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Soil remediation with a microbial community established on a carrier: Strong hints for microbial communication during 1,2,4-Trichlorobenzene degradation

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HIGHLIGHTS

- During 1,2,4-TCB mineralization signal molecules are produced.
- Microbial communities attached on a carrier establish a cell-to-cell communication.
- Efficient cell-to-cell communication results in high 1,2,4-TCB mineralization.
- Quantity and quality of signaling molecules depend on the type of inoculation.
- Cell-to-cell communication occurs via interspecies communication.

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ABSTRACT

The objective of the present study was to get more insight into the mechanisms that govern the high mineralization potential of a microbial community attached on a carrier material, as we found in an earlier study (Wang et al., 2010). A 1,2,4-Trichlorobenzene (1,2,4-TCB) degrading microbial community - attached (MCCP) and non-attached (MCLM) on clay particles – was inoculated into a simplified mineral medium system. Signaling molecules (AHLs), cell growth and 1,2,4-TCB mineralization were measured at different sampling points. The production of AHLs in the MCCP system increased continuously with increasing key degrader (*Bordetella* sp.) cell growth and a positive correlation was observed between the production of AHLs and 1,2,4-TCB mineralization. In the MCLM system, however, 1,2,4-TCB mineralization was lower than in the MCCP system; the AHLs production per *Bordetella* cell was higher than in MCCP and there was no correlation between AHLs and mineralization. Moreover, in the MCCP system less different AHLs were produced than in the MCLM system. These results indicate that a microbial community attached on a carrier material has an advantage over a non-attached community: it produces signaling molecules with much less energy and effort to achieve a well-directed cell-to-cell communication resulting in a high and effective mineralization.

1. Introduction

Numerous reports exist worldwide on the contamination of soils with organic chemicals and resulting risks for human health, and therefore many scientists were and are still discussing the pros and cons of various bio-augmentation approaches for decontaminating soils. Remarkable reviews on this wide topic have been presented (Gentry et al., 2004; Fantroussi and Agathos, 2005). When using microbial induced approaches for soil decontamination, various inoculation techniques are available: (i) isolated microbial strains are applied to soils; (ii) a combination of various and very efficient strains is used; (iii) specific microbial communities which were extracted from an adapted soil are inoculated; (iv) the microorganisms are introduced into soils as free cells or (v) as cells established on carrier materials.

One of the main problems of such bio-augmentation approaches is their sustainability. In laboratory experiments the survival rate of inoculated microbes whether inoculated as free cells or as cells established on a carrier into sterilized soil materials even

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at relatively low chemical concentrations is not critical because of the lacking competition between inoculated and native soil microbes, and thus an effective degradation of the chemical by the inoculated microbial strain can take place (Siripattanakul et al., 2009). But as soon as contaminated soils still containing their native microbial population are inoculated, the survival of the added microbes becomes a critical factor. The survival rate of microbes inoculated as free cells into soils mainly depends on a certain selection pressure caused by high (>1000 mg kg^{-1} soil) or middle (50–1000 mg kg^{-1} soil) contaminant concentrations. Under such conditions even horizontal gene transfer from inoculated donor microbes to native soil receptor cells can be observed (Dejonghe et al., 2000) enabling some native microbes to effectively degrade a certain contaminant. Thus, a successful and sustainable inoculation of single strains is feasible in high or middle contaminated soils (Gomes et al., 2005; Lima et al., 2009).

But when considering the sustainability of such inoculation approaches in soils with low contaminant concentrations, like in agricultural soils, an important question arises: is it possible to achieve a long term establishment of an isolated strain after an initially successful phase as soon as the contaminant concentration is considerably reduced? As it can be seen e.g. from the results of Lima et al. (2009) the number of inoculated cells can decrease with time and thus long-term survival of those cells in soils might be questionable. Reasons for such cell reduction include the competition between native and inoculated cells for limited nutrients, along with antagonistic interactions and predation by protozoa and bacteriophages (Gentry et al., 2004). But how to withstand such a trend, and how in general to proceed if even low contamination levels should be avoided e.g. in the case of pesticide residues in agricultural soils? In our previous work (Schroll et al., 2004; Grundmann et al., 2007; Wang et al., 2010), we tested several inoculation approaches and we could show that the use of microbial communities extracted from soils effective in contaminant degradation which were artificially attached to a soil-like carrier material was the most efficient and sustainable approach (Wang et al., 2010). Thus, the question arises: What are the reasons for such very efficient pollutant degradation even at relatively low concentrations of the contaminant in soils?

In soils, bacteria rarely exist as single, isolated entities, but rather as communities comprised of many other species (Atkinson and Williams, 2009; Lowery et al., 2009). To survive in competitive environments, micro-organisms have developed elaborate tactics such as the formation of biofilms (Lowery et al., 2009). In such biofilms microbes have several advantages that are not enjoyed by discrete bacteria; e.g. organisms within biofilms can withstand shear forces, nutrient deprivation, pH changes and antibiotics in a more pronounced manner (Bhinu, 2005; Singh et al., 2006). Biofilms generally consist of cell aggregates, in which a cell-to-cell communication can take place via the exchange of specific signaling molecules (Parsek and Greenberg, 2005). In gram-negative bacteria such signal molecules are formed by N-acyl-homoserine lactone (AHL) structures (Shiner et al., 2005; Camilli and Bassler, 2006; Uroz et al., 2009; Teplitski et al., 2011). The 1,2,4-Trichlorobenzene (1,2,4-TCB) degrading microbial community isolated in our previous study (Wang et al., 2010), consists of gram-negative bacteria; therefore, in the present study we focus on the detection of AHLs.

It was our strategy to identify these signaling molecules in relation to the mineralization of the contaminant 1,2,4-TCB. Positive findings and correlations could be considered as a hint for (i) the existence of a biofilm and (ii) for the higher effectiveness of a biofilm in mineralizing contaminants. We could not conduct this study in soil because there exist a high number of bacteria which produce a large amount of signal molecules, and those would cover the AHLs produced by the inoculated microbial community. Therefore, we performed this study in a more simplified system – liquid cultures – knowing that the out-coming results must be carefully interpreted. It was our main goal to compare the mineralization of 1,2,4-TCB with the formation of AHLs in liquid cultures inoculated (i) with a 1,2,4-TCB degrading microbial community attached to a carrier material (MCCP) and (ii) with the same microbial community without any carrier material (MCLM). To underline and strengthen our conclusions, some results of our former publication (Wang et al., 2010) were included as well.

2. Materials and methods

2.1. Bacteria, medium and chemicals

A 1,2,4-TCB-mineralizing bacterial community containing *Bordetella* sp. as the only 1,2,4-TCB-degrader (Wang et al., 2010) was used. Mineral medium contained 3 g $Na_2HPO_4{\cdot}2H_2O$, 3.5 g KH_2PO_4, 1 g NH_4NO_3, 100 mg $MgSO_4{\cdot}7H_2O$, 50 mg $Ca(NO_3)_2{\cdot}4H_2O$ per liter of ultrapure water (Millipore) with pH 6.5.

Uniformly ^{14}C-ring-labeled 1,2,4-TCB obtained from International Isotope (Munich, Germany) was mixed with non-labeled 1,2,4-TCB (Dr. Ehrenstorfer, Augsburg, Germany) for all experiments resulting in a specific radioactivity of 3.79 Bq μg^{-1}. Scintillation cocktails were obtained from Packard (Dreieich, Germany).

For immunoassay analysis of AHLs, 3-oxo-C_{10}-HSL, Protein G from *Streptococcus*, hydrogen peroxide (H_2O_2), 3,3′,5,5′-tetramethylbenzidine (TMB), and Dimethylsulfoxide (DMSO, 99%), were purchased from Sigma–Aldrich (Steinheim, Germany). All other chemicals and solvents were of analytical grade and were purchased from Merck (Darmstadt, Germany).

2.2. Establishing the microbial community on clay particles

To establish the microbial community on clay particles, a liquid culture experiment with 18 sets was performed in 50 mL mineral medium spiked with 1 g of sterile expanded clay particles and with 1 mL liquid medium containing the 1,2,4-TCB mineralizing bacterial community with $(1.4 \pm 0.1) \times 10^7$ *Bordetella* sp. cells (Wang et al., 2010). An amount of 750 μg ^{14}C-1,2,4-TCB dissolved in 25 μL acetone was applied to serve as the carbon source. The liquid culture was incubated in the dark at 100 rpm at 20 ± 1 °C. During the incubation the cultures were aerated twice per week for 1 h at an air exchange rate of 1 L h^{-1}. In order to keep the TCB concentration in the liquid culture nearly constant, 25 μL ^{14}C-1,2,4-TCB (750 μg) were reapplied after each aeration. After 2 weeks, the clay particles were separated from the liquid culture and washed twice with 20 mL PBS (Bertaux et al., 2007) before inoculation.

2.3. Biodegradation experiments

In general, these experiments were conducted (i) to quantify the mineralization of 1,2,4-TCB, (ii) to quantify AHL production during the mineralization process; and finally (iii) to determine the pattern of the AHLs for a selected sampling point at day 4. The comparative experiments were conducted in liquid cultures with 50 mL mineral medium and 25 μL acetonic ^{14}C-1,2,4-TCB (750 μg)-solution inoculated with (i) 1 g clay particles with attached microbial community (MCCP) and (ii) 1 mL liquid medium containing the microbial community (MCLM). Incubation, aeration and reapplication of the liquid cultures were the same as described in Section 2.2. The evolved $^{14}CO_2$ and volatile ^{14}C-substances were trapped separately, followed by a measurement of radioactivity via scintillation counting (Wang et al., 2010). Each set started with 18 replicates. On days 1, 4, 7, 14, 21 and 28 three replicates of each treatment were sacrificed for sample analysis: cell counting via

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FISH and determination of the sum of AHLs via immunochemical analysis. The replicates from day 4 were additionally analyzed for the pattern of the AHLs via FT-ICR-MS.

In the MCCP variants the clay particles were separated from the liquid medium and subsequently washed twice with 10 mL PBS. Aliquots of clay particles were taken for counting the *Bordetella* sp. cells and the remaining clay was used for AHLs extraction for immunoassay analysis and FT-ICR-MS analysis. From the liquid medium of the MCLM and MCCP variants aliquots were taken for cell counting and the rest of the liquid medium was centrifuged at 6000 rpm for 30 min. Aliquots of the supernatant were taken for immunoassay analysis of AHLs, and for AHLs extraction for FT-ICR-MS analysis. In the MCCP variants the respective aliquots (clay particles and liquid medium) were pooled together prior to analysis (see Section 2.4).

2.4. Cell counting with fluorescent in situ hybridization (FISH)

The bacteria were counted using a modified protocol from Bertaux et al. (2007) as described in Wang et al. (2010). In the MCLM variants, a 1 mL aliquot of the liquid culture was mixed with 3 mL 4% PFA solution and incubated for 2 h for fixation. In the MCCP variants, the microbes were counted in aliquots as follows: firstly, an aliquot of clay particles was taken, and microbes were extracted with 9 mL extraction solution (0.1 g L^{-1} NaCl, 0.02 g L^{-1} $CaCl_2{\cdot}2H_2O$, 0.2 g L^{-1} $MgSO_4{\cdot}7H_2O$, 5.0 g L^{-1} Tween 80) using a ball mill (Retsch, Haan, Germany) with an achievement of 80% for 7 min. The extract was transferred into a 15 mL centrifuge tube followed by ultrasonication for 4×30 s with each 1 min rest in between. After sedimentation for 15 min, 1.8 mL supernatant were taken for fixation followed by purification with 1 mL Nycodenz (1.3 g mL^{-1}, Gentaur, Belgium). The purified bacteria were finally combined with fixed bacteria from 1 mL liquid phase of the MCCP to count the cells in the total system – liquid phase plus clay particle phase. For both variants, the fixed bacteria were immobilized on polycarbonate membrane filters (GTTP 0.2 μm, Millipore, Germany) for hybridization with specific probes for *Bordetella* sp. (Wang et al., 2010) followed by enumeration using a Zeiss Axioplan II epifluorescence microscope (Zeiss, Oberkochen, Germany).

2.5. Extraction of AHLs

2.5.1. Extraction of AHLs from the MCLM variants

For FT-ICR-MS analysis 45 mL of centrifuged liquid phase was subjected to SPE (Mega Bond Elut C18, Varian, Darmstadt, Germany) to extract the AHLs according to Li et al. (2006).

2.5.2. Extraction of AHLs from the MCCP variants

Aliquots of clay particles were dried at 30 °C for 3 h followed by ultrasonication extraction of AHLs twice with 5 mL acetonitrile (ACN) for 15 min, respectively. Both 5 mL ACN extracts were combined and concentrated to about 1 mL on a rotation evaporator at 48 °C; further evaporation to dryness was conducted under a gentle stream of nitrogen. The samples were dissolved in 1 mL MQ/ACN (v/v, 9/1) and filtered through 0.2 μm PTFE membrane filters (VWR, West Chester, PA, USA). The filtered extracts were recombined with 45 mL centrifuged liquid medium and 1 mL of the combined phases was removed for immuno assay analysis. The remaining solution was extracted with SPE as described previously (Li et al., 2006) for FT-ICR-MS analysis.

2.6. Immunochemical analysis of AHLs

Samples from both variants and all sampling points were subjected to immunochemical analysis. From the MCLM variants aliquots were taken after centrifugation and directly measured via immunochemical analysis. The MCCP variants were treated as described in Section 2.5.2 before measuring AHLs using the enzyme tracer format ELISA (enzyme-linked immunosorbent assay) as described previously (Chen et al., 2010). Briefly, 2 μg mL^{-1} protein G from *Streptococcus* was used to coat the microtiter plate (200 μL $well^{-1}$ in 50 mM carbonate buffer, pH 9.6) over night at 4 °C. After washing the plate with PBST buffer, 150 μL mAb HSL1/2-2C10 (400 ng mL^{-1}) was added and incubated for 2 h at 25 °C. The plate was washed again, a sample volume of 100 μL was added to a serial of 3-oxo-C_{10}-HSL standards in 1,2,4-TCB mineral medium (0, 0.5–20,000 μg L^{-1}) and incubated for 1 h. Subsequently 50 μL 1:1000 diluted HSL3-HRP enzyme-tracer solution was added and the mixture was shaken at 200 rpm for 30 min. After washing, 150 μL TMB was used for enzymatic reaction for 15 min, followed by stopping with 50 μL 2 M H_2SO_4. The absorbance measurements were performed with a Spectra Max M5^e (Molecular Devices, Palo Alto, USA) microplate reader at 450 nm (reference 650 nm). A blank, consisting of all buffers, was subtracted from all values. The results were calculated by SOFTmax Pro and expressed as 3-oxo-C_{10}-HSL equivalents. Four replicates were performed for each sample. The AHLs were analyzed within a detection range of 90–9000 μg L^{-1} according to the 3-oxo-C_{10}-HSL standard curve.

2.7. AHL identification by FT-ICR-MS

For the samples which were sampled after 4 d of incubation, identification of AHLs was performed using high-resolution mass spectra (FT-ICR-MS) as described previously (Fekete et al., 2007, 2010). Prior to analysis the AHLs were extracted from the MCLM and MCCP variants as described above (Section 2.5). FT-ICR mass spectra analyses of AHLs were acquired on a Bruker Daltonics (Bremen, Germany) Apex Qe 12T system equipped with an APOLLO II electrospray ionization source. The samples were diluted in methanol and introduced into the electrospray source at a flow rate of 120 μL h^{-1} with a nebulizer gas pressure of 20 psi and a drying gas pressure of 15 psi. The spectra were externally calibrated based on arginine clusters (5×10^{-5} M). The spectra were acquired with a time domain of 1 MW over a mass range of 100–1000 *m*/*z*. One hundred scans were accumulated for each analyzed sample.

2.8. Data analysis and statistics

Analysis of *Duncan's Multiple Range Test* was performed using the software package SPSS 13.0 at the significant level of $p < 0.05$.

3. Results and discussion

3.1. Production of signaling molecules

AHL signaling molecules were produced in different amounts and showed different kinetics in the two systems. In the system where the microbial community was not attached on a carrier (MCLM) the AHLs concentration increased within the first day, decreased between day 1 and day 4, increased again from day 4 to a maximum at day 14 and then started to decrease again (Fig. 1a). In the MCCP system where the microbial community was attached on clay particles, the cells produced less AHLs at the beginning compared to the MCLM system, but the AHLs production increased continuously and after 21 d it was higher than in the MCLM system (Fig. 1a). The *Bordetella* cells increased continuously in both test systems whereby the increase in the MCCP system was twofold higher than in the MCLM system (Fig. 1b).

By detecting signal molecules in the liquid cultures we got strong hints for cell-to-cell communication within the 1,2,4-TCB degrading microbial community. But the communication in liquid

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culture was strongly dependent on the kind of inoculation – whether the microbial community was transferred directly (MCLM) into the medium or attached on clay particles (MCCP). In both systems the cell counts increased and at the beginning the production of AHLs also increased. Most probably the microbial community was already used to cell-to-cell communication in its natural soil environment and after inoculation to liquid culture it started to continue with communication. But later on, the MCLM system reduced the production of AHLs since this effort for communication was not effective enough and the mineralization of 1,2,4-TCB was lower than in the MCCP system. It seems that the microbial cells in the MCLM system were not able to establish a well-directed communication towards 1,2,4-TCB degradation. This could be due to the fact that the microbial cells in the MCLM system were distributed in a larger volume than the cells in the MCCP system. The initial conditions in both systems were as follows: In the MCLM system the cells were distributed in a volume of 50 mL liquid medium. In the MCCP system the microbial cells were attached on clay particles. The clay particles had a total volume of around 1 cm^3 and a pore volume of ca. 50% and if we suppose that the microbial cells were exclusively distributed in the inner pore space, the volume that was available for the cells amounted to 0.5 mL. This means that the concentration of the microbial cells in the MCLM system was 100 times lower than in the MCCP system and therefore the spatial distances between the cells were much greater in the MCLM system. We suppose that because of the lower concentration and the greater distances the communication between the cells in the MCLM system was hampered and therefore they reduced the energy consuming production of AHLs. From the present data we do not have any information how the reduction of AHLs production proceeds with time, we only could observe a considerable reduction of AHLs production after day 14.

Due to the fluctuating AHLs production in the MCLM system we could not find any correlation with the cell counts (Fig. 2a). However, in the MCCP system where an effective cell-to-cell communication was established we found a good correlation between AHLs production and cell counts (Fig. 2b).

3.2. Relationship between 1,2,4-TCB mineralization and signaling molecules

Though 1,2,4-TCB was rapidly mineralized in both inoculated liquid cultures, the MCCP system showed a 1.5-fold higher TCB mineralization than the MCLM system. The key degrader cells (*Bordetella* sp.) in the MCCP variant grew twice as fast as the cells in the MCLM variant ($p < 0.05$). In both test systems, there was a positive correlation between cell counts and 1,2,4-TCB mineralization (MCLM: $y = 88.80x$, $R^2 = 0.96$; MCCP: $y = 67.05x$, $R^2 = 0.92$).

From these results, one could suppose that 1,2,4-TCB mineralization is governed by the amount of cells: high cell counts lead to high mineralization; and the observed higher mineralization in the MCCP system would just be due to the higher amount of cells in

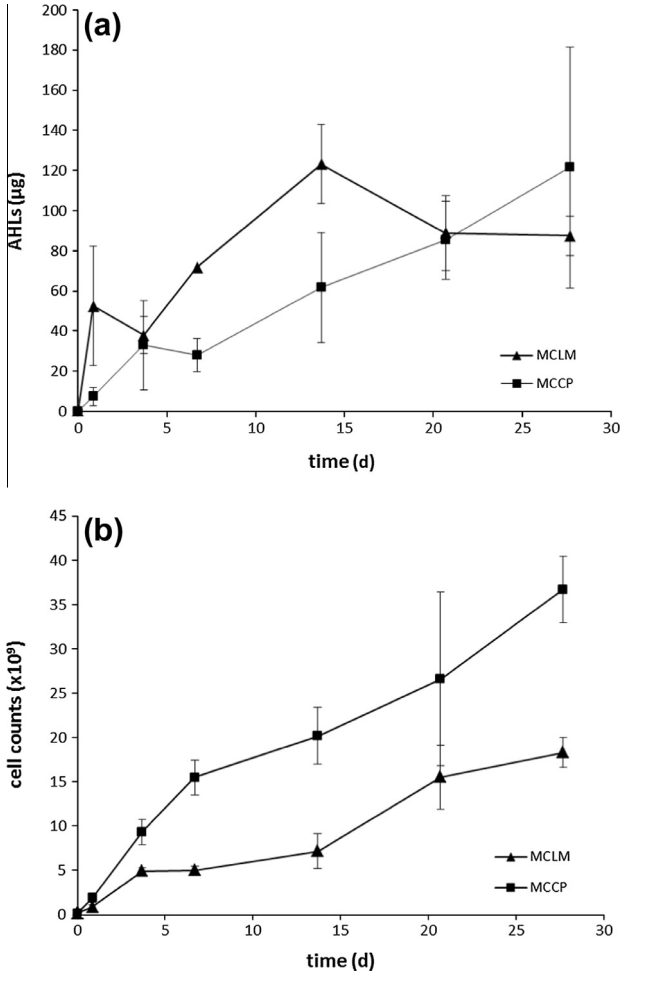

Fig. 1. Liquid culture with microbial community in liquid medium (MCLM) and liquid culture with microbial community attached on clay particles: (a): production of AHLs; and (b): growth of *Bordetella* cells. $n = 3$.

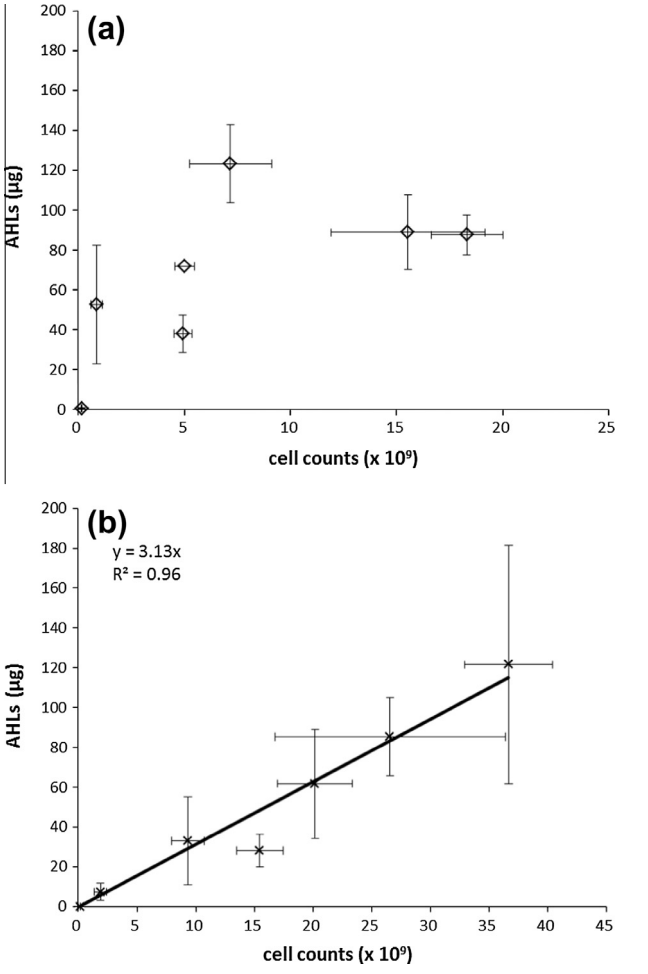

Fig. 2. Correlation between AHLs production and amount of *Bordetella* sp. cells: (a): liquid culture with microbial community in liquid medium (MCLM); and (b): liquid culture with microbial community attached on clay particles (MCCP). $n = 3$.

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this system. But, there is another important result which counteracts this argumentation: we found a highly significant positive correlation between 1,2,4-TCB mineralization and AHLs production during the whole incubation period of 28 d exclusively in the MCCP system (Fig. 3b) – in the MCLM system we could not find any correlation between AHLs production and mineralization (Fig. 3a). This important finding indicates that communication between the microbes plays a role for a more efficient TCB mineralization in the MCCP system.

Nevertheless, there was also 1,2,4-TCB mineralization in the MCLM system, where the microbial community was applied without a carrier. This can be explained by the presence of the key degrader cells (*Bordetella* sp.) in the microbial community, which have the ability to degrade TCB even when there is no efficient communication between the cells. But in the MCCP system – where the microbial cells were organized on a carrier, where the distances between the cells were lesser and where a well-directed communication was established – we observed a higher degradation capacity of the microbes than in the MCLM system. Thus, cell-to-cell communication within the microbial community on the carrier material led to a higher cell growth and finally to a more effective utilization of the C source, 1,2,4-TCB, resulting in a higher mineralization.

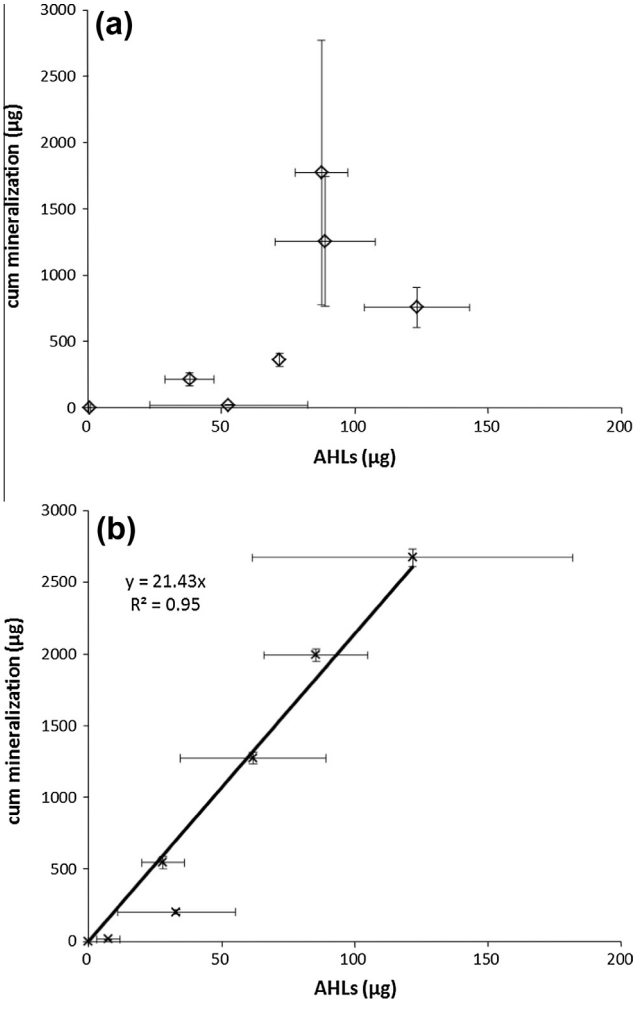

Fig. 3. Correlation between 1,2,4-TCB mineralization and AHLs production: (a) liquid culture with microbial community in liquid medium (MCLM); and (b): liquid culture with microbial community attached on clay particles (MCCP). $n = 3$.

3.3. Quality of signaling molecules

After 4 d of incubation, not only the sum of AHLs was measured but also the pattern of AHLs was determined to get an idea about the composition of the signaling molecules produced in the two different systems. Table 1 summarizes the putative AHLs in MCLM and MCCP. In the system without clay particles (MCLM) 10 different AHLs were detected ranging from C_{10} to C_{16}, whereas only 6 different AHLs – ranging from C_{10} to C_{14} – were detected in the variant with clay particles (MCCP). There was no big difference in the intensities of the AHLs for both treatments, but nevertheless, the MCCP system showed lower intensities of all AHLs than the MCLM system. 3-oxo-$C_{12:1}$-HSL showed the highest intensity in both systems.

Since bacteria not only synthesize the signal molecules but also enzymes that degrade the signal molecules (Zhang et al., 2002; Uroz et al., 2008), we additionally analyzed homoserines – degradation products which originate from hydrolysis of AHLs. In the MCLM system, 11 homoserines (HSs) ranging from C_8 to C_{16} were detected (Table 2), whereas in the system with clay particles (MCCP) only 5 HSs ranging from C_8 to C_{14} were detected. In both systems, more HSs were detected than AHLs. The concentration of most of the HSs was lower in the MCCP system than in the MCLM variant – this trend was already observed for the AHLs. But for the HSs there was one exception: the intensity of 3-oxo-$C_{12:1}$-HS was significantly higher in the MCCP system as compared with the MCLM system. Additionally, 3-oxo-$C_{12:1}$-HS was the HSs derivative that was present in the highest intensity of all HS in both systems. The corresponding precursor chemical – 3-oxo-$C_{12:1}$-HSL – was also the dominant AHL derivative in both systems (Table 1). This indicates that 3-oxo-$C_{12:1}$-HSL is probably an important signaling molecule which contributes to a highly efficient 1,2,4-TCB mineralization. Since we analyzed the quality of the AHLs at just one sampling point this is only a rough indication that must be further investigated by conducting detailed kinetic experiments.

The results show that not only the amount of AHLs was different in the two systems but also the quality of the signal molecules. In the MCCP system, the AHL derivatives produced were less than those produced in the MCLM system. This, again, indicates that there is a different and more efficient communication between cells when the cells are closer to each other and when spatial distances are not as great as in the MCLM system.

3.4. Intra-species communication or inter-species communication?

The question arises whether the communication of the 1,2,4-TCB degrading microbial community is a kind of intra- or inter-species communication? The results from Wang et al. (2010) are very helpful in answering this question and they also support our argument. In the study of Wang et al. (2010), an agricultural soil was inoculated with (i) the single strain (*Bordetella* sp.), attached on clay particles, and (ii) the microbial community, attached on clay particles: when applying the microbial community, the 1,2,4-TCB mineralization in the agricultural soil was enhanced to a big extent, whereas the application of the single strain alone was by far not as successful. The results of our present study show that 1,2,4-TCB mineralization correlates well with the concentration of signaling molecules in the liquid medium, when the microbial community is attached on clay particles. By combining these results from the two studies, one can argue that there is a well-directed communication between the cells of the community on clay particles resulting in high 1,2,4-TCB mineralization whereas the cell-to-cell communication between the single *Bordetella* cells on the clay particles is not so effective because it results in just a minor mineralization capacity. Since the single *Bordetella* sp. cells are not so efficient in 1,2,4-TCB degradation, there is a strong

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Table 1
Putative AHLs at day 4 in the liquid culture with microbial community in liquid medium (MCLM) and liquid culture with microbial community attached on clay particles (MCCP).

Detected mass (m/z)[a]	Formula structure[b]	Putative AHL-Na	Intensity ($\times 10^7$)	
			MCLM	MCCP
276.1569	$C_{14}H_{23}NO_3Na$	$C_{10:1}$-HSL	0.47	–[c]
278.1727	$C_{14}H_{25}NO_3Na$	C_{10}-HSL	0.73	–[c]
		3-oxo-C_{10} and/or		
292.1519	$C_{14}H_{23}NO_4Na$	3-OH-$C_{10:1}$-HSL	1.92	0.86
290.1362	$C_{14}H_{21}NO_4Na$	3-oxo-$C_{10:1}$-HSL	2.60	1.41
306.2039	$C_{16}H_{29}NO_3Na$	C_{12}-HSL	2.98	0.37
318.1675	$C_{16}H_{25}NO_4Na$	3-oxo-$C_{12:1}$-HSL	4.41	3.00
334.2352	$C_{18}H_{33}NO_3Na$	C_{14}-HSL	0.87	–[c]
		3-oxo-C_{14} and/or		
348.2145	$C_{18}H_{31}NO_4Na$	3-OH-$C_{14:1}$-HSL	0.90	0.50
350.2301	$C_{18}H_{33}NO_4Na$	3-OH-C_{14}-HSL	1.38	0.48
362.2665	$C_{20}H_{37}NO_3Na$	C_{16}-HSL	1.23	–[c]

[a] The m/z values provide information on elemental composition with an average error <1 ppm.
[b] The various AHLs are listed as their sodium adducts measured by FT-ICR-MS.
[c] "–" = not detected.

Table 2
Putative homoserines (HS) at day 4 in the liquid culture with microbial community in liquid medium (MCLM) and liquid culture with microbial community attached on clay particles (MCCP).

Detected mass (m/z)[a]	Formula structure[b]	Putative HS-Na	Intensity ($\times 10^7$)	
			MCLM	MCCP
266.1362	$C_{12}H_{21}NO_4Na$	$C_{8:1}$-HS	0.65	–[c]
268.1519	$C_{12}H_{23}NO_4Na$	C_8-HS	1.92	2.17
294.1675	$C_{14}H_{25}NO_4Na$	$C_{10:1}$-HS	30.55	2.59
324.2145	$C_{16}H_{31}NO_4Na$	C_{12}-HS	2.10	1.49
336.1782	$C_{16}H_{27}NO_5Na$	3-oxo-$C_{12:1}$-HS	48.78	72.81
		3-oxo-C_{12} and/or		
338.1937	$C_{16}H_{29}NO_5Na$	3-OH-$C_{12:1}$-HS	0.86	–[c]
352.2458	$C_{18}H_{35}NO_4Na$	C_{14}-HS	1.30	–[c]
364.2094	$C_{18}H_{31}NO_5Na$	3-oxo-$C_{14:1}$-HS	11.23	2.56
		3-oxo-C_{14} and/or		
366.2250	$C_{18}H_{33}NO_5Na$	3-OH-$C_{14:1}$-HS	0.97	–[c]
368.2407	$C_{18}H_{35}NO_5Na$	3-OH-C_{14}-HS	0.41	–[c]
378.2614	$C_{20}H_{37}NO_4Na$	$C_{16:1}$-HS	3.71	–[c]

[a] The m/z values provide information on elemental composition with an average error < 1 ppm.
[b] The various AHLs are listed as their sodium adducts measured by FT-ICR-MS.
[c] "–" = not detected.

indication for an inter-species rather than an intra-species communication.

4. Conclusions

In the present study we got further indications that microbial communities attached on a carrier material produce signaling molecules thereby establishing an effective inter-species communication. This communication empowers the microbial community for high 1,2,4-TCB mineralization in soil, as already shown in our earlier study (Wang et al., 2010). It seems that the application of microbial communities for remediation of soils is a more promising approach than the application of isolated key degrader strains alone. Therefore, we suggest the isolation of active microbial communities, rather than active single strains, and their application on a carrier material as the most effective soil remediation approach.

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References

Atkinson, S., Williams, P., 2009. Quorum sensing and social networking in the microbial world. J. Roy. Soc. Interface 6, 959–978.
Bertaux, J., Gloger, U., Schmid, M., Hartmann, A., Scheu, S., 2007. Routine fluorescence *in situ* hybridization in soil. J. Microbiol. Method 69, 451–460.
Bhinu, V.S., 2005. Insight into biofilm-associated microbial life. J. Mol. Microbiol. Biotechnol. 10, 15–21.
Camilli, A., Bassler, B.L., 2006. Bacterial small-molecule signaling pathways. Science 311, 1113–1116.
Chen, X., Buddrus-Schiemann, K., Rothballer, M., Kramer, P.M., Hartmann, A., 2010. Detection of quorum sensing molecules in *Burkholderia cepacia* culture supernatants with enzyme-linked immunosorbent assays. Anal. Bioanal. Chem. 398, 2669–2676.
Dejonghe, W., Goris, J., El Fantroussi, S., Höfte, M., De Vos, P., Verstraete, W., Top, E.M., 2000. Effect of dissemination of 2,4-dichlorophenoxyacetic acid (2,4-D) degradation plasmids on 2,4-D degradation and on bacterial community structure in two different soil horizons. Appl. Environ. Microbiol. 66, 3297–3304.
Fantroussi, S.E., Agathos, S.N., 2005. Is bioaugmentation a feasible strategy for pollutant removal and site remediation? Curr. Opin. Microbiol. 8, 268–275.
Fekete, A., Frommberger, M., Rothballer, M., Li, X.J., Englmann, M., Fekete, J., Hartmann, A., Eberl, L., Schmitt-Kopplin, P., 2007. Identification of bacterial N-acylhomoserine lactones (AHLs) with a combination of ultra-performance liquid chromatography (UPLC), ultra-high-resolution mass spectrometry, and in-situ biosensors. Anal. Bioanal. Chem. 387, 455–467.
Fekete, A., Kuttler, C., Rothballer, M., Hense, B.A., Fischer, D., Buddrus-Schiemann, K., Lucio, M., Muller, J., Schmitt-Kopplin, P., Hartmann, A., 2010. Dynamic

regulation of N-acyl-homoserine lactone production and degradation in *Pseudomonas putida* IsoF. FEMS Microbiol. Ecol. 72, 22–34.

Gentry, T., Rensing, C., Pepper, I., 2004. New approaches for bioaugmentation as a remediation technology. Crit. Rev. Environ. Sci. Technol. 34, 447–494.

Gomes, N.C.M., Kosheleva, I.A., Abraham, W.-R., Smalla, K., 2005. Effects of the inoculant strain *Pseudomonas putida* KT2442 (pNF142) and of naphthalene contamination on the soil bacterial community. FEMS Microbiol. Ecol. 54, 21–33.

Grundmann, S., Fuss, R., Schmid, M., Laschinger, M., Ruth, B., Schulin, R., Munch, J.C., Schroll, R., 2007. Application of microbial hot spots enhances pesticide degradation in soils. Chemosphere 68, 511–517.

Li, X.J., Fekete, A., Englmann, M., Gotz, C., Rothballer, M., Frommberger, M., Buddrus, K., Fekete, J., Cai, C.P., Schröder, P., Hartmann, A., Chen, G.N., Schmitt-Kopplin, P., 2006. Development and application of a method for the analysis of N-acylhomoserine lactones by solid-phase extraction and ultra high pressure liquid chromatography. J. Chromatogr. A 1134, 186–193.

Lima, D., Viana, P., André, S., Chelinho, S., Costa, C., Ribeiro, R., Sousa, J.P., Fialho, A.M., Viegas, C.A., 2009. Evaluating a bioremediation tool for atrazine contaminated soils in open soil microcosms: The effectiveness of bioaugmentation and biostimulation approaches. Chemosphere 74, 187–192.

Lowery, C.A., Park, J., Gloeckner, C., Meijer, M.M., Mueller, R.S., Boshoff, H.I., Ulrich, R.L., Barry, C.E., Bartlett, D.H., Kravchenko, V.V., Kaufmann, G.F., Janda, K.D., 2009. Defining the mode of action of tetramic acid antibacterials derived from *Pseudomonas aeruginosa* quorum sensing signals. J. Am. Chem. Soc. 131, 14473–14479.

Parsek, M.R., Greenberg, E.P., 2005. Sociomicrobiology: the connections between quorum sensing and biofilms. Trends Microbiol. 13, 27–33.

Schroll, R., Brahushi, F., Doerfler, U., Kuehn, S., Fekete, J., Munch, J.C., 2004. Biomineralisation of 1,2,4-trichlorobenzene in soils by an adapted microbial population. Environ. Pollut. 127, 395–401.

Shiner, E.K., Rumbaugh, K.P., Williams, S.C., 2005. Interkingdom signaling: deciphering the language of acyl homoserine lactones. FEMS Microbiol. Rev. 29, 935–947.

Singh, R., Paul, D., Jain, R.K., 2006. Biofilms: implications in bioremediation. Trends Microbiol. 14, 389–397.

Siripattanakul, S., Wirojanagud, W., McEvoy, J.M., Casey, F.X.M., Khan, E., 2009. Atrazine removal in agricultural infiltrate by bioaugmented polyvinyl alcohol immobilized and free *Agrobacterium radiobacter* J14a: a sand column study. Chemosphere 74, 308–313.

Teplitski, M., Mathesius, U., Rumbaugh, K.P., 2011. Perception and degradation of N-acyl homoserine lactone quorum sensing signals by mammalian and plant cells. Chem. Rev. 111, 100–116.

Uroz, S., Oger, P.M., Chapelle, E., Adeline, M.T., Faure, D., Dessaux, Y., 2008. A *Rhodococcus* qsdA-encoded enzyme defines a novel class of large-spectrum quorum-quenching lactonases. Appl. Environ. Microbiol. 74, 1357–1366.

Uroz, S., Dessaux, Y., Oger, P., 2009. Quorum sensing and quorum quenching: the yin and yang of bacterial communication. ChemBioChem 10, 205–216.

Wang, F., Dörfler, U., Schmid, M., Fischer, D., Kinzel, L., Scherb, H., Munch, J.C., Jiang, X., Schroll, R., 2010. Homogeneous inoculation vs. microbial hot spots of isolated strain and microbial community: What is the most promising approach in remediating 1,2,4-TCB contaminated soils? Soil Biol. Biochem. 42, 331–336.

Zhang, H.-B., Wang, L.-H., Zhang, L.-H., 2002. Genetic control of quorum-sensing signal turnover in *Agrobacterium tumefaciens*. Proc. Natl. Acad. Sci. USA 99, 4638–4643.

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