



# Sulfate-Reducing ElectroAutotrophs and Their Applications in Bioelectrochemical Systems

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Electroautotrophs are microbes able to perform different biocathodic reactions by using CO<sub>2</sub> as sole carbon source and electrochemical reducing power as a sole energy source. Electroautotrophy has been discovered in several groups of microorganisms, including iron-oxidizing bacteria, iron-reducing bacteria, nitrate-reducing bacteria, acetogens, methanogens and sulfate-reducing bacteria. The high diversity of electroautotrophs results in a wide range of Bioelectrochemical Systems (BES) applications, ranging from bioproduction to bioremediation. In the last decade, particular research attention has been devoted toward the discovery, characterization and application of acetogenic and methanogenic electroautotrophs. Less attention has been given to autotrophic sulfate-reducing microorganisms, which are extremely interesting biocatalysts for multiple BES technologies, with concomitant CO<sub>2</sub> fixation. They can accomplish water sulfate removal, hydrogen production and, in some case, even biochemicals production. This mini-review gives a journey into electroautotrophic ability of sulfate-reducing bacteria and highlights their possible importance for biosustainable applications. More specifically, general metabolic features of autotrophic sulfate reducers are introduced. Recently discovered strains able to perform extracellular electron uptake and possible molecular mechanisms behind this electron transfer capacity are explored. Finally, BES technologies based on sulfate-reducing electroautotrophs are illustrated.

**Keywords:** bioelectrochemical systems, electroautotrophs, sulfate-reducing microorganisms, biocathodes, bioremediation, bioproduction

## INTRODUCTION

During the last decade, much research focused toward the use of electroautotrophic microorganisms in Bioelectrochemical Systems (BES). While exoelectrogens have evolved to use extracellular insoluble minerals or electrodes as terminal electron acceptors, electroautotrophs are able to acquire energy by taking up electrons from extracellular solid compounds or electrodes, while using carbon dioxide (CO<sub>2</sub>) as inorganic carbon source (Tremblay et al., 2017).

Electroautotrophy was first discovered in the model exoelectrogen genus *Geobacter* (Gregory et al., 2004; Gregory and Lovley, 2005). As the majority of exoelectrogenic biocatalysts are dissimilatory iron-reducing bacteria, researchers hypothesized that iron-oxidizing bacteria could be able to accept electrons from a cathodic electrode. Indeed, *Acidithiobacillus ferrooxidans*, *Mariprofundus ferrooxydans* PV-1, and *Rhodospseudomonas palustris* have been designated

as electroautotrophs (Carbajosa et al., 2010; Summers et al., 2013; Bose et al., 2014). Moreover, the chemolithoautotrophic archaea *Methanococcus maripaludis* and *Methanobacterium*-like archaeon strain IM1, isolated with metallic iron as sole electron donor, are able to perform electromethanogenesis (Lohner et al., 2014; Beese-Vasbender et al., 2015a). Several acetogenic bacteria, including *Sporomusa ovata*, *Sporomusa silvacetica*, *Sporomusa sphaeroides*, *Sporomusa acidovorans*, *Sporomusa malonica*, *Clostridium ljungdahlii*, *Clostridium aceticum*, and *Moorella thermoacetica*, can utilize the cathodic current for CO<sub>2</sub> reduction to organic acids (Nevin et al., 2011; Aryal et al., 2017). Also, some autotrophic sulfate-reducing microorganisms (SRM) have shown the ability to consume electrons from the cathode to accomplish sulfate reduction and hydrogen (H<sub>2</sub>) production (Rodrigues and Rosenbaum, 2014; Beese-Vasbender et al., 2015b). However, overall fairly little research has been devoted toward this last group of electroautotrophic biocatalysts.

Pioneering discoveries regarding cathodic electron consumption of SRM are related to anaerobic microbial induced corrosion (MIC) studies (Widdel, 1992). SRM can stimulate not only a chemically-influenced corrosion of iron through the production of corrosive hydrogen sulfide (Widdel, 1992), but also an electrochemical-induced corrosion by the consumption of “cathodic hydrogen” formed on iron in contact with water (von Wolzogen Kühr and van der Vlugt, 1934; Pankhania, 1988), or by directly uptaking electrons from iron (Dinh et al., 2004; Gu et al., 2009; Gu and Xu, 2010; Xu and Gu, 2011). The readers are referred to several excellent reviews on to role of SRM in MIC (Enning and Garrelfs, 2014; Anandkumar et al., 2016; Li et al., in press).

It should be noticed that early BES studies with SRM have been focused on their anodic exploitation for electricity generation and sulfate removal using organic substrates (Habermann and Pommer, 1991; Liang et al., 2013; Zheng et al., 2014). However, it was soon clarified that the production of electricity with SRM-based anodes was mainly due to the abiotic oxidation of biologically produced sulfide to elemental sulfur (Zhao et al., 2008). Consequently, sulfur-oxidizing bacteria have started to be applied in anodic oxidation processes for current generation (Sun et al., 2009; Gong et al., 2013; Lee et al., 2014; Zhang et al., 2014).

Another very recent and promising BES application of SRM is the cathodic electrofermentation of short chain organic acids into more valuable compounds as alcohols and acetone (Sharma et al., 2013a,b, 2014, 2015).

**Figure 1A** summarizes the application of the different metabolic capabilities of SRM in various BES. Despite these different attractive SRM-based BES technologies, the specific focus of this mini-review is to summarize the current understanding and trends in biocathodic applications of electroautotrophic SRM, using CO<sub>2</sub> as inorganic carbon source (highlighted with a red box in **Figure 1A**).

## SULFATE REDUCING MICROORGANISMS

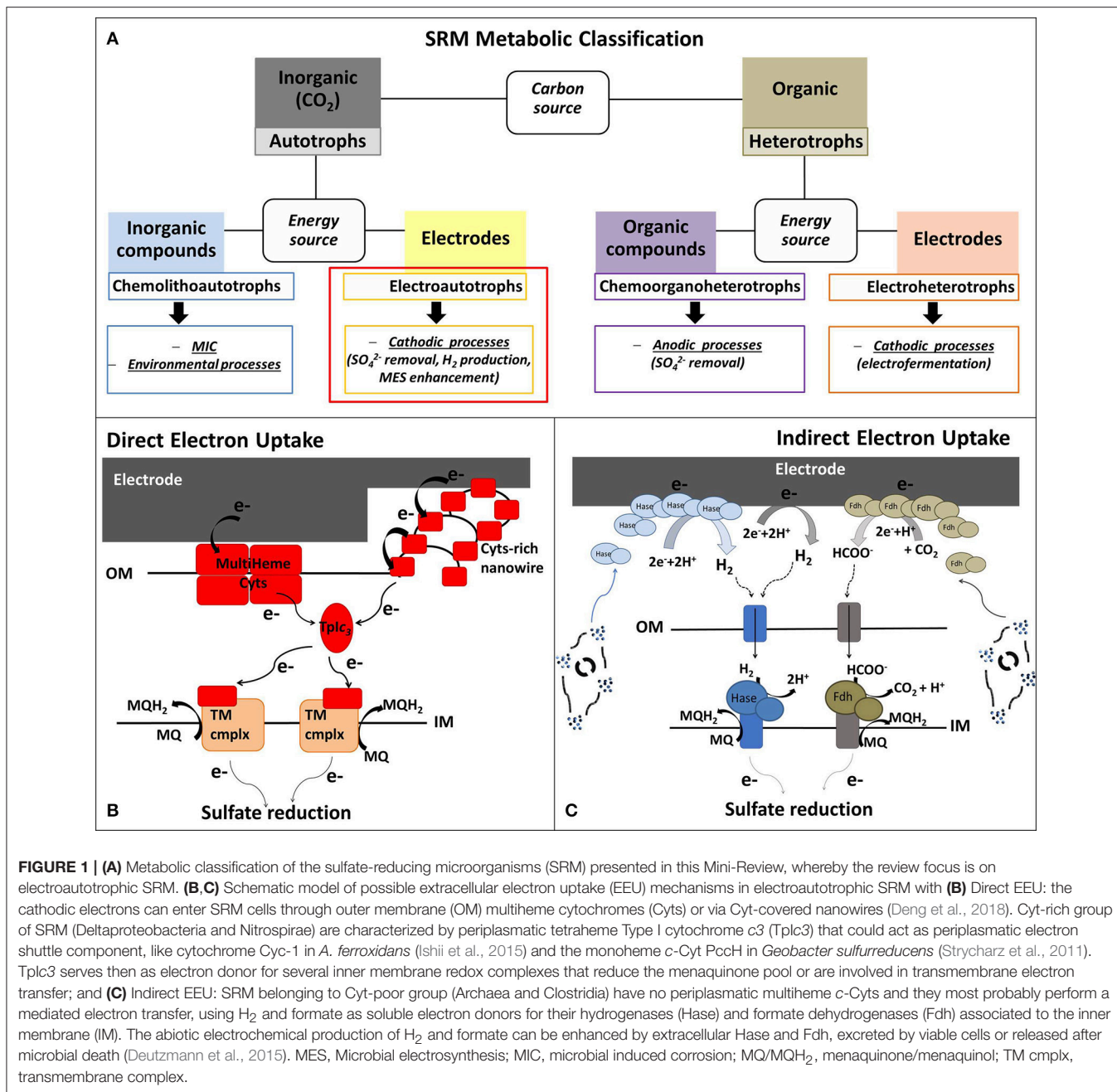
SRM are a heterogeneous group of anaerobic microorganisms, widely distributed in anoxic environments with essential roles in

the global cycling of carbon and sulfur (Jørgensen, 1982). Most cultured SRM belong to four bacterial (Deltaproteobacteria, Nitrospirae, Firmicutes, Thermodesulfobacteria) and two archaeal phyla (Euryarchaeota, Crenarchaeota) (Rabus et al., 2006; Muyzer and Stams, 2008). SRM have the ability to use sulfate, the most oxidized sulfur specie, as terminal electron acceptor for the oxidation of organic compounds or hydrogen in a process named dissimilatory sulfate reduction (Widdel and Hansen, 1991). This is an intracellular pathway that requires an eight-electron reaction for the reduction of sulfate to sulfide, with sulfite as intermediate. After crossing microbial membranes, sulfate is “activated” to form adenosine 5'-phosphosulfate (APS) by the enzyme Adenosine Triphosphate (ATP) sulfurylase (Peck, 1959). APS is then reduced to sulfite in a two-electron reaction performed by the enzyme APS reductase (AprBA) (Lampreia et al., 1994). The final step of sulfite reduction to sulfide is catalyzed by the dissimilatory sulfite reductase complex (Dsr), with the involvement of an energy-conserving membrane complex (DsrMKJOP or DsrMK) (Fike et al., 2016).

## Carbon Assimilation and Electron Donors

SRM can grow on more than one hundred organic compounds, including monocarboxylic acids, dicarboxylic acids, sugars, alcohols, ketones, amino acids, aromatic compounds, and hydrocarbons (Barton and Fauque, 2009).

SRM can either perform incomplete or complete oxidation of organic compounds (Rabus et al., 2006). Incomplete oxidation of organic substrates results in the excretion of acetate as main product, due to a deficiency for the terminal oxidation of acetyl-CoA (Widdel, 1988). On the contrary, complete oxidizers degrade organic compounds to CO<sub>2</sub>, oxidizing acetate with two different pathways: a modified citric acid cycle (e.g., *Desulfobacter* spp., Brandis-Heep et al., 1983), or the acetyl-CoA pathway (e.g., *Desulfobacterium* spp., Schauder et al., 1986). Both of these groups can also utilize H<sub>2</sub> as electron donor during sulfate reduction. Despite the prospects for chemolithoautotrophic growth on H<sub>2</sub>, most SRM require acetate in addition to CO<sub>2</sub> for growth (Badziong et al., 1979). Nevertheless, true autotrophic growth with H<sub>2</sub> as electron donor was discovered in some SRM (Pfennig et al., 1981; Jansen et al., 1985; Klemps et al., 1985; Brysch et al., 1987; Rozanova et al., 1988; Schauder et al., 1989). Of these facultative chemolithoautotrophic SRM all but *Desulfosporosinus orientis* are complete oxidizers (Brysch et al., 1987). Thereby, CO<sub>2</sub>-fixation proceeds through reverse reactions of the pathways used by SRM for acetyl-CoA oxidation during heterotrophic growth. *D. hydrogenophilus* assimilates CO<sub>2</sub> via a reductive citric acid cycle (Schauder et al., 1987), while *D. autotrophicum* and *D. orientis* use the reductive acetyl-CoA pathway (Wood Ljungdahl-pathway) (Schauder et al., 1989), the only autotrophic microbial route able to simultaneously fix CO<sub>2</sub> and yield ATP by converting acetyl-CoA to acetate (Wood et al., 1986; Fuchs, 2011). This pathway consists of two separate branches: one molecule of CO<sub>2</sub> is reduced to carbon monoxide (CO) in the carbonyl branch and another CO<sub>2</sub> molecule is reduced to a methyl group in the methyl branch. The acetyl-CoA is generated from the combination of the CO and methyl group with the coenzyme A (Wood et al., 1986). Recent reviews



**FIGURE 1 | (A)** Metabolic classification of the sulfate-reducing microorganisms (SRM) presented in this Mini-Review, whereby the review focus is on electroautotrophic SRM. **(B,C)** Schematic model of possible extracellular electron uptake (EEU) mechanisms in electroautotrophic SRM with **(B)** Direct EEU: the cathodic electrons can enter SRM cells through outer membrane (OM) multiheme cytochromes (Cyts) or via Cyt-covered nanowires (Deng et al., 2018). Cyt-rich group of SRM (Deltaproteobacteria and Nitrospirae) are characterized by periplasmic tetraheme Type I cytochrome c3 (Tplc3) that could act as periplasmic electron shuttle component, like cytochrome Cyc-1 in *A. ferroxidans* (Ishii et al., 2015) and the monoheme c-Cyt Pcch in *Geobacter sulfurreducens* (Strycharcz et al., 2011). Tplc3 serves then as electron donor for several inner membrane redox complexes that reduce the menaquinone pool or are involved in transmembrane electron transfer; and **(C)** Indirect EEU: SRM belonging to Cyt-poor group (Archaea and Clostridia) have no periplasmic multiheme c-Cyts and they most probably perform a mediated electron transfer, using H<sub>2</sub> and formate as soluble electron donors for their hydrogenases (Hase) and formate dehydrogenases (Fdh) associated to the inner membrane (IM). The abiotic electrochemical production of H<sub>2</sub> and formate can be enhanced by extracellular Hase and Fdh, excreted by viable cells or released after microbial death (Deutzmann et al., 2015). MES, Microbial electrosynthesis; MIC, microbial induced corrosion; MQ/MQH<sub>2</sub>, menaquinone/menaquinol; TM cmplx, transmembrane complex.

give an exhaustive biochemical description of these CO<sub>2</sub>-fixation pathways (Berg, 2011; Fuchs, 2011; Schuchmann and Müller, 2014).

### Energy-Conservation and Electron Transport Pathways

In chemolithoautotrophic SRM, sulfate reduction must be coupled to energy conservation by oxidative phosphorylation. This implies an electron transport chain that allows the production of a transmembrane proton motive force for the

chemiosmotic synthesis of ATP (Thauer et al., 2007; Grein et al., 2013). While APS and sulfite reduction are two strongly exoergonic reactions, the enzymes responsible, AprBA and DsrAB/DsrC, are cytoplasmic soluble reductases and, thus, cannot be directly involved in the formation of a transmembrane proton gradient. Instead, the quinone-interacting membrane-bound oxidoreductase complex (QmoABC) (Pires et al., 2003) and the DsrMKJOP complex (Pires et al., 2006) represent the membrane complex candidates that can act as electron donor for AprBA and DsrAB/DsrC, respectively (Ramos et al., 2012; Grein et al., 2013). Both complexes are strictly conserved across

SRM (Pereira et al., 2011). Other energy-conserving membrane complexes, capable of ion translocation, are present in SRM, but they are less conserved (Pereira et al., 2011).

Due to the high variability of organic and inorganic electron donors used by SRM, there is no unifying theory for their electron transport chain. However, one classification method is based on the content of periplasmic *c*-type cytochromes (*c*-Cyt) (Rabus et al., 2015). The cytochrome-rich group has numerous multiheme *c*-Cyt and includes SRM belonging to Deltaproteobacteria (e.g., *Desulfovibrio* spp., *Desulfobulbus* spp., *Desulfomicrobium* spp.) and Nitrospira (e.g., *Thermodesulfovibrio* spp.), while the cytochrome-poor group has few or no *c*-Cyt and comprises Archaea (e.g., *Archaeoglobus* spp.) and Clostridia SRM (e.g., *Desulfosporosinus* spp., *Desulfotomaculum* spp.) (Pereira et al., 2011; Rabus et al., 2015). Cytochrome-rich SRM have soluble periplasmatic hydrogenases and formate dehydrogenases that lack an integral membrane subunit. These soluble enzymes use a periplasmatic multiheme *c*-Cyt, usually the tetraheme cytochrome *c*<sub>3</sub> (TpIc<sub>3</sub>), as electron acceptor (Louro, 2007; da Silva et al., 2012; Romão et al., 2012) (**Figure 1B**). These SRM also contain a set of inner membrane redox complexes that reduce the menaquinone pool (Qrc, Nhc and Ohc) or are involved in transmembrane electron transfer (Tmc and Hmc) (Rabus et al., 2015). On the contrary, cytochrome-poor SRM have membrane-bound hydrogenases and formate dehydrogenases associated to the inner membrane through a *b*-type cytochrome that directly reduce the menaquinone pool (Pereira et al., 2011) (**Figure 1C**).

## Electroautotrophic SRM

The discovery of direct electron uptake capacity of some Fe(0)-corroding SRM (Dinh et al., 2004; Gu et al., 2009; Gu and Xu, 2010; Xu and Gu, 2011) suggested the researchers to start employing these microorganisms for biocathodic BES applications, turning, thus, this negative metabolic feature into positive and sustainable biotechnological solutions. However, so far only few pure culture SRM are elucidated as electroautotrophs.

In 2008, *Desulfovibrio desulfuricans* ATCC 27774 was shown to form an electroactive cathodic biofilm at an applied cathodic potential ( $E_{\text{cath}}$ ) of  $-0.169$  V vs. SHE. A stable negative current was obtained after 20 days, but lactate was supplied as carbon source, not CO<sub>2</sub> (Cordas et al., 2008). Subsequently, other species of the genus *Desulfovibrio* were tested for cathodic current generation and H<sub>2</sub> production, using bicarbonate or lactate as carbon source and  $E_{\text{cath}}$  that allow abiotic H<sub>2</sub> evolution: *D. paquesii* and *D. caledoniensis* (Yu et al., 2011; Aulenta et al., 2012).

As first pure culture SRM to really show electroautotrophy, we identified *Desulfosporosinus orientis* and *Desulfovibrio piger* at  $E_{\text{cath}} = -0.31$  V vs. SHE, which is much more positive than the H<sub>2</sub> evolution redox potential at neutral conditions ( $E_{\text{H}^+/\text{H}_2}^0 = -0.41$  V vs. SHE), with gaseous CO<sub>2</sub> as sole inorganic growth substrate (Rodrigues and Rosenbaum, 2014). *D. orientis* is a spore-forming SRM in the class Clostridia and is able to perform anaerobic sulfate respiration but also acetogenesis. It can utilize a wide range of energy sources, such as H<sub>2</sub>/CO<sub>2</sub>, CO, formate, lactate, pyruvate, methanol, ethanol, and medium

chain fatty acids (Klempes et al., 1985; Robertson et al., 2001), and different terminal electron acceptors, such as sulfate, thiosulfate, sulfite, sulfur dioxide (Cypionka and Pfennig, 1986).

*D. piger* is a non-spore-forming, H<sub>2</sub>-oxidizing Gram-negative SRM Deltaproteobacterium. It can oxidize organic compounds, such as ethanol, lactate, and pyruvate, incompletely to acetate. Like for the other *Desulfovibrio* species, autotrophic growth on CO<sub>2</sub> was not reported before.

*Desulfofilia corrodens* strain IS4 is the first Fe(0)-corroding SRM characterized in BES (Beese-Vasbender et al., 2015b). This Gram-negative Deltaproteobacterium was isolated from marine sediment using metallic iron as the sole electron donor. Using iron as energy source, this strain is able to perform very rapid sulfate reduction and hydrogen production compared to the conventional hydrogen-scavenging *Desulfovibrio* species (Dinh et al., 2004). In BES, direct electron uptake was achieved at  $E_{\text{cath}} = -0.4$  V vs. SHE with CO<sub>2</sub> in the headspace as carbon substrate (Beese-Vasbender et al., 2015b).

Very recently, electroautotrophic activity was reported also in the fully sequenced sulfate-reducing bacterium *Desulfobacterium autotrophicum* HRM2, using a  $E_{\text{cath}} = -0.5$  V vs. SHE (Zaybak et al., 2018). This Deltaproteobacterium, isolated from marine mud, is a complete oxidizer SRM belonging to the *c*-Cyt rich group and harboring a bidirectional Wood-Ljungdahl pathway (Brysch et al., 1987; Strittmatter et al., 2009). *D. autotrophicum* HRM2 exhibited acetate bioelectrosynthesis ability, with an extremely high coulombic efficiency of  $83 \pm 6\%$  (Zaybak et al., 2018).

## EXTRACELLULAR ELECTRON UPTAKE IN SRM

Little is known about the molecular mechanisms beyond extracellular electron uptake (EEU) in SRM and in electroautotrophic microorganisms in general. As for the extracellular electron transfer (EET) from microbes to the anode, direct and indirect pathways can be employed. So far, direct EEU mechanisms have been elucidated in the oxygen-reducers *Shewanella oneidensis*, which directly uses electrons from the cathode via the reversed anodic Mtr pathway (Ross et al., 2011), and *Acidithiobacillus ferrooxidans*, which utilizes a cascade of outer membrane (OM) cytochrome reductases (Ishii et al., 2015). Indirect electron transfer pathway has been demonstrated for the anaerobic methanogen *Methanococcus maripaludis* (Choi and Sang, 2016). Here, the electron uptake seems dependent on extracellular formate dehydrogenases and hydrogenases that catalyze the cathodic production of hydrogen and formate, which act as soluble electron donors for microbial activity (Deutzmann et al., 2015).

## Indirect Mechanism

Generally, in BES for bioremediation and bioproduction a power input is given to reduce cathodic potential and drive thermodynamically unfavorable bioelectrochemical reductions. If the  $E_{\text{cath}}$  is more negative than the standard redox potential of the H<sup>+</sup>/H<sub>2</sub> couple, molecular H<sub>2</sub> is generated at the

cathode. The majority of acetogens, methanogens as well as SRM are able to use  $H_2$  as electron donor. Consequently, abiotic  $H_2$  can easily mediate the EET from cathode to hydrogenotrophic microorganisms. In addition, similar to the case of *M. maripaludis*, soluble enzymes, released by SRM after cell lysis, could increase the abiotic production rate of  $H_2$  and even formate by decreasing the overpotentials of these abiotic electroreduction processes (Figure 1C). Indeed, SRM are characterized by a high amount of soluble periplasmatic and cytoplasmatic hydrogenases and formate dehydrogenases (Rabus et al., 2006).

## Direct Mechanism

As discussed above, *D. orientis*, *D. piger*, and *D. corrodens* strain IS4 have shown ability to grow in cathodic environments with applied potentials too positive for the abiotic  $H_2$  evolution, suggesting a direct EEU requiring a physical interaction between the electrode and the microbial cells (Figure 1B).

*D. piger* and *D. corrodens* strain IS4 belong to the cytochrome-rich group of SRM, both having the periplasmatic TpIc3, but not *D. orientis* (Rabus et al., 2006). Nevertheless, the OM proteins that permit the entrance of extracellular electrons inside SRM cells and the subsequent reduction of periplasmatic redox components have yet to be elucidated. Electrochemical and infrared spectroelectrochemical analyses identified *c*-Cyt as redox active components associated with the OM of *D. corrodens* strain IS4 and are, thus, possibly involved in direct EEU (Beese-Vasbender et al., 2015b). These results are supported by the very recent study of the electron uptake mechanism of another iron-corroding SRM, *Desulfovibrio ferrophilus* IS5 (Deng et al., 2018). This strain was isolated from marine sediment with *D. corrodens* IS4 (Dinh et al., 2004). Biochemical, transcriptomic, and microscopic analyses of *D. ferrophilus* IS5 pointed out a high expression of different OM multiheme cytochromes in response to organic electron donor limitation. Moreover, transmission electron microscopy revealed segmented nanowire structures, strongly positive for cytochrome staining and very similar to the ones of *S. oneidensis*, suggesting that also *D. ferrophilus* IS5 can use nanowires for EEU (Deng et al., 2018).

## BES TECHNOLOGIES BASED ON ELECTROAUTOTROPHIC SRM

With growing interest in Microbial Electrolysis Cell (MEC) (Logan and Rabaey, 2012), SRM have started to be applied in biocathodic systems. This section outlines the recent applications of electroautotrophic SRM-based biocathodes, using  $CO_2$  as inorganic carbon source.

### Sulfate-Rich Waters Treatment

Sulfate-rich wastewaters require treatment before being discharged to the environment, as this anion may create acute laxative effects in humans and it may increase dissolution of metals in water resources (Gomez et al., 1995). Biological sulfate removal technologies are based on the exploitation of SRM. Sulfate-rich wastewaters are usually deficient in organic matter and, thus, external electron donors are required to

achieve complete sulfate reduction in bioreactors (Liamleam and Annachatre, 2007). While methanogens are generally more competitive to use organic electron donors, autotrophic SRM are generally more efficient in  $H_2$  utilization. However, the application of hydrogen reports several disadvantages, like cost and safety aspects of  $H_2$  storage. BES can overcome some of these limitations since the production/consumption of  $H_2$  occur in the same reactor, without  $H_2$  waste, and with operations at atmospheric temperature and pressure. Electricity-driven autotrophic sulfate-reduction has been reported by several authors (Su et al., 2012; Coma et al., 2013; Luo et al., 2014; Pozo et al., 2015, 2017a; Blázquez et al., 2017) and an overview is presented in Table 1. The first sulfate-reducing biocathode used a mixed culture originated from wastewater and at  $E_{cath} = -0.2$  V vs. SHE a maximum sulfate reduction rate of  $0.02$  g  $L^{-1}$  day $^{-1}$  was achieved (Su et al., 2012). Thereafter, many studies, mostly with *Desulfovibrio*, have investigated different  $E_{cath}$ , BES operation conditions cathodic electrode materials, inoculum source and start-up strategies (Table 1). To date, the highest bioelectrochemical sulfate reduction rate of  $5.6$  g  $L^{-1}$  day $^{-1}$  was obtained with a mixed microbial community collected from previous sulfate-reducing BES reactors at an  $E_{cath}$  of  $-1.1$  V vs. SHE (Pozo et al., 2017a).

Sulfide is the principal product of biological sulfate respiration and it may lead to significant issues such as corrosion, bad odors and human health toxicity. For real application of BES in sulfate-rich water treatment, a second step of sulfide transformation to elemental sulfur should be integrated. Two recent studies have combined the bioelectroreduction of sulfate to sulfide with the recovery of elemental sulfur ( $S^0$ ) through the use of sulfur-oxidizing bacteria in the anodic chamber of a separate BES, resulting in a  $S^0$  recovery of 74% (Pozo et al., 2017b), or in the same biocathodic system by using part of the anodic-produced oxygen that partially diffuse to the cathode through the ion exchange membrane (Blázquez et al., 2016).

### Hydrogen Production

$H_2$  was the first value product generated through BES technology (Liu et al., 2005; Rozendal et al., 2006). Compared to conventional methods (dark fermentation, biophotolysis, water electrolysis and water photolysis),  $H_2$  production with MECs shows multiple advantages. Firstly, MECs can theoretically produce hydrogen with an energy input much lower than for industrial electrolyzers,  $1$  kWh  $m^{-3}H_2$  (Rozendal et al., 2008) vs.  $4.5$ – $5$  kWh  $m^{-3}H_2$  (Wang et al., 2014). Secondly, no precious metals catalysts are needed since both anodic and cathodic reactions can be catalyzed by electroactive microorganisms (Jafary et al., in press). Thirdly, cathodic biocatalysts can use the  $CO_2$  originating from organic matter as inorganic carbon source for cathodic  $H_2$  production. MEC biocathodes are usually inoculated with the effluent of running BES or by directly transferring used bioanodes or biocathodes to new cathodic chambers (Hasany et al., 2016). Microbial community analysis of several  $H_2$ -producing biocathodes revealed SRM as amongst the dominant bacteria (Table 1). SRM, indeed, have an extremely high hydrogenase activity and in sulfate limitation conditions can produce  $H_2$

**TABLE 1** | Overview of mixed-community electroautotrophic SRM-based biocathodes for sulfate removal and H<sub>2</sub> production.

SRM-Biocathodes for sulfate-rich water treatment						
Inoculum	Dominant species	Operation mode	E <sub>cath</sub> (V vs. SHE)	SO <sub>4</sub> <sup>2-</sup> reduction rate (g L <sup>-1</sup> day <sup>-1</sup> )	CE sulfate%	References
Enriched WWT sludge	<i>Desulfohalobus propionicus</i> , <i>Geobacter</i> spp.	Fed-batch	-0.2	0.015	72	Su et al., 2012
MFC effluent	-	Continuous	-0.26	0.06	-	Coma et al., 2013
Enriched WWT sludge	-	Continuous	-0.6	0.19	47	Luo et al., 2014
Non-acclimated consortia + autotrophic acetate-producing biocathode	<i>Methanobacterium</i> <i>Desulfovibrio</i> <i>Desulfomicrobium</i>	Fed-batch Continuous	-0.9 -1.1	0.19 5.6	5 78	Pozo et al., 2015 Pozo et al., 2017a
Acclimated sediment	<i>Desulfovibrio</i>	Fed-batch	-0.7	0.03	16	Teng et al., 2016
Lab-scale sewer	<i>Desulfovibrio</i>	Fed-batch	-1	0.7	85	Blázquez et al., 2017
Sediment	<i>Desulfovibrio</i>	Fed-batch	-0.7	0.06	25	Luo et al., 2017
Enriched river sediment	<i>Desulfovibrio</i> <i>Acetobacterium</i>	Fed-batch	-0.85	0.15	56	Hu et al., 2018
SRM-Rich Biocathodes for H <sub>2</sub> production						
Inoculum	Dominant species	Operation mode	E <sub>cath</sub> (V vs. SHE)	H <sub>2</sub> production rate (m <sup>3</sup> m <sup>-3</sup> day <sup>-1</sup> )	CE H <sub>2</sub> %	References
Marine sediment MFC	<i>Eubacterium limosum</i> , <i>Desulfovibrio</i> sp.A2, <i>Rhodococcus</i>	Batch	-0.54	0.08 mmoles	-	Pisciotta et al., 2012
Effluent of 4 year old MFC and MEC	<i>Desulfovibrio vulgaris</i>	Continuous	-0.7	0.63	-	Croese et al., 2011
MEC effluent	<i>Hydrogenophaga</i> <i>Desulfovibrio</i>	Continuous	-0.7	2.7	-	Croese et al., 2014
Palm oil mill effluent enriched in SRM	-	Batch	R <sub>ext</sub> 1Ω	1.85	-	Jafary et al., 2017

Except for the work of Jafary et al. (2017), all the BES studies reported in the table operated in MEC mode. CE, coulombic efficiency; R<sub>ext</sub>, external resistance.

fermentatively (Rabus et al., 2006). Very recently, Jafary and co-workers have purposely enriched a palm oil mill effluent sample for autotrophic SRM and then used this as inoculum source for biocathodic H<sub>2</sub> production. The SRM enriched-biocathode was able to generate 1.85 m<sup>3</sup> H<sub>2</sub>/ (m<sup>3</sup>·d) in acidic catholyte conditions (pH = 4) (Jafary et al., 2017).

It should be highlighted that the sulfate concentration in the catholyte of SRM-based MEC has to be limited not only to encourage SRM fermentative metabolism, but also to avoid the generation of a harmful off-gas mixture of H<sub>2</sub>S and H<sub>2</sub>. Researchers should start to focus their attention on the purification of the produced H<sub>2</sub>, especially in the case of mixed-community biocathodes.

## Microbial Electrosynthesis Enhancement

BES research on biocathodic production of alternative fuels and higher value chemicals from CO<sub>2</sub> has caught much attention in the last years. Methane and acetate are, usually, the main products of this microbial electrosynthesis (MES), particularly

in pure culture-based systems (Tremblay and Zhang, 2015). For practical implementation, the generation of molecules with higher value than acetate, such as longer carbon-chain organic compounds and alcohols, is desirable. Approaches with mixed-culture MES that exploit intermediate metabolite transfer and microbial cooperation show some success to extend the product spectrum to *n*-butyrate, propionate, ethanol, isopropanol and caproate (Ganigué et al., 2015; Arends et al., 2017; Batlle-Vilanova et al., 2017; Jourdin et al., 2018). In addition, the low rate EEU of electroautotrophic acetogens and methanogens still constitute a big limit for application on a commercial scale. Especially in undefined mixed community biocathodes, high negative potentials are applied to allow H<sub>2</sub>-mediated bioproduction processes, resulting in high energetic efficiency losses.

Very recently, researchers have evaluated the enhancement of MES rates through syntrophic growth of SRM with acetogens or methanogens (Deutzmann and Spormann, 2017; Song et al., 2017; Xiang et al., 2017). By adding low concentration of sulfate in the catholyte of a MES, Xiang et al. enriched the

biocathodic community in *Desulfovibrionaceae* (37%), resulting in a 2.7-fold increase in acetate production in comparison to a MES with lower abundance of SRM (*Desulfovibrionaceae* 7.3%). As proof of concept, the Spormann group evaluated two defined co-culture biocathodes: *D. corrodens* strain IS4 as high rate electron uptaking and H<sub>2</sub> producing strain was combined with *Acetobacterium woodii*, as acetogenic biocatalyst, or with *M. maripaludis*, as methane producer (Deutzmann and Spormann, 2017). *A. woodii* is not able to directly consume electrons from the cathode to produce acetate (Nevin et al., 2011), but in this case the co-culture showed acetate production rate of 0.21–0.23  $\mu\text{mol cm}^{-2} \text{h}^{-1}$  at  $E_{\text{cath}} = -0.4 \text{ V vs. SHE}$ . The *D. corrodens*-*M. maripaludis* co-culture exhibited a methane production 20-times higher (0.6–1.2  $\mu\text{mol h}^{-1} \text{cm}^{-2}$ ) (Deutzmann and Spormann, 2017) compared to a pure culture *M. maripaludis* cathode poised at  $-0.6 \text{ V}$  (0.05  $\mu\text{mol h}^{-1} \text{cm}^{-2}$ ) (Lohner et al., 2014). This study opens the door for the exploitation of defined co-cultures for microbial electrosynthesis of higher value chemicals from CO<sub>2</sub>. Thereby, naturally efficient electroautotrophic strains, can be coupled with acetogenic engineered strains. Importantly, this combination of an efficient cathodic EEU catalyst with an efficient bioproduction catalyst in the bulk liquid allows for a better use of the entire volume of the cathodic reactor. For the real scale-up of the MES process using SRM, researchers should consider also undesired MIC activities of these biocatalysts and, thus, avoid the use of metallic materials in the reactor design.

## CONCLUDING REMARKS AND OUTLOOK

With the aim to mitigate climate change, much research efforts have been initiated toward the development of new biotechnologies able to convert CO<sub>2</sub>-rich waste gases into valuable products. MES represents one of these technologies. Thereby, the exploitation of autotrophic SRM in CO<sub>2</sub>-based cathodic bioprocesses has just begun. With this mini-review, we highlight that SRM-based biocathodes represent a very promising technology for sustainable and environmentally friendly bioremediation and bioproduction applications. Further

understanding and characterization of these electroautotrophic biocatalysts will enable successful realization of SRM-based BES technologies. First of all, more effort should be addressed toward the screening of other Fe(0)-corroding strains, in order to likely discover SRM with superior EEU rate capacity. Moreover, the complete elucidation of the molecular mechanisms beyond the EEU transport chain will allow genetic and metabolic engineering of these biocatalysts for the incrementation of their natural electron uptake rate and to extend the product spectrum. From an engineering point of view, the most critical challenge to achieve the commercialization of BES technologies is the development of a cost-effective and scalable reactor design. The combination of the recovery of multiple value-added products could possibly help to cut down the implementation cost. The versatility of electroautotrophic SRM could positively contribute in accomplishing this goal. For example, the sulfide produced by SMR biocathodes can be exploited for the precipitation of heavy metals, combining, thus, the treatment of sulfate-rich wastewaters and metal-rich industrial effluents with the recovery of precious metals. Thus, with the highlighted new developments and emerging technologies the formerly considered destructive process of MIC might open new ways to biotechnological productions and environmental engineering strategies.

## AUTHOR CONTRIBUTIONS

VA performed the literature search for this review and prepared the first draft of the manuscript. MR discussed the content and structure and revised the review manuscript.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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