Microbial Ecology of Biofiltration Units Used for the Desulfurization of Biogas

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Abstract: Bacterial communities’ composition, activity and robustness determines the effectiveness of biofiltration units for the desulfurization of biogas. It is therefore important to get a better understanding of the bacterial communities that coexist in biofiltration units under different operational conditions for the removal of H2S, the main reduced sulfur compound to eliminate in biogas. This review presents the main characteristics of sulfur-oxidizing chemotrophic bacteria that are the base of the biological transformation of H2S to innocuous products in biofilters. A survey of the existing biofiltration technologies in relation to H2S elimination is then presented followed by a review of the microbial ecology studies performed to date on biotrickling filter units for the treatment of H2S in biogas under aerobic and anoxic conditions.

Keywords: biogas; desulfurization; hydrogen sulfide; sulfur-oxidizing bacteria; biofiltration; biotrickling filters; anoxic biofiltration; autotrophic denitrification; microbial ecology; molecular techniques

1. Introduction

Biogas is a promising renewable energy source that could contribute to regional economic growth due to its indigenous local-based production together with reduced greenhouse gas emissions [1]. Biogas can be used for heat and electricity generation, and after an upgrading process, as a natural gas substitute or as transportation fuel. Biogas is obtained from the decomposition of urban, industrial, animal or agricultural organic wastes under anaerobic conditions, a process called anaerobic digestion (AD) [2].

Biogas is a mixture typically composed of methane (CH4) (50–75%) and carbon dioxide (CO2) (25–50%) along with hydrogen sulfide (H2S), ammonia (NH3), aromatic, organochlorinated or organofluorated compounds and water vapor [3]. It contains H2S at significant concentrations ranging from 0.005 to 2% (v/v) (50–20,000 ppmv) depending on the raw material used and the conditions of the AD process [4] (Table 1).

Biogas upgrading refers to the removal of CO2, H2S, H2O and other trace contaminants such as siloxanes, halocarbons, O2 and N2. The type of upgrading process depends on the final use of biogas [5]. The removal of H2S, the most significant reduced sulfur compound in biogas, is necessary for environmental, technical and health reasons. The combustion of non-desulfurized biogas leads to the emission of SOx that are precursors of acid rain and the presence of H2S provokes the corrosion of combustion engines. Moreover, this gas emits a very unpleasant rotten-egg like odor which is detectable at very low concentrations (0.00047 ppmv) [6] and is highly toxic at concentrations of 50 ppmv and lethal at 300 ppmv [7]. H2S inhibits cellular respiration after entering the bloodstream.

where it binds to and inhibits the cytochrome C oxidase in complex IV, the terminal enzymatic complex of the mitochondrial respiratory chain, leading to pulmonary paralysis, sudden collapse and death [7].

Table 1. \( \text{H}_2\text{S} \) content in biogas produced by anaerobic digestion (AD) of different wastes. Adapted from [2].

<table>
<thead>
<tr>
<th>Biogas From</th>
<th>( \text{H}_2\text{S} ) (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wastewater AD plants</td>
<td>0–4000</td>
</tr>
<tr>
<td>Household waste</td>
<td>72–648</td>
</tr>
<tr>
<td>Agrifood industry</td>
<td>288</td>
</tr>
<tr>
<td>Agricultural waste</td>
<td>2160-7200</td>
</tr>
<tr>
<td>Landfill sites</td>
<td>0–100</td>
</tr>
<tr>
<td>Natural gas (^{\text{a}})</td>
<td>1.1–5.9</td>
</tr>
</tbody>
</table>

\(^{\text{a}}\) Although it is not produced by AD, the \( \text{H}_2\text{S} \) content of natural gas is shown for comparison.

Several physical/chemical and biological technologies are available for \( \text{H}_2\text{S} \) removal (desulfurization). Physicochemical technologies including absorption, adsorption, chemical oxidation and membrane separation have been traditionally used for desulfurization. However, most of these technologies are characterized by the intensive use of energy and chemicals with the associated increase in operational costs and environmental impact due to the generation of emissions and hazardous by-products that must be treated and disposed of [5,8]. These characteristics have led to an intensification of the research on biological alternatives for biogas desulfurization.

Biological technologies (biotechnologies) operate at low temperatures and pressures and are based on the ability of certain microorganisms (i.e., sulfur-oxidizing bacteria, SOBs) to oxidize \( \text{H}_2\text{S} \) to innocuous products such as elemental sulfur (\( \text{S}^0 \)) and sulfate (\( \text{SO}_4^{2-} \)) in the presence of \( \text{O}_2 \) or nitrate (\( \text{NO}_3^- \)) as the final electron acceptor (see Section 2). Biological desulfurization processes have become more popular due to their advantages compared to conventional technologies, including low energy requirements, the generation of harmless by-products and low investment and operation costs. The final products are non-hazardous: \( \text{SO}_4^{2-} \) can be directly discharged to receiving water bodies while \( \text{S}^0 \) can be separated and recovered to be used as a raw material for industrial and agricultural purposes [9].

The biological removal of \( \text{H}_2\text{S} \) in biogas has been conducted in gas-phase biological filter reactors (biofilters) and in algal-bacterial photobioreactors using the \( \text{O}_2 \) photosynthetically produced by microalgae or, in situ, in the headspace of AD vessels through the injection of micro-quantities of \( \text{O}_2 \) to stimulate the growth and activity of SOBs [5]. However, the main bioprocess employed has been biofiltration due to its high \( \text{H}_2\text{S} \) removal efficiencies, up to 99–100% depending on the concentration of \( \text{H}_2\text{S} \) at the inlet, and experience in full-scale implementation for waste gas treatment [10]. The objective of this review was to gather and discuss the current knowledge on microbial ecology in biofiltration units used for the removal of \( \text{H}_2\text{S} \) from biogas. Microbial ecology is the study of microorganisms in their natural environment and how microorganisms interact with each other and with the environment. The two main components of microbial ecology are biodiversity and microbial activity studies [11].

The novelty of this review article is to focus on microbial aspects of biogas desulfurization and depict the specific biofiltration technologies that could be used to treat high and variable loads of \( \text{H}_2\text{S} \) in biogas during extended periods of time, considering that the treated biogas is used in different applications after desulfurization. Such conditions are different from those found in the classical applications of biofiltration technologies for odor control in wastewater treatment plants (WWTPs) or other industrial processes.

The biological sulfur cycle is briefly presented, and the main characteristics of SOBs related to biofiltration are then reviewed with emphasis on their morphological and physiological diversity and metabolic versatility. The molecular techniques that have been used to characterize bacterial communities in biofilters are then briefly presented followed by a review on the current knowledge on microbial communities in biofiltration units used for biogas desulfurization under aerobic and
anoxic conditions. The present article is the first presenting a review on the microbial ecology aspects of biogas desulfurization.

2. The Biological Sulfur Cycle and the Sulfur-Oxidizing Bacteria

Sulfur is the tenth most abundant element in the Earth’s crust [12]. It is mainly found in the lithosphere, hydrosphere and atmosphere, in terrestrial and deep-sea hot springs, volcanic areas, mines, caves, seawater, in the form of sulfides (H₂S, HS⁻ and S²⁻), sulfate minerals (gypsum, CaSO₄), sulfide minerals (pyrite, FeS₂), S⁰ and SO₄²⁻ [12,13]. Human activities have impacted directly or indirectly by increasing the atmospheric emissions of sulfur in the form of H₂S (eutrophic marshes, sewage systems, several industries) and SOx (burning of fossil fuels) [13]. Sulfur is also important in the biosphere where it is incorporated into amino acids and proteins, hormones, lipids and vitamins. Biomass, which includes living and dead organic matter, constitutes a minor, but actively cycled, reservoir of sulfur [12].

Figure 1 shows a simplified version of the biological sulfur cycle and sulfur reservoirs, and highlights the importance of microorganisms, especially prokaryotes, in the cycling of inorganic sulfur compounds.

SO₄²⁻ is the fully oxidized species of sulfur. It is reduced and assimilated by plants, fungi and bacteria to form amino acids and proteins (organic sulfur) that later become part of the sulfur-containing amino acids for humans and other animals. The decomposition of proteins during the mineralization of organic matter leads to the release of H₂S that reenters the cycle. H₂S is also produced by sulfate-reduction in anoxic habitats where sulfate-reducing bacteria (SRB) use SO₄²⁻ as the terminal electron acceptor for the oxidation of organic matter. This process is an anaerobic respiration, similar to aerobic respiration in which O₂ is used as the terminal electron acceptor, producing H₂O instead of H₂S as the metabolic by-product. SRB use SO₄²⁻ to generate energy in the cell, not to synthesize organosulfur compounds that become part of the cell material. In this sense, sulfate-reduction is a dissipimilatory process. In the presence of SO₄²⁻ in biogas production processes, SRB can outcompete methanogenic bacteria for acetate and hydrogen (H₂), which are common substrates for both groups of bacteria, leading to the production of H₂S [14].
Thiomargarita

The phototrophic oxidation of sulfur is performed by green and purple sulfur bacteria. These bacteria incorporate carbon in the form of CO₂ or organic compounds using light energy, but instead of oxidizing H₂O to O₂ (oxygenic photosynthesis) they oxidize H₂S to S⁰ and S⁰ to SO₄²⁻ (anoxygenic photosynthesis). Dissimilative SOBs are chemolithotrophs that oxidize inorganic reduced sulfur compounds such as H₂S and S⁰, using these compounds as electron donors for energy generation (ATP), typically with O₂ as the electron acceptor (aerobic respiration). Aerobic heterotrophic bacteria and fungi oxidize sulfur to thiosulfate or sulfate; however, the heterotrophic sulfur oxidation pathway has not been clearly elucidated yet [12] and even though fungi have been used in biofiltration applications, their application has mainly been for the elimination of volatile organic compounds (VOCs) [15]. More detailed information on sulfate-reduction and phototrophic or chemolithotrophic sulfur oxidation can be found elsewhere [11,16].

Figure 2 presents a brief summary of microbial trophic types and how cells convert carbon, energy and electrons to precursor metabolites, ATP, reducing power and new cell material. Dissimilatory sulfate-reduction, chemolithotrophic and phototrophic sulfur oxidation are forms of metabolism that only members of the domains Bacteria and Archaea can perform.

Chemolithotrophic SOBs are found in many natural and engineered environments where the sulfur cycle is active such as in marine sediments, sulfur springs, hydrothermal systems, sewage systems, anaerobic digesters and mines [18–21]. SOBs have been found in 2.5 billion year old fossils, prior to the Great Oxidation Event, when H₂S was an abundant energy source for microbial life [22]. H₂S is not toxic to these bacteria because their respiratory oxidase cytochrome bd is resistant to H₂S inhibition [23]. These bacteria are the base of the removal of H₂S from gases and airstreams by biofiltration and other environmental biotechnologies that have been extensively reviewed [10,24–26].

Currently, chemolithoautotrophic SOBs are the most predominant bacteria used for the biodegradation of H₂S [10,25,26] due to their versatility to operate in a wide range of environmental conditions (e.g., pH and temperature), low nutrients requirements, high sulfide tolerance, and slower growth rates than heterotrophs thus leading to less biomass accumulation. SOBs are morphologically, physiologically, phylogenetically and metabolically diverse.

For instance, some Sulfurimonas species (Epsilonproteobacteria) have a small cell size (0.66 × 2.1 µm) while Thiomargarita (Gammaproteobacteria), with an average cell diameter of 750 µm, is the largest bacterium discovered to date [27,28]. Thiothrix (Gammaproteobacteria) forms ensheathed filamentous multicellular structures that form rosettes under certain environmental conditions [29].

Thiobacillus sp. (Betaproteobacteria) and related genera (Halothiobacillus) are the best-studied chemolithotrophic SOBs and are known as the colorless sulfur bacteria in contrast to the phototrophic green and purple sulfur bacteria. The oxidation of H₂S and S⁰ to SO₄²⁻ by Thiobacillus leads to
the production of sulfuric acid (H$_2$SO$_4$) that acidifies the medium. Thus, many *Thiobacillus* species are acidophilic. The most acidophilic SOB, *Acidithiobacillus thiooxidans*, is typically found in acid mine drainage and corroded concrete (refer to Section 4). Haloalkaliphilic SOBs, *Thioalkalivibrio* and *Thioalkalimicrobium* sp. (*Gammaproteobacteria*) have been isolated from soda lakes and thrive at high pH (7.5 to 10.5) and high salt concentrations (1.5–4.3 M total Na$^+$) [30].

Many SOB species deposit the S$^0$ produced by the oxidation of H$_2$S in intracellular or extracellular granules for later use as an electron donor when H$_2$S is depleted. Bacterial sulfur globules show clear difference in the speciation of sulfur depending on the type of SOB, reflecting possible ecological and physiological properties [31]. *Thiomargarita namibiensis* forms intracellular sulfur rings and *Acidithiobacillus thiooxidans* extracellular polythionates.

Most SOBs are aerobic, however, some species of *Thiobacillus*, *Sulfurimonas* or *Thioalkalivibrio*, among others, can grow anaerobically with nitrate (NO$_3^-$) or nitrite (NO$_2^-$) as the electron acceptor. NO$_3^-$ is sequentially reduced to NO$_2^-$, nitric oxide (NO), nitrous oxide (N$_2$O) and nitrogen gas (N$_2$), depending on the bacterial species and environmental conditions (refer to Section 3) [32]. This process is known as sulfur-oxidizing autotrophic denitrification. Most SOBs are obligate chemolithotrophs and can only use inorganic compounds as electron donors. Some SOBs are facultative chemolithotrophs and can grow either lithotrophically (autotrophs) or organotrophically. Bacteria that can simultaneously assimilate carbon CO$_2$ and organic sources are called mixotrophs (see Figure 2), for example *Thiothrix* sp. (refer to Section 4) [16].

No universal mechanism or pathway exists for sulfur oxidation in prokaryotes. Table 2 shows examples of the set of genes involved in sulfur oxidation in selected chemolithotrophic SOBs related to biofiltration applications. SOBs differ in the set of sulfur oxidation genes they contain, although some genes, but not all of them, are repeatedly found in different SOB species [33]. Well-studied examples include the complex SOx enzymatic system that catalyzes the oxidation of H$_2$S and S$^0$ to SO$_4^{2-}$ while the DSR system is related to the formation of sulfur globules and the SQR enzyme to the oxidation of H$_2$S to S$^0$ [18]. Interestingly, some genes are found both in anaerobic and aerobic photo- and chemotrophic SOBs [18,33]. Some SOBs form symbiotic intra- or extracellular associations with marine invertebrates [34]. The deep-sea clams *Calyptogena* spp., which are found clustered near hydrothermal vents, harbor symbiotic chemolithoautotrophic SOBs in their gills’ epithelial cells. These clams accumulate sulfide from the environment into their blood through their highly vascularized, muscular foot. The sulfide is transported via the blood to the gills where SOBs oxidize this reduced sulfur compound using it as an energy source for autotrophic growth and providing fixed carbon for the eukaryotic host. It has been shown that, in *Calyptogena*, key enzymes from five different sulfur oxidation pathways are equally expressed under three different environmental conditions (aerobic and semioxic) indicating that all pathways may function simultaneously to support intracellular endosymbiotic life [35]. This may be an advantage in an environment where the H$_2$S concentration rapidly fluctuates. No other reports are found concerning the expression of sulfur oxidation genes under different environmental conditions.

<table>
<thead>
<tr>
<th>SOB</th>
<th>Optimum pH Range</th>
<th>Anaerobic/Aerobic</th>
<th>Sulfur Oxidation Genes or Enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Thiobacillus denitrificans</em></td>
<td>6.8–7.4</td>
<td>AN/AE</td>
<td>$\text{sqr, fcc, sox without soxCD, dsr, apr}$</td>
</tr>
<tr>
<td><em>Acidithiobacillus</em> spp.</td>
<td>2–2.5</td>
<td>AN/AE *</td>
<td>$\text{tet, tsp, sox without soxCD}$</td>
</tr>
<tr>
<td><em>Thioalkalivibrio</em></td>
<td>9–10</td>
<td>AN/AE</td>
<td>$\text{sox without soxCD}$</td>
</tr>
<tr>
<td><em>Sulfurimonas</em> denitrificans</td>
<td>7</td>
<td>AN/AE</td>
<td>$\text{sox, spr}$</td>
</tr>
</tbody>
</table>

*Acidithiobacillus thiooxidans* is only aerobic, taken from reference [36]. *$\text{tst}$* Taken from references [37,38]. *$\text{hdr}$* Taken from reference [39].
3. Biofiltration Technologies

Since H₂S content in biogas may reach values up to 7000 ppmv, biological technologies should be able to withstand high and variable H₂S loads for extended periods of time. In the last few years, different biotechnologies addressing H₂S abatement in biogas and industrial waste gases have been proposed based on the activity of chemolithotrophic SOBs. In the case of large-scale application, most of them have been used for odor control in WWTPs and industrial processes. These waste gases are typically characterized by high flow rates and low contaminant concentrations that make them amenable to biological treatment [40].

Biological oxidation of H₂S using O₂ as the electron acceptor for the growth of colorless chemotrophic SOBs has been extensively reviewed [41]. Thiobacillus species together with Sulfolobus, Thiovulum, Thiothrix and Thiospira have been identified as representative genera [26]. The stoichiometry of H₂S oxidation reactions by chemolithotrophic SOBs in the presence of O₂ are as follows:

\[
\begin{align*}
H₂S + 0.5 O₂ & \rightarrow S^0 + H₂O & (-209.4 \text{ kJ/reaction}; O₂/H₂S = 0.5) \quad (1) \\
S^0 + 1.5 O₂ + H₂O & \rightarrow SO₄²⁻ + 2H⁺ & (-587 \text{ kJ/reaction}; O₂/H₂S = 1.5) \quad (2) \\
H₂S + 2O₂ & \rightarrow SO₄²⁻ + 2H⁺ & (-798.2 \text{ kJ/reaction}; O₂/H₂S = 2.0) \quad (3)
\end{align*}
\]

S⁰ is an intermediate compound formed under O₂-limited conditions, yielding less energy than the complete oxidation to SO₄²⁻. The O₂/H₂S ratio will affect the final products obtained. Values slightly higher than the stoichiometry value, typically around 0.7, will lead to the formation of S⁰ as the main final product, while ratios >1 will result in the significant formation of SO₄²⁻ [26].

Biological oxidation of H₂S can be also performed under anoxic conditions using oxidized forms of nitrogen (NO₃⁻ or NO₂⁻) as the terminal electron acceptor instead of O₂ for the growth of autotrophic denitrifying SOBs. Some representative species are found in the following genera: Thiobacillus, Thiomicrospira and Thiothrix [32,42–44]. Although most of the autotrophic denitrifying SOBs are facultative (e.g., the final electron acceptors can be O₂ or NO₃⁻/NO₂⁻), some strict anaerobes (e.g., Sulfurimonas denitrificans) have been reported [39,45]. In autotrophic denitrification coupled to sulfur oxidation, NO₃⁻ and/or NO₂⁻ are converted to N₂ through the following steps NO₃⁻→NO₂⁻→NO→N₂O→N₂ as described for traditional heterotrophic denitrification. Thiobacillus denitrificans, Paracoccus versutus and Sulfurimonas denitrificans can perform complete denitrification leading to the formation of N₂ while other species like Thiobacillus thioparus and Thiobacillus delicatus only reduce NO₃⁻ to NO₂⁻ [41,46]. Similarly to aerobic sulfur oxidation, the N/S ratio also affects the final products obtained [25,47–49].

\[
\begin{align*}
S²⁻ + 0.4 NO₃⁻ + 2.4 H⁺ & \rightarrow S⁰ + 0.2 N₂ + 1.2 H₂O \quad (191.0 \text{ kJ/reaction}; N/S = 0.4) \quad (4) \\
S²⁻ + NO₃⁻ + 2 H⁺ & \rightarrow S⁰ + NO₂⁻ + H₂O \quad (130.4 \text{ kJ/reaction}; N/S = 1) \quad (5) \\
S²⁻ + 1.6 NO₃⁻ + 1.6 H⁺ & \rightarrow SO₄²⁻ + 0.8 N₂ + 0.8 H₂O \quad (-743.9 \text{ kJ/reaction}; N/S = 1.6) \quad (6) \\
S²⁻ + 4 NO₃⁻ & \rightarrow SO₄²⁻ + 4 NO₂⁻ \quad (-62.7 \text{ kJ/reaction}; N/S = 4) \quad (7) \\
S²⁻ + 0.67 NO₂⁻ + 2.67 H⁺ & \rightarrow S⁰ + 0.33 N₂ + 1.33 H₂O \quad (-240.3 \text{ kJ/reaction}) \quad (8) \\
S²⁻ + 2.67 NO₂⁻ + 2.67 H⁺ & \rightarrow SO₄²⁻ + 1.33 N₂ + 1.33 H₂O \quad (-920.3 \text{ kJ/reaction}) \quad (9)
\end{align*}
\]

As for aerobic sulfur oxidation, either S⁰ or SO₄²⁻ will be formed depending on the N/S ratio. NO₃⁻ can be reduced to either NO₂⁻ or N₂. At a N/S ratio of 1.6, a complete oxidation of H₂S and reduction of NO₃⁻ to N₂ could be achieved with the highest energy yield [26]. However, the formation of S⁰ and NO₂⁻ is frequently observed as the oxidation and reduction processes do not evolve to completion [10]. When N/S ≤ 0.4, the end products are S⁰ and N₂ [47–49], If nitrates are
stochiometrically in excess (N/S > 4), the final products are nitrites, which accumulate and may also be used as electron acceptors [48,49].

Currently, the desulfurization of both biogas and industrial gas streams is mainly conducted in one of these bioreactors configurations: conventional biofilter, biotrickling filter (BTF) and suspended growth bioscrubber (Figure 3).

![Biofiltration unit configurations: (a) Biofilter; (b) biotrickling filter (BTF); (c) Bioscrubber.](image)

In biofilter and BTF systems, the contaminated gas stream is passed through a packed bed where the SOBs are immobilized in a biofilm. In conventional biofilters (Figure 3a), the packed bed is only periodically spread with water (or eventually nutrients) while in BTFs (Figure 3b) the packed bed is continuously trickled with a nutrient solution. The process is complex and involves several simultaneous physical, chemical and biological interactions. In conventional biofilters the pollutant (here H2S) is transferred by absorption into the biofilm where diffusion and sulfur oxidation take place releasing $S^0$ or $SO_4^{2-}$ (Figure 4). In BTFs, the difference is the presence of a continuous liquid phase that the pollutant first has to transfer into before transferring to the biofilm. Bioscrubbers are two-stage systems that consist of a gas scrubber and a biological reactor (Figure 3c). The H2S is first transferred from the gas phase to an alkaline aqueous phase in an absorption column (gas scrubber) and then the resulting aqueous stream, containing the dissolved H2S, is directed towards an agitated bioreactor where the H2S is put into contact with SOBs (biological reactor). Thus, in bioscrubbers, the SOBs develop their activity in a stirred liquid while in biofilters and BTFs, the bioreaction takes place in a biofilm. The absorption and the biological oxidation reaction are physically separated in conventional bioscrubbers.

![Schematic representation of the complex interactions that take place in biofilters.](image)
Conventional biofilters have the simplest configuration. They have been used in large-scale gas applications for the control of H₂S and other odorous emissions from WWTPs and other industries [40]. The main benefits are the low operation cost, low-energy and chemical requirements, as well as high removal efficiencies (REs), usually above 99% [50].

In conventional biofilters, the terminal electron acceptor is O₂ from the air since there is no continuously circulating liquid which could provide a constant supply of NO₃⁻ or NO₂⁻ as electron acceptors. Natural organic materials have normally been preferred for packing conventional biofilters (i.e., compost, peat, wood bark and soil among others) since they provide large specific areas with high porosity, low pressure loss, lightweight, low-cost, buffering and water-retaining capacity, intrinsic nutrient content as well as the presence of indigenous microbial consortia [51,52].

The drawbacks of conventional biofilters, especially under long-term operation, are (a) the accumulation of biomass and S⁰ which may lead to bed clogging episodes (i.e., the reduction of inter-particle void space) causing preferential flow in the biofilter bed and pressure drop with the consequent reduction of the available mass transfer area; (b) acidification of the packing material due to the generation of SO₄²⁻ which leads to the formation of H₂SO₄, especially at the inlet area where the H₂S concentration and oxidation rate are higher, which may decrease the pH to values <1 causing inhibition of the microbial activity and decrease in the mass transfer rate into the biofilm; (c) compaction and degradation of the packing material provoking a reduction of media porosity and buffering capacity [53–57].

Conventional biofilters do not seem to be the most suitable technology for biogas desulfurization. The production of acid may result in the degradation of the packing material and the formation of small particles of degradation products contributing to bed clogging while, on the other hand, the accumulation of S⁰ may also result in biofilter clogging [58], the main drawback of conventional biofilters, as mentioned above.

BTFs are a more sophisticated and controlled variation of conventional biofilters in which the aqueous phase is continuously trickled over the packed bed. Continuous trickling provides a better buffering capacity than in conventional biofilters and avoids excessive acidification in the packing bed through the continuous washout of SO₄²⁻ [59]. However, continuous nutrients supply and operation under higher loading rates than in conventional biofilters usually result in clogging caused by biomass growth and S⁰ accumulation, both leading an increase in back pressure, bed channeling, formation of anaerobic zones and a decrease in RE [60,61]. Therefore, the control of biomass growth and S⁰ accumulation arise as crucial operating parameters in BTFs. Different strategies have been used for limiting biomass overgrowth including the control of air supply to promote the oxidation of sulfide to SO₄²⁻, the use of appropriate packing materials, the application of biomass predators, periodical bed backwashing and the control of nutrient supply [26,61]. Increasing the quantity of injected air results in higher O₂ levels favoring the formation of SO₄²⁻ thus alleviating the clogging problem due to S⁰ accumulation [26]. However, this strategy reduces the off-gas quality and increases the risks of explosion. Optimizing the flow rate of the trickled liquid over the packing bed combined with recurrent draining and the application of a fresh trickling liquid have also been used to overcome clogging episodes during the treatment of VOCs in BTFs [61].

Another difference is the packing material, BTFs are usually packed with inert or synthetic materials including plastic rings, polyurethane foam (PUF), granular activated carbons, porous ceramics and lava rock and there are few applications reporting the use of natural materials [61–63]. Compaction and degradation of the packing material is therefore not a significant problem in BTFs. Moreover, synthetic packing beds maintain a relatively constant pressure drop, lower than natural materials. The open structure and high porosity of PUF results in a low pressure drop under conditions of high gas flow rates; additionally, PUF characteristics may also favor a faster biofilm formation in comparison to plastic materials and thus reducing the biofiltration start-up period [61].
Since the final product of the H$_2$S biological oxidation can be either S$^0$ or SO$_4^{2-}$, the O$_2$ mass transfer from gas to aqueous phase and biofilm (Figure 4) is one of the major parameters of this technology to ensure local stoichiometric level of O$_2$ in the biofilm [64].

Biogas desulfurization in BTFs through H$_2$S oxidation under chemolithoautotrophic denitrifying conditions has been recently recognized as a promising option [65–68]. The supply of NO$_3^-$ or NO$_2^-$ in the trickling liquid enables this biological process. Anoxic conditions eliminate the risk of explosion in CH$_4$/O$_2$ mixtures and dilution of the biogas with air. Moreover, there are no mass transfer limitations in the supply of NO$_3^-$/NO$_2^-$. In aerobic and anoxic BTFs, the generation of S$^0$ has to be controlled in order to avoid clogging effects, therefore the O$_2$/H$_2$S and N/S ratios are fundamental operational parameters.

Bioscrubbers have several advantages in comparison to biofilters and BTFs for biogas desulfurization. No O$_2$ is injected into the polluted gas stream, avoiding dilution effects and risks of explosion. Bioscrubbers can deal with fluctuating H$_2$S inlet loads, mostly because of the longer residence time of H$_2$S in the bioreactor [69] and easier management and control of the bioreactor operational condition, ensuring a stable and efficient operation [26]. Another advantage is that S$^0$ can be recovered from bioscrubbers and that there are no clogging problems. However, investment and operational costs are higher due to the presence of two separated operational units and large caustic consumption to maintain an efficient absorption, respectively. The Thiopaq® process (Paques, The Netherlands) and Sulfothane™ process (Biothane, USA) are bioscrubber-based systems that have been successfully developed at industrial large-scale for the aerobic desulfurization of biogas [7,25,26].

The main advantages and drawbacks of conventional biofilters, BTFs and bioscrubbers in relation to the treatment of H$_2$S in gas streams are summarized in Table 3. Among these three biotechnologies, the ability of BTFs to treat moderate to high H$_2$S loads has been demonstrated in numerous studies focused on the removal capacity and RE of BTFs treating H$_2$S at different loading rates, after shock loads, using different packing materials, under liquid flow patterns, as well as under different gas flow rates [70]. Hereafter, examples of studies combining the effect of different operational conditions and microbial diversity analyses, mainly in BTFs used in the removal of H$_2$S from biogas, are presented.

### Table 3. Main advantages and drawbacks of biofiltration technologies used for H$_2$S abatement.

<table>
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<tr>
<th></th>
<th>Conventional Biofilters</th>
<th>BTFs</th>
<th>Bioscrubbers</th>
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<tr>
<td><strong>Advantages</strong></td>
<td>Low investment and</td>
<td>Medium capital and low</td>
<td>Treatment of high flow rates</td>
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<td>operational costs</td>
<td>operating costs</td>
<td>at different H$_2$S</td>
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<td>Easy operation and</td>
<td>Effective removal of H$_2$S</td>
<td>gas concentration</td>
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<td>maintenance</td>
<td>at high concentrations</td>
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<td>Effective removal at</td>
<td>Easy process control</td>
<td>Operational stability</td>
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<td>low H$_2$S concentrations</td>
<td>(for example pH via trickled media)</td>
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<td>Durability of the</td>
<td>Low pressure drop</td>
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<td>packing materials</td>
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<td><strong>Drawbacks</strong></td>
<td>Bed clogging due to</td>
<td>Risk of bed clogging due to</td>
<td>Relatively complex</td>
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<td>biomass growth and S$^0$</td>
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<td>accumulation</td>
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<td>Secondary pollution</td>
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<td>Packing material</td>
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<td>generation (biomass and</td>
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<td>compaction</td>
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<td>liquid waste streams)</td>
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<td>Difficult to control pH</td>
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<td>drop due to SO$_4^{2-}$</td>
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<td>Need of filter bed</td>
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<td>replacement</td>
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4. Microbial Ecology Studies in Biofiltration Units for H$_2$S Removal

4.1. Molecular Techniques for Characterizing Bacterial Communities in Biofilters

Biofilters and BTFs make use of microorganisms embedded in a biofilm for the treatment of gaseous streams containing H$_2$S. Bacteria are the principal agents of sulfur oxidation. Since the performance of biofiltration processes depends on the activity and robustness of the bacterial communities involved, it is fundamental to characterize the bacterial populations that coexist in biofilters under different operational conditions in order to understand the sulfur cycling in these systems and propose potential improvements.
Classic microbiological methods based on cultivation favor the growth of certain groups of microorganisms. Artificial culture media, either liquid or solid, cannot reproduce the natural growth conditions present in biofilters, on the contrary, some nutrients may favor the growth of non-representative organisms only present in small amounts. Chemolithoautotrophic SOBs have slow growth rates and are usually closely associated in consortia containing several physiological and metabolic types of bacteria [71]. The limitations of culture techniques have been well documented, and it is now accepted that 99% of the microorganisms present in an environmental sample cannot be cultivated [72,73].

The advent of molecular techniques based on the small subunit of the ribosomal RNA gene (16S rRNA) has allowed the characterization of microbial communities from diverse environments such as humans, soils, oceans, engineered environments, bioreactors, bioremediation processes and, in the last 15 years, biofilters, among others. Phylogenetic analysis based on the 16S rRNA gene sequence is a gold standard for describing bacterial communities. This gene has been selected since the revolutionary studies of Carl Woese on the evolutionary history of cells (the three domains of life) and first applied by Norman Pace to the survey of natural microbial assemblages based on nucleic acid-based techniques [74,75]. This gene is ideal for bacterial phylogeny studies (evolution) and taxonomy purposes (classification) for the following reasons: (1) it is found in all prokaryotes, in single or multiple copies; (2) the function of this gene over time has not changed suggesting that changes in the sequence of this gene are a measure of evolution; (3) it offers sufficient resolution for discerning bacteria at the phylum to genus level; (4) it contains conserved regions for the design of general primers and probes; (5) it also has variable regions for the design of specific primers and probes and for phylogenetic studies; (6) it is large enough (1500 bp) for precise bioinformatic analyses [72–75]. Operational taxonomic units (OTUs) are used to categorize bacteria based on the similarity of their 16S rRNA gene sequences.

Millions of copies of these genes can be produced by using the polymerase chain reaction (PCR). The total DNA of a microbial community is used as the template in the PCR reaction. The mixture obtained after PCR amplification contains millions of copies of 16S rRNA sequences from every bacterium present in the original sample. These genes must then be separated, and their sequences determined in order to identify the corresponding bacteria. Several approaches have been used as fingerprinting methods, 16S rRNA clone libraries and direct amplicon sequencing without cloning using next generation sequencing technologies such as 454-pyrosequencing, now discontinued, and Illumina platforms [76–78]. Among fingerprinting methods, denaturing gradient gel electrophoresis (DGGE) and single-strand conformation polymorphism (SSCP) allow the separation of 16S rRNA amplicons by denaturing and non-denaturing gel electrophoresis, respectively, while terminal restriction fragment length polymorphism (T-RFLP) allows the separation of fluorescently labelled restriction fragments of 16S rRNA amplicons. Fluorescence in situ hybridization (FISH) is a 16S rRNA targeted in situ hybridization technique used to label, visualize and enumerate whole cells in samples with PCR amplification [79].

4.2. Aerobic Biofiltration

The first study describing the dynamics of microbial communities in BTFs treating H$_2$S appears in 2005, in relation to odor abatement [80]. The bacterial communities present in a two-stage BTF system designed for the simultaneous removal of H$_2$S and dimethyl sulfide (DMS) are described using the DGGE fingerprinting method. The first BTF, aimed at removing H$_2$S, was inoculated with a pure culture of the acidophilic bacterium Acidithiobacillus thiooxidans and operated without pH control while the second BTF, aimed at removing DMS, was inoculated with the VS. strain of Hyphomicrobium, a neutrophilic bacterium, and operated at neutral pH. This configuration was chosen to overcome the limitations encountered when treating gases containing mixtures of reduced sulfur compounds in which H$_2$S is preferentially degraded over organic sulfur compounds, especially at a low pH.
In the case of DMS (second BTF), the RE was found to be sensitive to lower values of the empty bed residence time (EBRT) and pH, H$_2$S overload and starvation times. However, the bacterial community in this BTF was found to be stable even on fluctuating DMS removal efficiencies. A high diversity was observed as shown by the multiple bands in the DGGE patterns and *Hyphomicrobium VS.* was no longer dominant, representing approximately only 10% of the established bacterial populations. A different trend was observed in the BTF inoculated with *Acidithiobacillus thiooxidans* (first BTF). The removal of H$_2$S was not affected by the operational conditions except when the H$_2$S load was increased from 1220 to 4037 ppmv. The pH, which was not controlled, maintained its value between 2 and 3 during the 117 days of operation and $S^0$ visibly accumulated. The bacterial diversity in this BTF was limited due to the low pH and only one prominent band corresponding to *Acidithiobacillus thiooxidans* was observed. This bacterium is the most acidophilic SOB and has been considered an ideal inoculum for the biofiltration of H$_2$S in biogas [50,81]. Its pH range for growth is between 0.5 and 5.5 with an optimum at pH 2–3 [36].

DGGE has also been used to study the evolution of bacterial populations in a two-stage BTF system for the simultaneous treatment of H$_2$S, methylmercaptan (MM), DMS and dimethyl disulfide (DMDS) for an odor abatement application [82]. The packing material consisted of polyurethane foam (PUF) cubes and the stream to be treated was generated in a compressor and enriched with H$_2$S and the above-mentioned organic reduced sulfur compounds. In the first BTF inoculated with *Acidithiobacillus thiooxidans* and operated at a low pH (2.5) for the removal of H$_2$S, the inoculated population remained constant at the different operation conditions tested (23 to 1320 ppmv of H$_2$S with and without different proportions of organic sulfur compounds). This result can also be explained by the low pH that restricted the colonization of this BTF by other bacteria. As reported by Sercu et al., (2005), the bacterial populations changed in the second BTF originally inoculated with *Thiobacillus thioparus* and operated under neutral conditions (pH 7.0), however the bacterial diversity in this BTF was low (one DGGE band observed) in spite of the permissive conditions of pH [80].

In the work of Sercu et al., (2005), the bacterial diversity in the recirculating liquid from the second BTF (for DMS removal) was also examined and it was observed that the planktonic bacterial community was quite different from the BTF community (biofilm), which highlights the importance of focusing on the biofilm [80].

A more extensive study on the diversity and spatial distribution of bacteria in a lab-scale BTF treating high H$_2$S loads (2000 ppmv) in a simulated biogas (“biogas mimic”) was published in 2009 [83]. In addition to a fingerprinting molecular method (T-RFLP), a 16S rRNA gene clone library was constructed in order to further investigate the identity of the bacteria present in the BTF. Full length sequences of the 16S rRNA gene ($\approx$1500 pb) can be retrieved from clone libraries contrary to DGGE and T-RFLP that only analyze relatively small fragments from which phylogenetic affiliation of bacterial species cannot be precisely inferred. The BTF was packed with high density polypropylene grids (HD Q-Pack) and inoculated with a sulfur-oxidizing culture at pH 1.6 obtained from a full-scale biogas desulfurization column, enriched using Na$_2$S as the energy source and progressively acclimated to pH 6 [84]. An artificial biomass sample “representative” of the total community in the BTF was obtained by mixing samples (1:1) taken at the inlet and outlet parts of the reactor for constructing the gene clone library.

74% of the obtained sequences belonged to *Proteobacteria*, among which 49.4, 15.6 and 9.1% were *Gamma-, Beta- and Epsilonproteobacteria*, respectively. Of the 75 clones sequenced, 60% were related to SOB species, namely, *Thiothrix* sp. (*Gammaproteobacteria*); *Sulfurimonas denitrificans* (*Epsilonproteobacteria*); *Thiobacillus denitrificans*, *Thiobacillus sajanensis* and *Thiobacillus plumbophilus* (*Betaproteobacteria*). *Thiothrix* is a filamentous SOB that thrives in wastewaters characterized by high organic loads and elevated concentrations of low molecular weight fatty acids and reduced sulfur compounds, low dissolved oxygen (DO) concentrations and deficit in phosphorus and nitrogen [85]. The overgrowth of *Thiothrix* is related to activated sludge bulking in WWTPs. The presence of *Thiothrix* in the BTF was probably due to its ability to grow under heterotrophic, lithotrophic or mixotrophic conditions and use the
organic matter, biomass and cellular debris accumulated in the BTF during its 6 months of operation. Sulfur-oxidizing mixotrophs can use reduced sulfur compounds as electron donors to produce ATP and CO₂ (autotrophy) or organic carbon (heterotrophy) to produce biomass. *Sulfurimonas* is a ubiquitous SOB found across the globe, in terrestrial and marine environments [86]. This bacterium is a strict chemoautotroph. *Sulfurimonas denitrificans* can use both sulfide and sulfur as electron donors and nitrate, nitrite and oxygen as electron acceptors [39]. *Thiobacillus* species are the typical colorless SOBs, with a strict chemolithoautotrophic metabolism. Among these, *Thiobacillus denitrificans* is a facultatively anaerobic bacterium, which can couple the oxidation of reduced sulfur compounds to denitrification [87].

Concerning the spatial distribution of bacterial populations, studied by T-RFLP, *Thiothrix* was more abundant in the outlet than in the inlet of the bioreactor. This finding is in agreement with niche differentiation patterns observed among sulfur-oxidizing populations in natural environments such as natural caves where three dimensions are critical, namely the sulfide, oxygen and water flow [88]. In such environments, *Thiothrix* is preferentially found in less turbulent or slowing flowing waters, with low sulfide and high oxygen. In BTFs, these conditions are met at the outlet. This is because, as in other plug flow types reactors, a gradient of H₂S and DO concentrations forms in BTFs, with higher H₂S/O₂ ratio in the inlet (bottom) and lower H₂S/O₂ ratio at the top (outlet). The conditions are therefore less severe in terms of H₂S concentration at the outlet.

Later on, the same group reported on the composition of the bacterial communities present in a similar laboratory scale BTF treating a biogas mimic, consisting of a mixture of H₂S and N₂, which was determined by using 16S rRNA clone libraries and FISH [89]. The H₂S concentration in the inlet was kept constant at 2000 ppm and the pH was controlled at 6.5–7 in the recirculating liquid. DO and pH were monitored online in the recirculating liquid. A 95% RE was achieved 3 days after inoculation of the BTF with an aerobic sludge from a municipal WWTP, indicating that this inoculum already contained a significant population of SOBs. An RE over 99% was obtained throughout the operation 10 days after start-up.

Equal amounts of biomass samples collected at days 42 and 189 from the inlet (bottom) and outlet (top) zones of the BTF were mixed to create “representative” samples from the total microbial community. The 16S rRNA clone libraries obtained 42 and 189 days after startup had a different composition indicating that the total bacterial communities present in the system changed throughout the operation, although the RE was above 99% throughout the study. Therefore, shuffling in the bacterial populations did not affect the performance of the BTF. A wide phylogenetic diversity was found in both libraries. After 42 and 189 days, 39 and 51% of the retrieved clones were affiliated with bacteria related to the sulfur cycle. The authors claim that using a biogas mimic is valid although it does not contain CH₄ or gaseous hydrocarbons since previous reports have indicated that the degradation of H₂S by lithotrophic bacteria is not affected by the presence of organic carbon sources. However, the presence of CH₄ in a real biogas may boost the growth of methanotrophic and heterotrophic bacteria that could compete for oxygen especially in areas of the bioreactor where DO is limiting. Diversity indexes indicated that bacterial diversity and evenness were lower at the longer operation time. So, a “simplification” or “metamorphosis” of bacterial communities towards more specific and dominant SOB communities was favored over long operation times in the presence of high H₂S loads.

*Thiobacillus* (*Betaproteobacteria*) was the most abundant genus detected (40%) at day 42 of operation while *Thiothrix* (*Gammaproteobacteria*) became the dominant genus (44%) after 189 days. Typical yellowish *Thiothrix*-like mats were observed at this time. The percentage of *Thiobacillus* sp. related bacteria decreased (7%) after 189 days of operation and the species detected at this time were different. As in the previous study, after some months of operation, more organic materials were present in the BTF and *Thiothrix* species displaced *Thiobacillus* sp. due to their ability to grow under heterotrophic, lithotrophic or mixotrophic conditions (see above for more information on *Thiothrix*). In contrast, *Thiobacillus* species are strictly chemolithotrophic. Other genera of SOBs were also detected, such as *Thiomonas, Sulfuricurvum, Acidithiobacillus* and *Halothiobacillus* at day 42 and *Sulfurimonas* at day 189.
Thiomonas species form a distinct phylogenetic cluster of SOBs capable of mixotrophic growth, which means they can incorporate organic substrates in the presence of an oxidable sulfur compound, this physiological feature clearly differentiates them from Thiobacillus species [90,91]. The potential of Thiomonas sp. for the removal of H2S in a gas-bubbling reactor has been reported [92]. Some authors have reported that mixotrophic biofilters performed better than autotrophic biofilters based on H2S RE after start-up, although most biofiltration processes have preferred autotrophic organisms due to their simplicity of operation and low biomass yield [92]. Sulfuricurvum is a strict chemolithotrophic SOB, that belongs to the Epsilonproteobacteria, a class that comprises other SOBs, such as Sulfurimonas (see above), with an important ecological and biogeochemical role in marine and terrestrial sulfidic habitats [93]. Low clone coverage was obtained (less than 49%) in both 16S rRNA libraries, which means that some SOB species were probably not detected.

Additionally, the authors used the FISH technique to follow the temporal and spatial dynamics of the SOBs in the BTF. For this, they used probes designed to detect the neutrophilic SOBs found in the clone libraries. Contrary to clone libraries, FISH is a quantitative technique for counting specific bacterial populations in environmental samples. It is not based on PCR, which is biased by the fact that rRNA operons have different copy numbers in different bacterial species. For example, according to the National Center for Biotechnology Information (NCBI) genome database [94], Acidithiobacillus thiooxidans and Thiomonas intermedia have one copy of the rRNA operon, Thiothrix sp., Thiobacillus denitrificans and Halothiobacillus neapolitanus have two copies and Thiomonas intermedia four copies. Additionally, in traditional PCR (end-point PCR), the proportion of dominant amplicons does not necessarily reflect the abundance of specific sequences, due to preferential amplification of certain sequences and accumulation of amplicons in the plateau phase of amplification, irrespective of their original abundance. In FISH with rRNA probes, single microbial cells are fluorescently stained and individually counted using a fluorescence microscope, independently of their rRNA content.

FISH revealed that Thiolithrix, Sulfurimonas denitrificans, Halothiobacillus neapolitanus, Thiobacillus denitrificans and Thiomonas intermedia were present from day 0 at the inlet. Thiomonas intermedia notably increased at day 105 but was replaced by Thiolithrix at day 189. A careful look at the monitored parameters at day 105 and 189 shows that the DO was globally lower at day 189, which may explain the difference in bacterial populations, although the DO was monitored in the liquid, not in the BTF, which may not be representative of local DO variations. The system was cleaned up after taking the sample at day 189 and, again at day 229, Thiomonas intermedia abundance increased, showing that this species may be a primary colonizer of the system in absence of significant quantities of organic matter. Thiolithrix was more abundant at the outlet while the abundance of T. intermedia increased at the inlet compared to outlet, meaning that this species may be more adapted to high H2S concentrations. After reactor clean up, Thiomonas intermedia was the most abundant SOB. As the pH was controlled in this system, the authors did not attribute the changes in microbial populations to pH but to DO, H2S concentration and sulfur or organic matter accumulation. Thiomonas intermedia is a slightly acidophilic SOB that has been found in corroding sludge digesters and sewage systems [21,95].

In absence of pH control, the pH drops to very low values <2.0 in biofilters treating H2S. The bacterial communities in pilot-scale BTFs packed with ceramic or volcanic rock, inoculated with an activated sludge from a municipal WWTP and operated at different EBRT (20, 15, 10 and 5 s) without pH control were studied [6]. The operation time at each EBRT was relatively short (15 days). The gas to be desulfurized proceeded from the WWTP with an H2S load of 2.84 mg/m3, corresponding to ≈2.0367 ppmv, a low load. The biomass samples for 16S rRNA gene pyrosequencing were collected in the bottom zone of the BTFs at the end of the first and third stages, corresponding to EBRTs of 20 and 10 s, respectively. The pH drops registered in the two BTFs were from 7.0 to 3.5 and 7.0 to 1.5 at EBRTs of 20 and 10 s, respectively. The volcanic rock BTF presented a 99% RE at any of the EBRTs tested while the RE of the ceramic BTF decreased from 94 to 60% at the lowest EBRT. The activity of the ceramic BTF was restored after washing with fresh medium, which means that it was more affected by pH than the volcanic rock BTF. Members of the Thiomonas genus were abundant in both BTFs and the abundance
of this genus even increased under the most drastic conditions (a pH drop from 7 to 1.5) showing the acidophilic nature of this bacterium (see *Thiomonas* characteristics above). Only the volcanic BTF was colonized by *Acidithiobacillus thiooxidans* at the most extreme operating condition (EBRT of 10 s) with an abundance of 27.9%. In the ceramic BTF, the abundance of *Acidithiobacillus thiooxidans* was low (<2%) at any of the tested EBRTs. *Acidithiobacillus thiooxidans* was therefore clearly responsible for the performance of the volcanic rock BTF under acidic conditions. This bacterium has been proposed as ideal for inoculating biofilters operating under stable conditions of low pH as mentioned above. The influence of the nature of the packing material was evidenced here since the volcanic rocks, which have a higher porosity and specific surface area than the ceramic granules, allowed colonization by *Acidithiobacillus thiooxidans* and achieved high RE even at very low pH values. The ceramic BTF could not be colonized by *Acidithiobacillus thiooxidans*. It has been reported that the use of cell attachment promoters enhanced the performance of BTFs packed with PUF cubes and treating H$_2$S polluted airstreams [96]. The attachment of *Acidithiobacillus thiooxidans*, the dominant bacterium in these BTFs, to the PUF cubes was enhanced after coating the cubes with polyethyleneimine. It has been shown that *Acidithiobacillus thiooxidans* preferentially colonized concrete compared to glass in long incubation times, showing that biofilm formation by this bacterium may be material-dependent [97].

Concerning the influence of the packing material, there is only one report comparing the bacterial communities and the H$_2$S RE of two large-scale biofilters (not BTFs) packed with different inorganic materials, marble or pozzolan supplemented with limestone, and treating waste gases from the same WWTP [98]. The 16S rRNA gene clone libraries were constructed to compare the bacterial diversity in the two biofilters. In this study, the marble biofilter was very acidic (pH < 3), had a better H$_2$S removal performance and a higher bacterial diversity. The main OTUs detected in relation to sulfur oxidation were a betaproteobacterium from a sulfidic cave biofilm (not identified at the genus level) and *Thiobacillus sajanensis* with a reported optimum pH range of 6.8–9.5 [99]. Surprisingly, the biofilter packed with pozzolan, a material of volcanic origin, was less acidic (pH 5.7–6.8) and harbored different microbial populations such as the thermoacidophilic red algae *Cyanidium caldarium* and *Acidithiobacillus* sp. The authors attributed the increase of pH and lower H$_2$S RE to the presence of limestone.

The characterization of bacterial communities at three layers (bottom, middle and top) of a pilot scale peat biofilter treating gases emitted from a WWTP is described [40]. Although the system is not a BTF and the application is in odor abatement, the results are interesting in some aspects related to the influence of pH in biofilters at the pilot scale level. The molecular technique used here was SSCP. Peat is like a “self-inoculated” packing material with high organic content. The H$_2$S inlet concentrations vary from 227 to 1136 mg/m$^3$ (163–815 ppmv). During operation, the registered decrease of pH in the bottom, middle and upper zones was from 7.8 to 2.5, 7.5 to 3.3 and 7.3 to 6, respectively. These results are consistent with the highest pH drop at the inlet where H$_2$S biodegradation is the highest due to the accumulation of SO$_4^{2-}$. In this study, cell counts were performed at the different layers, however the media used were directed to heterotrophic bacteria and fungi not SOBs. The presence of fungi was noted at the bottom, consistent with the tolerance of fungi to acidic conditions. The bottom layer presented the highest bacterial diversity according to the obtained SSCP patterns and each layer was dominated by a few bacterial species, different in each layer, consistent with a stratification of the bacterial populations observed in other biofiltration systems, independently of the type of packing material and contaminant treated [100]. The few clones sequenced in this study allowed retrieving *Pseudomonas* and other heterotrophic bacteria instead of typical colorless SOBs. This is not surprising for this type of packing material.

The pH transition to acidic values drastically reduced the microbial diversity in a BTF packed with stainless steel Pall rings and inoculated with aerobic sludge from a local municipal WWTP [101]. A reference synthetic gas containing 2000 ppmv of H$_2$S was used. A gradual and controlled pH shift was established from pH 6.5 to 2.75 (in the recirculating liquid) between days 440 and 600 of operation. The total DNA was extracted from biomass collected at days 245 (neutral pH) and 586 (acidic pH) from three sampling ports (bottom 1/3, middle 2/3 and top 3/3) and mixed in equal proportions.
for 16S rRNA gene amplicons pyrosequencing. At an acidic pH, the community was enriched in \textit{Gammaproteobacteria} where the acidic SOBs (\textit{Acidithiobacillus} sp.) group instead of \textit{Betaproteobacteria} where most neutrophilic SOBs are found. \textit{Acidithiobacillus thiooxidans} was found in the communities developed under neutral conditions (4.1\% of the OTU) together with \textit{Thiomonas} and \textit{Thiobacillus}. The abundance of \textit{Acidithiobacillus} sp. increased up to 57.4\% under acidic conditions. \textit{Acidiphilium} was also detected (11.4\%). This bacterium can grow under mixotrophic or chemolithotrophic conditions and is able to oxidize H$_2$S or elemental sulfur under oxygen limiting conditions. It is often found together with \textit{Acidithiobacillus thiooxidans} in natural and engineered environments such as acid mine drainage and corroded concrete [102,103].

One of the most extensive studies, from a microbial ecology point of view, is perhaps the one described by Tu et al., (2015) [104]. In this study, two identical bench scale BTFs packed with volcanic rock, seeded with the same inoculum and acclimated for one month under steady state conditions at pH 4.0 (BTFa, a for acidic) or pH 7.0 (BTFn, n for neutral) were compared in terms of operational performance and microbial populations. The inoculum was obtained by mixing an aerobic sludge from a municipal WWTP and a microbial consortium enriched in the presence of thiosulfate from a sample collected in a landfill leachate treatment plant. The EBRTs tested were 60, 30 and 15 s for 14 h each, during which the H$_2$S load was gradually increased from 175 to 5858 mg/m$^3$, 169 to 5028 mg/m$^3$ and 69 to 1029 mg/m$^3$, respectively. Samples of the packing material were taken at the bottom (b), middle (m) and upper (u) layers of the BTFs at the end of each stage for pH measurements and MiSeq sequencing of 16s rRNA gene amplicons. The RE is also determined at the three layers for each BTF. The obtained results can be summarized in Table 4.

\textbf{Table 4.} Comparison of the operational behavior, pH and bacterial populations in two BTFs operated under acidic (a) and neutral conditions (n). EBRT: empty bed residence time.

<table>
<thead>
<tr>
<th>Operational Behavior</th>
<th>Biofilter BTFa (pH 4)</th>
<th>Biofilter BTFn (pH 7)</th>
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<tr>
<td>EBRT (s)</td>
<td>60</td>
<td>60</td>
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<td>Maximum RE at the inlet (%)</td>
<td>99</td>
<td>87.5</td>
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<th>Average pH and Bacterial Populations</th>
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<td>Layer</td>
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<tr>
<td>pH</td>
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<tr>
<td>Abundance of $\beta$-proteobacteria (%)</td>
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<td>Abundance of $\beta$-proteobacteria (%)</td>
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According to the RE values, BTFa had a better performance than BTFn. So, the most acidic condition (BTFa) generated the most effective BTF. Although the pH of the recirculating liquids was controlled at 4.0 and 7.0, gradients of pH formed into the two BTFs. As expected, in both BTFs, the pH was lower at the inlet layers where H$_2$S biodegradation is maximum due to the formation of SO$_4^{2-}$. The abundance of \textit{Gammaproteobacteria}, where the acidic SOBs are found, was higher in the acidic BTF (46.7\%). The predominant bacterial genus was \textit{Acidithiobacillus thiooxidans}. The abundance of \textit{Betaproteobacteria} (where neutrophilic SOBs are found) was between 19.1 and 32.9\%. The most abundant genera were \textit{Thiomonas}, \textit{Thiobacillus} and \textit{Halothiobacillus}. Considering these results, \textit{Acidithiobacillus} was again found to be the key bacterium for H$_2$S biodegradation. The inoculation of biofilters with \textit{Acidithiobacillus} species is proposed as an alternative to reduce acclimation times during H$_2$S biofiltration. Principal components analysis of diversity index values clearly separated the two BTFs and, for each BTF, the vertical stratification was clear with samples from the bottom layers being the most distant. This is another example of the influence of the operational conditions, pH and gradients of H$_2$S and O$_2$ that influence the composition and diversity of bacterial communities in BTFs for H$_2$S removal.
4.3. Anoxic Biofiltration

Fernandez et al. (2013) have reported the elimination of H$_2$S under anoxic conditions in a lab-scale BTF packed with Pall rings and operated at pH 7.4–7.5 [105]. The biogas was produced on-site in an upflow anaerobic sludge blanket (UASB) reactor and the H$_2$S content was gradually increased with a H$_2$S generation system. The inoculum consisted of biomass collected from a previous BTF packed with open polyurethane foam, operated under neutral pH conditions and initially seeded with nitrate-reducing bacteria and SOBs. Reduction of the methane and CO$_2$ content in the biogas were not observed during the 104 days of operation. The BTF was operated under nitrate limiting conditions (N/S ratio of 0.77 mol/mol), so the main product formed was elemental sulfur. The formation of this product could be reduced 25% if the N/S ratio was increased to 1.52 mol/mol. Analysis of the bacterial communities by DGGE was performed but it is not indicated at what time and how the sample was taken. Although the DDGE bands were not identified, a low bacterial diversity was observed. The same four bands were present at the inlet and at the outlet of the BTF while the recirculating liquid presented three bands, two of them also present in the BTF samples, meaning that some of the bacterial population were strongly attached to the packing material and that the nature of the inoculum, the anoxic conditions and the high H$_2$S loads (1400–14,000 ppmv) favored the establishment of a specialized bacterial community.

The effect of gas-liquid flow patterns on the performance and bacterial diversity and dynamics in a pilot-scale BTF treating a real biogas effluent from the AD of a WWTP was studied [65]. The BTF was packed with PUF cubes, inoculated with wastewater from the degritter-degreasing unit of the WWTP and operated for 415 days in five different operation modes: (1) day 1–297, counter-current flow with increasing H$_2$S loads; (2) day 298–360, co-current flow with increasing H$_2$S loads; (3) day 361–367, biogas supply cut; (4) steady state counter-current flow with liquid recirculation; and (5) steady state counter-current flow without liquid recirculation. Biomass samples were taken at the top and bottom of the BTF at day 343 (phase 2) and at the bottom at day 415 (phase 5) for massive pyrosequencing of short 16S rRNA gene amplicons. In counter-flow mode, biofilm growth rate is usually higher at the bottom part of BTFs where H$_2$S degradation is at its maximum. S$^0$ usually accumulates at the bottom where the ratio of electron acceptors (NO$_3^-$ or O$_2$/H$_2$S) is the lowest. The slightly lower performance under co-current flow was attributed to the redistribution of biomass and S$^0$ along the packed bed as observed by visual inspection. This redistribution, however, promoted by alternate flow patterns, was favorable with respect to clogging and pressure drop problems. In addition, biogas supply cuts allowed the S$^0$ to be consumed by the microorganisms.

Concerning bacterial diversity temporal and spatial dynamics, a first observation is the high proportion of unclassified species found here, between 22 and 43% of the sequences, which is a drawback of the single-end sequencing of small 16S rRNA amplicons in the pyrosequencing technique. Presently, paired-end sequencing allows the sequencing of both ends of a fragment and generates high-quality, aligned sequence data, even with small fragments, in addition to high sequencing coverage. Bacterial diversity was similar at the bottom and top parts of the BTF operated under co-current flow (samples taken at day 343), with a strong dominance of Proteobacteria. Sedimenticola (Gammaproteobacteria) was the most dominant genus in both samples with a relative abundance of almost 49.5 and 44.2%. Sedimenticola is a versatile SOB comprising species that can grow lithoautotrophically under hypoxic and anaerobic conditions using a variety of anaerobic electron acceptors such as NO$_3^-$, (per)chlorate or chlorate and can also use organic compounds as a source of energy and electrons [106]. Bacteria from the Rhizobiales and Rhodobacteraceae were also detected at a significant abundance, 4.5 and 8.3%, respectively. Rhizobiales can fix N$_2$ while Rhodobacteraceae comprise chemoorgano- and photoheterotrophs putatively active in anoxic, nitrate-dependent sulfide oxidation [107]. The bacterial community structure drastically changed under counter-current flow without liquid recirculation. The community was more diverse, and most sequences were related to unclassified genera. It was therefore not possible to detect any SOBs. The performance of the BTF significantly decreased under single-pass flow (no liquid recirculation) at the two nitrate concentrations tested. The presence of so many unclassified sequences may be
related to the growth of phototrophic eukaryotic microorganisms in this BTF since it was made of fiberglass, which shows some transparency to light. Moreover, the presence of nitrate may boost the growth of algae, which may further contribute to clogging problems. The presence of such organisms contaminates the samples with chloroplastic and mitochondrial 16S rRNA sequences which group along with unclassified sequences at the domain level. The degree of contamination depends on several factors, the DNA extraction and purification protocol, the primers and region of the 16S rRNA gene that was amplified and the bioinformatics pipeline used to analyze the data [108]. Overall, the obtained results indicate that the flow pattern has an influence on the composition of bacterial communities in BTFs and their vertical distribution, affecting the performance of the system and life span of the packed bed.

In the last year, several studies have reported the characterization of bacterial communities in anoxic BTFs for the removal of H₂S in gas streams. As expected and discussed above, the bacterial communities in anoxic BTFs are different from those of aerobic BTFs due to the use of different electron acceptors [109]. The packing material, open pore PUF (OPUF) or polypropylene Pall rings, did not influence the composition of the bacterial communities in BTFs seeded with the same inoculum (a sample from a previous OPUF biofilters) and treating 1400 to 14,600 ppmv H₂S concentrations as shown by the almost identical DGGE banding patterns [109]. The bacterial diversity was low probably because the inoculum had already been acclimated for biofiltration. The following genera and species were detected in relation to sulfur oxidation and autotrophic denitrification, *Thiobacillus thiophilus*, *Thiohalophilus* sp. and *Thiomonas intermedia*. *Thiobacillus thiophilus* has been described as an obligately chemolithoautotrophic and facultatively anaerobic bacterium, growing with either oxygen or nitrate as the electron acceptor [45]. *Thiohalophilus* sp. is an SOB that has been isolated under microoxic conditions and found to be capable of sulfur-driven anaerobic growth with NO₂⁻ [110]. As mentioned in Sections 2 and 3, NO₂⁻ is an intermediate in autotrophic and heterotrophic denitrification that can accumulate in biofilters when NO₃⁻ is used as the electron acceptor. The role of *Thiomonas intermedia* in sulfur oxidation has already been highlighted in aerobic biofilters, however, it is not clear if this bacterium can use NO₃⁻ as the electron acceptor. NO₂⁻ has been successfully used as the electron acceptor in a BTF treating a synthetic biogas containing H₂S concentrations of 952 to 3564 ppmv with a mineral medium as the recirculating liquid phase [111]. Although the bacteria were not identified, the authors report that the bacterial diversity was reduced during the progressive adaptation from NO₃⁻ to NO₂⁻, however the DGGE banding patterns were similar, indicating that the same bacterial community was involved in sulfur-driven autotrophic denitrification with both electron acceptors. Finally, Khanongnuch et al. (2019) have just reported the anoxic desulfurization of a gas stream containing low H₂S concentrations (100–500 ppmv) at high EBRT values (3.5 min) using a synthetic nitrified wastewater as the recirculating liquid [68]. Using chemical sources of NO₃⁻ increases the operating costs of anoxic biofilters and the authors claim that using a nitrified wastewater as the trickling liquid would be a practical option if the H₂S treating BTF is located near a nitrification bioreactor. The obtained results indicated that H₂S elimination (RE >99%) via autotrophic denitrification was possible using nitrified wastewater and that *Thiobacillus* sp. was the only sulfur-oxidizing nitrate-reducing bacterial genus detected by DGGE. When the nitrified wastewater was amended with an organic compound to simulate the presence of residual organics, the H₂S RE drastically decreased to values between 60 and 80%. NO₃⁻ consumption increased due the growth of heterotrophic/mixotrophic denitrifying bacteria that probably outcompeted the autotrophic denitrifying SOBs leading to an increased accumulation of biomass. The detected heterotrophic denitrifiers were *Brevundimonas* and *Rhodocyclales*.

The main outcomes of microbial ecology studies in aerobic and anaerobic biofilters and BTFs for the abatement of H₂S in gas streams extensively reviewed in Sections 4.2 and 4.3, respectively, are summarized below in Table 5.
Table 5. Main outcomes of molecular microbial ecology studies on conventional biofilters and BTFs used for the removal of H$_2$S in odor abatement and biogas desulfurization applications. A.t: Acidithiobacillus thiooxidans; DGGE: denaturing gradient gel electrophoresis; DMS: dimethyl sulfide; FISH: fluorescence in situ hybridization; RSC: reduced sulfur compound; SSCP: single-strand conformation polymorphism; T-RFLP: terminal restriction fragment length polymorphism; UASB: upflow anaerobic sludge blanket; WWTP: wastewater treatment plant.

<table>
<thead>
<tr>
<th>Application</th>
<th>Scale</th>
<th>Process Type</th>
<th>Inlet Gas</th>
<th>H$_2$S Load (ppmv)</th>
<th>Packing Material</th>
<th>Inoculum</th>
<th>Molecular Technique</th>
<th>Main Outcome from Microbial Ecology Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odor abatement</td>
<td>Lab</td>
<td>Aerobic BTF</td>
<td>Air supplemented with pure H$_2$S and DMS</td>
<td>1220–4037</td>
<td>Polyethylene carrier rings</td>
<td>A.t</td>
<td>DGGE</td>
<td>Limited bacterial diversity Predominance of A.t</td>
</tr>
<tr>
<td>Odor abatement</td>
<td>Lab</td>
<td>Aerobic BTF</td>
<td>Air supplemented with H$_2$S and other organic RSC</td>
<td>23–1320</td>
<td>Polyurethane foam</td>
<td>A.t</td>
<td>DGGE</td>
<td>No change in bacterial diversity during operation Predominance of A.t</td>
</tr>
<tr>
<td>Biogas desulfurization</td>
<td>Lab</td>
<td>Aerobic BTF</td>
<td>Biogas mimic (mixture of H$_2$S, N$_2$ and air)</td>
<td>2000</td>
<td>High density polypropylene grids</td>
<td>Culture from full-scale biogas desulfurization column (pH 1.6) adapted to pH 6</td>
<td>-</td>
<td>T-RFLP</td>
</tr>
<tr>
<td>Biogas desulfurization</td>
<td>Lab</td>
<td>Aerobic BTF with pH control at 6.5–7 in the recirculating liquid</td>
<td>Biogas mimic (mixture of H$_2$S, N$_2$ and air)</td>
<td>2000</td>
<td>High density polypropylene grids</td>
<td>Aerobic sludge from a municipal WWTP at pH</td>
<td>-</td>
<td>16S rRNA gene amplicons pyrosequencing 16S gene clone library</td>
</tr>
<tr>
<td>Odor abatement Pilot</td>
<td>Pilot</td>
<td>Aerobic BTF</td>
<td>Waste gases from municipal WWTP</td>
<td>2.037</td>
<td>Ceramic volcanic rocks</td>
<td>Activated sludge from municipal WWTP</td>
<td>-</td>
<td>16S rRNA gene amplicons pyrosequencing 16S gene clone library</td>
</tr>
<tr>
<td>Odor abatement Large</td>
<td>Large</td>
<td>Conventional biofilter</td>
<td>Used air from stabilizer or primary decanter of WWTP</td>
<td>&gt;500</td>
<td>Pozzolan added with calcareous material Marble</td>
<td>Not described</td>
<td>-</td>
<td>Vertical stratification of microbial populations related to pH gradient from top to bottom of the biofilter bed Diversity of heterotrophic bacteria and presence of fungi instead of typical SOBs</td>
</tr>
<tr>
<td>Odor abatement Pilot</td>
<td>Pilot</td>
<td>Conventional biofilter</td>
<td>Odorous gas from a WWTP</td>
<td>≥163–815</td>
<td>Peat “Self-inoculated”</td>
<td>SSCP</td>
<td>-</td>
<td>A.t dominates at low pH</td>
</tr>
<tr>
<td>Biogas desulfurization</td>
<td>Lab</td>
<td>Aerobic BTF</td>
<td>Reference synthetic gas</td>
<td>2000</td>
<td>Steel pall rings</td>
<td>Aerobic sludge from a local municipal WWTP</td>
<td>16S rRNA gene amplicons pyrosequencing</td>
<td>-</td>
</tr>
</tbody>
</table>

**Notes:**
- DGGE: Denaturing gradient gel electrophoresis
- DMS: Dimethyl sulfide
- FISH: Fluorescence in situ hybridization
- RSC: Reduced sulfur compound
- SSCP: Single-strand conformation polymorphism
- T-RFLP: Terminal restriction fragment length polymorphism
- UASB: Upflow anaerobic sludge blanket
- WWTP: Wastewater treatment plant
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</tr>
</thead>
<tbody>
<tr>
<td>Odor abatement</td>
<td>Bench</td>
<td>Aerobic BTFs</td>
<td>Synthetic polluted gases generated by mixing H₂S vapors with fresh air</td>
<td>121–4200</td>
<td>Volcanic rock</td>
<td>Microbial consortium from biofilter treating landfill leachate waste gases + activated sludge from WWTP</td>
<td>MiSeq sequencing of 16S rRNA gene amplicons</td>
<td>Vertical stratification of bacterial populations related to pH gradient in the BTFs Bacterial community structure shaped by H₂S loading and pH of the recirculating nutrient solution</td>
</tr>
<tr>
<td>Biogas desulfurization</td>
<td>Lab</td>
<td>Anoxic BTF at neutral pH NO₃⁻ as electron acceptor</td>
<td>Biogas from UASB reactor</td>
<td>1400–14,000</td>
<td>Polypropylene Pall rings</td>
<td>Biomass from open-pore polyurethane foam of a previous BTF</td>
<td>DGGE</td>
<td>Specialized bacterial community</td>
</tr>
<tr>
<td>Biogas desulfurization</td>
<td>Pilot</td>
<td>Anoxic BTF at neutral pH NO₃⁻ as electron acceptor</td>
<td>Biogas split from anaerobic digester from WWTP</td>
<td>4490</td>
<td>Open-pore polyurethane foam</td>
<td>Wastewater from degritter-degreasing unit of WWTP</td>
<td>16S rRNA gene amplicons pyrosequencing</td>
<td></td>
</tr>
<tr>
<td>Biogas desulfurization</td>
<td>Lab</td>
<td>Anoxic BTFs at neutral pH NO₃⁻ and NO₂⁻ as electron acceptors</td>
<td>Biogas from UASB reactor</td>
<td>Not reported</td>
<td>Open-pore polyurethane foam Polypropylene Pall rings</td>
<td>Biomass from open-pore polyurethane foam of a previous BTF</td>
<td>DGGE</td>
<td>No influence of the packing material and operation time on bacterial diversity</td>
</tr>
<tr>
<td>Biogas desulfurization</td>
<td>Lab</td>
<td>Anoxic BTFs at neutral pH NO₃⁻ and NO₂⁻ as electron acceptors</td>
<td>Synthetic biogas (N₂ and H₂S)</td>
<td>710–3564</td>
<td>Polypropylene Pall rings</td>
<td>Not described</td>
<td>DGGE</td>
<td>Bacterial diversity reduced during the progressive adaptation from NO₃⁻ to NO₂⁻</td>
</tr>
<tr>
<td>Odor abatement</td>
<td>Lab</td>
<td>Anoxic BTF at neutral pH Autotrophic or mixotrophic conditions Synthetic nitrified wastewater as trickling liquid</td>
<td>Mixture of N₂ gas and H₂S generated using solutions of Na₂S and H₂SO₄</td>
<td>100–500</td>
<td>Polyurethane foam</td>
<td>Biofilm from a Thiobacillus-dominated lab-scale moving bed biofilm reactor</td>
<td>DGGE</td>
<td>Heterotrophic/mixotrophic denitrifying bacteria outcompete autotrophic denitrifying SOBs leading to an increased accumulation of biomass and decrease in the RE under mixotrophic conditions</td>
</tr>
</tbody>
</table>
5. Conclusions and Perspectives

Biofiltration appears to be a suitable biotechnology for the removal of H$_2$S from biogas. Different studies have shown that BTFs, contrary to conventional biofilters, are able to withstand high and variable loads of H$_2$S for extended periods of time, which is a prerequisite for application to the desulfurization of biogas. Large-scale applications of biofiltration for the removal of H$_2$S from gas streams, not necessarily biogas, make use of chemolithotrophic SOBs to oxidize H$_2$S to innocuous products such as SO$_4^{2-}$ and S$^0$ in the presence of O$_2$. S$^0$ is formed as an intermediate. Although SO$_4^{2-}$ is the preferred end product, S$^0$ generally accumulates in biofilters as the result of limited O$_2$ supply in the sulfur-oxidizing biofilm. S$^0$ accumulation causes clogging episodes, a main challenge for the application of BTFs, which can be alleviated by the periodical shutdown of BTF units to allow the biological oxidation of the accumulated S$^0$ to SO$_4^{2-}$ in absence of H$_2$S.

Chemolithotrophic SOBs are part of the natural biogeochemical sulfur cycle where they play a fundamental role in the elimination of the H$_2$S produced by SRB in natural environments. Many of these bacteria are autotrophic, which is advantageous due to their low biomass production. They are very diverse from the morphological, phylogenetic, physiological and metabolic point of view, allowing them to thrive under a variety of environmental conditions. The ability of certain species of SOBs to use NO$_3^-$ as the electron acceptor for the oxidation of H$_2$S is the base of anoxic biofiltration in BTFs. This new and less studied technology has been recognized as a promising option that would avoid the dilution of biogas and explosion risks due to the introduction of O$_2$.

As biofiltration is based on the activity of bacteria, it is important to reach a better understanding of the bacterial communities that populate BTFs under biogas desulfurization conditions. In the last 15 years, some studies describing the bacterial communities in aerobic and anoxic BTFs with the use of molecular biology tools based on the 16S rRNA gene sequence have been published in relation to biogas desulfurization (Table 5). These studies have shown that the environmental conditions imposed by the operational conditions have a direct impact on bacterial communities’ diversity, structure and dynamics. Anoxic bacterial communities have been less studied and require a more intensive sequencing effort to more precisely determine the phylogenetic affiliation of the involved SOBs under different operational conditions. The robustness of the biological oxidation process is shown by the fact that the H$_2$S RE is maintained over extended periods of time in BTFs, even under fluctuating operational conditions as well as a change in the electron acceptor. Although a shift is observed in the bacterial communities’ composition and structure, the performance of the BTF is maintained, showing the versatility, resilience and plasticity of bacterial sulfur-oxidizing communities. Vertical stratification of bacterial populations has been observed in aerobic BTFs, this spatial stratification is related to the H$_2$S/O$_2$ ratio along the packed bed. Extreme acidification due to the production of SO$_4^{2-}$ that leads to the formation of H$_2$SO$_4$, does not inhibit the process as new SOB populations able to grow under extreme acidity progressively replace neutrophilic SOBs.

However, the 16S rRNA gene-based phylogenetic analysis does not identify the functional features of SOBs and there is still insufficient knowledge of the physiology and functional role of the key populations involved under different operational conditions. Recent advances in next generation sequencing technologies and bioinformatics has allowed the analysis of environmental metagenomes without PCR amplification to survey both the taxonomic and functional properties of microbial communities. The availability of complete genome sequences of different SOBs allows probes to be designed for sulfur oxidation genes for quantitative PCR applications. Finally, metatranscriptomic and metaproteomic approaches would allow a more complete picture of the metabolic role and activity of different SOBs to be obtained for better control and optimization of the biofiltration process.

Future research directions for biogas desulfurization should be focused on scaling-up the major outcomes found at the laboratory scale for anoxic BTFs to pilot-scale in order to determine the performance limits of these systems and the behavior of the involved microbial populations, especially for long-term operation using real biogas instead of biogas mimics. Additionally, aerobic and anoxic biotrickling filtration technologies should be submitted to detailed economic and environmental
assessments using life cycle analysis-based approaches and, on this basis, compared to bioscrubber and physical/chemical technologies.

**Author Contributions:** S.L.B. proposed the structure and goal of the manuscript. S.L.B. and G.B. conceived and wrote the “1. Introduction”, “2. The Biological Sulfur Cycle and the Sulfur-Oxidizing Bacteria” and “5. Conclusions and Perspectives” sections. G.B. conceived and wrote the “3. Biofiltration Technologies” section. S.L.B. conceived and wrote the “4. Microbial Ecology Studies in Biofiltration Units for H\textsubscript{2}S Removal” section.

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