Microbiological versus Chemical Reductive Sulfdation: An Experimental and Theoretical Study

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ABSTRACT: Microbiological reductive sulfdation (RS) has rarely been documented, although it represents an efficient strategy for thiol formation. In this work, we reported on the sulfate-respiring bacterium Desulfovibrio sp.86 that has previously demonstrated RS activity toward the pesticide chlordecone. The purpose of this study was to assess its substrate versatility using a set of 28 carbonyls, to compare with chemical RS and to rationalize the observed trends using a dual experimental and theoretical approach. The chemical RS generally proceeds in two steps (S/O exchange using a sulfur donor like P₄S₁₀ reduction of the thione intermediate). Intriguingly, chlordecone was found to be converted into chlordecthiol following the first step. Hence, we designed a protocol and applied it to the 28 substrates to assess their propensity to be directly converted into thiols with the P₄S₁₀ step. Hence, we designed a protocol and applied it to the 28 substrates to assess their propensity to be directly converted into thiols with the P₄S₁₀ step. Finally, we performed density functional theory calculations on these carbonyls and their thiocarbonyl derivatives to build a set of structural, electronic, and thermodynamic parameters. The results showed that chemical and microbiological RS probably involved two distinct mechanisms. Chemically, we observed that several carbonyls, possessing electron-withdrawing groups and/or aromatic rings, were directly transformed into thiols in the presence of P₄S₁₀. The correlation obtained with the electron affinity of the thiones led us to conclude that a probable single-electron reductive transfer occurred during the first step. We also found that Desulfovibrio sp.86 transformed a variety of aldehydes and ketones, without ever detecting thiones. No significant correlation was observed with the calculated parameters, but a relationship between aldehyde RS biotransformation and bacterial growth was observed. Differences in selectivity with chemical RS open the way for further applications in organic synthesis.

1. INTRODUCTION

Among the different strategies for creating a C–S bond,1–9 reductive sulfdation (also referred as reductive thiolation) is one of the most appropriate. It consists in a two-step process: (i) thionation of carbonyls with the use of sulfur donors (e.g., Berzelius reagent (P₄S₁₀), hydrogen sulfide, thiophosphoryl chloride, bis(trimethylsilyl)sulfide, rhodamine, or 2,4-bis(p-methoxyphenyl)-1,3-dithiadiaphosphetane-2,4-disulfdide (Laweson’s reagent));6,10 (ii) reduction of the thione into the target thiol using NaBH₄ or LiAlH₄ for example.11,12 One-step reductive sulfdation has rarely been observed. A few papers have reported this direct transformation using H₂S. However, in these cases, the required conditions that limit the substrate spectrum have precluded the widespread use of this strategy.13–15 More recently, we have discovered that the single application of P₄S₁₀ on chlordecone congeners resulted in a significant amount of chlordecthiols.16

In biology, the formation of C–S bonds can be catalyzed by several types of enzymes.17,18 However, only a few enzyme commission (EC) numbers refer to enzymatic reductive sulfdation processes: (i) conversion of a cysteine into a 3-oxo-alanine residue in the active site of sulfatases (EC 1.8.98.7 and EC 1.8.3.7) and (ii) oxidation of methanethiol into formaldehyde (EC 1.8.3.4). These enzymes, specific to a single metabolic substrate, actually promote the backward direction, i.e., the formation of aldehydes. More generally, some papers referenced the enzymatic transformation of carbonyls into thiones. For example, 4-thiouridine was obtained from uridine via cysteine, ATP, and several proteins.19 In rare cases, the thiones formed were spontaneously isomerized into thiol moieties via tautomerization steps, just as in the biosynthesis of 2-thioglucose-6-phosphate20 or molybdenum cofactor.21 Interestingly, within the biosynthesis of thienodolin by Streptomyces sp. FXJ1.172, a formal reductive sulfdation occurred to yield 6-CI-thiotryptophan from 6-chloroindole-3-pyruvic acid. The
we hypothesized that similar structural and/or electronic particularities of chlordecone (12a), we selected a series of aldehydes and ketones containing electron-withdrawing groups (1a−2a, 13a−15a) (known to be partially or completely hydrated)\textsuperscript{25−29} and several cyclic ketones to explore the possible effect of the ring constraints (20a−28a). Finally, we added aliphatic, conjugated, and aromatic aldehydes and ketones to enlarge the structural diversity (3a−11a, 16a−19a). An additional lactone (29a) was also tested.

2.2. Chemical Reductive Sulfitation of Carbonyl Compounds Using P\textsubscript{4}S\textsubscript{10} and NaBD\textsubscript{4}. To mimic the bacterial reductive sulfitation observed with \textit{Desulfovibrio} sp.86, a standardized chemical protocol using P\textsubscript{4}S\textsubscript{10} was applied to each selected carbonyl compound. The chemical reaction conditions were adapted from already published protocols\textsuperscript{11,16} for the first reaction step, P\textsubscript{4}S\textsubscript{10} was used in acetonitrile at room temperature, instead of the most popular condition (refluxing pyridine)\textsuperscript{10,30} Acetonitrile is also reported for this type of reaction,\textsuperscript{30} and its use at room temperature could prevent chloral (1a) or similar compounds from hydrolysis or dehalogenation, possibly enhanced by high temperatures and basic conditions.\textsuperscript{29} At the end of this step, the thione intermediate was observed in most cases (Figures S7−S31). The second step consisted of the reduction of the thiones using deuterated sodium borohydride in anhydrox methanol-\textit{d}_4. All reactions were monitored using either headspace gas chromatography coupled to mass spectrometry (HS-GC-MS) or GC-MS using liquid injection (see Figures S7−S31). In each case, the peak area of the deuterated thiol was compared to the peak area of the nondeuterated thiol so as to assess the proportion of thiol already produced in the first
step versus the thiol formed after the addition of the reductant NaBD₄ in the second step.

Interestingly, polychloroaldehydes (1a, 2a), chlordecone (12a), and 10-monohydrochlordecone (13a) were almost completely transformed into their thiol derivatives after the first step. For most of the carbonyl compounds (3a−5a, 7a−8a, 11a, 14a−17a, 19a−27a), the deuterated thiols proved to be the predominant end-products. The thione intermediates were thus mainly reduced during the second step.

In the case of hexane-2,5-dione (18a), citral (9a), citronellal (6a), and cyclopent-2-en-1-one (28a), the expected thiol products were not observed. For each of these four compounds, we proposed a structure based on the analysis of the in-source GC-MS fragmentation and comparison with the existing mass spectra database and/or the literature (Table S6). Treatment of hexane-2,5-dione with P₄S₁₀ resulted in the appearance of one new GC-MS peak that remained unchanged after the reduction step. Based on the analysis of its mass spectrum (Figure S13), we assigned it as 2,5-dimethylthiophene (18b, Figure 2D). Indeed, it has already been reported that hexane-2,5-dione was transformed into dimethylthiophene by P₄S₁₀ via a Paal−Knorr mechanism.

For 2,3. Versatility and Specificity of Desulfovibrio sp.86

Reductive Sulfdation toward Carbonyls. We modified the incubation conditions previously reported for the reductive

Figure 2. Description of the detected products obtained by bacterial and chemical reductive sulfdation. (A) Carbonyl compounds not stable under bacterial conditions. (B) Carbonyl compounds probably metabolized by Desulfovibrio sp.86. (C) Ketones and lactone not transformed under bacterial conditions. (D) Aldehydes and ketones that similarly react under chemical and bacterial conditions. (E) Substrates that do not yield the same final products under chemical and bacterial conditions. The round-flask symbol represents products detected under chemical conditions, and the green bacterium symbol is associated with products detected under bacterial conditions. The following colors are associated with the four clusters established to describe microbiological transformation: green color for group a (12a, 13a), blue color for group b′ (4a, 5a, 6a, 7a, 8a, 9a, 10a, 11a), light green color for group c′ (18a, 20a, 21a, 22a, 23a, 24a, 25a) and yellow color for group d′ (17a, 19a, 26a, 27a, 29a).

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con 98/2), in a 100 mL sealed vial, thus corresponding to a electron donor, respectively (see Section 4). The bacterium consisted in supplying the culture in higher concentrations of identi fication of the expected thiols were facilitated by the presence of the previous P4S10/NaBD4 transformation state only refers to the transformation of the carbonyl into the target thiol (d and m stand for decomposition and metabolization, incubation time indicates the time required to observe a complete disapearance of the target carbonyl compounds by GC-MS analysis. The transformation state only refers to the transformation of the carbonyl into the target thiol (d and m stand for decomposition and metabolization, respectively).

sulfidation of chlordecone to test the panel of compounds in the presence of Desulfovibrio sp. sp.86.16 The present protocol consisted in supplying the culture in higher concentrations of sulfate (25 mM) and lactate (50 mM) as electron acceptor and electron donor, respectively (see Section 4). The bacterium and the substrate were then incubated in anaerobiosis (N2/He, 98/2), in a 100 mL sealed vial, thus corresponding to a confined atmosphere system (CA). The search and the identification of the expected thiols were facilitated by the thiols obtained chemically using the previous P4S10/NaBD4 sequence. Every transformation was monitored by GC-MS analysis (Figures S32–S52).

Unexpected behaviors were observed for a number of target carbonyl compounds: (i) trichloroacetaldehyde (chioral, 1a), dichloroacetaldehyde (2a), 1,3-difluoropropan-2-one (14a), and 1-fluoropropan-2-one (15a) disappeared within a few hours in both biotic experiments and abiotic controls; (ii) acetone (16a) and acetaldehyde (3a) were no longer detected in the biotic incubations after 1 week, while no thiol derivatives could be observed at the same time. Indeed, it is known that chloral (1a) decomposes in water to chloroform and methanoic acid at moderate temperature and under basic conditions.29 In our case, chloroform was detected in both experiments that involved chloral (1a), confirming the previous report. We thus assumed that chloral (1a) and dichloroacetaldehyde (2a) as well as 1,3-difluoropropan-2-one 14a and 1-fluoropropan-2-one (15a) underwent hydrolysis during incubation. Acetone (16a) and ethanol (3a) are probably used by Desulfovibrio sp. sp.86 as carbon sources and/or involved in other metabolic pathways as it has already been observed under anaerobic conditions for other sulfate-reducing bacteria.39,40

After incubation of 2 months, no trace of thiol could be detected for the linear ketone (17a), enone (19a), seven-membered ring ketone (26a), indanone (27a), and lactone (29a), while the substrates were still present in the culture. All other cyclic ketones were transformed into their thiol derivatives (20a–25a) but incompletely after the same period of time. For cyclopent-2-en-1-one 28a, the reduction of the C==C double bond was first evidenced with the detection of cyclopentanone (21a), which was finally transformed into cyclopentanethiol (21b). The promising high diasterosimetric excess (70–95%) observed for the reductive sulfidation of nor-camphor (22a), 2-methylcyclopentanone (24a), and 2-methylcyclohexanone (25a) unfortunately did not correspond to a complete conversion. Finally, chlordecone (12a), 10-monohydrochlordecone (13a), and all aldehydes have completely disappeared to give rise to their thiol derivatives (4b–13b). It turned out that citral and citronellal (9a, 6a) gave rise to two GC-MS peaks sharing the same fragment

| theoretical parameters | chemical results | microbiological results |
|-------------------------|-----------------|------------------------|
| substrate | $\Delta G_1$ (kJ/mol) | $\Delta G_2$ (kJ/mol) | log($K_{mol}$) | log($A_{C-SH}/A_{H-C-SH}$) | de (%) | transformation state | de (%) | incubation time (days) |
| 1a | −422.1 | −120.8 | 3.81 | −1.03 | d | | | |
| 2a | −403.9 | −120.5 | 3.98 | −1.72 | d | | | |
| 12a | −388.2 | −115.7 | 8.03 | −0.34 | 13 | fully transformed | 40 | |
| 13a | −380.0 | −115.0 | 7.69 | −0.68 | 10 | fully transformed | 40 | |
| 7a | −370.7 | −70.5 | −1.79 | 0.17 | | | |
| 14a | −344.7 | −63.5 | 3.21 | 0.51 | d | | | |
| 5a | −344.6 | −78.8 | −2.22 | 0.01 | | | |
| 8a | −349.4 | −67.9 | −2.88 | 0.27 | | | |
| 9a | −328.6 | −76.7 | −3.62 | | | | |
| 27a | −306.1 | −44.1 | −5.75 | 0.4 | | | |
| 15a | −300.0 | −82.4 | −0.78 | 1.26 | | | |
| 3a | −284.6 | −87.4 | 0.03 | 1.52 | m | | |
| 10a | −296.4 | −96.9 | 0.41 | 1.10 | d | | |
| 4a | −294.5 | −89.7 | −0.10 | 0.94 | | | |
| 11a | −296.9 | −89.8 | −0.30 | 1.04 | | | |
| 6a | −292.7 | −92.8 | −1.73 | | | | |
| 20a | −272.0 | −71.8 | −2.50 | 0.47 | | | |
| 21a | −270.9 | −64.2 | −3.41 | 0.54 | | | |
| 26a | −268.1 | −62.6 | −3.27 | 0.92 | | | |
| 22a | −268.0 | −59.3 | −6.54 | 1.66 | 19 | fully transformed | 70 | |
| 24a | −267.4 | −57.3 | −1.53 | 1.92 | 44 | partially transformed | 95 | |
| 23a | −266.5 | −64.6 | −1.00 | 0.80 | | | |
| 18a | −288.7 | −67.7 | −3.51 | | | | |
| 16a | −262.8 | −68.3 | −2.77 | 2.44 | m | | |
| 19a | −264.1 | −67.7 | −3.63 | 1.20 | not transformed | | |
| 25a | −263.5 | −63.5 | −2.89 | 0.66 | 15 | fully transformed | 76 | |
| 17a | −260.6 | −68.5 | −3.84 | 1.70 | | | |
| 29a | −225.7 | −16.3 | −9.27 | | | | |

$A_{C-SH}/A_{H-C-SH}$ represents the GC-MS peak area ratio of the deuterated thiol over the nondeuterated thiol. de stands for diastereomeric excess. Incubation time indicates the time required to observe a complete disapearance of the target carbonyl compounds by GC-MS analysis. The transformation state only refers to the transformation of the carbonyl into the target thiol (d and m stand for decomposition and metabolization, respectively).
losses with molecular ions m/z 168 and 170, respectively. The mass spectra did not correspond to the expected thiol.
However, the analysis of their in-source fragmentation mass spectra showed strong similarities to those from isopiperitenol and isopulegol (Figures S43 and S44).\textsuperscript{33,41} We thus assumed that a ring-closing reaction occurred, leading to cyclic thiols (6c, 9c, Figure 2E). Finally, dimethylthiophene (18b) was detected when hexane-2,5-dione (18a) was incubated.

2.4. Chemical versus Microbiological Reductive Sulfidation. At that stage, the results of bacterial and chemical reductive sulfidation were compared. First of all, we noted that four carbonyl compounds possessing electron-withdrawing groups (1a, 2a, 14a, 15a) that were not stable in liquid cultures reacted under chemical conditions to give the expected thioiols (1b, 2b, 14b, 15b) (Figure 2A). Therefore, we could not conclude on their fate in the presence of Desulfovibrio sp.86. Acetaldehyde (3a) and acetone (16a) were apparently metabolized by this bacterium but in unidentified products (Figure 2B). A set of five carbonyl substrates (17a, 19a, 26a, 27a, 29a) were chemically transformed but remained unchanged under microbiological conditions (Figure 2C). A total of six aldehydes 4a, 5a, 7a, 8a, 10a, and 11a and nine ketones 12a, 13a, 18a, 20a, 21a, 22a, 23a, 24a, and 25a reacted similarly both under chemical and bacterial conditions (Figure 2D), whereas the two aldehydes 6a and 9a and the enone 28a did not lead to the same final products depending on the conditions (Figure 2E).

Interestingly, even if microbiological transformations were not total, their diastereomeric excesses were systematically higher than those obtained under chemical conditions (Table 1). Furthermore, when the biotransformation of carbonyl compounds to thiol derivatives was effective, no trace of the possible thione intermediate was detected, contrary to the chemical reductive sulfidation. In addition, all negative controls performed without bacteria never showed any reductive sulfidation activity. It is noteworthy that the microbiological reductive conditions were reached through the use of Na\textsubscript{2}S. We thus concluded that this possible sulfur donor reagent, known to act on several ketones in organic media,\textsuperscript{12} is not reactive in the present case with any of the compounds studied.

To account for bacterial findings, we focused on 18 carbonyl substrates that showed the same reactivity toward chemical and microbiological transformation and discarded the others. Four categories were established according to the transformation degree of the carbonyl compounds: (a) fully transformed after several weeks (12a, 13a), (b) fully transformed after a few days (4a, 5a, 7a, 8a, 10a, 11a), (c) partially transformed (20a−25a), and (d) not transformed (17a, 19a, 26a, 27a).

As shown in Figure 3, group (a) consisting of chlordecone (12a) and 10-monohydrochlordecone (13a) had a specific behavior: they were completely transformed after 40 days by Desulfovibrio sp.86 into chlorodethiols 12b and 13b, while the same products were predominantly formed after a simple treatment with P\textsubscript{4}S\textsubscript{10}. However, no other significant profile could be identified from the chemical and microbiological data. Indeed, pairing of groups (b)−(d) did not lead to any statistical difference (Figure 3). From this set of data, it seems that the mechanism of chemical and bacterial reductive sulfidation may differ significantly.

2.5. Calculation of Structural, Electronic, and Thermodynamic Parameters. To account for the results of chemical and microbiological reductive sulfidation, we performed a series of quantum chemical calculations at the DFT level. We used the relative calculation approach to estimate the carbonyl hydration constants K\textsubscript{hyd} and K\textsubscript{hyd} were computed from the Gibbs free energy differences of the reaction exchange, \(\Delta G\textsubscript{ex}\), and the thermodynamic definition of K\textsubscript{hyd} (Scheme 1).

![Scheme 1. Thermodynamic Definition of the Hydration Constant K\textsubscript{hyd}](http://pubs.acs.org/journal/acodf)

\[
\log K_{\text{hyd}} = \log K_{\text{hyd}}(\text{CH}_2\text{CHO}) \cdot \frac{\Delta G_1}{\text{In}10 \text{RT}}
\]

The exchange reaction includes acetaldehyde as the reference compound. Its K\textsubscript{hyd} value is well known experimentally (\(\log K_{\text{hyd}}(\text{CH}_2\text{CHO}) = 0.03\)).\textsuperscript{43,44} Numeric data of all of these calculations are available in the Supporting Information (Tables S1−S3). Additional parameters relative to the carbonyl moiety were calculated, such as the lowest unoccupied molecular orbital (LUMO) energy level, geometrical features, and partial atomic charge (Table S5).

In the absence of detailed experimental information on the mechanisms of the bacterial and chemical reductive sulfidation reactions, two energy parameters have been calculated to estimate the ease of thione reduction, based on the reactions displayed in Schemes 2 and 3.

![Scheme 2. Hydrogenation of the Thione](http://pubs.acs.org/journal/acodf)

The Gibbs free energy of reaction shown in Scheme 3, \(\Delta G_3\), which corresponds to the electron affinity of the thione reactant, measures the stability of a monoelectronic reduced

![Scheme 3. Electron Affinity of the Thione](http://pubs.acs.org/journal/acodf)
intermediate along the reduction process. $\Delta G_2$ measures the thermodynamics of the full reduction process.

The LUMO energy level of the thione has been used as another parameter to evaluate the thione reducibility. Additional parameters such as the $C\equiv S$ bond length $d(C\equiv S^\bullet)$ of the thioketyl radical anion and the spin density of the $C\equiv S$ moiety were also calculated (Table S4).

### 2.6. Rationalization of Carbonyl Reactivity toward P$_4$S$_{10}$ with Selected Calculated Parameters

Here, we compared the ratio of GC-MS peak areas of deuterated thiols and nondeuterated thiols obtained by chemical reductive sulfoxidation with a selection of calculated parameters. For this part, data arising from 24 chemical experiments were exploited. The logarithm operator was applied to the ratio of GC-MS peak areas to minimize the effect of experimental fluctuations and mimic the relationship between the Gibbs free energy and the concentrations in the law of mass action. The logarithmic dataset reflected at a semiquantitative level the propensity of the thione intermediates to be easily reduced and thus made it possible to carry out correlation tests.

In view of the high level of hydration in chlordecone and congeners, we first examined the possible relationship between the hydration constant of the carbonyl substrates and their ability to be spontaneously reduced. Even if we could show a trend among the carbonyl compounds possessing electron-withdrawing substituents (Cl and F atoms, Figure S5) and known to be mainly present in the gem-diol form, this parameter could not explain the overall phenomenon. It means that the chemical reductive sulfoxidation is not very sensitive to the thermodynamics of the hydration reaction (Figure 4D). In addition, no specific relationship with any other selected parameters related to the carbonyl substrate could be detected (Table S5; Figure S5).

We then focused on the $C\equiv S$ bond reactivity. No trend could be established between the enthalpy of the $C\equiv S$ hydrogenation reaction and the chemical dataset (Figure 4B). More interestingly, we found a correlation with the electron affinity of $C\equiv S$ (Figure 4A). In our case, the greater the energy of electron affinity was, the greater the proportion of thiol formed in the presence of P$_4$S$_{10}$ became. As we could see, the substitution with electron-withdrawing groups including Cl and F atoms as well as the presence of aromatic rings conjugated with the thionyl moiety tends to increase this energy. It seems that the propensity of thiones to be reduced monoelectronically is the parameter driving their spontaneous reduction in the presence of P$_4$S$_{10}$. This assumption was supported by the significant relationship observed between the $C\equiv S^\bullet$ bond length $d(C\equiv S^\bullet)$ of the thioketyl radical anion and the set of experimental data (Figure 4C). Indeed, for some molecules, the added electron is trapped almost exclusively in the $C\equiv S$ bond, as revealed by the spin density on the $C\equiv S$ moiety, which is greater than 0.9 (Table S4). In these cases, the elongation of the $C\equiv S$ bond upon reduction (0.09–0.1 Å) is strong as the electron is located in the $\pi^*$ orbital. It corresponds to nonconjugated ketones and aldehydes deprived of electron-withdrawing substituents that did not show any
spontaneous reduction. For conjugated molecules, the added electron is partially delocalized in the π system (lower C=S elongation, lower spin density on C=S), which stabilizes it more, resulting in a greater electron affinity. For chlorinated and fluorinated molecules, a part of the added electron goes to adjacent C–X bond (X = Cl or F). Again, this stabilizes the reduced form and leads to a greater electron affinity. DFT calculations thus show that the presence of electron-withdrawing groups and/or conjugated systems plays a critical role in the stabilization of thioketyl radicals, which thus appear to be the key intermediates in the spontaneous reduction of thiones.

All of these observations are in agreement with what has been previously reported on cyclic and steroid ketones. In their paper, the authors studied the propensity of thione intermediates to be spontaneously reduced using Na2S and LiAlH4. Finally, they managed to show that the reduction process was achieved via a single-electron transfer mechanism by trapping the radical in an intramolecular cyclization reaction.

On the basis of these results, we therefore proposed the following sequence for the chemical reductive sulfidation without additional reductant (e.g., NABH4 or LiAlH4): (i) P4S10 initially reacted with carbonyl compounds to form four-membered ring intermediates, which decomposed into the corresponding thiones as previously assumed; (ii) then, radical and/or reducing species such as H2S present in the reaction mixture reacted with these thiones via a single-electron transfer mechanism; and (iii) finally, the protonation of the resulting C=S radical led to the observed thiolis (Scheme 4). For the carbonyl compounds involved in a conjugated system or possessing electron-withdrawing groups, the electron would be partially delocalized in the π-system or in the C–X bond, which would highly enhance the reduction of the thione intermediates.

2.7. Influence of Molecular Parameters on Bacterial Reductive Sulfidation of Carbonyls. In contrast to the chemical results, no (semi-)quantitative data could be retrieved from the microbiological experiments. We therefore decided to exploit the clustering previously introduced with four levels of transformation, varying from level 1 (no transformation) to level 4 (complete transformation within several days). Contrary to previous observation, four molecules were added to these groups: 6a and 9a to cluster (b’), 18a to cluster (c’), and 29a to cluster (d’). We applied statistical tests (analysis of variance (ANOVA)) to assess the significant differences between the clusters for each selected parameter. Since cluster (a) included only two compounds (chlordecone 12a and 10-monohydrochlordecone 13a), the statistical tool was not applicable in this case.

We first compared the different clusters with respect to the hydration constant of carbonyl substrates. As in the case of chemical reactions, we could not observe any clear influence of this parameter on the level of the observed biotransformation (Figure 5C). The singly occupied molecular orbital (SOMO) and LUMO energies of the thiones, the bond length of C=S, as well as the C=S and HC–SH radicals did not correlate better with the microbiological results (Table S4 and Figure S6). However, the calculation of the partial charge of the oxygen atom on the C=O bond enabled to show a slight distinction between groups (c’) and (d’) (Figure 5D). With respect to the electron affinity of the C=S bond and the Gibbs free energy of the C=S hydrogenation, we found significant differences between clusters (b’) and (c’) and also between clusters (b’) and (d’) (Figure S5A,B). Even so, among the selected parameters, none of them could discriminate between groups (c’) and (d’). It is noteworthy that the previous papers describing bacterial reductive sulfidation22,23 never mentioned any trace of the possible thione intermediate just as in our case. It may be the reason why these parameters focusing on the C=S bond did not correlate well with the qualitative trend presently observed.

Interestingly, we could correlate the biotransformation of groups (a) and (b’) to Desulfovibrio sp.86 growth. Indeed, while a couple of days were needed to achieve complete transformation of aldehydes, several weeks were required to fully convert chlordecone (12a) and 10-monohydrochlordecone (13a) into their thiol derivatives (Figure 6). As can be seen in Figure 6C–H, the reductive sulfidation of the aldehydes was correlated with the growth phase, whereas in Figure 6A,B, the biotransformation of group (a) occurred mainly during the stationary phase. These differences could be explained by the very low solubility of chlordecone (12a) and 10-monohydrochlordecone (13a) in water, estimated around 2 mg/L at pH 7 and 25 °C,55 and/or by the involvement of two distinct mechanisms depending on the nature of the substrate.

3. CONCLUSIONS

Bacterial reductive sulfidation has been rarely documented, although it represents an efficient strategy for the formation of thiois. In the present work, we studied the substrate versatility of the reductive sulfidation activity of Desulfovibrio sp.86 and compared it to a two-step chemical procedure using the classical sulfur donor P4S10. We showed that Desulfovibrio sp.86 transforms a variety of substrates (most preferably aldehydes or ketones substituted with electron-withdrawing groups and/or included in a ring system). Notable differences compared to chemical reductive sulfidation offer new perspectives for the selective synthesis of thiolated compounds.

A number of target thiois were partially or completely formed in the presence of P4S10 without the additional reduction step required according to the literature. Several parameters (electron affinity of the C=S bond, length and spin density of the C=S moiety of the thioketyl radical anion) obtained by quantum chemical calculation rationalize these observations. Indeed, DFT results demonstrate that the presence of electron-withdrawing groups and/or aromatic rings, which experimentally promote the reductive sulfidation, can stabilize the thioketyl radical through partial delocalization of the single electron over σ and/or π bonds. We therefore concluded that a single-electron transfer to the thione intermediate was likely to occur. The same correlation was not observed for the bacterial reductive sulfidation. Its
mechanism, whether or not involving the thione, may differ according to the type of substrates (aldehydes or ketones).

4. EXPERIMENTAL SECTION

4.1. Chemicals and Media. Chlordecone was obtained from Azur Isotopes (purity, 98%). Na$_2$S($\geq$ 98%), phosphorus pentasulfide (99%), aldehydes, ketones, and lactone were purchased from Sigma-Aldrich. Acetonitrile (MeCN, LC-HRMS grade) and acetone (>99.9%) were obtained from VWR Chemicals. The chemicals used for microbiological media were obtained from Sigma-Aldrich.

4.2. Anoxic Microbial Incubations. The anoxic incubation conditions used for *Desulfovibrio* sp.86 have already been described. They were carried out in MMD medium consisting of the MM-enriched mineral medium previously described supplemented with lactate as a carbon source (50 mM), yeast extract (1 g/L), Na$_2$SO$_4$ (25 mM), and Na$_2$S as a reducing agent (0.4 g/L). *Desulfovibrio* sp.86 cultures were incubated at 30 °C, in an oven, under anaerobic conditions (N$_2$/H$_2$ (98/2; V/V)). They were carried out in sealed culture vials, closed with butyl rubber septa. *Desulfovibrio* sp.86 cultures were inoculated at the onset of an experiment with 0.5 mL of preculture pregrown in the oven for 24 h.

All microbiological experiments were performed in 100 mL vials filled with 50 mL of MMD inoculated with an actively growing *Desulfovibrio* sp.86-1 culture (1/v/v) in duplicate, and an abiotic control was included. Carbonyl compounds were added to a final concentration of 40 mg/L in each vial.

4.3. Extraction/Sampling for Microbiological Culture Monitoring. Chlordecone and 10-monohydrochlordecone transformations were monitored by GC-MS analysis. Benzaldehyde, cinnamaldehyde, and indan-1-one transformations were monitored by GC-MS and HS-GC-MS analyses. The transformations of other carbonyl compounds were recorded by HS-GC-MS analysis.

For GC-MS analysis, 500 μL of the liquid medium was collected and extracted twice using 250 μL of isooctane. The combined organic layers were then analyzed by GC-MS analysis.

When HS-GC-MS was required, 1 mL of cultures was sampled and put into a 10 mL Chromacol 10-HSV vial (Agilent). The headspace gas was then analyzed.

4.4. Analytics. GC-MS analyses were carried out using a Thermo Fisher Focus GC coupled to a single quadrupole mass spectrometer (Thermo Fisher DSQ II). The instrument was equipped with a nonpolar 30 m × 0.25 mm × 0.25 μm DB-5MS column (Agilent J&W) and a split/splitless injector. Ionization conditions and GC program used for monitoring chlordecone and 10-monohydrochlordecone were described elsewhere (method GC-MS-1).

For benzaldehyde, cinnamaldehyde, and indan-1-one, the GC program started at 30 °C (hold time 6 min), continued with 15 °C/min to 90 °C (hold time 8 min), and followed by 10 °C/min to 200 °C (hold time 1 min) (method GC-MS-2).

HS-GC-MS analyses were performed on a Thermo Fisher Trace 1300 coupled to an ISQ 7000 VPI single quadrupole mass spectrometer. The instrument was equipped with a 30 m × 0.25 mm × 0.25 μm DB-624-UI column (Agilent J&W), a split/splitless injector, and an automatic sampler TriPlus RSH coupled to a Headspace tool. For mass spectrometry (MS) analyses, the following standard working conditions were applied: electronic impact ionization; positive mode detection;
ion source at 220 °C; detector voltage, 70 eV; full scan mode, m/z 33–300 (scan time 0.20 s). The temperatures of the injection and transfer lines were set at 200 and 280 °C. After the incubation time, 1 mL of the headspace gas was injected each time with a filling speed of 10 min/mL, an injection speed of 10 mL/min, and a penetration speed of 10 mL/s.

For acetone, ethanal, and 1-fluoroacetone monitoring, the vials were incubated for 1 min at 40 °C and sampled with a syringe at 40 °C. The split mode was applied (30 °C, flow rate at 16.7 mL/min, with a ratio of 33.4). The carrier gas was helium at 0.5 mL/min until reaching 1 mL/min (hold time, 9 min). The GC program was isocratic at 24 °C for 20 min (HS-GC-MS-1 method).

For the other carbonyl compounds, monitoring vials were incubated for 5 min at 50 °C and sampled with a syringe at 50 °C. The splitless mode was applied at 150 °C. The carrier gas was helium at a constant flow rate of 0.5 mL/min. The GC program started at 30 °C (hold time 6 min), continued with 15 °C/min to 130 °C (hold time 0.5 min), and followed by 7 °C/min to 250 °C (hold time, 10 min) (HS-GC-MS-2 method).

Figure 6. Reductive sulfdation of selected carbonyl compounds, over time, by Desulfovibrio sp.86, in MMD medium and CA conditions, monitored by GC-MS analysis. (A) Chlordecone (12a) transformation (adapted with permission from ref 16. Copyright 2020 SPRINGER NATURE), (B) 10-monohydrochlordecone (13a) transformation, (C) benzaldehyde (5a) transformation, (D) cinnamaldehyde (7a) transformation, (E) citral (9a) transformation, (F) citronellal (6a) transformation, (G) cyclopentanal (11a) transformation, and (H) furfural (8a) transformation. OD600 corresponds to the optical density at 600 nm. “A” corresponds to the GC-MS peak area.
4.5. Chemical Reductive Sulfdation of Carbonyl Compounds. Phosphorus pentasulfide (20 mg, 4.5 × 10⁻⁵ mol, 4.5 equiv) was added to a solution of carbonyl compound (1.0 × 10⁻⁵ mol, 1 equiv) in acetonitrile (1 mL). The reaction mixture was stirred under N₂ at room temperature for 12 h. After removal of acetonitrile under reduced pressure, 500 μL of methanol containing NaBD₄ at 1.0 × 10⁻¹ M was added (5.0 × 10⁻⁵ mol, 5 equiv) and stirred for 12 h at room temperature.

4.6. DFT Calculations. Calculations were carried out with the Gaussian 09 package. All structures were fully optimized without any symmetry constraints at the DFT level by means of the PBE0⁴⁷,⁴⁸ (PBE1PBE keyword in G09) and M06-2X⁴⁹ functionals. The 6-31+G(d,p) basis set was applied for all atoms for geometry optimization. Geometries have been first optimized in the gas phase at the PBE0/6-31+G(d,p) and M06-2X/6-31+G(d,p) levels. To ensure that solvation has almost no influence on the geometry, the geometries were reoptimized at the PBE0/6-31+G(d,p) level and including a continuum solvation method (integral equation formalism version of the polarizable continuum model (IEFPCM) for water). Each stationary point has been characterized with frequency analysis and shows no negative eigenvalues, as required for local minimum. Final energy calculations at the PBE0 and M06-2X levels associated with the 6-311++G(2d,2p) basis set, both in the gas phase and including solvation effect, have been achieved on the optimized geometries. To get accurate geometries and energies, the self-consistent field method (SCF) convergence criterion was systematically tightened to 10⁻⁸ au, and the force minimizations were carried out until the rms force became smaller than (at least) 1 × 10⁻⁵ au (“tight” optimization keyword in Gaussian 09). The “UltraFine” grid (99 radial shells and 590 angular points per shell) was used throughout the calculations, as recommended when using Gaussian 09. Unrestricted formalism (UDFT) was used for radicals.

The results obtained show an excellent agreement between PBE0 and M06-2X data and a minor influence of the implicit solvation on the optimized geometries (Figure S2). Comparison of the DFT results with post-HF CCSD(T) calculations (Figure S1) does not reveal any particular deficiencies in the functionals used, even in the case of the study of radicals.⁵⁰ Furthermore, comparison with experimental data (Figure S3) shows a slightly better agreement for M06-2X data. Therefore, only M06-2X data are presented in the text. The Gibb's free energies presented in this article are thus IEFPCM(water)-M06-2X/6-311++G(2d,2p)//M06-2X/6-31+G(d,p) electronic energies (which include solvation-energy corrections from the IEFPCM method) modified with thermal and entropy corrections from M06-2X/6-31+G(d,p) calculations.

4.7. Statistics. Statistical tests have been performed using RStudio, according to the following methods: normality was checked with a Shapiro test. If the distribution was normal, then the homogeneity of variances was checked with a Bartlett test and a two-way ANOVA test was performed. If the distribution was not normal, a nonparametric test coming from the ARTool package⁵¹ was used. Tukey analysis was subsequently used as a post hoc analysis.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c06041.
mass spectrometry; HS, headspace; hyd, hydration; LUMO, lowest unoccupied molecular orbital; MM, mineral medium; OD, optical density; RS, reductive sulfitation; SOMO, singly occupied molecular orbital

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