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# Advances in the biological removal of sulphides from aqueous phase in anaerobic processes: A review

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**Abstract**

In this paper, we review the latest developments in biological methods used in the removal of hydrogen sulphide, present in the liquid phase in anaerobic reactors. Also the toxicity of $H_2S$ on methane-forming microorganisms and the problems caused by the presence of this compound in the biogas generated during this process as well as the main causes of hydrogen sulphide generation in anaerobic processes of wastes. We specially discuss the fundamentals in applying micro-aerobic conditions in order to remove dissolved hydrogen sulphide from the aqueous phase of an anaerobic reactor. The alternative technology of simultaneous removal of sulphide, nitrate and organic matter is under recent investigation. Therefore, this review paper study and analyze the microbiological basis of this technology, the physical - chemical factors that influence the process and the potential application of this technology on different types of wastewaters and situations. Also considered are the fundamentals of both biofilm reactors and microbial fuel cells

desulphurization. Because relatively few studies on modeling desulphurisation processes are available, we discuss the advances made in the area.

**Keywords**: anaerobic, denitrifying sulphide, desulphurization, microaerobic, modeling.

## 1. Introduction

The different ways to remove hydrogen sulphide generated during the degradation of organic matter via anaerobic digestion has been an important subject in many studies. In recent years, several works have been written on the application of biological processes in the removal of hydrogen sulphide, including different reviews in microbiology on the sulphur cycle (Tang et al. 2009), sulfate conversion in wastewater treatment (Hao et al. 2014) and the simultaneous removal of nitrogen-sulphur-carbon (Show et al. 2013). Even though these works thoroughly review sulphur removal, their efforts have focused on the removal of $H_2S$ from the gas phase while sulphide removal from the liquid phase has been scarcely analyzed. Due to this reality and their potential, it is beneficial to analyze specifically the application of these processes. This paper begins by establishing the various problems that hydrogen sulphide presence and production generate in anaerobic processes; this background is extremely important in order to develope strategies to reduce their production. The second part of this work focuss in the foundations of the main biological desulphurization processes studied and recently applied on different scales: microaerobic desulphurization, autotrophic denitrifying, microbial fuel cells (MFCs) and biofilm reactors for desulphurization. Due to the potential applicability of process modeling, this subject has also been included in this paper.

## 2. $H_2S$ production, toxicity and their concerns in anaerobic processes

The application of anaerobic processes in the treatment of liquid and solid waste has increased significantly in recent years, mainly due to the upflow anaerobic sludge blanket (UASB) reactor developed by Gatze Lettinga in the Netherlands (Lettinga, et al. 1980). The main advantage that anaerobic processes has over aerobic processes is that the transformation of organic matter is achieved using a low power consumption technology.

When compared results that during the aerobic processes approximately 60% of the energy was consumed during the synthesis of new biomass and 40% of the energy is lost as reaction heat while during the anaerobic processes almost 90% of the energy that originally exist in the substrate is retain as biogas and only 7% of the initial energy is lost as reaction heat. During the aerobic processes approximately 50% of the carbon in the substrate was converted into biomass and 50% was converted to $CO_2$;while during the anaerobic processes approximately 95% of organic matter was converted to biogas ($CH_4$, $CO_2$) and only 5% is converted to biomass. Therefore, the production of biogas generate or recover energy instead of just save energy. This reduce operational costs when compared with aerobic processes with lower nutrient requirements with optimum C:N:P ratio of 100:0.5:0.1, which is approximately tenth than necessary in aerobic processes (Converti et al. 2009; Kothari et al. 2014; Semblante et al. 2014; Yang et al. 2014).

One of the main drawbacks of anaerobic digestion is hydrogen sulphide. $H_2S$ is generated from the reduction of sulfate in anaerobic digestion, causing inhibitory effects. Therefore it should be taken into account when wastewaters containing high sulfate concentrations are treated. (e.g. wastewater from fishery, tannery, food processing, distillery, pulp and mill, mining, metalurgical, chemical, pharmaceutical and oil refinery industries and livestock farming (Janssen et al. 1999; Jarvis and Younger 2000; Lens et al. 2003; Altaş and Büyükgüngör 2008; Kaksonen and Puhakka 2007; Zheng et al. 2009; Hiibel et al. 2011; Shakir et al. 2012; Klok et al. 2013; Hao et al. 2014; Searmsirimongkol et al. 2011). The toxicity problem of hydrogen sulphide is extremely complicated due to the complex roles this compound plays as a nutrient as well as an inhibitor of microorganism activities. Moreover, $H_2S$ is a volatile malodorous compound whose presence causes downstream corrosion and damage in equipment, for example, in combined heat and power biogas engines. Therefore, $H_2S$ must be removed from biogas if is used in energy generation (Peu et al. 2012).

Hydrogen sulphide generated by sulfate reducing bacteria (SRB), in the presence of organic matter, appears partially dissociated as $HS^-$ and $H^+$, depending on the pH of the liquid bulk (Sawyer et al. 2003, Simbualhong et al. 2007). The non-ionized form of sulphide is the molcule responsable for the inhibition process (Visser et al. 1993; Valdés et al. 2006). The pH value plays a fundamental role in the degree of inhibition, since it determines the equilibrium between ionized and non-ionized sulphide forms as can be seen in Figure 1.

From Fig. 1, it can be inferred that as pH values approach 6, the ionized form predominates. For this reason, it is recommended that in wastewater treatment with high concentrations of sulfates, operating pH must be maintained at relatively high values. The mechanism of inhibition indicates that the non- ionized hydrogen sulphide molecule, is able to penetrate the methanogenic archaea (MA) cell membrane and interfere with disulphide bridges between polypeptide chains, obstructing coenzyme activities (Vahdati 2007) and preventing sulphur assimilation process by the MA (Chen et al. 2008).

From a thermodynamic and kinetic point of view (Tables 1 and 2), a sulfate reduction process is more favourable than methanogenesis. This fact implies that SRB can out-compete MA in the presence of unlimited sulfate concentrations for several substrates such as hydrogen, formate, acetate, propionate, butyrate, ethanol and sucrose (Stams 1994; Colleran et al. 1995; Omil et al. 1996; Greben et al. 2000; Muyzer and Stams 2008). SRB does not compete with MA for some organic substrates, such as, trimethylamine, or methionine (Oremland and Polcin 1982). SRB and MA at mesophilic temperatures compete for methanol utilization, but at temperatures above 65 ºC SRB will out-compete methanogens for this substrate (Weijma et al. 2000).

The influent chemical oxygen demand (COD) – sulfate ratio ($COD/SO_4^{2-}$) is the most important parameter concerning the competition between SRB and MA and other anaerobic bacteria (Velasco et al. 2008). Reducing 1g of $SO_4^{2-}$ equals 0.67 g COD (Eq. 1 and 2), which means that for every kg of $SO_4^{2-}$- that is reduced, the production of $CH_4$ decreases in 0.23 $m^3$. If microorganism growth is taken into account, much higher ratios of 0.67 are needed to reduce $SO_4^{2-}$. There is extensive evidence supporting this behavior. Table 3 contains examples of COD removal variations dependent on $COD/SO_4^{2-}$ ratio.

$$SO_4^{2-} \rightarrow S^{2-} + 2O_2 \quad (1)$$

$$96 \text{ g } SO_4^{2-} \rightarrow 64 \text{ g } O_2 \quad (2)$$

As the $COD/SO_4^{2-}$ ratio increases, organic matter removal also increases (as shown in Table 3). However, the most conclusive results are shown by Choi and Rim (1991), they observed that SRB and MA were very competitive at COD/ $SO_4^{2-}$ ratio from 1.7 to 2.7; also that MA predominated at high $COD/SO_4^{2-}$ ratios, while SRB predominated when the value of this ratio decreased. On the other hand, Prasad et al. (1988)

observed that MA prevailed over SRB at $COD/SO_4^{2-}$ ratio around 1. Vossoughi et al. (2003) working with an anaerobic baffled reactor (ABR) treating synthetic wastewater (3000 mg COD/L) at 35ºC, observed that when $COD/SO_4^{2-}$ ratios change from 16.7 to 6 with increasing sulfate concentration from 180 to 500 mg/L, a slight increase in COD removal was achieved.

Although studies vary in their results, it is noteworthy to mention that in most cases, $H_2S$ production increases with decreasing of $COD/SO_4^{2-}$ ratio, decreasing production of $CH_4$. Some studies even show that this ratio is not decisive on the performance of UASB reactors (Callado and Foresti 1992). One must also take into account that when this ratio reaches values greater than 10, an important part of $H_2S$ formed is stripped from the liquid phase due to a much larger gas production. Moreover in different studies is been observed that the behavior of the anaerobic process is not only influenced by the $COD/SO_4^{2-}$ ratio, but also by the initial concentration of sulfates and sulphides. Inlet $SO_4^{2-}$ concentration of 150 mg/L caused a degree of inhibition in anaerobic processes (Silva et al. 2002). In other different studies (Cohen et al. 1982; Rinzema and Lettinga 1988; Nanqi et al. 2002), carried out in digesters operating with acetates, propionates, lactates and glucose concluded:

-Levels of dissolved sulphide of 64 - 200 mg of dissolved sulphide/L caused “stress” in completely mixed systems and at higher values total failure ocurred in systems operated with acetates and propionates.

-Levels of hydrogen sulphide of 100 – 150 mg of sulphur/L and dissolved sulphide of 200 – 400 mg of sulphur/L can be tolerated in anaerobic systems fed with lactate and glucose, operated with significantly lower efficiency level (50 – 70 % of COD removal, 40 – 80 % of sulfate conversion). Under similar conditions operating with lactate and glucose versus acetate and propionate, higher levels of dissolved sulphide and hydrogen sulphide are achieve in the anaerobic digester operating with lactate and glucose.

-Anaerobic packed bed reactors can withstand much higher concentrations of dissolved hydrogen sulphide than the completely mixed systems. In this type of reactors, fed with propionates, the hydrogen sulphide levels above 200 mg/L did not cause inhibition and levels of dissolved sulphides near 1000 mg sulphur/L could be tolerated with minor negative effects.

-In a packed reactor fed with acetate, the hydrogen sulphide levels in excess of 125 mg sulphur/L, caused no inhibition. In these same studies, in assays carried out with acetates and propionates using chemostats, it was

observed that hydrogen sulphide levels of 50 – 80 sulphur mg/L caused damage to anaerobiosis.

From the above studies, it is evident that there are difficulties involved when setting sulphide concentrations so no inhibition of the anaerobic process occurs, however, there is a general consensus that anaerobic inhibition begins to occur at values of 50-250 mg sulphur/L. Although, there have been studies that not only obtain good performances from anaerobic reactors operating at higher concentrations than those identified above (Iza et al. 1986), but it has also been suggested that increased concentrations of sulphur can enhance the biological sulfate reduction (Greben et al. 2005).

As previously commented, hydrogen sulphide cannot only cause inhibition in the anaerobic process with consequent loss of organic matter removal efficiency, but also when the undissolved part of biogas is considered it often limits significantly the use of this gas; there have been values of up to 17000 ppm of $H_2S$ reported in the biogas (Chaiprapat et al. 2011). However, this level of concentration is highly unusual; the expected concentration is no greater than 5000 ppm (Namgung et al. 2012) and in many cases this concentration is in the range of 1000 – 2500 ppm (Srichareon 2007; Pipatmanomai et al. 2009).

In summary, the $H_2S$ content in biogas depends on various factors such as wastewater pH, waste carbon source composition and operational conditions (Noyola et al. 2006). They will determine the existence of different substances which can serve as donor electrons for sulfate reduction such as: $H_2$/CO (Sipma et al. 2007), $H_2/CO_2$ (Liamlean and Annachhatre 2007), $CH_4$ (Zhang et al. 2010a), formate (Bitjmans et al. 2008), acetate (Koschorreck et al. 2004), lactate (Bertolino et al. 2011), glucose/acetate (Erdirencelebi et al. 2007), molasses (Teclu et al. 2009), cheese whey (Jiménez – Rodríguez et al. 2010) and animal manure (Gibert et al. 2004). Consequently, different SRB genera act in sulfate reduction depending on electron donors; 16 genera belong to incomplete organic oxidizers that produce acetate and $H_2S$ and 22 genera are complete oxidizers that produce $CO_2$, $H_2O$ and $H_2S$ (Hao et al. 2014).

Some authors set the maximum allowable amounts of $H_2S$ in biogas for use in 100-500 mg/$Nm^3$ biogas (65 – 330 ppmv) if the biogas is to be used in combined heat and power installations (Peu et al. 2012). Others indicate that the sulphide content in biogas should not be more than 1000 and 0.1 ppmv in internal combustion engines and molten carbonate fuel cells respectively (Rasi et al. 2011). Likewise, in combined heat and power plants, which are mainly implemented for the utilization of biogas, levels below 250 ppmv are

required (Weiland 2010). Duangmanee (2009) informed that the maximum $H_2S$ concentration for utilization in steam boiler and internal combustion motor must be 1000 and 100 ppmv, respectively. $H_2S$ concentration in biogas, higher than 0.03% (v/v), can cause acid rain due to high $SO_x$ generation in the combustion engine. The corrosive effect of $H_2S$ gas, 0.05 – 2% (v/v), significantly reduces the lifetime of pipe work and other installations (Deublein and Steinhauser 2011; González et al. 2014). Deublein and Steinhauser (2011) also stated that for vehicles the content must be lower than 5 mg/$Nm^3$.

$H_2S$ can also cause health problems. Several laws and regulations have been issued in different countries to minimize its presence in all part of biogas plants, including in digesters, gasholders, storage tanks, etc (Deublein and Steinhauser 2011). Small amounts of $H_2S$ in biogas (0.01 % v/v) emanate an odour reminding rotten eggs. Levels of $H_2S$ greater than 10 ppm in the air can affect human health, while levels more than 600 ppm can cause death (Droste 1997). Other authors stated that concentracions of 0.2% of $H_2S$, in the air is fatal to humans exposed for a few minutes and is also explosive at concentrations of 4.3 – 4.5% (Camargo 1986).



## 3. Sulphide biological removal technologies

### 3.1 General aspects

Due to the previously mentioned difficulties caused by the presence of hydrogen sulphide in the biogas, different technologies have been applied to purify biogas (Cirne et al. 2008). Therefore, a wide range of physical, chemical and biological methods exist (Abatzoglou and Boivin 2009; Kobayashi et al. 2012; Lin et al. 2013). Since sulphideis toxis for MA in liquid phase and causes the inhibition of the anaerobic process; therefore, it is convenient to remove the sulphides in the liquid phase. The physico-chemical method most applied for hydrogen sulphide removal from the liquid phase in an anaerobic process has been precipitation with metals, mainly with $Fe^{3+}$ (McFarland and Jewell 1989). A simplified reaction of hydrogen sulphide with $Fe^{3+}$ is as follows (Parameshwaran and Hills 1984):

$$Fe_2O_3 + 3H_2S \rightarrow Fe_2S_3\downarrow + 3H_2O \qquad (3)$$

However, this practice has several important limitations. It is expensive, complicated from an operational standpoint and generates sludge that may contain amounts of iron that complicates final disposal (McFarland and Jewell 1989).

In contrast, biological methods have lower operational costs with lower or no utilization of chemicals (Syed et al. 2006; Mahmood et al. 2007). Several biological methods, for the removal of sulphide from the aqueous phase of an anaerobic digester, have been studied. Recently, microaerobic, autotrophic denitrification, microbial fuel cells and biofilms processes have been studied at different levels.

### 3.2 Microaerobic desulphurisation

Microaerobic desulphurisation consists in injectiing small amounts of oxygen or air into the liquid phase of anaerobic reactors (Jenicek et al. 2008). Some authors have pointed out that $H_2S$ removal takes place both biologically and chemically (Kleinjan 2005, Díaz et al. 2011). The final products of biological oxidation depend on the amount of oxygen available for sulphide oxidising bacteria (SOB), in accordance with the following reactions (Tang et al. 2009):



$$H_2S + 1/2\ O_2 \rightarrow S^o + H_2O \qquad \Delta G^o = -209.4\ kJ \qquad (4)$$

$$S^o + H_2O + 3/2\ O_2 \rightarrow SO_4^{2-} + 2H^+ \qquad \Delta G^o = -587.1\ kJ \qquad (5)$$

$$H_2S + 2\ O_2 \rightarrow SO_4^{2-} + 2H^+ \qquad \Delta G^o = -798.2\ kJ \qquad (6)$$

The predominance of elemental sulphur or sulfate as the final product of oxidation depends on the availability of oxygen; thus, in limited oxygen conditions (microaerobic), elemental sulphur is the main product (Janssen et al. 1995). Consequently, depending on the substrate and operational conditions (mainly oxygen content available), microorganisms responsible for the $H_2S$ oxidation belong to very large and different genera and species (Chaiprapat et al. 2011; Ramos et al. 2013, 2014a; Yu et al. 2014).

The results of several studies show the benefits of applying a microaerobic process to anaerobic digestion. However, recently there have been some concerns regarding a possible process failure due to the damage oxygen could cause to strict anaerobes, for example, methanogenic archea. But different authors have found evidence to support the possibility of no inhibitory effects of oxygen on anaerobic microorganisms. Information presented by Botheju and Bakke (2011) highlights that strict anaerobes have several deterrence mechanisms to tolerate microaerobic conditions. Other authors have found that granular sludges protect, to some extent, strict anaerobes from the effects of oxygen in the medium (Kato et al. 1994; Durán et al. 2008). A study carried out by Krayzelova et al. (2014), at laboratory scale, demonstrated that the microaerobic procedure did not impair the quality of granular sludge in an UASB reactor; the specific methanogenic activity (SMA) of the sludge achieved was that of 0.389 and 0.336 ml $CH_4$/g TSS·d for UASB reactors with and without microaeration, respectively. Also, there were no inhibitory effects found on suspended sludge (Estrada-Vázquez et al. 2003) Jenicek et al. (2011) reported similar results.

As previously mentioned, the limiting operational parameter of microaerobic desulphurisation in practical conditions is the oxygen supply, because other parameters such as temperature are fixed (generally 35 – 37ºC). Both organic load and hydraulic retention time (HRT) depend on the type of reactor used in each specific installation. There have been reports on different oxygen amounts applied to the anaerobic process and they vary widely; there is no set of parameters or general indicators to compare the results of different studies objectively. An alternative could be the the use of the $O_2/H_2S_{supplied}$ ratio (Fortuny et al. 2008; Ramos et al. 2013), allowing for the normalization of the oxygen application or simply knowing the specific amount of oxygen being used. Another alternative could be the use of the parameter $O_2/SO_4^{2-}{}_{supplied}$, taking into account that in most cases $H_2S$ in an anaerobic process comes from $SO_4^{2-}$ reduction. $O_2$ added volume/reactor volume.minute (vvm) could also be a comparative parameter for different microaerobic studies.

Ramos et al. (2014b) operated a pilot anerobic sludge digester at HRT of 22- 24 days with an initial 1% (v/v) $HS^-$ , supplying 0.21 – 0.28 NL $O_2/L_{sludge\ feed}$ achieving $CH_4$, $H_2S$ and $O_2$ concentrations (% v/v) of 95.3, 0.03 and 0.86 , respectively. In other studies (Ramos et al. 2013), a pilot anaerobic sludge digester was operated at 14 – 18 days of HRT working with oxygen flow rates of 4.4 – 6.2 NL $O_2/m^3$.d, achieving concentrations (% v/v) of $H_2S$ in biogas of 0.02-0.03. Whereas, when oxygen was not supplied, $H_2S$ concentration (% v/v) in biogas was 0.34. The high efficiency of microaerobic desulphurization was also

demonstrated in studies performed by Díaz et al. (2011); in pilot sludge anaerobic digester where $H_2S$ concentration of 1.5% (v/v) in biogas was obtained after the application of 0.25 NL $O_2/L_{fed\ sludge.}$ The resulting $H_2S$ concentration was very close to zero most of the time (more than 98 % $H_2S$ removal was achieved).

Montalvo et al. (2014a) used natural zeolite in a microaerobic procedure (0.08 ppmv) into a UASB reactor. They found that the use of natural zeolite helped the granulation process and start up of the UASB reactor; with zeolite there was a time decrease of 50% to complete the granulation compared to that of the UASB reactor without zeolite. The anaerobic process enhancement has been shown in various studies (Fernández et al. 2007, Montalvo et al. 2012, Montalvo et al. 2014b). Hydrogen sulphide removal in the UASB reactor with natural zeolite and micro-aeration was not largely affected neither by different HRTs applied to the operation of the reactor nor by high volumetric organic loads (VOL). When operating at a HRT of 2.4 h and a VOL of 18.6 kg COD/$m^3$/d, there was no evident decrease in sulphide removal. The average hydrogen sulphide removal was higher than 94.56 ± 4.71%, confirming that the micro-aeration system is reliable to operate under conditions in which shocks of organic matter or sulfate concentrations in the reactor may happens without a reduction in their efficiency. In this study, when an excess of $O_2$ was applied to assays carried out in batch reactors, there was a re-conversion of $H_2S$ to $H_2SO_4$.

Pure $O_2$ or air (21% $O_2$ and 79% $N_2$) can be injected in anaerobic reactors in order to promote a microaerobic environment. Air is a costless oxygen source; however, the effect of introducing nitrogen results in calorific power dilution of the biogas. Thus, it is very important to know what will be the end use of the biogas. Díaz et al. (2011) carried out research in order to compare microaerobic – anaerobic process behavior when air is injected into a reactor. They found that similar removal efficiencies were achieved when using oxygen and air, but air slightly lowered the methane concentration in the biogas because of nitrogen dilution, yet the biogas maintained its fuel qualities. Montalvo et al. (2014a, b) also found that using air in microaerobic – anaerobic process, biogas also maintained its fuel qualities. Díaz et al. (2011) stated that when air is used therefore diluteing biogas with nitrogen in microaerobic process, a redution in engine efficiency might be expected. Considering that in many cases biogas is not used to generate electricity or moving internal combustion engines, the use of air in microaerobic processes becomes more applicable. Porpatham et al. (2007) demonstrated that “diluted” biogas could be used in a combustion engine, they found that a

decrease in methane concentration from 70% to 50% only reduced the spark-ignition engine energetic performance by 0.9% for the same mass methane flow.

A common aspect in all studies about microaerobic desulphurization is that the dissolved $O_2$ concentration in liquid media always remains as dissolved oxygen below 1 mg/l.

One aspect that is more complex to analyze in microaerobic desulphurization is the balance of sulphur compounds, because of the use of oxygen in a liquid medium containing sulphides where different sulphur chemical species exist according to the following reactions (Duan et al. 2005):

$$H_2S \rightarrow S^o \rightarrow S_2O_3^{2-} \rightarrow S_4O_6^{2-} \rightarrow S_3O_6^{3-} \rightarrow SO_3^{2-} \rightarrow SO_4^{2-} \quad (7)$$

The balance of sulphur compounds is very important, because the hydrogen sulphide content and dissolved hydrogen sulphide content in the biogas is of interest. The mentioned interest is not only due to the inhibition that it may cause on the anaerobic process, but also because their presence in the liquid effluent of the digester can consumption a significant amount of oxygen in their final disposal. This is of preponderant importance, especially if its final disposal is in rivers or lackes. Finally, the formation about elemental sulphur is one aspect that can have a great impact on process maintenance, because they generate solid deposits inside the digesters.

The various streams leaving the microaerated anaerobic reactor that contain sulphur compounds, also affects the sulphur balance: 1) total sulphur compounds in the effluent, 2) total sulphur compounds in the excess of biomass, 3) hydrogen sulphide in biogas, 4) deposition of elemental sulphur in the reactor headspace, 5) total sulphur compounds in the effluent solids. For example, De Graff et al. (2012), in order to calculate the elemental sulphur concentration, used the following mass balance under steady-state conditions:

$$[S^\circ] = [\text{Influent S}] - [SO_4^{2-}] - 2[S_2O_3^{2-}] - [HS^-] \quad (8)$$

It has been proven that the balance can be carried out with minimal error, if its only consider in practical conditions of microaeration the following chemical species in the streams leaving the reactors: $H_2S$ dissolved and in the biogas, Sº present in the biomass, in the headspace and in the effluent solids and $SO_4^{2-}$ in the effluent. It is also very important to know the sulfate concentration that may be in the liquid effluent from the digester, because concentration of this chemical species has regulated values when discharged into watercourses.

It is known that the solubility of oxygen in a liquid medium is relatively low, hence, a substantial part of the supplied oxygen will remains in gas phase which results in: 1) a certain amount of $O_2$ will incorporate itself into the biogas leaving the digester and 2) another amount of oxygen will be involved in the oxidation of hydrogen sulphide present in the biogas. This desulphurization results in Sº deposition in the reactor headspace which in turn requires periodic cleaning in order to prevent clogging problems (Díaz and Fernández–Polanco 2011). Ramos et al. (2013) observed that Sº accumulates in the surface near to the liquid media (liquid surface and wall and ceiling of the digester). Sº also settled at the digester bottom. Ramos et al. (2014a) found that a cleaning interval of 14 months was necessary in order to maintain good process efficiency. They also found that once microaerobic conditions were restored after being cleaned, all $H_2S$ was rapidly removed from the biogas.

The application of microaeration in anaerobic process not only induc $H_2S$ removal, but also enhanced hydrolysis by increasing the synthesis and activity of extracellular hydrolytic enzymes (Johansen and Bakke 2006; Zhu et al. 2009; Botheju and Bakke 2011). This improve the anaerobic process mainly when sludge is treated, because t hydrolysis is the bottleneck of the anaerobic process due to the high organic suspended solid content of this residue (Myint et al. 2007; Lillo et al. 2014).

### 3.3 Autotrophic denitrification

Sulphide can be present in wastewater together with carbon and nitrogen compounds and their interactions between the biological cycles of the three elements can be used to remove each other (Figure 3).

The biological interaction between sulphur and nitrogen cycles is given by autotrophic denitrification which consists in the oxidation of sulphide (or other reduced sulphur compounds such as $S_2O_3^{-2}$ and Sº) by

nitrogen oxides ($NO_3^-$ and/or $NO_2^-$) producing sulfate (Equations 9, 10 and 11) which is less harmful than $S^{-2}$, particularly when the effluent is disposed in a marine environment.

$$5\ S^{-2} + 8\ NO_3^- + 8\ H^+ \rightarrow 5\ SO_4^{-2} + 4\ N_2 + 4\ H_2O \quad (9)$$

$$5\ S_2O_3^{-2} + 8\ NO_3^- + H_2O \rightarrow 10\ SO_4^{-2} + 4\ N_2 + 2\ H^+ \quad (10)$$

$$5\ S^\circ + 6\ NO_3^- + 2\ H_2O \rightarrow 5\ SO_4^{-2} + 3\ N_2 + 4\ H^+ \quad (11)$$

Sulphur denitrifying bacteria are members of the phylum *Proteobacteria*. The microorganism best studied, able to carry out autotrophic denitrification using reduced sulphur compounds, is *Thiobacillus denitrificans* (*β-Proteobacteria* class) and it is known as colourless sulphur bacteria (Robertson and Kuenen 1992). It is rod-shaped, gram-negative with polar flagella motile or non-motile bacteria and it grows under mesophilic conditions. *Thiobacillus thiophilus* has also been recently reported as an autotrophic denitrifying bacterium that uses thiosulfate and nitrate (Kellermann and Griebler 2009). Another major bacterium performing the autotrophic denitrification is *Sulphurimonas denitrificans* (*Epsilonproteobacteria*). It is a rod-shaped, non-motile bacteria and it is able to oxidize $S_2O_3^{2-}$ and $S^{-2}$ into sulfate coupled to the reduction of nitrate (Gadekar et al. 2006; Takai et al. 2006; Tandukar et al. 2009).



### 3.3.1 Kinetic and stoichiometric parameters of sulphur denitrifying bacteria

For autotrophic denitrification, bacteria growth and substrate consumption rates can be described by Monod equation (Oh et al. 2000). The majority of the kinetic studies have been conducted with pure cultures of *Thiobacillus denitrificans* and *Thiomicrospira denitrificans*. The estimated kinetic and stoichiometric parameters from the different studied bacterial populations present a wide range of values (Table 4) which indicates their large diversity.

### 3.3.2 Key operational parameters for sulphur denitrification

There are some basic operational parameters to consider during the application of autotrophic denitrification for the treatment of wastewater containing sulphide and nitrogen compounds, such as:

- Temperature and pH

Autotrophic denitrifying bacteria have been found in mesophilic environments (25-35ºC); their optimum temperature being around 35ºC. When temperature is higher than 40 ºC (Oh et al. 2000) or lower than 15 ºC (Yamamoto-Ikemoto et al. 2000), the autotrophic denitrification rate is negligible. The optimal pH range for this kind of bacteria is 7-8 (Oh et al. 2000; Claus and Kutzner 1985). In this range of pH values, the end products of denitrification are $N_2$ and sulfate. While at pH values below 7 the denitrification process is incomplete and intermediate products such as nitrite and/or elemental sulphur are detected. At pH values under 6 or over 9, a complete inhibition of denitrification is observed (Oh et al. 2000; Moon et al. 2004).

- Oxygen

Oxygen and nitrate are electron acceptors in the oxidation of sulphide. The oxidation of $S^{-2}$ in the presence of oxygen is thermodynamically more favourable than the oxidation using nitrate. Therefore, its presence should be avoided. Several research works agree that the minimum concentration of dissolved oxygen which does not cause the inhibition of autotrophic denitrification is between of 0.1-0.3 mg $O_2$/L. Above these concentrations denitrification is inhibited (Sublette et al. 1998; Kimura et al. 2002; Gu et al. 2004).

- Presence of inhibitory compounds

Inhibition of autotrophic denitrification by substrates (nitrate, nitrite and sulphide) has been reported. Nitrate exerts inhibitory effects at concentrations of 660 mg $NO_3^-$-N/L, while nitrite and sulphide appear to be strong inhibitors of denitrification even at low concentrations (36-60 mg $NO_2^-$-N/L and 200 mg S-$S^{-2}$/L) (Oh et al. 2002, Fajardo et al. 2014). The inhibitory effect of sulphide can be avoided by applying specific sulphide loading rates lower than the specific sulphide removal rate of the biomass (Fajardo et al. 2012) or maintaining an influent S/N ratio lower than the stoichiometric ratio. The last strategy is not advisable since sulphur limitation generally causes the accumulation of nitrite that is also a strong inhibitor of the

denitrification process and, in addition, elemental sulphur that is retained causes the accumulation of inorganic solids inside the system (Fajardo et al. 2012).

The inhibition of autotrophic denitrification, by heavy metals such as Zn and Cu at concentrations of 0.5 and 1.0 mg/L, has also been reported (Claus and Kutzner 1985; Krishnakumar and Manilal 1999; Oh et al. 2000; Moon et al. 2006). Organic matter has no inhibitory effect on the process, but it affects the oxidation of sulphur species, decreasing the formation of sulfate (Kim and Son 2000; Oh et al. 2002). Sulfate is a product of the process and has been reported to provoke partial inhibition at concentrations of 500 mg $SO_4^{-2}$-S/L and total activity depletion at 6400 mg $SO_4^{-2}$-S/L (Claus and Kutzer 1985; Campos et al. 2008).

### 3.3.3 Potential applications of autotrophic denitrification

Autotrophic denitrification can be considered as a suitable process to remove sulphide from wastewater (Vaiopoulou et al. 2005; Fajardo et al. 2014) or even in removing $H_2S$ from biogas generated during the anaerobic digestion of effluents containing sulfate (canneries, petrochemical industries, tanneries, among other) or fluel gas (Kleerebezem and Méndez 2002; Syed et al. 2006; Baspinar et al. 2011; Qian et al. 2015). However, in spite of its advantages, up to now, this process has been scarcely applied on a full scale (Garuti et al. 2001; Sahinkaya et al. 2014). The following potential applications of autotrophic denitrification using sulphur compounds can be highlighted:

- Industrial wastewater treatment

Industrial effluents generally contain large quantities of organic matter and if treated by anaerobic digestion can result in a significant source of energy. However, anaerobic digestion only removes organic matter and, then, effluents with low C/N are generated. The post-treatment of these effluents by conventional nitrification–denitrification processes is not economically feasible since additional carbon source is needed to carry out denitrification. On the other hand, part of these industrial effluents can contain high concentrations of sulfate, which is converted into sulphide during anaerobic digestion (Tandukar et al. 2009). Depending on the operational conditions, the sulphide generated could remain in the liquid phase or transfer to the biogas.

In the case of sulphide being predominantly in the liquid phase, a predenitrifying configuration should be used to remove both nitrogen and sulphide (Fig. 4a) (Tandukar et al. 2009). In this configuration, the effluent from the anaerobic digester is fed into the denitrifying reactor and later a nitrification is carried out. A stream from the aerobic tank containing nitrate and/or nitrite is recirculated to the first unit to carry out denitrification. Therefore, the nitrogen removal efficiency depends on the recycling ratio. The post denitrifying configuration is advisable when sulphide is mainly present in the biogas. In this case, the effluent of the anaerobic digester is fed into the nitrifying unit and its effluent enters an absorption tower where biogas is supplied in order to transfer sulphide to the liquid phase. Afterwards, sulphur and nitrogen compounds are removed in the denitrifying reactor. This configuration is very simple, easy to control and no recycling is needed (Fig. 4b) (Fajardo et al. 2013).

- Sewage treatment

When seawater is used for toilet flushing, concentrations around 500 mg/L of sulfate can be expected in sewage (Wang et al. 2009a). In this case, if an anaerobic digester is used to remove organic matter, most of it is consumed by sulfate-reducing bacteria, instead of be converted into methane, and an effluent with a high sulphide concentration is generated. In this case, ammonia can be removed by applying nitrification and autotrophic denitrification units in a predenitrifying configuration (SANI process; Lu et al. 2009).

- $H_2S$ emissions control in sewers systems

Hydrogen sulphide generation by anaerobic microorganisms in sewer systems is generally associated with biogenic corrosion of concrete and release of odors to the urban atmosphere (Zhang et al. 2008). There are several chemicals inhibiting $H_2S$ formation or removing sulphide from wastewater, such as, oxygen, hydrogen peroxide and ferric salts. Nevertheless, the addition of nitrate seems a very atractive option due its high solubility, low consumption rate and low operational costs compared to those of the other chemicals (Park et al. 2014).

The addition of nitrate in a septic wastewater oxidizes biologically dissolved sulphide, via autotrophic denitrification by sulphur denitrifying bacteria and also promotes the development of heterotrophic denitrifying bacteria, competing with SRB for organic matter (Fig. 5).

### 3.4 Sulphide removal from liquid streams using biofilm reactors

Even though a traditional suspended growth bioreactor, such as activated sludge, is commonly used in wastewater treatment, it has problems associated with its high solid retention time (SRT) and lower HRT, which strongly relies on effective settling of the final clarifier. In order to avoid this problem, immobilized cell technology has been applied in sulphide biological treatment (Yang et al. 1997). This technology has several advantages such as: (i) Biomass is easily retained and no recirculation is required, allowing higher biomass concentration. (ii) The system can tolerate higher hydraulic or organic loads because of higher biomass concentration in the reactor. (iii) The coexistence of aerobic, anoxic, and anaerobic environments becomes possible, because of the interaction between the microbial oxygen demand and molecular oxygen transfer. This method can provide for more diversified microorganism species within the system (Kuo and Shu 2004).

The biological sulphide removing studies, with immobilized biomass, use either photoautotrophic or chemolithotrophic SOB. Photoautotrophs use $CO_2$ as the terminal electron acceptor, while with chemolithotrophs oxygen (aerobic species) or nitrate and nitrite (anaerobic species) serve as terminal electron acceptors (Tang et al. 2009). Bioreactors using chemotrophic SOB generally achieve higher sulphide loading rates than photoautotrophic systems (Krishnakumar et al. 2005; Tang et al. 2009). The simpler nutritional requirements and higher sulphide tolerance of chemotrophic organisms also favoured their application in biological sulphide oxidation systems (Krishnakumar et al. 2005). Indeed, after 2006 there are no publications of phototrophic technology applied to sulphide removal. A number of studies have been conducted using chemotrophic bacteria to convert $H_2S$ to $S^0$, using different electron acceptors since Tang et al. (2009) summarized from the research works done in this area prior to 2009. Therefore, only chemotrophic SOB will be analyzed in this review. Removals and main characteristics of biofilm systems are shown in Table 5.

Different kinds of support biofilm and bioreactors have been proposed recently. Sarti et al. (2009) proposed the use of a bench-scale anaerobic sequencing batch biofilm reactors (ASBBR) containing mineral coal as inert support for removal of sulphide and organic matter (ethanol) from sulfate reduction process effluents. Using oxygen under micro-aeration conditions as an acceptor electron, showed that the ASBBR at bench scale ($ASBBR_{BS}$) could obtain a COD removal efficiency of up to 90%, while effluents total sulphide

concentrations ($H_2S$, $HS^-$, $S^{2-}$) remained in the range of 1.5 to 7.5 mg/L during the 50 days of operation (25 cycles). The use of an ASBBR at pilot scale ($ASBBR_{PS}$) provided only significant results in terms of COD removal (88%), with a low total dissolved sulphide (TDS) removal (57%). However, they mentioned that TDS removal can be improved by the optimization of operational strategies applied to the ASBBR configuration. Moghanloo et al. (2010) studied sulphide removal using *Thiobacillusthioparus* TK-1 in a biofilm airlift suspension reactor (BAS), with oxygen as acceptor electron. They evaluated the relationship between biofilm formation and changes in inlet loading rates. Optimal treatment performance was obtained at loading rate of 4.8 mol $S^{2-}/m^3/h$ with a conversion efficiency as high as 100%. The main product of $H_2S$ oxidation in the BAS-reactor was sulfate, because of high oxygen concentrations in the airlift reactor. The maximum sulphide oxidation rate was 6.7 mol $S^{2-}/m^3/h$ at a hydraulic residence time of 3.3 h in the mineral medium. Midha et al. (2012) used a continuous fluidized bed bioreactor (FBBR) with nylon support particles to treat synthetic sulphide wastewater at different hydraulic retention times. The microorganisms used came from an activated sludge, taken from the effluent of a tannery treatment plant. They demonstrated that almost 90–92% sulphide oxidation was achieved at all hydraulic retention times, being the highest sulphide oxidation (92%) obtained at a hydraulic retention time of 75 min and upflow velocity of 14 m/h. This study also explored the use of a statistical model that included the upflow velocity, hydraulic retention time and reactor operation time, which could explain data within 94% variability. Liu et al. (2013) proposed a new support (polyethylene semi-soft packing), in order to obtain a more cost-effective technology. They indicated that the activity of bacteria reached the highest value at pH 7.8–8.2, with a maximal sulphide removal load of 7.25 $kg/m^3/d$, using 4.80 mg/L of dissolved oxygen (DO). The increase in the DO value corresponds to a decrease in the sulphur yield, obtaining its highest sulphide removal load and sulphur yield at 2.55 mg/L DO. On the other hand, HRT had little effect on desulphurization efficiency with constantsulphide removal load. Finally, the sulphide removal load decreased from 2.85 to 0.51 $kg/m^3/d$) with increasing salinity from 0.5% to 2.5% (w/w).

Other electron acceptors than oxygen for sulphide removal are also proposed. Beristain-Cardoso et al. (2009) studied the simultaneous autotrophic-heterotrophic denitrification with phenol as the organic matter in a microbial consortium attached on a polyethylene support. They showed through a mass balance the complete removal of phenol, sulphide and nitrate, and the products were nearly stoichiometrically recovered

as bicarbonate, sulfate and $N_2$, respectively. Based on the results of microbial biofilm community analysis, they sugested that the simultaneous oxidation of phenol and sulphide coupled to nitrate reduction might be carried out at least by two different microbial genera. Tang et al. (2010) studied the autotrophic and heterotrophic denitrification processes in biofilm reactors using microbial cultures from an oil reservoir. They indicated that the use of this kind of microorganisms led to a marked improvement of sulphide and nitrate removal rates (both autotrophic and heterotrophic) when compared with those reported in literature. They also showed that the application of biofilms improved sulphide and nitrate removal rates significantly when compared with freely suspended cells, with maximum sulphide and nitrate removal rates under autotrophic conditions of 30.0 and 24.4 mM/h, respectively (residence time: 0.5 h). In this study, the conversion of sulphide to sulfate increased as nitrate to sulphide molar loading ratio was increased. On the other hand, Moraes et al. (2011) evaluated the effect of sulphide concentration on autrotrophic denitrification using nitrate and nitrite as electron acceptors in vertical fixed-bed reactors. The reactors' bed consisted of 0.5 cm polyurethane foam cubic matrices, in which the biomass was immobilized. Two sulphide concentrations were tested with each electron acceptor: excess of electron donor (molar N/S ratios of 0.9 and 1.5, for nitrate and nitrite respectively) and close to the required stoichiometrically (molar N/S ratios of 1.7 and 2.8 for nitrate and nitrite, respectively), both considering complete oxidation to sulfate. Sulphide concentration influenced the formation of final oxidation products. Higher sulphide concentrations led to a larger formation of intermediary sulphur compounds. Finally, it was found that microorganisms use nitrite more readily when compared to nitrate, information that might be useful for planning and optimizing the first step of nitrogen removal from effluents produced by anaerobic reactors applied to domestic sewage treatment. Moraes et al. (2013) investigated the feasibility of simultaneous nitrification/denitrification (SND) coupled with sulphide oxidation sequencing fed-batch biofilm reactors intermittently aerated for the post treatment of the effluent from an UASB reactor. The main objective was to evaluate two start-up alternatives and feeding strategies for the establishment of nitrification and denitrification. The fed-batch mode with sulphide application in excess was the best feeding strategy only in the anoxic periods, providing average efficiencies of 85.7% and 53.0% for nitrification and denitrification, respectively. However, the low overall nitrogen removal efficiency and some operational constraints indicated that autotrophic denitrification using sulphide in a single SBR was not suitable for SND under the assayed conditions. Liang et al. (2013) also investigated autotrophic partial

nitrification/denitrification and simultaneous sulphide removal by using synthetic wastewater in a vertical submerged biofilm reactor. Influent ammonium nitrogen and sulphide concentrations ranging from 54.6 to 129.8 mg /L and from 52.7 to 412.4 mg/L, respectively, were used. The results demonstrated that the working parameters were more stable when the sulphur/nitrogen ratio was set at 3:2, which yielded the maximum sulphur conversion. Furthermore, batch experiments with different phosphate concentrations proved that a suitable phosphate buffer solution to control pH values could improve process performance by synchronous desulphurization denitrification. Chen et al. (2014) presented the integrated simultaneous desulphurization and denitrification (ISDD) using an expanded granular sludge bed (EGSB) reactor, exploring the effect of the $COD/SO_4^{2-}$ ratio on the performance of ISDD process. At $COD/SO_4^{2-}$ in the range1.5-2:1, the granules were formed to retain most functional strains in the reactor. At $COD/SO_4^{2-} > 2:1$, the excess sulphide yielded SRB, which inhibited the activities of heterotrophic denitrifiers (hNRB) and autotrophic denitrifiers (aNRB) to deteriorate reactor performance. At $COD/ SO_4^{2-} < 1:1$, the hNRB group would out-compete the SRB group with the limited organic electronic donors, therefore, the $S^{2-}$ was not sufficiently produced with limited activity of aNRB.

In addition to microorganisms, enzymes are also an option for sulphide removal. Zhang et al. (2009b) proposed the use of a bioreactor packed with an enzyme (sulphide-oxidase) immobilized on chitosan beads, using oxygen as an electron acceptor, showing that this technology could remove up to 99% of inlet sulphide. Volumetric loading, space velocity and airflow rate had significant effects on the efficiency of sulphide removal. The most important finding was the prediction of the performance of the bioreactor using operational equations.

Regarding the study of the microbial community, biofilm systems have also been investigated for sulphide removal. Vannini et al. (2008) characterized the microbial community in an experimental membrane bioreactor for sulphide oxidation and the selected microbial community was characterized by constructing 16SrRNA gene libraries and subsequent screening of clones. Fluorescence in situ hybridization (FISH) was then used to assess the relative abundance of different bacterial groups. After the start-up phase, the process proceeded in a very stable manner, as long as the influent sulphide concentrations did not exceed 900 mg/L with a 79% of sulphide removal. Nevertheless, membrane fouling was relatively fast, needing weekly washing. Both analysis of clone libraries and FISH experiments revealed that the dominant operational

taxonomic unit (OTU), in the bioreactor, was constituted by Gamma proteobacteria belonging to the *Halothiobacillaceae* family.

### 3.4.1 Sulphide removal in microbial fuel cells

MFCs enable the direct capture of the energy contained in biodegradable organic matter in the form of electricity. The basis of this technology relates to the fact that electron transfer is inherent to the nature of microbial metabolism, as bacteria derive their energy from electrons transfered from a substrate to an electron acceptor at a higher redox potential. Microbial fuel cells provide a new approach for wastewater treatment, allowing electricity generation from the degradation of organic and inorganic matter (Logan et al., 2006). In a microbial fuel cell, the bacteria are stimulated to transfer their electrons to an electrode, from which the electrons flow to the external electrical circuit (*I*). On the basis of this principle, MFCs have been developed first for organic compounds and from 2006, with the work presented by Rabaey et al. (2006), for sulphide compounds. Sulphide is oxidized under standard conditions to elemental sulphur at potentials of at least higher than -0.274 V versus standard hydrogen electrode (SHE). Increasing the potential can further oxidize elemental sulphur. The work of Rabaey et al. (2006) has also been mentioned in another review (Zhang et al., 2008)). Here further findings will be analyzed. Table 6 shows the main characteristics of the MFC used for sulphide removal.

Sun et al. (2009) studied sulphide oxidation coupled with electricity generation, demonstrating that both electrochemical reactions and microbial catalysis were involved in a complex sulphide oxidation process in the anode of a MFC. They also proposed the sulphide oxidation pathways where the oxidation of sulphide to $S^0/S_x^{2-}$ and further to $S_4O_6^{2-}/S_2O_3^{2-}$ occurred spontaneously as electrochemical reactions produced electricity. Meanwhile, the bacteria in the MFC anode, generating $SO_4^{-2}$, accelerate the formation of $S^0/S_x^{2-}$ and $S_2O_3^{2-}$. Finally, it was noted that the microbe-assisted production of $S_2O_3^{2-}$ and $SO_4^{2-}$ resulted in a persistent current from the MFC. Zhang et al. (2009a) proposed the simultaneous removal of sulphide (including organics) and Vanadium (V) removal with electricity generation. During a 72 h operation, a sulphide removal rate of up to 84.7 ± 2.8% was achieved, with a Vanadium (V) reduction rate of 25.3 ± 1.1%, while MFCs produced a maximum power output of 572.4 ± 18.2 mW/m$^2$. Furthermore, a 20.7 ± 2.1% of the organics in sulphide

containing wastewater could also be removed alongside sulphide. An important improvement from this research was obtaining solid sulphur without controlling the anode potential and the use of cathode materials composed of carbon without any need for a plating of noble metal, therefore reducing the material costs.

Zhang et al. (2010b) examined the operating parameters such as initial concentration, conductivity, pH and external resistance for the sulphide removal using Vanadium (V) as an electron acceptor. It was found that the anode potential decreased as the initial sulphide concentration increased, resulting in the increase of the power output. The maximum power density obtained in this section was in the range of 500 – 700 $mW/m^2$. On the other hand, increasing the anode electrolyte conductivity up to a threshold value (12.3 mS/cm here), considerably raised the sulphide removal rate and quantity. For anode electrolyte conductivities ranging from 7.4 to 12.3 mS/cm, the sulphide removal rate remained above 91%. However, the maximum power density rose to a peak, then, declined with increasing anode electrolyte conductivity. Regarding pH and external resistance, it was demonstrated that lower pH increasesulphide removal and power generation, while the sulphide removal increased with lower resistance values. Zhao et al. (2009) studied a MFC that uses an activated carbon cloth plus carbon fibre veil anode composite, air-breathing dual cathodes and the sulfate-reducing species *Desulfovibriodesulphuricans*. Compared with other membrane types, proton (cation) exchange membrane and nafionionomer at the catalyst, enabled the cathode assembly to achieve high performance. The anode performance is controlled by the sulphide concentration, which was nearly completely removed from the wastewater during MFC operation. Lee et al. (2012a) applied a pure culture, an autotrophic denitrifier, *Pseudomonas* sp. C27, to start up a two-chambered MFC using sulphide as the sole electron donor. The MFC can successfully convert sulphide to elementary sulphur with electricity generation at a maximum power density of 40 $mW/m^2$. The addition of acetate interfered biofilm activity of electricity generation from sulphide. Nitrate was revealed as a more powerful electron acceptor than anode in the MFC.

Lee et al. (2012b) started up a microbial fuel cell using enriched sulfate-reducing mixed culture as anodic biofilms and applied the MFC for treating sulfate or sulphide-laden wastewater. The sulfate-reducing bacteria in anodic biofilm effectively reduced sulfate to sulphide, which was then used by neighboring anode respiring bacteria (ARB) as an electron donor for electricity production. The presence of organic carbon enhanced MFC performance since the biofilm ARB are mixotrophs that need organic carbon to grow. In the presence of lactate, sulfate in water change from 248 mgL to 39.3 mg/L as S in 3 days, with 84.1%

conversion to $S^0$. With or without the addition of lactate, the MFC effectively oxidized sulphide in water to $S^0$. The MFC produced electricity from sulfate or sulphide-laden wastewater in the presence of lactate. Lee et al. (2014) applied the microbial fuel cell with sulfate-reducing bacteria plus sulphide oxidizing bacteria in the anodic biofilm for treating the sulfate plus organic carbon wastewater. According to the results, the cell efficiently converted sulfate to $S^0$ at an open-circuit cell voltage of 730 mV and maximum power density ($P_{max}$) of about 62 mW/m$^2$. Sulphide ions produced by SRB from sulfate were the key metabolite that determined the cell performance. Without biofilm, the anodic surface cannot efficiently oxidise sulphide. With biofilm, SRB converted sulfate to sulphide and then the formed sulphide diffused to neighboring SOB for oxidation and release of excess electrons.

Rakoczy et al. (2013) studied a two-chambered microbial fuel cell in order to treat sulfidic-benzene-contaminated groundwater. With this system, the total electron recoveries for benzene and sulphide were between 18% and 49%, implying incomplete oxidation of benzene and sulphide at the anode. Even though there was very little removal, this work demonstrated the feasibility of removing undesired substances through enrichment of groundwater microorganisms in MFC systems. Zhang et al. (2013b) proposed the removal of sulphide in MFC using corn stover filtrate (CSF) as a co-substrate. They showed that CSF concentrations and electrolyte conductivities had significant improving effects on the performance of the MFCs. The presence of organic compounds did not affect the sulphide removal also degrading organics present in CSF with almost 52% of COD removed.

Regarding the microbial communities, Sun et al. (2010) explored their roles in the sulphide conversion and electricity generation. Community analysis of the sulphide-fed MFC showed a great diversity of bacteria in the anodic chamber, including exoelectrogenic bacteria and sulphur-related bacteria. The anode-attached and planktonic communities shared similar richness and diversity, while their structures were significantly different according to the LIBSHUFF analysis. Furthermore, the anode-attached planktonic communities could perform catalysis independently, and synergistic interactions occurred when the two communities worked together. Exoelectrogenic, sulphur-oxidizing and sulfate-reducing bacteria were found in the MFC anodic chamber. The discovery of these bacteria was consistent with the community characteristics for electricity generation from sulphide oxidation. The exoelectrogenic bacteria are present both on the anode and in the solution. The sulphur-oxidizing bacteria are present in greater abundance on the anode than in the

solution, while the sulfate-reducing bacteria preferably lived in the solution. Zhang et al. (2013a) presented the principles of sulphide removal as well as the bacteria involved in the MFCs with sulphide and glucose as the complex substrate. Community analysis shows a great diversity of bacteria on the anode surface, including the exoelectrogenic bacteria and sulphur-related bacteria. They are present in greater abundance than those in the MFCs fed with only sulphide and responsible for the effective electricity generation and sulphide oxidation in the above proposed MFCs. In this system, Bacteroidetes was most frequently found in the anode biofilms (11%), involved in electricity generation in the MFCs. In addition, Lentisphaerae (10%) and Armatimonadetes (2%) were new electricigens that appeared on the anode, demonstrating more electrochemically activated bacteria in this system than those reported by Sun et al. (2010) probably due to the complex substrate (sulphide and glucose) used in this study.

### 3.4.2 Modeling of sulphide removal process

The literature on the carbon, nitrogen and sulfate removal processes is abundant, but for sulphide treatment, further investigation is still needed. The sulphur cycle in wastewater and gas treatments lack of modelling tools, where the oxidation of sulphide is complex to predict, because it can be biologically (and chemically) oxidized to either elemental sulphur or sulfate, depending on the operating conditions (Mannucci et al. 2012). On the other hand, authors have used single-substrate kinetic models taking into account microbial growth rates associated only to a single pollutant biodegradation (Monod, Haldane and other kinetic equations) to describe biological processes (Mora et al., 2015). A drawback of single-substrate kinetic models is the inability to describe the potential limitations of other species such as nutrients or the electron acceptors. Also, models based on single-substrates can hardly describe the formation of multiple end-products in complex biological processes such as biological denitrification and desulphurization processes (Klok et al. 2013).

Even though $H_2S$ gas treatment kinetics is well re´ported, few articles focusing on $H_2S$ in liquid phase are available. In gas treatment, there is mass transfer limitation of $H_2S$ from the gas phase to the liquid phase, which is regularly included in the model. With respects to the bio-kinetics, the most used model has been Monod, including also some inhibitions. Gonzalez-Sanchez et al. (2009) proposed a multisubstrate function, where the kinetics depends on $H_2S$ concentration (type Haldane kinetic) and oxygen (Monod kinetic). They

calibrated their model using respirometry, reporting values of the biokinetic parameters in the same order of magnitude than those commonly reported for neutrophilic microorganisms. However, the value of maximum Oxygen Uptake Rate ($OUR_{max}$) was much lower than reported for specialized sulphide oxidizing strains. Mannucci et al. (2012) proposed a non-competitive model including only the $H_2S$ as substrate and the $SO_4^{2-}$ as inhibitor. Soreanu et al. (2010) proposed a statistical model for the $H_2S$ gas treatment, but using $NO_3^-$ as an electron acceptor. Although the key factors in the control of biofilter performance were demonstrated to be the biogas flow-rate and $H_2S$ concentration, the results of this study indicate that the influence of $H_2S$ concentration on the removal efficiency is more significant, under the experimental conditions specified in the paper.

Aqueous phase bio-oxidation of sulphide has been commonly applied to autotrophic denitrification (and related processes) but has rarely been studied using $O_2$ as an electron acceptor. Bio-oxidation of sulphide using $O_2$ as an acceptor electron was studied by Gadekar et al. (2006) using a novel sulphide-oxidizing bacterium *Thiomicrospira* sp. CVO. In this study, experimental data of sulphide removal was fitted to Monod, Tessier, Moser and Contois expressions and the value of various coefficients were determined through nonlinear regression. The model that represent the biological behavior of the system was the Moser model. Jing et al. (2010) studied the effect of nitrate and nitrite as electron acceptors on the performance of the anaerobic sulphide oxidizing process (ASO process). In this study, when the substrates were nitrate and sulphide, the inhibition of sulphide removal was weaker, which could be explained by the Monod equation with respect to sulphide and nitrate. While using the substrates nitrite and sulphide, the inhibition was strong and this fits better to the Haldane equation. This implies that the tolerance of activated sludge to influent substrate was sulphide > nitrate > nitrite. Moraes et al. (2011) evaluated the fundamentals and kinetics of sulphide-oxidizing autotrophic denitrification in batch reactors containing suspended and immobilized cells. They showed that, for nitrate concentration, zero-order models adjust better to profiles obtained for suspended cell reactors, whereas first-order models were more adequate for immobilized cell reactors. However, in the latter, mass transfer physical phenomena had a significant effect on kinetics based on biochemical reactions. Furthermore, they assumed that the sulphide concentration was not in low concentration, and that nitrogen compounds ($NO_x$) were the limiting substrates.

Roosta et al. (2011) presented a mathematical modell of sulphide oxidation with oxygen in a fed-batch reactor. In this case, complete oxidation of $HS^-$ to $SO_4^{2-}$ was reached, using the $S^0$ as intermediated product. They indicated that the first step ($HS^-$ to $S^0$) depends on $HS^-$ and $O_2$, both following Monod kinetics. The second step ($S^0$ to $SO_4^{2-}$) depends on $S^0$, $O_2$ also on pH ($OH^-$ concentration). Through this kinetic model, they showed that the rate of sulphur production ($r_1$) is independent of DO values except at very low DO and that the rate of sulphur oxidation to sulfate ($r_2$) increases with an increase in DO value. Thus, at low DO values, $r_2$ is lower than $r_1$ and consequently, sulfate production is low and the main product is sulphur particles. As DO value increases, the reaction rate of $r_2$ increases while $r_1$ remains constant, thus more parts of produced sulphur convert to sulfate.

The simultaneous removal of sulphide, nitrate and COD, known as denitrifying sulphide removal (DSR), has been recently studied and the kinetic removal of sulphide has also been proposed. The first attempt was made by Wang et al. (2009b), using an artificial neural networks as a tool. Later, Wang et al. (2010) presented a kinetic model of the DSR process in a batch system based on Activated Sludge Model N° 1 (ASM1). This model has seven microbial steps: (1) growth of heterotrophic denitrifier, (2) growth of autotrophic denitrifier, (3) decay of heterotrophic denitrifier, (4) decay of autotrophic denitrifier, (5) ammonification of organic nitrogen, (6) hydrolysis of particulate organic carbon and (7) hydrolysis of particulate nitrogen. Removal of sulphide by autotrophic denitrification obeys a multiple Monod kinetics, depending on $HS^-$ and $NO_3^-$. They also incorporated a switch function in order to describe the competition between the autotrophic and heterotrophic denitrifiers. Xu et al. (2014), following the same approach, improved the model including the $NO_2^-$, oxygen and $SO_4^{2-}$ reduction in the process. All the biological processes obey Monod kinetics. Lee and Wong (2014) proposed a novel kinetic diagram, based on mass and electron balances, to graphically interpret the system kinetics and identify the accessible regime where DSR reactions can be applied. Reduction–oxidation reactions incorporate all chemical reactions with oxidation state changes of the involved reactants.

## 4. Conclusions

- Hydrogen sulphide is an undesirable product from anaerobic digestion, produced when sulfate is present in the influent. Until recently, there was no practical strategy based on varying operational conditions used to avoid sulfate reduction. Taking into account thermodynamic and kinetic parameters, this process is more favourable than methanogenesis. Part of $H_2S$ is transferr to biogas, causing corrosion problems during methane combustion due to $SO_x$ compounds formation. Therefore, the most efective way to avoid the negative effects caused by $H_2S$ is to remove it.
- Sulfate reduction causes a double negative effect on methane production during the anaerobic process. On one hand, sulfate reduction consumes part of COD then; less organic matter is available for methanogens. On the other hand, the $H_2S$ generated inhibits the activity of methanogens. This last effect could be minimized by oxidizing $H_2S$ to elemental sulphur under microaerobic conditions inside the anaerobic reactor with a decrease in caloric value largely due to the increase of nitrogen present in the air.
- To reduce oxygen demand it is necessary to remove the $H_2S$ present in the effluent of the anaerobic digester. Oxidation of hydrogen sulphide is accieved in biofilm reactors using oxygen or nitrate (autotrophic denitrification), autotrophic denitrification being the most advisable option when nitrogen removal is required.
- Currently, biofilms using chemolithotrophic sulphide oxidizing bacteria is recommended, due to a higher sulphide loading, simpler nutritional requirements and higher sulphide tolerance.
- Biofilm systems used for sulphide removal, utilizing different kinds of electron acceptors (nitrate, nitrite, oxygen) have been proposed, highlighting an important potential for their use at an industrial scale. The sulphide removal efficiencies in these systems were most of the time superior to 90%.
- Microbial fuel cell is a new technology used in the removal of sulphide at the laboratory scale and has been applied since 2006. This technology achieves values of sulphide removal superior to 80% while also generating power between 40 W $m^{-2}$ and 740 W $m^{-2}$.
- Regarding the modeling of sulphur removal, further investigation is still needed. The kinetics mainly involves sulphur and an electron acceptor. The main kinetics model used has been Monod, but Moser kinetics has also been studied and reported**.**

- Considering that in all biological desulfurization processes different species of microorganisms are involved that use similar substrates generating different metabolic products to achieve a more precise control of these processes further and deeper microbiological studies is required.

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**Ethical Statement**

All the information used in the development of this manuscript was obtained from widespread and public publications, and have been properly referenced in this work.

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## TABLES

**Table 1.** Some thermodynamic values of hydrogen and acetate of SRB and MA (Alphenaar et al. 1993).

| Thermodynamic equations | $\Delta G^o$ (kJ) |
|---|---|
| $4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O$ | - 38.0 |
| $4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$ | - 32.7 |
| $CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$ | - 28.2 |
| $CH_3COO^- + SO_4^{2-} \rightarrow HS^- + 2HCO_3^-$ | - 39.5 |

**Table 2.** Kinetic parameters of hydrogen and acetate conversion of SRB and MA.

| | $\mu$ ($d^{-1}$) | Y (g VSS/mol) | Reference |
|---|---|---|---|
| **Hydrogen kinetics** | | | |
| *Desulfovibrio vulgaris* | 5.52 | 1.00 – 1.25 | Thauer et al. (1977) |
| *Desulfovibrio sp.* | 1.37 | 0.85 | Thauer and Badziong (1978) |
| *Desulfovibrio gigas* | 1.37 | 1.75 – 2.00 | Lupton and Zeikus (1984) |
| *Methanobacter formicicum* | 2.00 | 0.80 | Thauer and Brandis (1981) |
| *Methanobacter hungatei* | 1.20 | 0.20 | Thauer and Brandis (1981) |
| *Methanobacterium sp.* | - | 0.60 | Thauer and Badziong (1978) |
| **Acetate kinetics:** | | | |
| *Desulfobacter postagei* | 1.03 | 2.56 | Tiedje and Robinson (1984) |
| *Desulfotomaculum acetoxidans* | 0.55 | 5.52 | Brandis (1983) |
| *Desulfonema limicola* | 0.55 | - | Pfenning and Widdel (1981) |
| Mixed culture of SRB | 0.51 | 3.72 | Middleton and Lawrence (1977) |
| *Methanothrix soehengenii* | - | 1.47 | Huser (1980) |
| *Methanosarcina barkuse* | 0.21 | - | Thauer and Brandis (1981) |
| Mixed cultura of MA | 0.24 | 3.24 | Huser (1980) |

**Table 3.** COD removal variations dependend on $COD/SO_4^{2-}$ ratio

| $COD/SO_4^{2-}$ ratio | COD removal (%) | Observations | References |
|---|---|---|---|
| 3 | 40 - 60 | Wastewater with concentrated oils | Escriba et al. 1998 |
| 3-4 | 90 | Acidogenic – Methanogenic completely mixed anaerobic reactors in series | Nanqi et al. 2002 |
| 3 | 88 | | |
| 2 | 80 | | |
| > 2.5 | 90 | UASB reactor | Silva et al. 2002 |
| 1.1-0.9 | 40 | | |
| 3.3 | 77 | Tannery wastewater | Guerrero et al. 2013 |
| 1.66 | 60 | Batch reactors | |
| 1.0 | 43 | High sulfate concentration (2 – 10.4 g/L) | |
| 0.77 | 32 | | |
| 0.63 | 25 | | |
| 4 | 65 | UASB reactor | Lopes et al. 2007 |
| 1 | 25 - 35 | Glucose substrate | |
| 6.67 | 95 | Anaerobic baffled reactor (ABR) | Sipma et al. 2000 |

**Table 4.** Kinetic parameters of sulphur oxidizing bacteria.

| | $\mu_{max}$ $h^{-1}$ | $r_{max}$ $h^{-1}$ | $K_s$ mg $N \cdot L^{-1}$ | $Y$ mg VSS/mg $NO_3^-N$ | Reference |
|---|---|---|---|---|---|
| Enriched sludge | 0.12-0.2 | 0.3-0.4 | 3-10 | 0.4-0.5 | Oh et al. (2000) |
| *Thiobacillus denitrificans* | 0.11 | | 0.2 | 0.4-0.57 | Claus and Kutzner (1985) |
| *Thiomicrospira denitrificans* | 0.19-0.22 | 0.36 | 0.22* | 0.5** | Gadekar et al. (2006) |
| *Thiobacillus denitrificans* | 0.02-0.08 | | | | Justin and Kelly (1978) |
| Enriched sludge | 0.006 | | 0.398 | 0.81-1.1 | Zeng and Zang (2005) |

*mg $S \cdot L^{-1}$ **mg VSS/mg $S^{-2}$-S

**Table 5.** Operating conditions and removal in biofilms reactors with chemolithotrophic sulphide oxidizing bacteria

| Reference | Culture source | Biorector | Biofilm support | Electron acceptor | Temperature | pH | Treated influent | Sulphide Efficiency removal, % | End product |
|---|---|---|---|---|---|---|---|---|---|
| Sarti et al. (2009) | anaerobic sludge | Anaerobic sequencing batch biofilm reactor | Irregular pieces of mineral Coal | $O_2$ | 32-36 | 6.1-7.5 | Effluents from the sulfate reduction process | 57 | $S^0$ |
| Beristain-Cardoso et al. (2009) | denitrifying sludge | Inverse fluidized bed reactor | Low density polyethylene | Nitrate | 30 ± 1 | 7 | Phenol, sulphide and nitrate | 100 | $SO_4^{2-}$ |
| Moghanloo et al. (2010) | *Thiobacillus thioparus*TK-1 | Biofilm airlift suspension reactor (BAS) | Basalt | $O_2$ | 25-45 | 7 | $S^{2-}$ | 100 | $SO_4^{2-}$ |
| Midha et al. (2012) | Tannery effluent treatment plant | Fluidized bed reactor | Nylon particles | $O_2$ | 30 ± 2 | 5.5-6.5 | $S^{2-}$ | 90% | $S^0$ and $SO_4^{2-}$ |
| Tang et al. (2010) | Cultures enriched from the produce | Up-flow biofilm reactor | quartz sand | $NO_3^-$ | 23–25 | 7-7.5 | $S^{2-}$, $NO_3^-$ and acetate | 97.6–99.7 | $S^0$ and $SO_4^{2-}$ |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | water of the Coleville oil field | | | | | | | | |
| Moraes et al. (2012) | Anaerobic sludge | Vertical fixed-bed reactor | polyurethane foam cubic matrices | $NO_3^-$ and $NO_2^-$ | 30 ± 1 | 8.2-8.8 | $S^{2-}$, $NO_3^-$ and $NO_2^-$ | 99% | $S^0$ and $SO_4^{2-}$ |
| Moraes et al. (2013) | Anaerobic sludge | Chemostat | cubic matrices of polyurethane foam | $O_2$, $NO_3^-$ and $NO_2^-$ | 30 ± 1 | 8.5-8.9 | COD, $NH_3$ and $S^{2-}$ | 99% | --- |
| Liang et al. (2013) | --- | Fixed-bed biofilm | --- | $NO_3^-$ and $NO_2^-$ | 30 ± 1 | 7.0-10.9 | $S^{2-}$ | 80-92 | --- |
| Liu et al. (2013) | municipal sludge | fixed-bed biofilm | Polyethylene semisoft packing | $O_2$ | 30 | 6.5-9.2 | $S^{2-}$ | 87.6 | $S^0$ and $SO_4^{2-}$ |
| Chen et al. (2014) | anaerobic sludge | Plexiglass expanded granular sludge bed | --- | $NO_3^-$ | 28 ± 1 | 8 ± 0.3 | $S^{2-}$, COD and $NO_3^-$ | 29.4-100 | $S^0$ and $SO_4^{2-}$ |

**Table 6.** Removal rate and the maximum current produced in MFCs

| Reference | Culture source | Type of MFC | Removal Efficiency, % | Maximum Power output | End product |
|---|---|---|---|---|---|
| Rabaey et al. (2006) | mixed aerobic sulphide-oxidizing | Square-type MFCs with granular graphite as anodic electrode (projected surface between 817 and 2720 $m^2m^{-3}$) | > 99 | 18 mW $L^{-1}$ total anode compartment | $S^0$ |
| Sun et al. (2009) | anaerobic sludge | Square-type MFC with plain carbon paper (3 × 7.5 cm, not wet proofed) as anodic electrode | --- | 112 mA $m^{-2}$ | $S_2O_3^{2-}$ and $SO_4^{2-}$ |
| Zhang et al. (2009a) | anaerobic granular sludge | Double-chamber MFCs in a cylindrical geometry with carbon fiber felt of 16 $cm^2$ as the anodic electrode. | 84.7 ± 2.8 | 572.4 ± 18.2 $mWm^{-2}$ | $S^0$ and $SO_4^{2-}$ |
| Zhao et al. (2009) | *Desulfovibriodesulphuricans* | A single chamber, air- | 91-86 | 2.68 mW | $S^0$ |

| | | | | | |
|---|---|---|---|---|---|
| | | breathing dual cathode assembly, and continuous flow type MFC. Activated carbon cloth (60 $cm^2$) as anode. | | | |
| Zhang et al. (2010) | anaerobic granular sludge | H-type MFCs in cylindrical geometry with carbon fiber felt of 16 $cm^2$ as the anodic electrode. | 95.2 to 47.5, depending of sulphide initial concentration | 500–700 mW $m^{-2}$ | $S^0$ and $SO_4^{2-}$ |
| Lee et al. (2012a) | *Pseudomonas* sp. C27 | Two cilindrical chambers. Anode was made of carbon felt (area, 6 $cm^2$) | 98.4 | 40 mW $m^{-2}$ | $S_2O_3^{2-}$ and $S^0$ |
| Lee et al. (2012b) | waste activated sludge | Two cilindrical chambers. Anode was made of carbon felt (area, 6 $cm^2$) | 84.1 | 200–300 mW $m^{-2}$ | $S^0$ |
| Lee et al. (2014) | waste activated sludge | Dual MFC comprising anode and cathode cylindrical | 77.9-47.6 | 61–63 $Wm^{-2}$ | $S^0$ |

| | | | | | |
|---|---|---|---|---|---|
| | | chambers. Anode was made of carbon felt (area, 9 $cm^2$) | | | |
| Rakoczy et al. (2013) | benzene- and sulphide-contaminated groundwater, composed of several different phylotypes affiliated to anaerobic microorganisms. | Two cylindrical glass chambers. Anode was graphite fibers with 94 $m^2$ area. | 99-87 | --- | $SO_4^{2-}$ |
| Zhang et al. (2013a) | anaerobic sludge | Four cubic single-chamber MFCs. Anode was carbon fiber felt. | Up to 92 | 744 mW $m^{-2}$ | $S^0$ and $SO_4^{2-}$ |

## FIGURE CAPTIONS

**Fig. 1.** Ionized and non-ionized sulphide forms depending on the pH of the aquatic environment (Sawyer et al., 2003).

**Fig. 2.** Scheme of competition between SBR and MA.

**Fig. 3.** Biological interactions between carbon, nitrogen and sulphur cycles.

**Fig. 4.** Schematic representation of: a) Predenitrifying configuration; b) postdenitrifying configuration.

**Fig. 5.** Biological transformations of organic and sulphur compounds when nitrate is added to a sewer system (Adapted from Jiang et al., 2009).

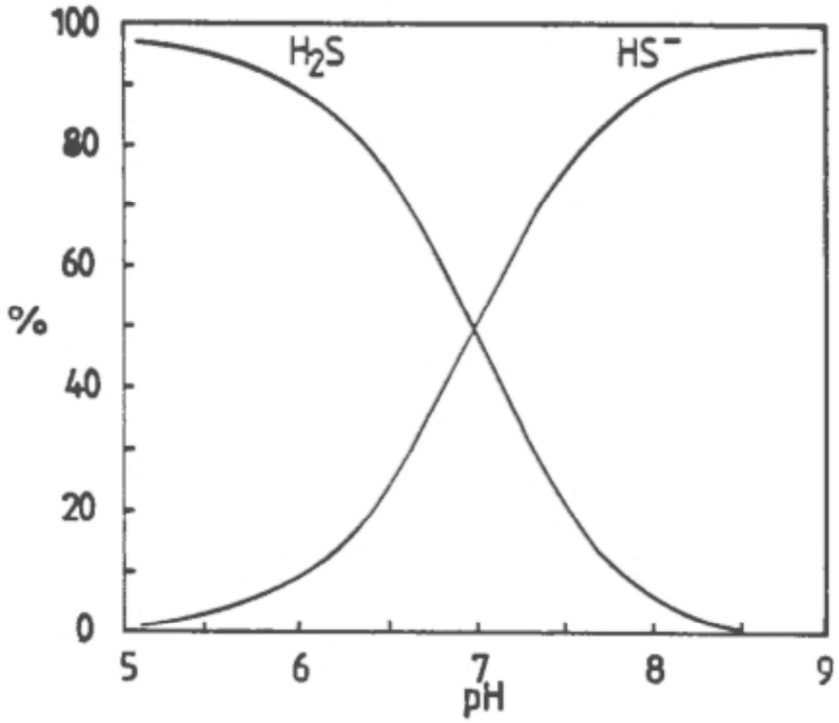





**Figure 1**

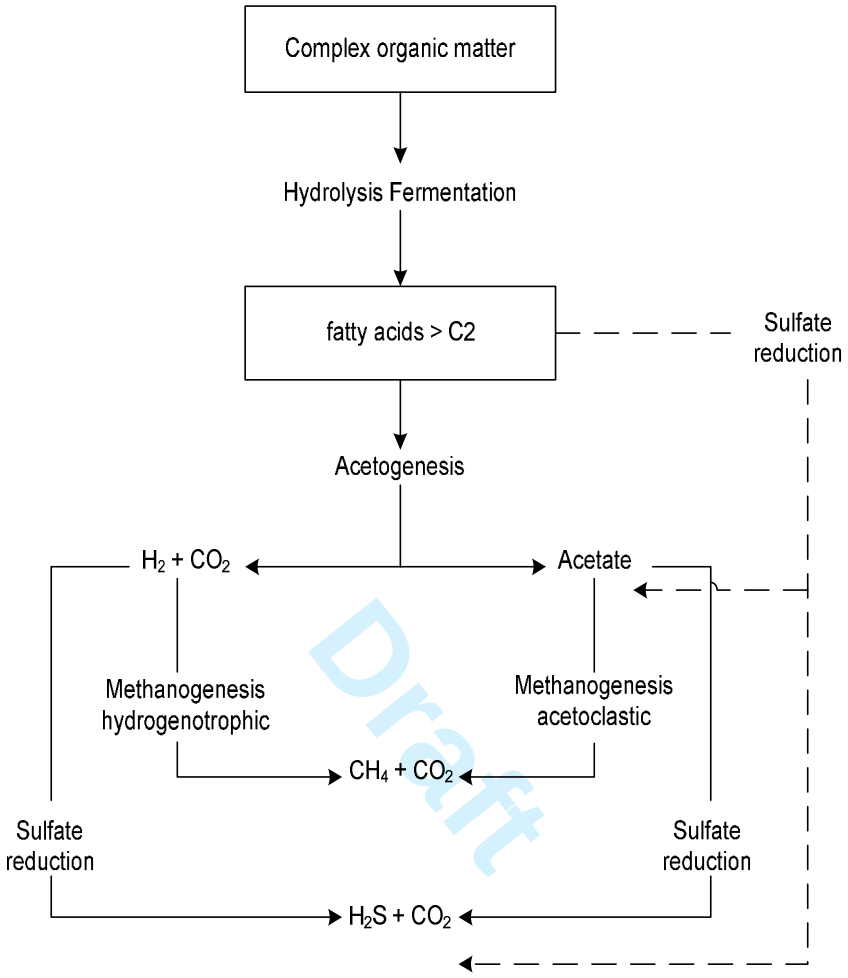

Complex organic matter
Hydrolysis Fermentation
fatty acids > C2
Sulfate reduction
Acetogenesis
$H_2 + CO_2$
Acetate
Methanogenesis hydrogenotrophic
Methanogenesis acetoclastic
$CH_4 + CO_2$
Sulfate reduction
Sulfate reduction
$H_2S + CO_2$

**Figure 2**

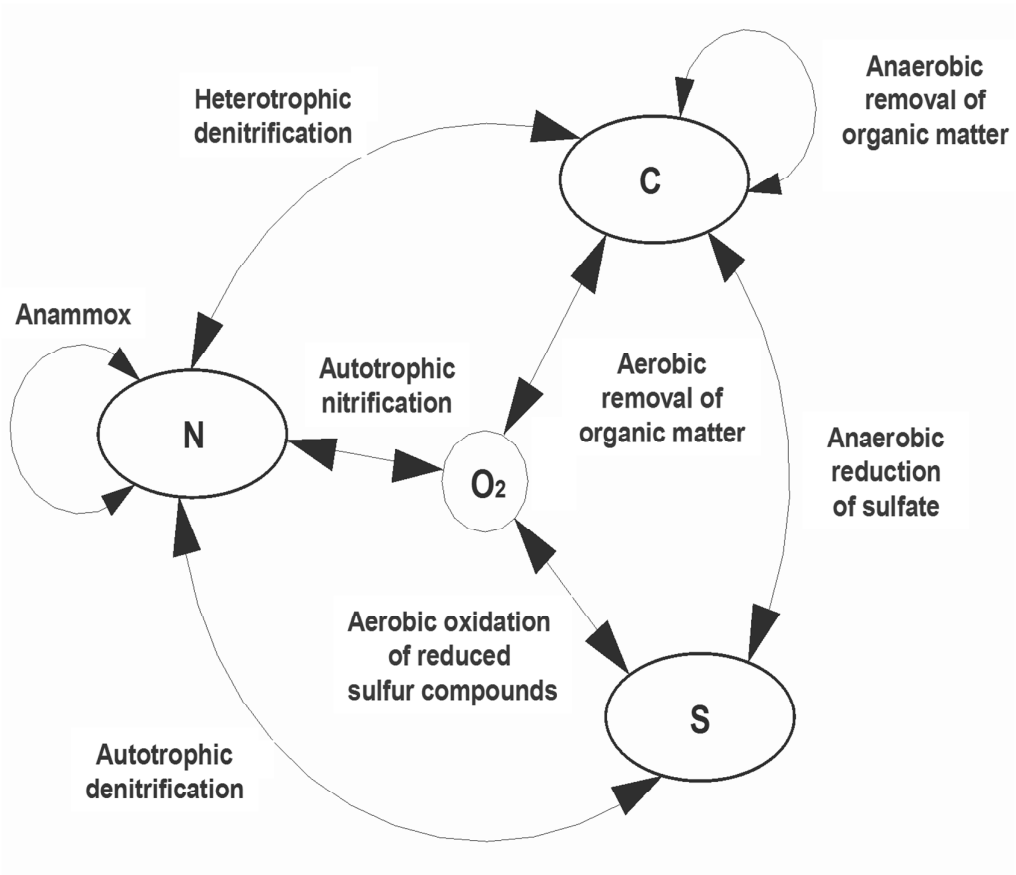
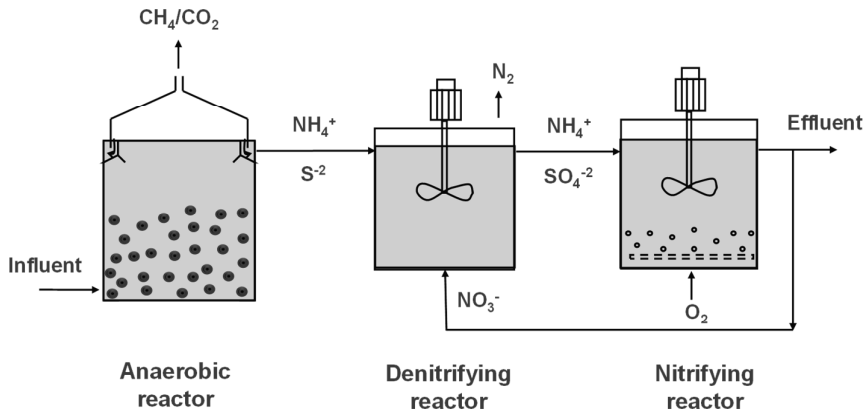

Heterotrophic denitrification
Anaerobic removal of organic matter
C
Anammox
Autotrophic nitrification
Aerobic removal of organic matter
N
O2
Anaerobic reduction of sulfate
Aerobic oxidation of reduced sulfur compounds
S
Autotrophic denitrification

**Figure 3**

a)


$CH_4/CO_2$
$N_2$
$NH_4^+$
$S^{-2}$
$NH_4^+$
$SO_4^{-2}$
Effluent
Influent
$NO_3^-$
$O_2$
Anaerobic reactor
Denitrifying reactor
Nitrifying reactor

b)

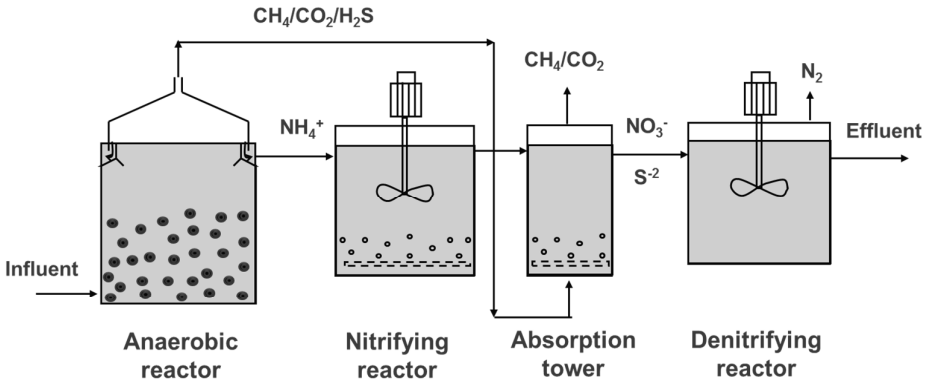

$CH_4/CO_2/H_2S$
$CH_4/CO_2$
$N_2$
$NH_4^+$
$NO_3^-$
$S^{-2}$
Effluent
Influent
Anaerobic reactor
Nitrifying reactor
Absorption tower
Denitrifying reactor

**Figure 4**

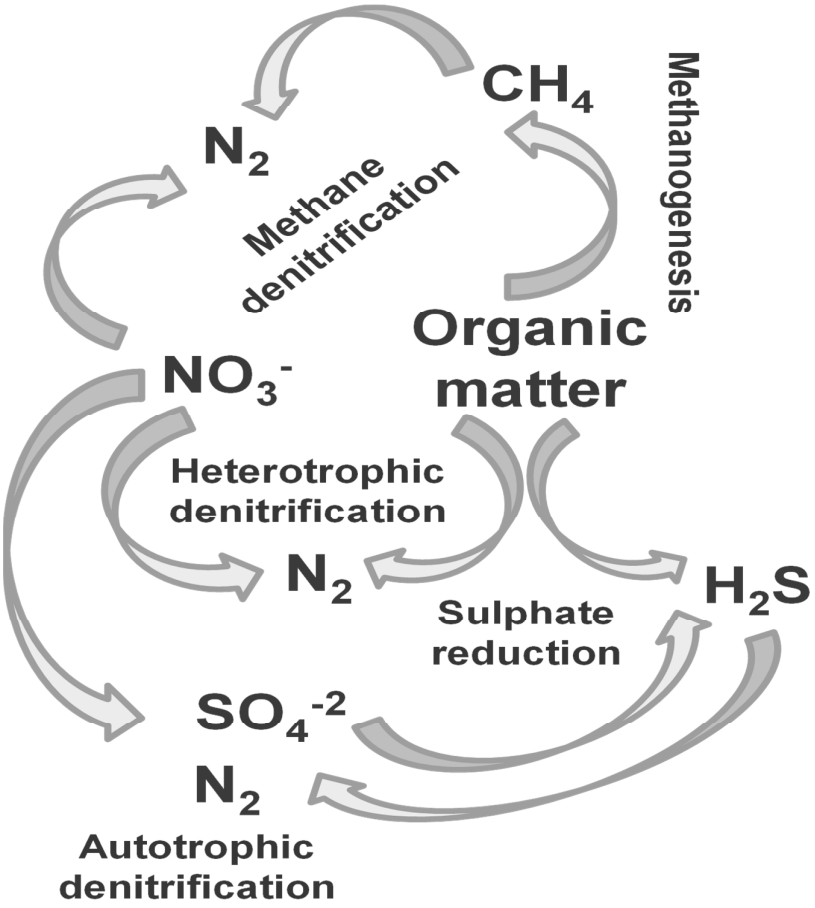

CH4
Methanogenesis
N2
Methane denitrification
Organic matter
NO3-
Heterotrophic denitrification
N2
H2S
Sulphate reduction
SO4-2
N2
Autotrophic denitrification

Figure 5

## ABBREVIATIONS

ABR: anaerobic baffled reactor.

aNRB: authotropic denitrifiers.

ARB: anode respiring bacteria.

ASBBR: anaerobic sequencing batch biofilm reactor.

$ASBBR_{BS}$: ASBBR at bench scale

$ASBBR_{PS}$: ASBBR at pilot scale

ASM1: Activated Sludge Model N° 1.

ASO process: anaerobic sulphide oxidizing process.

BAS: biofilm airlift suspension reactor

COD: chemical oxygen demand

CSF: corn stover filtrate.

DO: dissolved oxygen.

DSR: denitrifying sulphide removal.

EGSB: expanded granular sludge bed reactor.

FBBR: fluidized bed bioreactor.

FISH: Fluorescence in situ hybridization.

hNRB: heterotrophic denitrifiers.

HRT: hydraulic retention time.

ISDD: integrated simultaneous desulphurization and denitrification

$K_S$: saturation constant.

MA: methanogenic archaea.

MFCs: microbial fuel cells.

OTU: operational taxonomic unit.

$OUR_{max}$: maximum Oxygen Uptake Rate.

$P_{max}$: maximum power density.

$r_1$: rate of sulphur production.

$r_2$: rate of sulphur oxidation to sulfate.

$r_{max}$: maximum substrate removal rate constant.

$S^o$: elemental sulfur.

SHE: standard hydrogen electrode.

SMA: specific methanogenic activity.

SND: simultaneous nitrification/denitrification.

SOB: sulfide oxidizing bacteria.

SRB: sulfate reducing bacteria.

SRT: solids retention time.

TDS: total dissolved sulfide.

UASB: Upflow anaerobic sludge blanket reactor.

VOL: volumetric organic load.

Y: microorganisms growth yield.

$\mu_{max}$: maximum specific growth rate of the microorganisms.