ADSORPTION OF COLIPHAGES TO PARTICULATES


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ABSTRACT

Adsorption characteristics of coliphages (host cell: E. coli B) to particulates (kaolin, sand, soil, microbial particulates in an oxidation pond) were investigated using batch experiments under various conditions of pH, concentrations of cations and concentrations of dissolved oxygen. The coliphages showed no resistance to acid (pH 3) and weak resistance to alkali (pH 10). Under neutral pH conditions, sodium ions did not have a large effect on the adsorption of coliphages to the solid surfaces of sand. Divalent cations (Mg**, Ca**) had no effect on the adsorption to sand at concentrations below 0.01 mol/l but some effect at 0.05 and 0.1 mol/I. The presence of kaolin had very little effect on removal of coliphages from the water phase under any conditions. Formation of flocs such as Mg-hydroxides in alkaline conditions enhanced coliphage removal from the water phase. Coliphage chemical adsorption to particulates in natural waters would probably be low except in estuarine and sea waters. However, the adsorption of coliphages to microbial particulates occurred in aerobic conditions. The desorption of coliphages was observed under anaerobic conditions. This adsorption-desorption process was reversible. The biological adsorption appears to be the dominating cause of coliphage adsorption in natural waters containing microbial particulates.

KEYWORDS

Coliphage; sand; soil; kaolin; cation; adsorption; pH dependency; biological adsorption.

INTRODUCTION

Background

Increasing water reuse has required an understanding of the inactivation processes or survival mechanisms of enteric viruses in natural waters, for preventing waterborne diseases due to pathogenic viruses. The inactivation processes of enteric viruses are affected by many factors (temperature, pH, turbidity, sunlight intensity and cation concentration). There is not enough information on the effects of these various factors on inactivation. The adsorption of viruses to particulates removes the viruses from water, but the adsorbed viruses are then protected from various inactivation factors and this can enhance the persistence of viruses in natural aquatic environments. As this phenomenon may allow long survival of viruses in waters, an investigation into adsorption of viruses is required for management of human wastes. In
addition, the problems of movement and survival of microbial pathogens in soils must be taken into account in assessing the potential public health hazards associated with land disposal.

Mahdy (1979) pointed out that human viruses had been readily and repeatedly detected in sewage effluents which, after various degrees of treatment, enter waterways and become a part of the rivers and streams that are the sources of drinking water for many communities. Smith et al. (1978) and Dahling and Safferman (1979) demonstrated the long survival of enteric viruses under natural conditions. Gerba and Schaiberger (1975) and Gerba et al. (1978) studied the behavior of solids-associated viruses in natural waters. Lipson and Stotzky (1984) reviewed adsorption of viruses to particulates as a possible major factor affecting virus persistence.

Coliphages as Indicators of Viral Survival

The use of coliphages and other bacteriophages as indicators for the survival of pathogenic enteric viruses has been discussed. Some recent studies showed the possibility of using coliphages as a viral model in water pollution (Kott et al., 1974, 1978; Grabow et al., 1984), because coliphages outnumber enteric viruses in normal water environments, they are more resistant to unfavorable environmental conditions, they are detectable by simple and economical techniques which yield results within one day, and they create no risk to human health. However, different viruses have different characteristics, therefore, one group of viruses, such as coliphages, cannot be representative of all pathogenic enteric viruses. Some studies have indicated that coliphages are not suitable for all criteria under all circumstances (Bell, 1976; Neimi, 1976). The controversial results are no doubt caused by differing methods, media and phage strains. Thus, further investigation is required to develop a precise standard method and assess the advantages and/or disadvantages of coliphages as a viral indicator. Information on the fate of coliphages in natural waters would be useful, not only for routine virological surveillance, but also for further insight into virus transport and survival in aquatic environments.

Objectives

This study was designed to investigate the degree of adsorption of coliphages to particulates (soil, sand, kaolin, microbial particulates in an oxidation pond), using batch experiments under various conditions which occur in natural aquatic environments.

MATERIALS AND METHODS

Coliphages

Media. The phage agar for the isolation of coliphages and coliphages assay consisted of 0.8% nutrient broth (Difco, or 0.1% Bacto peptone, 0.3% beef extract), 0.5% NaCl, 0.02% MgSO₄·7H₂O, 0.005% MnSO₄·4H₂O, 0.15% dextrose and 1.1% Bacto agar (Difco, adjusted to a final pH of 7.2). All plates were dried at 45°C before use. The formula of the soft agar was the same as above, except that 0.6% instead of 1.1% Bacto agar was used. The phage broth had the same composition as the phage agar but without the Bacto agar. This medium was used for culturing the host strains (Escherichia coli B) and also for diluting the phage samples.

Bacterial culture. Escherichia coli B was employed in a pure culture as the host bacterium for quantitative determination and propagation of the coliphages. Escherichia coli B was obtained from the Division of Agricultural and Food Engineering, Asian Institute of Technology, Thailand. The culture of E. coli B was inoculated into 10 ml of the phage broth and incubated at 37°C for 3.5-4.0 hrs before being used for phage isolation and plaque formation.
Isolation of coliphages. Natural water was collected from a canal, Klong Nueng, in Bangkok. This water sample (10 ml) was centrifuged at 12,000 rpm for 10 mins at 4°C. The supernatant was then filtered through a 0.45 μm membrane filter. Coliphages in the filtrate were sought by the agar-layer method. Equal volumes (0.1 ml) of filtrate were inoculated on the surface of the phage agar, and then 4 ml of melted soft agar, including 0.3 ml of young bacterial cultures and supplemented with 0.2 ml of 0.1 mol/l CaCl₂, were added. After thorough mixing the plates were incubated at 37°C overnight. The coliphages were purified by repeated single-plaque isolation as follows: single plaques with surrounding medium were transferred by a sterilized loop to 2 ml of the phage broth and incubated at 37°C for 4 hrs. The broth was centrifuged at 12,000 rpm for 10 mins at 4°C. The supernatant was then serially diluted ten-fold and plated as before but using the appropriate E. coli B host cells. The second and the third single-plaque isolations followed the same procedure.

Propagation of coliphages. After the third single-plaque isolation, single plaques of the same size were transferred into the phage broth (ratio = one plaque to 2 ml) and this phage broth was mixed with the bacterial culture (volume ratio = 20:1). The mixture was then incubated at 37°C for 2-3 hrs until clear. The mixture was centrifuged at 12,000 rpm for 10 mins at 4°C and filtered by a sterilized 0.45 μm membrane filter. The filtrate was kept at 4°C as stock phage solution. Propagation of coliphages by this method yielded 10⁸-10⁹ plaques/ml.

Determination of coliphage concentration. Coliphage concentration measurements were carried out by the agar-layer method (plaque forming method). A 0.1 ml sample, 0.3 ml of young bacterial cultures, 0.2 ml of CaCl₂ (CaCl₂·2H₂O, 15 g in 1000 ml of distilled water) and 4.0 ml of melted soft agar were mixed and poured over the surface of an ordinary phage agar plate and allowed to harden to form a thin layer. After incubation at 37°C overnight, each plaque appeared as a clear area in the opaque lawn of E. coli B. A suitable dilution was made to obtain from 30 to 300 plaques on a plate. A plaque forming unit (PFU)/sample volume was used as the unit of coliphage concentration in this study for convenience of data handling.

Particulates

Soils. Two different soil materials were used in this study: a paddy field soil and a lateritic soil sampled from Rangsit Patumthani and Pitsanuloak, in Thailand, respectively.

Sand. The particle size of sand used was between 0.45 and 0.55 mm. The sand was washed with 6N HCl and rinsed well with tap water and distilled water.

Particulates in the oxidation pond. The particulates (mainly algae) were collected from an oxidation pond (retention time = 20 days) at the Asian Institute of Technology, Thailand.

Experimental Procedures

Effects of pH. Buffer solutions of pH 4.4, 5.0, 6.0, 8.0 and 10.0 were used. After sterilizing the solutions by an autoclave, 1 ml of the coliphage suspension was added to each buffer solution. Coliphage concentration was determined after shaking for 1 hr at 100 rpm.

Adsorption to sand and soil. Coliphage adsorption to sand and soil was studied in batch experiments. Water containing different concentrations of ions (NaCl, MgCl₂ or CaCl₂) at a specific pH was added to 25 g of sand or soil in separate Erlenmeyer flasks. The mixtures were sterilized by an autoclave at 121°C, 15 psi for 15 mins. A few millilitres of stock coliphage solution containing 10⁷ to 10⁸ PFU/ml were introduced into each flask to make the total volume of liquid equal to 25 ml. Then the mixtures were agitated by an orbital shaker for 1 hr at 100 rpm. After this, the supernatants were assayed.
for coliphage determination. Control samples were treated in the same manner without sand or soil.

Adsorption to kaolin and elution method. A kaolin (300 mg/l) solution containing different concentrations of ions (MgCl₂, CaCl₂) at a specific pH was shaken at 30 rpm for 30 mins using a horizontal shaker. The kaolin and flocs were then centrifuged at 12,000 rpm for 20 mins at 4°C, and the supernatant was used for measurement of coliphage concentration. The residue was resuspended with 3% beef extract (the ratio of beef extract to sample = 1:10 to 1:20) at pH 9.0 and was then shaken for 10 mins (75 rpm) at room temperature (about 25°C). After shaking, it was filtered through a 0.45 μm membrane filter. The filtrate (eluate) was used for measurement of coliphage concentration. This elution method for solids-combined coliphages was based on Seeley's method (Seeley and Primose, 1979).

Adsorption to microbial particulates in the oxidation pond. A solution containing 65 mg/l of microbial particulates (mainly algae) was aerated in 300 ml bottles by an air pump for 3 hrs, after addition of the proper amount of coliphage stock solution. After aeration, the bottles were allowed to stand until the next aeration. All bottles were covered with aluminium foil. The dissolved oxygen concentration was measured by a DO-meter or the Azide-modification method. Coliphage concentration in the liquid phase and the amount of coliphages adsorbed to particulates were determined by the same method as the experimental procedure for kaolin.

RESULTS AND DISCUSSION

Effect of pH on Inactivation and Fate of Coliphages in Soil

The inactivation of coliphages at different pHs is shown in Fig. 1. The coliphages used in this research were stable in the pH range of 6.0-9.0. At this pH range, the inactivation of coliphages was between 5% and 30%. It can be seen that the coliphages are more sensitive on the acidic side than on the alkaline side. The inactivation of coliphages was greater than 99.9% at a pH of 4.4. The removal of viruses during water softening precipitation was investigated by Thayer and Sproul (1966). They showed that Bacteriophage T-2 was stable in the pH range of 7.0-9.2 and inactivation in excess of 99.9% occurred at pH 10.8.

![Fig. 1. Effect of pH on coliphage inactivation](image)

*The dotted line shows the minimum detectable count
**Initial coliphage concentration = 3.3 x 10⁷ PFU/26 ml

Experiments were performed to investigate the adsorption of coliphages onto two soils (paddy field soil and lateritic soil). The results are shown in Table 1. Coliphages could not be detected in the supernatant of either the paddy field soil or the lateritic soil after shaking for 1 hr. The pH of the supernatants of the paddy field soil and the lateritic soil were 3.4 and 4.4 respectively. Therefore, all coliphages would be inactivated by the low pH of the soil-water mixtures.
TABLE 1 Fate of Coliphages in Soil

<table>
<thead>
<tr>
<th>Sample</th>
<th>Coliphages remaining in supernatant (PFU/25 ml)</th>
<th>Inactivation (%)</th>
<th>Final pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.9 x 10^7</td>
<td>13.3</td>
<td>8.1</td>
</tr>
<tr>
<td>Lateritic soil</td>
<td>0</td>
<td>100</td>
<td>4.4</td>
</tr>
<tr>
<td>Paddy field soil</td>
<td>0</td>
<td>100</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Input coliphages = 4.5 x 10^7 PFU/25 ml

Adsorption to Sand

Effect of monovalent cation (Na\(^+\)). The experiment to determine the effect of sodium ions upon coliphage adsorption on sand was conducted at pH 8.0, by varying the concentration of NaCl. The results are illustrated in Fig. 2. These results indicate that coliphage adsorption on sand surfaces did not occur even though the concentration of NaCl was as high as 0.2 mol/l. Lefler and Kott (1974) reported that sodium ions had little effect on the mechanism of virus removal by sand, even though the sodium ion concentration was as high as 0.5 mol/l. In the pH range of natural water (pH 6.8-8.4), viruses and sand are negatively charged. Jenkins (1980) reported that there exists an electrostatic repulsive force between the negatively charged viruses and sand that inhibits the sand from removing the viruses.

![Fig. 2. Effect of NaCl on coliphage adsorption to sand](image)

Effect of divalent cations (Mg\(^++\) and Ca\(^++\)). The data presented in Fig. 3 illustrate the effect of MgCl\(_2\) at pH 7.0 on coliphage removal by sand. Removal of coliphages from the liquid phase increased with MgCl\(_2\) concentration in the presence of sand at pH 7. There was no removal of coliphages in the solution without sand even at 0.1 mol/l MgCl\(_2\) concentration. It may be concluded that, at pH 7.0, there was no coagulation-flocculation of the viruses with Mg ions. Coliphage removal in the system containing sand at 0.1 mol/l MgCl was 15% of the coliphage input. It is considered that the coliphages were removed from the water by adsorption on the sand.

![Fig. 3. Effect of MgCl\(_2\) on coliphage adsorption to sand](image)

The effect of CaCl\(_2\) at pH 7.0 is shown in Fig. 4. The reduction of coliphages in the system increased with increasing concentration of CaCl\(_2\). In the system containing both sand and CaCl\(_2\), the percent removal of coliphages (almost 90% removal at 0.05 M CaCl\(_2\)) exceeded the removal in the system without sand. It appears that the coliphages were removed by coagulation-flocculation and
adsorption. Lefler and Kott (1974) showed that divalent cations considerably increased the removal efficiency of a sand column for Poliovirus 1 and Coliphage f2. Fuhs et al. (1979) indicated that the presence of Ca++ greatly enhanced adsorption to unreactive surfaces, such as silica sand, in neutral media. The data obtained by Carlson et al. (1968) showed that approximately 10 times more sodium ions than calcium ions were required to bring about the maximum adsorption with the particular ion. Jenkins (1980) and Taylor (1981), also reported similar observations and interpretations. Their observations are consistent with our data.

Adsorption to Kaolin and Effect of Flocculation

Effect of divalent cations at pH 7. The effect of Mg++ and Ca++ ions on adsorption of coliphages to kaolin is shown in Fig. 5. The presence of kaolin had very little effect on the concentration of coliphages in both supernatant and eluate. This implies that the coliphages were not adsorbed to kaolin at pH 7. The increase of the PFU value in the eluate at 0.05 mol/l and 0.1 mol/l would arise from coagulation-flocculation.
Effect of flocculation. The effect of Mg$^{2+}$ ions on adsorption of coliphages to kaolin at pH 10 is shown in Fig. 6. The increase in turbidity with concentration of MgCl$_2$ implies floc formation (Fig. 6(a)). The concentration of coliphages in the supernatant and the eluate was not affected by the presence of kaolin. The results in Fig. 6(b) and (c) showed that most of the coliphages (more than 99%) were captured by the flocs at 0.05 and 0.1 mol/l. The amount of floc-associated coliphages did not reach the initial amount of coliphages due to the low recovery efficiency of the elution method. This result implies that flocculation has a major effect on the fate of coliphages in water.

![Graphs showing effect of flocculation and MgCl$_2$ on adsorption of coliphages to kaolin at pH 10](https://iwaponline.com/wst/article-pdf/18/7-8/267/97902/267.pdf)

**Fig. 6.** Effect of flocculation and effect of MgCl$_2$ on adsorption of coliphages to kaolin at pH 10

Adsorption of Microbial Particulates in the Oxidation Pond

Dissolved oxygen (DO) concentration is shown in Fig. 7(a). After aeration was stopped, the DO decreased and reached zero after 3-4 hrs, due to the respiration of algae and other microbial organisms. During aeration, the coliphage concentration in the supernatant decreased and the coliphage concentration in the eluate increased due to adsorption of coliphages to algae and other microbial particulates (Fig. 7(b)). On the other hand, when the DO decreased, the coliphage concentration in the supernatant increased and the coliphage concentration in the eluate decreased due to desorption of coliphages from algae and other microbial particulates. During anaerobic conditions, the coliphage concentration in the supernatant regained its initial value. The second aeration showed the same result as the first. The imbalance in the change of coliphage concentration between the supernatant and the eluate (Fig. 7(b)) was due to the low recovery efficiency of the elution method. However, both coliphage concentrations changed simultaneously. This means that a reversible adsorption-desorption process was the dominating cause of change in the coliphage concentration in this system. A similar phenomenon in the activated sludge process was reported by Shimohara et al. (1984). They showed that a nitrogen gas injection instead of aeration stopped the virus removal by activated sludge.
SUMMARY

Adsorption characteristics of coliphages to particulates (kaolin, sand, soils, microbial particulates in an oxidation pond) were studied under various conditions of pH, concentrations of cations and concentrations of dissolved oxygen.

Escherichia coli B was used as the host cells for isolation and propagation of the coliphages. The coliphages were assayed by the plaque forming technique (PFU method). The coliphages which were used in this study had no resistance to acid (pH 3) and weak resistance to alkali (pH 10). The monovalent cation Na⁺ did not have a large effect on the adsorption of coliphages to the solid surfaces of sand. Divalent cations (Mg²⁺, Ca²⁺) had no effect on the adsorption of coliphages to sand at pH 7.0 and below a concentration of 0.01 mol/l. However, coliphages were removed partially from the water phase by adsorption to sand at 0.05 and 0.1 mol/l at pH 7.0. The presence of kaolin had very little effect on the removal of coliphages from the water phase. On the other hand, formation of flocs such as Mg hydroxides under alkaline conditions enhanced coliphage removal from the water phase. The adsorption-coagulation of coliphages to kaolin and flocs was confirmed by the elution method with beef extract. Therefore, coliphage chemical adsorption to particulates in natural waters (neutral pH, low concentrations of cations and low turbidity) would probably be low except in estuarine and sea waters which contain about 0.05 mol/l Mg²⁺ and 0.01 mol/l Ca²⁺.

However, the adsorption of coliphages to the microbial particulates (mainly algae) in the oxidation pond occurred under aerobic conditions. The desorption of coliphages from the particulates was observed under anaerobic conditions. The adsorption-desorption process was reversible and was controlled by the DO concentration. This indicates that aerobic conditions would be required for adsorption of coliphages to microbial particulates, and that biological adsorption appears to be the dominating cause of the coliphage adsorption phenomenon in natural waters containing microbial particulates. Viruses
combined with particulates should be considered in assessing virological pollution.

REFERENCES


