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# Application of Mössbauer spectroscopy for studying chemical effects of environmental factors on microbial signalling: Redox processes involving iron(III) and some microbial autoinducer molecules

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ABSTRACT

Diverse processes of microbial remote intercellular communication as well as the exchange of molecular signals between microbial cells and their host macroorganisms, involving specific low-molecular-mass diffusible substances (used as a 'chemical language'), are at the peak of current research in biosciences. This fundamental interest is due to the unique possibility of controlling the microbial behaviour and metabolism by influencing merely their signalling pathways. On the other hand, abiotic impact of the environment (medium) on extracellular molecular signals is also of great importance, as any of their chemical interactions (e.g., complexation or oxidation) represent direct interferences in the process of 'signal delivery' through the medium. In this work, chemical interactions of microbial extracellular molecular signals (alkylresorcinols (AR), homoserine lactone (HL) – chemical analogues of microbial autoregulatory substances) with iron(III) were monitored using freeze-quench $^{57}$Fe Mössbauer spectroscopy in moderately acidic aqueous solutions as well as in the dried solids obtained thereof. The conditions applied were designed to simulate possible processes occurring in soils, where ferric iron is commonly ubiquitous. Gradual reduction of iron(III) by AR was observed, coupled to oxidative degradation of the organics, in solution, while iron(II) also remained dominant upon drying, whereas for HL, some iron(II) was detected in the dried solid only. The iron(III) reduction rate in solution for AR with a longer alkyl chain (4-*n*-$C_6$) was found to be much higher than that for the methyl (5-$C_1$)-substituted derivative, pointing to the importance of the structure (i.e., the position and/or the nature of the alkyl substituent) of the alkylresorcinol molecule for the redox process rate. The results obtained indicate that ARs can be readily oxidised abiotically by soil iron(III) in moderately acidic media, thus being excluded from signalling pathways, which is equal to "message undelivery", directly affecting microbial autoregulation.

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## 1. Introduction

It has now been well documented that diverse microbial metabolic processes and behavioural features are regulated via intercellular remote chemical signalling which involves a variety of special low-molecular-mass diffusible molecules (see, e.g. recent reviews [1–6]). The microbial producer cells respond to their own signals ("autoinducers" or "autoregulators") if the latter accumulate up to a threshold concentration, reflecting the cells' perception of their 'population density' sufficient for concerted actions ("quorum sensing") [1–4,6–8], mass transfer properties of the medium ("diffusion sensing") or a combination of these two, including also spatial cell distribution, as a unifying hypothesis ("efficiency sensing") [5]. Thus, the microbial metabolism and behaviour may be regulated by influencing not the whole consortium but barely its signalling pathways [8–10]. Note that some naturally occurring mechanisms of suppressing (quenching) microbial quorum sensing (i.e., "quorum quenching") have been found to play important roles in microbe–microbe and pathogen–host interactions [8,11–13]. In biomedicine, such studies can lead to a novel therapeutic approach, with new generations of antimicrobial agents aiming at the quorum-sensing circuitry of pathogens (which controls their pathogenicity and biofilm formation) but not at their growth *per se* [8,14,15].

On the other hand, possible abiotic (physicochemical) effects of the environment on extracellular signalling molecules, including any chemical interactions, also represent direct interferences in the signalling processes [16,17]. Besides hydrolytical transformations dependent on pH, temperature and the nature of the molecular signal [16], the chemical reactivity of particular metal species (or their possible catalytic effects) should also be

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considered to be of importance [17]. It could result in complexation or redox degradation of biogenic organics involved in microbe–host interactions or intercellular microbial communication, i.e. in the aforementioned remote exchange of molecular signals, autoinducers, nutrients; quorum (efficiency) sensing within microbial communities, etc. [17–21]. Such processes are likely to take place, e.g. in metal-polluted environments, especially in the case of relatively acidic soils which are of relatively frequent occurrence [22].

In our earlier studies (see, e.g. [19–21] and references therein), it has been found that even under aerobic conditions in moderately acidic aqueous media, iron(III) can be gradually reduced by some auxin phytohormones including indole-3-acetic acid (IAA). The latter, produced also by many soil microorganisms and excreted into the environment, plays a pivotal role in plant–microbe interactions and signalling [23]. In this work, the processes of abiotic iron(III) reduction in moderately acidic aqueous solutions by some chemical analogues of microbial autoinducers in air were directly evidenced using $^{57}$Fe Mössbauer spectroscopy. As iron(III) is ubiquitous in most soils, sediments and aquifers, such redox processes could occur, e.g. in moderately acidic environments, resulting in oxidative degradation of the biomolecules involved in cell–cell communication, thus directly affecting the relevant signalling pathways.

## 2. Experimental

### 2.1. Reagents

As chemical analogues of bacterial autoregulatory substances, the following reagents were used: orcinol (also known as orcine, or 5-methylresorcinol, or 1,3-dihydroxy-5-methylbenzene) monohydrate ($C_7H_8O_2{\cdot}H_2O$; obtained from "Fluka", No. 75420); 4-*n*-hexylresorcinol (1,3-dihydroxy-4-*n*-hexylbenzene, $C_{12}H_{18}O_2$; "Sigma", No. 209465), as well as homoserine lactone hydrobromide ((*S*)-(−)-α-amino-γ-butyrolactone hydrobromide; $C_4H_7NO_2$ HBr; "Sigma", No. 471429). Structural formulas of the organic compounds studied are shown in Fig. 1.

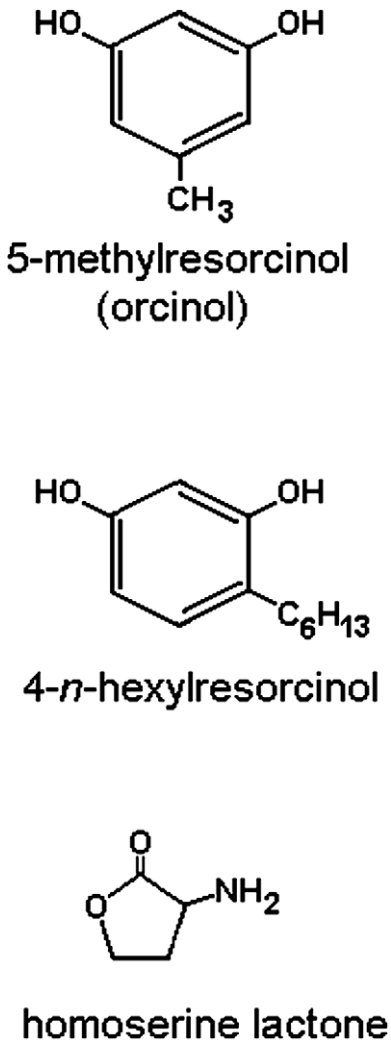


**Fig. 1.** Molecular structures of the organic substances under study (5-methylresorcinol, 4-*n*-hexylresorcinol and homoserine lactone).

Stock solutions of the reagents (1.5 ml each in closed Eppendorf tubes) were prepared as 60 mM aqueous solutions (for well water-soluble orcinol and homoserine lactone) or as a 25%(v/v) ethanol–water solution (for 4-*n*-hexylresorcinol, corresponding to ca. 20% ethanol in the Fe-containing sample solution prepared for measurements, in order to increase its solubility which is relatively low in purely aqueous solutions). Note that special comparative Mössbauer spectroscopic measurements for 5-methylresorcinol in aqueous and 20% ethanol–water solutions showed negligible differences in the $Fe^{III}$ reduction rate at pH ~ 3 (data not shown). Stock iron(III) aqueous solution contained 0.1 M $^{57}FeCl_3$ (enriched with $^{57}$Fe up to 90% to increase the intensity of resonant absorption of γ-radiation). Special Mössbauer spectroscopic measurements confirmed the absence of iron(II) in the $^{57}FeCl_3$ stock solution used.

### 2.2. Sample preparation procedures

For Mössbauer measurements, 0.50 ml of an organic reagent were mixed with 0.10 ml of the stock iron(III) solution, so that the iron(III)-to-organics molar ratio was 1:3. The initial pH was brought from ~1.5 to the final value of pH ~ 3 by immediately adding 30 μl (for each of the alkylresorcinols) or 60 μl (for homoserine lactone hydrobromide) of 1 M KOH under stirring. The resulting mixture was kept at room temperature for a required period of time (closed to prevent evaporation for lengthy periods) and then rapidly frozen within a few seconds in liquid nitrogen (freeze-quench method). Solid samples were prepared by drying similar aliquots of the sample solutions in air at ambient temperature in the same sample holder of the spectrometer.

### 2.3. Mössbauer spectroscopic measurements

All Mössbauer spectroscopic measurements were performed at $T \sim 80$ K, with each sample kept in a "cold-finger" cryostat filled with liquid nitrogen, using a conventional constant-acceleration Mössbauer spectrometer and a $^{57}$Co(Rh) source kept at room temperature. Statistical treatment of the spectral data was performed using the MOSSWINN 3.0 program [24], with the assumption of Lorentzian line shapes. The calculated parameters were the isomer shift ($\delta$, mm/s), quadrupole splitting ($\Delta$, mm/s), experimentally observed line width (full width at half maximum, $\Gamma_{exp}$, mm/s) and partial resonant absorption area ($S_r$,%) for each spectral component. The latter parameter ($S_r$) was used to represent the relative content of the related iron form (type of microenvironment), reasonably assuming a common recoilless fraction for all forms in a sample contributing to the spectrum at low temperature. In Mössbauer spectra, relative transmission of γ-radiation (in fractions of unity) was plotted against relative velocity ($v$, in mm s$^{-1}$) of the $^{57}$Co(Rh) 14.4-keV γ-radiation source versus the absorber (a $^{57}$Fe-containing sample), which corresponds to the energy scale according to the Doppler effect (i.e., with ±1 mm s$^{-1}$ corresponding to ±48.1 neV),

calibrated using α-Fe foil at room temperature. All isomer shift values discussed in this paper are reported relative to α-Fe at room temperature.

## 3. Results and discussion

It should be noted that surprisingly little attention has been paid so far to the chemical turnover and fate of extracellularly released microbial signalling molecules as a function of the environmental properties around the producer microorganisms [16,17]. For *N*-acylated homoserine lactones (AHL) representing a large class of quorum-sensing signalling molecules widely spread in many Gram-negative bacteria and known to regulate a variety of physiological processes [1–5], Yates et al. [16] experimentally studied their pH- and temperature-dependent lactonolysis. The conclusion was drawn that, owing to the latter process of chemical degradation of the AHL molecular signals, the rate at which a population of bacteria could reach a "quorum" for concerted actions would greatly depend on the local pH and temperature, as well as on the AHL acyl chain length. Recently, Götz et al. [25], who experimentally studied the uptake and degradation of AHLs (with $C_6$ to $C_{10}$ acyl chains) in plants, reported that AHL adsorption onto the solid phase (glass beads) contributed only in a minor way to the decline of their concentrations in the plant-free growth media; other abiotic processes (e.g. photo-catalysed oxidation or hydrolysis) resulted in the degradation of less than 10% of the AHLs (at 15 °C and pH ~ 5.7) within 17 days. In the present work, we studied possible interactions of iron(III) at pH ~ 3, which might occur, e.g. in moderately acidic soils (or environments acidified by excreted metabolic products of plant roots or microorganisms) [17,21,22], with the unacylated AHL analogue, homoserine lactone (see Fig. 1). This 'general bacterial signal of starvation' [26] is also known to induce non-species-specific effects on the growth and development of different bacteria suggesting its regulatory functions (see [27] and references reported therein).

Alkylhydroxybenzenes (AHB), largely represented by alkylresorcinols (i.e., alkyl-substituted *m*-dihydroxybenzenes), comprise a group of natural phenolic substances that have a wide range of known biological functions [28]. In particular, alkylresorcinols with varying alkyl chain length, excreted by many microorganisms into the environment, have been documented to perform autoregulatory and adaptogenic functions under unfavourable conditions [27,29,30]. Besides that, they were reported to induce DNA structural transitions [28,31], to exhibit bacteriostatic activity [28] as well as to be capable of binding to proteins and other biopolymers [28], stabilising enzymes in aqueous media and either increasing [32] or inhibiting their catalytic activity [28]. Such remarkably diverse biochemical and physiological activities of alkylresorcinols, including their important role in microbial extracellular remote signalling, infer the importance of their fate in the environment related to possible abiotic (e.g., chemical) effects of the latter. There have been solitary reports, e.g. on $Cu^{2+}$- and oxygen-dependent oxidation of 5-alkylresorcinols in alkaline aqueous media resulting in trihydroxylated alkylbenzene derivatives [33] or on radiation-induced oxidation of alkylresorcinols in aerated neutral aqueous solutions giving a sophisticated mixture of products (see [34] and references therein).

In our work, the possibility of redox transformations involving iron(III) and the aforementioned alkylresorcinols was also tested in aqueous media and upon drying. The whole experimental set was designed to simulate acidic soil conditions with varying humidity [18]. Transmission $^{57}$Fe Mössbauer spectroscopy was used as a very sensitive technique for the qualitative and quantitative monitoring of chemical transformations of high-spin iron(II) and iron(III) species [18–21,35]. A rapid freezing of the solutions under study (which is necessary for measuring Mössbauer spectra of Fe-containing liquid phases [36]) in liquid nitrogen allowed any ongoing chemical processes to be ceased at a certain point (the so-called freeze-quench method which can be applicable even to much faster processes [35]). In order to compare the Mössbauer parameters of the dried solids obtained from the initial solutions by drying in air at ambient temperature, measurements for the dried residues were performed under the same conditions (frozen in liquid nitrogen and measured in the cryostat at $T \sim 80$ K; see Section 2).

In aqueous mixtures of 5-methylresorcinol and [$^{57}$Fe]-iron(III) salt at pH ~ 3, the appearance of iron(II) was clearly noticeable in the Mössbauer spectrum already after a few minutes (Fig. 2a), with its gradual accumulation within a few hours (cf. the spectra in Fig. 2a and b; all the Mössbauer spectroscopic parameters calculated from the spectroscopic data are listed in Table 1). The parameters of the ferrous species formed upon iron(III) reduction in the solutions (in particular, isomer shift $\delta \sim 1.4$ mm s$^{-1}$ and quadrupole splitting $\Delta \sim 3.2$ mm s$^{-1}$ at $T = 80$ K) most closely correspond to hexaaquo iron(II) coordination [20,21,36].

When the mixture of 5-methylresorcinol and iron(III) was kept open in air to dry under ambient conditions, the resulting dried solid gave a more complicated Mössbauer spectrum (Fig. 2c), with two different forms of iron(II) species (altogether comprising over 3/4 of the total iron), along with a residual ferric form (see Table 1). The parameters of the two ferrous forms evidently represent different complexes of iron(II) with the possible residuary 5-methylresorcinol and/or its oxidation product(s), which might include 1,2,4-trihydroxy-6-methyl- and/or 1,2,3-trihydroxy-5-methylbenzenes or the relevant hydroxylated quinones [28,33]. Thus, these Mössbauer measurements provide unambiguous evidence that iron(III) is gradually reduced by 5-methylresorcinol in moderately acidic (pH ~ 3) aqueous solutions in air.

Remarkably, under the same conditions 4-*n*-hexylresorcinol showed a significantly higher iron(III) reduction rate: already 5 min after mixing the reagents, about a third of the total iron(III) was reduced to $Fe^{II}$ represented by a doublet with the parameters typical for hexaaquo iron(II) complex (Fig. 3a, see also Table 1). As iron(III) ions at pH ~ 3 are well known to be prone to hydrolytic polymerisation, in this case the residual iron(III), which gives virtually the same Mössbauer parameters as those for the aqueous 5-methylresorcinol solution 10 min after mixing (see Table 1), represents, most probably, hydrolysed polymeric and/or colloidal hydroxo species [36] (note that some participation of the organic molecules or their oxidation products in $Fe^{III}$ coordination cannot be ruled out merely on the basis of $Fe^{III}$ Mössbauer parameters; for instance, the residual $Fe^{III}$ in the aqueous 5-methylresorcinol solution kept for 5.5 h after mixing gave a slightly lower quadrupole splitting).

After similarly drying the mixture of 4-*n*-hexylresorcinol and iron(III) in air under ambient conditions, the resulting dried solid gave a yet more complicated Mössbauer spectrum, with *three* different forms of ferrous species (altogether comprising about 2/3 of the total iron), along with a residual ferric form (Fig. 3b; see also Table 1). The parameters of the ferrous forms in the dry residue, except probably for form 3 (which are rather close to those for hydrated $Fe^{2+}$ ions in solid ferrous chloride hydrates at $T \sim 80$ K [36]), evidently correspond to different iron(II) complexes with the possible residuary 4-*n*-hexylresorcinol and/or its oxidation product(s). Note, however, that these parameters and, consequently, the $Fe^{2+}$ microenvironments (i.e., type and arrangement of donor atoms in the ligands) differ from those for the dried 5-methylresorcinol solution (see above and Table 1). Different positions of the alkyl substituents in the 1,3-dihydroxybenzene moieties might be expected to lead to different oxidation products of the 5- and 4-alkyl-substituted resorcinols [28,33]. This factor, possibly together with the length of the alkyl group, might be a reason for the signif-

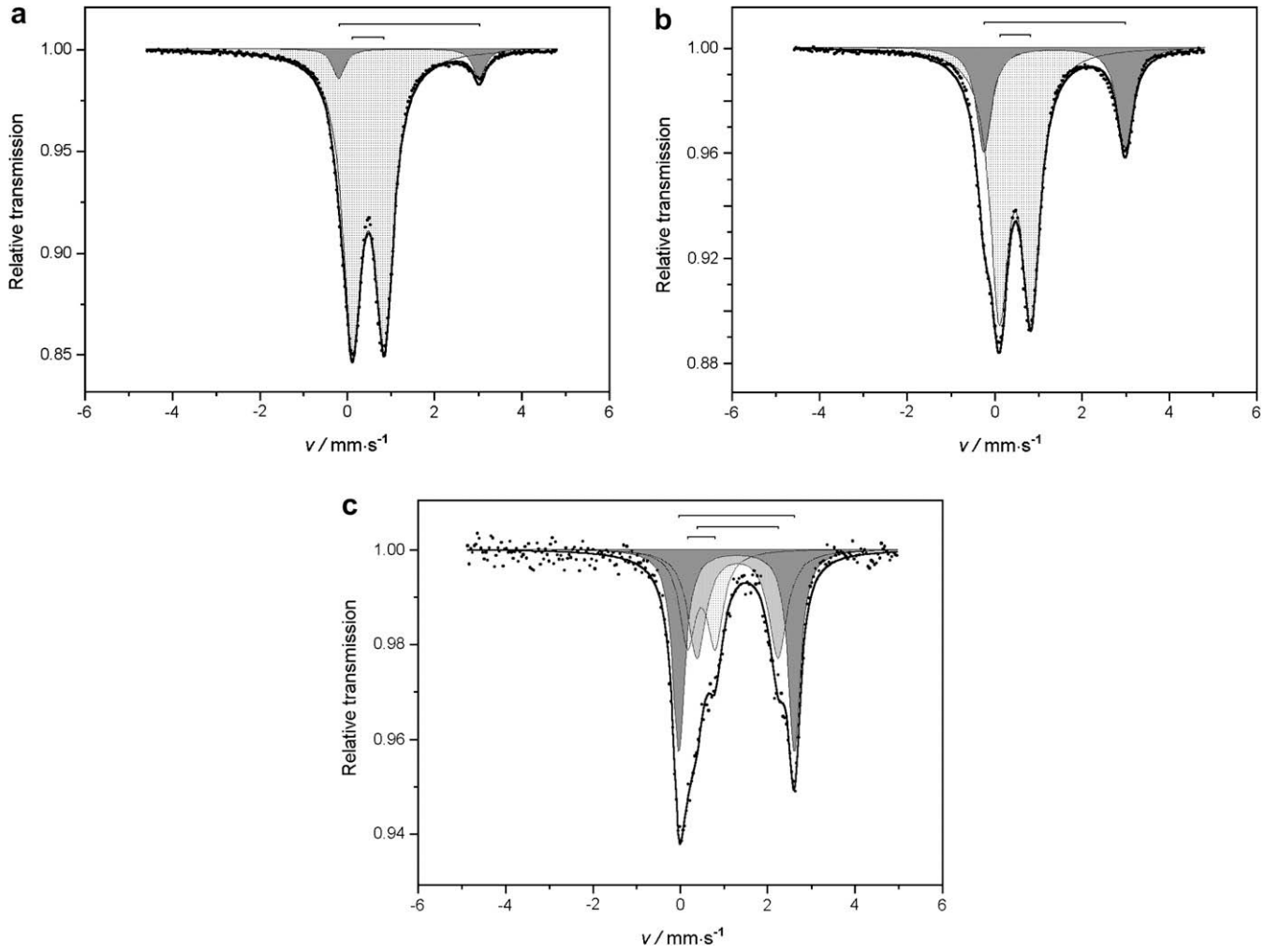


**Fig. 2.** Mössbauer spectra of $^{57}$Fe(III)-containing aqueous solutions of 5-methylresorcinol (orcinol) rapidly frozen (a) 10 min and (b) 5.5 h after mixing, as well as of (c) the solid residue obtained by air-drying the solution at room temperature (all spectra measured at $T = 80$ K). The shaded areas (quadrupole doublets) represent contributions of $Fe^{III}$ (the most lightly shaded area) or $Fe^{II}$ (darker shaded areas) to the whole spectrum area (defined by the outer solid-line envelope); in spectrum (c), the two more dark-shaded areas correspond to two different iron(II) species (forms 1 and 2, respectively; see Table 1). The position of each quadrupole doublet is shown by a square bracket above the spectra (the same for Figs. 3 and 4).

icant difference in the iron(III) reduction rate observed in the present study for 5-methyl- (i.e., 5-$C_1$-alkyl; a gradual redox process) and 4-*n*-hexyl-substituted resorcinol (4-*n*-$C_6$-alkyl; a much more rapid redox process).

Under similar conditions (see Section 2), homoserine lactone (an unacylated analogue of *N*-acylhomoserine lactone common microbial signalling molecules involved in quorum sensing in a variety of bacteria [1–5]) did not show any noticeable reduction of iron(III) in solution (Fig. 4a), although some minor amount (under 3%) of iron(II) was yet detected in its dried residue (Fig. 4b; see also Table 1). Note that similar trends of iron(III) reduction in going from the autoinducer solutions to their air-dried solids have been obtained for 5-methylresorcinol (orcinol), 4-*n*-hexylresorcinol and homoserine lactone, respectively, also at slightly lower pH ~ 1.5 (data not shown; to be discussed in more detail elsewhere).

The Mössbauer parameters of the residual iron(III) forms for all the *dried solids* studied (see Table 1) differed slightly but distinctly from each other in either isomer shift (ranging from 0.48 to 0.54 mm $s^{-1}$) or quadrupole splitting values (ranging from 0.63 to ca. 0.77 mm $s^{-1}$). However, these parameters for high-spin $Fe^{III}$ are rather nonspecific and at this step do not allow the chemical nature of the ferric species to be revealed (e.g., to distinguish between possible different organic complexes and different hydroxylated dimeric/polymeric moieties [19–21]), which would require additional studies.

Nevertheless, it has to be specially emphasized that the redox behaviour of all the microbial autoinducers studied in this work (in particular, of both the alkylresorcinols) *upon drying* was totally different from that of indole-3-acetic acid (auxin) aqueous solutions studied earlier. Indole-3-acetic acid had as well been found to be capable of gradually reducing iron(III) in moderately acidic $NO_3^-$-containing and/or aerated aqueous media, up to total reduction within 2 days [19–21,37,38], but upon subsequent drying in air, all iron(II) had been found to get reoxidised back to iron(III) [38]. In contrast, in dried solutions of the alkylresorcinols under study, the resulting iron(II) was evidently not reoxidised upon drying in air; moreover, it was in fact found to be dominating in the dried solid (see Table 1). For instance, for iron(III)-containing 5-methylresorcinol aqueous solution, the relative content of iron(II) in the final dried residue was ca. 3 times higher than that in the solution 5.5 h after mixing; the same trend was observed for 4-*n*-hexylresorcinol (see Table 1). Even for homoserine lactone, which did not show any signs of iron(III) reduction in aqueous solution, some iron(II) was still detected in the dried solid (see above). These findings point to significantly stronger reducing and/or binding

**Table 1**
Mössbauer parameters[a] for $^{57}Fe^{III}$-containing aqueous solutions of 5-methylresorcinol (orcinol; see also Figs. 1 and 2), 4-*n*-hexylresorcinol (containing 20% v/v ethanol; see also Figs. 1 and 3) and homoserine lactone (see also Figs. 1 and 4) at pH ~ 3 (total [Fe] = 16 ± 1 mM; 1:3 Fe-to-organics ratios), rapidly frozen after specified periods of time, and for their solid residues obtained by drying in air at ambient temperature (measured at $T$ = 80 K).

| Organics mixed with $^{57}Fe^{III}$ in solution (sample measured) | Time[b] | Fe oxidation state | $\delta$[c] (mm s$^{-1}$) | $\Delta$[d] (mm s$^{-1}$) | $\Gamma_{exp}$[e] (mm s$^{-1}$) | $S_r$[f] % |
|---|---|---|---|---|---|---|
| 5-Methylresorcinol (*solution*) | 10 min | +3 | 0.48(1) | 0.74(1) | 0.52(1) | 93.3 |
| | | +2 | 1.41(1) | 3.22(1) | 0.34(1) | 6.7 |
| | 5.5 h | +3 | 0.48(1) | 0.72(1) | 0.50(1) | 74.1 |
| | | +2 | 1.38(1) | 3.23(1) | 0.42(1) | 25.9 |
| 5-Methylresorcinol (*dried solid*) | – | +3 | 0.48(1) | 0.63(2) | 0.43(2) | 24.5 |
| | | +2 (*form 1*) | 1.31(1) | 1.86(3) | 0.50(3) | 33.3 |
| | | +2 (*form 2*) | 1.29(1) | 2.65(1) | 0.34(1) | 42.2 |
| 4-*n*-Hexylresorcinol (*solution*) | 5 min | +3 | 0.475(3) | 0.757(4) | 0.49(1) | 68.8 |
| | | +2 | 1.402(3) | 3.388(5) | 0.34(1) | 31.2 |
| 4-*n*-Hexylresorcinol (*dried solid*) | – | +3 | 0.54(1) | 0.65(1) | 0.48(2) | 34.9 |
| | | +2 (*form 1*) | 1.43(1) | 1.55(3) | 0.35(3) | 9.0 |
| | | +2 (*form 2*) | 1.29(1) | 2.54(2) | 0.45(3) | 23.7 |
| | | +2 (*form 3*) | 1.359(3) | 3.10(1) | 0.34(1) | 32.4 |
| Homoserine lactone (*solution*) | 10 min | +3 | 0.483(1) | 0.785(1) | 0.374(1) | 100 |
| Homoserine lactone (*dried solid*) | – | +3 | 0.484(2) | 0.766(4) | 0.52(1) | 97.6 |
| | | +2 | 1.45(1) | 2.75(1) | 0.52(1) | 2.4 |

[a] Errors (in the last digits) are given in parentheses.
[b] Period from mixing the reagents until rapid freezing of the solution.
[c] Isomer shift (relative to α-Fe at ambient temperature).
[d] Quadrupole splitting.
[e] Full line width at half maximum.
[f] Partial resonant absorption areas of spectral components which represent relative contents of the corresponding Fe forms assuming a common recoilless fraction for all forms (for $S_r$, relative error is ca. ±4% of the given values).

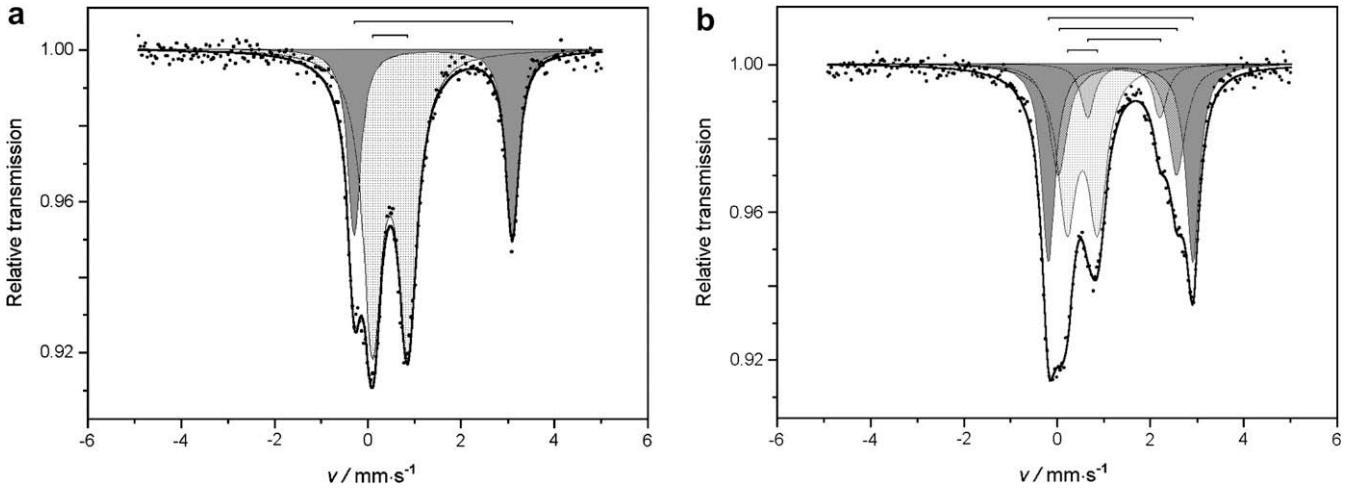


**Fig. 3.** Mössbauer spectra (a) of $^{57}Fe(III)$-containing aqueous solution of 4-*n*-hexylresorcinol rapidly frozen 5 min after mixing and (b) of the solid residue obtained by air-drying the solution at room temperature (both spectra measured at $T$ = 80 K). The shaded areas (quadrupole doublets) represent contributions of $Fe^{III}$ (the most lightly shaded area) or $Fe^{II}$ (darker shaded areas) to the whole spectrum area (defined by the outer solid-line envelope); in spectrum (b), the three more dark-shaded areas correspond to three different iron(II) species (forms 1–3, respectively; see Table 1).

(coordinating) power of the autoinducers or their oxidation products as compared to those of auxin.

Finally, it should be mentioned that these findings showing substantial iron(III) reduction by alkylresorcinols are still more interesting, considering the report of Pracht et al. [39]. They did not find any reduction of iron(III) by resorcinol (i.e., an unalkylated *m*-dihydroxy analogue, 1,3-dihydroxybenzene, named "resorcine" in [39]) in acidic aqueous solutions, whereas both its *o*- and *p*-dihydroxy isomers, catechol and hydroquinone, respectively, substantially reduced iron(III) within the first hour [39]. Note that electronic coupling of the *o*- and *p*-dihydroxy substituents via resonance with the aromatic moiety yields facile and in many instances reversible electron transfer [40]. In contrast, *m*-substituted forms (resorcinol derivatives) do not possess this level of resonance, are not involved in reversible two-electron transfer (and hence are not classified as quinones) and are therefore less prone to oxidation [40]. Note also for comparison that two other *m*-dihydroxy isomers, 2,4- and 2,6-dihydroxybenzoic acids (at $10^{-5}$ M), in the presence of $2.0 \times 10^{-4}$ M $FeCl_3$ in 20 mM sodium acetate buffer (at pH 4.5) were recently reported by Aguiar and Ferraz [41] to reduce ca. 0.9% and 4.4% of all iron(III) to iron(II) in 30 min, whereas catechol and hydroquinone under those conditions gave ca. 12.4% and 9.8% reduction, respectively. These facts, in line with our experimental observations in this study, point to the importance of the structure (i.e., the nature and position of an organic substituent relative to the *m*-dihydroxy moiety in the benzene ring) of a substituted resorcinol, which deter-

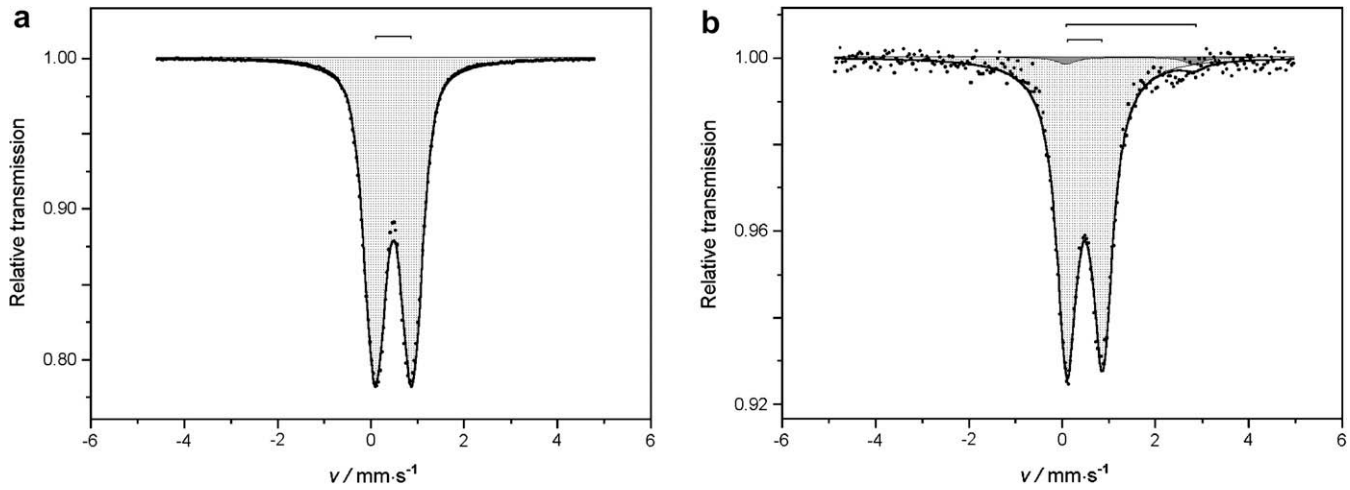


**Fig. 4.** Mössbauer spectra (a) of $^{57}$Fe(III)-containing aqueous solution of homoserine lactone rapidly frozen 10 min after mixing and (b) of the solid residue obtained by air-drying the solution at room temperature (both spectra measured at $T = 80$ K). In spectrum (b), the dark-shaded area (weak doublet with a larger quadrupole splitting) corresponds to iron(II) species (see Table 1).

mines the reducing power of the resulting organic molecule with respect to iron(III).

## 4. Conclusions

The results obtained unambiguously imply that microbial alkylresorcinol autoinducers can be abiotically oxidised by soil iron(III) in moderately acidic media, which can be of ecological significance with regard to microbial signalling processes. Note that metal ion complexation *per se*, although changing the properties of the resulting complex as compared to the free ligand, still does not mean degradation of the latter. On the contrary, some of the signalling molecules can relatively easily be oxidised by direct action of redox-active metal species (or via their catalytic activity), similar to the aforementioned processes involving iron(III) and auxins [19–21,37,38] or alkylresorcinols (observed in this study). In such cases, the resulting product is completely another substance with different properties. Thus, an oxidation process involving a particular molecular signal is very likely to exclude the latter from any signalling pathways, which is equal to "message undelivery", directly affecting microbial autoregulation and, generally, intercellular communication within the microbial consortium.

It should also be noted that the iron(III) reduction rate in moderately acidic solutions of 4-*n*-hexylresorcinol was found to be much higher than that of 5-methylresorcinol. Thus, according to the results of this study and their comparison with some data available from the literature [39–40], the structure of the alkylresorcinol molecule (in particular, the position and nature of an alkyl substituent) influences the ability of the organics to be oxidised, e.g. by iron(III) in moderately acidic aqueous media.

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