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Evaluation of Decentralized Wastewater Treatment Systems: Sulfur as a Redox Indicator

Master Thesis

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Annotation:

In this study, 13 decentralized wastewater treatment systems representing 4 design types were monitored during the summer and winter season. Inlet and outlet samples were analyzed for common water-quality parameters. Attention was paid to redox indicators, especially to sulfur oxidation forms.

Prohlášení:

Prohlašuji, že svoji diplomovou práci jsem vypracovala samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

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1. LITERATURE SURVEY

1.1. Nutrients in role of pollutants

The most substantial surveyed contaminants in municipal and domestic wastewaters are suspended solids, organic matter, dissolved nutrients and pathogens. Wastewater treatment systems that have been developed for this type of wastewater thus focus on maximizing of their removal performance. Appropriate treatment can also improve physicochemical parameters such as pH, redox potential and electric conductivity or temperature which in extreme values can be harmful to the environment. Furthermore, wastewater might contain other pollutants that are not regularly looked at; they could include: surfactants, pharmaceuticals, personal care products, metals and others. Major macronutrients are in focus of our study therefore possible mechanisms of their disposal are described below.

Organic matter content is usually expressed as chemical oxygen demand COD, biological oxygen demand BOD or dissolved organic carbon DOC. High values of these quantities indicate substantial organic load. Organic matter decomposition causes oxygen depletion in aqueous environment, which is harmful to aquatic aerobic organisms. Organic matter can be degraded in both aerobic and anaerobic conditions. However, energy yield of aerobic respiration is much higher than fermentation so the decomposition rate in oxygen presence is greater (Schlesinger, 1997). Degradation of biopolymers is a multi-step process and wetland environment provides a wide scale of conditions. Therefore high functional diversity is attributed to wetland microbial assemblage. Generally, a product of one biochemical pathway is a source for another and this net ends up with simple inorganic molecules as final products of organic matter decomposition (CO_2, CH_4, H_20). Decomposition reactions using different electron acceptors occur under various conditions, which are specified later in the chapter of inorganic indicators of redox properties. They include aerobic respiration, denitrification, manganese, iron and sulfate reduction and methanogenesis.

Nitrogen is a crucial nutrient with many possible oxidation states (+5 to -3) and several inorganic forms (NH_4^+, NO_3^-, NO_2^-, gaseous N_2, N_2O, NO_2, N_2O_4 and NH_3). High nitrogen load can threaten balanced oligotrophic nitrogen-limited ecosystems by eutrophication; moreover, resulting from N transformations, pH can be affected. Different processes take place in variable conditions because of the many possible oxidation states of nitrogen. The number of possible N transformations in the environment is adequate to nitrogen numerous forms. However, only sequence of certain processes leads to complete N disposal from solution. Biochemical pathway frequently considered as major mechanism of N removal happens in three major steps (Vymazal, 2007):

- Mineralization = ammonification: organic N (amino acids) is turned into ammonia by aerobic, facultative or obligate anaerobic microflora.

oxidative: amino acids → imino acids → keto acids → NH_3
reductive: amino acids → saturated acids → NH_3

- Nitrification: ammonia is oxidized to nitrate, which is done predominantly by aerobic chemoautotrophs. Nitrification passes in two steps processed by different microbial groups via nitrite as a semi-product.

summary equation: $NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$

- Denitrification: nitrate is converted via nitrite, nitric oxide and nitrous oxide into N_2. Denitrifying bacteria are obligate anaerobes, mostly chemotrophs.

summary equation: $6(CH_2O) + 4NO_3^- \rightarrow 6CO_2 + 2N_2 + 6H_2O$

Other possible ways of N removal:

ANAMMOX = anaerobic ammonium oxidation where nitrate or nitrite serve as electron acceptor. This process probably plays more or less important role in N removal in constructed wetlands; according to experimentally found stoichiometry is ANAMMOX much less energy demanding than nitrification/denitrification

Ammonia volatilization is also possible way of N removal. However, according to conditions for equilibrium between gaseous and hydroxyl forms, losses are insignificant at $pH < 8$. Ammonia volatilization can be substantial at $pH = 9.3$ where ratio of both forms is 1:1. (Vymazal, 2007).

Phosphorus surplus input into oligotrophic ecosystem is, similarly to nitrogen, cause of eutrophication. Phosphorus occurs as phosphate in organic as well as inorganic compounds. Consequently, P removal does not depend on change of oxidation states. Main natural disposal mechanisms are sorption on substrate matrix, precipitation with Fe, Al, Ca and Mg, microbial or plant uptake and soil or peat accretion (Vymazal, 2007).

1.2. Wastewater treatment (WWT)

Pollution prevention is a duty of modern civilization to the environment. The progress in goods production and the increasing living standards call for modern technologies to treat the by-product and waste disposal. Waste management represents a substantial part of sustainable development of current society.

Wastewater from densely populated regions or big cities is usually transported via sewers and treated in large wastewater treatment plants based on mechanical pretreatment and biochemical nutrient removal generally by activated sludge. However, this solution is not economically viable for sparsely populated regions or seasonally used dwellings. Multiple alternative technologies have been developed in order to protect effluent-receiving water bodies, groundwater and soil from eutrophication and pollution.

In Denmark, additionally to sparse population, stringent legal constraints for discharged contaminant content (Brix and Arias, 2005) require efficient and simple decentralized wastewater treatment systems that can be used for remote single households or small communities.

1.2.1. Types of biological WWT systems

Objects of study

Among the accepted decentralized systems to treat wastewater in Denmark, we chose four types which are frequently used as secondary treatment following mandatory primary treatment in sedimentation tank (see Fig. 1 in chapter 3.2):

- Horizontal subsurface flow constructed wetlands (HFCW)
- Vertical subsurface flow constructed wetlands (VFCW)
- Biological sand filters (BSF)
- Compact biofilters (BF) filled with light expanded clay aggregates (LECA) – in two variations

Constructed wetlands of present design have been used since pilot system tested by Seidel in 1956 (cited in Brix, 1994), are extensively used and more than 5000 systems have been built. Infiltration, percolation in biological sand filters is a traditional design used for more than hundred years (Healy et al., 2007), while biofilters with LECA filling represent the newest technology originated in Norway (Johansson, 1997).

Constructed wetlands consist of a media filled bed, planted with wetland vegetation. The two types differ in water regime; HFCW filter bed is constantly saturated with water that permeates the substrate in horizontal direction (Brix, 1994), whereas in VFCW, wastewater is intermittently homogenously loaded on the top of the substrate and the water trickles down through an unsaturated filter bed which. Similarly, BSF includes unsaturated filter bed, however, it is unplanted. Compact BFs comprise of a fiber glass dome, half-filled with LECA where water is intermittently pressured on the surface by sprinkler nozzle. Consequently, the water percolates through the filling and in type Natusan® is ready to be discharged while in Filtralite® continues horizontally through another saturated bed with LECA substrate (Arias and Brix, 2006).

Natural biochemical processes participating in contaminant removal occur under different conditions relevant to living requirements of appropriate microbe or microbial assemblage. Nitrifying bacteria thrive in the presence of oxygen; on the other hand, denitrifying microbes flourish in anaerobic environment. Hence, the whole gradient of conditions is needed for efficient and complete performance of system (nitrification-denitrification). Unsaturated beds provide mostly aerobic conditions so they can reach high efficiency in nitrification. On the contrary, anaerobic conditions prevail in saturated bed and thus promote denitrification, but cannot occur effectively if the nitrification step is not achieved. Even if both extremes are usually present within each system, one of them is always dominant.

If the basic design of systems cannot meet the strict legal effluent discharge constraints, some improvements can be implied to reach sufficient performance (Brix and Arias, 2005). Higher N removal efficiency can be achieved by recirculation of treated effluents (nitrified). A part of the nitrified effluent is recycled back to the sedimentation tank where N disposal is completed, to some extent, by denitrification process (Brix et al., 2003; Marti et al., 2003; Arias et al., 2005). P removal in these systems is dependent on the media capacity to bind, adsorb or precipitate P. The capacity is restricted and not sustainable through time. In order to enhance P removal efficiency, chemical precipitation device can be installed in sedimentation tank (Brix and Arias, 2005). Polyaluminum chloride (PAX 14) is dosed in the inlet and clot sediments in the pretreatment.

Other biological WWT systems

Free water surface constructed wetlands (FWS CW) are based on flooded substrate and can use all types of vegetation mentioned later in the chapter Plants as part of wastewater treatment. However, the water surface is exposed to the atmosphere. Such systems have been built as polishing steps for previously treated wastewater (Brix, 1994; Vymazal et al., 2006).

Hybrid systems including saturated as well as unsaturated stages combine advantages of both horizontal and vertical subsurface flow. In result, they have been designed particularly for more efficient nitrogen removal (Vymazal, 2005; Vymazal et al., 2006). Modern pioneer constructed wetlands by Seidel at the Max Planck Institute, Krefeld, Germany in 1956 (in Brix, 1994) consisted of several parallel intermittently loaded VF beds in two stages followed by two HF beds in series. Similar VF-HF systems in modifications have been used over the Europe and nowadays are getting more attention. Two-stage type later introduced by Johansen and Brix (1996) comprises of first large HF bed and second small VF bed. HF-VF system is supplemented with recirculation circuit in order to denitrify effluent from VF. Other types of hybrid systems include also FWS CW and might be combined even with conventional systems. E.g. wastewater treatment plant described by Maehlum (1995) consists of anaerobic pond, aerated lagoon, HFCW and FWS CW, respectively. In fact, also design of compact biofilter Filtralite® takes the advantage of shift from unsaturated dome to following saturated filter bed (Arias and Brix, 2006).

Willow systems have been developed in Denmark for on-site domestic wastewater treatment (Gregersen and Brix, 2001; Brix and Gregersen, 2002). The system consists of impermeable basin filled with soil and planted with willows (*Salix* sp.). These facilities have the advantage of no discharge because all the received water is lost to the atmosphere via evapotranspiration. Size of planted basin is based on local specific climatic characteristics as annual sum of precipitation or evapotranspiration rate and on water balance. Moreover, a part of willow trees is harvested annually in order to keep young stems and high demand of nutrients and water. Thus the nutrients incorporated into the biomass are removed and the harvested trees can be used as fuel, material for wickerwork, etc.

Substrate materials and clogging

Different materials are used as filling media. HFCW have been traditionally filled with soil or gravel (Brix, 1994). However, requirements for enhanced phosphorus removal provoked experiments on more efficient substrate; currently, sand (Arias et al., 2001), shellsand, wollastolite, LECA (Johansson, 1997), cracked limestone or crystalline slug are utilized, as well.

Hydraulic conductivity of substrate is affected by size of grain and porosity and impacts hydraulic retention time (HRT) of wastewater in unsaturated or saturated bed. HRT expresses the time when treated water stays in touch with the substrate and the biofilm growing on the substrate. Nutrient removal, especially in the way of sorption, also depends on chemical composition of the substrate material. Negatively charged surface of the substrate (e.g. clay) increases cation exchange capacity and vice versa, which helps to decline electrical conductivity in treated water (Jarboui et al., 2008). Phosphorus removal is promoted by presence of Ca^{2+}, Fe^{3+} and Al^{3+}. Available Ca^{2+} results in calcium phosphate precipitation at pH > 7 while content of Al^{3+} and Fe^{3+} ions improves phosphorus sorption at lower pH (Arias et al., 2001). Nevertheless, the phosphorus binding capacity is limited by abundance of these ions on substrate surface and the possibility of contact with the treated water.

Substrate clogging is naturally coupled with the process of filtration and biofilm growth and might be a serious problem for filter beds. Clogged layer of substrate can cause hydraulic shor circuits or ponding which substantially changes conditions especially in unsaturated filter bed. In any case, ponding obstructs wastewater contact with substrate; consequently, pollutant removal might be limited (Langergraber et al., 2003).

1.2.2. Multiple use of biological WWT systems

Biological treatment systems have been used for quality improvement of various types of wastewater mainly for small but also for large sources of water pollution. There are many examples of treating municipal and domestic wastewater, industrial (petrochemical and chemical factories, tannery, textile, pulp and paper industries, abattoirs and food processing, winery and distillery) and agricultural effluents (Vymazal, 2009), landfill leachates (Bulc, 2006) and storm water runoffs (Weiss et al., 2006), as well as wastewaters with high content of endocrine disruptors or pharmaceuticals (Matamoros et al., 2009).

1.3. Plants as part of wastewater treatment

Wetland and aquatic plants suitable for various constructed wetlands are generally divided in following functional groups (reviewed in Vymazal, 1998):

- Emergent macrophytes - originally littoral species - tolerate saturated and even submersed substrate (sp. frequently used in Europe: *Phragmites australis, Phalaris arundinacea, Typha latifolia, Glyceria maxima*)

- Floating-leaved macrophytes - rooted deep in submersed substrate (*Nuphar lutea, Nymphaea odorata*)

- Submerged macrophytes – grow in water column pervaded with light (*Ceratophyllum demersum, Myriophyllum spicatum*)

- Freely floating macrophytes – not rooted in sediment (*Lemna minor, Eichhornia crassipes*)

Wetland plants are well-adapted to flooding conditions that cause lack of oxygen in rhizosphere. Aerenchyma, tissue permeable for gasses, enables active gas exchange between above- and below-ground parts, which can happen via simple diffusion (Armstrong et al., 2000), pressurized throughflow or convective flow (Armstrong et al. 1991; Bendix et al., 1994). Even if barriers from dense cells are present in hypodermis oxygen is concurrently radially lost from roots, due to active apical growth especially from their tips (Armstrong et al., 2000). Radial oxygen loss (ROL) is thus creating a microhabitat for aerobic microorganisms on the very surface of roots or in immediate vicinity.

Nevertheless, it is questionable how substantial is the oxidative effect of roots in comparison with oxygen amount required for biodegradation (Brix, 1997). However, Síma et al. (2009) proved higher abundance of oxidized forms of different redox indicators in the rooted layer of HFCW vegetated beds, in the contrary to deeper layers. Dušek et al. (2008) also recorded periodical diurnal as well as annual changes during *in-situ* measurement of redox potential in HFCW. Regime of redox potential changes corresponded to plant photosynthetic activity driving the convective flow. Choi et al. (2006) focused on wetland sediment cycling of sulfur and metals and revealed that oxygen released by plants via roots into the sediment during the growing season reoxidized metal sulfides and caused mobilization of metals and sulfide oxidative change to dissolved sulfate form. Langergraber et al. (2003) compared planted and unplanted pilot scale VFCW but no significant difference in nitrification/denitrification was noticed. On the other hand, Kaseva (2004) recorded better performace of vegetated filter beds of HFCW in ammonia and COD removal than unvegetated. However, such disagreement is explainable by design difference between VFCW and HFCW. It is expected, that plant effect is more limited in intermittently loaded unsaturated bed if oxygen release is the main topic. It is suggested that plants will contribute in maintaining hydraulic characteristics.

Additionally, to O_2 release, Karathanasis et al. (2003) suggested large surface and released organic exudates, carbon source for microbes, as significant effects of rhizosphere that has to be taken into account. Data presented by Edwards et al. (2006) support this hypothesis when reporting correlation between growth of below-ground plant biomass and microbial nutrient accumulation. Additionally, nutrient plant uptake has been investigated in many studies as removal mechanism. It is efficient in tropical countries (Konnerup et al., 2009) but in temperate climate, there is not sufficient amount of biomass that can be produced so that the nutrient removal would be substantial (Edwards et al., 2006; Vymazal and Kröpfelová, 2009).

Summary of plant impacts on wetland soil processes:

- ROL providing aerobic microhabitat for microorganisms
- Organic root exudates as easily degradable carbon source
- Large surface for microbes set-up or filtration
- Nutrient uptake

1.4. Redox potential

1.4.1. Quantity, unit, traditional way to measure

Redox potential (*E*, frequently stated in unit mV) is physicochemical property of water which expresses capability of aqueous solution to accept or provide electrons (to be reduced or oxidized). *E* gradient ranges from the highest positive values in oxygen saturated solution to the lowest negative values in the most anaerobic conditions (Schlesinger, 1997). *E* can be measured as balanced potential between indicator electrode made from precious metal (Pt, Au) and standard hydrogen electrode.

1.4.2. Inorganic indicators of redox properties

Wetlands are characterized by long-lasting oxygen lack deeper in flooded soils/sediments and wide range of redox conditions within the system depending on the place. Redox properties vary a lot in rhizosphere and upper layers. Oxygen supply throughout the water column or the soil profile depends on its diffusion from atmosphere and radial oxygen loss from roots; however, none of these two has long reach. Oxygen is rapidly used in microbial respiration as electron acceptor of the highest reducing potential. Limited oxygen supply is generally overwhelmed by available amount of assimilable organic compounds. Microbial catalyzed oxidation of organic matter in oxygen absence utilizes sequence of other inorganic electron acceptors, which are basically systems of different oxidation state forms of one element susceptible to redox potential changes. Decreasing redox potential goes along with descending order of electron acceptors: NO_3^-, Mn^{IV}, Fe^{III}, SO_4^{2-} and last process is methanogenesis. As organic matter is being decomposed, one electron acceptor after another is reduced. Traditional hypothesis represented e.g. by Vymazal (1998) implies that organic matter oxidation is controlled by this sequential reduction chain. In the contrary, a new study by Alewell et al. (2008) infers that wetland sites with large source of dissolved organic carbon as electron donor do not follow the sequential reduction chain because no competition for carbon source is triggered. Consequently, processes thought to be strictly divided appear simultaneously because processes attributed to low redox potential are not out-competed. This approach considers sequence of electron acceptors to occur only in sites with low organic carbon content.

Further following the traditional hypothesis, intervals of redox potential have been experimentally found for each process (Tab. 1). Consequently, determination of oxidized/reduced form concentration ratio of a suitable electron acceptor can provide information about redox properties. Nevertheless, other elements, whether linked with electron acceptors cycles (metals) or other substances sensitive to redox changes can be used as redox indicators. Biogeochemical balance of all compartments of wetland or sediment redox system dynamically follows ongoing outer changes. Thus, determination of inorganic redox indicators gives a view on long-term state in the environment in comparison to instant potentiometrical quantity *E*.

Table 1: Redox processes coupled with microbial respiration

Microbially catalyzed process	Scheme of change	Range of *E* (mV)[1]	Range of *E* (mV)[2]	Energy yield from glucose oxidation (kJ mol^{-1} glucose)[3]
aerobic respiration	$O_2 \rightarrow H_2O$	> 300	320* to 700	2 887
denitrification	$NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$	100 to 300*	220 to 340*	2 721
manganese reduction	$Mn^{4+} \rightarrow Mn^{2+}$	100 to 300*	220 to 120	2 027
iron reduction	$Fe^{3+} \rightarrow Fe^{2+}$	-100 to 100	120 to -150	441
sulfate reduction	$SO_4^{2-} \rightarrow S^{2-}$	-200 to -100	-150 to -250	382
methanogenesis	$CO_2 \rightarrow CH_4$	< -200	-250 to -300	

[1] Reddy and D´Angelo (1994) cited in Vymazal (1998); [2] Gambrell and Patrick (1978) in Kadlec and Knight (1996); [3] Reddy and DeLaune 2008; [1] and [2] values are related to pH=7; *overlapping borders

General terminology for redox conditions arose from oxygen availability. Dissolved oxygen is freely available in an environment designated as aerobic (*E* > 300 mV), while any dissolved oxygen is absent in anaerobic conditions (E < -100 mV). The transitional stage where dissolved oxygen is close to zero is termed as anoxic (Kadlec and Knight, 1996).

1.4.3. Importance of redox indicators

As explained above, some elements respond to redox conditions in sediment. Their cycles are coupled with the distribution of dissolved oxygen and sulfide concentrations which depend on organic carbon loading and microbial decomposition rate. In study of Macdonald et al. (2008), abundance of redox sensitive elements (Mn, Cd, U, Re, Mo) has been measured to evaluate impact of municipal wastewater loading on coastal sediments.

Lewis et al. (2007) focused on short-term description of physical mixing processes and resulting vertical distribution of redox indicators (sulfide, Mn^{2+}, Fe^{2+}) in water column of Chesapeake Bay. The study confirms delay of changes in redox sensitive chemical parameters behind ambient oxygen level. They suggest redox tracers data to be used to infer previous storm events injecting oxygen into bottom water while there is no evidence of it in surface water anymore.

Directly related to alternative wastewater treatment, Fe, S, Mn and N oxidation forms were monitored in a reed bed of HFCW in several points from inflow zone to outflow zone to evaluate redox conditions that are crucial for organic matter biodegradation and nutrient removal (Diáková et al., 2006; Šíma et al., 2007; Šíma et al., 2009).

1.5. Sulfur forms and its role in life- and nutrient cycles

1.5.1. Global sulfur cycle

Lithosphere represents major global pool of sulfur of which main part occurs in sedimentary rocks mostly as FeS_2 – pyrite (Schlesinger, 1997). The second largest reservoir is dissolved as SO_4^{2-} in oceans and consequently, its chemical precipitation or biogenic formation of sedimentary rocks contributes back to the lithosphere mostly as $CaSO_4$ (gypsum) and $MgSO_4$. In comparison to that, only little reservoirs are represented by atmosphere, which contains evaporates from sea and emissions from volcanoes, soil and biomass. Sulfur is essential to all organisms but in the contrary to other nutrients as C and N, it is in most cases sufficiently abundant in all environments. An exception can happen in freshwater wetlands or flooded soils as mentioned below.

Human activities also inconsiderably contribute to global sulfur cycle. Due to combustion of fossil fuels naturally containing sulfur, SO_2 is emitted into the atmosphere. Sequentially, SO_2 in reaction with atmospheric moisture produces H_2SO_4 which is the main cause of acid rain. Metal ore mining of metal sulfide minerals is another substantial anthropogenic intervention where metal sulfides are exposed to oxidizing conditions and consequently, acid mine drainage characterized by dissolved metals, sulfates and high acidity is produced (Gazea et al., 1996). Content of sulfates in drinking water, namely $CaSO_4$ and $MgSO_4$, causes permanent water hardness which, if undesired, can be removed by chemical precipitation.

1.5.2. Sulfur transformations

Sulfate SO_4^{2-} is a predominant inorganic sulfur species in oxidizing conditions whereas sulfur occurs as elemental sulfur S^0 or sulfides S^{2-} in reducing environment. However, sulfur has also other natural less abundant species:

- sulfite SO_3^{2-}, thiosulfate $S_2O_3^{2-}$, tetrathionate $S_4O_6^{2-}$
- gaseous forms: SO_2, H_2S, dimethylsulfide DMS, dimethyldisulfide DMDS, methanethiol MeSH, carbonyl sulfide COS and carbon disulfide CS_2

Due to many possible oxidation states, it acts also as an electron transporter in microbial respiration pathways (chapter 4.2). Sulfate reduction can run under anaerobic conditions if redox potential decreases to the range between -100 and -200 mV (Lovley, 1991; Vymazal, 1998) or -150 and -250 mV (Kadlec and Knight, 1996). Nielsen et al. (2005) reported sulfide production even at -50 mV but it is exceptionally high in overall literature. At that point, pool of oxidized forms of electron acceptors with energetically higher potential as O_2, NO_2^- /NO_3^-, Fe^{III} and Mn^{IV} have been depleted and changed to reduced forms. According to sulfur distribution diagram, sulfur in oxygen absence occurs as volatile H_2S in pH lower than 7 and thus can be emitted into the atmosphere. However, form of HS^- prevails in pH higher than 7 (Fig. 1). If metals are present in conditions of low redox potential, sulfur precipitates as metal sulfides (S^{2-}), which is a principle of metals immobilization from polluted water. Precipitation with iron has been observed in domestic wastewater treatment (Nielsen et al., 2005), in freshwater wetlands (van der Welle, 2006), as well as in flooded soils (Prietzel et al., 2009). Also other trace elements (As, Cd, Co, Cu, Mn, Ni, Pb, Zn) take part in sulfides precipitation. Their removal happens as co-precipitation with FeS, adsorption on FeS or formation of separate sulfides (Huerta-Diaz et al., 1998; Canavan et al., 2007). Sulfur is highly reactive under anaerobic conditions and, theoretically, binds almost all metals in sulfides, except of Au and Pt (Sturman et al., 2008). Contrary, bioleaching of metals using the microbial sulfur cycle can be used as a mechanism for remediation of soils polluted by heavy metals (Tichý, 1998).

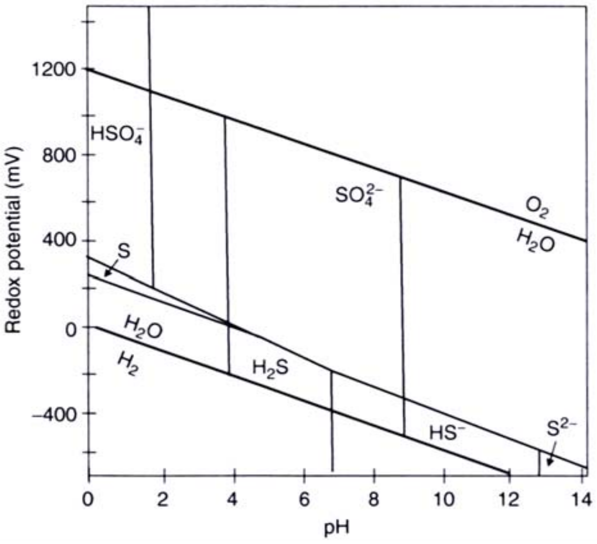

Figure 1: Distribution diagram of sulfur: sulfur forms occurring in different pH and redox conditions (according to Reddy and DeLaune, 2008)

Sulfur is incorporated in life cycle as an essential element of biomass, especially as a constituent of amino acids. However, sulfides are toxic for plants as well as for animals in the environment (Gonzalias et al., 2007; van der Welle, 2008). The toxicity is caused whether directly as inhibiting important biochemical reactions or indirectly as a lack of essential amount of biogenic sulfur and metals (Zn, Cu) resulting from metal sulfides precipitation and thus immobilization. Sulfide toxicity is harmful also for other microbial species taking part in nutrient removal (Gonzalias et al., 2007; Wiessner et al., 2008). Therefore some techniques have been tested and applied to reduce sulfides generating in wastewater treatment systems (discussed below in part about S versus NO_3^- and Fe interactions).

1.5.3. Microbial pathways of sulfur cycle

Overview of microbial sulfur transformations (Reddy and DeLaune, 2008):

- **Mineralization** is general transformation where sulfur is relieved from organic compounds and transferred into inorganic forms. Sulfur mineralization is naturally bound with the overall decomposition of organic matter.

- **Dissimilative sulfate reduction** ($SO_4^{2-} \rightarrow S^{2-}$) is served by sulfate as electron acceptor in simple organic compound oxidation.
- **Dissimilatory sulfur reduction** ($S^0 \rightarrow S^{2-}$) is analogy to the previous one.
- **Assimilative sulfate reduction** where sulfate is used as nutrient source and thus reduced to organic sulfhydryl groups (R-SH) is usual nutrient uptake of prokaryotes, fungi, eukaryotic algae and vascular plants.
- **Sulfur compounds oxidation** is group of processes where different reduced sulfur compounds serve as energy source for CO_2 fixation.

Most abundant pathways in detail

Dissimilative sulfate reduction: In dissimilative sulfate reduction, sulfate acts as an electron acceptor and is utilized in energy production by obligate anaerobes. Carbon source for sulfate reducing bacteria (SRB) are simple organic compounds as lactate, pyruvate, ethanol, malate, acetate and other short-chain organic acids.

Sulfate reducers of group I: incomplete oxidizers (*Desulfovibrio*, *Desulfomonas, Desulfotomaculum*) oxidize lactate while producing acetate.

Sulfate reducers of group II: complete oxidizers (*Desulfotomaculum, Desulfobacterium, Desulfococcus, Desulfonema, Desulfocacrcina*) oxidize acetate while releasing CO_2.

SRB are not able to decompose organic substrate of higher molecular weight therefore they thrive in presence of fermenting bacteria. SRB are unique among the others in c_3 cytochrome serving as electron carrier in their biochemical pathways. However, sulfate reduction is inhibited by presence of electron acceptors of higher reduction potential because it provides only about 13% of energy yield under aerobic conditions (Tab. 1); in result, SRB are outcompeted by microbes with more efficient strategy. Similarly, methanogenic bacteria are suppressed by SRB until most of sulfates are reduced. Moreover, oxygen has been found to be toxic for SRB (Reddy and DeLaune, 2008). On the other hand, Fortin et al. (2000) observed active SRB in oxic layer of sediment, which suggests that some SRB are oxygen-tolerant or that anoxic microenvironments were present. These results support new approach to sequential reducing chain (Alewell et al., 2008) discussed in chapter dealing with redox potential. Anyway, no substantial rate of abiotic sulfate reduction has been ever reported in any natural environment under temperature 200°C and higher pressure (Reddy and DeLaune, 2008).

Some studies imply that sulfate reduction rate varies throughout the year; higher sulfate reduction rates were observed in summer, lower in winter (Howarth and Giblin, 1983). This trend is probably dependent on oxygen solubility in water. Oxygen is easier dissolvable in cold water and thus its diffusion has longer reach down in the profile where sulfate reduction is inhibited; at warm conditions vice versa. Moreover, oxygen consumption by decomposers is faster in summer, which promotes the summer lack of oxygen in sediment. However, Fortin et al. (2000) reported SRB thriving in colder months with highest populations in winter at the temperature 1°C.

Sulfur compounds oxidation: In the contrary to sulfate reduction, reduced sulfur compounds can be oxidized in biotic as well as abiotic way. Reduced sulfur forms play a role of electron donor in microbial pathways. Sulfur compounds oxidation is located on interfaces:

- in oxic-anoxic interface, abiotic and chemolithotrophic oxidation can take place. Sulfur oxidizing bacteria (SOB) use reduced sulfur as energy source for CO_2 fixation. Colorless sulfur bacteria (*Thiobacillus, Beggiatoa*) utilize oxygen as an electron acceptor. However, nitrates play this role in respiration of nitrate-reducing sulfur-oxidizing bacteria (NR SOB).

- in light-dark interface in oxygen absence, anoxygenic photosynthesis is likely to occur. Involved green (*Chlorobium*) and purple (*Chloromatium*) sulfur bacteria use light as energy source while sulfides act as electron donors for CO_2 fixation.

Sulfide oxidation can happen in a few steps:

$$HS^- \rightarrow S^0 \rightarrow S_2O_3^{2-} \rightarrow S_4O_6^{2-} \rightarrow SO_3^{2-} \rightarrow SO_4^{2-} \qquad \text{(Paul and Clark, 1996)}$$

Different SOB species can utilize partial processes, e.g. green and purple bacteria deposit elemental sulfur extracellularly and intracellularly, respectively, as a product of sulfide oxidation. This resource can be further oxidized in case of later sulfides lack (Sturman et al., 2008). On the other hand, chemolithotrophic bacteria can use besides hydrogen sulfide also elemental sulfur or thiosulfate as an electron donor (Reddy and DeLaune, 2008).

Interactions with contiguous processes

Sulfate reduction is bound with bacterial nitrate, manganese and iron reduction as well as with methanogenesis, first because they are immediate processes competing for carbon sources and second because their products occasionally interact (Sturman et al., 2008).

Denitrification

NO_2^- as first-step-product of denitrification inhibits SRB (Sturman et al., 2008). On the other hand, NR SOB beneficially connect nitrogen and sulfur transformations. They thrive on microhabitats where sulfides diffuse from zone of sulfate reduction to layer rich on nitrate. Experiments with additional nitrate supply in order to control sulfide generation in WWTPs have been conducted using NR SOB genera *Thiomicrospira denitrificans* (Garcia de Lomas et al., 2007) and *Pseudomonas stutzeri* (Aguilar et al., 2008).

Manganese reduction

Manganese sulfide is more soluble than iron sulfide ($pK_s(FeS) = 17.2$; $pK_s(MnS) = 9.6/12.6$ - different forms). Therefore MnS precipitates under higher Mn^{2+} concentration (in comparison to Fe^{2+}) where sulfide concentration is constant.

Iron reduction

Fe^{II} as iron reduction product rapidly reacts with S^{2-} followed first by formation of amorphous black clot FeS. Further contact with sulfide leads to formation of Fe_3S_4 (greigite) and finally FeS_2 (pyrite). Pyrite is the most stable and re-dissolution resistant form accountable for most of Fe and S immobilization (Sturman et al., 2008). Bacterially assisted pyrite transformation has been proved as more efficient than abiotic processes (Donald and Southam, 1999). Once FeS appears at aerobic conditions its half-life period is 11.7 h whereas half-life period of dissolved sulfide is typically less than 1 h (Nielsen et al., 2005). Microbial sulfur-oxidizing Fe^{III} reducers are able to utilize elemental sulfur as electron donor and Fe^{III} as electron acceptor under aerobic conditions (Lovley, 1991; Nielsen et al., 2005) (*Thiobacillus thiooxidans, Sulfolobus acidocaldarius*). Moreover, dissolved sulfides have a potential to reduce Fe^{III} as well as Mn^{IV} even abiotically. However, sulfate reduction and methanogenesis are restricted where Fe^{III} is being used as electron acceptor in organic matter oxidation resulting from competition for carbon sources (Lovley, 1991). Consequently, addition of Fe^{III} salts was suggested as sulfide control strategy in sewer networks (Nielsen et al., 2005).

Methanogenesis

Sulfate reduction is a dominant organic matter respiration pathway in coastal sediments. Due to unlimited pool of sulfate as electron acceptor in sea water, sulfate reduction restrains methanogenesis and thus prevents CH_4 emissions from salt marshes (Howarth and Giblin, 1983). However, in deeper anaerobic sediment on a methane-sulfate transition zone, originating CH_4 can be used by SRB as highly energetic substrate. This process of anaerobic methane oxidation has been found deep in seabed (Niewöhner et al., 1998).

1.5.4. Intentional removal of sulfur from wastewater

Some industry wastewaters carry high load of sulfur (textile industry effluents, acid mine drainages etc.). In these cases, wastewater treatment is besides the usual performance parameters also focused on sulfur removal to prevent sulfur pollution of river and consequently sea, which can result in further decrease of redox potential in sediments (Winter and Kickuth, 1989). Satisfactory 82% sulfur removal efficiency from textile industry effluent was achieved by traditional Root Zone Method which is an original expression for HFCW (Winter and Kickuth, 1989). Other methods have been applied according to the nature of desired process, e.g. nitrates or Fe^{3+} salts were added as sulfides forming control in order to avoid sulfide toxicity or occasional undesired smell caused by emitted H_2S; as already mentioned above (Nielsen et al., 2005; Garcia de Lomas et al., 2007; Aguilar et al., 2008). It can be summarized that following mechanisms of sulfur removal take place in wastewater treatment systems: (i) H_2S emission, (ii) precipitation with metals and (iii) plant and microbial uptake.

1.6. References

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2. OBJECTIVES

This survey has been conducted to compare performance of four selected types of systems for decentralized wastewater treatment in winter and summer. In addition, we also assessed redox conditions at inlets and outlets and the impact on performance.

Partial aims:

- Determine main sulfur oxidation forms (sulfides, sulfates) in samples and use their proportion as an indicator to describe redox conditions at the inlets and outlets
- Assess removal efficiencies for TSS, COD, NH_4^+, PO_4^{3-}, which are legally limited; additionally, determine nitrates/nitrites, total sulfur and metals (Cd, Ni, Cr, Cu, Zn)
- Measure in situ physicochemical parameters as temperature, oxygen saturation, pH, conductivity and redox potential to gather supplementary data
- Collect samples in summer and winter to evaluate seasonal impact on all monitored parameters

3. MANUSCRIPT DRAFT

Evaluation of decentralized wastewater treatment systems: sulfur as a redox indicator

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