Title: Anaerobic expanded granular sludge bed (EGSB) reactor for the removal of sulphide by autotrophic denitrification

Authors: Carlos Dinamarca

Article citation: Dinamarca, C. (2014). Anaerobic expanded granular sludge bed (EGSB) reactor for the removal of sulphide by autotrophic denitrification. *The International Journal of Energy and Environment, 5*(1), 111-118.

INTERNATIONAL JOURNAL OF
ENERGY AND ENVIRONMENT

Volume 5, Issue 1, 2014 pp.111-118

Journal homepage: www.IJEE.IEEFoundation.org

Anaerobic expanded granular sludge bed (EGSB) reactor for the removal of sulphide by autotrophic denitrification

Carlos Dinamarca

Department of Process, Energy and Environment; Faculty of Technology; Telemark University College
Kjølnes ring 56, 3918 Porsgrunn, Norway.

Abstract
The Removal efficiency, load and N/S molar ratio, of an EGSB reactor for autotrophic sulphide denitrification operated for 96 days, were studied. The reactor was operated at high inlet sulphide concentrations between 0.25 to 3.00 g HS^--S/L equivalents to loads between 5 to 250 g HS^--S/m^3·h. Sulphide removals higher than 99 % were achieved. At a N/S molar ratio of 0.3 and 12 hours HRT the process was stable even during transition periods of influent sulphide concentration and pH (9.0-12.1). At N/S molar ratio of 1.3, granules lost some of their sedimentation properties and appeared to disintegrate. On average 94 ± 4 % of the equivalent inlet sulphur ended as elemental sulphur.

Keywords: Granular sludge; Nitrate; Sulphide removal; Sulphur recovery.

1. Introduction

Sulphide species (H_2S, HS^-, S^{2-}) are derived from the anaerobic oxidation of organic matter and sulphate reduction. H_2S is a poisonous and corrosive substance that needs to be removed from wastewaters before discharge and from energy-rich gases before combustion or transfer to natural gas pipelines. Physical-chemical methods (e.g. wet air oxidation) are used for sulphide removal, but these methods are relatively expensive, have complex technology and can have a negative impact on the environment [1].

Biological sulphide removal appears to overcome the limitations of physical-chemical processes. Biological processes are generally less energy demanding, being operated at atmospheric pressure and mesophilic temperatures, and are not noxious to the environment [2]. The conversion of sulphides in bioreactors is performed by sulphur oxidizing bacteria (SOB) which are a highly diverse, both phylogenetically and metabolically, group of prokaryotes [3]. Dissimilatory sulphide removal with oxygen [4], nitrate [5] or iron [6] as electron acceptors has been extensively studied in different reactor configurations, such as batch reactors, continuous stirred tank reactors, biofilters, biotrickling filters and bioscrubbers [7]. Studies using granular sludge, as in UASB's, have been focusing on mixotrophic sulphide removal, simultaneous autotrophic and heterotrophic denitrification, with the addition of both nitrate and an organic carbon source [8-11].

Sulphides can be partially oxidized to elemental sulphur (S°) and then completely oxidized to sulphate (SO_4^{2-}) depending on the bio-availability of the electron acceptor. According to equations (1) and (2) sulphide can be oxidized to sulphate and/or sulphur depending on the ratio between NO_3^- and HS^- [5].

$$3HS^- + 3.9NO_3^- + 0.2NH_4^+ + HCO_3^- + 1.7H^+ \rightarrow CH_{1.8}O_{0.5}N_{0.2} + 1.9N_2 + 3SO_4^{2-} + 2.3H_2O \quad (1)$$

$$14.5HS^- + 5NO_3^- + 0.2NH_4^+ + HCO_3^- + 20.3H^+ \rightarrow CH_{1.8}O_{0.5}N_{0.2} + 2.5N_2 + 14.5S + 27.4H_2O \quad (2)$$

Studying granular sludge bed solutions is motivated by operational disadvantages of fixed biofilms for the treatment of sulphides. The incomplete oxidation of sulphides by anaerobic bacteria leads to the formation of sulphur (S°) which gradually accumulates in biofilm carries leading to overpressure and reactor failure. Fixed biofilm reactors need therefore periodic stops for the removal of accumulated elemental sulphur [12]. Reactors equipped with a circulation loop, to achieve granular biomass suspension, may not have this problem since fixed biofilm carriers are not needed. In UASB type reactors the elemental sulphur can instead accumulate in the walls, precipitate to the bottom of the reactor or be washed out with the effluent.

The aim of this study was to evaluate the use of granular sludge for the removal of sulphide at high inlet concentrations (0.25-3.00 g HS^--S/L) with nitrate as an electron acceptor and $NaHCO_3$ as the carbon source. Our work focuses on the effect of N/S molar ratio, sulphide removal efficiency, load and on the competitive advantages of high load EGSB, with respect to ease of operation and elemental sulphur recovery, compared to fixed biofilm reactors.

2. Materials and methods

2.1 Microbial inoculum

Granular biomass was obtained from a UASB methane reactor treating wastewater from the pulp and paper industry at "Norske Skog Saugbrugs" Halden, Norway. Granules size ranged between 1 and 4 mm. Average inflow concentration of SO_3^{2-}, SO_4^{2-} and HS^- were 166 mg/l, 234 mg/l and 31 mg/l, respectively.

2.2 Synthetic media

Na_2S feed solution was prepared at concentrations shown in Table 1 with $NaHCO_3$ added at variable concentration according to equations (1) and (2). Nitrate feed solution was based on concentrated HNO_3 and the following stock solutions in g/L A) NH_4Cl, 10; NaCl, 10; $MgCl_2 \cdot 6H_2O$, 10; $CaCl_2 \cdot 2H_2O$, 5. B) K_2HPO_4, 300. 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. D) A 10 times concentrated vitamin solution described by Wolin *et al.*[13]. Concentrated HNO_3 and 10, 2, 2, 1 ml/L of solutions A, B, C and D, respectively, were dissolved in distilled water. The EGSB reactor was fed at 80:20 v/v ratio between Na_2S sulphide and nitrate solution.

2.3 Experiment

The experimental plan and laboratory setup are summarized in Table 1 and Figure 1, respectively. The sulphide removal reactor was built from acrylic glass with an effective volume of 1.3 L. The reactor had a radius of 24 mm, and a height of 820 mm. 15 L glass bottles were used for the feed solutions and the waste material. Glass bottles were connected to the reactor through Tygon tubes fitted with Teflon connections. Peristaltic pumps of the type Ismatec Ecoline were used to feed the reactor (pumps 1 and 2) and of the type Watson Marlow for the recirculation. The liquid vertical velocity was adjusted between 4.5 m/h- 7.4m/h.

Table 1. Experimental plan. days 0-35 were considered as adaptation period

	EGSB Reactor			Feed bottles		Approximate molar ratio
Day	**S**	**HRT**	**Load**	**$Na_2S \cdot 9H_2O$**	**NO_3^-**	**N/ S**
	g S /L	h	g S/ $m^3 \cdot h$	g/L	g/L	
0- 35	0.25	48	5	2.34	0.8	0.3
36- 47	0.25	12	21	2.34	3.1	1.3
48- 66	0.25	12	21	2.34	0.8	0.3
67- 77	0.50	12	42	5.00	1.7	0.3
78- 85	2.00	12	167	18.71	6.7	0.3
86- 93	3.00	12	250	28.07	10.0	0.3
94- 96	3.00	6	499	28.07	10.0	0.3

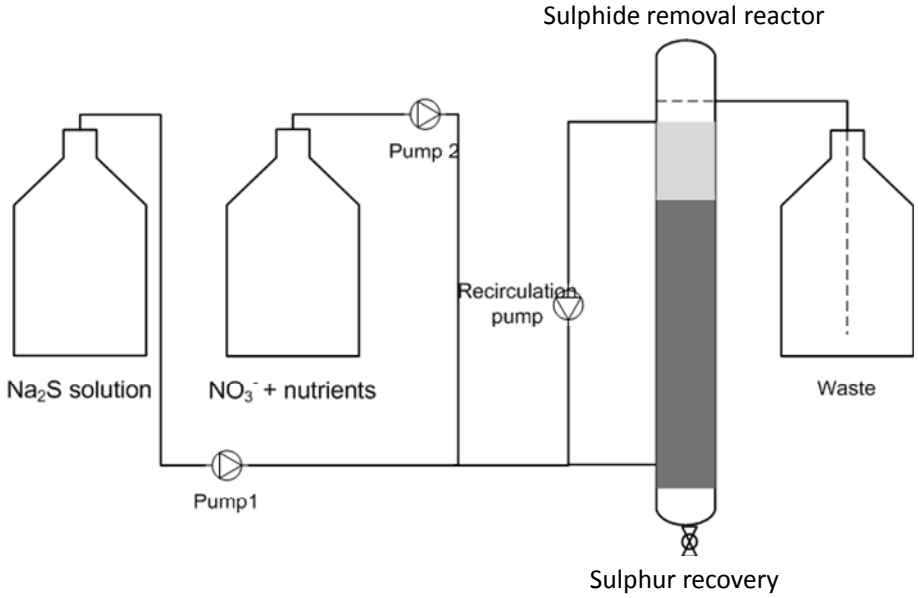

Figure 1. Schematic laboratory scale EGSB reactor

2.4 Analytical methods

Total solids (TS), total volatile solids (TVS) and settling properties were obtain according to US standard methods 2540B, 2540E, 2710 respectively. Effluent concentrations of sulphide (HS^-/S^{2-}), sulphate (SO_4^{2-}) and nitrate (NO_3^-) were analysed by test cuvettes HACH-LANGE LCK 653, LCK 153, and LCK 340, respectively measured with spectrophotometer DR 2008.

2.5 Sulphur recovery

The amount of elemental sulphur can be calculated from the difference between influent sulphide less the equivalent sulphate and sulphide at the effluent as show in equation (3).

$$S_{S^\circ}=S_{[HS]-\_in}-S_{[SO4]2-\_out}-S_{[HS]-\_out} \quad (3)$$

where: $S_{[HS]-\_in}$ = Equivalent sulphur input as sulphide, $S_{[SO4]2-\_out}$= Equivalent sulphur output as sulphate, $S_{[HS]-\_out}$ =Equivalent sulphur output as sulphide, S_{S° = Elemental sulphur.

3. Results and discussion

3.1 N/S molar ratio

To test the influence of electron acceptor concentration, the reactor was fed at a N/S ratio of 1.3 and 0.3 according to equations (1) and (2) respectively (Table 1). Shortly after the change in feed strategy from 0.3 to 1.3 N/S molar ratio (day 36), the granules present in the reactor cracked (Figure 2) and appeared to lose some of the sedimentation properties (suspended solids appearing in the recirculation loop and effluent). A constant N/S ratio of 0.3 was therefore applied from day 48 and no more disintegration of the granular sludge was observed.

Excess of the electron donor led to the highest turnover of SO_4^{2-}-S as shown in figure 3 at 22 g HS^--S/m^3·h load, and as conversion of sulphide to sulphate gives more energy for growth [5], it can be hypothesized that the disintegration of granules observed here was caused by better trophic conditions that favour growth of free cells rather than granule formation. Granules disintegration has been previously attributed to sudden variations in loading rates, influent concentrations or changes in pH [14] this agrees in part with our observations. Inlet nitrate concentration was changed from 0.8 g/L to 3.1 g/L while average pH was kept relatively constant at 9.9 ± 0.6.

The understanding of granule formation and disintegration is a key factor for successful operation of UASB type reactors [14]. High inlet NO_3^- concentration that triggered granule disintegration can, at the same time, be used as a strategy to avoid elemental sulphur formation when needed [12]. Future research should study in detail the N/S molar ratio effect on granule characteristics in sulphide removal reactors.

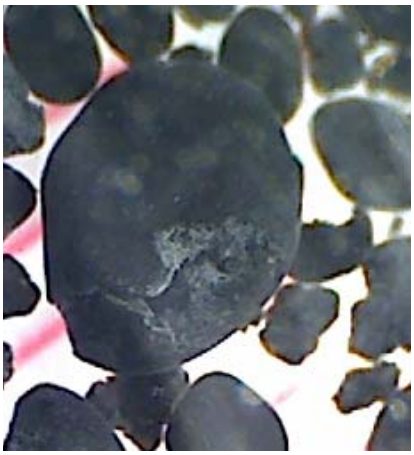

Figure 2. Granular sludge disintegrating, day 40 at N/S molar ratio = 1.3

3.2 Removal efficiency and load

The reactor was run at increasing sulphide concentrations (from 0.25 to 3.00 g HS^--S/L). The anaerobic EGSB reactor achieved between 99- 100 % removal efficiencies up to a load of 250 g HS^--S/m^3·h and no operational problems were experienced at a constant N/S molar ratio of 0.3. Sulphide concentrations found in the effluent ranged from 0.2 mg/L to 4.0 mg/L, while nitrate concentrations were found between 65 mg/L- 526 mg/L (Figure 3). pH ranged between 9.0 and 12.1. The reactor was exposed to an extreme load of 499 g HS^--S/m^3·h prior to stopping the experiment due to the end of the project time frame. Only 10 % removal efficiency was observed after 48 hours of this operation. Given the stability of the process during the previous 46 days we believe that the reactor could achieve much higher removal efficiencies than the measured 10 % even at 499 g HS^--S/m^3·h load given slower load increase and longer adaptation time.

Sulphide removal efficiencies in biofilters, biotrickling filters and bioscrubbers are reported also in the range between 90-100 % [7]. The loads reported are typically between 16 g HS^-_S/m^3·h [15] to 171 g HS^--S/m^3·h [16], with cases in between as reported by Wani *et al*. [17] and Duan *et al*. [18]. The highest load reported so far in the literature, by Fortuny and collaborators [12], is equivalent to 236-264 g HS^--S/m^3·h in trickling filters with oxygen as the final electron acceptor.

While the highest reported load (to our knowledge) with nitrate as electron acceptor is 167 g HS^--S/m^3·h with 95 % efficiency in a continuous bioscrubber fed biogas at flow rates between 5-25 m^3/h [19]. These authors also recognized that higher volumetric loads could be achieved by improving the gas absorption process. Our experiment was not limited to absorption processes but pH control could become a challenging aspect when reactors are operated at such high sulphides load.

A load of 200 g HS^--S/m^3·h has been reported by Chen et al. [8] in an EGSB operated 12 days under mixotrophic conditions. The reactor metabolized sulphide, nitrite and acetic acid at a stoichiometric ratio 1.5:1:0.5 for S:N:C respectively, their results show that autotrophic and heterotrophic denitrifiers cooperate as previously stated by Reyes-Avila et al. [20]. This is a valuable contribution knowing the synergistic effect of mixotrophic cultures; however it may be applicable in places with available degradable organic matter.

3.3 Comments on the ease of operation

The use of fixed biofilm in previously reported studies has shown to be difficult due to biofilm carriers clogging, caused by deposits of elemental sulphur on biofilm carriers. Reactors often are run at a high concentration of the electron acceptor to achieve the full oxidation of sulphide to sulphate (Eq. 1), intending to avoid clogging problems. For example Fortuny *et al*. [12] tested two different packing materials, foam cubes and HD Q-PAC, at high loading rates with oxygen as the final electron acceptor. The reactor with foam cubes was completely clogged after 3 months of operation and was shut down, while the reactor with HD Q-PAC biofilm carriers was operative until the end of the experiment (160 days). However, considerable accumulation of sulphur was observed, even when it was operated at a high concentration of electron acceptor.

Additionally, the production of excess SO_4^{2-} makes the use of chemicals for pH control, to achieve optimal operational conditions, necessary. High SO_4^{2-} concentrations at 1500 mg/L [21], 2000 mg/L [22]

and 6000 mg/L [23] also have shown inhibitory effects on the culture. The highest SO_4^{2-} concentration of 234 mg/L observed in the present study (Figure 3) at a load of 258 g $HS^-/m^3 \cdot h$ with a 99.9 % removal efficiency will cause no such inhibitory or pH control problems.

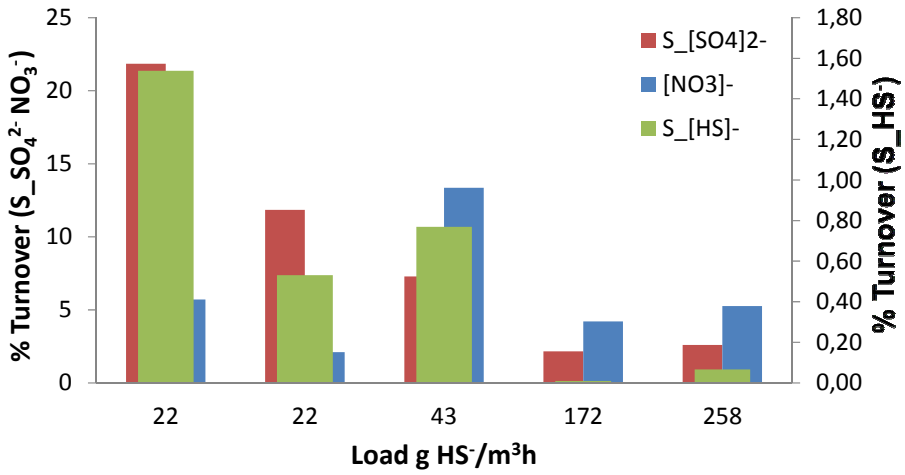

Figure 3. Pseudo-steady state concentration of sulphate, sulphide and nitrate in the effluent at different sulphide loads. Standard deviations for sulphate, sulphide and nitrate in the effluent are less than 0.5 %, 0.01 %, and 1 %, respectively of the input sulphur and nitrogen equivalent

We believe, based on the cited references that the use of oxygen, as the final electron acceptor, has some disadvantages such as the production of sulphur oxide compounds (SO_3^{-2}, $S_2O_3^{-2}$, SO_2), mass transfer limitations [24] and the need for more complicated control systems compared to nitrate as an electron acceptor. Also, the addition of air or oxygen into a system containing flammable gases may be hazardous, and is not allowed in some countries. It is argued that biofilm / biotrickling systems have the advantage that they can be seeded with any sludge from WWTP which makes the start-up process very easy [24]. We claim, based on the present study that it is at least as easy to start with granular biomass from any wastewater treatment plant. We used sludge from a UASB reactor treating wastewater from the pulp and paper industry simply because it is the only full scale granular sludge AD in Norway.

2.4 Sulphur (S°) recovery

Total solids (TS) and total volatile solids (TVS) analysis (Table 2) clearly show that a considerable amount of minerals were accumulated at the bottom of the reactor. An increase in 19 % of the mineral fraction, relative to the dry weight of biomass, was observed during the test period. This is also evident from Figure 4 which shows the elemental sulphur left after the ignition of dried settled sludge. It is estimated, based on the measured concentrations of SO_4^{2-} and HS^- in the effluent under pseudo steady-state conditions, according to Eq. (3), that on average 94 ± 4 % of the fed sulphide ended as elemental sulphur.

The recovery of elemental sulphur as settled particles is a great advantage compared to fixed film filters. Suspended systems with oxygen as an electron donor have been shown to be very effective in the recovery of elemental sulphur (e.g. THIOPAQ® process) in a range similar (higher than 90 % sulphur recovery) to our results with nitrate presented here. The commercially available designs require an external sedimentation unit for the separation of the sulphur, while it may be possible to separate sulphur directly at the bottom of an EGSB as proposed here.

Table 2. Biomass characterization at the start of feeding with a molar ratio N/S = 0.3 (day 48) and at the end of the experiment (day 96). Average TS and TVS (n = 3)

Day	TS (TVS)
	g/L (%)
48	45.8 (70 %)
96	49.9 (51%)

Figure 4. Elemental sulphur recovered at the bottom of the reactor, after the ignition of settled sludge at 550 °C

4. Further work

- Further develop the reactor (process) design for better separation of settled solids without disturbing granule performance which can result in long term and robust reactor operation.
- Study the effects, and eventually adaptation time, of high N/S molar ratio to the characteristics of granules.
- To study more deeply the pH range at which the process is viable. Here sulphide consumption was observed at effluent pH of 12.1. While in the cited articles the pH was between 8 and 10. Variation in the concentration of sulphides and nitrates affects the cation/anion balance and therefore the pH. More insight is needed into the relationship between alkalinity, sulphide load and N/S molar ratio. The study of pH gradients inside the granules is also important to explain further the results observed here.
- Identification of suitable control parameters. To further work on process control and automation.
- Identification of the bacterial community responsible for the oxidation of sulphide, but most important to study the community change during the adaptation period.

5. Conclusions

The anaerobic granular sludge reactor was able to remove more than 99 % of sulphide (HS^-) at 250 g HS^--S/m^3·h load. At this high load the average concentration of sulphate (SO_4^{2-}) was 234 mg/L representing no risk for inhibitory effects. 94 ± 4 % of the equivalent inlet sulphur ended as elemental sulphur (S°) and an important fraction was easily recovered as settled solids at the bottom of the reactor. This represents a considerable improvement of the process compared to fixed biofilm reactors. The system functioned well at a N/S molar ratio of 0.3.

Acknowledgements

The authors acknowledge the Research Council of Norway and Yara International AS for their financial support. Also we want to thank Clara Agustin for her assistance with the reactor operation and analytical work.

References

[1] Sublette K.L., Sylvester N.D. Oxidation of hydrogen sulfide by Thiobacillus denitrificans: Desulfurization of natural gas. Biotechnol. Bioeng. 1987, 29(2), 249-257.

[2] Gabriel D., Deshusses M.A. Retrofitting existing chemical scrubbers to biotrickling filters for H2S emission control. P. Nat. Acad. Sci. USA. 203, 100 (11), 6308-6312.

[3] Madigan M.T., Martinko J.M. Brock Biology of Microorganisms. 11th edition. Pearson Prentice Hall. 2006.

[4] Díaz I., Lopes A.C., Pérez S.I., Fdz-Polanco M. Determination of the optimal rate for the microaerobic treatment of several H2S concentrations in biogas from sludge digesters. Wat. Sci. Technol. 2011, 64(1), 233-238.

[5] Kleerebezem R., Mendez R. Autotrophic denitrification for combined hydrogen sulfide removal from biogas and post-denitrification. Wat. Sci. Technol. 2002, 45(10), 349-346.
[6] Guoqiang Z., Jie P., Yi Y., Shimin Z. Bacterial desulfurization of the H2S- containing biogas. Biotechnol. Lett. 1994, 16(10), 1087-1090.
[7] Syed M., Soreanu G., Falleta P., Béland M. Removal of hydrogen sulfide from gas streams using biological processes- A review. Ca. Biosyst. Eng. 2006, 48, 2.1- 2.14.
[8] Chen C., Wang A., Ren N., Lee D.J., Lai, J.Y. High-rate denitrifying sulfide removal process in expanded granular sludge bed reactor. Bioresource Technol. 2009, 100(7), 2316-2319.
[9] Chen C., Ren N., Wang A., Yu Z., Lee D.J. Simultaneous biological removal of sulfur, nitrogen and carbon using EGSB reactor. Appl. Microbiol. Biot. 2008, 78(6), 1057-1063.
[10] Beristain R., Texier A., Sierra R., Field J., Razo E., Gómez J. Simultaneous sulfide and acetate oxidation under denitrifying conditions using and inverse fluidized bed reactor. J. Chem. Technol. Biot. 2008, 83(9), 1197-1203.
[11] Sierra R., Guerrero F., Rowlette P., Freeman S., Field J. Comparison of chemo-, hetero- and mixotrophic denitrification in laboratory-scale UASBs. Wat. Sci. Technol. 2005, 52(1-2), 337-342.
[12] Fortuny M., Baeza J.A., Gamisans X., Casas C., Lafuente J., Deshusses M.A., Gabriel D. Biological sweetening of energy gases mimics in biotrickling filters. Chemosphere. 2008, 71(1), 10- 17.
[13] Wolin A., Wolin M., Wolfe R. Formation of methane by bacterial extracts. J. Biol. Chem.1963, 238(8), 2882- 2886.
[14] Alphenaar P.A. Anaerobic Granular Sludge: Characterization and Factors Affecting its Functioning. Ph.D. Thesis. Wageningen University dissertation no. 1806. 1994.
[15] Deng L., Chen H., Chen Z., Liu Y., Pu X., Song L. Process of simultaneous hydrogen sulfide removal from biogas and nitrogen removal from swine wastewater. Bioresource Technol. 2009, 100(23), 5600-5608.
[16] Duan H., Koe L.C.C., Yan R., Chen X. Biological treatment of H2S using pellet activated carbon as carrier of microorganisms in a biofilter. Wat. Res. 2006, 40(14), 2629-2636.
[17] Wani A.H., Lau A.K., Branion M.R. Biofiltration control of pulping odors- hydrogen sulfide: performance, microkinetics and coexistence effects of organo-sulfur species. J. Chem. Technol. Biot. 1999, 74(1), 9-16.
[18] Duan H., Koe L., Yan R. Treatment of H2S using a horizontal biotrickling filter based on biological activated carbon: reactor setup and performance evaluation. Appl Microbiol. Biotechnol. 2005, 67(1), 143-149.
[19] Baspinar A., Turker M., Hocalar A., Ozturk I. Biogas desulphurization at technical scale by lithotrophic denitrification: Integration of sulphide and nitrogen removal. Process Biochem. 2011, 46(4), 916-922.
[20] Reyes J., Razo E., Gómez J. Simultaneous biological removal of nitrogen, carbon and sulfur by denitrification. Wat. Res. 2004, 38(14-15), 3313-3321.
[21] Campos J.L., Carvalho S., Portela R., Mosquera A., Mendez R. Kinetics of denitrification using sulphur compounds: Effect of S/N ratio, endogenous and exogenous compounds. Bioresource Technol. 2008, 99(5), 1293-1299.
[22] Oh S.E., Kim K.S., Choi H.C., Cho J., Kim I.S. Kinetics and physiological characteristics of autotrophic denitrification by denitrifying sulphur bacteria. Wat. Sci. Technol. 2000, 42(3-4), 59-68.
[23] Zhang Z., Lei Z., He X., Zhang Z., Yang Y., Sugiura N. Nitrate removal by Thiobacillus denitrificans immobilized on poly(vinyl alcohol) carrier. J. Hazard Mater. 2009, 163(2-3): 1090-1095.
[24] Fortuny M., Gamisans X., Deshusses M.A., Lafuente J., Casas C., Gabriel D. Operational aspects of the desulfurization process of energy gases mimics in biotrickling filters. Wat. Res. 2001, 45(17), 5665-5674.

Carlos A. S. Dinamarca has a PhD in Environmental Biotechnology and is currently Associate Professor in the department of Energy, Process and Environmental Technology at the Telemark University College, Porsgrunn-Norway. He has more than 12 year experience in the field of water quality, process and environmental analysis. Related to both industry (experience from aquaculture to oil production) and scientific research. His main research activity is in the field of anaerobic processes, where he has several publications. Carlos is interested in syntrophic anaerobic phenomena, autotrophic metabolism and fermentation linked to practical applications.
E-mail address: Carlos.Dinamarca@hit.no