# Effectiveness of Composite Iron Matrix in Removing Viruses from Water

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## Abstract

**Effectiveness of Composite Iron Matrix in Removing Viruses from Water**

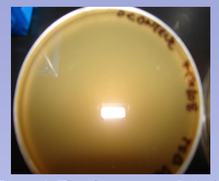
Composite Iron Matrix (CIM) was developed by Dr. Abul Hussam to remove arsenic from water in contaminated wells. Arsenic in drinking water causes bladder, lung and skin cancer, and may cause kidney and liver cancer. Arsenic can also harm the central and peripheral nervous systems, as well as heart and blood vessels, and may cause serious skin problems. It also may cause birth defects and reproductive problems. Similarly, viruses can be present in high numbers in drinking water. Water-borne viruses have become a major threat to numerous countries where water filtering is not properly used. In addition to removing arsenic from drinking water, published studies suggest that filtering materials such as CIM can remove viruses from water. We hypothesized that CIM could remove RNA viruses, such as the MS2 bacteriophage, from solution. This virus infects bacteria such as *E. coli* and is commonly used as a model virus for human pathogenic RNA viruses such as hepatitis and influenza.

The plaque assay was used to determine how much bacteriophage was present in a sample. In this assay, solid agar plates are overlaid with soft top agar containing bacteria. Serial dilutions of virus-containing samples are prepared and added to the top agar. After overnight incubation, lysis of bacterial cells is visualized by the presence of a clear area (the plaque). The numbers of plaques are counted to determine the phage concentration (plaque forming units). In our first experiment we combined 1 g of CIM, 10 ml water, and 100 µl of the appropriate phage dilution, then incubated for an hour and plated. There was a considerable decrease in the number of plaques on the plates, suggesting that the CIM is effective in removing viruses from water. A time dependency test was conducted with the CIM to determine the amount of time required to remove the virus. The virus was incubated for 1, 5, 10, 15, 30, 45, and 60 min. Although there wasn't any decrease in the number of plaques for the 1, 5, and 10 min incubations, a considerable decrease was seen starting from the 15 min incubation plate. The 45 min and 60 min incubation plates had no plaques. These initial experiments conducted with the CIM show promising results and there appears to be a likely chance that the CIM is able to remove viruses from water.

## Introduction

To determine the right amount of top agar for the plates, we plated different amounts of top agar and determined the optimal amount. The different amounts of top agar were 1.5, 3, 4.5, 6, 7.5, and 9. After overnight incubation, it was determined that 3 ml of top agar was the optimum amount of top agar. Next, to determine the right amount of bacteria, we plated different amounts of bacteria and then determined the right amount. The different amounts were 1, 2.5, 10, 25, 100, and 1000 µl. After overnight incubation, it was determined that 25 µl of bacteria was the right amount. In order for the bacteria to work during the experiment, it is important for the bacteria to be growing in the exponential stage, hence they had to be incubated for at least 3 hours in TSB broth in the shaking incubator. The phage propagation assay was used to determine the best way to add phage to the bacteria. The first method was to add phage separately onto a plate already containing top agar and bacteria. The second method was to add the phage directly to the bacteria and top agar in glass tubes in a water bath and then plate them. It was determined that the phage should be added directly to the bacteria and top agar in glass tubes. For the CIM experiments, we counted the number of plaques on each plate after overnight incubation. After the initial CIM experiments, it was determined that there was a considerable decrease in the number of plaques, indicating the CIM does have the ability to remove viruses from water.

## Methods



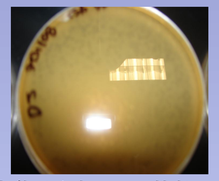
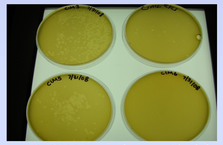


***Figure 1:*** **The plaque assay**- 3ml of top agar, 25 µl of bacteria and 100 µl of bacteriophage were added to agar plates and after overnight incubation, they were analyzed to see the formation of plaques (tiny, white clearings). The plaques indicated that the bacteriophage have successfully lysed the bacteria.

***Figure 2:*** **Serial Dilutions**-100 µl of 10- fold dilutions of the bacteriophage were used along with 10 µl of bacteria and 3 ml of top agar. They were plated and incubated overnight. As the dilutions increased, fewer plaques were seen, indicating that the greater the number of bacteriophage, the greater the number of plaques.



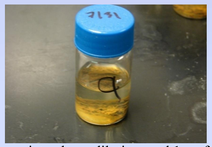


***Figure 3:*** **CIM Experiment**- 10 vials were filled with 10 ml water, 100 µl of appropriate phage dilution, and 1 g of CIM. They were shaken at room temperature for 1 hour, and 100 µl were added to 3 ml top agar and 25 µl of bacteria and then plated. After overnight incubation, they were analyzed for the number of plaques. If there is a significant decrease in the number of plaques in the plates, then it indicated that the CIM does have an effect on the bacteriophage.

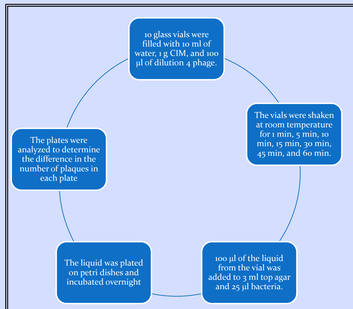



***Figure 4:*** **CIM Experiment with various lengths of incubation time**- 10 vials were filled with 10 ml water, 100 µl of dilution 4 phage, and 1 g CIM. They were shaken at room temperature for 1, 5, 10, 15, 30, 45, and 60 min. They were then added to 3 ml of top agar and 25 µl of bacteria and plated. After overnight incubation, they were analyzed for the difference in the number of plaques.

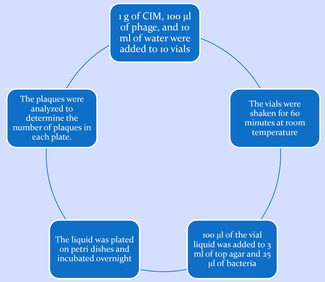



***Figure 5:*** **CIM Experiment with pond water**- 10 vials were filled with 10 ml of pond water, 100 µl of various dilution phage, and 1 g of CIM. They were shaken at room temperature for 60 minutes and then added to 3 ml top agar and 25 µl bacteria and plated. This experiment is more representative of water wells, where the water has numerous contaminants.

## Results

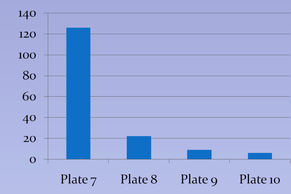



**Figure 1 Results:** ***Serial Dilutions***- Plates 1-6 had too many plaques to count, indicating that there are too many phage. Plate 7 had 126 plaques, plate 8 had 22 plaques, plate 9 had 9 plaques, and plate 10 had 6 plaques. This experiment proved that as the phage become more dilute, the number of plaques decrease.

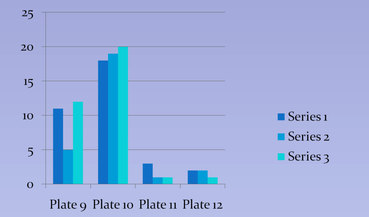
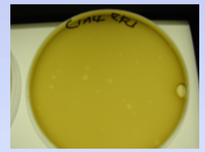
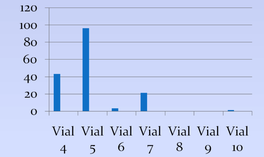



**Figure 2 Results:** ***Triplicate Dilutions***- Triplicate dilution experiments were performed to get more accurate results. Triplicates were done for dilutions 9-12. For plate 9, there were 11, 5, and 12 phage. For plate 10, there were 18, 19, and 20 phage. For plate 11, there were 3, 1, and 1 phage. For plate 12, there were 2, 2, and 1 phage.





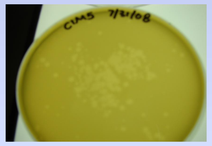



**Figure 3 Results:** ***CIM experiment***- Vial 4 (with dilution 2 phage) had 43 plaques, vial 5 (with dilution 3 phage) had 96 plaques, vial 6 (with dilution 4 phage) had 3 plaques, vial 7 (with dilution 5 phage) had 21 plaques, vial 8 (with dilution 6 phage) had 0 plaques, and vial 10 (with dilution 8 phage) had 1 plaque. In the absence of CIM, dilutions 2 through 6 had too many plaques to count. This shows that there is almost a 99.995% decrease in the number of plaque forming units compared to the serial dilutions. This shows that there is considerable reason to believe that CIM has an effect in removing viruses from water.

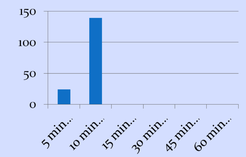



**Figure 4 Results:** ***Time Dependency Test***- There were 24 plaques for 5 min incubation time, 139 plaques for 10 min incubation time, 1 plaque for 15 min incubation time, and 0 plaques for 30 min, 45 min, and 60 min incubation time. This suggests that under the tested conditions it requires about 15 minutes for the CIM to remove the virus.

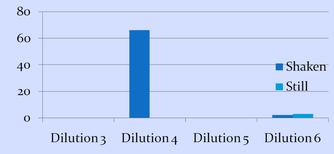



**Figure 5 Results:** ***CIM Experiment shaking vs. still***- For the shaken vials, dilution 3 had too many plaques to count, dilution 4 had 66 plaques, dilution 3 had 0 plaques, and dilution 6 had 2 plaques. For the plates that were still for 60 minutes, all the plates had too many plaques to count. The experiment demonstrates that the virus was required to come in contact with the CIM in order for it to be removed from solution.

## Conclusions

After the initial experiments with Composite Iron Matrix, there seems to be a considerable reason to believe that the CIM has an effect in removing viruses from water. These experiments were conducted using the MS2 bacteriophage as a model virus. Additional experiments are required to prove that the decrease of viruses from water is due to the CIM and not some other cause. In addition, we will expand the experiments to determine the effect of CIM on DNA viruses and intact bacteria.