Determination of Matrix Pore Size Distribution in Fractured Clayey Till and Assessment of Matrix Migration of Dechlorinating Bacteria

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ABSTRACT The pore structure and pore size distribution (PSD) in the clayey till matrix from three Danish field sites were investigated by image analysis to assess the matrix migration of dechlorinating bacteria in clayey till. Clayey till samples had a wide range of pore sizes, with diameters of 0.1–100 μm, and two typical peaks of pore sizes were observed in all clayey till samples. A large area fraction of the individual pores centered around 2 μm in diameter, and another fraction centered around 20 μm. In general, the typical macropore sizes (1 μm < D < 30 μm) in clayey tills determined by image analysis account for approximately 30–60% of the total porosity (20–26%), which is within the range of those reported for clayey soils and other clayey deposits in the literature. The pore size, PSD, and interconnectivity of pores in clayey till matrix may play an important role in evaluation of the migration of dechlorinating bacteria between fractures and clayey till matrix. Dechlorinating bacteria are small (0.3–1 μm) and may have the ability to morphologically adapt to space constraints. The results in this paper in combination with recent field data indicate that the migration of dechlorinating bacteria in fractures and into the clayey till matrix is likely, which is of significance for natural and stimulated degradation of chlorinated solvents by reductive dechlorination in clayey tills.

KEYWORDS backscatter scanning electron microscopy, dechlorinating bacteria, fracture aperture, fractured clayey till, pore size distribution (PSD)

INTRODUCTION

Chlorinated solvent contamination of the subsurface is a widespread problem and often occurs in low-permeability deposits such as clayey tills (Scheutz et al. 2010). Clayey tills are often fractured, which provides a preferential pathway for transport of chlorinated solvents into underlying aquifers (Chambon et al. 2011). The chlorinated solvents will also diffuse into the clayey matrix
from the fractures, and the clayey till matrix becomes a long-term secondary source of contamination due to back-diffusion and leaching of chlorinated solvents (Hønning, Broholm, and Bjerg 2007; Scheutz et al. 2010; Manoli et al. 2012). Enhanced reductive dechlorination (ERD) is a proven and relatively efficient technology for dissolved chloroethylenes in high-permeability aquifers (Lendvay et al. 2003; Lee et al. 2008; Scheutz et al. 2008). But for low-permeability deposits such as clayey tills, a major challenge for ERD is to achieve effective mixing and contact between the electron donor and dechlorinating bacteria and the contaminants trapped in the matrix (Scheutz et al. 2010; Christiansen et al. 2010).

Natural fractures in clay may be an important pathway for migration of bacteria and provide a residence for bacteria to colonize (Da and Sleep 2007). In unfractured clay, bacterial population densities may be very low (Beloin, Sinclair, and Ghiorse 1988; Sinclair et al. 1990). Recent studies reporting fracture aperture in clayey tills and other clayey deposits, which are summarized in Table S1 in Supplementary Material, indicate that the typical fracture aperture is in a range of 1–100 μm.

Migration of soil bacteria is often listed as imposing constraints to microbial activity (Chapelle and Lovley 1990; Fredrickson et al. 1997), as bacteria growth in clay is expected to be limited due to the small pore size (Scow and Johnson 1997), and to the scarcity of nutrients and electron acceptors necessary to maintain the bacterial population (Da and Sleep 2007). Rouquerol et al. (1994) recommended the pores in porous media to be characterized as three different types: macropores (diameter \(D > 0.05 \mu m\)), mesopores (\(D = 0.002–0.05 \mu m\)), and micropores (\(D < 0.002 \mu m\)). Some work has been done to investigate the matrix pore size in clayey soils and other relatively homogenous clayey deposits (see Table S2). Existing studies showed that the typical range of matrix pore size in clay soils and other clayey deposits is 0.01–0.3 \(\mu m\). However, there is a lack of information on the pore structure and pore size distribution (PSD) in heterogeneous clayey till matrix.

Scanning electron microscope (SEM), backscatter scanning electron microscope (BSEM), and transmission electron microscope (TEM) are mostly employed to get shape and sizes of any individual pores (also dead-ended pores) and fracture apertures in clayey tills (Tsakiroglou and Ioannidis 2008; Rosenbom et al. 2009; Tzovolou et al. 2009). These methods can be followed by image analysis, which has been well conducted in many studies to obtain the pore size distribution of porous media (Diamond and Leeman 1995; Aydilek, Oguz, and Edil 2002; Ziel, Haus, and Tulke 2008; Yang et al. 2009; Zhang et al. 2010). Mercury intrusion porosimetry (MIP) can also give information on pore throat size distribution, but it is of limited applicability for measurements of materials that have irregular pore geometry (Abell, Willis, and Lange 1999).

In order to evaluate ERD as a remediation technology in clayey tills, it is necessary to investigate the actual pore size distribution and pore structure in clayey till matrix, as well as fracture aperture, which would be further combined with other field data (dechlorinating bacteria distribution, degradation process, etc.) to assess the matrix migration of dechlorinating bacteria in the clayey till matrix.

The primary objectives of this paper are (1) to determine the matrix pore structure and pore size distribution in clayey tills from three Danish field sites; (2) to investigate the size of dechlorinating bacteria and their distribution in fractured clayey tills based on a literature review; and (3) to assess the matrix migration of dechlorinating bacteria in clayey tills by comparing our pore size data with published data for the three field sites regarding dechlorination and presence of dechlorinating bacteria.

**MATERIALS AND METHODS**

**Site Description**

All the clayey till samples investigated in this study were collected from three field sites (Vadsbyvej, Gl. Kongevej, and Sortebrovej) in Denmark. The location of the field sites is shown in Figure S1 in Supplementary Material. During the Pleistocene epoch, several till units with different characteristics were deposited in the eastern part of Denmark.

The Vadsbyvej site is dominated by two regionally distributed basal till units with a total thickness of 14–16 m. The tills are underlain by 2–3 m of fine sand and silt, and finally a primary limestone aquifer. The upper till is classified as a type B basal till, showing a high density of both vertical and horizontal fracture networks, and strong consolidation. The lower till (>7 m below surface [b.s.]) is interpreted as a type A basal till. It has only few vertical fractures and is more
permeable, due to a less consolidated matrix (Christiansen et al. 2008, 2010). In this study, the samples collected from the Vadsbyvej site are from the two basal clayey till units (Table 1).

At the Gl. Kongevej site, two systematically fractured clayey till deposits (2–8 m b.s., each with a thickness of 3–4 m) are saturated from approximately 2 m below ground surface (Lemming, Friis-Hansen, and Bjerg 2010; Damgaard et al. 2013a). The lower clayey till (the Copenhagen till) is more compact than the upper till. The two clayey till units are separated by discontinuous melt water sand lenses and underlain by the regional chalk aquifer. The clayey till units are basal tills. In this study, the samples collected from Gl. Kongevej site are from the two basal clayey till units (Table 1).

At the Sortebrovej site, the clayey till (0–40 m b.s.) is separated in four benches by sand layers at 10, 20–25, and 40 m b.s., respectively, and all three have interbedded sand stringers (Manoli et al. 2011). The upper clayey till is a flow till (<10 m b.s.), whereas the three underlying clayey tills were deposited as individual benches of basal clayey till in three glaciation events. In this study, the samples collected from Sortebrovej site are from the upper basal clayey till bench (10–20 m b.s.; Table 1).

### Sampling and Preparation of Specimens

Intact core samples were collected in stainless steel core tubes (inner diameter [ID] = 7 cm, length [L] = 50 cm) by a dry rotary drill rig and stored at 10°C. The 2–3 cm core samples of the intact cores were extruded and then sliced into three sections (Figure S2) in order to obtain pore size distribution in three dimensions (3-D). One section is the horizontal, and the other two sections are perpendicular vertical two-dimensional (2-D) surfaces. They are denoted as X-Y, Y-Z, and X-Z sections, respectively (see Figure S2b). A general description of each investigated clayey till sample is listed in Table 1.

### Preparation of Specimens for Image Analysis

Specimens for image analysis were prepared from polished epoxy-impregnated samples.

### Epoxy Impregnation

All samples were dried in an oven at 60°C for 24 h. They were cut into regular rectangle shape to fit to the plastic container where the samples were impregnated with epoxy. Epoxy (Ciba, Basel, Switzerland), which consists of resin (Araldit BDF, 5 parts) and hardener (HY 956, 1 part), was injected into the plastic container in a vacuum chamber until the surface of samples was covered. The air inside of the samples was replaced by plasticized resin and epoxy-impregnated samples were solidified at 40°C for 8 h.

### TABLE 1 General Description of All Investigated Clayey Till Samples

| Borehole and location | Depth (m b.s.) | Clayey till unit | Wet bulk density (g/cm³) | Porosity (%) | Lithology |
|-----------------------|---------------|------------------|--------------------------|--------------|-----------|
| N3, Vadsbyvej         | 3.25–3.28     | Upper            | 2.16a                    | 22%b         | Clayey till |
|                       | 6.45–6.49     | Upper            | 2.31                     | 21%          | Clayey till |
|                       | 10.35–10.38   | Lower            | 2.34                     | 21%          | Clayey till |
| C2, Gl. Kongevej      | 3.30–3.70     | Upper            | 2.31                     | 21%          | Clayey till |
|                       | 6.33–6.36     | Lower            | 2.34                     | 21%          | Clayey till |
|                       | 7.50–7.52     | Lower            | 2.18                     | 20%          | Clayey till |
|                       | 5.55–5.58     | Lower            | 2.43                     | 23%          | Clayey till |
| B370, Sortebrovej     | 13.46–13.50   | All upper basal clay till bench | 2.25 | 21% | Clayey till |
|                       | 13.60–14.20   |                  | 2.35                     | 22%          | Clayey till |
|                       | 14.38–14.40   |                  | 2.29                     | 23%          | Clayey till |
|                       | 16.20–16.23   |                  | 2.35                     | 23%          | Clayey till |
|                       | 16.78–16.80   |                  | 2.49                     | 26%          | Clayey till |
|                       | 17.57–17.60   |                  | 2.41                     | 26%          | Clayey till |

a,b: The average density and porosity, respectively, of clayey till samples from hot spot BDTU10, 11, and 12 in Vadsbyvej site. All densities and porosities were determined by gravimetric methods.
Polishing

First, the epoxy-impregnated samples were ground with three kinds of SiC (SiC 400, 600, and 1000, in order) in water on a glass disk to remove the grains from samples surface. Secondly, ground samples were polished on a polisher (λ Logitech) by three steps (see more in Supplementary Material).

Carbon Coater

After epoxy impregnation and polishing, the surface of specimens was coated with a conductive carbon film by carbon coater (agar SEM carbon coater, B7230; Agar Scientific, UK), in order to avoid electric charge accumulating on the surface of specimens during SEM image collection.

Image Analysis

A scanning electron microscope (JEOL JSM 5900LV; JEOL Inc., Japan) was employed to obtain the images of individual pore structures in the clayey till samples. BSEM images were recorded at 1280 × 960 pixels resolution following the operation manual (JEOL 2006). Two areas per specimen (duplicates) were selected randomly, and images at two different magnifications were obtained for each area, 753 × 565 μm and 64 × 48 μm (Figure S3). The higher-magnification image is the center part of the lower-magnification image. In total, 156 BSEM images (duplicates included) with two different magnifications were collected for image analysis with the software ImageJ (http://rsb.info.nih.gov/ij).

According to the definition of pore size recommended by Rouquere et al. (1994), only macropores can be resolved well by software Image J in this study, which is due to its resolution limit. The big macropores (1 < D < 100 μm) and small macropores (0.05 < D < 1 μm) were resolved well in the low- and high-magnification BSEM images, respectively. However, the mesopores and micropores cannot be resolved in the BSEM images. Therefore, in this study, only the macropores (0.05 < D < 100 μm) split into big and small macropores in the clayey till matrix were analyzed by ImageJ and discussed in subsequent sections. The procedure for image analysis of BSEM images is described in detail in Supplementary Material.

Data Processing

It was assumed that the pore size on the polished surface can be represented as the diameter of an equivalent circle area. To establish 3-D pore size distribution, the shapes of pores are simplified as cylinders extending normal to the plane of observation. Hence, the area fraction occupied by the pores was presented as the volume fraction of the whole specimen. Note that the assumption is not that any individual pore actually passes through the entire thickness of the specimen; it was only assumed that at any section a set of pores exists equivalent to those observed on the surface being analyzed. PSD data from image analysis are presented in the form of cumulative pore diameter distribution curves. The data were cumulated from largest pore diameter to the smallest diameter limit set by the ImageJ software (0.6 μm per pixel in the low-magnification image, 0.05 μm per pixel in the high-magnification image). The overlap of the PSD data between the images at two magnifications was replaced by the data from the image of larger magnification, which had higher resolution.

RESULTS AND DISCUSSION

Pore Structure, Pore Size Distribution, and Fracture Apertures in Clayey Tills

The 156 BSEM images representing the Vadsbyvej, Gl. Kongevej, and Sortebrovej field sites are presented in Figures 1–3 (see Figures S4–S6 for additional examples). It was found that the pore shapes are irregular, and the pore sizes vary between samples from the different field sites. Some microfractures can be observed in the low-magnification images (i.e., Figure 1b1, c1 and Figure 2g1).

The cumulative macropore size distribution (PSD) and individual PSD data in clayey till matrix obtained by image analysis are illustrated in Figure 4. In general, there was a variation of pore size distribution between duplicate BSEM image analyses. The cumulative PSD curve illustrated that the macropores of Vadsbyvej, Gl. Kongevej, and Sortebrovej sites varied in a range of 6–14%, 6–12%, and 6–16% in area fraction, respectively. The widths of microfractures in investigated clayey till samples determined by image analysis are generally smaller than 50 μm, which is consistent with data (approximate 1–100 μm) in the literature (Table S1).
For individual PSD, the diameters of macropores ranged between 0.1 and 100 μm, and two typical peaks of macropore sizes were observed in all investigated clayey till samples. One peak centered around 2 μm in diameter, where individual macropores constituted a significantly larger area fraction; and another peak centered around 20 μm. A smaller fraction of macropores with diameter from 0.01 to 1 μm was expressed in PSD of all investigated clayey till samples. Only little difference of individual PSD existed between field sites. Thus, the typical macropore sizes of all investigated clayey till samples in this study ranged from 1 to 30 μm. This corresponds to approximately 30–60% of the total porosity (20–26%, gravimetric method; Table 1). In the literature, the typical pore sizes in clayey soils and other clayey deposits were mostly determined by mercury intrusion porosimetry (MIP) and in a relatively large range of 0.001–75 μm (Table S2). So the typical macropore sizes of clayey tills in this study are within the range of those reported for clayey soils and other clayey deposits. Oven-drying could alter the pore size distribution. Diamond (1970) demonstrated that oven-drying may cause the shrinkage, consequently changing the pore size distribution of clays,
Gl. Kongevej site

FIGURE 2  BSEM images of clayey till samples at Gl. Kongevej site. Scale bar is shown in each image. Images marked d, e, f, and g are samples from 3.30–3.37, 6.33–6.36, 5.55–5.58, and 7.50–7.52 m b.s., respectively. The black part is pore space and other parts are the clayey till matrix.
FIGURE 3  BSEM images of clayey till samples at Sortebrovej site. Scale bar is shown in each image. Images marked h, i, j, k, l, and m are samples from 13.46–13.50, 13.75–13.78, 14.38–14.40, 16.20–16.23, 16.78–16.80, and 17.57–17.60 m b.s., respectively. The black part is pore space and other parts are the clayey till matrix.
but it should be noted that the range of pore sizes covered in this study extends over 4 orders of magnitude; therefore, small shifts in the experimental pore size distribution associated with shrinkage effects should not affect the general pattern of data. Abell, Willis, and Lange (1999) and Diamond (2000) suggested that MIP is useful only to provide the threshold diameters of pores and that image analysis shows a greater quantity of larger pores than MIP in cement-based materials with irregular pore geometry.

The distribution of interconnectivity of pores is not uniform and difficult to determine (Reszat and Hendry 2009). Although some pores or fractures could be dead-ended or isolated, the effective porosity in a glacial marine clay deposit was found to be approximately equal to the total porosity within an error of less than 10% (Sevee 2010). In this study, information about the 3-D pore network connectivity cannot be directly obtained by image analysis, only the data (e.g., macropore size, distribution, and the connectivity of macropores) in 2-D section surface were investigated through BSEM images. Thus, an alternative way was set up to estimate the 3-D connectivity by comparing the fraction of macropores between the three 2-D sections of each sample (see Figure S7). For clayey till samples at each depth, the difference in fraction of macropores was greater between the three directions than between duplicates for one direction. This indicates that macropores may not be homogeneously distributed in 3-D, consequently implying directional variation in connectivity in clayey tills.

In conclusion, the PSD or typical macropore sizes of clayey till samples in this study (1–30 μm) are within the range of those reported for clayey soils and other clayey deposits (0.001–75 μm) (Table S2), whereas the fracture apertures/sizes were in the range of the typical width of fracture apertures in clayey tills in the literature (1–100 μm; shown in Table S1).

**Distribution of Bacteria in Clayey Materials**

Bacterial population densities in unfractured clay have been expected to be very low (Beloin, Sinclair, and Ghiorse 1988; Sinclair et al. 1990) due to pore size restrictions. However, Lawrence et al. (2000) found that low numbers of bacteria were distributed...
heterogeneously within a thick aquitard complex, and Da and Sleep (2007) reported that organisms identified in their study were larger than the dominant pore size and were able to grow in the pores. They suggested that this was probably due to local heterogeneity within the clay matrix, including some larger connected pores or natural fractures. Fredrickson et al. (1997) suggested that subsurface bacteria (sulfate-reducing bacteria) require interconnected pore throats greater than 0.2 µm in diameter for sustained activity. Recently, Mannik et al. (2009) reported that bacteria can undergo morphological adaptation in narrow pore space, resulting in smaller size and elongated shape. Therefore, the characteristic shapes and sizes of bacteria may be considered as less important factors affecting the growth and migration of bacteria between fractures and clay matrix.

Size and Shape of Dechlorinating Bacteria

At least 18 dechlorinating bacteria have been isolated and identified, including *Dehalococcoides* sp., *Dehalobacter* sp., *Geobacter* sp., *Desulfitobacterium* sp., *Sulfurospirillum* sp., *Desulfomonile* sp., and *Desulfuromonas* sp. In particular, *Dehalococcoides* sp. can have functional genes encoded for synthesis of vinyl chloride reductase capable of complete dechlorination of chloroethylenes to ethane (Scheutz et al. 2008; Cheng and He 2009). The characteristics (size and shape) of the above-mentioned dechlorinating bacteria are summarized in Table 2 based on a literature review. Most of the bacteria were studied in pure cultures except for *Geobacter* sp., *Desulfitobacterium* sp., and *Methanomethylovorans* sp., which were also studied in the two mixed cultures, KB-1 and PCE1 (Table 2). In general, the typical diameter of dechlorinating bacteria ranged from 0.3 to 1 µm, illustrated in Figure 5. Electron micrographs of the reported dechlorinating bacteria showed that most of them are rod, coccoid, or disc shaped, and diameters are typically smaller than 1 µm (DeWeerd et al. 1990; Utkin, Woese, and Wiegel 1994; Scholz-Muramatsu et al. 1995; Bouchard et al. 1996; Krumholz, Sharp, and Fishbain 1996; Sanford et al. 1996; Wild, Hermann, and Leisinger 1996; Maymó-Gatell et al. 1997; Holliger et al. 1998; Adrian et al. 2000; He et al. 2003; Duhamel, Mo, and Edwards 2004; Cheng and He 2009).

Spatial Distribution of Dechlorinating Bacteria in Fractured Clayey Tills

The spatial distribution of dechlorinating bacteria in clayey tills has as discussed in this section only recently been reported at the three study sites (Manoli et al. 2012; Damgaard et al. 2013a,b) and at one additional clayey till site after hydraulic fracturing (Scheutz et al. 2010). The study site treatment and observations of dechlorinating bacteria and dechlorination activity in the clayey till matrix of these are summarized in Table 3. In addition, Takeuchi et al. (2011) reported that an organic-rich clay layer within a PCE-contaminated sandy aquifer was a major habitat zone for
| Bacteria/culture name | Species | Culture | Diameter (μm) | Shape | Electron donor | Electron acceptor | Reference |
|-----------------------|---------|---------|---------------|-------|----------------|-------------------|-----------|
| CBDB1                 | Dehalococcoides sp. | Pure | 1–2 | Coccoid | H₂, acetate | TCB, TeCB, PCDD | Adrian et al. (2000) |
| KB-1                  | Dehalococcoides sp., Geobacter, Methanomethylovorans, etc. | Mixture | ≤ 1 | Coccoid | H₂ | TCE, cis-DCE, VC | Duhamel et al. (2004) |
| BAV1                  | Dehalococcoides sp. | Pure | ≤ 0.8 | Disc-shaped | Acetate | trans-DCE, cis-DCE, 1,2-DCA, VC | He et al. (2003) |
| 195                  | D. ethenogenes | Pure | 0.5–0.7 | Irregular coccos | H₂ | PCE, TCE, 1,1-DCE, cis-DCE, 1,2-DCA | Maymo-Gatell et al. (1997) |
| MB                   | Dehalococcoides sp. | Pure | 1.0 | Disc-shaped | H₂ | PCE, TCE Pentachlorophenol (PCP) | Cheng and He (2009) |
| PCP-I                | Desulfitobacterium fiappieri | Pure | 0.7 | Rod | Pyruvate | TCE, TCE | Bouchard et al. (1996) |
| TT4B                 | Desulfuromonas acetexigens | Pure | 0.6 | Rod | Acetate | PCE, TCE | Krumholz et al. (1996) |
| JW/II-DCI            | Desulfobacterium dehalogenans | Pure | 0.5–0.7 | Slightly curved rod | Pyruvate, lactate, formate, H₂ | 3-C1-4-OHPA | Utkin et al. (1994) |
| Co23                 | Desulfitobacterium chlororespirans | Pure | 0.5–1 | Curved bacillus | Pyruvate, lactate, formate, H₂ | 2,3-dichlorophenol | Sanford et al. (1996) |
| DCB-2                | Desulfitobacterium hafniense | Pure | 0.6–0.7 | Rod | Pyruvate, tryptophan | 3-C1-4-OHPA | Christiansen and Ahring (1996) |
| PCE1                 | Desulfitobacterium, Geobacter, etc. | Mixture | 0.6–0.8 | Curved rod, 4 lateral flagella | Pyruvate, butyrate, formate, etc. | TCE, 3-C1-4-OHPA, 2-chlorophenol, etc. | Gerritse et al. (1995) |
| TEA                  | Dehalobacter restrictus | Pure | 0.2–0.3 | Rod, 1–4 lateral flagella | H₂ | PCE, TCE | Wild et al. (1996) |
| PER-K23              | Dehalobacter restrictus | Pure | 0.3–0.5 | Rod | H₂ | PCE, TCE | Holliger et al. (1998) |
| DCB 1                | Desulfomonile tiedjei | Pure | 0.8–1 | Rod | Pyruvate | Meta-halobenzoates | DeWeerd et al. (1990) |
| 2CP-1                | Myxobacteria | Pure | 0.5 | Rod | Acetate | 2-Chlorophenol | Cole et al. (1994) |
| D. multivorans       | Dehalospirillum multivorans | Pure | 0.45 | Rod | Pyruvate, lactate, ethanol, H₂, etc. | PCE | Scholz-Muramatsu et al. (1995) |

Note. DCE = dichloroethylene; TCE = trichloroethylene; VC = vinyl chloride.
| Site/Activity | Contamination | Treatment | Specific degraders | Quantity | PSD data |
|---------------|---------------|-----------|-------------------|----------|----------|
|               |               |           |                   | A        | B        | C        | Macroporosity (%) | Typical pore size (µm) |
| Vasbyvej¹     | TCE, PCE, TCA, nonchlorinated organic solvents | No treatment applied | Dhb | $10^5$–$10^8$ | nd* | 6–14 | 1–30 |
|               |               |           | Dhc              | nd*      |          |           |                  |                      |
|               |               |           | vcr              | $10^4$–$10^9$ |          |           |                  |                      |
| Sortebrovej²  | PCE           | ERD       | Dhc              | $10^5$–$10^8$ |          |           |                  |                      |
|               |               |           | vcr              | nd        |          |           |                  |                      |
| Gl. Kongevej³ | PCE           | ERD       | Dhb              | $10^5$–$10^6$ | nd–$10^6$ | nd       | 6–12 | 1–30 |
|               |               |           | Dhc              | nd        | $10^5$–$10^6$ | $10^5$–$10^8$ |                  |                      |
|               |               |           | vcr              | nd        | $10^4$–$10^6$ | $10^5$–$10^8$ |                  |                      |

Note: 1: Damgaard et al. (2013b); 2: Manoli et al. (2012); 3: Damgaard et al. (2013a).

*Dhb = Dehalobacter (cells/g); Dhc = Dehalococcoides (cells/g); vcr = vinyl chloride reductase (genes/g); A = sections dominated by cis-dichloroethylene (cDCE) and with no vinyl chloride (VC) production; B = sections dominated by cDCE with some VC production; C = dominated by VC; nd = nondetected; TCA = trichloroethane; TCE = trichloroethylene.

*One small section with Dhc and vcr $10^4$–$10^7$ in some samples.
Damgaard et al. (2013a; Table 3) found Dehalococcoides, vinyl chloride reductase gene vcrA, and Dehalobacter sp., which had migrated from sand stringers, lenses, and fractures into the clayey till matrix, forming bioactive zones of up to 1.8 m thickness, after bioaugmentation with dechlorinating bacteria 4 years previously at the Gl. Kongevej site (Bioclear, Groningen, The Netherlands).

Assessment of Matrix Migration of Dechlorinating Bacteria in Clayey Tills

In this study, the typical macropore size in the clayey till matrix determined by image analysis is in the range of 1–30 μm, which is significantly larger than the typical diameter (0.3–1 μm) of dechlorinating bacteria. Moreover, the typical fraction of macropores accounts for 30–60% of the total porosity. These results reveal a potential ability of dechlorinating bacteria to migrate from the fractures into the clayey till matrix through the macropores in our investigated field sites. The macropore connectivity should be taken into account as another important factor that may effectively control the continuous microbial activity (incidence, pathway, etc.) of the dechlorinating bacteria. The evaluation of 3-D network connectivity in this study might imply directional variations, which may restrict the migration of dechlorinating bacteria further into clayey till matrix. Nevertheless, the dechlorinating bacteria may be able to spread to other macropores connected by smaller pores based on an ability of bacteria to morphologically adapt (Mannik et al. 2009). A significant number of specific degraders and genes (up to 10⁹ cells or genes/g) were found in the clayey till matrix at the three field sites (Table 3). This documents the presence of dechlorinating bacteria in the matrix, and the different types of dechlorinators present for different dechlorination steps in the sequential dechlorination (Table 3) support that the bacterial population is able to migrate and/or grow in the clayey till matrix.

CONCLUSION

The pore distribution was studied for clayey till samples from three Danish field sites. The typical macropore sizes (1 < D < 30 μm) in clayey tills determined by image analysis in this study are within the range of those reported for clayey soils and other clayey deposits. The macropores accounted for approximately...
30–60% of the total porosity. The size of dechlorinating bacteria is significantly smaller than the typical matrix macropore or fracture aperture in clayey tills. A large percent of macropores could provide space for the migration of dechlorinating bacteria from fractures into clayey till matrix and be habitats for their sustained microbial activities if electron donor, carbon sources, and other nutrients are available. The presence, potential migration, and growth of dechlorinating bacteria in the clayey till matrix is documented by specific observations at the three study sites and supported by recent reports in literature.

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SUPPLEMENTARY MATERIAL

Supplemental data for this article can be accessed on the publisher’s website.

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