-cat and Amine process (Hancock et al., 1998; Krishnakumar et al., 2005; Sanopoulos and Karabelas, 1997; Sassi and Gupta, 2008).

$\text{H}_2\text{S}/\text{HS}^-$  can be removed from gas and/or water stream by caustic washing, scrubbing, filtration, sedimentation or retention on packing material (i.e. grains). These methods are using the physical properties of the i.e. packing material such as physical state, surface area and density for removal purposes (McComas et al., 2001; Mohammad et al., 2016).

The chemical removal of  $\text{HS}^-$  is usually conducted by aeration, adsorption, ion exchange, coagulation and flocculation (Mohammad et al., 2016).  $\text{HS}^-$  can be neutralized by addition of chemicals like quick lime (CaO) sodium hydroxide (NaOH) or nickel (Ni) to the wastewater stream (Hancock et al., 1998; Salman, 2009). During the adsorption process, solid adsorbents like activated carbon are used to remove dissolved  $\text{HS}^-$  from wastewater. Activated carbon is used widely due to their large surface area and can be applied in granular and powdered form GAC and PAC, respectively. The operational parameters like pH, pollutant concentration in wastewater, contact time between adsorbent and treated substance, adsorbent mass concentration and temperature of the pollutant can significantly affect the efficiency of adsorption process (Salman, 2009).

#### 2.1.2 Biological sulphide removal

Biological sulphide removal is an attractive alternative to physicochemical removal methods. Physicochemical methods require higher energy input, chemicals addition and produce excessive amounts of sludge (An et al., 2010; Krishnakumar and Manilal, 1999; Li et al., 2009). Alternatively, biological methods are environmental friendly and more economic, what make them preferable for water/wastewater treatment purposes (Chen et al., 2009; Ravichandra et al., 2007; Sposob et al., 2017a).

Photoautotrophic and chemolithotrophic sulphide oxidizing bacteria (SOB) are two main microorganisms that can conduct the biological  $\text{HS}^-$  oxidation process (Krishnakumar et al., 2005).  $\text{HS}^-$  oxidation to  $\text{S}^0$  and/or  $\text{SO}_4^{2-}$  can be carried out by phototrophic and colorless sulphur bacteria (Yavuz et al., 2007). The biological oxidation of  $\text{HS}^-$  can be only conducted under  $\text{NO}_2^-/\text{NO}_3^-$  or  $\text{O}_2$  presence as the electron acceptor source. Yavuz et al., (2007) showed that  $\text{HS}^-$  removal rate is faster when  $\text{NO}_2^-/\text{NO}_3^-$  used as an electron acceptor rather than  $\text{O}_2$ .

*Thiobacillus denitrificans* is usually present during biological  $\text{HS}^-$  removal processes where  $\text{NO}_3^-$  is supplied as electron acceptor. During  $\text{HS}^-$  oxidation *Thiobacillus denitrificans* will reduce nitrogenous species to dinitrogen, what makes the simultaneous removal of  $\text{HS}^-$  and  $\text{NO}_2^-/\text{NO}_3^-$  possible (Mahmood et al., 2007a). Additionally, due to their low nutritional needs they are preferable solution for biological treatment (Krishnakumar and Manilal, 1999). However, the work provided by Chen et al., (2017) shows that simultaneous removal of  $\text{NO}_3^-$  and  $\text{HS}^-$  relies on different microorganisms such as heterotrophic nitrate-reduction bacteria, sulphate-reduction bacteria, sulphide-oxidation, nitrate-reduction bacteria. Hence, depends on



feed (reactor influent) properties a minor amount of *Thiobacillus denitrificans* can be present in biological process.

The main problems of biological treatment is sensitivity of the bacteria to high  $\text{HS}^-$  load (Mahmood et al., 2007a), temperature changes (Y. Chen et al., 2008; Sposob et al., 2017a), different N/S ratios (Cai et al., 2010; Sposob et al., 2017b) and amount of activated sludge (when  $\text{O}_2$  is the only electron acceptor) (Yavuz et al., 2007).

### 2.1.3 Nitrogen cycle

For biological removal purposes the most important processes of the nitrogen cycle (Fig. 2.1) is nitrification and denitrification processes. The nitrification is following up two steps.  $\text{NH}_4^+$  oxidizes to  $\text{NO}_2^-$  on the first step, continue with  $\text{NO}_2^-$  oxidation to  $\text{NO}_3^-$ . Nitrification provides under aerobic conditions and carried out by unrelated to each other ammonia and nitrite oxidizing bacteria (Beristain-Cardoso et al., 2009). This bacteria (e.g. *Nitrosomonas* and *Nitrobacter* (Gomez et al., 2000)) are belong to *Nitrobacteraceae* family (Prosser, 1990).

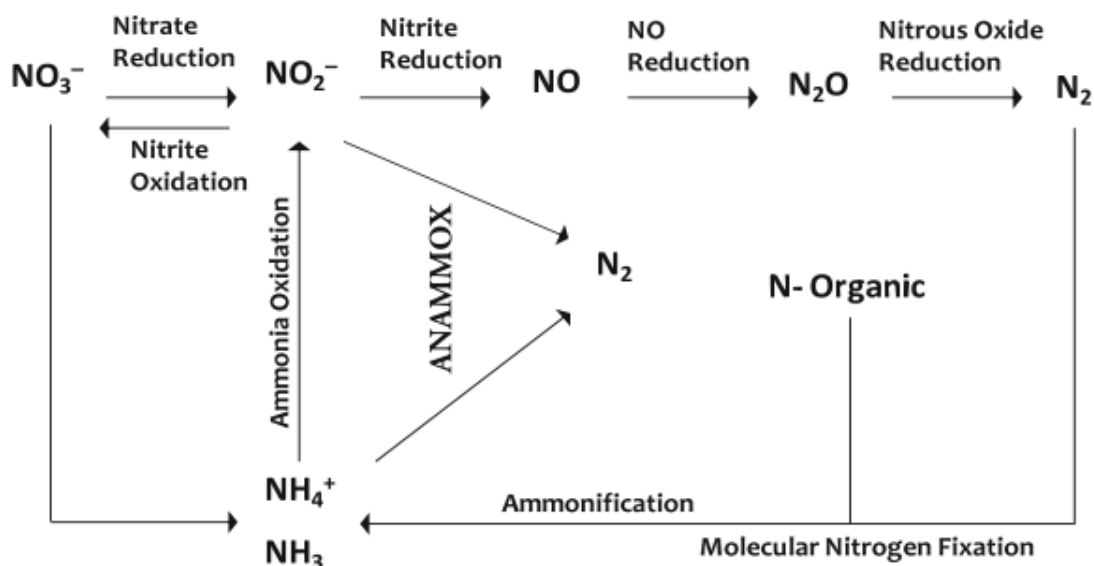


Figure 2.1: Biogeochemical nitrogen cycle (Beristain-Cardoso et al., 2009)

Nitrogen-containing wastewaters are generated in agricultural, food industries and during detergents production (Show et al., 2013). Nitrogen naturally exists in various oxidation states e.g.  $\text{NH}_4^+$ ,  $\text{N}_2$ ,  $\text{N}_2\text{O}$ ,  $\text{NO}_3^-$  and  $\text{NO}_2^-$ .  $\text{NO}_3^-$ , as well as  $\text{HS}^-$ , are poisonous to animals and humans. Presence of  $\text{NO}_3^-$  in blood leads to conversion of hemoglobin to methemoglobin. Blood cells in presence of methemoglobin will not transport  $\text{O}_2$  what can be lethal (Roozeboom et al., 2011). Because of high toxicity of  $\text{NO}_3^-$  it should be removed from drinking water as well (Show et al., 2013). Physicochemical properties of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  are presented in table below (Table 2.1) (WHO, 2011).

Table 2.1: Physicochemical properties of Nitrates (WHO, 2011)

Property	NO <sub>3</sub> <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>
Acid	Conjugate base of strong acid HNO <sub>3</sub> ; pK <sub>a</sub> =-1.3	Conjugate base of weak acid HNO <sub>2</sub> ; pK <sub>a</sub> =3.4
Salts	Very soluble in water	Very soluble in water
Reactivity	Unreactive	Reactive; oxidizes antioxidants, Fe <sup>2+</sup> of hemoglobin to Fe <sup>3+</sup> , and primary amines; nitrosates several amines and amides

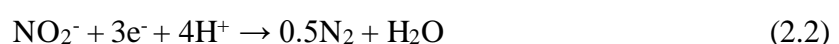
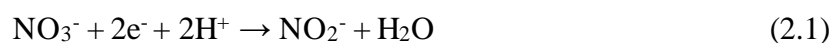
#### 2.1.4 Denitrification

Denitrification process is commonly applied in wastewater treatment. Domestic wastewater typically consists 10-40 mgN/L in a form of organic nitrogen or NH<sub>4</sub><sup>+</sup>. Amount of NO<sub>3</sub><sup>-</sup> in industrial wastewaters is significantly higher than in domestic sector. Presence of NO<sub>3</sub><sup>-</sup> in wastewater streams varies in different industries and the presence of chloride and HS<sup>-</sup> ions makes more complicated to determinate the exact amount of NO<sub>3</sub><sup>-</sup> (Lu et al., 2014). Mineral processing, fertilizers, metal finishing and explosive industries produce large quantities of NO<sub>3</sub><sup>-</sup> as byproduct in concentrations higher than 1 g/L (De Filippis et al., 2013).

Concentration of NO<sub>3</sub><sup>-</sup> in drinking water derived from surface should be less than 10 mg/L. However, for drinking purposes NO<sub>3</sub><sup>-</sup> concentration should be below 0.1 mg/L (WHO, 2011).

Denitrification is anaerobic process and it is the next stage of nitrogen cycle. During denitrification process oxidized nitrogen compounds (NO<sub>2</sub><sup>-</sup> or NO<sub>3</sub><sup>-</sup>) from nitrification stage converts to gaseous N<sub>2</sub> or N<sub>2</sub>O (Beristain-Cardoso et al., 2009). Denitrification process is mainly performed by heterotrophic bacteria (Carlson and Ingraham, 1983) e.g. presence of the *Pseudomonas* bacteria has been reported by Chen et al., (2008). Autotrophic denitrifiers also can be responsible for denitrification. As well as, some types of fungi (Shoun and Tanimoto, 1991).

Biological denitrification process consists a two main stages, and follows chemical reactions below (De Filippis et al., 2013):



During the first stage (Eq. (2.1)) NO<sub>3</sub><sup>-</sup> is reduced to NO<sub>2</sub><sup>-</sup>. Subsequently, NO<sub>2</sub><sup>-</sup> from first reaction is reduced to N<sub>2</sub> on a second stage (Eq. (2.2)) (De Filippis et al., 2013).

Operating conditions are critical for denitrification process, solids retention time (SRT), pH, dissolved O<sub>2</sub> can highly influence the denitrification process. For example, pH level for proper floc formation during denitrification process must range from 6.5 to 8.5. O<sub>2</sub> inhibits denitrification process therefore, presence of O<sub>2</sub> during denitrification is undesirable and should not exceed 0.2-0.5 mg/L. Temperature has been reported as a key factor, which influence overall denitrification efficiency. Range of temperatures between 20 – 30 °C is acceptable range for denitrification, values outside the range slows down the denitrification process (Lu et al., 2014).

In a past few years' greenhouse emission control became a challenge for wastewater denitrification, due to release of a nitrous oxide (N<sub>2</sub>O) during denitrification process, which is 300 times more harmful than carbon dioxide. Data about microbial ecology of denitrifying systems must be used for improving biochemical denitrification models and process design (Lu et al., 2014).

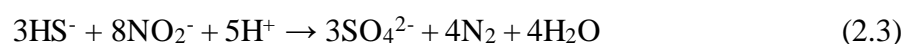
Coexistence of different removal processes is possible in most of wastewater treatment systems. Denitrification process is tightly connected with HS<sup>-</sup> removal process (Krishnakumar and Manilal, 1999). This is due to the fact that NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> can serve as electron acceptors for sulphides (Sposob et al., 2017a).

## 2.2 Simultaneous removal of sulphide and nitrate

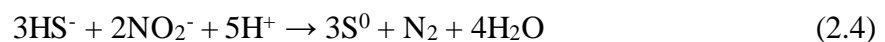
NO<sub>3</sub><sup>-</sup> and HS<sup>-</sup> can be simultaneously removed which simplifies removal process flows and reduces operational costs (Chen et al., 2017). Operating conditions during simultaneous removal process influence overall efficiency. Easy and fast control of such parameters is crucial for high level performance.

### 2.2.1 Nitrogen/ sulphide ratio effect

NO<sub>3</sub><sup>-</sup> / NO<sub>2</sub><sup>-</sup> used as electron acceptors for HS<sup>-</sup> oxidation. Depending on the amount of nitrate, HS<sup>-</sup> will oxidize to S<sup>0</sup> and/or SO<sub>4</sub><sup>2-</sup>. Eq. (2.1) and (2.2) are introducing overall catabolic reaction for different NO<sub>2</sub><sup>-</sup> / HS<sup>-</sup> ratios during HS<sup>-</sup> oxidation (Mahmood et al. 2007).



$$\Delta G^{\theta'} = -2944 \text{ kJ/mol}$$

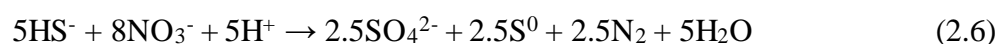


$$\Delta G^{\theta'} = -917 \text{ kJ/mol}$$

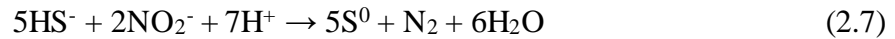
The anoxic sulphide-oxidizing (ASO) process follows reactions below (depending on the NO<sub>3</sub><sup>-</sup> / HS<sup>-</sup> ratios) (Cai et al., 2008):



$$\Delta G^{\theta'} = -3848 \text{ kJ/mol}$$



$$\Delta G^{\theta'} = -2564 \text{ kJ/mol}$$

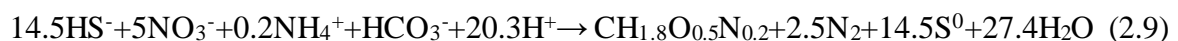


$$\Delta G^\theta = -1264 \text{ kJ/mol}$$

Presented equations have different standard free Gibbs energy values ( $\Delta G^\theta$ ) due to different molar ratios between substrates:  $\text{HS}^-$  and  $\text{NO}_3^-/\text{NO}_2^-$ . For Eq. (2.3) and (2.5)  $\text{SO}_4^{2-}$  is the main reaction product. After changing the N/S ratio in Eqs. (2.4), and (2.7) on lower one the  $\text{S}^0$  become the main product of the reaction, which is more preferable from recourse recovery point of view (Cai et al., 2008). In the absence of  $\text{HS}^-$  granulated  $\text{S}^0$  tends to disappear (Shively, 1974).

To judge the process efficiency, the comparison between influent and effluent concentrations of contaminants was studied. Cai et al., (2008) showed that the best removal efficiency has been obtained at the ratio between  $\text{NO}_3^-$  and  $\text{HS}^-$  equal to 2:5 (Eq. (2.7)). When this ratio has been applied, electrons offered by  $\text{HS}^-$  were in excess compare to electrons accepted by  $\text{NO}_3^-$ . Such case demands extra electron acceptor e.g.  $\text{O}_2$ . The effluent concentration of  $\text{HS}^-$  reached values less than 1 mg/L, where  $\text{NO}_3^-$ -N has been not detected. Under 8:5 N/S ratio, donor electrons were equal to acceptor electrons, and no extra  $\text{O}_2$  acceptor were needed, thus simultaneous anaerobic removal reactions were dominant with 8:5 ratio.

Effect of the different N/S molar ratios in EGSB reactor have been described by Dinamarca, (2014). At the N/S = 1.3 granules in the reactor cracked and lost part of the sedimentation properties. Under N/S = 0.3 no negative effect has been observed in terms of granules properties. Molar ratios 1.3 N/S and 0.3 N/S were applied in Eqs. (2.8) and (2.9) respectively (Kleerebezem and Mendez, 2002).



In the work of Sposob et al., (2017a), applied molar N/S ratios of 0.35 and 1.30 at 10 °C showed almost no difference in  $\text{HS}^-$  removal, with 89% and 87% efficiency, respectively. Removal effectiveness were decreasing at ratios between 0.35 and 1.30. At N/S = 0.6 efficiency has been the lowest 77%. Increasing concentration of the electron acceptor ( $\text{NO}_3^-$ ) led to increase in  $\text{SO}_4^{2-}$  production and decrease of  $\text{S}^0$  fraction.

Different N/S ratios may have an impact on alkalinity, which can affect the process efficiency and stability. For simultaneous removal it became obvious that influent N/S ratio is very important factor and can be used as a controlling factor (Cai et al., 2008).

### 2.2.2 pH effect

pH is an important parameter in biological  $\text{HS}^-$  treatment. Under different pH sulphides occurs in different forms. Sulphide dependence on pH is presented on the Figure 1.2.

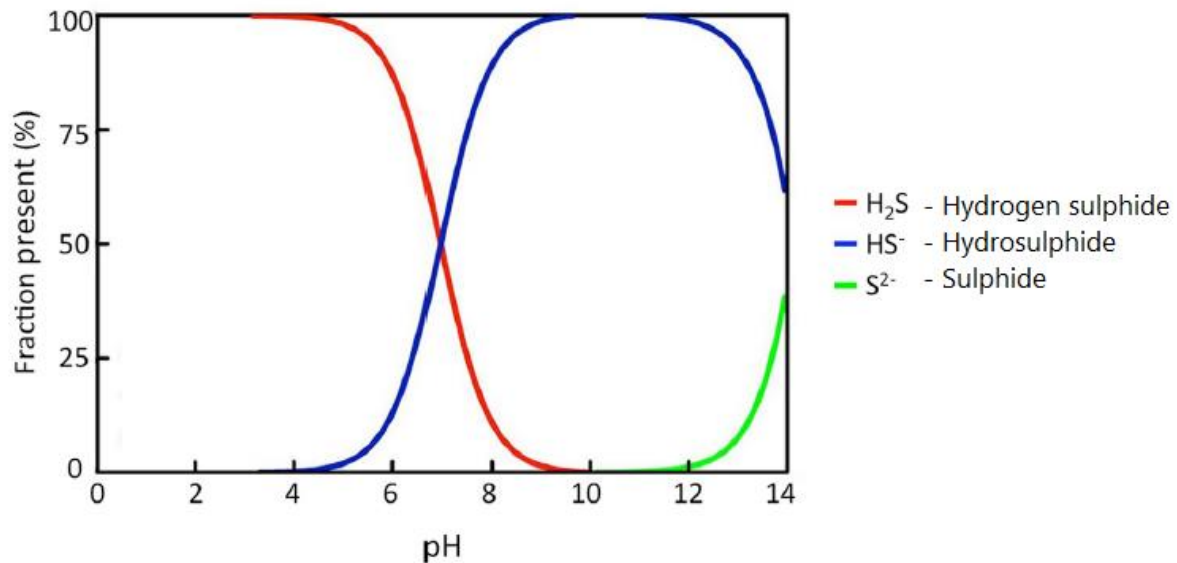


Figure 1.2: Sulphide solubility chart (Holmer and Hasler-Sheetal, 2014)

$\text{H}_2\text{S}$  is partially soluble gas which can be present in wastewater along with  $\text{HS}^-$  and  $\text{S}^{2-}$  ions in equilibrium.  $\text{H}_2\text{S}$  gas has unpleasant rotten egg odor and can also cause corrosion. Around 50% of  $\text{H}_2\text{S}$  gas can be released to the atmosphere at pH 7. On the other hand, at pH 9  $\text{H}_2\text{S}$  presence less is lower than 1%.

During denitrification process every  $\text{NO}_2^-$  mole reduced to  $\text{N}_2$  gas, consuming 0.6 acid equivalents which are turned into alkalinity during anoxic sulphide oxidation (ASO) (Mahmood et al., 2007b). Thus, pH level in the system increases and may have an impact on the overall process efficiency. Literature reports different values of optimal pH, however, the variations are slight. In most cases appropriate pH appears to be in a range from 7 to 8 (Mahmood et al., 2007b) or 6.5-8.5 (Lu et al., 2014). The pH outside these ranges can cause problems in the system. If pH level is higher than 8.0 the  $\text{NO}_2^-$  will tend to accumulate in alkaline environment, what is undesirable.

Mahmood et al. (2007b) studied influence of pH on the ASO reactor performance. Different loadings tests, showed that the effluent pH was usually around 7-9.5. That pH level seems to be related to the activity of denitrifying bacteria. At  $\text{pH} > 8.0$  removal efficiencies of the both  $\text{HS}^-$  and  $\text{NO}_2^-$  decreased. During hydraulic retention time (HRT) tests wastewater with higher  $\text{NO}_3^-$  and  $\text{HS}^-$  concentrations was used and pH around 7-7.5 was applied. Reactor operated at steady state and outlet pH value raised above 9, however, the removal efficiency remained stable (96%). It appeared that bacterial communities in reactor were more sensitive to low (acidic) pH.

In study under different influent pH it has been showed that after decreasing pH below 4 the  $\text{NO}_3^-$  and  $\text{HS}^-$  removal efficiency decreased significantly. The same situation happened when pH has been increased from 8 to 11, however then the  $\text{NO}_3^-$  removal efficiency decreased slowly and gradually. The maximum removal efficiency was achieved at pH level around 8.0, however, it is possible to operate ASO reactor in pH range 5-11 (Mahmood et al., 2007b).

HS<sup>-</sup> removal rate is also a function of pH. At pH level above recommended the HS<sup>-</sup> removal rate will decrease (Yavuz et al., 2007).

In Cai et al. (2010) study HS<sup>-</sup> and NO<sub>3</sub><sup>-</sup> removal efficiency were investigated under very high ('shock') pH and high loads of substrates. Study showed that at high pH and/or substrates load the reactor performance is influenced. Substrates high loads are not affecting microbiological activity in reactor directly, what means that the 'shock' loads less harmful in comparison to pH. However, continuous overloading increase pH level. Based on the experimental results, it is possible to recover to previous substrate concentrations (520 mg/L and 91mg/L of HS<sup>-</sup> and NO<sub>3</sub><sup>-</sup> respectively), from high substrates load (2-3.5 times higher than initial) and high pH load (8-10) in about 30 hours.

### 2.2.3 HRT effect

Depending on the reactor design (e.g. UASB, EGSB or CSTR) HRT may or may not has an impact on HS<sup>-</sup> removal efficiency during anoxic nitrate biooxidation. Reactors as UASB and EGSB have a very high solids retention time (SRT) as a result there is a great biomass concentration, therefore HRT has a little impact on HS<sup>-</sup> removal efficiency. Based on Mahmood et al. (2007) study where HRT in UASB reactor has been changed from 1.5 to 0.10 days any changes in terms of HS<sup>-</sup> removal percentage were observed. Removal efficiency was stable and >99%. Nevertheless, removal efficiency dropped down to 96% when HRT has been decreased to 0.08 day. The same phenomena occurred for effluent NO<sub>3</sub><sup>-</sup>, its removal efficiency remained at 80% when HRT has been decreased to 0.10 day, further decreasing to 0.08 day had negative impact on NO<sub>3</sub><sup>-</sup> removal performance (55%). Therefore, it is possible to conclude that HRT has an impact on the reactor (USAB) performance only when its value is very low.

### 2.2.4 Temperature effect

The temperature effect at HS<sup>-</sup> removal process is rarely studied. In most cases, autotrophic denitrification process operates at temperature range between 20-30 °C (Di Capua et al., 2015). Generally, increasing temperature positively affects the bacterial growth rate (Chen et al., 2008). Decreasing temperatures lead to decrease in process efficiency. It was investigated that during simultaneous S<sup>0</sup> and NO<sub>3</sub><sup>-</sup> removal when temperature was 5-10 °C, efficiency dropped more than half (Di Capua et al., 2017). In another study, in EGSB reactor, at the low temperatures (15-10 °C) increase of S<sub>2</sub>O<sub>3</sub><sup>2-</sup> and SO<sub>4</sub><sup>2-</sup> concentrations was observed. Additionally, removal of previously incorporated S<sup>0</sup> in the reactor was detected (Sposob et al., 2016). Low temperatures can cause metabolic changes in BSR process. Sposob et al., (2017a) observed that temperatures (<25 °C) impacts simultaneous biological removal of NO<sub>3</sub><sup>-</sup> and HS<sup>-</sup>. Decreasing temperatures reflects in metabolic shift from S<sup>0</sup> to SO<sub>4</sub><sup>2-</sup> production. As a result of high SO<sub>4</sub><sup>2-</sup> production also increased biomass yield production. Biological removal processes operation at low temperatures (<20 °C) decrease operational and capital costs (Di Capua et al., 2017). This problem is typical for countries with cold climate (Sposob et al., 2017a). However, denitrifying bacteria can be active even at 1 °C (Zou et al., 2016) and complete denitrification under S<sub>2</sub>O<sub>3</sub><sup>2-</sup> presence was observed at 3 °C (Di Capua et al., 2017).

## 2.3 EGSB reactor

Biological  $\text{HS}^-$  removal using electron acceptor ( $\text{O}_2$  or  $\text{NO}_3^-/\text{NO}_2^-$ ) is applicable for different types of reactors e.g. continuous stirred tank reactors (CSTR), batch reactors, biofilters and bioscrubbers (Dinamarca, 2014). One more way for biological  $\text{HS}^-$  removal is  $\text{HS}^-$  autotrophic denitrification using anaerobic expanded granular sludge bed (EGSB) reactor. EGSB reactor operates at high upflow velocity without losing biomass (Chen et al., 2009). This reactor's advantage prolongs the SRT. In EGSB reactor under appropriate N/S ratio  $\text{S}^0$  can be incorporated in granules which can be retained in the reactor (Sposob et al., 2016). Accumulated  $\text{S}^0$  on the walls and bottom of the reactor can be removed easily or be wash out with the effluent (Dinamarca, 2014).

In granular sludge reactors for better performance used both - autotrophic and heterotrophic denitrification approaches. With a limited  $\text{NO}_3^-$  supply autotrophic and heterotrophic microorganisms competing between each other for available nitrates in reactor and both of them will remove  $\text{HS}^-$  and  $\text{NO}_3^-$  (Chen et al., 2009; Y. Chen et al., 2008).

USN in cooperation with YARA ASA international has developed a technology for sulphides removal from wastewaters using the EGSB reactor. Reactor design described in a Chapter 3.3 of the report.

## 3 Materials and methods

### 3.1 Inoculum

The inoculum was taken from an UASB methane producing reactor treating pulp and paper industry at Norske Skog Saugbrugs, Halden, Norway. Sludge with a volume of 0.25 L and solid content of 59.9 g/L and 86% of organic fraction was added to the reactor. Sludge color was characterized as completely black. EGSB reactor was fed continuously with the same influent composition for one month at 25 °C, in order to acclimatization period of the bacteria. No methane production and no sulphur presence at the effluent was observed due to lithoautotrophic conditions (Sposob et al., 2017a).

### 3.2 Synthetic wastewater

Synthetic wastewater was prepared according to research provided by Sposob et al. (2017). Synthetic feed contained  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  (100 mg S/L) with  $\text{NaHCO}_3$  at concentration according to Eq. (2.9). High concentrated nitric acid ( $\text{HNO}_3$ ) was used as a nitrate (electron acceptor) source at concentration according to Eq. (2.9). Potassium phosphate was used as a buffer. Nitrate feed contained next stock chemical solutions (A)  $\text{NH}_4\text{Cl}$  (10 g/L),  $\text{MgCl}_2\cdot 6\text{H}_2\text{O}$  (10 g/L),  $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$  (10 g/L); (B)  $\text{K}_2\text{HPO}_4$  (300 g/L); (C)  $\text{MnSO}_4\cdot \text{H}_2\text{O}$  (0.04 g/L),  $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$  (2.7 g/L),  $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$  (0.055 g/L),  $\text{NiCl}_2\cdot 6\text{H}_2\text{O}$  (0.1 g/L),  $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$  (0.088 g/L),  $\text{CoCl}_2\cdot 6\text{H}_2\text{O}$  (0.05 g/L),  $\text{H}_3\text{BO}_3$  (0.05 g/L); (D) biotin (20 mg/L), folic acid (20 mg/L), pyridoxine hydrochloride (100 mg/L), riboflavin (50 mg/L), thiamine (50 mg/L), nicotinic acid (50 mg/L), pantothenic acid (50 mg/L), vitamin  $\text{B}_{12}$  (1 mg/L), *p*-aminobenzoic acid (50 mg/L), thioctic acid (50 mg/L) (Wolin et al., 1963), 10 times concentrated.  $\text{HNO}_3$  and stock solutions A (10 ml/L), B (2 ml/L), C (2 ml/L) and D (1 ml/L) were dissolved in distilled water.  $\text{HNO}_3$  and  $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$  were fed from different bottles to prevent feed pollution and avoid chemical reaction in the feed bottles.

### 3.3 Experimental setup

Experimental setup was developed in USN (Figure 3.1). EGSB reactor has been made from polycarbonate tube an inner diameter 32 mm and height of 620 mm, which gives a working volume of 0.5 L. For maintaining temperature in recirculation loop of the reactor was used cold plate cooler (TE Technology, Inc.) Different temperatures from 25 to 35 °C were tested during the experiment. Temperature change was provided step-by-step and temperatures changed when effluent composition results become stable. Peristaltic pump used for pumping synthetic influent and recycling pump used to mix influent with sludge and expand the sludge bed. Also set-up equipped with ORP/pH sensors, data from sensors can be read from two pH/ORP Meters HI98183 (Hanna Instruments). Reactor pH was maintained at range 8.0-9.0.



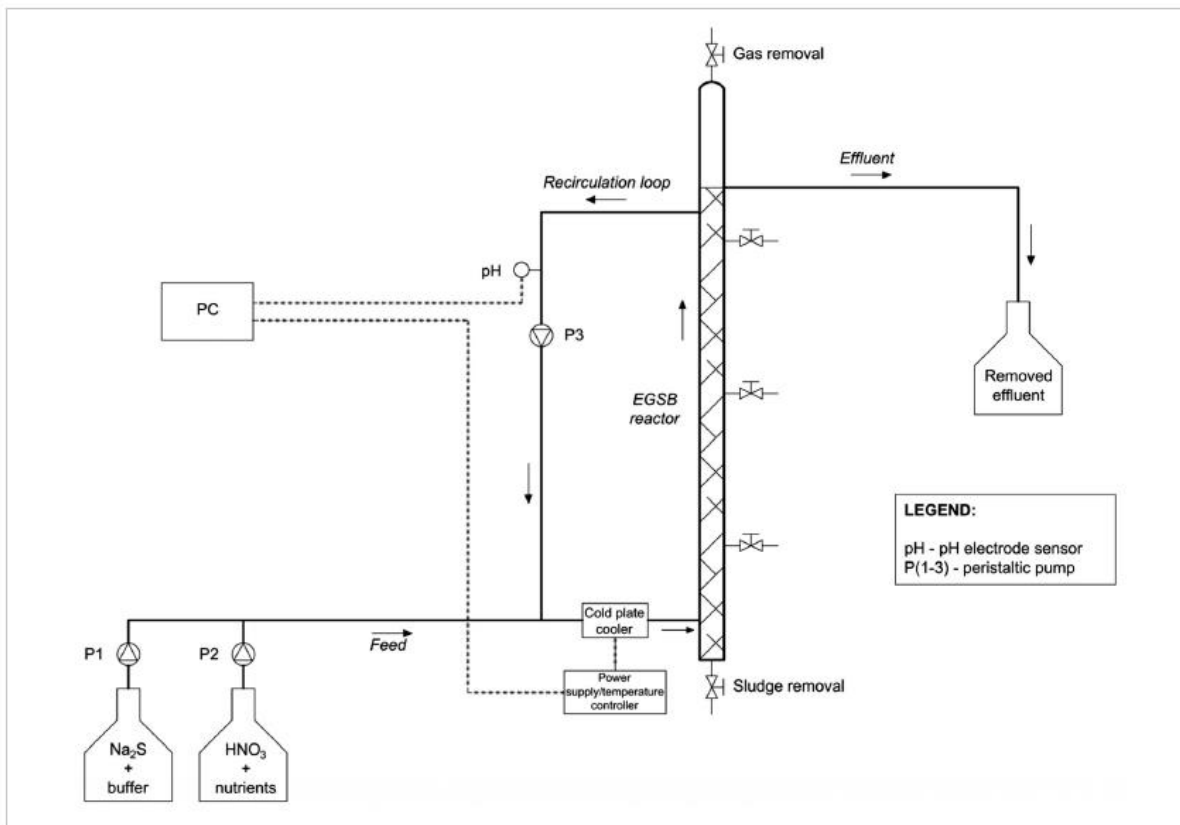


Figure 3.1: Experimental set-up (Sposob et al., 2017a)

### 3.4 Analytical procedure

Effluent wastewater samples were collected at least 3 times per week. Samples were analyzed on the same day when they were taken.  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{HS}^-$  and  $\text{S}_2\text{O}_3^{2-}$  in collected liquid samples (following  $0.45\mu\text{m}$  filtration) were measured by ion chromatography (Dionex ICS-5000). Procedure for determination of  $\text{HS}^-$  concentration developed by Sposob et al. (2017b). Concentration was determined indirectly by addition of permanganate oxide ( $\text{KMnO}_4$ ) to the filtered sample. Sample elution has been performed using an IonPac AS11-HC2 mm analytical column with potassium hydroxide (KOH) as an eluent.

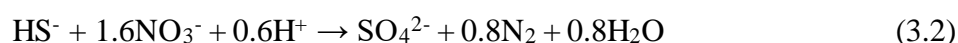
### 3.5 Data evaluation

Obtained experimental data was evaluated using Microsoft Excel (2016) software. Concentrations of  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{HS}^-$  and  $\text{S}_2\text{O}_3^{2-}$  ( $\text{S}^0_{\text{ss}}$ ) were collected in Excel for processing. The plots showing concentration according to day when sample has been analyzed were generated. Additionally, plots include temperature scale which reflects temperature changes during the experiment.

Excel functions 'AVERAGE' and 'DSTDEV' were used to calculate average and standard deviations, respectively. Plots which show a relation between average concentrations and different temperatures/ratios were generated.

### 3.6 Gibbs energy calculations

Denitrification process in the experiment followed the chemical reactions below (Eq. (3.1), Eq. (3.2)):



To calculate and use standard Gibbs energy the information about free energies of formation for each individual component of the reaction is required (Rittmann and McCarty, 2001). Energy of formation for the substrates and products used in Eq. (3.1) and Eq. (3.2) are listed below.

Table 3.1: Free energies of formation at 25 °C (Rittmann and McCarty, 2001)

Class	Substance	Form	kJ/mol
Bisulphide	HS <sup>-</sup>	Aq.	12.05
Nitrate	NO <sub>3</sub> <sup>-</sup>	Aq.	-111.34
Hydrogen Ion	H <sup>+</sup>	Aq.	0
Sulphur	S <sup>0</sup>	C.	0
Thiosulphite	S <sub>2</sub> O <sub>3</sub> <sup>2-</sup>	Aq.	-513.40
Nitrogen	N <sub>2</sub>	C.	0
Water	H <sub>2</sub> O	L.	-237.18

The standard free Gibbs energy is calculated as a sum of the free energies of the reaction products minus the sum of the free energies of the reactants (Rittmann and McCarty, 2001).

$$\Delta G^{\theta'} = \sum \Delta G^{\theta'}_{\text{products}} - \sum \Delta G^{\theta'}_{\text{reactants}} \quad (3.3)$$

For Eq. (10) free Gibbs energy:

$$\Delta G^{\theta'} = (0.2 \cdot 0 + (-237.18) \cdot 1.2) - (12.05 + (-111.34 \cdot 0.4)) = -252.13 \text{ kJ/mol} \quad (3.4)$$

For Eq. (11) free Gibbs energy:

$$\Delta G^{\theta'} = (-744.63 + 0.8 \cdot 0 + (-237.18 \cdot 0.8)) - (12.05 + (-111.34 \cdot 1.6)) = -768.28 \text{ kJ/mol} \quad (3.5)$$

### 3.7 Thiosulphate measurements

During sample analysis,  $S_2O_3^{2-}$  and  $SO_4^{2-}$  were detected in the effluent. These  $HS^-$  oxidation products are the main constituents of the effluent sulphur concentration. Oxidation of  $HS^-$  to  $S_2O_3^{2-}$  not supposed to occur under applied  $NO_3^-/HS^-$  ratio (0.35 and lower). However, according to the applied analytical procedure (Sposob et al., 2016)  $S_2O_3^{2-}$  was detected during the sample analysis. Following this procedure  $S^0$  in the liquid phase possibly oxidized by alkaline eluent to  $S_2O_3^{2-}$ . Therefore, it is possible to say that the measured effluent  $S_2O_3^{2-}$  is actually  $S^0$  denoted as  $S_{ss}^0$ .

### 3.8 Elemental sulphur balance

The amount of incorporated sulphur in the reactor was calculated using indirect method based on an overall sulphur mass balance. Indirect method was used due to difficulties of applying direct  $S_{acc}^0$  quantification method.

$S^0$  in the reactor can be presented in two forms – incorporated into granules (bacterial inclusion bodies) (Shively, 1974) and the liquid phase (Chen et al., 2008; Krishnakumar and Manilal, 1999).

Concentration of the  $S_{acc}^0$  is possible to calculate as a difference between concentrations of the  $HS^-_{inf}$ ,  $HS^-_{eff}$ ,  $SO_4^{2-}_{eff}$  and  $S_{ss}^0$  (Eq. 3.6).  $H_2S$  gas not included in the balance due to alkaline pH in the reactor.

$$S_{acc}^0 = HS^-_{inf} - HS^-_{eff} - SO_4^{2-}_{eff} - S_{ss}^0 \quad (3.6)$$

### 3.9 Experiment scenario and process parameters

Reactor performance under different temperatures and different ratios was recorded over 70 days where 51 data points were obtained. Main part of the experiment (temperature impact) lasted 49 days with 36 data points. Additionally, experiment was prolonged for 21 days to investigate the effect of the under-stoichiometric ratios (Chapter 4.5). 15 data points was obtained in this part of the experiment. The experiment scenario is included in Table 3.2.

The temperature related experiment was conducted under  $NO_3^-/HS^- = 0.35$  ratio and at constant sulphur load = 0.40 kgS/m<sup>3</sup>d. General information about process parameters are summarized in a Table 3.3.

### 3.10 Microscopy analysis

Reactor sludge was collected after finishing the experiment (day 70). To investigate sludge surface microscope analysis was performed for separate sludge granules, sludge sample dried at room temperature and sludge sample dried at 550 °C oven (30 minutes). All samples were analyzed by Nikon SMZ745 zoom stereomicroscope.

Table 3.2: Experiment scenario

	Days	Temperature °C	N/S ratio
Temperature experiment (49 days)	1-6	25	0.35
	7-18	27	
	19-28	29	
	29-43	32	
	44-49	35	
N/S ratio experiment (21 days)	50-56	35	0.32
	57-64	35	0.275
	65-70	35	0.245

Table 3.3: Overall process parameters

N/S molar ratio	0.35-0.245
Temperatures °C	25-35
HRT (h)	6
Influent pH (average)	8-9
Vertical velocity (m/h)	6
Load of S <sup>2-</sup> (kgS/m <sup>3</sup> d)	0.40
S <sup>2-</sup> influent (mg/L)	100
NO <sub>3</sub> <sup>-</sup> influent (mg/L)	66-47

## 4 Results and discussion

### 4.1 Reactor performance

The average effluent pH was  $8.6 \pm 0.3$ . Variations of pH (7.6-9.0) were observed during the whole experimental period. It was noticed that with increasing temperature, pH tended to decrease (Fig.4.1). Highest pH (9.0) was detected at 27 °C. The average pH = 8.6, at this temperature, was the highest compare to the other studied temperatures. The lowest pH was observed at the last day of 25 °C period.

During biological treatment  $\text{HS}^-$  can be removed with high efficiency of 90 to 100% (Syed et al., 2006). High removal efficiency of the  $\text{NO}_3^-$  and  $\text{HS}^-$  was achieved during the main experiment at all tested temperatures. The electron acceptor was not detected in the effluent, except two occasions at 27 °C. The average removal of electron donor ( $\text{HS}^-$ ) at different temperatures was at 92% level. Measured concentration of  $\text{HS}^-$  according at different temperatures is presented at Fig. 4.2. The highest (95%) and the most stable  $\text{HS}^-$  removal was achieved at 27 °C. Mahmood et al. (2007b) suggests that bacterial species in the reactor are less sensitive to alkaline pH what can explain high  $\text{HS}^-$  removal efficiency. Although, removal efficiency at high temperatures (29-35 °C) was high (average of 92%), concentration of the effluent  $\text{HS}^-$  varied during this period.

The lowest  $\text{HS}^-$  removal efficiency (91%) was observed at 25 °C. The highest  $\text{HS}^-$  concentrations 17.4 and 18.0 mgS/L were detected at 25 and 32 °C, respectively. Process outcome is summarized in a Table 4.2. Sum of all sulphur components in the effluent presented as a total effluent sulphur.

At 25 °C, collected effluent samples were colorless. After temperature increase to 27-29 °C samples were characterized by 'light' yellow color appearance. Colored effluent can be explained by the presence of  $\text{S}^0_{\text{ss}}$  in the liquid phase (Chen et al., 2008; Krishnakumar and Manilal, 1999).

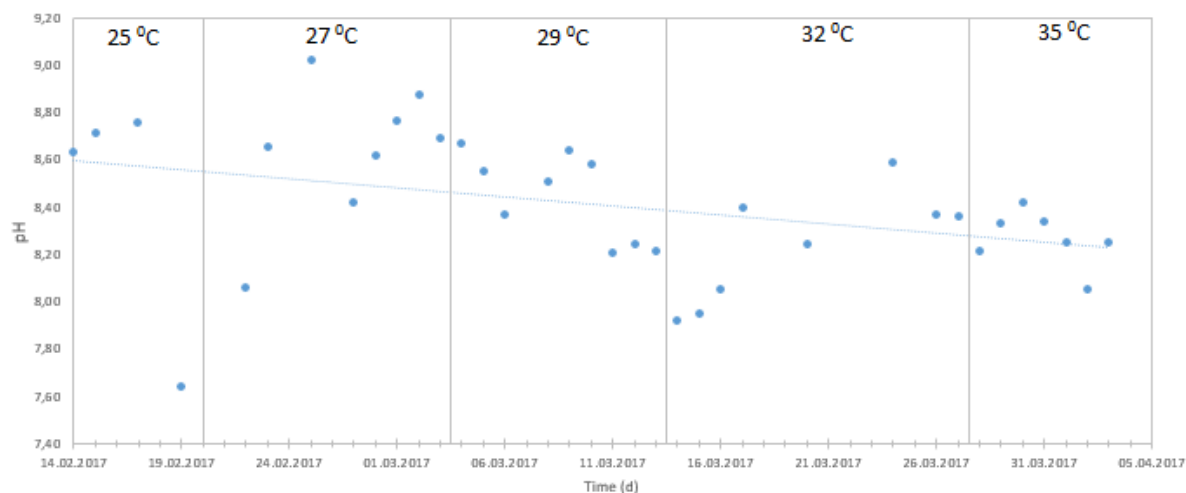


Figure 4.1: pH profile according to date and temperature (25 - 35 °C)

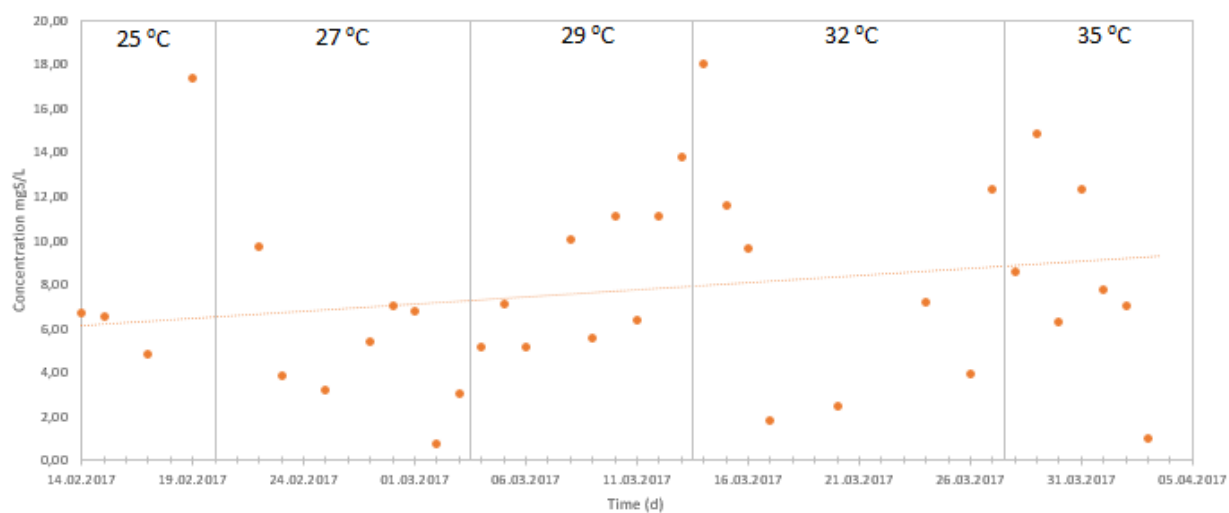


Figure 4.2: HS<sup>-</sup> profile according to date and temperature (25 - 35 °C)

Table 4.2: Process outcomes at different temperatures

Temperature °C	Solutions (mgS/L; mgN/L)		Products (mgS/L)	
	NO <sub>3</sub> <sup>-</sup> (% removal efficiency)	HS <sup>-</sup> (% removal efficiency)	Total effluent sulphur (% sulphur suspended in the effluent)	Accumulated sulphur (% of sulphur accumulated in the reactor, S <sup>0</sup> <sub>acc</sub> )
25	n.d. (>99%)	8.87±5.73 (91%)	48.71±6.10 (49%)	51.29±6.10 (51%)
27	0.66±1.31 (99%)	4.99±2.85 (95%)	56.03±9.08 (56%)	43.97±9.08 (44%)
29	n.d. (>99%)	8.38±3.18 (92%)	59.67±6.55 (60%)	40.33±6.55 (40%)
32	n.d. (>99%)	8.38±5.59 (92%)	56.33±12.27 (56%)	43.67±12.27 (44%)
35	n.d. (>99%)	8.27±4.45 (92%)	50.88±5.77 (51%)	49.12±5.77 (49%)

## 4.2 SO<sub>4</sub><sup>2-</sup>, S<sup>0</sup><sub>ss</sub>, NO<sub>3</sub><sup>-</sup> formation

Effluent concentration of S<sup>0</sup><sub>ss</sub> was predominantly higher than SO<sub>4</sub><sup>2-</sup> during all temperature experiment, except 35 °C temperature period, where average concentration of SO<sub>4</sub><sup>2-</sup> (23.60±4.85 mgS/L) was slightly higher than S<sup>0</sup><sub>ss</sub> (22.98±6.38 mgS/L). In presented experiment production of SO<sub>4</sub><sup>2-</sup> was increasing with temperature increase (Table 4.3). For comparison under the same experimental conditions Spodob et al., (2017a) reported that share of SO<sub>4</sub><sup>2-</sup> in the effluent of the EGSB reactor also was increase but with temperature decrease. Such results can be explained by bacteria communities adaptation to increasing temperatures which reflects in shifting to SO<sub>4</sub><sup>2-</sup> production energy pathway (Eq. 2.8).

S<sup>0</sup><sub>ss</sub> production varied through-out the experiment. Rapid increase in S<sup>0</sup><sub>ss</sub> effluent concentration has been observed at 27 °C where S<sup>0</sup><sub>ss</sub> production reached it highest average value of 40.03±7.59 mgS/L in comparison to other temperature periods. Such changes may be related to high average pH (8.64±0.29) obtained at this temperature. It was reported that sulphide oxidation process under alkaline pH leads to S<sub>2</sub>O<sub>3</sub><sup>2-</sup> (S<sup>0</sup><sub>ss</sub>) accumulation (Krishnakumar et al., 2005; Steudel, 1996). Further temperature increase characterized stable S<sup>0</sup><sub>ss</sub> decrease where achieved data not varied significantly.

Complete NO<sub>3</sub><sup>-</sup> removal was observed during whole experiment, except days 9-10 (Figure 4.3). Average concentration of detected NO<sub>3</sub><sup>-</sup> was 0.66±1.31 mgN/L. Presence of NO<sub>3</sub><sup>-</sup> in the effluent says about incomplete use of the available electron acceptor at this period. While efficiency of denitrification at 27 °C was slightly lower than 100%, HS<sup>-</sup> removal reached 95%, which is the best result among all different temperatures. Figure 4.3 shows NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, S<sup>0</sup><sub>ss</sub> and total effluent sulphur concentrations at the effluent

Table 4.3: Side effluent products at different temperatures

Temperatures °C	SO <sub>4</sub> <sup>2-</sup> mgS/L	S <sup>0</sup> <sub>ss</sub> mgS/L	NO <sub>3</sub> <sup>-</sup> mgN/L
25	15.05±1.83	28.33±2.68	n.d.
27	15.27±6.58	40.03±7.59	0.66±1.31
29	18.75±2.52	35.12±2.84	n.d.
32	23.05±3.10	27.95±8.11	n.d.
35	23.60±4.85	22.98±6.38	n.d.

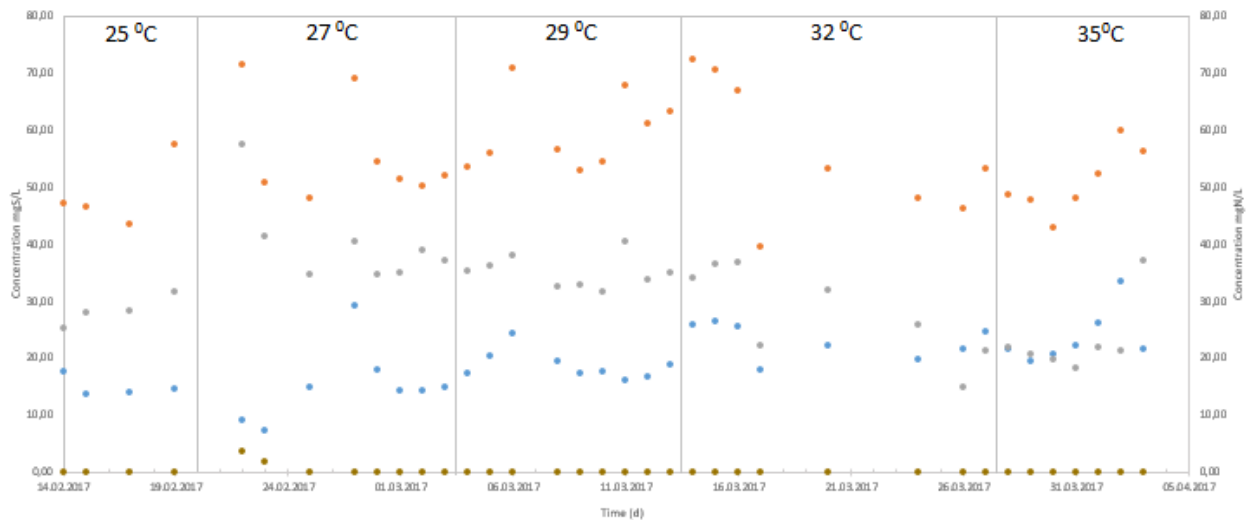


Figure 4.3: Substrate ( $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{S}^0_{\text{ss}}$ , Total effluent Sulphur) concentration with time under different temperatures

### 4.3 $\text{S}^0_{\text{ss}}$ and $\text{S}^0_{\text{acc}}$

Sulphide can be oxidized to  $\text{SO}_4^{2-}$  or  $\text{S}^0$  depending on electron acceptor availability (Kleerebezem and Mendez, 2002; Mahmood et al., 2007a). In the experiment, under 0.35 N/S molar ratio, the reaction should follow  $\text{S}^0$  production energy pathway (Eq. 2.9).

Amount of total effluent sulphur varied slightly at different temperatures through-out the experiment (Table 4.2). Highest presence of total effluent sulphur was detected at 27, 29, 32 °C. Peak of sulphur presence in the effluent ( $59.67 \pm 6.55$  mgS/L) was at 29 °C. To exclude the possibility of secondary pollution from total effluent sulphur different recovering approaches possible to apply (e.g. sedimentation, slow rate filtration process).

The rest of the S which was not discharged with the effluent, was retained inside the reactor. In EGSB reactor  $\text{S}^0$  accumulates ( $\text{S}^0_{\text{acc}}$ ) inside sulphur oxidizing bacteria which attached to the sludge granule surface. This is an advantage compare to different biofilm reactors, where accumulated  $\text{S}^0$  need to be periodically removed to avoid reactor failure as in biotrickling filters (Fortuny et al., 2008).

In literature different amount of  $\text{S}^0$  yield (50-88%) has been reported (Beristain-Cardoso et al., 2009; Krishnakumar and Manilal, 1999; Li et al., 2009). In present experiment amount of accumulated  $\text{S}^0$  varied through-out the experiment (Fig.4.5). During temperature changes at the beginning of the experiment (25-29 °C)  $\text{S}^0$  yield tended to decrease and at 29 °C reached lowest value of  $40.33 \pm 3.18$  mgS/L. After continue of increasing temperatures  $\text{S}^0$  recovered almost to initial amount ( $49.12 \pm 5.77$  mgS/L (49% of total)). Such behavior possibly related to adaptation of the bacteria to the higher temperature regime.

Overall from 40 to 51% of produced  $\text{S}^0$  was accumulated inside the reactor.



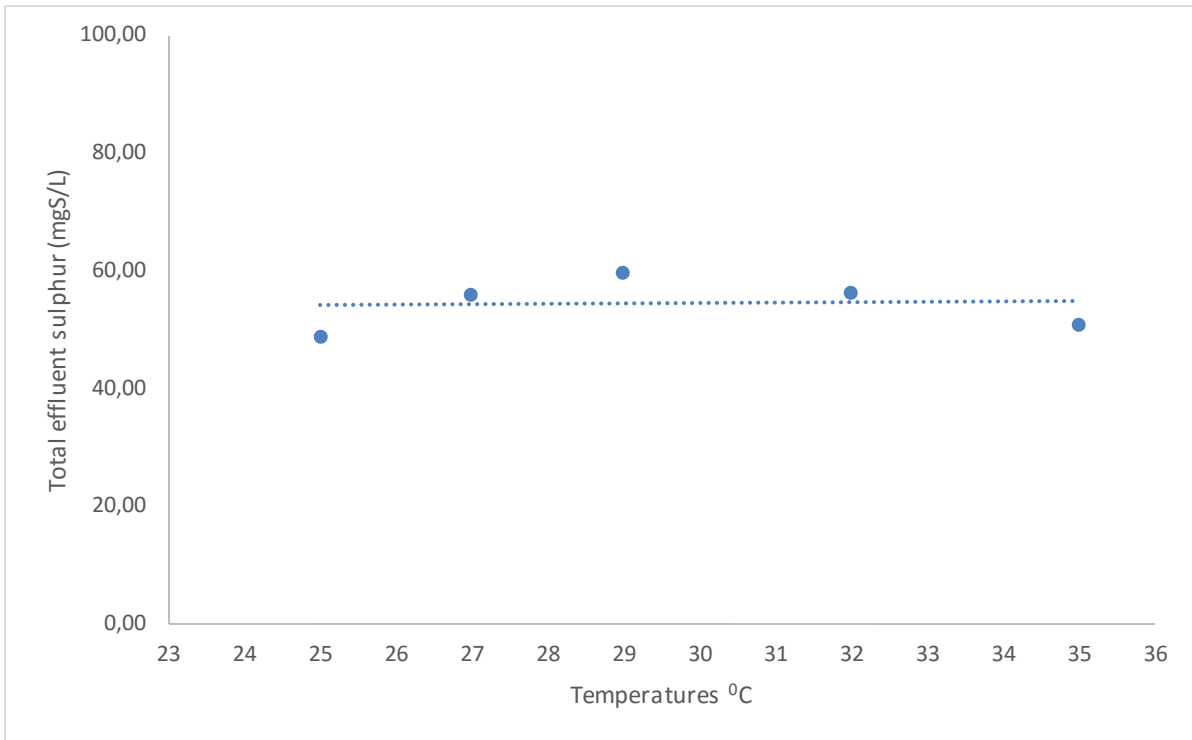


Figure 4.4: Average  $S^0_{ss}$  in the effluent at different temperatures

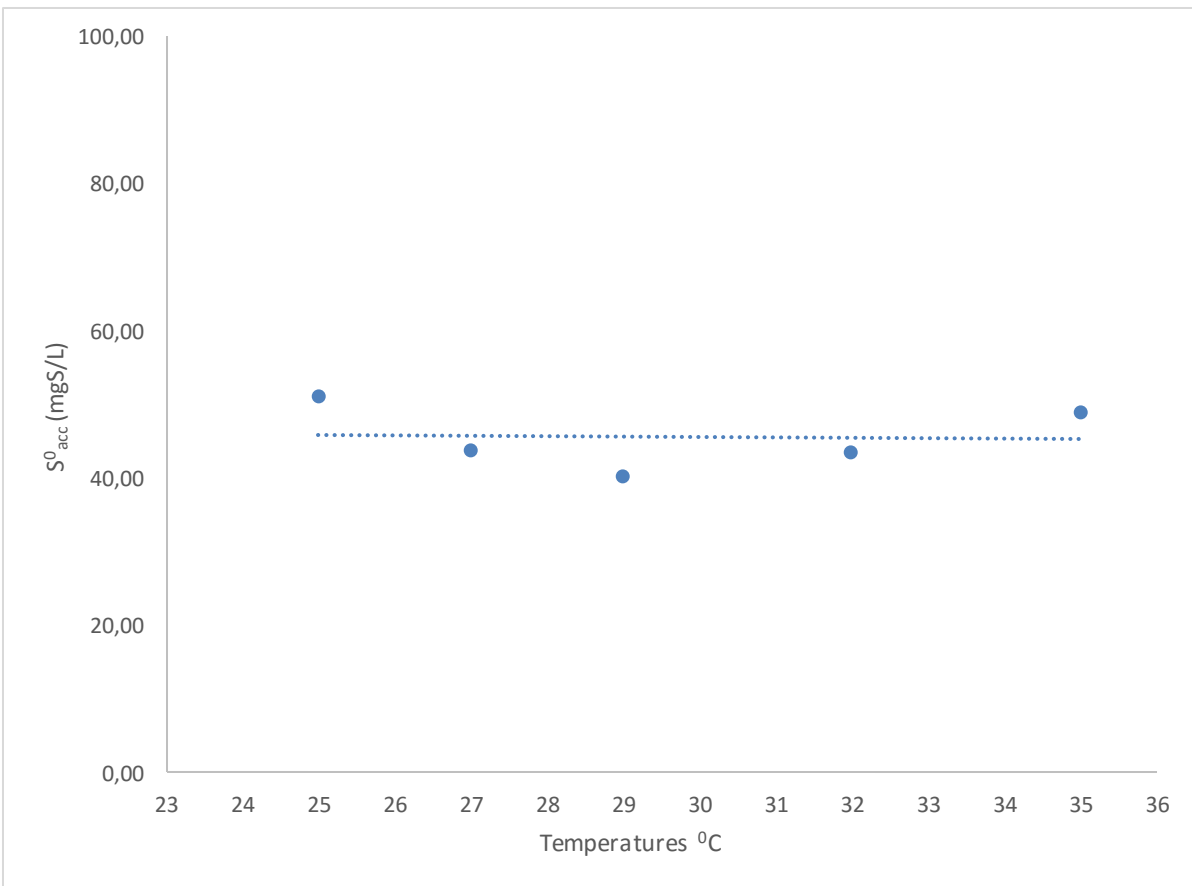


Figure 4.5: Average  $S^0_{acc}$  inside the EGSB reactor at different temperatures

## 4.4 Mass balance

Amount of sulphur components observed in the effluent summarized as average values for each temperature period in Table 4.4. As long as achieved experimental data were used for calculations in the process reactions all results were converted into mM/L dimensions.

Theoretically according to energy pathway in Eq. (2.9) influent  $\text{HS}^-$  (3.12 mM/L) supposed to fully convert into the same amount of  $\text{S}^0$ . According to experimental results  $\text{HS}^-$  was detected in the effluent. It means that not all influent  $\text{HS}^-$  reacted, so it is possible to exclude it from mass balance (Eq.3.6). Real amount of  $\text{HS}^-$  that participated in the reaction is a sum of  $\text{S}^0_{\text{ss}}$  and  $\text{S}^0_{\text{acc}}$ . Except  $\text{S}^0_{\text{ss}}$  and  $\text{S}^0_{\text{acc}}$  in the effluent always was present  $\text{SO}_4^{2-}$ .

Experimental results showed that production of the  $\text{SO}_4^{2-}$  is higher than amount of  $\text{NO}_3^-$  which could cover this oxidation (Eq.2.8). Part of  $\text{HS}^-$  still has been oxidized (to  $\text{SO}_4^{2-}$ ) by only one present electron acceptor ( $\text{NO}_3^-$ ) (Table 4.4). The rest oxidation can be explained by biological or/and abiotic factors. Further oxidation of  $\text{S}^0_{\text{acc}}$  is possibly a reason of  $\text{SO}_4^{2-}$  production (Li et al., 2009). On the other hand, contact with extra electron acceptor (i.e. atmospheric  $\text{O}_2$ ) during sampling procedure may be the reason of  $\text{SO}_4^{2-}$  presence in the effluent (Cai et al., 2008). Amount of  $\text{HS}^-$  not oxidized by  $\text{NO}_3^-$  was calculated as a difference between effluent  $\text{SO}_4^{2-}$  and amount of  $\text{SO}_4^{2-}$  produced by  $\text{NO}_3^-$  (Table 4.4).

Table 4.4: Reactor effluent composition

Temperature °C	Reactants (mM/L)		Products (mM/L)				
	$\text{NO}_3^-$	$\text{HS}^-$	Elemental sulphur		$\text{SO}_4^{2-}$	$\text{SO}_4^{2-}$ produced by $\text{NO}_3^-$	$\text{SO}_4^{2-}$ produced not by $\text{NO}_3^-$
			$\text{S}^0_{\text{ss}}$	$\text{S}^0_{\text{acc}}$			
25	n.d.	0.28±0.18	0.89±0.04	1.60±0.19	0.47±0.06	0.17	0.30
27	0.05±0.09	0.16±0.09	1.25±0.12	1.37±0.28	0.48±0.21	0.14	0.34
29	n.d.	0.26±0.10	1.10±0.04	1.26±0.20	0.59±0.08	0.21	0.38
32	n.d.	0.26±0.17	0.87±0.13	1.36±0.38	0.72±0.10	0.24	0.48
35	n.d.	0.26±0.14	0.72±0.10	1.54±0.18	0.74±0.15	0.23	0.51

Abiotic factors possible to describe as accidental. But stable increase of the results considering partly  $\text{HS}^-$  oxidation to  $\text{SO}_4^{2-}$  by extra electron acceptor more possible tells about biological nature of such changes. Amount of  $\text{SO}_4^{2-}$  was increasing with temperature increase. It was noticed that the same trend kept for  $\text{SO}_4^{2-}$  produced by unknown electron acceptor. At high temperature (35 °C) amount of  $\text{HS}^-$  oxidized not by  $\text{NO}_3^-$  almost two times higher than at lowest experimented temperature (25 °C). In contrary, effluent concentration of  $\text{SO}_4^{2-}$  produced by the  $\text{NO}_3^-$  is not following this trend. Judging from the result it is possible to suggest that temperature increase may be the factor which can influence microbiological

community inside the reactor. Increasing temperatures may influence the grow rate and development of some microorganisms which are responsible for  $\text{HS}^-$  oxidation to  $\text{SO}_4^{2-}$ . Also higher temperatures ( $>25\text{ }^\circ\text{C}$ ) can influence the metabolism of available bacteria and slow down or increase the production of the side products in the effluent.

## 4.5 Free Gibbs energy

To investigate how different temperatures will affect process reaction energy, free Gibbs energy was calculated as an energy of  $\text{HS}^-$  oxidation by  $\text{NO}_3^-$  (Table 4.5).

Average  $\Delta G^\theta$  during the experiment was  $-316.67 \pm 17.52$  kJ/mol. Slow decrease of energy was observed with temperature increasement (except  $27\text{ }^\circ\text{C}$  when energy increased at 3% compare to initial temperature). Energy difference between lowest and highest temperature conditions constitute only 8%. Figure 4.6. reflects changes in free Gibbs energy of  $\text{HS}^-$  oxidation by  $\text{NO}_3^-$ . Slight variation in the removal efficiency of electron donor and acceptor has minor impact on energy changes (Table 4.5).

Table 4.5: Free Gibbs energy of  $\text{HS}^-$  oxidized by  $\text{NO}_3^-$  at different temperatures

Temperature $^\circ\text{C}$	$\Delta G^\theta$ kJ/mol	$\text{HS}^-$ removal efficiency %	$\text{NO}_3^-$ removal efficiency %
25	-304.58	91	>99
27	-293.18	95	>97
29	-320.49	92	>99
32	-334.82	92	>99
35	-330.31	92	>99

Observed changes in free Gibbs energy can be possibly associated with temperature changes. However, small change in energy output at the start and in the end of the experiment (only 8%) more likely related to impact of another factors e.g. changes in microbiological community or microorganism resistance to temperature changes. Also changes in free Gibbs energy can be explained by metabolic shift from primary ( $\text{S}^0$ ) to secondary ( $\text{SO}_4^{2-}$ ) pathway. To check this assumption, percentage share of electron acceptor use between two energy pathways were made (Table 4.6). Achieved results showed increasement of  $\text{NO}_3^-$  use in pathway leading to  $\text{SO}_4^{2-}$  production (5-6% at  $25\text{ }^\circ\text{C}$  to 9-10% at  $35\text{ }^\circ\text{C}$ ). This results can be compared to the results in Table 4.4 - where  $\text{SO}_4^{2-}$  production by  $\text{NO}_3^-$  tended to increase with temperature. Overall free Gibbs energy for relative percentage share of  $\text{NO}_3^-$  between  $\text{SO}_4^{2-}/\text{S}^0$  pathways decreased stably (on 26%) with temperature increase (Figure 4.6). Such energy change make reaction more exothermic. Increasing use of the electron acceptor on  $\text{SO}_4^{2-}$  pathway also was observed when temperature was gradually decreased ( $25\text{-}10\text{ }^\circ\text{C}$ ) (Sposob et al., 2017a).

Table 4.6: Electron acceptor share between two energy pathways in relation to electron donor availability

Temperature °C	% by SO <sub>4</sub> <sup>2-</sup> pathway	% by S <sup>0</sup> pathway	ΔG <sup>0</sup> kJ/mol
25	6	94	-237.07
27	5	95	-277.93
29	8	92	-293.42
32	10	90	-303.75
35	9	91	-298.60

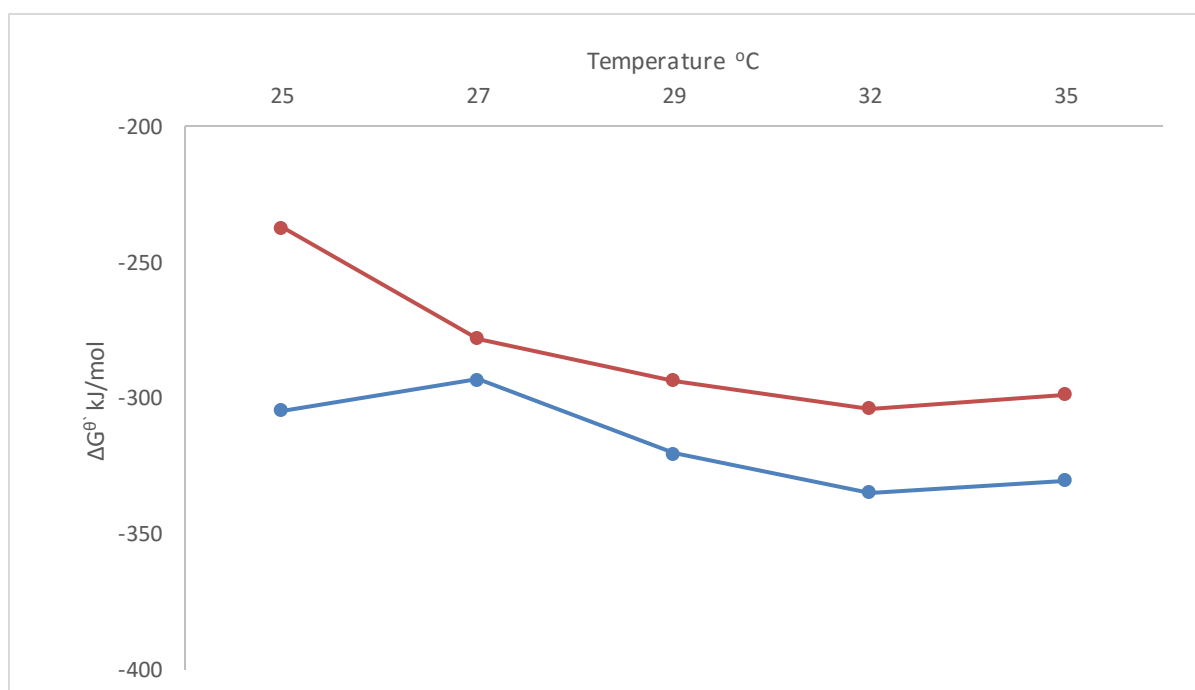


Figure 4.6 – Changes in Gibbs energy with temperature

Shift to SO<sub>4</sub><sup>2-</sup> production energy pathway (Eq.2.8) cause the increasement of biomass yield compare to S<sup>0</sup> pathway (Eq. 2.9). Increasing biomass yield helps to achieve almost stable free Gibbs energy for catabolic reactions, even if removal efficiency of HS<sup>-</sup> decreasing. (Sposob et al., 2017a). Shift to more energy rich pathway may be dictated by bacteria adaptation to unusually high temperature conditions (29-35 °C). SO<sub>4</sub><sup>2-</sup> increasement in the effluent related to a temperature changes (25-10 °C) was also observed by Sposob et al. (2017b).

## 4.6 Changing N/S ratio

Additionally, to the temperature effect on biological sulphide oxidation, were decided to investigate how different N/S ratios influence the removal efficiency. Temperature during this part of the experiment remained stable (35 °C). Three decreasing of the  $\text{NO}_3^-$  concentration were made, thereby different lower than stoichiometric N/S ratios has been achieved (Table 4.7).

Average pH level was 8.3 and remained stable during the experiment.

$\text{NO}_3^-$  was detected in the effluent only twice (Figure 4.8) after reactor maintaining period. Presence of  $\text{NO}_3^-$  in the effluent was minor and has no significant impact on overall  $\text{NO}_3^-$  removal efficiency. It is possible to say that changes in N/S ratio, to lower than stoichiometric has no influence on electron acceptor removal during the experiment. At the same time stable decreasing of electron donor removal efficiency was observed - 92% at 0.35 ratio to 88% at 0.245 ratio. It is clearly saying about that fact that it was not enough of electron acceptor to provide the oxidation reaction. Thus, more influent  $\text{HS}^-$  left unreacted ( $11.55 \pm 2.23$  mgS/L) at lowest N/S ratio.

Table 4.7: Process outcomes at different N/S ratios

N/S ratio	Solutions (mgS/L; mgN/L)		Products (mgS/L)	
	$\text{NO}_3^-$ (% removal efficiency)	$\text{HS}^-$ (% removal efficiency)	Total effluent sulphur (% sulphur suspended in the effluent, $\text{S}^0$ )	Accumulated sulphur (% of sulphur accumulated in the reactor, $\text{S}^0_{\text{acc}}$ )
0.35	n.d. (>99%)	$8.27 \pm 4.45$ (92%)	$50.88 \pm 5.77$ (51%)	$49.12 \pm 5.77$ (49%)
0.32	n.d. (>99%)	$7.87 \pm 1.14$ (92%)	$48.07 \pm 4.40$ (48%)	$51.93 \pm 4.40$ (52%)
0.275	n.d. (>99%)	$10.92 \pm 2.02$ (90%)	$55.57 \pm 2.68$ (55%)	$44.43 \pm 2.68$ (45%)
0.245	n.d. (>99%)	$11.55 \pm 2.23$ (88%)	$55.01 \pm 2.76$ (55%)	$44.99 \pm 2.76$ (45%)

First new low ratio was 0.32. No significant changes in the  $\text{HS}^-$  removal efficiency were observed. In contrary changes of the presence of  $\text{SO}_4^{2-}$ ,  $\text{S}^0_{\text{ss}}$  and  $\text{NO}_3^-$  in the effluent were noticed (Table 4.8). Stable decreasing of  $\text{SO}_4^{2-}$  and increasing of  $\text{S}^0_{\text{ss}}$  was investigated. Average amount of  $\text{SO}_4^{2-}$  and  $\text{S}^0_{\text{ss}}$  at 0.35 ratio was almost the same. However, after decreasing N/S ratio (0.32-0.245), changes in presence of the sulphur components were observed.  $\text{S}^0_{\text{ss}}$  become the main sulphur component in the effluent at lower ratios (Figure 4.8).

Such change may be related to changes in amount of accumulated sulphur in the reactor (Table 4.7). After temporary increase of  $S^0_{acc}$  at 0.32 ratio, amount of retained sulphur started to decrease. Thus,  $S^0_{acc}$  in the reactor may be an origin of the side products in the effluent. In lack of electron acceptor  $S^0$  granules could started to break, which possibly caused increase of  $S^0_{ss}$ . As a fact, achieved samples at lower ratios characterized by high amount of black sludge particles which indicates that sludge granules in the reactor started to crack. Decreasing of  $SO_4^{2-}$  in the effluent may be explained by lack of available electron acceptor which is responsible for  $HS^-$  oxidation.

Table 4.8 – Side effluent products at different ratios

N/S ratios	$SO_4^{2-}$ mgS/L	$S^0_{ss}$ mgS/L	$NO_3^-$ mgN/L
0.35	23.60±4.85	22.98±6.38	n.d.
0.32	19.22±3.18	24.03±2.78	n.d.
0.275	15.96±3.32	33.32±3.65	0.33±0.66
0.245	12.34±2.86	36.02±2.46	0.17±0.39

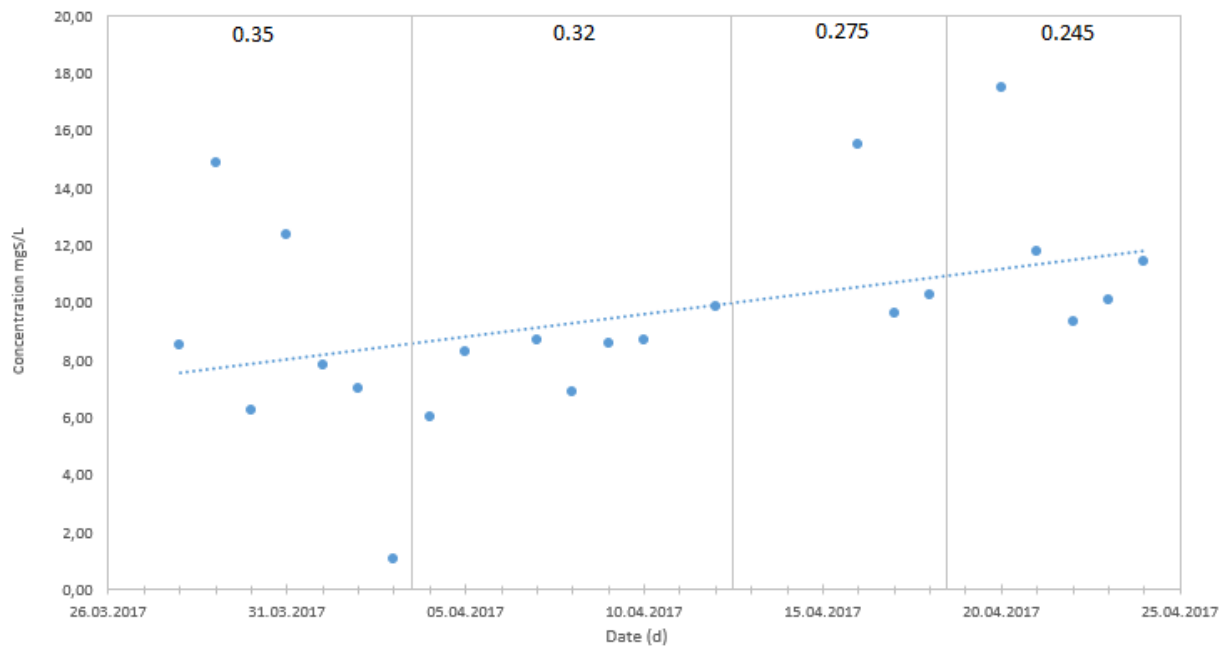


Figure 2.8:  $HS^-$  profile according to date and N/S ratio (0.35, 0.32, 0.275, 0.245)

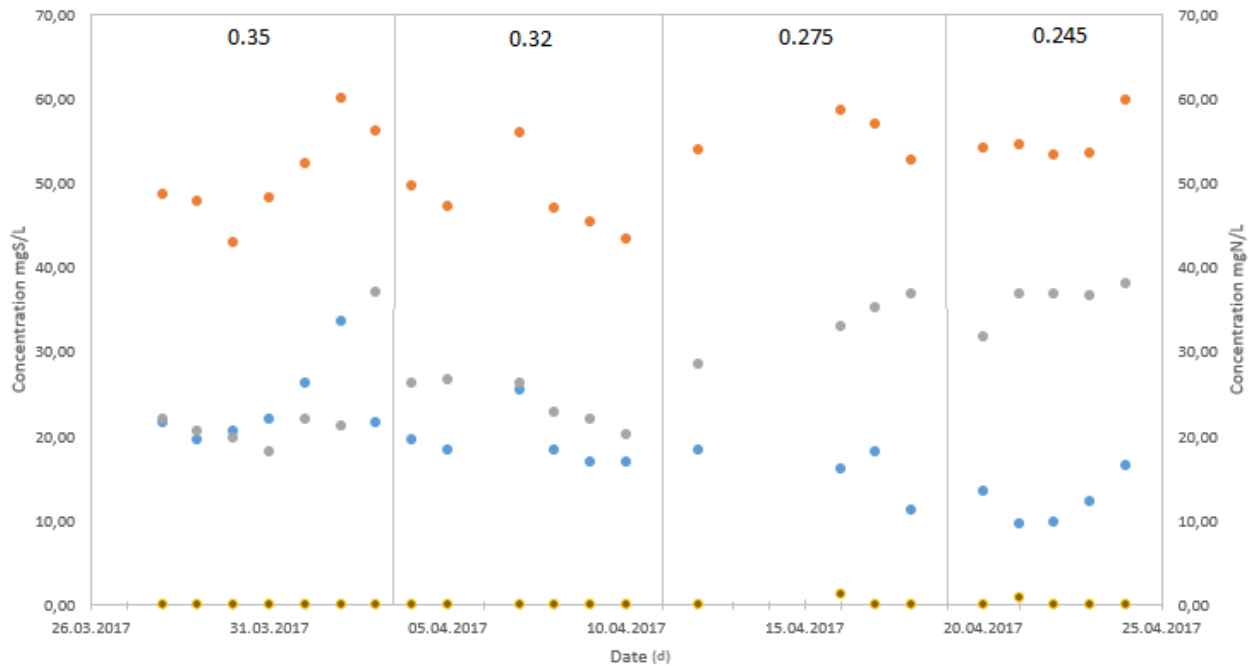


Figure 4.7: Substrate ( $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{S}^0_{\text{ss}}$ , Total effluent sulphur) concentration with time under different N/S ratios

## 4.7 Microscopy

After experimental trial finished, the sludge was collected. Due to observed changes of the sludge color, which changed from black to a grey (Figure 4.9), it was decided to perform microscopy analysis using Nikon SMZ745 zoom stereomicroscope.

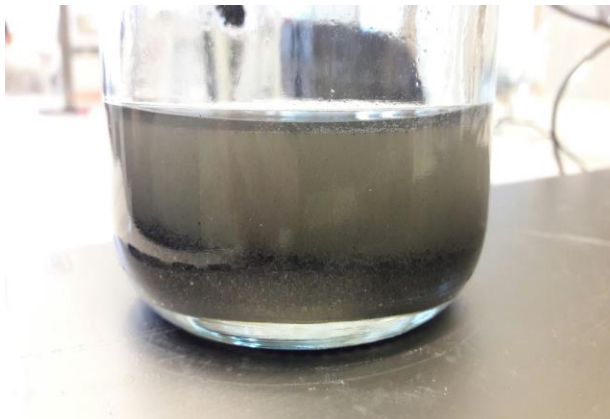


Figure 4.9: Collected sludge from the reactor

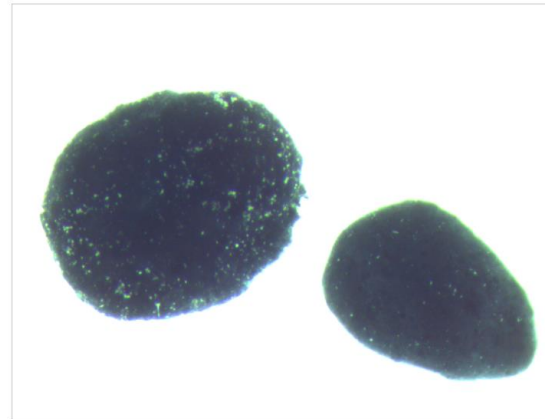


Figure 4.10: Separate sludge granules

Magnified sludge granules (Figure 4.10) had mostly oval shape and porous surface. Granules surface was covered with white spots of unknown origin, what can explain the sludge color changes. The sludge structure was also observed after drying the sludge sample at room temperature (Figure 4.12). The dried sample was also characterized by inclusions of a white color.

The sludge started to change its color during the lowering N/S ratio at 35 °C. It can possibly suggest that the origin of a white inclusions on the surface of a sludge granules is related to the ratio changes or/and continuous influence of high temperature.

Based on the basic microscopic evaluation it can be suggested that white spots on the sludge granules are sulphur-based components i.e.  $S^0$ . Two forms of sulphur that could attach to a granules surface exist: one is sulphur in form of iron sulphide ( $FeS$ ) and another one is biologically-driven sulphur following Eq. 2.9 (Sposob et al., 2016). Low concentrations of electron acceptor may be the reason for formation of biologically-driven sulphur as attached particles to the sludge granule surface.

Change in  $S^0$  accumulation probably can be explained by temperature impact. Suggestion is that  $S^0$  granules may have low temperature resistance and cannot be biologically formed at high temperatures. Thus, sulphur components attached to the sludge in form of separate particles.

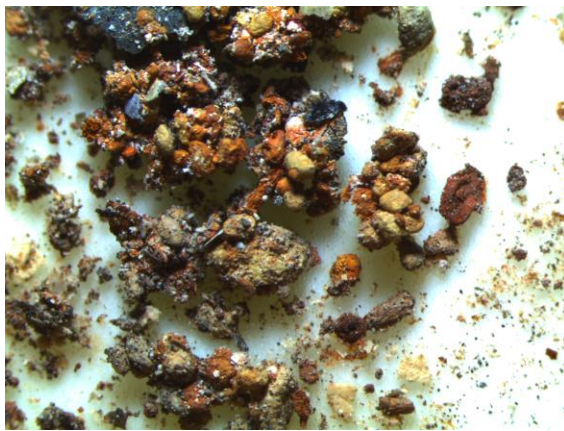


Figure 4.11: Sludge dried at 550 °C

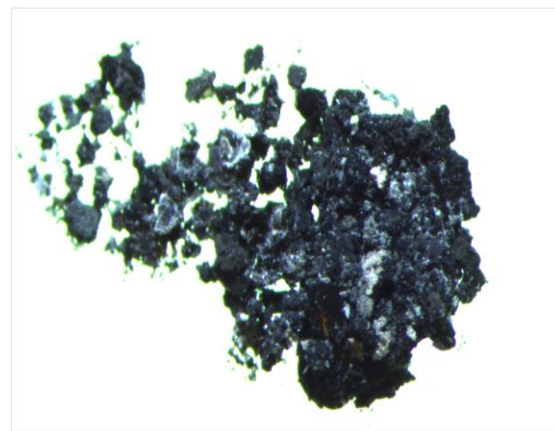


Figure 4.12: Sludge dried at room temperature

Additionally, part of the sludge was dried at 550 °C oven during 30 minutes (Figure 4.11). Oven dried sludge characterized by different colors (red, black, orange, yellow, white, grey). Also dried sludge was burned over the open fire, to investigate presence of flammable materials. No sludge ignition was observed.

Red-orange particles could possibly have mineral and metal origin. In study provided by Sposob et al., (2016) iron and phosphorus presence on a granule surface were confirmed. Feed includes iron-containing compound ( $FeSO_4 \cdot 7H_2O$ ) which could be the reason of red-orange particles in the burned sludge. Attached to the sludge granular surface sulphur in form of  $FeS$  was exposed to the air and high temperatures when it was drying inside the oven. Thus  $FeS$  was oxidized and as a result dried sludge achieved characteristic to iron oxides red (rust) color. Yellow and white particles are probably  $S^0$  accumulated during the experiment.



## 5 Conclusion

- Microbiological communities could be affected by high temperatures (29-35 °C) as well as low temperatures (15-10 °C) and respond to such changes in form of shifting to more energy rich pathway ( $\text{SO}_4^{2-}$  pathway).
- Applied biological sulphide oxidation method of  $\text{S}^{2-}$  removal with  $\text{NO}_3^-$  as an electron acceptor showed high removal efficiency of 92 and 99% of  $\text{S}^{2-}$  and  $\text{NO}_3^-$ , respectively
- Changing temperature regimes (27-35 °C) have insignificant effect on  $\text{S}^{2-}$  and  $\text{NO}_3^-$  removal efficiency.
- Increasing temperatures caused stable increasing of  $\text{SO}_4^{2-}$  production.
- Up to 51% of produced  $\text{S}^0$  were retained inside the reactor during the experiment while the rest was discharged with effluent
- Amount of incorporated sulphur varied with temperatures changes and was the lowest  $40.33 \pm 6.55$  mgS/L (40%) at 29 °C, nonetheless production of accumulated sulphur were recovered to initial level at the end of temperature experiment (35 °C).
- Increasing temperatures (25-35 °C) cause no significant changes in overall free Gibbs energy.
- Decreasing of N/S ratio caused decrease in overall  $\text{HS}^-$  removal efficiency (92% to 88% at 0.35 and 0.245 N/S ratio respectively)
- $\text{S}^0_{\text{ss}}$  became the dominate specie in the effluent at low N/S ratios (<0.35)
- Through-out the experiment reactor sludge changed its color from completely black to grey.
- White spots on a sludge granule surface probably are sulphur based components.
- Red-orange particles observed in the dried (550 °C) reactor sludge possibly tells about presence of iron or/and phosphate components.
- Low N/S ratio or/and high temperature (35 °C) may be the reason of non-efficient  $\text{S}^0$  granular formation.

# References

- An, S., Tang, K., Nemati, M., 2010. Simultaneous biodesulphurization and denitrification using an oil reservoir microbial culture: Effects of sulphide loading rate and sulphide to nitrate loading ratio. *Water Res.* 44, 1531–1541.
- Beristain-Cardoso, R., Texier, A.C., Razo-Flores, E., Méndez-Pampín, R., Gómez, J., 2009. Biotransformation of aromatic compounds from wastewaters containing N and/or S, by nitrification/denitrification: A review. *Rev. Environ. Sci. Biotechnol.* 8, 325–342.
- Cai, J., Zheng, P., Mahmood, Q., 2010. Influence of transient pH and substrate shocks on simultaneous anaerobic sulfide and nitrate removal. *J. Hazard. Mater.*
- Cai, J., Zheng, P., Mahmood, Q., 2008. Effect of sulfide to nitrate ratios on the simultaneous anaerobic sulfide and nitrate removal. *Bioresour. Technol.* 99, 5520–5527.
- Carlson, C.A., Ingraham, J.L., 1983. Comparison of denitrification by *Pseudomonas stutzeri*, *Pseudomonas aeruginosa*, and *paracoccus denitrificans*. *Appl. Environ. Microbiol.* 45, 1247–1253.
- Chen, C., Ren, N., Wang, A., Yu, Z., Lee, D.J., 2008. Simultaneous biological removal of sulfur, nitrogen and carbon using EGSB reactor. *Appl. Microbiol. Biotechnol.* 78, 1057–1063.
- Chen, C., Wang, A., Ren, N., Lee, D.J., Lai, J.Y., 2009. High-rate denitrifying sulfide removal process in expanded granular sludge bed reactor. *Bioresour. Technol.* 100, 2316–2319.
- Chen, C., Xu, X.-J., Xie, P., Yuan, Y., Zhou, X., Wang, A.-J., Lee, D.-J., Ren, N.-Q., 2017. Pyrosequencing reveals microbial community dynamics in integrated simultaneous desulfurization and denitrification process at different influent nitrate concentrations. *Chemosphere* 171, 294–301.
- Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: A review. *Bioresour. Technol.* 99, 4044–4064.
- De Filippis, P., Di Palma, L., Scarsella, M., Verdone, N., 2013. Biological denitrification of high-nitrate wastewaters: A comparison between three electron donors. *Chem. Eng. Trans.* 32, 319–324.
- Di Capua, F., Milone, I., Lakaniemi, A.-M., N.L. Lens, P., Esposito, G., 2017. High-rate autotrophic denitrification in a fluidized-bed reactor at psychrophilic temperatures. *Chem. Eng. J.* 313, 591–598.
- Di Capua, F., Papirio, S., Lens, P.N.L., Esposito, G., 2015. Chemolithotrophic denitrification in biofilm reactors. *Chem. Eng. J.* 280, 643–657.
- Dinamarca, C., 2014. Anaerobic Expanded Granular Sludge Bed (EGSB) reactor for the removal of sulphide by autotrophic denitrification. *Int. J. Energy Environ.* 5, 111–118.
- Fortuny, M., Baeza, J.A., Gamisans, X., Casas, C., Lafuente, J., Deshusses, M.A., Gabriel, D., 2008. Biological sweetening of energy gases mimics in biotrickling filters. *Chemosphere* 71, 10–17.
- Geldenhuis, a. J., Maree, J.P., de Beer, M., Hlabela, P., 2003. An integrated limestone/lime process for partial sulphate removal. *J. South African Institue Min. Metall.* 345–354.

- Gomez, J., Mendez, R., Lema, J.M., 2000. Kinetic study of addition of volatile organic compounds to a nitrifying sludge. *Appl. Biochem. Biotechnol.* 87, 189–202.
- Grady Jr, C.P.L., Daigger, G.T., Love, N.G., Filipe, C.D.M., 2011. *Biological wastewater treatment*. CRC press.
- Hancock, F.E., King, F., Flavell, W.R., Islam, M.S., 1998. Catalytically Enhanced Absorption of Sulphur Species from Odorous Air Streams: A New Technology for Odour Abatement. *Catal. Today* 40, 289–296.
- Holmer, M., Hasler-Sheetal, H., 2014. Sulfide intrusion in seagrasses assessed by stable sulfur isotopes: a synthesis of current results. *Front. Mar. Sci.* 1, 1–12.
- Kleerebezem, R., Mendez, R., 2002. Autotrophic denitrification for combined hydrogen sulfide removal from biogas and post-denitrification. *Water Sci. Technol.* 45, 349–356.
- Krishnakumar, B., Majumdar, S., Manilal, V.B., Haridas, A., 2005. Treatment of sulphide containing wastewater with sulphur recovery in a novel reverse fluidized loop reactor (RFLR). *Water Res.* 39, 639–647.
- Krishnakumar, B., Manilal, V.B., 1999. Bacterial oxidation of sulphide under denitrifying conditions. *Biotechnol. Lett.* 21, 437–440.
- Li, W., Zhao, Q., Liang, Liu, H., 2009. Sulfide removal by simultaneous autotrophic and heterotrophic desulfurization-denitrification process. *J. Hazard. Mater.* 162, 848–853.
- Lu, H., Chandran, K., Stensel, D., 2014. Microbial ecology of denitrification in biological wastewater treatment. *Water Res.* 64, 237–254.
- Mahmood, Q., Zheng, P., Cai, J., Wu, D., Hu, B., Islam, E., Azim, M.R., 2007a. Comparison of anoxic sulfide biooxidation using nitrate/ nitrite as electron acceptor. *Environ. Prog.* 26, 169–177.
- Mahmood, Q., Zheng, P., Hayat, Y., Islam, E., Wu, D., Ren-cun, J., 2007b. Effect of pH on anoxic sulfide oxidizing reactor performance. *Bioresour. Technol.* 99, 3291–3296.
- McComas, C., Sublette, K.L., Jenneman, G., Bala, G., 2001. Characterization of a novel biocatalyst system for sulfide oxidation. *Biotechnol. Prog.* 17, 439–446.
- Mohammad, A.B., Mohammadi, S., Mohammad, Talaeiyan Araghi; Ravanbakhsh, S., Ghaffari, H.R., 2016. Suggestion and feasibility of appropriate treatment methods for the removal of sulfate and organic matter from petrochemical wastewater effluent - a case study. *Int. J. Pharm. Technol.* 1, 173–177.
- Prosser, J.I., 1990. Autotrophic Nitrification in Bacteria. *Adv. Microb. Physiol.* 30, 125–181.
- Ravichandra, P., Gopal, M., Gangagni Rao, A., Ramakrishna, M., Annapurna, J., 2007. Continuous operation of fluidized bed reactor for biogenic sulfide oxidation using immobilized cells of *Thiobacillus* sp. *J. Appl. Sci.* 7, 2188–2193.
- Rittmann, B., McCarty, P., 2001. *Environmental biotechnology: principles and applications*. New York McGrawHill 400.
- Roozeboom, K., Blasi, D., Mengel, D., 2011. *Nitrate Toxicity*. Kansas State University Agricultural Experiment Station and Cooperative Extension Service.
- Salman, M.S., 2009. Removal of Sulfate from Waste Water by Activated Carbon. *Al-Khwarizmi Eng. J.* 5, 72–76.

- Sanopoulos, D., Karabelas, A., 1997. H<sub>2</sub>S Abatement in Geothermal Plants: Evaluation of Process Alternatives. *Energy Sources* 19, 63–77.
- Sassi, M., Gupta, A.K., 2008. Sulfur recovery from acid gas using the Claus process and High Temperature Air Combustion (HiTAC) technology. *Am. J. Environ. Sci.* 4, 502–511.
- Shively, J.M., 1974. Inclusion Bodies of Prokaryotes. *Annu. Rev. Microbiol.* 28, 167–188.
- Shoun, H., Tanimoto, T., 1991. Denitrification by the fungus *Fusarium oxysporum* and involvement of cytochrome P-450 in the respiratory nitrite reduction. *J. Biol. Chem.* 266, 11078–11082.
- Show, K.Y., Lee, D.J., Pan, X., 2013. Simultaneous biological removal of nitrogen-sulfur-carbon: Recent advances and challenges. *Biotechnol. Adv.* 31, 409–420.
- Sposob, M., Bakke, R., Dinamarca, C., 2017a. Metabolic divergence in simultaneous biological removal of nitrate and sulfide for elemental sulfur production under temperature stress. *Bioresour. Technol.* 233, 13.
- Sposob, M., Bakke, R., Dinamarca, C., 2017b. Effects of N / S molar ratio on products formation in psychrophilic autotrophic biological removal of sulfide. (Submitted)
- Sposob, M., Dinamarca, C., Bakke, R., 2016. Short-term temperature impact on simultaneous biological nitrogen-sulphur treatment in EGSB reactor. *Water Sci. Technol.* 74, 1610–1618.
- Studel, R., 1996. Mechanism for the Formation of Elemental Sulfur from Aqueous Sulfide in Chemical and Microbiological Desulfurization Processes. *Ind. Eng. Chem. Res.* 35, 1417–1423.
- Syed, M., Soreanu, G., Falletta, P., Beland, M., 2006. Removal of hydrogen sulfide from gas streams using biological processes - A review. *Can. Biosyst. Eng.* 48, 2.1-2.14.
- Wolin, E.A., Wolin, M.J., Wolfe, R.S., 1963. Formation of methane. *J. Franklin Inst.* 176, 737.
- World Health Organization, 2011. Nitrate and nitrite in drinking-water. *Backgr. Doc. Dev. WHO Guidel. Drink. Qual.* 37, 227–231.
- Yavuz, B., Turker, M., Engin, G.O., 2007. Autotrophic removal of sulphide from industrial wastewaters using oxygen and nitrate as electron acceptors. *Environ. Eng. Sci.* 24, 457–470.
- Zhou, W., Sun, Y., Wu, B., Zhang, Y., Huang, M., Miyanaga, T., Zhang, Z., 2011. Autotrophic denitrification for nitrate and nitrite removal using sulfur-limestone. *J. Environ. Sci.* 23, 1761–1769.
- Zou, G., Papirio, S., Lakaniemi, A.M., Ahoranta, S.H., Puhakka, J.A., 2016. High rate autotrophic denitrification in fluidized-bed biofilm reactors. *Chem. Eng. J.* 284, 1287–1294.