Review

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Electron donors for autotrophic denitrification

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Abstract

Autotrophic denitrification (AuDen) is an efficient, convenient and eco-friendly biological process for the treatment of nitrate-contaminated organic-deficient waters. AuDen can be applied as a unique process or complement the conventional denitrification with organics, reducing the risk of organic carbon breakthrough in the effluent and formation of undesirable byproducts downstream (e.g. trihalomethanes). A wide range of inorganic compounds can act as electron donor for AuDen. The most used electron donors include hydrogen gas and reduced sulfur compounds, i.e. elemental sulfur, sulfide and thiosulfate. Recently, the denitrification potential of certain contaminants (such as sulfite, thiocyanate, arsenite and manganese) and inorganic wastes (such as biogenic elemental sulfur from biogas upgrading) has been revealed and attracted interest for developing technologies that combine nitrate removal with water detoxification. This paper critically reviews the state of the art of the most used electron donors for AuDen and highlights recent advances on the application of novel inorganic compounds, reactor configurations and microorganisms to support denitrification. Criteria and guidelines for the selection of a suitable electron donor are provided.

Keywords

autotrophic denitrification; denitrifying bacteria; electron donors; hydrogen; nitrate removal; reduced sulfur compounds.

1 Introduction

Nitrate (NO_3^-) is one of the most common water contaminants and a serious environmental concern in many areas of the world [1]. Over the last decades, NO_3^- concentrations in American and European rivers have dramatically increased, resulting in up to 15-fold higher concentrations than 100 years ago [2]. NO_3^- contamination of the aquatic environments is mainly due to anthropogenic activities, e.g. the massive use of fertilizers in agriculture [3] and nitrogen-based chemicals (e.g. ammonium nitrate and nitric acid) in the industry [4] as well as intense livestock farming and on-site sewage disposal [5]. NO_3^- in drinking water is a threat to human health, since an excessive intake increases the risk of severe diseases such as infantile methemoglobinemia [6], non-Hodgkin's lymphoma [7], gastric cancer [8] and cardiac diseases [9]. In order to prevent potential health issues linked to nitrate contamination, the European Commission (Drinking Water Directive 98/83/EC) and the US Environmental Protection Agency [10] have set the maximum admissible NO_3^- concentration in drinking water to 11.3 and 10 mg $N\text{-}NO_3^-$ L^{-1}, respectively.

Biological denitrification is a more cost-effective and environmentally friendly NO_3^- removal method than physicochemical processes such as ion exchange, adsorption and reverse osmosis as it requires a limited amount of energy and chemicals [11]. On the other hand, denitrifying microorganisms can be very sensitive to the physical and chemical parameters of the contaminated water, which cannot be too far from the optimal values. Nevertheless, biological denitrification has been widely used for the treatment of several nitrate-rich industrial wastewaters [12–14]. Additionally, biological denitrification may be intended to oxidize excess organic matter and/or toxic pollutants such as hydrogen sulfide (H_2S),

trivalent arsenic (As^{3+}) or cyanide (CN^-), thus reducing their toxicity or completely remove these pollutants.

Denitrifying bacteria can be heterotrophs, autotrophs or mixotrophs, depending on whether they gain their energy from the oxidation of organic or inorganic compounds, or both. Methanol, ethanol and acetic acid are readily degradable organic substrates particularly suitable for the denitrification of drinking water [15,16]. In the last years, hydrogen gas (H_2) and reduced inorganic sulfur compounds (RISCs), such as elemental sulfur (S^0), sulfide (S^{2-}) and thiosulfate ($S_2O_3^{2-}$), have been widely used as electron donors for autotrophic denitrification (AuDen) in batch and continuous systems [17]. The effectiveness of pyrite (FeS_2) [18], thiocyanate (SCN^-) [19], ferrous iron (Fe^{2+}) [20] and As^{3+} [21] as energy sources for denitrifiers has been explored as well. The use of sulfite (SO_3^{2-}) as electron donor for AuDen has recently gained increasing attention as well [22].

The choice of the electron donor strongly affects the biological denitrification kinetics. The electron donor concentration, microbial affinity, hydrophilicity and particle size must be carefully evaluated in order to achieve the desired denitrification rate and efficiency [23,24]. Additionally, the choice of the inorganic carbon source, i.e. carbonate (CO_3^{2-}), bicarbonate (HCO_3^-) or carbon dioxide (CO_2), is strictly related to the selected electron donor, microbial culture and reactor configuration [25].

The past few years have seen an increasing interest and important advances in investigating the feasibility and effectiveness of novel electron donors for AuDen [22,26], combination of electron donors for mixotrophic denitrification [27] and improving the denitrification kinetics and process feasibility under harsh conditions [28–30].

This paper overviews the electron donors for autotrophic denitrification by discussing chemical and biological aspects of twelve different inorganic compounds, i.e. hydrogen gas,

elemental sulfur, sulfide, thiosulfate, sulfite, biogenic elemental sulfur, pyrite, thiocyanate, zero-valent iron, ferrous iron, arsenite and manganese. Their advantages, drawbacks, bioreactor applications and criteria for appropriate selection are discussed as well.

2 Overview of denitrification

2.1 Microbiology and reaction stoichiometry

Biological denitrification is a respiratory process (also known as dissimilatory NO_3^- reduction to N_2) occurring in four sequential steps catalyzed by specific enzymes (**Fig. 1**). Unlike heterotrophic denitrification, AuDen produces acidity except when H_2, S^{2-}, SCN^- or Mn^{2+} are used as electron donors (**Table 1**). Autotrophic denitrifiers are randomly distributed within the α-, β-, γ-, and ε-*Proteobacteria* and have been isolated from both natural habitats and anthropogenic environments [31]. **Table 2** provides a non-restricted list of identified *Proteobacteria* able to support AuDen with various inorganic electron donors.

Fig. 1.

Table 1.

Table 2.

2.2 Environmental parameters

Anoxic conditions are required for denitrification since dissolved oxygen (DO) can inhibit the synthesis of the denitrification enzymes (**Fig. 1**). In particular, nitrite reductase (*NiR*) and nitrous oxide reductase (*NoS*) are more sensitive to O_2 than nitrate reductase (*NaR*) and their inhibition at high DO concentrations results in the accumulation of denitrification intermediates, i.e. NO_2 and nitrous oxide (N_2O) [73–75]. Denitrification usually occurs at redox potentials (ORP) between -100 and +100 mV, although stable and complete AuDen has been reported at ORP values lower than -200 mV as well [76]. However, ORP values below -250 mV should be avoided as they may trigger the bacterial competition for common electron

donors, e.g. H_2 [77]. Temperatures around 30°C are usually optimal for denitrifiers, while lower temperatures may significantly reduce the denitrification rate and efficiency [78]. Acidic pH values also limit the denitrification rate and increase the concentration of harmful intermediates such as N_2O [79,80]. Excess pH increase and water hardness can also affect bacterial metabolism and cause mineral precipitation [81].

2.3 Inorganic electron donors for denitrification

Drinking water from rivers, lakes, aquifers and man-made reservoirs is commonly organic-deficient. Similarly, wastewaters from industrial activities such as mineral processing, electroplating, semiconductor manufacturing and power plants contain negligible concentrations of organic compounds [11]. As a result, the addition of external organic carbon may be required in conventional wastewater treatment plants. The addition of supplemental organic carbon may result in a higher sludge production and potential formation of extremely toxic organic compounds during chlorination, such as trihalomethanes (THMs) and haloacetic acids (HAAs) [82]. Thus, performing denitrification with autotrophic bacteria is an appealing alternative.

Table 3 lists the denitrification rates observed with different inorganic electron donors and bioreactor systems. The choice of the electron donor and bioreactor has a substantial impact on the denitrification kinetics. High denitrification rates have been achieved using H_2 or RISCs as electron donor [83,84], whereas much lower rates have been observed with other inorganic compounds [85,86].

Sulfur-containing contaminants such as H_2S, $S_2O_3^{2-}$, SO_3^{2-} and microbially produced S^0 can be removed with NO_3^- in wastewater treatment plants by coupling biogas desulfurization with denitrification [87]. Minerals such as S^0 and Mn have low cost and are readily available as mined materials. Additionally, AuDen can also be applied to oxidize toxic compounds such

as As^{3+} and SCN^-, which can occur both in drinking and process water due to natural biogeochemical reactions [88] and mining activity [89], respectively.

Autotrophic denitrification is usually performed in attached-growth systems such as membrane biofilm reactors (MBfR), biofilters and fluidized bed reactors (FBRs) (**Table 3**) as they can assure extremely high biomass concentrations and a sheltered environment to the slow-growing autotrophic denitrifers [17]. Biomass yield depends on the electron donor used and is usually lower when using inorganic electron donors (**Table 1**).

Table 3.

3 Reduced inorganic sulfur compounds as electron donors for AuDen

RISCs such as S^0, S^{2-}, $S_2O_3^{2-}$ and SO_3^{2-} are often regarded as environmental pollutants. For this reason, the ability of some bacteria to use these compounds as electron donors for NO_3^- reduction has gained increasing interest. **Table 1** reports the complete denitrification reactions with the three most used electron donors among RISCs, i.e. S^0, S^{2-} and $S_2O_3^{2-}$. The main drawback of sulfur-based denitrification is SO_4^{2-} production. Different strategies to limit the SO_4^{2-} concentration in denitrification effluents are also discussed in this section.

3.1 Sulfide

Sulfide can exist in water as hydrogen sulfide (H_2S) and/or non-volatile ionic species, i.e. hydrosulfide (HS^-) and sulfide (S^{2-}) ions depending on pH. Even at low concentrations, H_2S represents a health and environmental concern because of its toxicity, odor and corrosive properties [96]. H_2S generation by sulfate reducing bacteria (SRB) is a major problem in water distribution pipes, sewers, municipal wastewater treatment plants [97], water injected oil reservoirs [98] and sediments in urban areas [99].

Eqs. 1-4 show the complete and partial biological oxidation of S^{2-} to SO_4^{2-} and S^0, respectively, with either NO_3^- or NO_2^- as the electron acceptor [93]. NO_2^- can also be used as

electron acceptor for sulfide oxidation, resulting in the so-called autotrophic denitritation [100]. The complete oxidation of sulfide to SO_4^{2-} (**Eqs. 1** and **2**), transferring eight electrons per atom of sulfur, is among the most energetically attractive processes for chemoautotrophs [68,101]. When sulfide-rich anoxic sediments were treated by NO_3^- addition to remove recalcitrant organic residues, autotrophic denitrifiers dominated the whole process [99,102].

$$5\ HS^- + 8\ NO_3^- + 3\ H^+ \rightarrow 5\ SO_4^{2-} + 4\ N_2 + 4\ H_2O \quad (1)$$

$$3\ HS^- + 8\ NO_2^- + 5\ H^+ \rightarrow 3\ SO_4^{2-} + 4\ N_2 + 4\ H_2O \quad (2)$$

$$5\ HS^- + 2\ NO_3^- + 7\ H^+ \rightarrow 5\ S^0 + N_2 + 6\ H_2O \quad (3)$$

$$3\ HS^- + 2\ NO_2^- + 5\ H^+ \rightarrow 3\ S^0 + N_2 + 4\ H_2O \quad (4)$$

Many authors have reported the formation of S^0 during sulfide-based denitrification depending on NO_3^- or NO_2^- concentration. The complete oxidation of S^{2-} to SO_4^{2-} is thermodynamically more favorable than the partial oxidation to S^0 (**Eqs. 3** and **4**), although the occurrence of S^0 as a transient product in the oxidation pathway to SO_4^{2-} has been frequently observed [93,103]. Sulfide conversion to S^0 consumes four times less NO_3^- and NO_2^- than its complete oxidation to SO_4^{2-} (**Eqs. 1-4)**, resulting in a preferential process under electron acceptor limitation. In these conditions, the oxidation of excess sulfide is favored due to its higher bioavailability to denitrifiers compared to S^0, which accumulates over time. Cardoso et al. [103] observed complete sulfide oxidation to SO_4^{2-} when NO_3^- was supplemented at stoichiometric or higher N/S ratios, whereas limiting NO_3^- concentrations resulted in the formation of a colloidal S^0 precipitate. Transient S^0 accumulation with both NO_3^- and NO_2^- as electron acceptors has been reported also at stoichiometric N/S ratios [93]. Evidence suggested that the produced S^0 was being used as complementary electron donor. High sulfide loadings also promote partial sulfide oxidation to S^0. Mahmood et al. [100] observed an increasing S^0 accumulation and a decreasing SO_4^{2-} production in a sulfide-

oxidizing UASB reactor operated at nearly stoichiometric N/S ratios when the sulfide concentration was increased stepwise from 32 to 1920 mg L^{-1}.

Sulfide supplementation at increasing loading rates under mixotrophic conditions was shown to increase the rates of both autotrophic denitrification and S^0 accumulation as well as the yield of sulfide conversion to S^0 [104]. A possible explanation is that acetate and partial sulfide oxidation, being faster than S^0 oxidation to SO_4^{2-}, could drive both the NO_3^- and NO_2^- reduction steps. The activity of heterotrophic denitrifiers such as *Pseudomonas* sp. C27 might have a primary role in sulfur oxidation under mixotrophic conditions, as they are capable of partial sulfide oxidation [105]. S^0 accumulation by these so-called heterotrophic sulfide-oxidizing nitrate-reducing bacteria (h-soNRB) was observed to be enhanced at high sulfide and acetate concentrations, whereas at low-sulfide conditions complete sulfide oxidation by autotrophic sulfur-oxidizing bacteria such as *T. denitrificans* preferably occurs [106].

Unlike the other RISCs, sulfide results in alkalinity production when used as electron donor, which can be advantageous for the treatment of slightly acidic wastewaters. The complete reduction of 1 mg $N\text{-}NO_3^-$ L^{-1} produces 1.66 mg L^{-1} of alkalinity as $CaCO_3$ (**Table 1**). Additionally, the complete oxidation of sulfide produces less SO_4^{2-} compared to both S^0 and $S_2O_3^{2-}$ as only 5.58 mg SO_4^{2-} L^{-1} is produced per mg $N\text{-}NO_3^-$ L^{-1}, which makes sulfide a promising electron donor for drinking water denitrification if excess sulfide is prevented.

Despite its high potential as an electron donor, sulfide is a known inhibitor of both heterotrophic [107] and autotrophic [103] denitrification. Low sulfide levels can significantly impact the reduction of N_2O, a potent greenhouse gas [80], whereas less influence has been observed on NO_3^- and NO_2^- reduction. Pan et al. [107] showed 50% inhibition of N_2O reduction by methanol-utilizing denitrifiers exposed to 0.04 and 0.1 mg $S\text{-}H_2S$ L^{-1} before and after adaptation to sulfide, respectively. A 50% inhibition on NO_2^- reduction was observed at only 2.0 mg $S\text{-}H_2S$ L^{-1}, which however did not affect NO_3^- reduction. In another study, S^{2-}

concentrations of 240 and 320 mg L^{-1} completely inhibited autotrophic NO_3^- reduction in batch bioassays [103]. As a result, sulfide levels in denitrifying bioreactors must be controlled carefully to avoid process inhibition. Nitrogen loadings in sulfide-fed denitrifying bioreactors are generally lower than those applied to S^0-based biofilm reactors to limit sulfide levels. This means that at high NO_3^- concentrations bioreactors should be operated under sulfide limitation, which would result in incomplete denitrification. In this condition, sulfide concentration in the effluent would be extremely low and post-treatment for excess sulfide removal not necessary.

3.2 Elemental sulfur

3.2.1 Chemically synthesized S^0

Chemically synthesized S^0 (S^0_{chem}) has been the most used electron donor for AuDen among RISCs so far, because it is inexpensive, easy to handle and transport and is able to act both as a source of energy and biomass support [17]. S^0_{chem} is poorly soluble in water and commonly used in granules forming the carrier material of packed-bed bioreactors. According to the stoichiometry of S^0-based denitrification (**Table 1**), the reduction of 1 mg $N\text{-}NO_3^-$ generates 7.83 mg SO_4^{2-} L^{-1} and consumes 3.36 mg L^{-1} of alkalinity as $CaCO_3$. Alkalinity consumption may result in a large pH decrease, which could inhibit the process if the pH drops below 6.

The addition of an external buffer is often required to counteract the pH decrease. Limestone has been widely used in reactive barriers both as pH buffer and inorganic carbon source for autotrophic growth. The sulfur-limestone autotrophic denitrification (SLAD) process has been widely used both for on-site and off-site ground and waste water treatment, since it combines ease of use, good efficiency and convenience [17]. Several types of solid-phase sulfur (sulfur chips, flakes, lentils and pop-corn sulfur) and sources of inorganic carbon (limestone, marble chips and crushed oyster shells) have been used in SLAD columns [108,109]. However, excess $CaCO_3$ supplementation increases hardness and induces

phosphorus precipitation, which may limit bacterial growth. Bicarbonate is a soluble and more versatile alternative to limestone both as alkalinity and carbon source [17]. Recently, mixotrophic denitrification has gained increasing attention as a way to limit alkalinity consumption and sulfate production as well as increase the denitrification kinetics [110,111].

The main drawback related to the use of S^0_{chem} as electron donor is its extremely low aqueous solubility (0.16 μM in pure water at 25°C), which severely limits sulfur mass transfer from the solid to the aqueous phase and, thus, the rates of S^0_{chem} biological oxidation [23,112]. The size of S^0_{chem} granules affects the rates of sulfur oxidation both in suspended or attached growth systems as increasing the specific surface area (SSA) of the particles enhances sulfur mass transfer from the solid to the liquid phase and provides more available surface to microorganisms for biofilm development [113]. Sierra-Alvarez et al. [23] quantified the increase in denitrification rate normalized to the S^0_{chem} surface at 26.4 mmol NO_3^-/m^2 d in a SLAD column, while Di Capua et al. [24] calculated a size normalized denitrification rate of about 1 μg $N\text{-}NO_3^-$ L^{-1} d^{-1} per μm of S^0_{chem} particle size in suspension.

The use of S^0_{chem} also results in a higher NO_2^- accumulation compared to S^{2-}, with potential inhibition on denitrification [103]. An important aspect to consider when operating S^0-packed biofilters is that S^0_{chem} may undergo biological disproportionation into SO_4^{2-} and H_2S due to anaerobic conditions (absence of free and molecular oxygen) potentially occurring under low NO_3^- loading rates [109]. In full scale SLAD reactors (**Fig. 2**), S^0_{chem} disproportionation may result in considerable H_2S emissions into the environment as well as clogging and head loss downstream due to colloidal S^0 precipitation [114]. Pre-deaeration (**Fig. 2**) can mitigate bed clogging as it avoids the N_2 supersaturation and reduces aerobic growth in the sulfur bed. Similarly, effluent recirculation (**Fig. 2**) can reduce bed clogging as it increases the upflow water velocity and, thus, the S^0_{chem} solubility [115].

Fig. 2.

3.2.2 Biogenic S^0

Biogenic S^0 (S^0_{bio}) is largely produced as a waste product during biological H_2S removal from natural gas or industrial waste streams, e.g. wastewater from metal refineries, flue-gases from coal-fired power plants and biogas from anaerobic digestion [117]. The process is carried out by a community of *Thiobacillus* or *Acidithiobacillus* bacteria that partially oxidize the H_2S to S^0 particles of approximately 0.1-1 μm, which are stabilized by organic matter and form aggregates [118]. S^0_{bio} can also be produced under anaerobic denitrifying conditions under low N/S ratios and high sulfide loadings, as described in **Section 3.1.** The accumulation of S^0_{bio} in bioreactors is responsible for pipe blockage and secondary pollution and, thus, S^0_{bio} isolation is required [119]. Plain sedimentation is the cheapest method to isolate S^0_{bio}, although flocculation, filtration, extraction and flotation have shown higher efficiency due to the colloidal properties of S^0_{bio} particles [120].

The S^0_{bio} particles produced by chemotrophic bacteria are composed of a core of orthorhombic S^0 rings covered by a layer of long-chain polymers (i.e. polythionates, polysulfides and proteins) with hydrophilic properties, which disturb the particle aggregation and increase their dispersion in solution [121]. This particular structure provides a higher SSA, solubility and colloidal stability compared to S^0_{chem} particles. These properties enhance the S^0 oxidation rates especially in suspension and have been primarily exploited to enhance metal bioleaching from contaminated sediments [117].

In a recent study, Di Capua et al. [24] investigated the potential of S^0_{bio} produced by *Acidithiobacillus* bacteria to sustain AuDen by a suspended culture of *T. denitrificans* in batch tests. Results showed that 1.7-fold faster NO_3^- removal and 3-fold higher SO_4^{2-} production were achieved with S^0_{bio} compared to S^0_{chem} powder. These results were attributed to the particle structure of S^0_{bio}, which enhanced S^0_{bio} bioavailability to microorganisms. Unlike S^0_{chem}, S^0_{bio} is suitable for suspended-growth denitrifying bioreactors as it forms

colloidal dispersions due to its hydrophilic properties. As a waste and non-toxic product, S^0_{bio} is also a more convenient and eco-friendly choice as an electron donor than other RISCs. However, the use of S^0_{bio} for denitrification may result in high NO_2^- accumulation [24], which limits its application to high-strength NO_3^- contaminated wastewaters and demands an accurate control of the NO_2^- concentrations in the effluent.

Although the use of S^0_{bio} can improve the rates of S^0-based denitrification with suspended cultures, much higher denitrification rates were obtained with more soluble RISCs, i.e. $S_2O_3^{2-}$ and S^{2-}. Solid/liquid mass transfer thus has an important role in determining the denitrification rate also with S^0_{bio}. It is possible that the increase of the denitrification rate observed with S^0_{bio} mainly relies on the oxidation of the sulfur-containing polymers adsorbed on the surface of S_8 crystals. Polysulfides and polythionates (e.g. tetrathionate $S_4O_6^{2-}$) are in fact intermediates in the oxidation of RISCs by chemolithotrophic and photoautotrophic bacteria and represent a pool of linear bioavailable sulfur utilized in both oxidative and reductive sulfur metabolism [122]. Further studies are required to elucidate the effect of oxidative processes on the structure of S^0_{bio} particles and on the fate of both core and superficial sulfur compounds.

3.3 Thiosulfate

$S_2O_3^{2-}$ is usually discharged in industrial effluents as a product of sulfide oxidation. Concentrations as high as 3 g $S_2O_3^{2-}$ L^{-1} are produced during the processing of natural gas from offshore installations by the oxidation of the H_2S present in the natural gas through a caustic oxidation process [123]. $S_2O_3^{2-}$ is also used in the mining industry as an alternative lixiviant to cyanide for gold leaching and can co-occur with NO_3^- in mining effluents [124].

Anoxic thiosulfate oxidation with NO_3^- as electron acceptor (**Table 1**) has been recently investigated in biofilm reactors under different operating conditions. $S_2O_3^{2-}$ showed no

inhibitory effects on AuDen up to 2.2 g L^{-1} [24] and can be successfully used as electron donor for the removal of high NO_3^- concentrations. Autotrophic denitrifiers and denitrifying biofilms cultivated on $S_2O_3^{2-}$ demonstrated outstanding performance, robustness and resiliency. Complete thiosulfate-driven denitrification and denitritation were performed in FBRs at $S_2O_3^{2-}$ loading rates as high as 600 and 228 mg L^{-1} h^{-1}, respectively [83]. Di Capua et al. [28,30] demonstrated that high-rate thiosulfate-driven denitrification in a continuous FBR was feasible at pH and temperatures as low as 4.75 and 3°C, respectively. Concentrations of free and EDTA-complexed nickel as high as 200 and 100 mg L^{-1} were tolerated by a *T. denitrificans*-dominated FBR biofilm [29], higher than those tolerated by FBR-cultivated heterotrophic denitrifiers [125]. Pethkar and Patnikar [126] cultivated a *T. thioparus* culture that tolerated 350 g L^{-1} silver while oxidizing the $S_2O_3^{2-}$ contained in photofilm processing wastewater to sulfate and elemental sulfur. Khanongnuch et al. [127] showed that the efficiency of anoxic $S_2O_3^{2-}$ oxidation with NO_3^- as electron acceptor in a FBR recovered to 80% within 3 days after long-term biofilm cultivation under severe NO_3^- starvation (N/S ratio of 0.1). However, SO_4^{2-} production limits the use of high concentrations of $S_2O_3^{2-}$ since 11.067 mg SO_4^{2-} L^{-1} are produced by the reduction of 1 mg N-NO_3^- L^{-1}, which is 1.4 and 2.0 times higher than the sulfate produced using S^0 and H_2S as electron donors, respectively.

3.4 Pyrite

Pyrite is an ubiquitous mineral in the terrestrial crust and represents a major sink within the global biogeochemical cycles of sulfur and iron [128]. The aerobic oxidation of pyrite-bearing rocks by chemolithotrophic bacteria can generate an enormous amount of sulfuric acid and is responsible for serious environmental issues, especially in mining areas where mined rocks exposed to oxygen and rainwater generate an extremely acidic surface runoff known as acid mine drainage.

Although pyrite is considered stable under anoxic conditions [129], pyrite-driven denitrification emerged as a dominant pathway in NO_3^- removal from groundwater even in the presence of organic matter. Jørgensen et al. [18] showed that 50% of NO_3^- removal from pyrite-bearing groundwater sediments could be ascribed to anoxic pyrite oxidation. As a result, pyrite-driven denitrification can control nitrate concentrations in groundwater and protect shallow aquifers from anthropogenic nitrate contamination. The complete anoxic nitrate-dependent pyrite oxidation (**Table 1**) results from the following two reactions [130]:

$$5\ FeS_2 + 14\ NO_3^- + 4\ H^+ \rightarrow 5\ Fe^{2+} + 7\ N_2 + 10\ SO_4^{2-} + 2\ H_2O \quad (5)$$

$$2\ NO_3^- + 10\ Fe^{2+} + 12\ H^+ \rightarrow N_2 + 10\ Fe^{3+} + 6\ H_2O \quad (6)$$

Partial NO_3^- reduction to NO_2^- may also occur as follows [38]:

$$2\ FeS_2 + 15\ NO_3^- + 7\ H_2O \rightarrow 2\ Fe(OH)_3 + 15\ NO_2^- + 4\ SO_4^{2-} + 8\ H^+ \quad (7)$$

The reduction of 1 mg $N\text{-}NO_3^-$ L^{-1} by pyrite-driven denitrification produces 4.61 mg SO_4^{2-} L^{-1} and consumes 1.19 mg L^{-1} as $CaCO_3$ of alkalinity, which are 41% and 65% lower than the values obtained by S^0-based denitrification (**Table 1**). Moreover, the SO_4^{2-} concentration in pyritic aquifers has often been found much lower than the stoichiometric amount [131,132]. This may be related to the occurrence of side processes such as the partial oxidation of the sulfur moiety of FeS_2 to S^0 [132].

Pyrite is insoluble in water and commonly applied as granular medium in biofilter columns to allow the formation of a denitrifying biofilm [17]. Kong et al. [13] compared AuDen with S^0 and FeS_2 in two double stage (aerobic/anoxic) biofilters performing the removal of organics, total nitrogen and phosphorus. Despite a 9% lower NO_3^- removal, the biofilter containing pyrite showed a higher ability to maintain the pH and produced 44% less SO_4^{2-} than the biofilter packed with S^0. From this point of view, denitrification with FeS_2 is potentially more feasible than S^0-based denitrification for the treatment of nitrate-contaminated groundwater.

However, the potential release of toxic metals entrapped in the pyrite minerals might limit the use of FeS_2 for denitrification of drinking water. Pu et al. [133] observed that acid pretreatment of pyrite improved the rate of AuDen, probably due to the removal of iron and sulfur impurities (potentially toxic to microorganisms) as well as to the roughening of the pyrite surface, which facilitates microbial attachment and biofilm formation. In addition, the produced $Fe(OH)_3$ might help in the sequestration of toxic metals [95].

Huang et al. [95] recommend the use of FeS over both FeS_2 and S^0 to support AuDen, as FeS was more efficient than FeS_2 as electron donor and releases less free sulfide than S^0. According to Brunet and Garcia-Gil [134], high free sulfide levels might disrupt denitrification by driving part of the electron flow from S^{2-} to NH_4^+ and induce dissimilatory nitrate reduction to ammonium (DNRA).

The limited acid production by pyrite-based denitrification makes the use of limestone in biofilters unnecessary and thus results in cost savings. Moreover, pyrite can be used to control pH in basic environments (pH > 7.4), since it is generally oxidized producing $Fe(OH)_2$ precipitates which buffer the pH of the system [135]:

$$FeS_{2\,(s)} + 18\ OH^-_{(aq)} \rightarrow Fe(OH)_{2\,(s)} + 2\ SO_4^{2-}{}_{(aq)} + 14\ e^- + 8\ H_2O_{(aq)} \quad (8)$$

Due to this property, FeS_2 addition is effective in limiting the pH increase during in situ groundwater denitrification with organic supplementation [136] and Fe^0-assisted hydrogenotrophic denitrification [135].

3.5 Thiocyanate

Thiocyanate in water can be formed biologically during cyanide detoxification [137] or chemically, e.g. during gold cyanidation as a result of the interaction of free cyanide with various RISCs present in the ore [138]. The possible industrial uses of SCN^- include the production of insecticides and herbicides as well as chemical synthesis [35].

SCN^- can be used as an electron donor by sulfur-oxidizing neutrophilic bacteria both under aerobic and anaerobic conditions [35]. De Kruyff et al. [139] firstly reported that anaerobic growth of certain *Thiobacillus* species on SCN^- is possible by using NO_3^- as electron acceptor. In particular, *T. denitrificans* is able of complete denitrification with SCN^- as energy source.

Thiocyanate-driven denitrification consumes acidity and produces NH_3, SO_4^{2-} and N_2 (**Table 1**). Besides *T. denitrificans*, the ability to grow on SCN^- by complete denitrification was observed in a few haloalkaliphilic and halophilic bacterial species belonging to the genus *Thialkalivibrio* and *Thiohalomonas* [35,66]. Bacterial growth on SCN^- was observed to be 3.3-fold slower than on $S_2O_3^{2-}$ under both aerobic and anaerobic conditions [35]. Nevertheless, the ability of certain denitrifiers to utilize SCN^- as secondary energy source may result in a competitive advantage over other sulfur-oxidizing bacteria when more effective sulfur-containing electron donors, such as $S_2O_3^{2-}$ and H_2S, are limited or not available.

Thiocyanate-driven denitrification could be potentially advantageous for the treatment of effluents from mining activities, in which SCN^-, NO_3^- and acidity often co-occur [4,138]. Although the reported thiocyanate-utilizing bacteria are alkaliphiles or neutrophiles [35,66], Broman et al. [19] showed that thiocyanate-driven denitrification is feasible at pH and temperatures as low as 3.5 and 8°C in an anaerobic continuous culture reactor dominated by *Thiobacillus*. On the other hand, biodegradable but potentially toxic compounds may inhibit SCN^- biodegradation. Sahariah and Chakraborty [140] reported that SCN^- removal via NO_3^- reduction significantly dropped in an anoxic moving bed biofilm reactor (MBBR) after increasing the phenol concentration from 350 to 500 mg L^{-1}.

NH_4^+ accumulation has been observed during anoxic SCN^- oxidation in the presence of NO_3^-. Broman et al. [19] reported that NH_4^+ was produced in an anoxic bioreactor fed with SCN^-

and NO_3^- as a result of two potential pathways: 1) biological denitrification via SCN^- oxidation to CNO^- and 2) abiotic CNO^- degradation to CO_2 and NH_4^+. A decrease in temperature from 20°C to 8°C and pH from 8-8.5 to 3.5 significantly decreases both NH_4^+ production and SCN^- oxidation [19].

3.6 Sulfite

SO_3^{2-} occurs in nature from chemical or biological reactions involving inorganic and organic sulfur compounds as well as from the anaerobic mineralization of organic matter by dissimilatory sulfate reduction [141]. SO_3^{2-} is a strong reductant and rapidly reacts with oxygen, especially in the presence of transition metal catalysts such as Fe^{2+} and Cu^{2+} [142]. Despite its high reactivity and potential toxicity, sulfite can be oxidized by microorganisms belonging to a large and phylogenetically very diverse group of bacteria and archaea able to use RISCs for their chemotrophic or phototrophic growth [143]. The microbial oxidation of SO_3^{2-} to SO_4^{2-} can occur through a direct or indirect pathway transferring 2 electrons per atom of sulfur [22]. Because SO_3^{2-} is an intermediate in the oxidation pathway of other RISCs, a wide variety of sulfur-oxidizing bacteria possess an enzymatic system for sulfite oxidation.

Although there is evidence that SO_3^{2-} can serve as electron donor for denitrification, very little research has been conducted on this topic. Adams et al. [144] were the first to report the feasibility of sulfite-mediated denitrification. In their study, a pure culture of *T. denitrificans* could reduce NO_3^- to gaseous nitrogen products by using SO_3^{2-} as electron donor, which was the most effective compound for gas production except sulfide. The highest rates of sulfite-based denitrification were observed at a pH of 8.5.

SO_3^{2-} supplementation to activated sludge systems or denitrifying biofilters could be carried out via dissolution of salts as sodium sulfite (Na_2SO_3) or bubbling sulfur dioxide (SO_2), which dissolves in water forming HSO_3^- and SO_3^{2-}. As SO_3^{2-} rapidly reacts with oxygen, care

must be taken to avoid abiotic oxidation of SO_3^{2-} stocks. Similarly, SO_3^{2-} overdosing should be avoided as it will demand for downstream oxygen and chlorine. On the other hand, excess SO_3^{2-} can be easily removed by air stripping. According to Sabba et al. [22], a potential configuration to implement sulfite-based denitrification could be a MBfR to which sulfur dioxide (SO_2) is supplied by means of a hollow fiber membrane with a denitrifying biofilm on the exterior. This configuration would allow a nearly 100% gas utilization efficiency by retaining the gas within the biofilm and serving as a substrate for denitrification.

The potential application of sulfite as supplemental electron donor for biological denitrification would address certain challenges due to the anti-microbial properties of SO_3^{2-}. While $S_2O_3^{2-}$ can be self-inhibitory at high concentrations, little is known about SO_3^{2-} toxicity on dominant microorganisms in activated sludge systems (e.g. nitrifying and denitrifying bacteria) and the inhibitory thresholds have not yet been identified [22]. Lai et al. [145] showed that 0.5 mM SO_3^{2-} increased phosphate uptake and intracellular polyphosphate accumulation in activated sludge by 17%, probably due to an increased abundance of chemolithotrophic sulfur oxidizers in the sludge biomass or to a stress-response in polyphosphate-accumulating bacteria as a result of SO_3^{2-} addition [146]. Interestingly, this effect was not observed with other RISCs. The selection for sulfite-oxidizing microorganisms might also impact the settling properties of the activated sludge [22] and requires further investigation.

4 Hydrogen gas

H_2 is mainly produced by reformation of hydrocarbon fuels (e.g. natural gas, oil and coal) and water electrolysis, but it can be also biologically produced via dark or photo fermentation [147]. H_2 is one of the most thermodynamically favorable electron donors for denitrification [148] and its high diffusivity through biofilms promotes NO_3^- removal in attached-growth

systems. Moreover, H_2 is easy to remove by air stripping due to its low solubility in water (0.182% vH_2/vH_2O at 20°C and 1 atm) [81]. On the other hand, the relatively high cost, low solubility and hazardous handling and storage limit the use of H_2 in full-scale plants.

H_2 can be used as electron donor by different types of microorganisms, depending on the redox potential of the system [77]. ORP values below -250 mV allow the use of H_2 by methanogenic, sulfate-reducing and homoacetogenic bacteria, whereas ORP values higher than -50 mV favor denitrification. *Proteobacteria* such as *Paracoccus denitrificans* (**Table 2**) have been widely reported to perform H_2-driven denitrification [84,149–151], along with *Flavobacteria* [152] and *Sphingobacteria* [84]. Pure cultures of *Ralstonia metallidurans* [52,153] and *Rhodocyclus sp.* [56] have also been used to perform hydrogenotrophic denitrification in biofilm reactors. The genera *Pseudomonas* [45] and *Acinetobacter* [154] have been reported to dominate mixed cultures of hydrogenotrophic denitrifiers.

Optimal pH values for hydrogenotrophic denitrifiers are in the range of 7.6-8.6 [77,155–157]. Lower pH values may result in inorganic carbon limitation, whereas higher values may inhibit the hydrogenotrophic activity [77,158]. An increase in hardness and alkalinity can be detrimental for the process as it can result in pH values exceeding the inhibitory threshold and lead to carbonate precipitation which, in turn, may limit H_2 and NO_3^- mass transfer to the biomass. An alkalinity concentration of 1.1 g $NaHCO_3$ L^{-1} was reported as an optimal condition for the process [156]. As hydrogenotrophic denitrification consumes acidity, it can be advantageous for the treatment of slightly acidic waters if sufficient inorganic carbon is present. Optimal temperatures for hydrogenotrophic denitrifiers are between 25 and 35°C, as severe NO_2^- accumulation was observed at 25°C and temperatures above 35°C resulted in low denitrification rates [159].

4.1 Direct H_2 supply

H_2 can be delivered directly to bioreactors by bubbling via gas permeable membranes or undergo gas/liquid absorption in an external tank (**Fig. 2**) prior to being delivered in the liquid phase to the bioreactor [17]. Alternatively, H_2 can be electrolytically produced directly in the bioreactor [81]. An efficient H_2 utilization is fundamental to achieve high denitrification rates and reduce the costs related to hydrogen supply. H_2 concentrations above 0.2 mg L^{-1} should be maintained in the bioreactor, since lower concentrations can lead to NO_2^- accumulation [53].

4.2 Fe^0-assisted hydrogenotrophic denitrification

An alternative strategy for H_2 production is the anaerobic zero-valent iron (Fe^0 or ZVI) corrosion. Fe^0 is thermodynamically unstable in water and produces cathodic hydrogen under anaerobic conditions (**Eq. 9**). If NO_3^- is present, Fe^0 stimulates the chemical reduction of NO_3^- to NH_4^+ (**Eq. 10**), N_2 (**Eq. 11**) or NO_2^- (**Eq. 12**). All three reactions consume acidity and the ratios among the nitrogenous products depend on chemical (e.g. pH) and structural (e.g. particle size) factors [36,160].

$$Fe^0 + 2\ H_2O \rightarrow H_2 + Fe^{2+} + 2\ OH^- \quad (9)$$

$$4\ Fe^0 + NO_3^- + 7\ H_2O \rightarrow 4\ Fe^{2+} + NH_4^+ + 10\ OH^- \quad (10)$$

$$5\ Fe^0 + 2\ NO_3^- + 6\ H_2O \rightarrow 5\ Fe^{2+} + N_2 + 12\ OH^- \quad (11)$$

$$Fe^0 + NO_3^- + 2\ H^+ \rightarrow Fe^{2+} + NO_2^- + H_2O \quad (12)$$

Abiotic NO_3^- and NO_2^- reduction has been observed at pH values ranging from 2 to 11 with increasing rates at decreasing pH values [160]. In particular, negligible reduction rates were observed at pH ≥ 5 [161]. This can be mainly attributed to H^+ limitation and precipitation of iron oxides forming a physical barrier on reactive Fe^0 sites [160]. The Fe^0 particle size also influences the rate and efficiency of abiotic NO_3^- reduction. Choe et al. [36] showed that

complete abiotic Fe^0-assisted denitrification is possible by using chemically synthesized nanoscale Fe^0 (particle size 1-100 nm). In contrast, the use of larger Fe^0 particles commonly results in a mixture of NH_4^+ and N_2 as end-products [162,163].

Eq. 11 describes the combined anoxic Fe^0 corrosion and biological hydrogenotrophic denitrification, which is thermodynamically more feasible than the abiotic NO_3^- reduction to NH_4^+ with Fe^0 described by **Eq. 10** [164]. As NH_4^+ is a pollutant, Fe^0-assisted hydrogenotrophic denitrification with N_2 as the end product is preferable (**Eq. 11**). However, the abiotic reduction of NO_3^- to NH_4^+ may occur in parallel with biological denitrification, resulting in a substantial production of NH_4^+. In order to minimize NH_4^+ production, Till et al. [164] separated Fe^0 from NO_3^- and a pure culture of *Paracoccus denitrificans* in a dual-flask apparatus where H_2 could diffuse from one flask to another (**Fig. 3**). This configuration resulted in the complete removal of 25 mg $N\text{-}NO_3^-$ L^{-1} within 5-7 days at pH values from 5 to 9. Biswas and Bose [163] observed that decreasing the concentration of the Fe^0 particles reduced the abiotic NH_4^+ production. On the other hand, lower Fe^0 concentrations may also limit the hydrogenotrophic denitrification rates and an increase of the HRT of the bioreactors may be necessary for complete denitrification.

Fig. 3.

Anaerobic iron corrosion (**Eq. 9**) and both abiotic (**Eq. 10**) and biological (**Eq. 11**) Fe^0-assisted NO_3^- reduction produce alkalinity and may increase the pH of the system. Hydrogenotrophic activity is inhibited at a pH of 10 or higher [164]. Thus, it is necessary to buffer the system against a large pH increase. pH buffering can be accomplished through CO_2 addition, which provides a supplementary carbon source for hydrogenotrophic denitrifiers. The use of Fe^0 sources with a low SSA (e.g. steel wool) is advantageous as they promote more favorable conditions for denitrifiers by limiting the pH increase due to iron corrosion and the abiotic NO_3^- reduction to NH_4^+ [163].

The combination of both abiotic and biotic Fe^0 oxidation has potential advantages for denitrification. If oxygen is present, the aerobic Fe^0 corrosion can rapidly induce anoxic conditions which are favorable for denitrification. Additionally, Fe^0 may favor microbial activity by removing potential inhibitors such as Cr(VI) [165]. In turn, bacteria can degrade the byproducts of the abiotic transformations involving Fe^0, e.g. dichloromethane produced by carbon tetrachloride hydrogenolysis with Fe^0 [166].

5 Ferrous iron

Ferrous iron acts as electron donor in many chemical and biological processes with free oxygen as electron acceptor [167]. However, biological and abiotic Fe^{2+} oxidation occur also under anoxic conditions [168], although the abiotic NO_3^- reduction via Fe^{2+} oxidation is not prevalent in typical natural environments [169].

In the last 20 years, many denitrifying microorganisms using Fe^{2+} as electron donor at circumneutral pH have been identified (**Table 2**). Straub et al. [37] showed that denitrifying strains grown on aromatic substrates and NO_3^- as well as *Thiobacillus denitrificans* and *Pseudomonas stutzeri* can oxidize Fe^{2+} as sole or supplemental electron donor in the presence of acetate, whereas *Thiomicrosprira denitrificans* and *Paracoccus denitrificans* were unable of Fe^{2+}-mediated denitrification. Nielsen and Nielsen [20] showed that NO_3^- was reduced concomitantly with Fe^{2+} oxidation in a wastewater treatment plant performing chemical phosphorus removal with $FeCl_3$. According to the authors, the alternation of aerobic, anoxic and anaerobic conditions as well as a short sludge retention time (4-10 days) promoted AuDen linked to Fe^{2+} oxidation. A significant pH dependency was also observed, the Fe^{2+} oxidation rate at pH 8 being about four times higher than at pH 6.

The iron biochemistry is strongly influenced by the redox potential of the Fe^{2+}/Fe^{3+} couple, which depends on the solution pH and presence of complexing agents. At circumneutral pH,

the $FeCO_3/Fe(OH)_3$ redox potential is sufficiently low (+200 mV) to enable the use of NO_3^- and NO_2^- as electron acceptors instead of O_2. However, the low redox differential between electron donor and acceptor (+230 mV) limits the amount of energy available for the anoxic metabolism [170]. As a result, the denitrification rates obtained using Fe^{2+} as electron donor (**Table 1**) are generally lower than those obtained with other organic and inorganic electron donors. Devlin et al. [171] observed a slower NO_3^- removal with Fe^{2+} compared to acetate, H_2 and S^0. Additionally, Baeseman et al. [79] reported that the addition of both Fe^{2+} and Fe^{3+} in sediments from AMD-impacted streams decreased the denitrification rate, most likely due to iron complexation with organic matter which reduced the bioavailability of organic carbon.

6 Arsenite

Groundwater contamination by arsenic is a serious issue worldwide and particularly in Bangladesh, India and Vietnam [172–174]. The main sources of arsenic in aquatic environments are the oxidation of As-bearing rocks [88] and arsenic release caused by anthropogenic activities [21]. Arsenic is present in nature as arsenate (As^{5+}) or arsenite (As^{3+}), with As^{3+} being more toxic (100 times), mobile and bioavailable than As^{5+} [175]. The main mechanism of immobilizing arsenic is by adsorption onto a solid phase, e.g. clay minerals [176]. Since As^{5+} can form stronger bonds than As^{3+} to clay and minerals with aluminum oxides [177–179], the conversion of As^{3+} to As^{5+} is highly desirable to achieve arsenic immobilization [180,181].

Arsenic removal processes such as coagulation, filtration, adsorption and reverse osmosis usually require a pre-oxidation step, as As^{3+} is thermodynamically stable and non-ionic (H_3AsO_3, pKa=9.22) in most natural environments [182]. As^{3+} can be oxidized chemically using potent oxidants, such as ozone, chlorine or hydrogen peroxide. However, the use of chemicals results in additional pollution and higher treatment costs [183].

Biological As^{3+} oxidation has been identified as a cost-effective method for arsenic removal from As-contaminated waters [183,184]. Many studies on arsenic oxidation focused on heterotrophic applications, which require the addition of organic substrates [185]. Recently, chemolithotrophic oxidizers able to fix CO_2 coupled to As^{3+} oxidation to As^{5+} have been isolated and classified according to their ecology, phylogenetic relationship and physiological characteristics [186–189]. Microorganisms able to oxidize As^{3+} under anoxic conditions in the presence of NO_3^- [21,39,190] or selenate (SeO_4^{2-}) [191] prevail in As-contaminated lakes [190,192], soils [39] as well as sludge and sediments not previously exposed to arsenic [21]. Microbial oxidation of As^{3+} can occur under anoxic conditions as long as the oxidant has a higher redox potential than the reductant. Denitrification linked to As^{3+} oxidation to As^{5+} (**Table 1**) is feasible as the redox potential of the As^{5+}/As^{3+} couple is +139 V, while that for NO_3^-/N_2 is 747 mV, which equates to a $\Delta G°'$ of -117.3 kJ/mol As^{3+} for complete denitrification [21].

Arsenite-driven denitrification is of high interest for public health and economy, since it can simultaneously remove arsenic and nitrate and increase drinking water availability in those areas of the world affected by severe arsenic contamination of groundwater. However, only a few lab-scale applications have been operated so far. In continuous reactors, arsenite-driven denitrification is a stable and efficient process over long-term periods [86,193]. However, As^{3+} concentrations in the range of 3.5-5 mM are reported to completely inhibit the activity of autotrophic denitrifiers [21]. As a result, the As^{3+} concentration is the main limitation affecting the arsenite-driven denitrification rates. The use of As^{3+} as complementary electron donor by the addition of a primary organic or inorganic energy source for denitrifiers is a potential solution to overcome this limitation and achieve a satisfactory combined arsenic and nitrate removal.

7 Manganese

Manganese in excess is removed from drinking water as it alters water quality and causes operational issues to water distribution systems. Insoluble manganese species impart turbidity and dark color to drinking water and form deposits in plumbing and water-using appliances, resulting in a negative economic outcome. As a result, the simultaneous removal of Mn^{2+} and NO_3^- from drinking water is of great interest as the excess of both compounds is undesirable.

Mn^{2+} has been tested for the first time as electron donor for AuDen in batch experiments performed by Su et al. [194]. Bacteria belonging to the genera *Acinetobacter* and *Pseudomonas* exhibited efficient AuDen ability using Mn^{2+} as energy source, resulting in denitrification rates as high as 3.12 mg $N\text{-}NO_3^-$ L^{-1} h and NO_3^- removal efficiencies of 77% in a MBBR with a 40% filling ratio [26]. Response surface methodology (RSM) experiments revealed that the highest rates of Mn^{2+}-based denitrification by *Acinetobacter* sp. SZ28 can be achieved at a Mn^{2+} concentration of 143.6 mg L^{-1}, a C/N ratio of 6.8, an initial pH of 5.17 and a temperature of 34.3°C [194]. Lower rates were obtained with the same *Acinetobacter* strain using S^{2-} as electron donor, where growth on Fe^{2+} resulted in faster NO_3^- removal [69].

Based on preliminary research, Mn^{2+}-based denitrification appears as a promising process for the treatment of circumneutral or slightly acidic wastewaters contaminated by NO_3^- and Mn^{2+}, e.g. groundwater [195] and acidic mine effluents [4]. However, excessive manganese exposure affects protein and other macromolecular stability and was shown to repress the genes in the anaerobic (e.g. NO_3^- and NO_2^-) respiratory pathways of *E. coli* cells [196]. Further research is needed to determine manganese toxic thresholds for autotrophic denitrifiers and provide more specific kinetic and stoichiometric information on Mn^{2+}-based denitrification, i.e. specific growth rates of NO_3^- and NO_2^- reducers, specific Mn^{2+} utilization rate, half-saturation constants, biomass yields and substrate inhibition thresholds.

8 Selection of the electron donor

The selection of the substrate to be used as electron donor for AuDen should be based on the evaluation of a wide range of parameters, including substrate bioavailability to microorganisms, microbial affinity and energetic yield, which determine the rate and efficiency of denitrification as well as process cost and effluent water quality.

Table 4 lists the specific substrate utilization (SSU) values calculated for all inorganic electron donors used for denitrification. Two organic compounds (ethanol and acetic acid) are also included for comparison. H_2 shows a 6- and 9-fold lower SSU than ethanol and acetic acid, respectively, and from 5- to 15-fold lower SSU than RISCs, resulting in a cost-competitive energy source despite its expensive price. S^0_{bio} is 6 times less efficient than H_2 for denitrification. However, as a waste product, S^0_{bio} is inexpensive and its use as electron donor results in a lower overall cost.

This section provides guidelines for the selection of a suitable electron donor for AuDen. Selection criteria focus on electron donor kinetics, potential application in bioreactors, environmental impact and cost. A schematic overview of the advantages and drawbacks related to the use of each inorganic compound is provided in **Table 5**.

Table 4.

8.1 Kinetics and potential application

The extremely low aqueous solubility and dissolution rate of S^0 (Section 3.1.2), H_2 (Section 3.2) and FeS_2 (Section 3.4) severely limit their availability to denitrifying microorganisms and the denitrification kinetics. As a result, biofilm systems are recommended for S^0-based, pyrite-driven and hydrogenotrophic denitrification as they allow a direct contact between electron donor and microorganisms [17]. The use of biofilm-coated gas-permeable membranes can further enhance H_2 dissolution by establishing counter fluxes of H_2 and NO_3^-

[199]. Similarly, biofilm development on S^0 granules enhances sulfur dissolution by utilization of surface-bound or extracellular enzymes hydrolyzing S^0 to more soluble forms, i.e. polysulfides. However, the hydrophobic nature and the low SSA of S^0 limit sulfur dispersion in solution and biofilm contact. SSA also regulates the H_2 generation rate and NO_3^- removal rates with ZVI, although pH was observed as the most important factor affecting denitrification kinetics of ZVI-assisted denitrification [160]. The slow denitrification rates observed with ZVI can be balanced using ZVI powder at acidic pH values, although the choice of the ZVI source and particle size should also take into account the potential abiotic conversion of NO_3^- to NH_4^+ (**Fig. 3**).

The H_2S in untreated biogas from anaerobic digestion might be directly used in biofilters, e.g. biotricking filters (BTFs), to remove NO_3^- from wastewater [200]. In these bioreactors, gas-liquid mass transfer and contact surface play a major role in determining denitrification kinetics. As a result, BTFs are usually operated with the gas and liquid phases (containing the electron donor and acceptor, respectively) flowing counter-currently. Complete oxidation of H_2S to SO_4^{2-} occurs if excess nitrate is available, while nitrate-limiting and stoichiometric conditions result in the production of S^0_{bio} [201], which can be recovered and effectively reused. Gas-permeable membranes are not recommended for H_2S delivery into a denitrifying bioreactor as S^0_{bio} precipitation would increase the cleaning frequency and maintenance costs.

S^0_{bio} has hydrophilic properties and forms colloidal dispersions in water, resulting in higher bioavailability and denitrification rates with suspended cultures compared to S^0_{chem} [24]. Nevertheless, 4.4-fold higher denitrification rates have been observed with $S_2O_3^{2-}$, which has been confirmed as the most effective electron donor among RISCs due to its high solubility, bioavailability, energetic yield and substrate inhibition threshold [24,103,112]. Biofilm systems fed with $S_2O_3^{2-}$ maintained high denitrification rates even under extreme conditions such as acidic pH (< 5), psychrophilic temperatures (3°C) and high heavy metal

concentrations (up to 200 mg Ni L^{-1}) [28–30]. H_2S is also characterized by high bioavailability and energetic yield, but its toxicity and gas-liquid mass transfer limitation can detrimentally affect the denitrification efficiency especially when treating high nitrogen loads.

Although the potential of SO_3^{2-} to serve as electron donor for denitrification has been revealed [22], further research is needed at bench and pilot scale to collect kinetic and stoichiometric information as well as investigate SO_3^{2-} inhibition on sulfur-oxidizing denitrifiers and other microbial populations (e.g. nitrifiers) potentially co-occurring in the purification basins. The use of other inorganic compounds as electron donors results in low denitrification rates and, in the case of AsO_3^{3-} and SCN^-, potential toxicity on denitrifiers at relatively low concentrations, which limit their use for AuDen.

8.2 Environmental impact

H_2 is the cleanest electron donor for denitrification, since no undesirable compounds are formed and hydrogenotrophic denitrifiers have low biomass yields. However, RISCs are preferred over H_2 in large-scale bioreactor applications due to their low cost and ease of use [115]. The main drawback of using RISCs as electron donors for denitrification is SO_4^{2-} production. As discussed in Section 3.1, $S_2O_3^{2-}$ oxidation produces more SO_4^{2-} than other RISCs, followed by S^0 and H_2S. Although no adverse effects of SO_4^{2-}on human health have been demonstrated, it can significantly alter the organoleptic properties of drinking water. For this reason, the EU (Council Directive 98/83/EC) and US EPA have set the water quality standard for SO_4^{2-} concentration in drinking water to 250 mg L^{-1}. Denitrification with S^0 and $S_2O_3^{2-}$ can also result in severe water acidification if not enough buffer is provided. On the other hand, the use of limestone as an inexpensive buffer increases the hardness and induces precipitation of calcium phosphate salts, which may limit phosphorus bioavailability and create operational issues downstream [92]. The addition of organics as supplemental electron

donors to RISCs can significantly reduce both SO_4^{2-} production and alkalinity consumption [110], although it arises concerns about byproduct formation and requires a more accurate quality control of the reactor effluents.

Denitrification coupled to SCN^- or As^{3+} removal is a potent means of water detoxification. Anoxic SCN^- oxidation results in the destruction of the cyanide molecules and production of innocuous compounds (**Table 1**). Similarly, As^{3+} oxidation to As^{5+} strongly reduces arsenic toxicity since As(V) can be easily immobilized on a solid matrix such as activated alumina (AA) or titanium dioxide (TiO_2) and thus removed from the water phase [21].

The use of ZVI and S^0_{bio} for denitrification is environmentally friendly as both electron donors are non-toxic waste products from the steel industry and biological desulfurization, respectively. The reuse of biological and industrial waste for denitrification embraces the principles of circular economy and would represent progress towards the realization of a zero-waste water treatment cycle [202]. Denitrification coupled to $S_2O_3^{2-}$ oxidation is particularly advantageous for the treatment of mining wastewater as residual $S_2O_3^{2-}$ can be simultaneously removed with NO_3^- in effluents from gold leaching. Similarly, the combined removal of Mn^{2+} and NO_3^- is advantageous for the treatment of drinking water as excess manganese is responsible for aesthetic and operational issues.

8.3 Cost

Table 4 compares the costs of all described inorganic compounds and those of widely used organic electron donors for heterotrophic denitrification, i.e. ethanol and acetate. The use of inorganic compounds as energy source for denitrification eliminates the need of external organics, avoids secondary carbon contamination and reduces the cost of sludge handling due to a decreased biomass production (**Table 1**). Autotrophic denitrification often requires the

supplementation of an inorganic electron donor. Excess electron donor should be avoided to reduce process cost and prevent secondary pollution.

H_2 is among the most expensive electron donors for AuDen. However, the low H_2 requirements for denitrification (only 0.41 kg H_2 are used per kg $N\text{-}NO_3^-$), coupled to the use of gas-permeable membranes allowing nearly 100% H_2 utilization, significantly reduce the cost of H_2 supply. Additionally, H_2 production has become more and more cost-competitive in the last years. According to James et al. [197], technologies integrating steam reforming and water electrolysis, e.g. reformed-electrolyzed-purifier (REP) systems, are the most economically viable for H_2 production, resulting in a cost below 3 USD/kg H_2. Nevertheless, membrane operation and cleaning can significantly increase the cost of H_2 utilization depending on wastewater strength and, therefore, a cost analysis is recommended especially for full scale applications.

Despite its higher applicability, $S_2O_3^{2-}$ is about 6 times more expensive than S^{2-} and chemically produced S^0. Other RISCs such as residual H_2S in biogas and S^0_{bio} originating from biogas cleaning and flue gas desulfurization are costless as well as iron scraps or filings from the metallurgical industry. SO_3^{2-} can be cost-effectively produced in scrubbers removing SO_2 from exhaust flue gases and originate from S^0 combustion. SCN^- and As^{3+} are potentially toxic compounds, with the latter featuring a high specific utilization (**Table 4**). Therefore, the use of these compounds for denitrification should be considered only if already present in nitrate-contaminated water.

Table 5.

9 Conclusions

Autotrophic denitrification can be carried out with a wide variety of inorganic compounds. The selection of the most suitable electron donor should be based on kinetics, cost,

availability, applicability, environmental sustainability and potential toxicity as general criteria. Wastewater characteristics such as pH, temperature and chemical composition are also highly relevant in the choice of the most suitable denitrification process. The availability of potential electron donors among waste products (hydrogen sulfide, biogenic sulfur and zero-valent iron), toxic compounds (thiocyanate and arsenite) and potential contaminants (sulfite and manganese) in ground and waste water should be exploited in order to combine denitrification with detoxification. Despite safety concerns, H_2 can be considered the most promising electron donor due to its fast kinetics, low biomass yield, eco-sustainability and reasonable price. RISCs are a good alternative to H_2, especially as an electron supplement for mixotrophic denitrification, which limits the effluent sulfate concentration and are suitable to treat a wide range of nitrate-contaminated waters. Implementation and scaling of novel denitrification bioprocesses are critical to compare AuDen-based systems to other existing technologies and unravel operational issues and costs on a real scale. Future research should also provide kinetic and stoichiometric characterization of novel electron donors (e.g. SO_3^{2-}, AsO_3^{2-} and Mn^{2+}) and investigate the efficiency of mixotrophic denitrification with diverse microbial communities and bioreactor configurations.

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Tables and Figures

Table 1 – Stoichiometry of denitrification reactions with organic and inorganic electron donors. Cellular yields are provided based on reaction stoichiometry.

Table 2 – Physiology of various identified autotrophic denitrifying *Proteobacteria*.

Table 3 – Denitrification performance of continuous bioreactors using different inorganic electron donors.

Table 4 – Price, utilization and cost of inorganic electron donors for autotrophic denitrification.

Table 5 – Benefits (+) and drawbacks (-) related to the use of inorganic electron donors for autotrophic denitrification.

Figure 1 – Complete denitrification pathway with the reductase enzymes and accepted electron equivalents for each step. *NaR* = nitrate reductase, *NiR* = nitrite reductase; *NoR* = nitric oxide reductase; *NoS* = nitrous oxide reductase.

Figure 2 – Full-scale applications of sulfur-limestone autotrophic denitrification (SLAD) [114,115] and hydrogenotrophic denitrification [116] for groundwater and wastewater treatment.

Figure 3 – Concept of Fe^0-assisted hydrogenotrophic denitrification as described by Till et al. [164]. The abiotic NO_3^- reduction to NH_4^+ can be reduced by separating the Fe^0 from NO_3^- and denitrifying bacteria into two anoxic compartments and allowing the cathodic H_2 to flow from one compartment to the other.

Table 1

Reaction	Biomass yield (g cells/g N-NO_3^-)	Reference
CH_3COOH + 1.18 NO_3^- + 1.18 H^+ → 0.12 $C_5H_7O_2N$ + 1.4 CO_2 + 2.5 H_2O + 0.53 N_2	0.82	[32]
CH_3OH + 0.926 NO_3^- + 0.926 H^+ → 0.060 $C_5H_7O_2N$ + 0.703 CO_2 + 2.26 H_2O + 0.432 N_2	0.52	
H_2 + 0.355 NO_3^- + 0.049 CO_2 + 0.355 H^+ → 0.010 $C_5H_7O_2N$ + 0.172 N_2 + 1.143 H_2O	0.23, 0.51[a]	[25]
S^0 + 0.876 NO_3^- + 0.343 H_2O + 0.379 HCO_3^- + 0.023 CO_2 + 0.080 NH_4^+ → 0.080 $C_5H_7O_2N$ + 0.824 H^+ + 0.44 N_2 + SO_4^{2-}	0.74	[33,34]
HS^- + 1.23 NO_3^- + 0.573 H^+ +0.438 HCO_3^- + 0.027 CO_2 + 0.093 NH_4^+ → 0.093 $C_5H_7O_2N$ + 0.866 H_2O + 0.614 N_2 + SO_4^{2-}	0.61	
$S_2O_3^{2-}$ + 1.24 NO_3^- + 0.45 HCO_3^- + 0.09 NH_4^+ + 0.11 H_2O → 0.09 $C_5H_7O_2N$ + 0.40 H^+ + 0.62 N_2 + 2 SO_4^{2-}	0.59	
SCN^- + 1.6 NO_3^- + 0.2 H_2O + 1.6 H^+ + HCO_3^- → SO_4^{2-} + NH_3 + 2 CO_2 + 0.8 N_2	0.19[b]	[35]
Fe^0 + 0.4 NO_3^- + 1.2 H_2O → Fe^{2+} + 0.2 N_2 + 2.4 OH^-	n.a.	[36]
Fe^{2+} + 0.2 NO_3^- + 2.4 H_2O → $Fe(OH)_3$ + 0.1 N_2 + 1.8 H^+	n.a.	[37]
FeS_2 + 3 NO_3^- + 2 H_2O → $Fe(OH)_3$ + 1.5 N_2 + 2 SO_4^{2-} + H^+	n.a.	[38]
H_3AsO_3 + 0.4 NO_3^- → 1.6 H^+ + $HAsO_4^{2-}$ + 0.2 N_2 + 0.2 H_2O	n.a.	[39]
Mn^{2+} + 0.4 NO_3^- + 0.8 H_2O → MnO_2 + 0.2 N_2 + 1.6 H^+	n.a.	[40]

n.a. = not available.

[a] growth yield obtained for a pure culture of *Paracoccus denitrificans* by Strohm et al. [41].

[b] calculated as g proteins g (N-NO_3^-)$^{-1}$ for the bacterium *Thialkalivibrio thiocyanodenitrificans*.

Table 2

Species	Class of *Proteobacteria*	Isolation site	Growth pH	Growth T (°C)	Electron donor	Electron acceptor	Denitrification products	References
Paracoccus denitrificans	α	soil, activated sludge	6.5-8.5	25-37	H_2, $S_2O_3^{2-}$, HS^-, Organics	O_2, NO_3^-	N_2, N_2O	[42]
Paracoccus ferrooxidans	α	denitrifying bioreactor	5-8.5	10-45	H_2, $S_2O_3^2$, SCN^-, $[Fe(II)EDTA]^{2-}$, Organics	O_2, NO_2^-, NO_3^-, N_2O, $[Fe(II)EDTA{\cdot}NO]^{2-}$	N_2	[43]
Paracoccus pantotrophus	α	denitrifying, sulfide-oxidizing effluent treatment plant	6.5–10.5	15-42	H_2, $S_2O_3^{2-}$, HS^-, Organics	O_2, NO_3^-	n.a.	[44]
Ochrobactrum anthropi	α	soil, denitrifying reactor, blood, urogenital tract, respiratory tract, eyes	7-8 (opt.)	7-40	H_2, Organics	O_2, NO_3^-	N_2	[45,46]
Azospirillum brasilense	α	soil and grass roots in tropical areas	7-8 (opt.)	37 (opt.)	H_2, Organics	O_2, NO_2^-, NO_3^-, N_2O	N_2	[47]
Bradyrhizobium japonicum	α	root tips of soy bean plants	4-9.5	27.7-35.2 (opt.)	H_2, Organics	O_2, NO_2^-, NO_3^-, N_2O	N_2, NO_2^-, N_2O	[48,49]
Thiobacillus denitrificans	β	pond, brackish mud, soil, marine sediment, sewage lagoon, digestion tank	6.8–7.4	28-32	HS^-, S^0, $S_2O_3^{2-}$, Fe^{2+}, FeS_2	O_2, NO_3^-, NO_2^-	N_2, NO_2^-, N_2O	[18,37,50]
Ralstonia metallidurans	β	sludge of a zinc decantation tank, gold grains, metal factory, sediments	7 (opt.)	4-41, 30 (opt.)	H_2, organics,	O_2, $NO_2^-NO_3^-$	N_2, NO_2^-	[51–53]
Thiobacillus thiophilus	β	oil-contaminated sediment	6.3–8.7	-2-30	$S_2O_3^{2-}$	O_2, NO_3^-	N_2	[54]
Rhodocyclus sp.	β	sewage-lagoon, drinking water aquifer	6.5-7.5	30 (opt.)	H_2, organics	O_2, NO_3^-	N_2, NO_2^-	[55,56]
Azospira oryzae	β	swine waste lagoons grass roots, rice	6.5 (opt.)	37 (opt.)	Fe^{2+}, organics	O_2, NO_3^-, ClO_3^-, ClO_4^-, $SeO4^{2-}$, SeO_3^{2-}	N_2	[57,58]
Acidovorax BoFeN1	β	lake sediments	6-9	4-37	Fe^{2+}, organics	NO_2^-, NO_3^-, N_2O	N_2, NO_2^-	[59,60]

Pseudogulbenkiania sp. strain 2002	β	freshwater lake sediments	6.75-8	15-40	Fe^{2+}, organics	O_2, NO_2^-, NO_3^-, N_2O	N_2	[61]
Thioalkalivibrio denitrificans	γ	soda lake sediment	6–10.5	30 (opt.)	S_2O3^{2-}, polysulfide	O_2, N_2O	N_2	[62,63]
Thialkalivibrio nitratireducens	γ	hypersaline soda lake sediment	8–10.5	30 (opt.)	HS^-, $S_2O_3^{2-}$, polysulfide	O_2, NO_3^-	NO_2^-	[64]
Thiohalomonas nitratireducens	γ	sediments of hypersaline lakes	8	30 (opt.)	$S_2O_3^{2-}$	O_2, NO_3^-	NO_2^-	[65]
Thiohalomonas denitrificans	γ	sediments of hypersaline lakes	6.5–8.2	30 (opt.)	HS^-, $S_2O_3^{2-}$	O_2, NO_3^-, NO_2^-	N_2, NO_2^-, N_2O	[64]
Thioalkalivibrio thiocyanodenitrificans	γ	hypersaline soda lake sediment	8–10.5	30 (opt.)	$S_2O_3^{2-}$	NO_2^-	N_2, N_2O	[35]
Thiohalophilus thiocyanoxidans	γ	sediments of hypersaline lakes	6.5–8.2	30 (opt.)	$S_2O_3^{2-}$	NO_3^-	N_2, NO_2^-, N_2O	[66]
Thioalkalispira microaerophila	γ	sediments of hypersaline lakes	8–10.4	30 (opt.)	HS^- , $S_2O_3^{2-}$	O_2, NO_3^- (no growth)	NO_2^-	[67]
Thiohalorhabdus denitrificans	γ	sediments of hypersaline inland lakes	6.5–8.2	33–35 (opt.)	$S_2O_3^{2-}$	O_2, NO_3^-	NO_2^-, N_2O	[68]
Acinetobacter sp. SZ28	γ	oligotrophic reservoir	6 (opt.)	30 (opt.)	Mn^{2+}, Fe^{2+}, S^{2-}	NO_3^-, NO_2^-	n.a.	[69]
Alkalilimnicola ehrlichi strain MLHE-1	γ	stratified soda lake	7.3-10 9.3 (opt.)	13-40 30 (opt.)	As^{3+}, H_2, S^{2-}, $S_2O_3^{2-}$	NO_3^-, O_2	NO_2^-	[70]
Sulfurimonas denitrificans	ε	estuarine mud	7 (opt.)	22 (opt.)	HS^- , $S_2O_3^{2-}$	NO_3^-, NO_2^-	N_2, NO_2^-, N_2O	[71]
Thiomicrospira CVO	ε	oil field	5.5–8.5	5–35	HS^-, S^0 , $S_2O_3^{2-}$	O_2, NO_3^-, NO_2^-	N_2, NO_2^-, N_2O	[72]
Sulfurimonas paralvinellae	ε	hydrothermal vent polychaetes	5.4–8.6	4–35	HS^-, S^0 , $S_2O_3^{2-}$	O_2, NO_3^-, NO_2^-	N_2, NO_2^-, N_2O	[71]

n.a. = not available.

Table 3

Electron donor	Bioreactor system	Influent type	Temperature [a] (°C)	pH [a]	Nitrogen loading rate [a] (g $N\text{-}NO_3^-$ L^{-1} d^{-1})	Denitrification efficiency (%)	Reference
H_2	membrane biofilm reactor	synthetic groundwater	n.a	9	0.96–1.2	93 (average)	[84]
H_2	series of biofilters	contaminated drinking water	27	6.8	1.97–6.2	>97.5	[90]
S^0	packed-bed reactor	synthetic wastewater	35	n.a.	1.64–2.46	>95	[91]
S^0	fluidized bed reactor	sanitary landfill leachate	20	7.2–8.2	2.68	>98	[92]
S^{2-}	packed-bed reactor	simulated nitrified domestic sewage	30±1	8.9–9.0	0.05	98.5 (average)	[93]
S^{2-}	completely mixed activated sludge reactor	synthetic wastewater	30	7.5	0.09–0.74	>98	[94]
$S_2O_3^{2-}$	fluidized bed reactor	synthetic wastewater	30	7	0.28–3.25	100	[83]
FeS	packed-bed reactor	contaminated drinking water	n.a.	~7.8	0.13	>95	[95]
Fe^{2+}	up-flow anaerobic sludge blanket	synthetic wastewater	30±1	6-6.6	0.041-0.117	>99	[85]
SCN^-	continuous stirred-tank reactor	synthetic wastewater	21	8–8.5	1.2 [b]	>90	[19]

n.a. = not available.

[a] only values associated to denitrification efficiencies above 90% and nitrate-limiting conditions (except [b]) are reported.

Table 4

Electron donor	Price [a] (USD/kg e^- donor)	Specific substrate utilization [b] (kg e^- donor/kg N-NO_3^-)	Cost (USD/kg N-NO_3^-)
Methanol (CH_3OH)	0.7-0.9	2.5	1.8-2.3
Acetic acid (CH_3COOH)	2.2	3.6	7.9
Hydrogen (H_2)	2.6–5.1	0.4	1.1–2.1
Elemental sulfur (S^0_{chem})	0.1	2.6	0.26
Biogenic sulfur (S^0_{bio})	0	2.6	0
Sulfide (S^{2-})	0.13	1.9	0.25
Thiosulfate ($S_2O_3^{2-}$)	0.21–0.26 [c]	6.5	1.4–1.7
Ferrous iron (Fe^{2+})	0.30	19.9	6.0
Zero-valent iron (Fe^0)	0	10.0	0
Pyrite (FeS_2)	0.4	2.9	1.2
Sulfite (SO_3^{2-})	0.18	n.a.	n.a.
Manganese (Mn^{2+})	0.20–0.21 [d]	9.8	2.0-2.1
Thiocyanate (SCN^-)	0	2.6	0
Arsenite (As^{3+})	0	21.9	0

n.a. = not available.

[a] the price of H_2 is based on the information reported by James et al. [197]. The price of organics is provided by Park and Yoo [11]. The price of S^0, S^{2-} and Fe^{2+} is provided by Zhu and Getting [198]. The price of SO_3^{2-} is as reported by Sabba et al. [22]. The price of SCN^- is based on a survey of chemical suppliers on US market. Fe^0, SCN^-, As^{3+} and S^0_{bio} were assumed free of cost being waste products and/or contaminants.

[b] calculated according to the equations listed in **Table 1**.

[c] based on the price of $Na_2S_2O_3 \cdot 5H_2O$ provided by ICIS (https://www.icis.com/).

[d] average price of manganese metallurgical ore (46-48% Mn content) based on a survey on the US market in 2017.

Table 5

Electron donor	Kinetics	Cost [a]	Handling [b]	Availability	Applicability [c]	Sustainability	Toxicity [d]
Hydrogen (H_2)	+	-	-	+/-	+	+	+
Elemental sulfur (S^0)	+/-	+	+	+	+/-	+/-	+
Biogenic sulfur (S^0_{bio})	+/-	+	+	+	+/-	+	+
Sulfide (S^{2-})	+/-	+	+	+/-	+	-	-
Hydrogen sulfide (H_2S)	-	+	-	-	+/-	+	-
Thiosulfate ($S_2O_3^{2-}$)	+	-	+	+/-	+	+/-	+
Sulfite (SO_3^{2-})	+/-	+/-	+	+/-	+	+/-	+/-
Thiocyanate (SCN^-)	-	+	-	-	-	+	-
Ferrous iron (Fe^{2+})	-	-	+	+/-	+/-	-	+
Zero-valent iron (Fe^0)	-	+	+	+	+/-	+	+
Pyrite (FeS_2)	-	+/-	+	+	+/-	+/-	+
Arsenite (As^{3+})	-	+	-	-	-	+	-
Manganese (Mn^{2+})	-	+/-	+	+/-	+/-	+/-	+

[a] arsenite and thiocyanate are considered inexpensive when already present in the influent water; evaluation on manganese and sulfite is based exclusively on their price.

[b] both safety and ease of handling are considered.

[c] electron donor applicability to different reactor configurations and NO_3^- contaminated water types is considered.

[d] potential toxicity to both humans and microorganisms is considered.

Figure 1

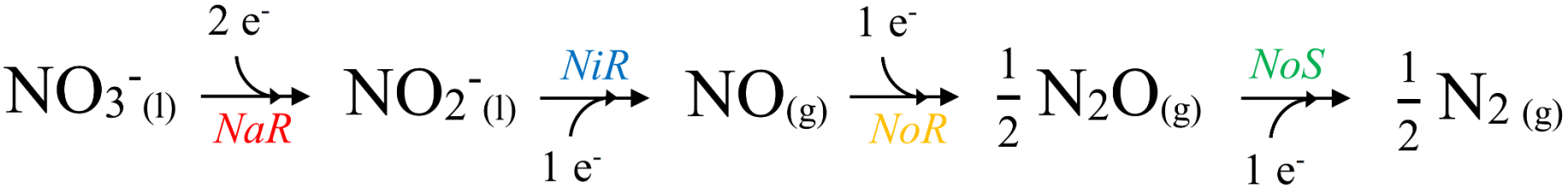


Figure 2

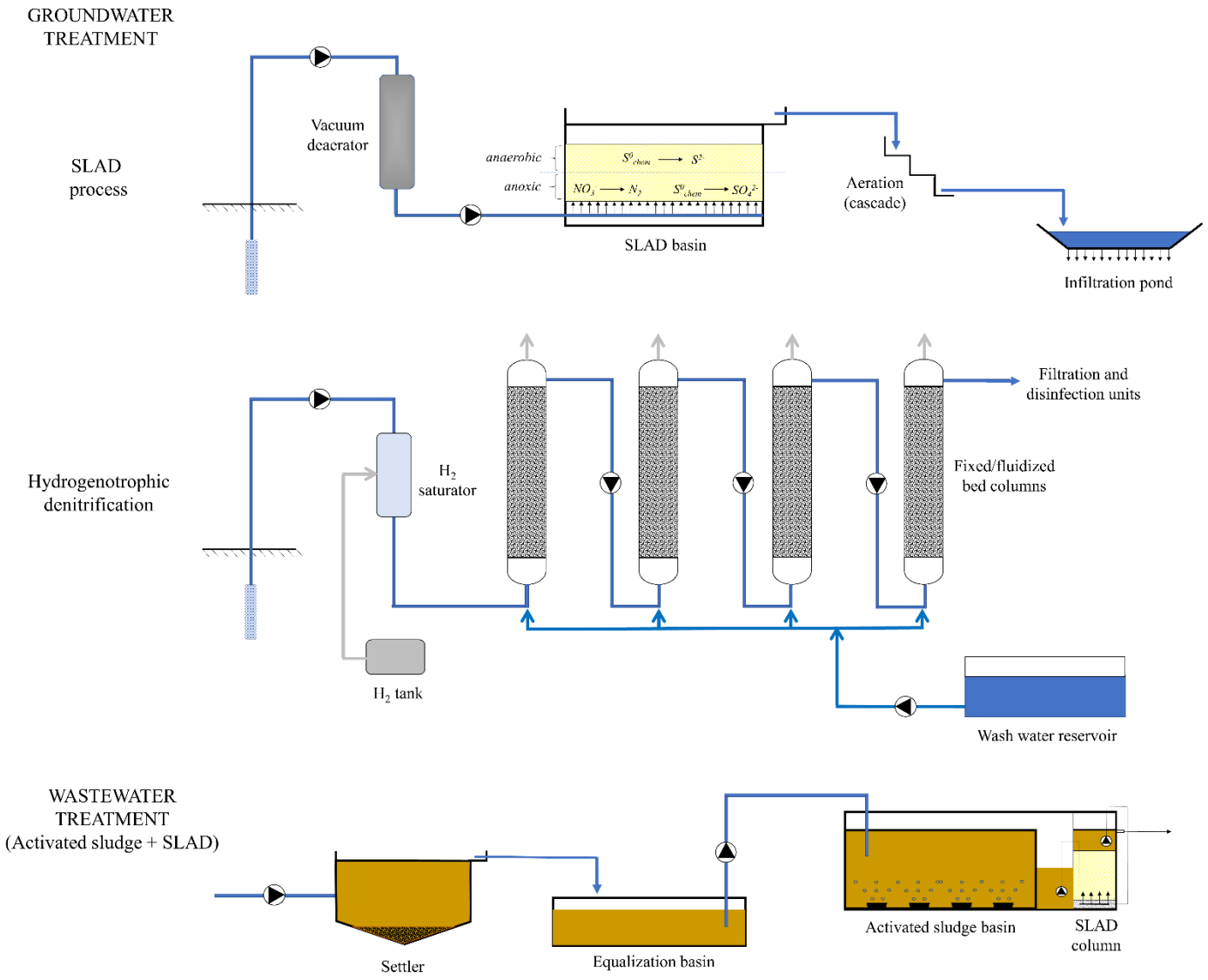
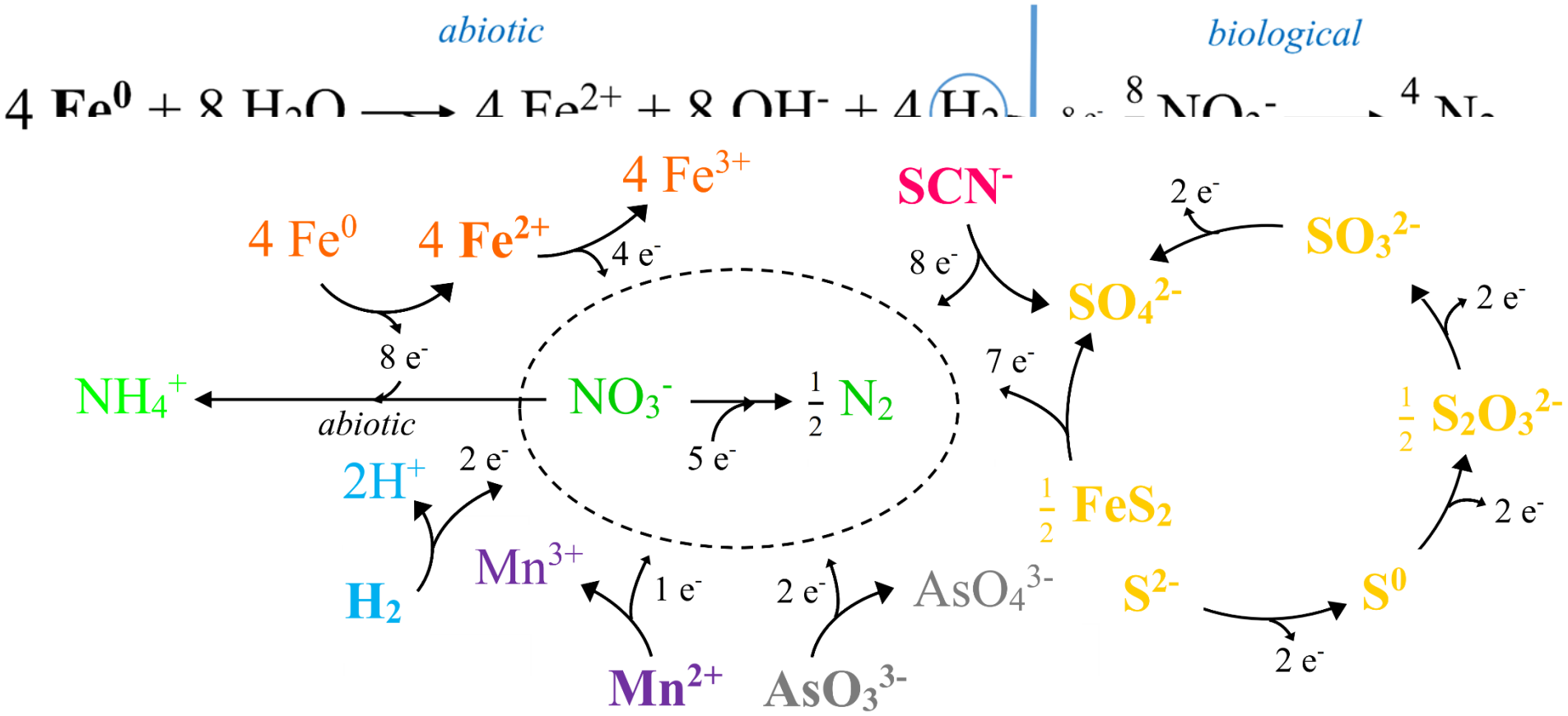


Figure 3



Graphical abstract

Highlights

- Twelve electron donors for autotrophic denitrification are critically reviewed.
- Biochemical aspects and microbiology of autotrophic denitrification are discussed.
- Novel insights on the use of inorganic compounds for denitrification are presented.
- Applications, cost and environmental impact of inorganic compounds are compared.
- Criteria and guidelines for electron donor selection are provided.