

Adsorption of Microorganisms to Sand and Diatomaceous Earth Particles Coated With Metallic Hydroxides*

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Abstract

Particles of diatomaceous earth and sand were coated with metallic hydroxides. The coating increased the concentration of metals associated with the particles, their zeta potential, and their ability to adsorb microorganisms in water. The maximum adsorption of microorganisms was achieved using particles coated with a combination of ferric hydroxide and aluminum hydroxide.

1. Introduction

Metallic hydroxides flocs (ferric hydroxide and aluminum hydroxide) have been used to treat water and waste water for many years (1). These flocs have been found to adsorb microorganisms and to remove them from the water following settling or filtration. Metallic hydroxide flocs have also been used in procedures to recover viruses from water as part of detection procedures (11, 12). In both applications, efficient adsorption of microorganisms has been observed.

Combining metallic hydroxide flocs with microporous or depth filters has had limited success in producing flow-through filters for adsorbing microorganisms in large volumes of water. The flocs are either not retained by the filters or clog the filters and greatly restrict the flow of water (5, 9).

It is also possible to coat sand or diatomaceous earth by using pre-formed flocs of metallic hydroxides or by forming the flocs in solutions that are in contact with the particles (2, 3, 4, 8). Coating particles with a metallic hydroxide by these procedures increases their ability to adsorb microorganisms (2, 3, 8) and metallic ions (4) from water. However, the coating procedure may have to be repeated many times (up to 15 times) or require a relatively long contact time (24 to 36 hrs) to ensure coating (2, 3, 4, 8). Also, the coatings formed may not be stable and may be detached from the particles in contact with water (2, 3).

In previous studies, we have found that forming metallic hydroxides directly on the surface of a particle

or inside the fibers of a filter produces a stably modified solid. Also, combinations of metallic hydroxides can be deposited on a particle (5, 6).

In this paper, we have expanded our previous studies by including sand and by determining the adsorption of bacteria along with viruses. Both sand and diatomaceous earth particles coated with a combination of ferric hydroxide and aluminum hydroxide efficiently removed bacteria and bacteriophages from water.

2. Materials and experimental procedure

Diatomaceous earth (grade 1) was obtained from Sigma Chemical Co., St. Louis, MO. Sand was obtained from local sources and sieved to 50 or 100 mesh. For coating with one metallic hydroxide, the sand or diatomaceous earth was mixed with sufficient solutions of ferric chloride, magnesium chloride or aluminum chloride to cover it for 30 minutes. The specific solutions are given in **Figures 1 and 2** and **Tables 2 and 3**. Excess liquid was drained and the solids were allowed to air dry. The samples were then mixed with approximately 2 volumes of 3 N ammonium hydroxide for 10 min, rinsed with deionized water to remove excess precipitates, allowed to dry and rinsed again. Particles were coated with combinations of metallic hydroxides as described above except that solutions with different concentrations of ferric chloride, magnesium chloride and aluminum chloride were used. Sequential coating of particles was accomplished by treating the particles with either ferric chloride, magnesium chloride or aluminum chloride then forming the corresponding hydroxide as described above and then repeating the procedure using a different salt for the second coating.

* Journal paper no. R-05459 from the Florida Agriculture Experiment Station, Gainesville

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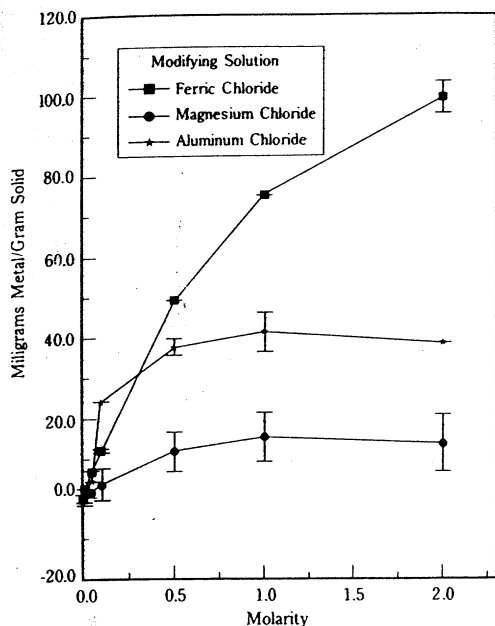


Fig. 1 Metal Composition of Modified Diatomaceous Earth

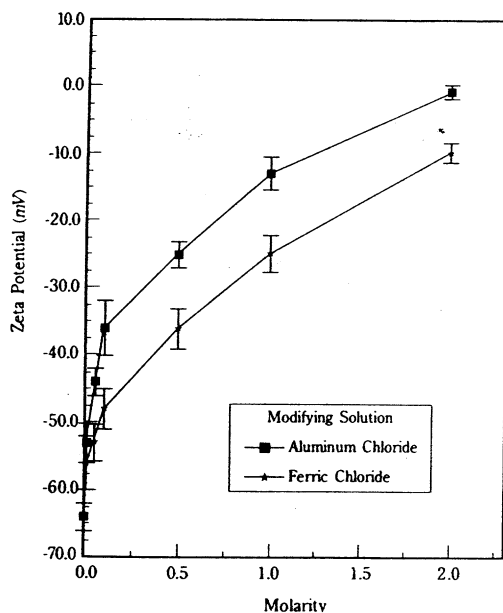


Fig. 2 Zeta Potential (mV) of diatomaceous Earth vs. Molarity of Modifying Solution

Table 1. Comparison of the removal of microorganisms by diatomaceous earth treated sequentially with two metal salts or a single metal salt¹.

Single Treatment	PRD-1 Removal (Log ₁₀)	Sequential Treatment Salt #1 → Salt #2	PRD-1 Removal (log ₁₀)
AlCl ₃	2.54 ± 0.04	0.25M FeCl ₃ → 0.1M AlCl ₃	2.95 ± 0.05
FeCl ₃	1.71 ± 0.03	0.25M FeCl ₃ → 0.5M AlCl ₃	3.56 ± 0.05

¹ Batch Adsorption experiments were conducted in buffer at pH 7 using 0.5g diatomaceous earth as described in the text.

Table 2. Removal of bacteria and bacteriophages in batch experiments by sequentially treated or untreated sand and diatomaceous earth from seeded pH 7 buffer¹.

Microorganism	Log ₁₀ Reductions For			
	Untreated Sand	0.5M FeCl ₃ → 1M AlCl ₃ Treated Sand ²	Untreated Diatomaceous Earth	0.25M FeCl ₃ → 0.5M AlCl ₃ Treated D.E. ²
<i>S. Aureus</i>	0.21 ± 0.03	3.34 ± 0.09	0.33 ± 0.01	3.91 ± 0.09
<i>S. Faecalis</i>	0.29 ± 0.02	2.76 ± 0.07	0.36 ± 0.02	3.31 ± 0.10
<i>E. Coli</i>	0.20 ± 0.02	2.18 ± 0.08	0.34 ± 0.03	2.27 ± 0.06
<i>S. Typhimurium</i>	0.32 ± 0.03	2.33 ± 0.12	0.53 ± 0.07	2.59 ± 0.11
MS-2	0.20 ± 0.01	3.26 ± 0.22	0.22 ± 0.02	5.06 ± 0.21
ΦX-174	0.09 ± 0.006	3.06 ± 0.11	0.13 ± 0.01	3.81 ± 0.12
PRD-1	0.18 ± 0.09	2.78 ± 0.04	0.21 ± 0.05	4.03 ± 0.14

¹ Sand or diatomaceous earth was soaked first in a ferric chloride solution, allowed to dry, soaked in 3M ammonium hydroxide, rinsed and dried, soaked in an aluminum chloride solution, dried, soaked in 3M ammonium hydroxide, then it was rinsed and dried.

² Sand or diatomaceous earth was soaked in a ferric chloride solution, allowed to dry, soaked in 3M ammonium hydroxide, rinsed and dried, soaked in an aluminum chloride solution, dried, soaked in 3M ammonium hydroxide, then it was rinsed and dried.

Table 3. Removal and recovery of bacteriophages from water by sand columns or diatomaceous earth filters¹.

Procedure	Bacteriophages		
	PRD-1	ΦX-174	MS-2
Diatomaceous Earth Filters			
Treated ²			
Percent Removal	99.934%	99.996%	99.987%
Percent Recovery	34%	40%	45%
Untreated			
Percent Removal	50.12%	27.31%	15.48%
Sand Columns			
Treated ³			
Percent Removal	61.690%	85.659%	72.683%
Percent Recovery	36%	43%	48%
Untreated			
Percent Removal	8%	11%	14%

¹ Twenty liters of dechlorinated tap water was seeded with indicated bacteriophage and passed through filters containing modified or unmodified diatomaceous earth or through columns containing modified or unmodified sand.

² Five grams of unmodified or diatomaceous earth modified sequentially with 0.25M ferric and 0.5M aluminum chloride as described in the text was packed in a series of two 47mm holders with a fiberglass filter sheet.

³ A 60 × 3 centimeter column was packed with either unmodified or sand modified sequentially with ferric and aluminum chloride as described in the text.

The amount of metal deposited on the particles was determined by first mixing samples of sand and diatomaceous with 5 volumes of 6 N HCl overnight. The solids were separated from the solution by settling (sand) or by centrifugation at 300 g for 5 min

(diatomaceous earth). The composition of the solutions were analyzed using an Inductively Coupled Argon Plasma (ICAP) spectrophotometer. Samples of tap water were also analyzed for metal content before and after passage through filters and columns, using the ICAP spectrophotometer.

The zeta potential of diatomaceous earth particles was measured in 0.02 M glycine 0.02 M imidazole buffer using a Lazer Zee model 501 zeta meter.

The following bacteria were used: *Streptococcus faecalis* (ATCC 19433), *Staphylococcus aureus* (ATCC 12600), *Salmonella typhimurium* (ATCC 19585), and *Escherichia coli* (ATCC 15597). The following bacteriophages and their hosts were used: MS2 (*Escherichia coli* C-3000); Φ X 174 (*E. coli*); and PRD-1 (*Salmonella typhimurium*). The bacteria were grown in 3% trypticase soy broth overnight and then diluted 1/1000 in buffer (0.02 M glycine + 0.02 M imidazole) for adsorption studies. Phage stocks were prepared and phage assays done using their host bacteria as previously described (10).

Adsorption studies were conducted by mixing 2 grams of sand or 0.5 grams of diatomaceous earth in buffer (0.02 M imidazole + 0.02 M glycine) with the appropriate virus or bacterium for 5 min. on a reciprocating shaker. The supernatant fractions were assayed after settling (sand) or after low-speed centrifugation (300 g for 5 min for diatomaceous earth). Adsorption was determined by comparing the number of microorganisms in the supernatant fractions with the number in control samples of buffer alone. Unless otherwise stated, adsorption experiments were conducted at pH 7 and room temperature (approximately 25°C).

Bacteria and viruses adsorbed to particles were recovered by mixing the samples with 20 ml of 3% beef extract, pH 7, for 5 min. The microorganisms were separated from the solids by settling or centrifugation, as described above. The fraction recovered was determined by comparing the number of microorganisms in the beef extract with the number adsorbed.

Adsorption of phages in tap water dechlorinated by the addition of sodium thiosulfate was studied using 5 g of diatomaceous supported by fiberglass filters (0.45- μ m, Filterite Corp., Timonium MD) in a series of two 47-mm holders or approximately 400 grams of sand in 3 by 60 cm columns. Water containing added phages was passed through the filters at 100 ml/min and through the columns at 1,000 ml/min. Adsorbed phages were recovered by mixing the diatomaceous with 20 ml of beef extract or mixing the sand with 200 ml of beef extract as described above.

3. Results

Increasing the concentration of the salts used for modification increased the amount of metal deposited on the diatomaceous earth (Figure 1). The amount of aluminum or magnesium deposited did not increase much after the concentration of modifying solution was increased past 0.5 M. The concentration of iron deposited on diatomaceous continued to increase as the concentration was increased to 2 M. Higher concentrations of salts were not used since these were hygroscopic and not easily dried.

The addition of the metallic hydroxides increased the zeta potential of the diatomaceous earth particles (Figure 2). The zeta potential continued to increase as the concentration of salts used for modification was increased. Similar results were obtained with diatomaceous earth particles treated with magnesium chloride (data not shown).

Changing the zeta potential of diatomaceous earth particles greatly increased the ability of the particles to adsorb bacteriophages in batch tests (Figure 3). Increasing the zeta potential to -10 mV permitted a reduction of greater than $2.5 \log_{10}$ in the numbers of the three phages tested. Data in Figure 3 were obtained using diatomaceous earth coated with aluminum hydroxide. Similar results were obtained with diatomaceous earth coated with ferric hydroxide or magnesium hydroxide (data not shown).

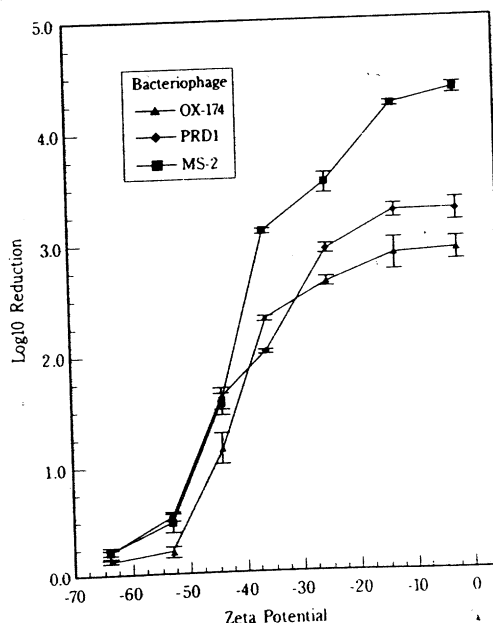


Fig. 3 Removal of Bacteriophage From Water vs. Zeta Potential of Modified Diatomaceous Earth.

The adsorption of bacteria to diatomaceous earth coated with aluminum hydroxide was similar to adsorption of phages in that increasing the zeta potential increased adsorption (**Figure 4**). However, untreated diatomaceous earth adsorbed more bacteria than phages. Also, the removal of *E. coli* and *S. typhimurium* ($1.5 \log_{10}$) was less than that observed for the phages and for *S. aureus* (greater than $2.5 \log_{10}$).

Only aluminum hydroxide and ferric hydroxide were used in most tests since these have lower solubility constants than magnesium hydroxide or other metallic hydroxides (7). Additional tests were conducted using diatomaceous earth treated with different combinations of aluminum and ferric chloride. Combinations of aluminum chloride and ferric chloride between 0.1 and 2 M were used in combination or sequentially to treat diatomaceous earth and sand particles. Diatomaceous earth sequentially treated with 0.25 M ferric chloride and 0.5 M aluminum chloride adsorbed more virus than did diatomaceous earth treated with a single salt or other combinations of salts. Representative data from these tests is shown in **Table 1**.

In a similar series of tests, it was found that treating sand with 0.5 M ferric chloride and 3 M ammonium hydroxide followed by 1 M aluminum chloride and 3 M ammonium hydroxide adsorbed more microorganisms than sand treated with other combinations (data not shown). Sand or diatomaceous earth treated with the combinations of ferric chloride and aluminum chloride described above adsorbed significantly more of the microorganisms tested than did the untreated materials (**Table 2**). Filters containing diatomaceous

treated with the combination of ferric chloride and aluminum chloride as described above removed greater than 99.9% of the bacteriophages from 20 liters of dechlorinated tap water (**Table 3**). However, less than 50% of the phages could be recovered using 3% beef extract as the eluting solution. Untreated diatomaceous earth removed from 15 to 50% of the phages. Sand columns removed 62 to 86% of the phages in dechlorinated tap water. Untreated sand removed less than 15% of the phages. As with the diatomaceous earth, less than 50% of the phages could be recovered using beef extract.

The concentrations of iron and aluminum in the tap water before filtering and in the effluent from the diatomaceous earth filters and sand columns was less than 0.1 mg/l.

4. Discussion

The ability of metallic hydroxide flocs to adsorb microorganisms has been useful both in treating water and in recovering viruses from water (1, 11, 12). This has led to studies on their immobilization on filters. Collecting preformed flocs on microporous filters has not led to the development of useful filters. Filters that retain the flocs are subject to clogging (5, 6, 9). Forming flocs in the presence of particles can result in coating of the particles (2, 3, 4, 8). These coating procedures may not be efficient. Several cycles (15) of floc-formation followed by adsorption may be necessary (4) or long periods (24 to 36 hrs) of contact between the particles and flocs may be necessary to ensure coating (8). Also, the coating may not be stable enough to permit the use of the coated particles in filters used for tap water (2, 3). Adsorption of a soluble metallic chloride by particles, followed by drying of the particles and then precipitation of the metals as the hydroxide leads to the formation of stable coatings. Also, coatings with multiple metallic hydroxides can be deposited on the particles (5, 6). Our results show that increasing concentration of salt used to modify the particles increases the concentration of deposited metal and the zeta potential of the particles. The reason for the increase in zeta potential after the concentration of metal deposited has become stable is not known.

It is clear that the zeta potential of the modified solids is directly related to the adsorption of both bacteria and bacteriophages. Efficient adsorption of these microorganisms occurs once the zeta potential is raised above approximately -30 mv (for phages) and -10 mv (for bacteria).

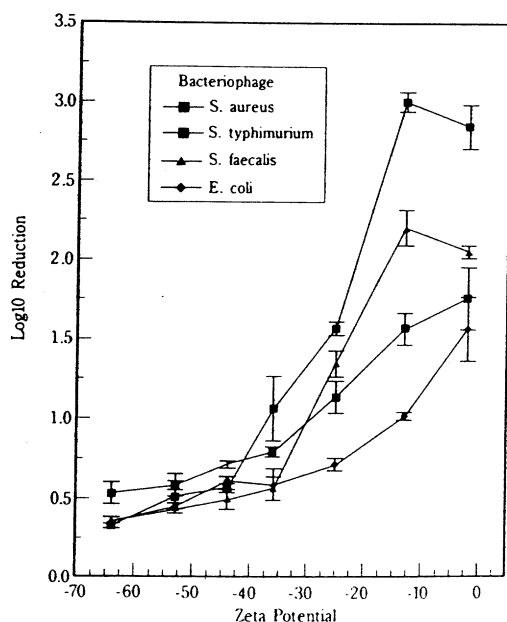


Fig. 4 Removal of Bacteria from Water vs. Zeta Potential of Modified Diatomaceous Earth.

Coating diatomaceous earth and sand particles with a combination of ferric hydroxide and aluminum hydroxide permitted greater removal than coating the particles with only one metallic hydroxide. Filters and columns composed of particles made with these modified solids removed significantly more microorganisms than did similar filters and columns composed of untreated particles.

Attempts to recover microorganisms adsorbed to the modified particles were only partially successful. Only 50% or less of the microorganisms removed from water could be recovered in the beef extract eluting solution. Improvements in the recovery rate would make the filters more useful for detecting microorganisms in large volumes of water.

None of the metals used for coating diatomaceous earth were detected in effluents from the filters. It does not appear that leaching of the metals from

the particles will produce toxic effluents or greatly limit the length of time that the filters could be used.

In summary, we have found that the removal of bacteria and phages can be improved by using diatomaceous earth or sand particles coated with a combination of ferric hydroxide and aluminum hydroxide. Future studies will concentrate on testing the ability of the filters to remove microorganisms from larger volumes of water and on improving procedures for recovering them from the adsorbed particles.

Acknowledgment

We would like to acknowledge the financial contribution of the Engineering Research Center (ERC) for Particle Science and Technology at the University of Florida, The National Science Foundation (NSF) grant #EEC-94-02989, and the Industrial Partners of the ERC.

Author's short biography



Jerzy Lukasik received his bachelor's degree in Microbiology and cell science from the University of Florida in 1993. Currently, he is working towards the completion of his Ph.D. in Microbiology and cell science. He is also a member of the advanced separations thrust group of the Engineering Research Centers.

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At Present Dr. Shah is the director of the Center for Surface Science and Engineering as well as the head of two Engineering Research Centers thrust groups in the area of advanced particle processes. He received his undergraduate training at the University of Bombay and his doctoral degree from Columbia University in 1965 under the direction of the late professor J.H. Shulman a pioneer in the field of Surface and Colloid Science. In 1970 he joined the University of Florida and has received numerous awards in his illustrious career spanning over 25 years. Most recently he was the recipient of the Charles A. Stokes Professorship of Chemical Engineering, Anesthesiology and Biophysics.

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