# Supplementary Information for

## Assessing the microbial impact on the performance of bentonite clay at different thermo-hydro-geochemical conditions

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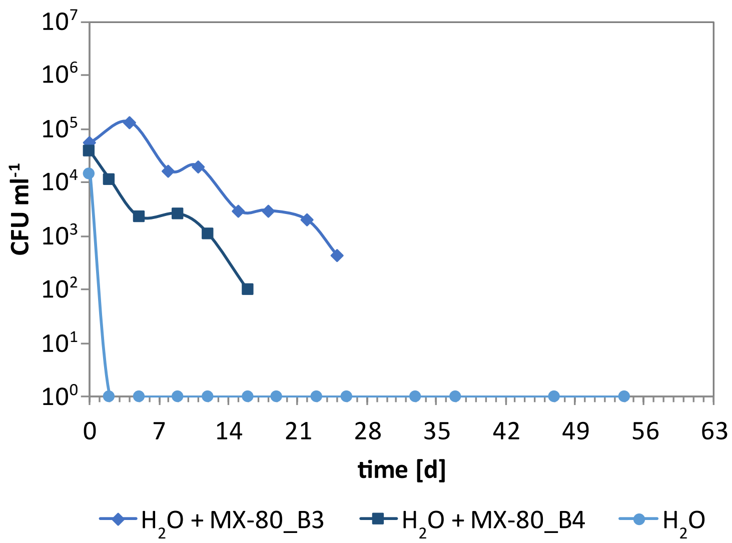
**This file includes:**

1 Supplemental results
2 Figures S1 to S8
3 Tables S1 to S4

**1. Supplemental results**

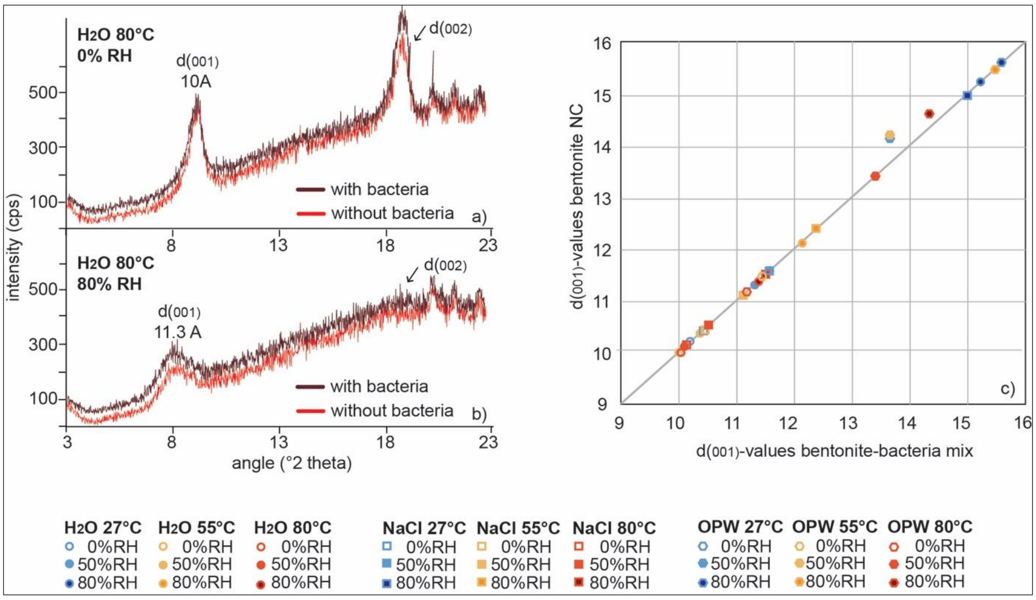
*Result S1: Bacterial growth with bentonite in $H_2O$*

In two $H_2O$ mesocosms with MX-80, the determination of CFU abundance was stopped after 16 and 25 days because of a contamination. Similarly to NaCl and OPW, the cell numbers slowly decreased until the occurrence of the contamination, from less than $10^5$ to $10^2$ CFU $mL^{-1}$. Like in NaCl, the number of CFU in $H_2O$ without MX-80 rapidly decreased after inoculation. After 2 days, no CFU could be determined (shown in below figure).



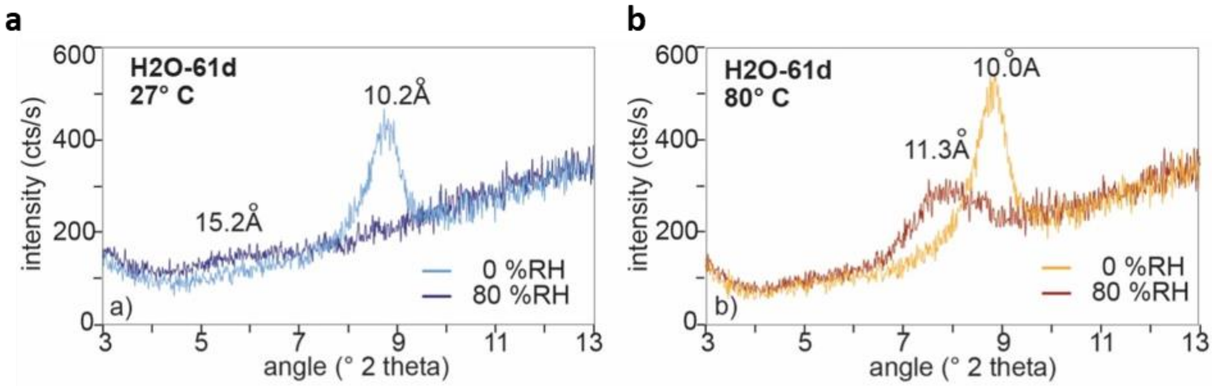

*Result S2: Swelling behaviour of bentonite in $H_2O$*

Despite the presence of *S. bentonitica* and the contaminant in $H_2O$, we did not observe a significant difference in the location of diffraction peaks, i.e., in the swelling behaviour. At 80 °C and 0% RH (below Figure a), the basal spacing $d_{001}$ peak of montmorillonite showed typical values of $d_{001}$=10.1 Å, reflecting the lack of water in the interlayer sheet. At 80 °C and 80% RH (below Figure b), the peak shifted to values of $d_{001}$=11.3 Å, showing the absorption of less than 1 water layer into the interlayer sheets. According to (1) and (2), this reflects the storage of probably no more than 4.5 wt% water.

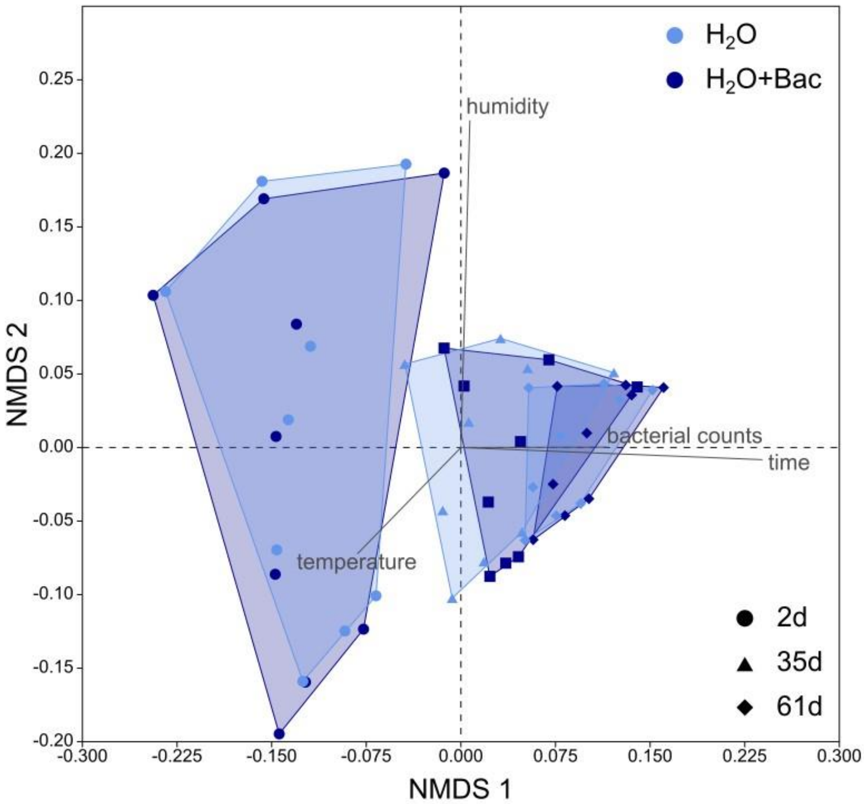



| | | *without bacteria (NC)* | | | *with bacteria (BAC)* | | |
|---|---|---|---|---|---|---|---|
| ***medium*** | **temperature (°C)** | **relative humidity (%)** | | | | | |
| | | **0** | **50** | **80** | **0** | **50** | **80** |
| | 27 | 10.2 | 11.2 | 15.2 | 10.2 | 11.2 | 15.2 |
| *$H_2O$* | 55 | 10.0 | 10.3 | 12.1 | 10.0 | 10.3 | 12.1 |
| | 80 | 10.0 | 10.1 | 11.3 | 10.0 | 10.1 | 11.3 |

After incubating bentonite with *S. bentonitica* and the contaminating strain in $H_2O$ for 61 days, the X-ray diffraction pattern at 27 °C shows a clear shift of the $d_{001}$ smectite peak from 10.2 Å at 0% RH to 15.2 Å at 80% RH, caused by the absorption of 2 water layers (ca. 15 wt% water after Ikari et al. [2]) into the interlayer sheet (Figure. a). A shift was also observed at 80 °C (Figure. b), but the d001 decreased to 1 water layer from $d_{001}$=10 Å at 0% RH to $d_{001}$=11.3 Å (ca. 4 wt% water). With increasing temperature, a loss of interlayer water of ca. 11 wt% was recognized. It can also be observed that both the d-values and the peak width (measured in full width at half maximum FWHM) display the number of water layers that formed in the interlayers of the bentonite clay. The peak widens during the formation of a new water layer while it tightens again when the formation is completed and a new hydration step is reached.




The diffractograms measured after 2, 35 and 61 d were visualized in a NMDS ordination. In accordance with the shift to the right side of the plot, i.e., with increasing time, MX-80 remained in $H_2O$ and the variance within a cluster along the y-axis decreased as the intensity of the montmorillonite peaks decreased. This shift and decrease in variance with time was particularly pronounced for MX-80 in $H_2O$. The cluster of MX-80 after 61 d in $H_2O$ strongly overlapped with the cluster of MX-80 after 2 d in NaCl (data not shown), indicating a similar swelling behaviour and peak intensity of both samples after different periods of time in solutions of different ionic strength.




**2. Supplemental Figures**

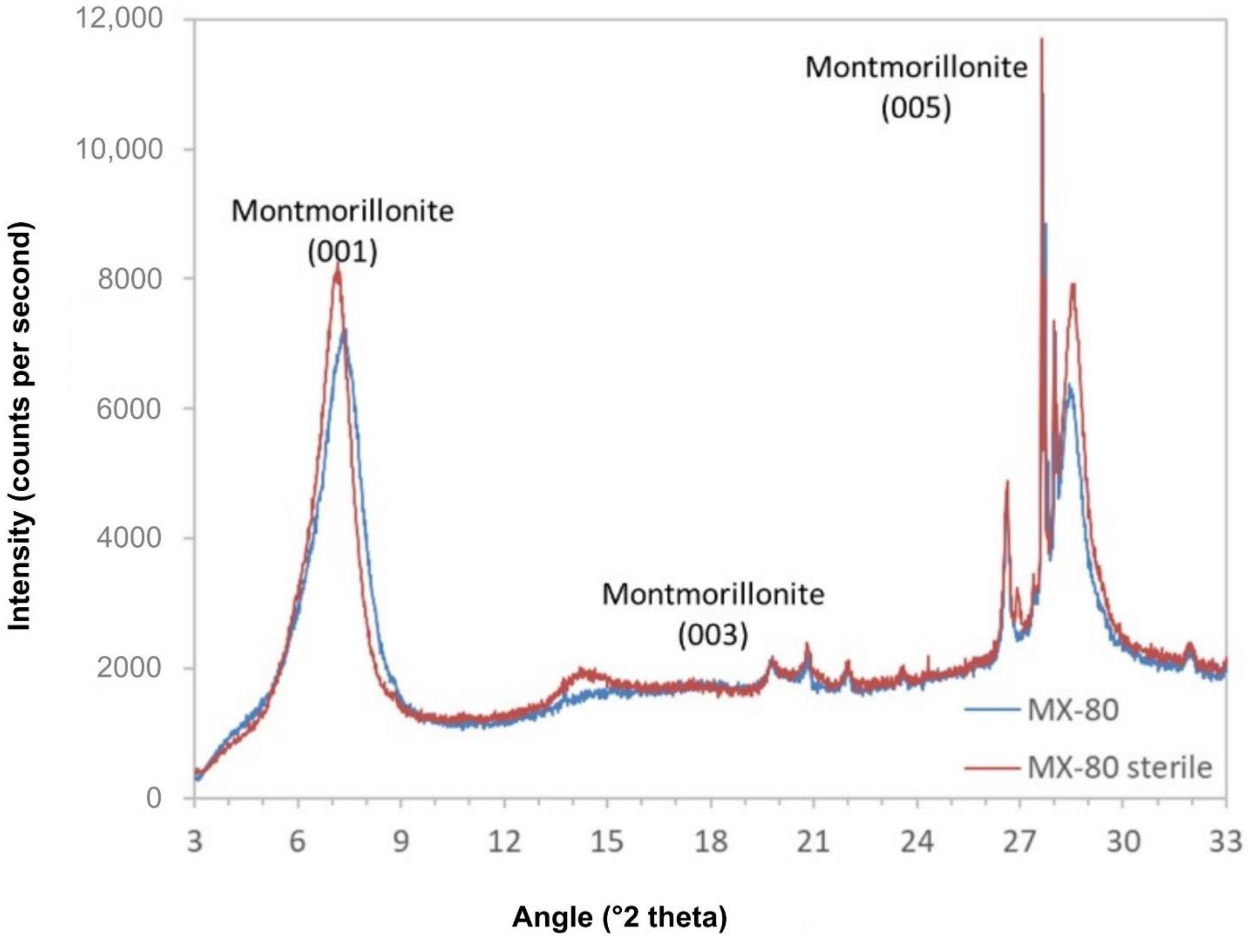



**Figure S1.** XRD spectra of bentonite MX-80 before and after sterilization via electron beam irradiation.

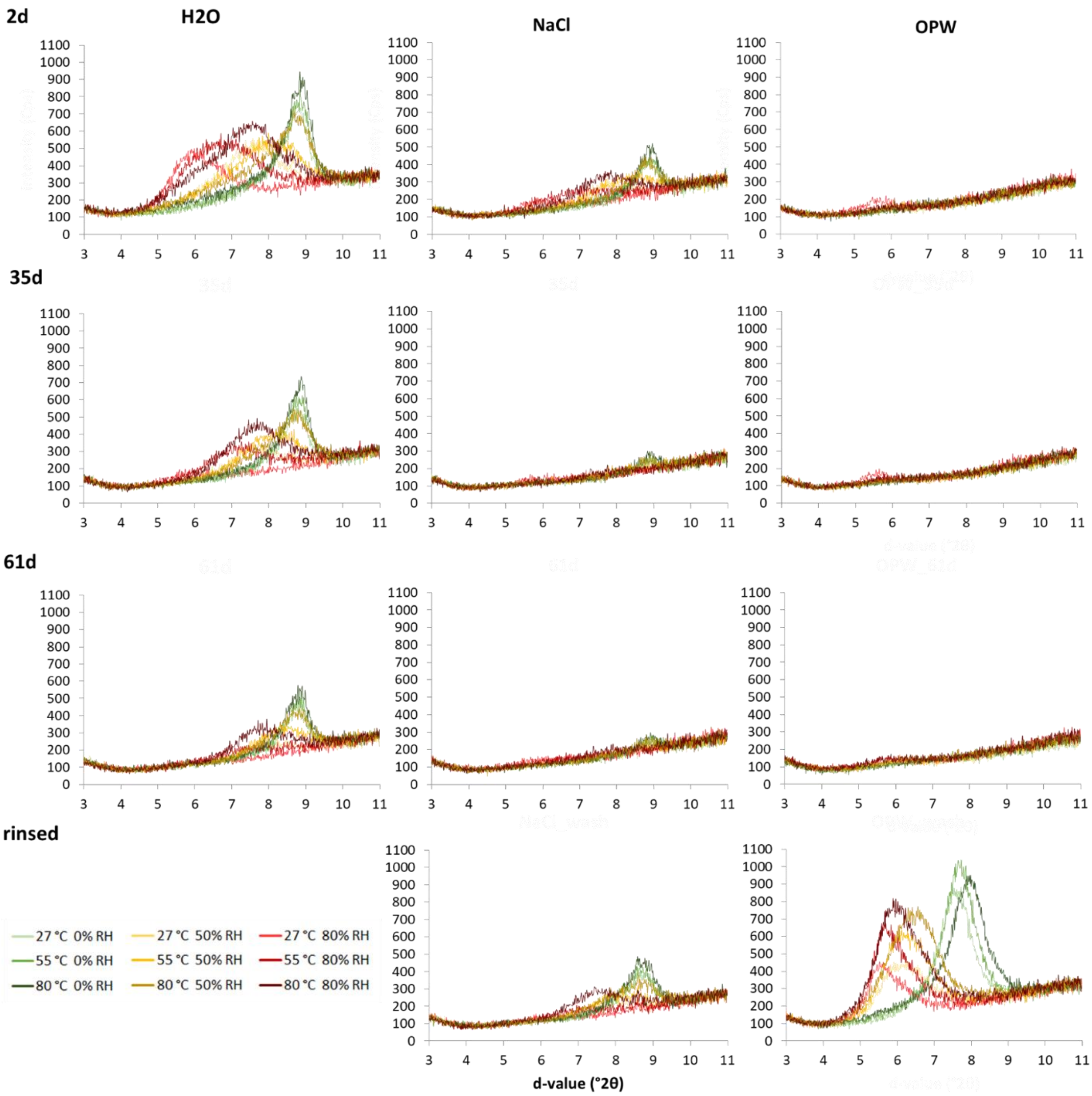



**Figure S2.** XRD spectra of MX-80 after 2, 35 and 61d of incubation in $H_2O$, NaCl or OPW solutions and after washing with ultrapure water.

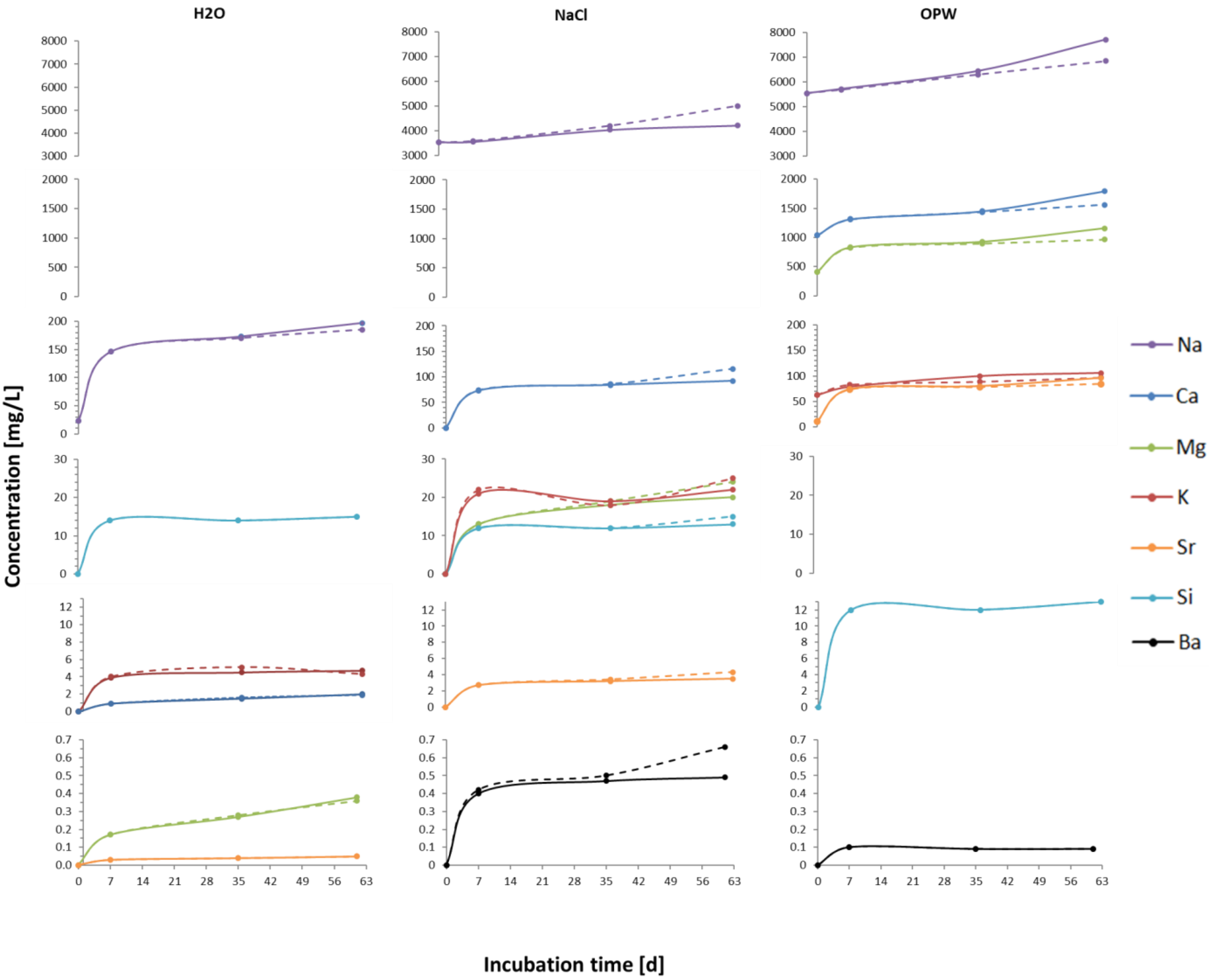



**Figure S3.** Element composition of the solutions $H_2O$, NaCl and OPW determined after 7, 35 and 61 days of contact with MX-80 bentonite. Solid lines reflect concentrations of mesocosms with *S. bentonitica* and dashed lines without bacteria.

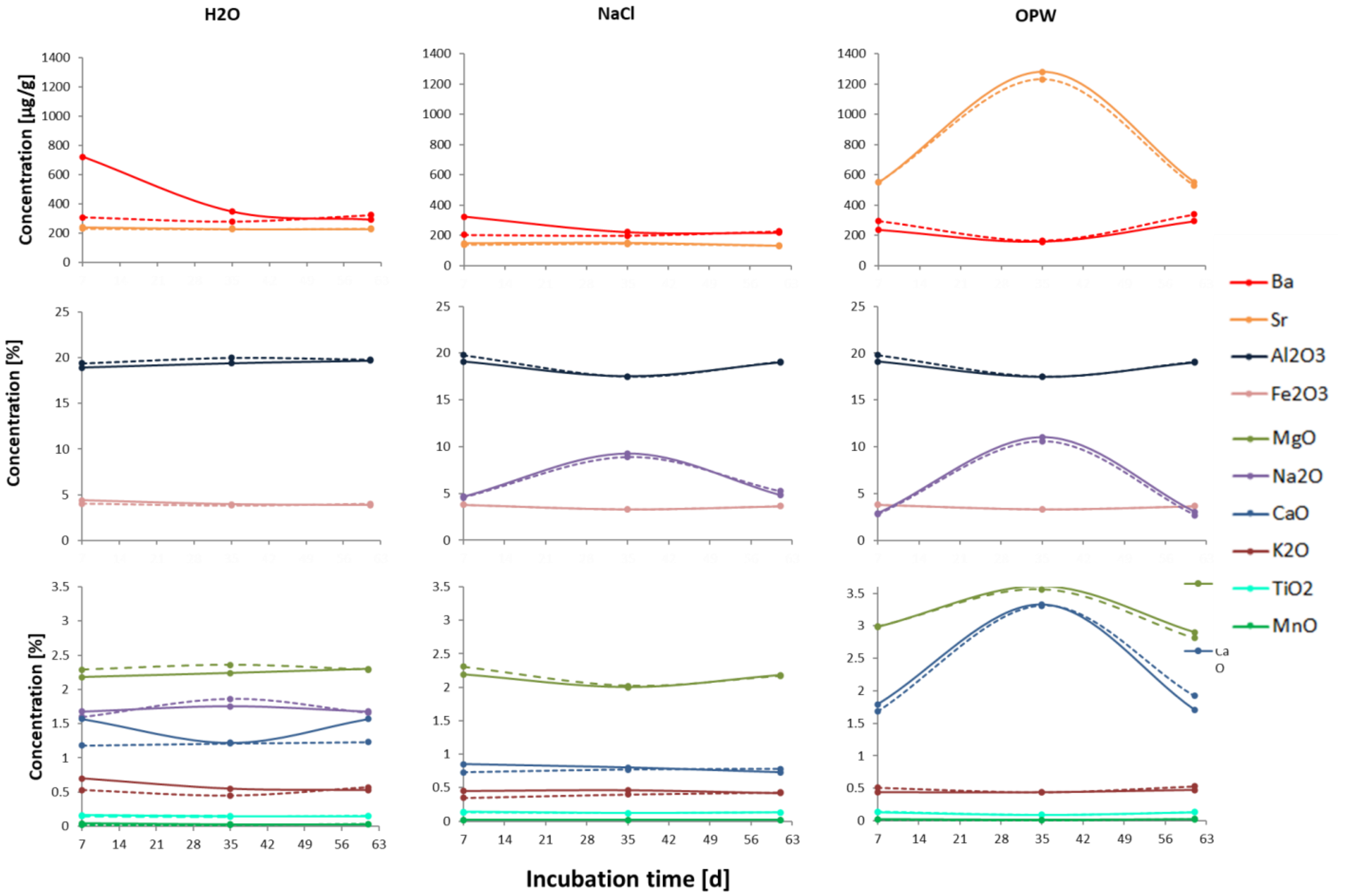



**Figure S4.** Element composition of MX-80 bentonite after 7, 35 and 61 days in $H_2O$, NaCl and OPW. Solid lines reflect concentrations of mesocosms with *S. bentonitica* and dashed lines without bacteria.

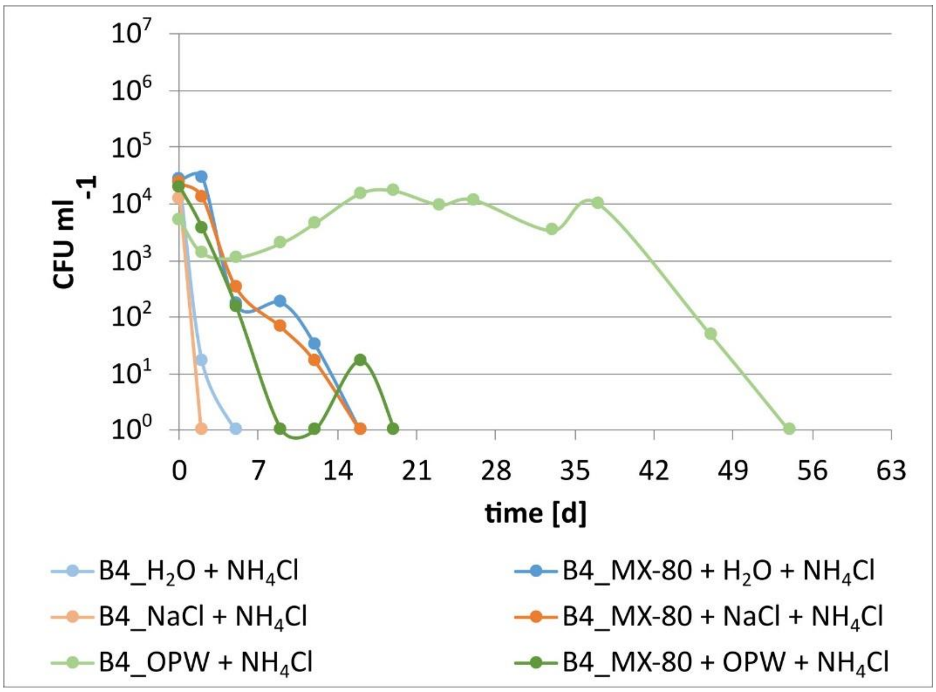



**Figure S5.** Growth of *S. bentonitica* determined as CFU $ml^{-1}$ in different solutions with and without the addition of MX-80 bentonite. All cultures were supplemented with $NH_4Cl$ as additional N-source.

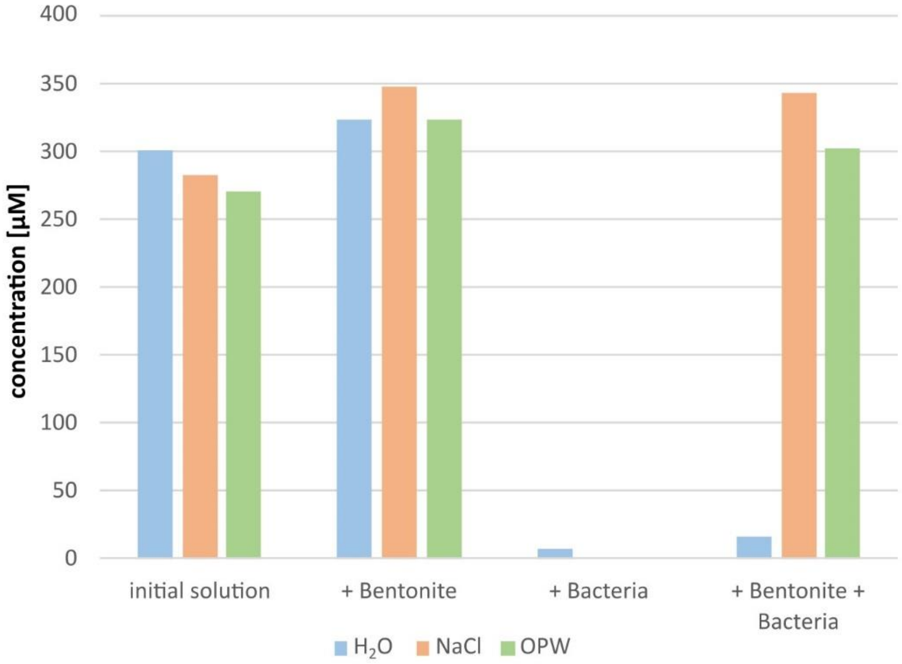



**Figure S6.** Acetate concentrations of $H_2O$, NaCl and OPW determined after 4 weeks in the initial solutions used for the batch experiments, in the control containing MX-80 but no *S. bentonitica* (+Bentonie), in the pre-cultures used to inoculate the mesocosms (+Bacteria) and in the experimental mesocosms containing *S. bentonitica* and MX-80 bentonite (+Bentonite +Bacteria).

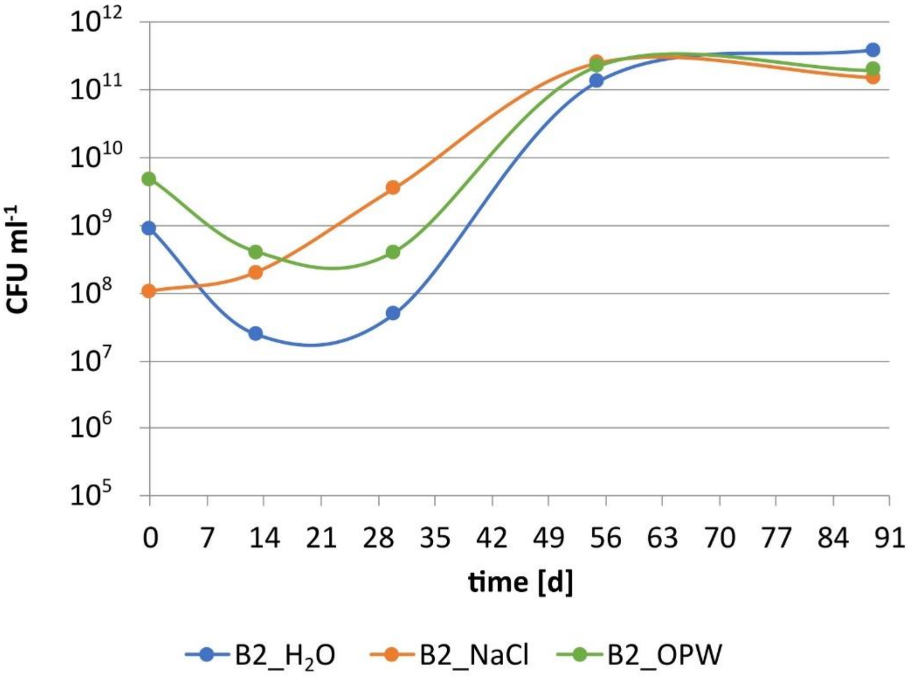



**Figure S7.** Growth of *S. bentonitica* determined as CFU $ml^{-1}$ in different solutions without MX-80 bentonite, incubated at room temperature, without agitation and aeration. These cultures were prepared by washing pre-cultures in the respective solution and then used for inoculating the mesocosms.

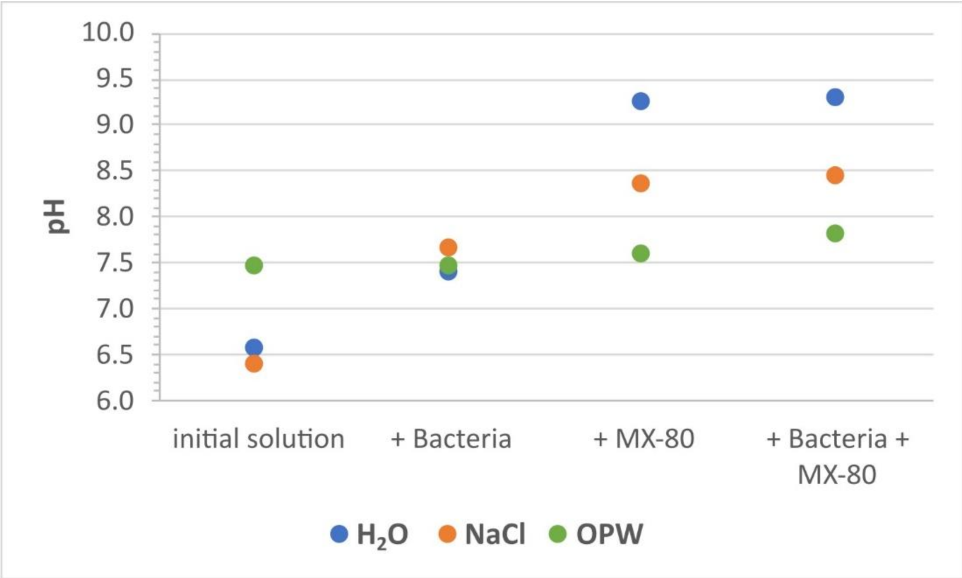



**Figure S8.** pH of the sterile filtered initial solutions used for the microcosms, pre-cultures that were not agitated, mesocosms containing MX-80 and mesocosms containing *S. bentonitica* and MX-80 after 69 days. Sample points represent the mean of duplicate or triplicate measurements.

## 3. Supplemental Tables

**Table S1.** $d_{001}$ values of montmorillonite after exposure to NaCl and OPW solutions in presence or absence of bacteria determined at different temperatures and relative humidity.

| | | *without bacteria (NC)* | | | *with bacteria (BAC)* | | |
|---|---|---|---|---|---|---|---|
| ***medium*** | **temperature (°C)** | **relative humidity (%)** | | | | | |
| | | **0** | **50** | **80** | **0** | **50** | **80** |
| NaCl | 27 | 10.3 | 11.6 | 15.0 | 10.2 | 11.6 | 15.0 |
| | 55 | 10.3 | 11.1 | 12.3 | 10.3 | 11.1 | 12.3 |
| | 80 | 10.2 | 10.5 | 11.6 | 10.0 | 10.5 | 12.0 |
| OPW | 27 | 10.2 | 14.2 | 15.7 | 10.2 | 13.6 | 13.2 |
| | 55 | 10.2 | 14.3 | 15.5 | 10.0 | 13.6 | 13.5 |
| | 80 | 10.2 | 13.4 | 14.7 | 10.0 | 13.4 | 12.4 |

**Table S2.** Pairwise PerMANOVA used to analyze the difference in XRD spectra of MX-80 incubated with (Bac) and without (NC) bacteria in $H_2O$, NaCl and OPW. The left panel of tables shows the *p*-value and the right panel of tables the F-values. Values in italics indicate p-values that were not significant after applying the Bonferroni correction. P-values of the grey shaded boxes are not significant, indicating no significant differences between the XRD spectra of MX-80 with and without bacteria.

| ***p-value*** | **NC_H2O_2d** | **NC_H2O_35d** | **NC_H2O_61d** |
|---|---|---|---|
| Bac_H2O_2d | **0.9769** | 0.0011 | 0.0001 |
| Bac_H2O_33d | 0.0001 | **0.4491** | 0.1012 |
| Bac_H2O_61d | 0.0001 | 0.0108 | **0.8375** |
| | **NC_NaCl_2d** | **NC_NaCl_35d** | **NC_NaCl_61d** |
| Bac_NaCl_2d | **0.9699** | 0.0001 | 0.0001 |
| Bac_NaCl_35d | 0.0002 | **0.0618** | 0.0034 |
| Bac_NaCl_61d | 0.0002 | 0.0442 | **0.4542** |
| | **NC_OPW_2d** | **NC_OPW_35d** | **NC_OPW_61d** |

| ***F-value*** | **NC_H2O_2d** | **NC_H2O_35d** | **NC_H2O_61d** |
|---|---|---|---|
| Bac_H2O_2d | 0.08903 | 6.627 | 14.99 |
| Bac_H2O_33d | 9.358 | 0.8479 | 2.161 |
| Bac_H2O_61d | 18.71 | 4.465 | 0.3332 |
| | **NC_NaCl_2d** | **NC_NaCl_35d** | **NC_NaCl_61d** |
| Bac_NaCl_2d | 0.2958 | 20.75 | 24.02 |
| Bac_NaCl_35d | 11.44 | 2.034 | 4.206 |
| Bac_NaCl_61d | 24.86 | 2.449 | 0.8697 |
| | **NC_OPW_2d** | **NC_OPW_35d** | **NC_OPW_61d** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Bac_OPW_2d | **0.1856** | 0.0001 | 0.0001 | Bac_APW_2d | 1.215 | 16.58 | 33.87 |
| Bac_OPW_35d | 0.0001 | **0.7011** | 0.0017 | Bac_APW_35d | 11.9 | 0.6912 | 5.667 |
| Bac_OPW_61d | 0.0001 | 0.0096 | ***0.0247*** | Bac_APW_61d | 14.37 | 2.908 | 2.924 |

**Table S3.** Calculation of the molar ionic strength *I* of the solutions $H_2O$, NaCl and OPW.

| | mM | M | no. of cat-ion | charge of cat-ion | no. of an-ion | charge of an-ion | |
|---|---|---|---|---|---|---|---|
| **H2O** | | | | | | | |
| $NaCH_3COO$ | 0.2 | 0.0002 | 1 | 1 | 1 | -1 | 0.0004 |
| | | | | | | ***I*** **=** | **0.0002** |
| **NaCl** | | | | | | | |
| NaCl | 154 | 0.154 | 1 | 1 | 1 | -1 | 0.308 |
| $NaCH_3COO$ | 0.2 | 0.0002 | 1 | 1 | 1 | -1 | 0.0004 |
| | | | | | | ***I*** **=** | **0.1542** |
| **OPW** | | | | | | | |
| NaCl | 212 | 0.212 | 1 | 1 | 1 | -1 | 0.424 |
| $CaCl_2$ | 26 | 0.026 | 1 | 2 | 2 | -1 | 0.156 |
| $Na_2SO_4$ | 14 | 0.014 | 2 | 1 | 1 | -2 | 0.084 |
| KCl | 1.6 | 0.0016 | 1 | 1 | 1 | -1 | 0.0032 |
| $MgCl_2$ | 17 | 0.017 | 1 | 2 | 2 | -1 | 0.102 |
| $SrCl_2$ | 0.51 | 0.00051 | 1 | 2 | 2 | -1 | 0.00306 |
| $NaHCO_3$ | 0.47 | 0.00047 | 1 | 1 | 1 | -1 | 0.00094 |
| $NaCH_3COO$ | 0.2 | 0.0002 | 1 | 1 | 1 | -1 | 0.0004 |
| | | | | | | ***I*** **=** | **0.3868** |

**Table S4.** Mantel test of different factors controlling the peak position and intensity of MX-80 bentonite in NaCl and OPW. Significant p-values are highlighted red.

| **MANTEL** | Correlation R: | p (uncorr): |
|---|---|---|
| **time** | **0.65** | **0.0001** |
| **ionic strength** | **0.12** | **0.0015** |
| **temperature** | **0.09** | **0.0130** |
| **humidity** | 0.03 | 0.2007 |
| **all** | **0.48** | **0.0001** |

## References


1. V. A. Colten-Bradley, Role of Pressure in Smectite Dehydration - Effects on Geopressure and Smectite-To-Illite Transformation. *Am. Assoc. Pet. Geol. Bull.* **1987**, *71*, 1414–1427.
2. M. J. Ikari, D. M. Saffer, C. Marone, Effect of hydration state on the frictional properties of montmorillonite-based fault gouge. *J. Geophys. Res. Solid Earth* **2007**, *112*, 1–12.