Removal of Waterborne Human Enteric Viruses and Coliphages with Oxidized Coal

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It is well known that water is the vehicle for the transmission of many viral diseases [9, 12]. More than 120 different viruses are excreted in human feces and subsequently find their way into sewage, where they become water pollutants [15, 19]. Among these waterborne viruses are members of the family Picornaviridae (including poliovirus, coxsackievirus, echovirus, and hepatitis A virus), members of the family Reoviridae (including rotavirus), and members of the adenovirus family. In contrast to many bacterial pathogens, the minimal infective dose for viruses is quite low, and a small number could be, under ideal conditions, capable of producing a full-blown infection in the appropriate host [3, 15].

Modern living standards and the concentration of populations into high-density zones during urbanization have necessitated the diversification of the sources of drinking water. Considering that surface water is a major source of subterranean water, viral contamination of subterranean water systems as a result of the surface shedding of human enteric viruses through feces and urine becomes a major health concern. In raw wastewater receiving fecal matter, virus concentrations are generally very high [5]. Procedures such as the activated sludge process [14, 18, 20], oxidation ponds [17], activated carbon treatment [13], filtration [4], and lime coagulation [10] eliminate between 50% and 90% of viruses present in wastewater sources. Nevertheless, viruses surviving these processes still pose a potential health risk.

Coal is one of the world's most abundant fossil resources [8], and it has been used previously for removal of viruses from water [4]. Oxidized coal (patent: oxicoal) is produced by the wet oxidation of low-grade bituminous coal [7]. In this process, the coal is mixed with an aqeous solution, producing a slurry of pH 4–9 which is in turn oxidized under specific temperature/pressure conditions with a gaseous oxidant composed of mixtures of oxygen and air [7]. The oxicoal product has previously been shown to possess coliphage removal properties during trial tests performed in our laboratory (unpublished). With the investigation described here, it was our aim to determine the effect of oxicoal for removing human enteric viruses from water. It was found that a variety of different pathogenic human viruses could be removed by oxicoal and that the removal was dependent upon the virus type, duration of treatment, and oxicoal concentration.

Materials and Methods

Bacterial strains, viral strains, and cell lines. *E. coli* C (ATCC 13706), Coliphage V1, the viral strains poliovirus I, poliovirus II, reovirus, echovirus I, coxsackie virus B1, and the BGM (Buffalo Green Monkey) cell line were obtained from W.O.K. Grabow (Department of Medical Virology, University of Pretoria). *E. coli* K12 was obtained from the culture collection of the Department of Microbiology and Plant Pathology of the University of Pretoria, and Coliphage SI1 used with *E. coli* k12 was isolated from raw sewage by the method of Adams [1].

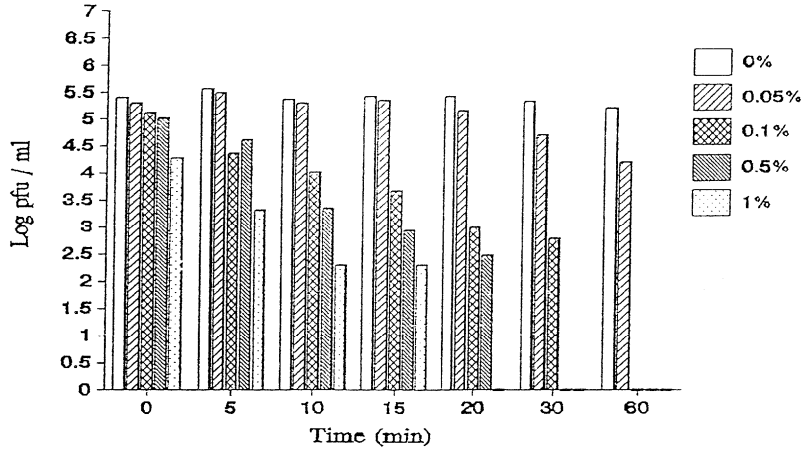

Fig 1. Removal of coliphage V1 with different concentrations of oxicoal. The number of plaque-forming units was determined at different intervals as described in Materials and Methods.

High-titer coliphage stocks were prepared by the plate method of Billing [2]. Bacterial strains were cultured in Nutrient broth (Biolab, E. Merck, Johannesburg) and plaque assays were carried out with the use of Nutrient agar plates (Biolab, E. Merck, Johannesburg). All dilution series were carried out with 1/4 strength Ringer's solution (E. Merck). BSE cell monolayers were cultured at 37°C on Eagle's minimal essential medium (MEM) supplemented with 10% newborn calf serum. All experiments were carried out in triplicate, and all results are the averages of three separate experiments.

Oxicoal concentration and exposure time: effect on removal of coliphages V1 and SI1. Oxicoal [7] was obtained from the Division of Energy Technology at the Council for Scientific and Industrial Research (CSIR), Pretoria, South Africa. Suspensions of oxicoal were prepared in 50 ml of sterile distilled water to obtain concentrations of 1%, 0.5%, 0.1%, and 0.05% oxicoal. Coliphage preparations were added to each of the oxicoal suspensions to a final concentration of 10^6 pfu/ml (plaque-forming units/ml). The suspensions were placed on a shaker at 100 rpm at ambient temperature. Two-milliliter aliquots were removed from each suspension at time intervals of 5 min, starting at t=0 min and ending at t=60 min. Aliquots were centrifuged at 5000 *g* for 2 min in a 360K Hermle bench-top centrifuge (Omniscience, Johannesburg) to remove the oxicoal. The coliphage titer in each supernatant was determined by the double-agar-layer technique [1, 2].

Effect of coliphage concentration on removal efficiency. Fifty-milliliter suspensions of oxicoal at a concentration of 0.1% were prepared, and suspensions were inoculated with coliphage V1 to obtain a final phage concentration of 10^3, 10^4, 10^5, 10^6, 10^7, and 10^8 pfu/ml. Suspensions were then placed on a shaker at 100 rpm at ambient temperature. Coliphage titers were determined as described in the previous section.

Oxicoal concentration and exposure time: effect on removal of human enteric viruses. Suspensions of oxicoal were prepared in 50 ml of sterile distilled water to obtain concentrations of 0.5% and 0.1% oxicoal. To each of the suspensions was added virus to a final concentration of 10^6 to 10^8 iu/ml (infectious units/ml). The suspensions were placed on a shaker at 100 rpm at ambient temperature. Two-milliliter aliquots were removed from each suspension at time intervals of 5 min, starting at t = 0 min and ending at t = 120 min. Aliquots were centrifuged at 5000 *g* for 2 min in a 360K Hermle bench-top centrifuge (Omniscience, Johannesburg) to remove the oxicoal, and virus titers in the supernatant were determined with a most-probable-number technique approach [16] under the following conditions: Six wells of a micro titer plate, containing cell monolayers previously washed with phosphate-buffered saline, were inoculated with 10 µl of a series of 10-fold virus dilutions. Thirty minutes were allowed for infection, after which MEM was added. The infected cultures were incubated at 37°C, and cells were microscopically examined for any cytopathic effect. Cytopathic effect within 3 days was recorded as a positive result at the relevant virus dilution.

Results and Discussion

Oxicoal concentration and exposure time: effect on removal of coliphages V1 and SI1. An oxicoal concentration of 0.05% was not sufficient to remove coliphages V1 and SI1, even after 60 min of exposure, although there was a 1-log decrease in coliphage V1 titer and a 4-log decrease in coliphage SI1 titer respectively (Figs. 1 and 2). This corresponds to 99.99% removal of coliphage SI1, compared with only 93.75% removal of coliphage V1. Oxicoal at concentrations of 0.1%, 0.5%, and 1.0% completely removed coliphage V1 within 60 min, 30 min, and 20 min respectively and removed coliphage SI1 within 20 min, 15 min, and 10 min respectively. Thus, oxicoal removed coliphage SI1 at a faster rate than coliphage V1, suggesting that coliphage V1 does not interact with the oxicoal as efficiently as does coliphage SI1. Coliphage SI1 is a wild strain isolated from the Daspoort activated sludge plant prior to the experiment, whereas coliphage V1, a typical isolate from a sewage-polluted river [11,13], was a laboratory strain. It is conceivable that the reactive groups (amino and carboxyl) [3] on the capsid surface of coliphage V1 altered over time and during routine laboratory passage, and may not interact as well as the wild strain with the reactive groups on the oxicoal surface (carboxyl and phenolic hydroxyl groups) [6]. Hence, we concluded that increased exposure time or oxicoal concentration resulted in a greater removal of coliphage.

Effect of coliphage concentration on removal efficiency. On *E. coli* C (ATCC 13706) cells, coliphage V1 produced relatively large plaques (ca. 2–3 mm in diameter), whereas coliphage SI1 produced very small plaques (ca. 1 mm diameter), which were more difficult to read. Given these observations as well as the results of our previous experiments, which showed that coliphage V1 is removed less efficiently of the two phage strains, it was decided to continue further experiments with phage V1 only. It was shown that coliphage V1 concentrations of up to 10^7 pfu/ml were completely removed in 0.1% oxicoal suspensions, within 60 min (Fig. 3). Coliphage concentrations of 10^8 pfu/ml decreased to 10^5 pfu/ml after 60 min exposure, the corresponding removal efficiency being 99.9%. There is thought to be a physical limit to the number of virus particles that can be absorbed by the oxicoal surface, and we concluded that the bacteriophage removal capacity for 0.1% oxicoal was 10^7 pfu/ml.

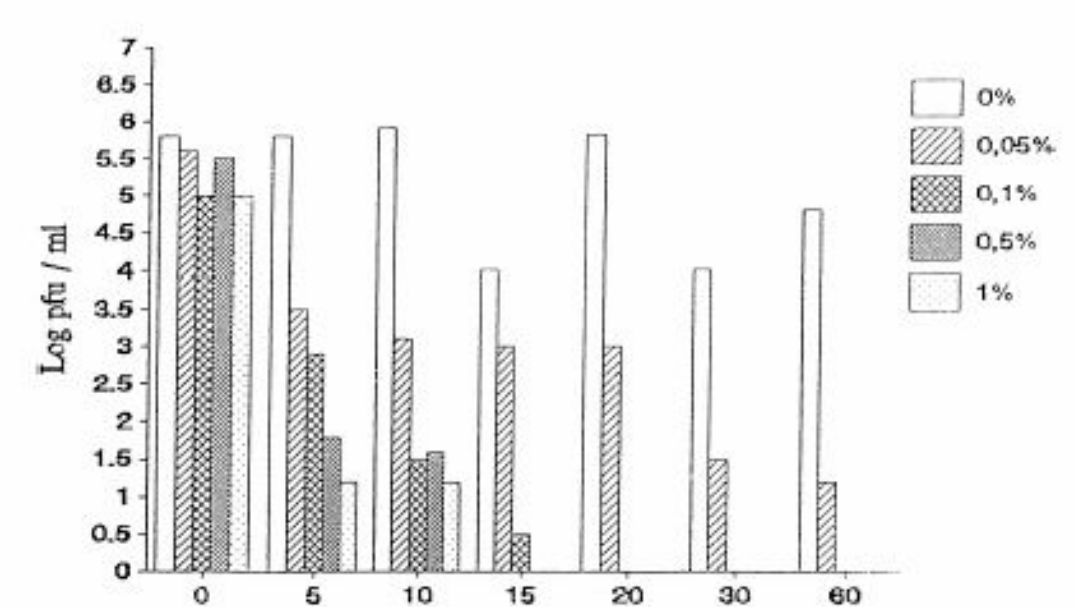

Fig. 2. Removal of coliphage SI1. The effect of different concentrations of oxicoal over a 60-min period was investigated, and removal efficiency was based on the decrease in plaque-forming units, determined as described in Materials and Methods.

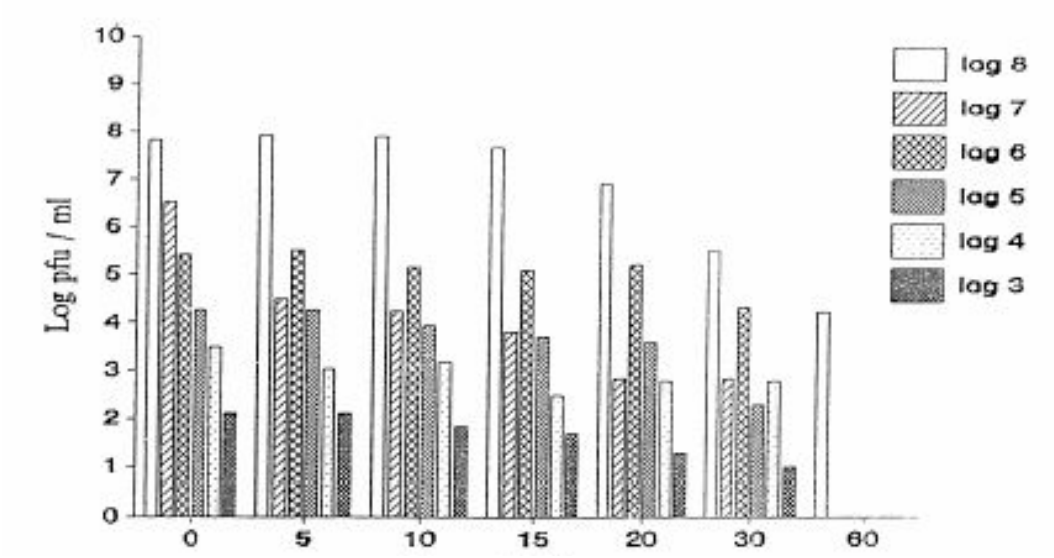

Fig. 3. Determination of the number of virus particles removed by a fixed amount of oxicoal. Removal of coliphage V1 as determined by the number of remaining plaque-forming units after exposing a series of virus dilutions, ranging in virus titer from 10^8 to 10^3, to 0.1% oxicoal for 60 min.

Oxicoal concentration and exposure time: effect on removal of human enteric viruses. Although a 0.1% oxicoal preparation was sufficient to remove up to 10^7 pfu/ml of coliphage V1 (Figs. 1, 3) within 60 min, poliovirus I or poliovirus II could not be removed with the same efficiency (Fig. 4). A decrease in virus titer was observed within the first 10 min of exposure, but was followed by an increase in titer over the next 20 min, although the overall virus numbers were reduced by ca. 2 log units after 60 min. The apparent increase that followed the initial virus titer

decrease could have been owing to virus adsorption to the glass surface of the flasks, followed by subsequent desorption. Such a phenomenon had been described previously [21].

An oxicoal concentration of 0.2%, on the other hand, was sufficient to remove poliovirus I, poliovirus II, and echovirus I within 60, 30, and 30 min respectively (Fig. 5). It was insufficient to remove coxsackievirus B1 over a period of 2 h, although it did effect a 3.8-log unit decrease in titer, which corresponds to a 99.98% removal. Thus, 0.2% oxicoal removed all enteroviruses tested, with the exception of coxsackievirus B1, within a period of 60 min. Oxicoal at 0.2% was also tested for its ability to remove reovirus particles and was found to do so within 60 min (Fig. 6). Once again, the phenomenon of a decrease in titer followed by a subsequent increase was observed within the first 5 min of exposure.

In conclusion, our results indicate that oxicoal is efficient in removing high titers of coliphages and human enteric viruses at concentrations of 0.1% and 0.2%. A linear relationship between oxicoal concentration and removal efficiency leads to the supposition that virus removal is due to adsorption of the viruses to the surface of the oxicoal. Thus, the greater the surface area of oxicoal available, the greater the potential for binding and removal of virions.

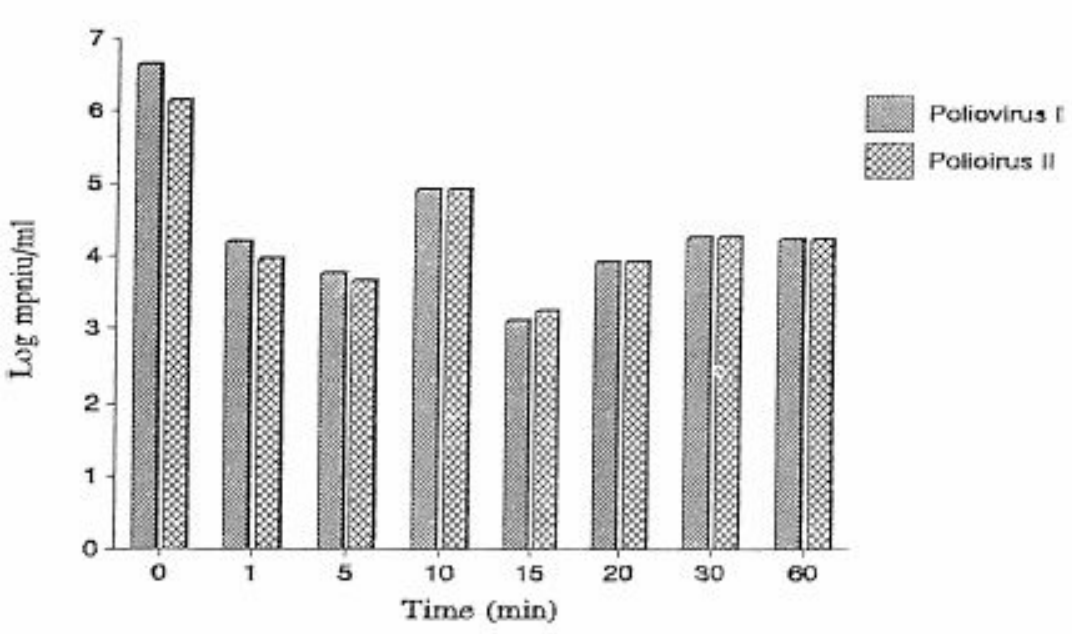

Fig. 4. Decrease in poliovirus titer following treatment with 0.1% oxicoal as described in Materials and Methods.

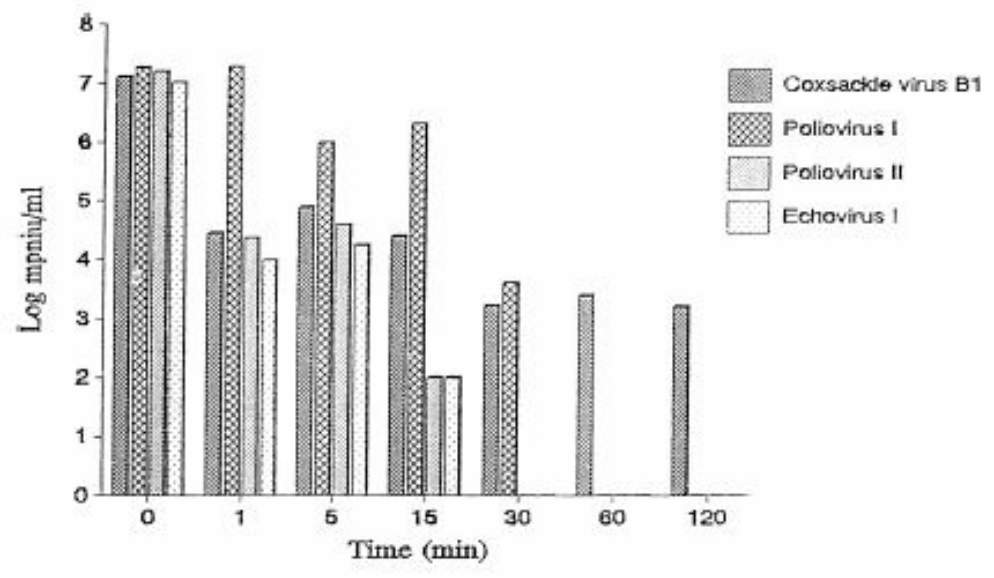

Fig. 5. Decrease in virus titers of different en-teroviruses after 0.3% oxicoal treatment over a 120-min period, as described in Methods.

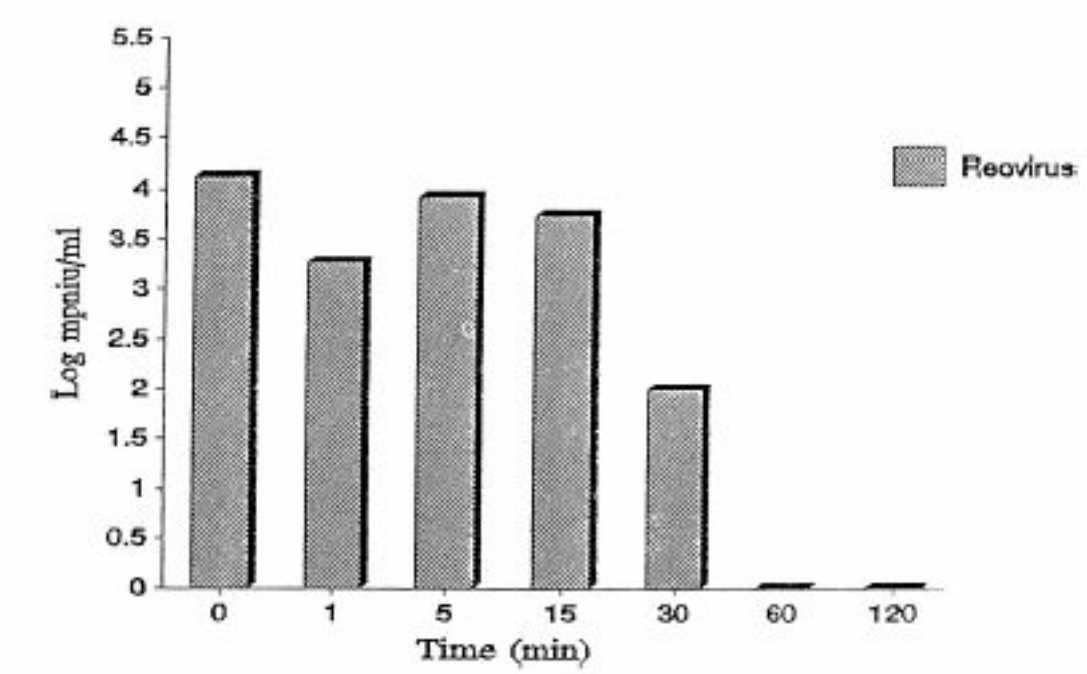

Fig. 6. Reovirus titers at different time intervals after treatment with 0.2% oxicoal as described in the text.

Although no specific conclusions can as yet be made as to the mechanism of virus removal by oxicoal, it is our belief that the use of oxicoal towards improving the biological quality of specific water resources may find future application.

ACKNOWLEDGMENTS

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