

The Final Report

Title: Reactions between contaminants and functionalized organic self-assembled monolayers in aqueous solutions

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Final Report

Contract Number FA5209-05-P-0402

Study of the reactions between contaminants and functionalised organic self-assembled monolayers in aqueous solutions

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Introduction

The subject of microbiological contamination in water has long been a major public concern, where microorganisms such as coliform bacteria, viruses and pathogenic protozoa incite various waterborne diseases ranging from mild gastroenteritis to severe diarrhea, cholera, typhoid, shigella and hepatitis. Waterborne diseases by microbial organisms infect approximately 250 million people each year causing 10-20 million deaths [1]. The majority of these cases occur in developing nations and rural areas where admissible sanitation is low and public health awareness less significant than in more developed countries. The health implications of drinking water quality and the continuing demand for aesthetic water has brought about a rapid proliferation in the types and numbers of point-of-use water treatment devices sold [2]. Various water treatment technologies available for purifying unpotable water include artificial UV radiation, chlorination, ozonation and solar heating [3], and though these methods prove beneficial for water disinfection, treatment can be costly, time consuming and require complex equipment. The use of self-assembled monolayers (SAMs) in this respect is to override these current issues to provide a cheaper option for water treatment that do not require energy to operate. While microbiological adhesions to SAMs have been investigated in areas such as infection of biomaterials [4], medicinal purposes [5,6] and food packaging [7], the application of functionalized SAMs for water treatment has not been studied widely.

A SAM is a closely packed, highly ordered array of chained hydrocarbon molecules containing various numbers of CH_2 -units [8]. The SAM is simply described as a hydrocarbon with the general formula $\text{A}-(\text{CH}_2)_n\text{-B}$. **B** represents the bonding group, such as trichlorosilyl ($-\text{SiCl}_3$) and trimethoxysilane ($\text{Si}(\text{OCH}_3)_3$) forming tightly covalent Si-O-Si-bonds to the surface atoms of silicon and silica. However, bonding with titanium and titania via Si-O-Ti bonds were also observed [9]. **A** denotes the head group, chosen from among a number of possible species, such as sulfonate ($-\text{SO}_3\text{H}$) and amine ($-\text{NH}_2$), respectively. The length of the hydrocarbon molecules and the related thickness of the SAM are calculated based on the numbers of CH_2 -units were calculated to vary between about 0.6 nm (three CH_2 -units) and about 2.5 nm (seventeen CH_2 -units). Immersed into aqueous solutions, some

of the head groups like sulfonate head groups tend to deprotonate and to form negatively charged surfaces. Head groups like NH_2 are known to deprotonate at high pH-values and to capture protons at low pH-values forming positively charged surfaces [10]. Therefore, by carefully choosing of the SAM and pH-value of the solution, in which the SAM is immersed, negatively as well as positively charged surfaces can be obtained.

Experimental

SAMs Synthesis

Amorphous silicon dioxide (SiO_2 , analytical reagent, Malinckrodt Chemical Works, grain size range: 3 – 90 μm with $d_{10}=7.6\ \mu\text{m}$, $d_{50}=24.3\ \mu\text{m}$, $d_{90}=49.1\ \mu\text{m}$, surface ratio.= 0.23 m^2/g) and crystalline quartz sand (grain size range: 100 to 350 μm with $d_{10}=121.9\ \mu\text{m}$, $d_{50}=239.3\ \mu\text{m}$, $d_{90}=330.6\ \mu\text{m}$, surface ratio = 0.02 m^2/g) was used as substrate powder. Before mixing with the modifying agent, the powder was dried in an oven at 450°C in air for several days in order to remove all adsorbed water from the surface, which is known to be stable up to about 450°C [10].

The powder was then immersed in 200 ml of a solution containing 70 vol% of H_2SO_4 and 30 vol% of H_2O_2 (30 wt%) for 5 min. Subsequently, the powder was separated from the solution using a Bucher funnel with a ceramic filter and rinsed with milli-q water until the pH of the water after rinsing was about 5

All organic chemicals were purchased from Aldrich. The following surfactants were used:

4-Trichlorosilylbutyronitrile ($\text{Cl}_3\text{Si}(\text{CH}_2)_3\text{CN}$) (CN-SAM) 97 %),

3-Aminopropyltrimethoxysilane ($\text{H}_2\text{N}(\text{CH}_2)_3\text{Si}(\text{OCH}_3)_3$) (NH_2 -SAM) (97 %), and

(3-Mercaptopropyl)trimethoxysilane ($\text{HS}(\text{CH}_2)_3\text{Si}(\text{OCH}_3)_3$) (SH-SAM) (95 %),

The deposition of the SAMs on the silica powder was performed by stirring the silica powder in a solution of anhydrous Toluene (99.8 %) containing 2 vol % of the surfactant for definite times (Table 1). Subsequently, the powders were filtered using Bucher funnel with a ceramic filter. 50 ml of solution was used for each experiment. After the removing of the powders from the solution, the wet powders were subsequently thoroughly washed in an ultrasonic bath first in chloroform (analytical reagent) and dried and secondly in milli-q water to remove all traces of excess surfactant. The washing treatment is known to be essential and effective

for removing of excess of the surfactant molecules, which are not attached to the surface [3,4,8,9]. The SH-functionality was converted into a SO₃H-functionality by immersing the powder in Oxone, which is a potassium hydrogen monopersulfate for about 4 h at room temperature, as described in [6-9]. Finally, the powder was dried in air at room temperature.

Table 1: Parameters for SAM deposition on silica particles.

SAM	Deposition time	Temperature	Atmosphere
CN	5 mins	Room Temp.	Dry nitrogen, glove box
NH ₂	5 min	Room Temp.	Air, fume chamber
SH	4 hrs	65 °C	Dry nitrogen, glove box
* SH → SO ₃ H	4 hrs	Room Temp.	Air, fume chamber

Electrophoresis measurements

Electrophoretic mobility have been measured using a Rank Brothers Mark II microelectrophoretic apparatus. For the measurements, high-purity water was produced with a Milli-Q[®] Reagent Water System by the following treatment. A reverse osmosis was done in two stages of mixed-bed ion exchange, which were an activated carbon treatment and a final filtering step through a 0.22 µm filter. The conductivity after this treatment was less than 0.5 µS cm⁻¹ and the surface tension was 72.8 mN m⁻¹ at 20° C.

A quantity of 500 cm³ of 0.125 w/v % sample was suspended in 0.001 M KNO₃ electrolyte and conditioned for 30 min at 20° C in a sealed, temperature controlled reaction vessel. The suspension was stirred continuously and nitrogen-purged during conditioning and measurement. Using small additions of KOH and HNO₃, the suspension pH was initially adjusted to 9, after which the pH was incrementally reduced from 9 down to 2 with 15-min time intervals to allow for equilibration between each two pH value measurements. Electrophoretic mobility was converted to zeta potential using the Smoluchowski equation [30].

FT-IR measurements

FT-IR measurements have been performed with a Nicolet Magna-IRTM Spectrometer 750 and a connected Omnic FT-IR Software. The measurements were all done in air in ATR mode using 5 mg of powder. As standard chemical KBr-powder for FT-IR measurements (Aldrich) was chosen. For the measurement of the samples, 5 % of the sample powder and 95 % of the KBr standard were mixed to assure the same measurement conditions.

Time-of-Flight-Mass-Spectrometry (ToF-SIMS)

ToF-SIMS measurements were performed using a PHI TRIFT 2100 ToF-SIMS Pectrometer (PHI, Chanhassen, USA) equipped with a gallium liquid metal ion gun (LMIG). Analysis of about 1 mg of the silica powder were performed using the spectroscopy mode of the unit

UV Absorption measurements

10 ml of the samples were measured at 279 nm using a UV-visible spectrometer (Phillips PU8470).

Albumin

20 mL of solutions containing 2 mg per Litre albumin (crystallized albumin from mouse serum, Sigma) were prepared. The pH of the samples was adjusted using appropriate amounts of HCl and NaOH, respectively. 5 g of SAMs coated silica powder (surface area of about 1 m²) per Litre water was added (50 mg per 20 mL) and the samples were stirred for various times using a magnetic stirrer at about 250 rpm. After treatment, the powders were separated from the water using 450 nm syringe filters.

Escherichia coli (E. coli)

Bacterial strains selected were *E. coli* ATTC 1175 and ATCC 1559 both enumerated in a similar manner. Prior to enumeration, pure cultures of each strain were obtained by employing the streak plate method. *E. coli* was cultured by taking a loopful of bacterial inoculum from the broth culture to a dry Tryptone Soya Agar (TSA) plate. The sample was streaked out to gain single colonies with incubation at 35°C, shaken at 150rpm. From an overnight culture, total culturable bacteria were estimated by performing serial dilutions in

Dulbecco's Phosphate Buffered Saline solution (DPBS, 100 μ L of *E. coli*, 900 μ L DPBS) and 100 μ L spread on dry TSA plates. Plates were inverted and incubated at 35°C for 24 hours. The viable plate count method was used to count the observed colonies and only counts between 20 and 300 colonies per plate were utilized for the calculation of bacterial numbers. The number of colony forming units (CFUs) per 100 μ L was calculated by multiplying the mean of the triplicate counts for the selected dilution by the reciprocal of the dilution. A final count per cm³ was calculated by a 10-fold multiplication.

To monitor the removal of *E. coli* in the presence of silica powder and sand particles (with and without SAMs) a 1cm³ aliquot of diluted *E. coli* culture (from 10² CFU/ μ L) was placed into 100cm³ of DPBS (pH $\approx$ 7.15) before 20cm³ was transferred from a sterile pipette into four individual 50cm³ beakers with varying concentrations for the substrate listed in table 1, each set on a magnetic stirrer. Sand particles were transferred into 50cm³ TTP tubes as an alternative to schott beakers and spun using a rotary wheel due to the weight of the sand.

Over a time interval of 0 to 100 minutes, 1cm³ of the sample was pipetted from solution every 20 minutes, placed in a 1.5cm³ eppendorf tube and centrifuged at 200rpm for 60 seconds. Samples were placed onto TSA plates (in triplicate) and spread evenly before subjection to overnight incubation at 35°C.

MS-2 bacteriophage

MS-2 bacteriophage stocks (ATCC 15597 B-1) were prepared in suspension (DPBS) using their host cells *E. coli* (ATCC 15597). Active cultures of the *E. coli* host were first cultivated for 24 hours at 35°C in the medium suggested by the American Type Culture Collection [12]. To enumerate, total culturable phage were estimated by preparing triplicate 10-fold serial dilutions of the phage stock suspended in DPBS. The addition of 30 μ L of an overnight *E. coli* culture and 100 μ L of phage suspension were transferred to pre-molten agar overlays and poured onto a dry TSA plate, shifting the plate until the overlay coats the surface, then incubated at 35°C for 24 hours. Plaque counts between 20 and 300 per plate were applied for calculation of page numbers. The number of phage forming units (PFUs) per 100 μ L was calculated by multiplying the mean of the triplicate counts for the selected dilution by the reciprocal of the dilution. A final count per cm³ was calculated by a 10-fold multiplication.

To examine the removal of MS-2 bacteriophage, 100 μ L of diluted phage culture (from 10⁵ PFU/100 μ L) was placed into 100cm³ of DPBS (pH $\approx$ 7.15) with 20cm³ of the solution placed into four separate 50cm³ beakers containing silica powder or sand particle concentrations listed in table 1. Beakers set on magnetic stirrers each (sand particles in 50cm³ TTP tubes, spun on a rotary wheel), 1cm³ of the sample was pipetted out of solution every 20 minutes (from 0 to 100 minutes), centrifuged for 60 seconds at 2000 rpm and in triplicate, 100 μ L transferred onto pre-molten agar overlays with the addition of 30 μ L of an overnight *E. coli* ATCC 15597 culture and poured onto dry TSA plates and set. Plates were incubated at 35°C for 24 hours.

Data Analysis

Statistical analysis was performed using Student's *t*-test on Microsoft Excel 2003. All *p* values <0.05 were considered to be significant.

Results and Discussion

The FT-IR measurements of the samples clearly show the presence of CH₂ in the samples (Figure 1). The asymmetric stretch mode at around 2926 $\pm$ 10 cm⁻¹ and also the symmetric stretch mode at around 2855 $\pm$ 10 cm⁻¹ of CH₂ of alkanes can be seen in most of the spectra very clearly [13]. Additional to these peaks, the asymmetric bending mode of alkanes at around 1460 $\pm$ 10 cm⁻¹ and the CH₂ bending vibrations of alkanes can be identified. These are the symmetric bending modes (umbrella modes) at 1375 $\pm$ 10 cm⁻¹. The determined CH₂-groups are believed to belong to the CH₂-units of the SAMs.

The functional head-groups of the SAMs provide pronounced absorption peaks in the FT-IR spectra of the samples. The spectrum of the SH-SAM shows a clear peak at 2564 cm⁻¹, which has its origin in the stretch mode of mercaptans and thiols and is, therefore, an indication for the SH-head-group of the SAM. The spectrum of the SO₃H-SAM contains a very small peak indicating mercaptans and thiols, but also a small peak at 1415 cm⁻¹, which belongs to SO₂ containing molecules. This also indicates that the transformation from the SH-SAM into the SO₃H-SAM is not fully completed. The spectrum of the CN-SAM shows a pronounced peak of C $\equiv$ N-groups at 2260 cm⁻¹. The spectrum of the NH₂-SAM contains a weak, but clearly

detectable peak due to scissors mode of primary amine groups at 1570 cm^{-1} . In addition, all spectra show a broad adsorption peak at about 1640 cm^{-1} , which can be attributed to OH bending of adsorbed water [14], supposedly caused by the final washing of the powder.

The ToF-SIMS measurements also clearly indicate the presence of the functional groups on the surface of the particles (Table 2). In case of the SO_3H -SAM coated powder, the data show that the transformation from the SH-SAM into the SO_3H -SAM was not complete, which is believed to be due to the fact that the used powder exhibits a much larger surface than the silicon wafers used in literature [6-9] and the published amounts of Oxone are, therefore, not sufficient to completely oxidize the SH-SAM at the surface of the particles.. The ratio between SH-groups and SO_3H -groups is about $\text{SH}/\text{SO}_3\text{H} = 30/70$. Prolonged oxidation with Oxone in order to increase the transformation rate were not successful. In addition, the S/Si ratio of the samples show that the transformation from SH to SO_3H results in a reduction of the sulphur content of about 80 %, which is believed to be equivalent to a reduction of about 80 % of the SAM coating. The reason for this phenomenon is believed to be caused by leaching of the SAM mainly due to oxidation of the CH_2 -units of the SAM by Oxone. Similar observations were made when H_2O_2 was used as the oxidation agent [15].

Table 2: ToF-SIMS analysis of the samples

	CN-SAM	NH₂-SAM	SH-SAM	SO₃H-SAM
Functional Group/Si ratio before SAM deposition/transformation	0	0.005	0.0002	0
Functional Group/Si ratio after SAM deposition/transformation	2.0771	0.0157	SH: 0.1333 SO ₃ H: 0.0005 S: 0.2409	SH: 0.0056 SO ₃ H: 0.0138 S: 0.0332

The results of the zeta-potential-measurements of the samples show clear variations depending on the type of SAM onto silica (Figure 2). Compared to the zeta-potential of the pure silica powder used in this study, the SH- and SO_3H -SAMs show a more negative, but similar potential over the measure pH range.

The zeta potential of the CN-SAM is similar to those of the SH- and SO₃H-SAMs at higher pH-values, but moves up into the positive region at a pH-value of about 3. Also the NH₂-SAM provides a positive surface charge at lower pH-values with an IEP at about 7 and depicts a pronounced positive surface charge with values at 30 to 40 mV at pH-values below 5.

The FTIR and ToF-SIMS measurements clearly indicate the presence of a SAM onto the silica particles of most of the samples. Most obvious is the presence of CH₂-groups in the spectra, which is believed to belong to the CH₂-groups of the chains of the SAMs. In addition, in the spectra of all samples peaks for the related head-groups of the different SAMs can be observed.

The more negative potential of the samples with SH- and SO₃H-SAM is due to deprotonation of the functional groups. Shyue et al. [16] have reported that SO₃H has a deprotonation rate of about 100 % at a pH value of 6.5 and above and a rate of about 50 % at a pH value of 2.5. The clear negative zeta-potential of SO₃H-SAM coated silica powder can, therefore, be easily explained by the deprotonation of the groups.

The ToF-SIMS measurements clearly show that the SH-functionality is not completely transformed into the SO₃H-functionality and a transformation rate of about 70% can be calculated from the ToF-SIMS data. In addition, the transformation results in a significant loss of the SAM coating. As the SO₃H-group has a more acid characteristic than the SH-group, a significant lower zeta-potential for complete transformed SO₃H-SAMs with a much higher coating density can be expected.

CN-groups are known to undergo hydrolysis in both, acidic and basic conditions [5]. In acidic conditions CN-groups are hydrolysed to CNH-groups and with further hydrolysis at low pH values eventually to NH₂-groups. The formation of NH₂-groups is believed to be the reason of the strong increase of the surface charge of the sample to positive values at low pH-values. In basic conditions the hydrolysis eventually results in the formation of COOH groups, which easily deprotonate forming COO⁻-groups resulting in an negatively charged surface of the particles at high pH values.

The chemistry of the amines is dominated by the ability of the lone pair on the nitrogen atom to capture protons forming NH_3^+ -groups. This phenomenon is the reason for the positive charge of the particles coated with NH_2 -SAM at pH values below about 7. Contrary to that, at pH values above about 7 the hydrogen of the amines is sufficient acidic to undergo deprotonation forming negatively charged NH^- -groups, which is the reason of the negative surface charge of the NH_2 -SAM coated particles at pH values above about 7.

Albumin removal

The measurements of the UV absorption at 279 nm of the obtained water samples clearly indicate a reduction of the concentration of albumin as a function of the treatment time and the pH value (Figures 3,4). The variation in the absorption of the starting samples (0 min) is due to slight variations in the concentration of albumin in the samples. Albumin absorption is about 0.5 per mg/ml. At the considered concentration of 2 mg per L the expected absorption is about 10^{-3} , which correlates with the measured values. The time dependence of the removal of albumin is believed to be mainly caused by the fact that at the very low concentration of both, albumin and powder, the powder particles need time to get in contact with the albumin molecules during stirring. For the optimum removal of albumin at a pH value of 6.5, 30 min treatment time, and the given concentration of albumin in water, a value of 0.06 mg albumin can be removed per m^2 SAMs coated surface and min.

The pH dependence of the removal is significant and is believed to be mainly caused by the variation of the surface charge of both, albumin and the amine functionality, at different pH values. The chemistry of the amines is dominated by the ability of the lone pair on the nitrogen atom to capture protons forming NH_3^+ -groups. This phenomenon is the reason for the positive charge of the particles coated with NH_2 -SAM at pH values below about 8. Contrary to that, at pH values above about 8 the hydrogen of the amines is sufficient acidic to undergo deprotonation forming negatively charged NH^- -groups, which causes a the negative surface charge of the NH_2 -SAM coated particles at pH values above about 8.

At pH 3.5 and 5 the bulk charge of albumin (i.e.p. at about pH 5.2) is positive like the amine functionality. However, it can be expected that negative binding sites, such as COO^- , are

available, which allow a limited interaction with the positively charged amine functionality. At a pH 6.5 the bulk charge of albumin is negative, whereas, that of the amine functionality is still positive. Therefore, their interaction is expected to be very strong, which would explain the significantly efficient removal of albumin from the water samples. At pH 8, albumin is negatively charged and the amine functionality is almost neutral. Therefore, only a very limited removal of albumin can be observed. At pH 9, both, albumin and the amine functionality is negatively charged. However, the removal of albumin has been observed to be as almost as efficient as at pH 6.5. The reason for this phenomenon is not clear, yet, but is most likely due to the formation of amide bonds between carboxylic acids and amines at elevated pH values in which the hydroxyl group of carboxylic acid has been replaced by an amine providing a chemical bonding between albumin and the SAMs [17].

Escherichia coli

With and without SAM coated silica powder

Figure 5 shows a combined graphical plot of *E. coli* removal in the presence of both silica powders with and without SAMs as a function of time. This data illustrates that removal of *E. coli* is achievable with each concentration of silica powder in respect to control observations, where experiments sustained bacterial values over the maximum 100 minute time point as no variation was found between the counts ($p > 0.05$) indicating no loss of bacteria due to experimental set up. Specifically, we found that though unmodified powder possessed the ability to remove bacteria, SAMs coated silica powder exhibited a much more efficient removal with the detection limit of the assay reached at 20 minutes with 20 m²/L, followed by 4 m²/L at 60 minutes, compared to unmodified powder with only a 1.4 log removal at the maximum concentration. With no statistical significance ($p > 0.05$) observed between concentrations 4 m²/L and 20 m²/L for unmodified silica powder, it can be postulated that no significant removal would be obtained at higher concentrations.

With and without SAM coated sand particles

Figure 6 displays the capability of sand particles with and without SAMs to remove *E. coli* from solution. The acquired consistency of the data resulting from all concentrations of unmodified sand particles together with the added insignificance of the data with the control observations ($p > 0.05$) demonstrates that the particles have no mechanistic ability to attract

the bacteria to its surface. In correlation to SAMs coated sand particles data, a steady increase in removal is observed, therefore demonstrating that bacteria removed in these conditions are attributed to the presence of the SAMs. With each concentration of the SAMs coated particles possessing larger removal at higher concentrations, only a maximum 1.7 log removal is obtained with 1 m²/L at the highest time point compared to SAMs coated silica powder reaching the detection limit at the same time. The latter could be explained by silica powders higher surface area (100 µm compared to 120 µm), allowing the bacteria to have greater access to the binding sites on the surface, which may also cause faster removal (also observed with unmodified powder) and hence, greater removal efficiency.

MS2-Bacteriophage

With and without SAM coated silica powder

(Removal analysis was also adapted with MS-2 bacteriophage, except a 60-minute duration was recommended due to results obtained from *E. coli* experiments)

A correlation between the binding natures of *E. coli* and MS-2 bacteriophage is evident with results obtained from both silica powders with and without SAMs in Figure 7. Both substrates succeed in removing bacteriophage, with silica powder coated SAMs reaching the detection limit at the first time interval of 20 minutes with 20 m²/L. If the sampling period were increased to 100 minutes, the presumption here is that a similar decline would be observed for the bacteriophage compared to *E. coli*, where an initial rapid decrease would occur, followed by a gradual decrease with all concentrations reaching a plateau over time and no binding sites left for increased removal of the microorganisms.

With and without SAM coated sand particles

Removal of bacteriophage using sand particles with or without SAMs resulted in the same outcome as *E. coli* illustrated in Figure 8. No significant difference was observed for unmodified sand particles ($p > 0.05$). Removal with SAMs coated sand particles followed a similar trend, though the rate of removal was slower with bacteriophage in relation to *E. coli* with only a maximum 1.2 log removal compared to a 1.7 log removal maximum for *E. coli*. This may be attributed to a large number of factors, including surface charge, surface roughness and surface area, not covered in the scope of this text.

SEM

A low-magnification scanning electron micrograph representative of two sites on the surface of one sand (with SAMs) particle sample is illustrated in Figure 9 (a) and (b). Investigations verified the presence of randomly distributed individual rod-shaped cells both the size (approximately 1 μm) and morphology of *Escherichia coli*, confirming their adhesion on the particle surface in the presence of SAMs. Focusing on figure 5 (b) exposes *E. coli* attachment with type 1 fimbriae (filamentous proteinaceous appendages) that act in adhesions involved in specific receptor recognition and colonization, characteristic of *E. coli* binding properties.

No MS-2 bacteriophage samples were analyzed with the scanning electron microscope as documentation [8] suggest identification of viruses do not preserve well as a result of drying procedures and small size ($\approx 30\text{nm}$). Distinction between a phage and an artifact at this level of magnitude would be difficult to discern.

Conclusion

The interaction of bacteria, virus, and proteins with functionalized surfaces has been studied widely and the results of this investigation do not contradict the existing knowledge [18,19]. However, the attachment of the organic matter onto SAMs coated particles in considerable agitated water is remarkable, because it indicates a strong interaction between the SAMs and the organic matter. At the pH of the water samples the organic compounds studied here are negatively charged, whereas, the NH_2 -terminated SAM is positively charged [5]. It is therefore assumed, that the removal of the organic matter is mainly due to a strong electrostatic attraction and immobilisation of the organic matter at the surface of the particles.

A very clear indication of the crucial role of the SAM is the fact that particles without SAM-coating (NO-SAM) have no influence on the organic matter as indicated by the experiments. Increasing the available surface by decreasing the grain size of the SAMs coated material significantly increases the efficiency of the removal. With SAMs coated silica powder a decrease of about two orders of magnitude of the amount of bacteria was observed after a treatment of 20 min, whereas, using coarser quartz sand a decrease of only about one order of magnitude was achieved.

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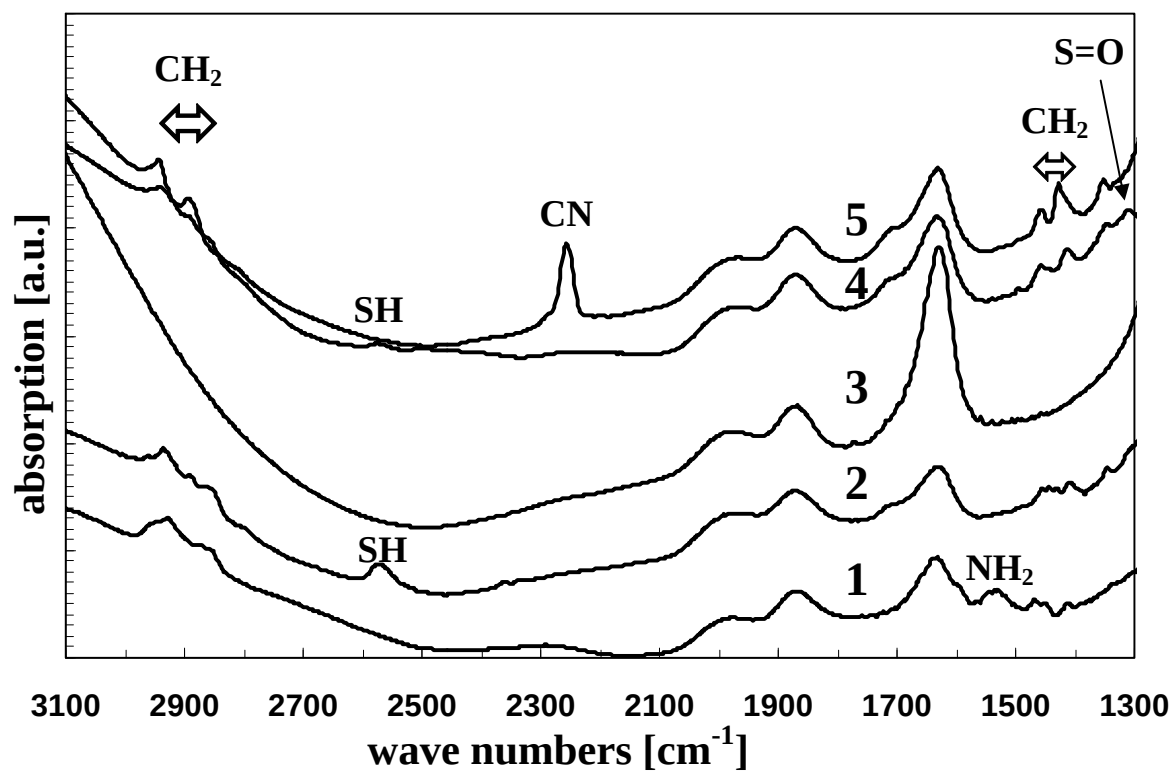


Figure 1: FTIR-measurements of the prepared samples

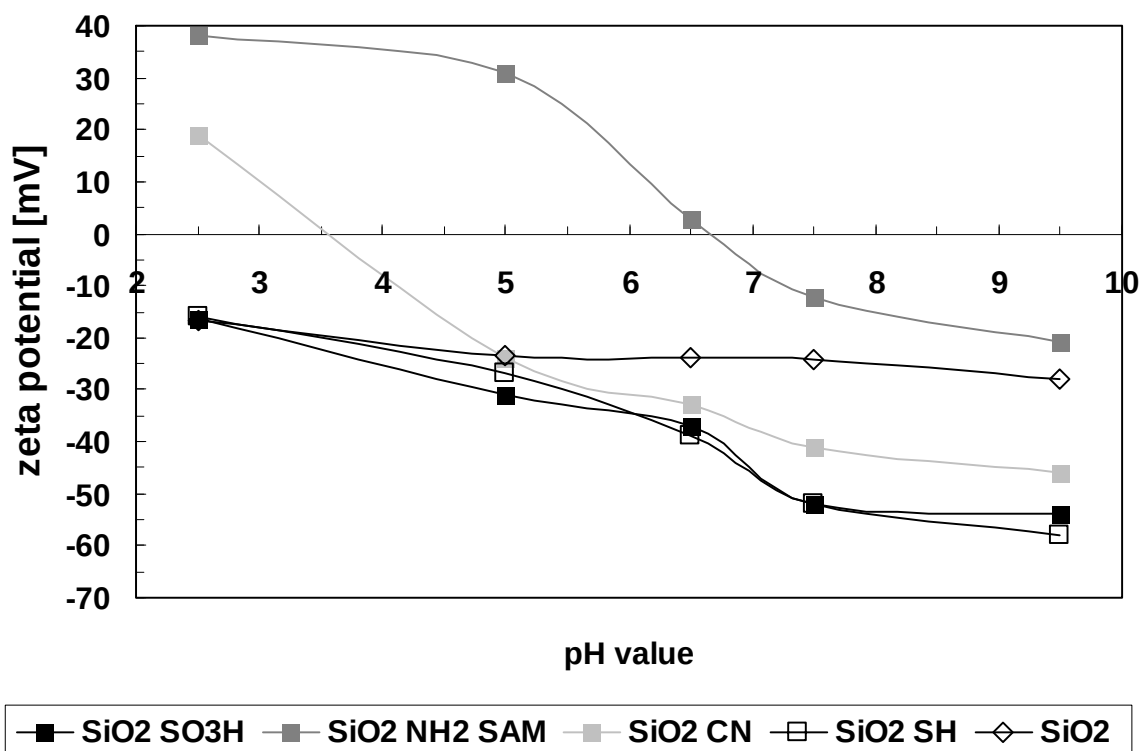


Figure 2: zeta-potential of silica, SH-SAM on silica, SO₃H-SAM on silica, CN-SAM on silica, and NH₂-SAM on silica.

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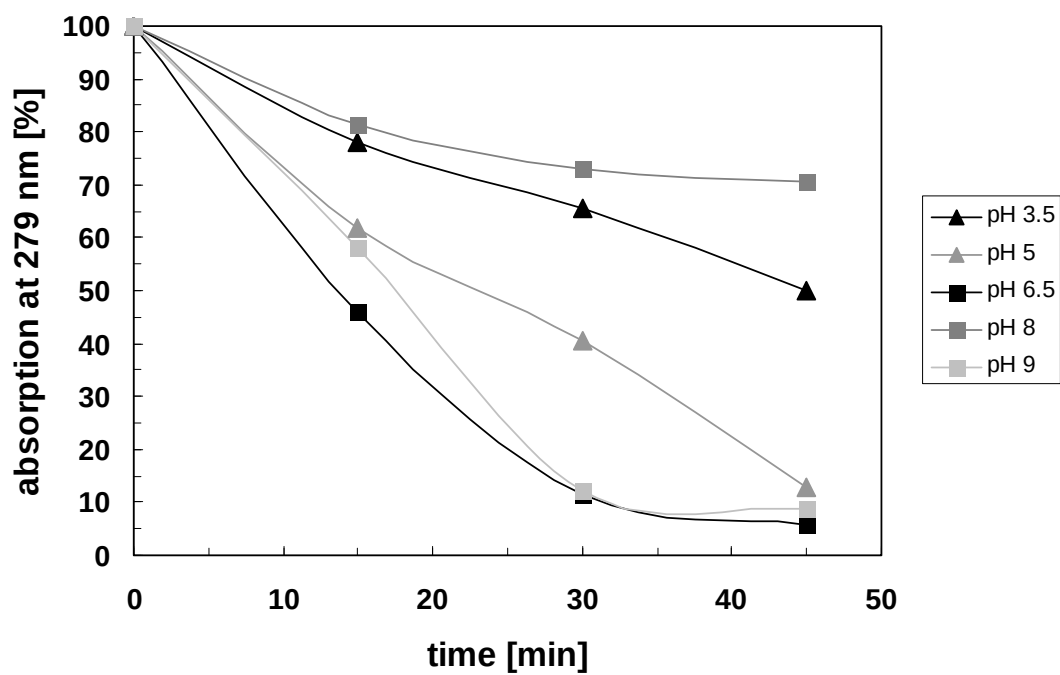


Figure 3: Absorbance of albumin at 279 nm in % of starting sample vs. treatment time.

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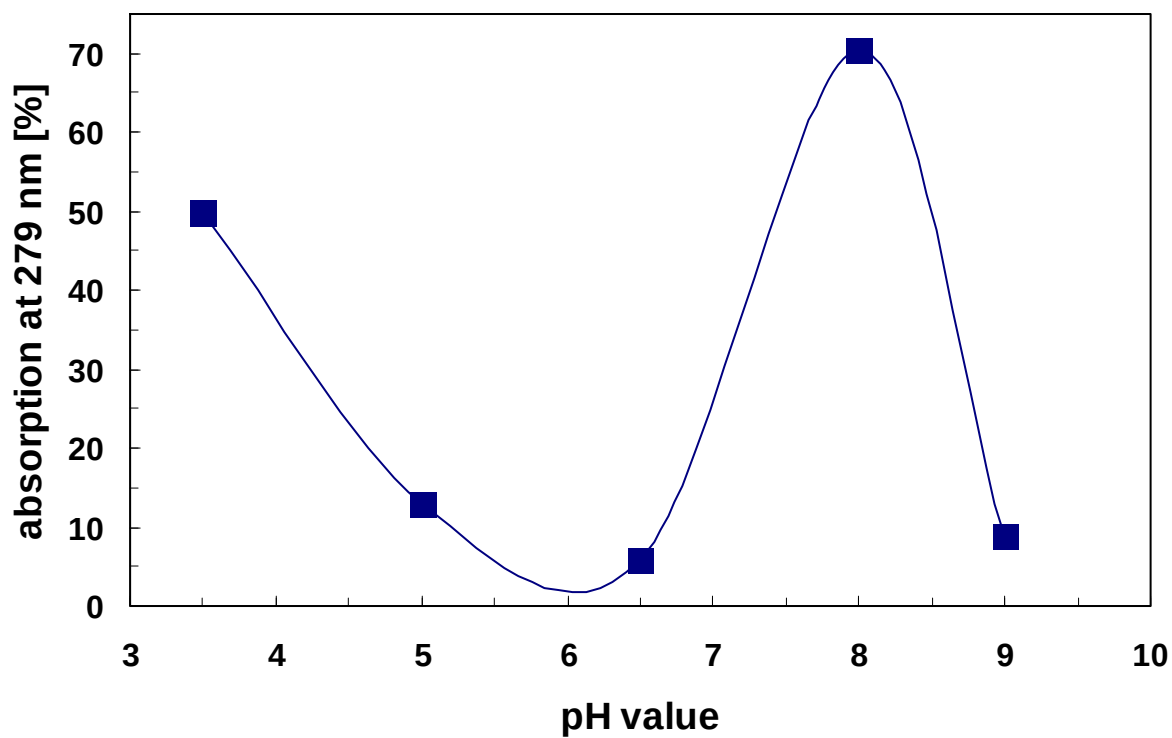


Figure 4: Absorbance of albumin at 279 nm in % of starting sample after a 45 min treatment vs. pH value.

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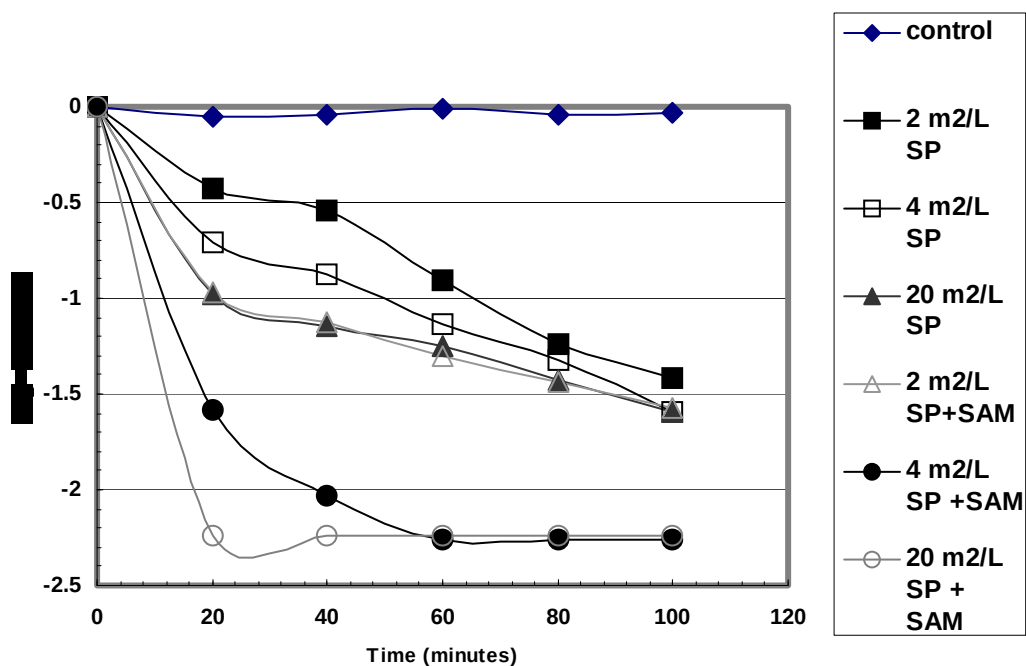


Figure 5: Graph of silica powders with and without SAMs *E. coli* log₁₀ removal vs. time

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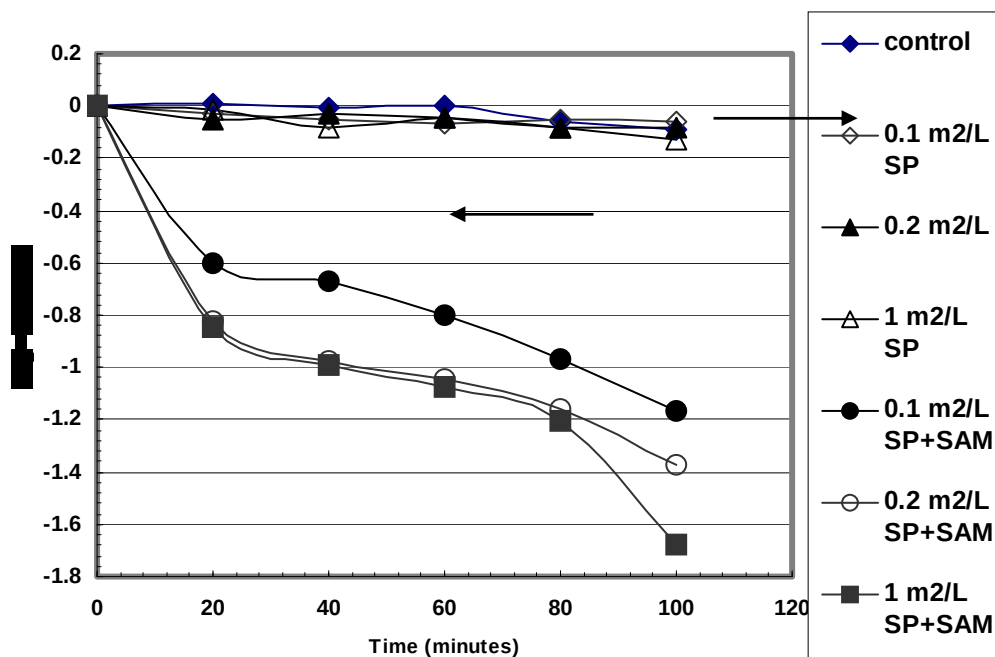


Figure 6: Graph of sand particles with and without SAMs *E. coli* log₁₀ removal vs. time.

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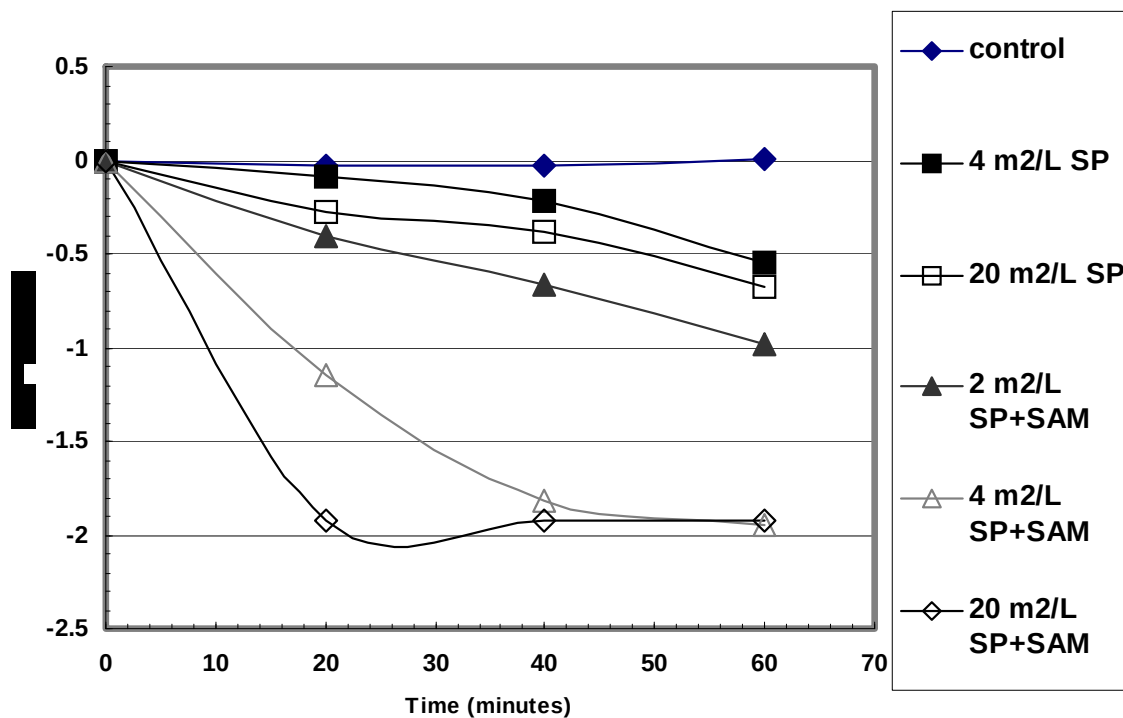


Figure 7: Graph of silica powders with and without SAMs phage log₁₀ removal vs. time

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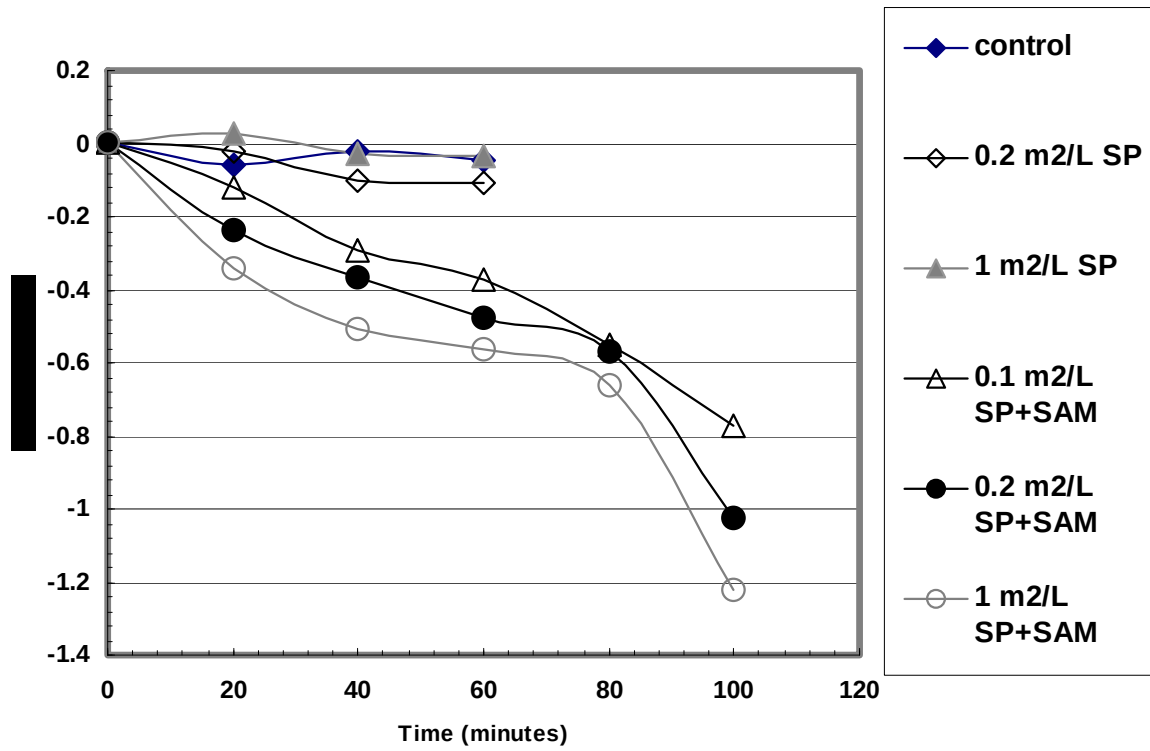


Figure 8: Graph of sand particles with and without SAMs phage log₁₀ removal vs. time

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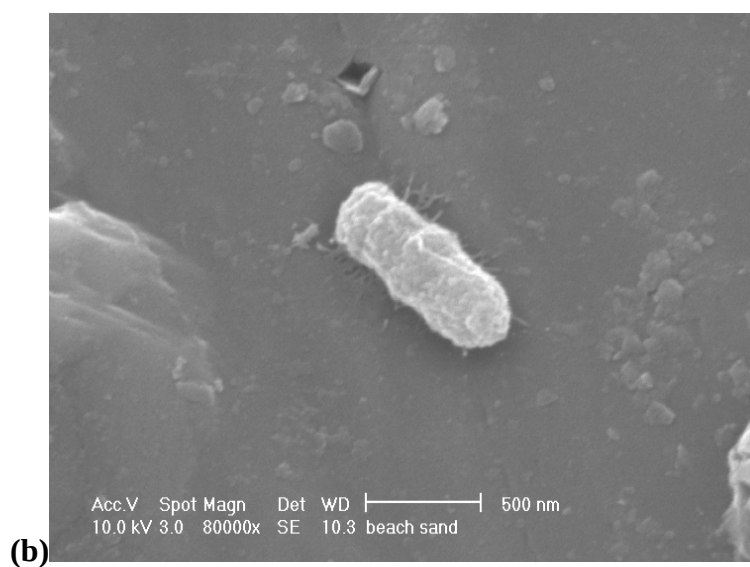
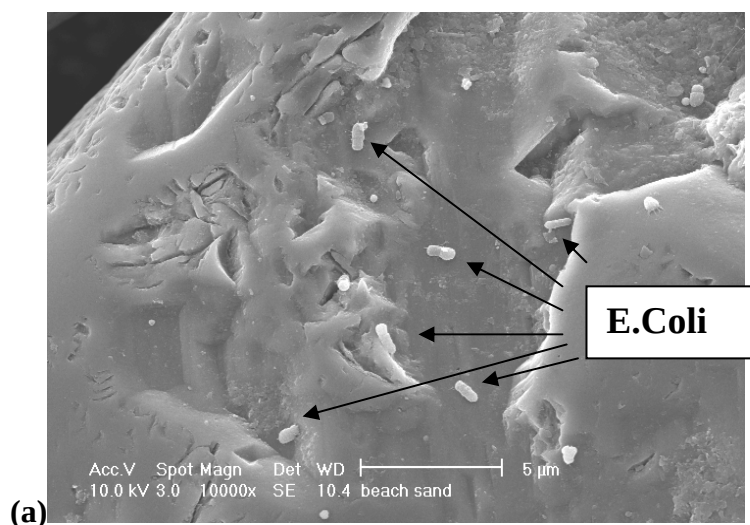


Figure 9 (a) & (b): SEM images from two different sites on sand particles (with SAMs)