1 Supplementary material

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3 **Determination of matrix pore-size distribution in fractured**
4 **clayey till and assessment of matrix-migration of**
5 **dechlorinating bacteria**

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1. Specimen Preparation for back-scatter scanning electronic microscope (BSEM)

1.1 Epoxy-impregnation

All samples were dried out in 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. Meanwhile, it is important to make the surface of cut sample flat. Epoxy (CIBA) 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.

1.2 Polishing

First, the surface of epoxy-impregnated samples was ground with 3 kinds of SiC (SiC 400, 600 and 1000 in order) in water on a glass disk to remove the grains from samples surface. About 250 µm thick surfaces were removed with SiC 400, 50 µm thick surfaces were removed with SiC 600 and 25µm thick surfaces were removed with SiC 1000. Cleaning the sample surfaces and glass disk between each step is necessary to remove all grains from previous step. Secondly, ground samples were polished on a polisher (λ LOGITECH) by 3 steps: 1) samples were polished with 3 µm diamonds on a fabric (cloth, PoliPan1 from Struers) and ethylene glycol was used as lubricant; 2) samples were polished with 1 µm diamonds on a fabric (cloth, MD-Dur) and ethylene glycol was used as lubricant; 3) samples were polished with

63 0.25 μ m diamonds on a fabric (DP-NAP) and distilled water was used as lubricant.
64 Each polishing step lasts less than 15 s.

65 **1.3 Carbon coater**

66 After Epoxy-impregnation and polishing, the surface of specimens was coated with
67 a conductive carbon film by Carbon coater (AGAR SEM CARBON COATER,
68 B7230), in order to avoid electric charge accumulated on the surface of specimens
69 during BSEM image collection.

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71 **2. Image analysis procedure**

72 In this paper, image analysis was carried out by software Image J
73 (<http://rsb.info.nih.gov/ij>). The image analysis procedure was sensitive to the choice
74 of porosity threshold. The threshold values were determined by setting the porosity
75 threshold at visually estimated maximum, minimum and best possible position (Alam
76 et al., 2010). In image analysis by software Image J, the deviation of area fraction of
77 pores in low magnification image varied from 1.5% to 1.8% per 10 threshold values,
78 while the deviation of area fraction of pores in high magnification image varied from
79 3.3% to 3.7% per 10 threshold values. Therefore, the threshold values of porosity of
80 clayey till samples in low and high magnification images in this study were set as
81 50-55 and 80-90, respectively. The procedure for measurements of pore size
82 distribution (PSD) is outlined in the following five steps.

83 1) The brightness/contrast of images were adjusted by equalize histogram function

- 84 in software to get an even gray scale distribution.
- 85 2) Segment gray scale image into features of interest by setting threshold values
- 86 manually and convert the gray scale image to a binary image.
- 87 3) Use the open/close option to smooth the binary images, fill in the small holes and
- 88 remove isolated pixels;
- 89 4) Set spatial scale of image and measurements to choose relevant parameters (such
- 90 as perimeter, area and shape descriptor, etc) in image analysis;
- 91 5) Run command of “Analyze particle” to get the data of image parameters.

TABLE S1 Width of fracture apertures in clayey tills and other clayey deposits

Deposits	Location	Diameter /(μm)	Methods	Reference
Glaciolacustrine clay	Southeastern Manitoba, C.A.	10-50	—	(Grisak and Cherry, 1975)
Lacustrine clay deposit	Mexico City	30	—	(Rudolph et al., 1991)
Silty-clay deposit	—	15-50	Modelling parameter	(Reynolds and Kueper, 2002)
Clay aquitard	—	30-100	—	(Falta, 2005)
Clay-rich till	Southwestern Ontario, C.A.	1-43	Tracer experiment	(McKay et al., 1993)
Clay-rich till	Skælskør, Denmark	13-120	Tracer experiment	(Hinsby et al., 1996)
Clayey till	Ontario, C.A.	1-100	Modelling parameter	(Parker et al., 1994)
Clayey till	—	30-50	Modelling parameter	(Slough et al., 1999)
Clayey till	Sarnia, C.A.	20-30	Tracer test	(Dastous et al., 1989)
Clayey till	—	10-30	Modelling parameter	(Harrison et al., 1992)
Clayey till	Havdrup, Denmark	39-84	Dye tracer experiment	(Jorgensen et al., 2002)
Clayey till	Hinnerup & Grundfor, Denmark	14-94	Dye tracer experiment	(Jorgensen et al., 2004)
Clayey till	—	75	Modelling parameter	(Mundle et al., 2007)

TABLE S2 Typical pore size in clayey soils and other clayey deposits

Deposits	Geological characteristics	Location	Diameter/(μm)	Methods	Reference
Clay soil	Grey soil of heavy texture	Western Australia	0.002-50	Mercury porosimetry	(Sills et al., 1974)
Clay soil	Gault clay and lower lias clay	Drayton and Compton	0.3-30	Mercury porosimetry	(Lawrence et al., 1979)
Clayey soil	Unweathered clayey silt till	Halton waste site	≤0.1	—	(Hrapovic and Rowe, 2002)
Clay soil	Fertilized with minerla fertilizers	Farm, Veneto, Italy	0.01-75	Mercury porosimetry	(Lugato et al., 2009)
Clay sediment	Marine sediment	Mol, Belgium	<0.2	—	(Boivin-Jahns et al., 1996)
Clayey sediment	Clayey confining beds	South Carolina	<0.05	—	(Chapelle and Lovley, 1990)
Clayey sediment	Clay layer above the aquifer	Yamagata Prefecture, Japan	<1	Mercury porosimetry	(Takeuchi et al., 2011)
Clay minerals	Illite, kaolinite, etc.	Wyoming, U.S.	0.03-0.2	Mercury porosimetry	(Diamond, 1970)

TABLE S3 Composition of pore size distribution (PSD) of 3D-section clayey samples at different depths, Vadsbyvej site (X-Y, Y-Z and X-Z mean the 2-D surfaces of clayey till sample; the sample No. is corresponding to each marked BSEM image in FIGURE S3)

Sample No.	Depth (m b.s.)	Grains	Macropores (%)			Meso-micropores ^a (%)	Total Porosity ^b (%)
			Big pore	Small pore	Total		
A1	3.25-3.28(X-Y)	78	2.40	8.10	10.50	11.50	22
A2		78	1.06	9.69	10.75	11.25	22
B1	3.25-3.28(X-Z)	78	2.50	5.73	8.23	13.77	22
B2		78	1.10	6.41	7.51	14.49	22
C1	3.25-3.28(Y-Z)	78	1.84	8.70	10.54	11.46	22
C2		78	2.73	8.76	11.49	10.51	22
D1	6.45-6.49(X-Y)	78	0.72	8.65	9.37	12.63	22
D2		78	2.08	11.40	13.48	8.52	22
E1	6.45-6.49(X-Z)	78	2.50	6.55	9.05	12.95	22
E2		78	0.87	12.02	12.90	9.10	22
F1	6.45-6.49(Y-Z)	78	0.76	9.09	9.85	12.15	22
F2		78	0.66	7.14	7.80	14.20	22
G1	10.35-10.38(X-Y)	78	0.91	8.99	9.90	12.10	22
G2		78	0.51	11.77	12.28	9.72	22
H1	10.35-10.38(X-Z)	78	1.29	9.87	11.16	10.84	22
H2		78	0.82	10.64	11.46	10.54	22
I1	10.35-10.38(Y-Z)	78	0.86	8.63	9.49	12.51	22
I2		78	1.33	10.72	12.05	9.95	22

^aMeso-micropores,% = Total porosity-Macropores. ^b The average porosity of clayey till samples from hot spot BDTU10, 11 and 12 in Vadsbyvej site. Total porosities were determined by gravimetric methods in laboratory. Macropores consist of big macropore ($D > 1 \mu\text{m}$) and small macropore ($0.1 \mu\text{m} < D < 1 \mu\text{m}$).

TABLE S4 Composition of PSD of 3D-section clayey samples at different depths, Gl. Kongevej site (X-Y, Y-Z and X-Z mean the 2-Dsurfaces of clayey till sample; the sample No. is corresponding to each marked BSEM image in FIGURE S4)

Sample No.	Depth (m b.s.)	Grains	Macropores (%)			Meso-micropores ^a (%)	Total Porosity ^b (%)
			Big pore	Small pore	Total		
A1	3.3-3.7 (X-Y)	79	1.9	9.5	11.3	9.7	21
A2		79	1.8	7.2	9.1	11.9	21
B1	3.3-3.7 (X-Z)	79	1.0	6.8	7.8	13.2	21
B2		79	1.2	13.2	14.4	6.6	21
C1	3.3-3.7 (Y-Z)	79	2.0	7.5	9.5	11.5	21
C2		79	2.4	6.0	8.3	12.7	21
D1	6.33-6.36 (X-Y)	79	2.6	5.2	7.9	13.1	21
D2		79	3.6	4.2	7.7	13.3	21
E1	6.33-6.36 (X-Z)	79	1.6	5.2	6.9	14.1	21
E2		79	1.7	6.0	7.7	13.3	21
F1	6.33-6.36 (Y-Z)	79	5.2	2.5	7.7	13.3	21
F2		79	2.9	4.9	7.9	13.1	21
G1	7.50-7.52 (X-Y)	80	4.0	6.3	10.4	9.6	20
G2		80	4.4	6.1	10.5	9.5	20
H1	7.50-7.52 (X-Z)	80	3.2	7.8	10.9	9.1	20
H2		80	2.1	5.5	7.6	12.4	20
I1	7.50-7.52 (Y-Z)	80	2.8	6.6	9.4	10.6	20
I2		80	2.1	6.5	8.6	11.4	20
J1	5.55-5.58 (X-Y)	77	2.1	9.0	11.0	12.0	23
J2		77	2.7	5.1	7.8	15.2	23
K1	5.55-5.58 (X-Z)	77	1.8	8.4	10.2	12.8	23
K2		77	3.6	6.4	9.9	13.1	23
L1	5.55-5.58 (Y-Z)	77	1.6	7.5	9.1	13.9	23
L2		77	0.9	6.9	7.8	15.2	23

^aMeso-micropores,% = Total porosity-Macropores. ^b The total porosity was determined by gravimetric method in lab. Macropores consist of big macropore ($D > 1 \mu\text{m}$) and small macropore ($0.1 \mu\text{m} < D < 1 \mu\text{m}$).

TABLE S5 Composition of PSD of 3D-section clayey samples at different depths, Sortebrovej site (X-Y, Y-Z and X-Z means the 2-D surfaces of clayey till sample; the sample No. is corresponding to each marked BSEM image in FIGURE S5)

Sample No.	Depth (m b.s.)	Grains	Macropores (%)			Meso-micropores ^a (%)	Total Porosity ^b (%)
			Big pore	Small pore	Total		
A1	13.46-13.50(X-Y)	79	2.71	3.91	6.62	14.38	21
A2		79	2.47	6.56	9.02	11.98	21
B1	13.46-13.50(X-Z)	79	2.86	4.27	7.13	13.87	21
B2		79	4.33	5.59	9.91	11.09	21
C1	13.46-13.50(Y-Z)	79	3.69	5.47	9.16	11.84	21
C2		79	3.35	6.53	9.88	11.12	21
D1	13.60-14.20(X-Y)	78	2.26	5.17	7.43	14.57	22
D2		78	1.37	8.20	9.57	12.43	22
E1	13.60-14.20(X-Z)	78	2.88	7.35	10.23	11.77	22
E2		78	0.98	7.29	8.27	13.73	22
F1	13.60-14.20(Y-Z)	78	2.85	5.47	8.32	13.68	22
F2		78	1.27	6.63	7.90	14.10	22
G1	14.38-14.40(X-Y)	77	3.33	5.59	8.92	14.08	23
G2		77	3.33	5.59	8.92	14.08	23
H1	14.38-14.40(X-Z)	77	6.07	4.47	10.54	12.46	23
H2		77	2.44	8.78	11.22	11.78	23
I1	14.38-14.40(Y-Z)	77	1.99	5.90	7.89	15.11	23
I2		77	1.35	7.44	8.78	14.22	23
J1	16.20-16.23(X-Y)	77	1.04	7.96	9.00	14.00	23
J2		77	2.36	5.47	7.83	15.17	23
K1	16.20-16.23(X-Z)	77	2.34	6.21	8.54	14.46	23
K2		77	5.68	4.73	10.42	12.58	23
L1	16.20-16.23(Y-Z)	77	4.08	4.48	8.56	14.44	23
L2		77	2.93	7.12	10.05	12.95	23
M1	16.78-16.80(X-Y)	74	2.95	6.22	9.16	16.84	26
M2		74	4.37	5.78	10.14	15.86	26
N1	16.78-16.80(X-Z)	74	2.85	5.42	8.27	17.73	26
N2		74	2.95	3.88	6.82	19.18	26
O1	16.78-16.80(Y-Z)	74	3.09	6.93	10.02	15.98	26
O2		74	3.16	6.53	9.69	16.31	26
P1	17.57-17.60(X-Y)	74	0.26	13.53	13.79	12.21	26
P2		74	0.68	12.26	12.94	13.06	26
Q1	17.57-17.60(X-Z)	74	0.91	12.41	13.32	12.68	26
Q2		74	0.50	13.31	13.81	12.19	26
R1	17.57-17.60(Y-Z)	74	1.11	14.22	15.33	10.67	26
R2		74	0.05	12.26	12.31	13.69	26

^aMeso-micropores,% = Total porosity-Macropores. ^b The total porosity was

determined by gravimetric method in lab. Macropores consist of big macropore ($D > 1 \mu\text{m}$) and small macropore ($0.1 \mu\text{m} < D < 1 \mu\text{m}$).

FIGURE S1. Map of clayey till field sampling sites in Denmark.

FIGURE S2. Sampling of core sample from Vadsbyvej field site, at 3.25-3.28 m below surface (m b.s.). (a) Core sample, (b) Three sections of piece of core sample.

FIGURE S3. Example BSEM images of resin-impregnated clayey till samples from Sortebrovej site, at 13.46-13.50 m.b.s.: (a-b) grey-scale and binary image (low magnification, 753 μm × 565 μm) and (c-d) grey-scale and binary image (high magnification, 64 μm × 48 μm).

FIGURE S4. BSEM images (including duplicates) of clayey till samples from Vadsbyvej site used for the analysis of pore size distribution and macroporosity. Images marked with uppercases are low magnification magnification images ($753 \mu\text{m} \times 565 \mu\text{m}$) and images marked with lower-cases are high magnification magnification images ($64 \mu\text{m} \times 48 \mu\text{m}$). Scale bar is $100 \mu\text{m}$ and $10 \mu\text{m}$ in low and

high magnification images, respectively. Marked label in images are corresponding to the samples No. in TABLE S3 where general information of clayey till samples are shown. The black part is the pore space and other parts are clayey till matrix.

FIGURE S4. continued

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FIGURE S4. continued

FIGURE S5. BSEM images (including duplicates) of clayey till samples from Gl. Kongevej site used for the analysis of pore size distribution and macroporosity. Images marked with uppercases are low magnification images ($753 \times 565 \mu\text{m}^2$) and images marked with lower-cases are high magnification images ($64 \times 48 \mu\text{m}^2$). Scale

bar is 100 μm and 10 μm in low magnification and high magnification images, respectively. Marked label in images are corresponding to the samples No. in TABLE S4 where general information of clayey till samples are shown. The black part is the pore space and other parts are clayey till matrix.

FIGURE S5. continued

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FIGURE S5. continued

FIGURE S6. BSEM images (including duplicates) of clayey till samples from Sortebrovej site used for the analysis of pore size distribution and macroporosity. Images marked with uppercases are low magnification magnification images ($753 \times 565 \mu\text{m}^2$) and images marked with lower-cases are high magnification

magnification images ($64 \times 48 \mu\text{m}^2$). Scale bar is 100 and 10 μm in low magnification and high magnification images, respectively. Marked label in images are corresponding to the samples No. in TABLE S5 where general information of clayey till samples are shown. The black part is the pore space and other parts are clayey till matrix.

FIGURE S6. continued

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FIGURE S7. Comparison of macroporosity between different sections of all clayey till samples from three field sites

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