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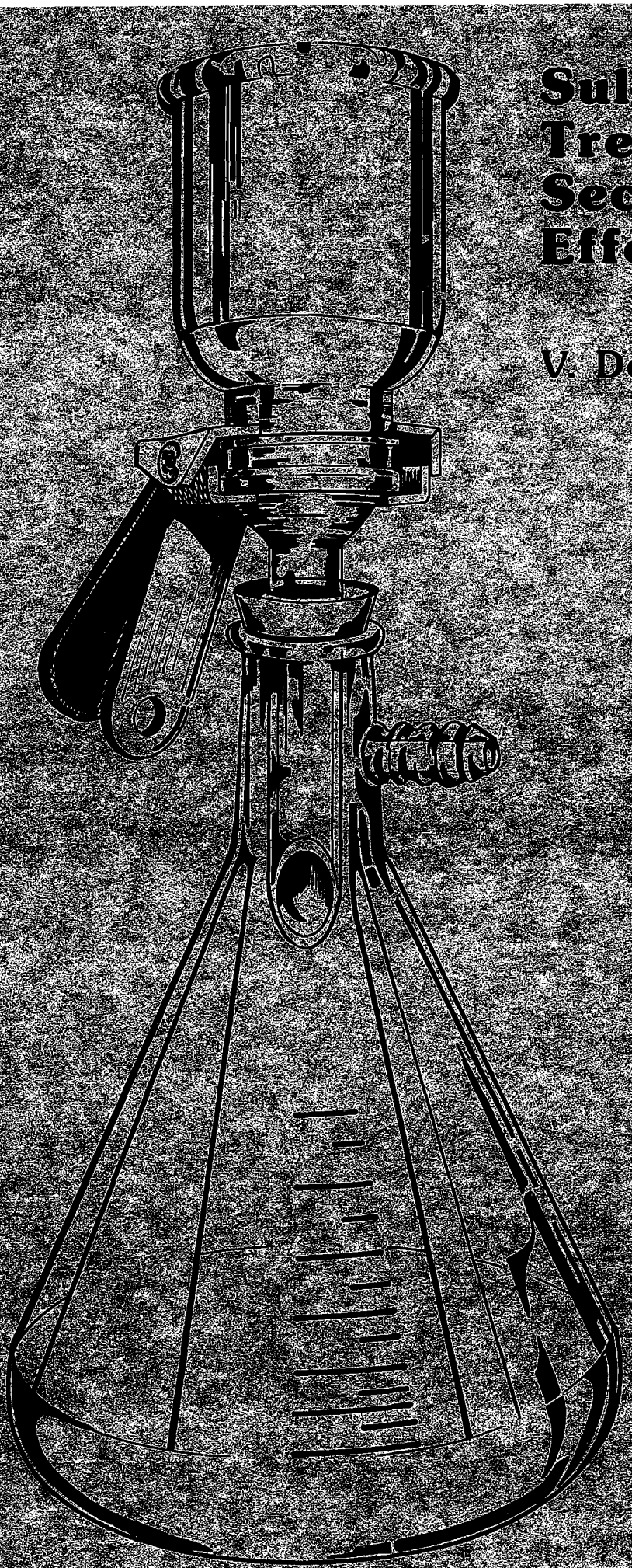

Sulfur Dioxide Treatment of Secondary Sewage: Effect on Viruses

V. Dean Adams

Report to:

International Environmental, Inc.
Salt Lake City, Utah

SULFUR DIOXIDE TREATMENT OF SECONDARY SEWAGE: EFFECT ON VIRUSES

by

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Report to

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Submitted by

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SULFUR DIOXIDE TREATMENT OF SECONDARY SEWAGE: EFFECT ON VIRUSES

VIRAL INACTIVATION

Recently there has been considerable concern regarding virus inactivation in wastewater using techniques such as chlorination, ozonation, ultraviolet light, etc. Some of the parameters required for inactivation of viral type microorganisms are shown in Table 1. Engineering aspects of chlorine contactor design have been reported by Longley (1978) and he concluded that conventional wastewater chlorination practice produced negligible viral inactivation at doses up to 17 mg/l. Use of turbulent mixing and plug flow contact chambers seem to improve treatment efficiency. It has been observed that the residual-contact time combinations for viral inactivation are greater in lake water than in distilled water and that water hardness or an associated water quality parameter, influenced sensitivity (Wedemeyer et al. 1978). Improved efficiency by using ultrasonic treatment during chlorination of f2 bacteriophage has been shown by Zotova et al. (1977) who attributed it to the destruction of viral aggregates. Repeated cycles of chlorination, dechlorination, and exposure of surviving progency (Poliovirus Type I (LSc)) at pH 7 resulted in apparently more resistant viral cultures (Bates et al. 1977).

Farooq et al. (1978) reviewed the literature on engineering design of ozone disinfection reactors and proposed contact times and residuals for appropriate operation. Small bubble size improved microorganism-bubble contact and increased gas-liquid interfacial area thus improving disinfection. Low pH enhanced ozone stability

Table 1. Reported parameters for inactivation of viral type microorganisms.

Organism	Medium	Disinfectant	Contact Time (min)	Dose (D) or Residual (R) mg/l	Criterion	Reference
Echovirus Type II	10% fetal calf serum	Chlorine	0.5	1200 (D)	3.5 logs Inactivation	Drulak et al. (1978b)
Infectious Pancreatic Necrosis Virus (IPNV)	?	Chlorine	5.0	4 (D)	4.5 logs Inactivation	Elliott and Amend (1978)
IPNV	Distilled water	Chlorine	1.0	0.1 (R)	?	Wedemeyer et al. (1978)
IPNV	Distilled water	Ozone	1.0	0.01 (D)	?	"
Infectious Hematopoietic Necrosis Virus (IHNV)	Distilled water	Chlorine	0.5	0.1 (R)	?	"
IHNV	Distilled water	Ozone	0.5	0.01 (D)	?	"
Poliovirus I and II Coxsackievirus A9 & B5 Echovirus 1 and 5 SV40 and Kilham Rat Virus	?	Chlorine	0.3-3.4 1.5-96	0.5 (R) pH 6 0.5 (R) pH 10	2 logs Inactivation	Engelbrecht et al. (1978)
6 Enteroviruses	Wastewater	Ozone	10	3.0 (D)	?	Evison (1978)
Poliovirus I	Sewage	Methylene blue	5	670 nm light 20 w/m^2 pH 10		Gerba et al. (1977)
Coxsackievirus Type B5	Buffer plus fetal calf serum	Chlorine	0.5	600-800 mg/l	99.999% Inactivation	Drulak et al. (1978a)

and thus improved inactivation efficiency, but inactivation by ultraviolet radiation catalysis was not significant.

Parrella et al. (1977) reported that chlorine dioxide was more effective than chlorine in wastewater disinfection containing Poliovirus Type I (Sabin) and bacteriophage T4. Dryden et al. (1979) studied the removal of viruses in model systems for tertiary wastewater treatment (coagulation, sand filtration, direct filtration, and carbon adsorption followed by chemical disinfection with either chlorine or ozone). All systems produced virus reductions of at least five orders of magnitude. Berg et al. (1979) compared chlorine dioxide with chlorine as a disinfectant for wastewater effluents and found that both were effective in reducing virus levels.

The efficiency of chlorine as a raw wastewater disinfectant was found to be dependent on pH, temperature, time, demand, and mixing; however, the quantitative prediction of performance was stated to be difficult (Irving 1980). Hajenian and Butler (1980) have found that bacteriophage f2 was more sensitive at low pH than at high pH, whereas no consistent effect of pH on the sensitivity of poliovirus to chlorine could be noted.

Ozone inactivation of six viruses in a continuous flow reactor was studied with a 40 fold difference existing between the most resistant and the most sensitive virus (Roy 1979). Keswick (1979) has found that BrCl was two to three times more effective at inactivating poliovirus than chlorine in pH 6 buffer. In three studies, chlorine dioxide has been found to be a more effective disinfectant than chlorine against bacteria and viruses in wastewater (Longley et al. 1980, Berg et al. 1980, Aieta et al. 1980).

Many resources and much effort has been devoted to viral inactivation studies due to potential serious health implications associated with viral contamination. As can be seen from the brief literature survey, many different types of virus microorganisms under a variety of conditions have been treated with the most commonly used disinfectants. Because of the complex nature of wastewater and the "stamina" of virus microorganisms there is no clear or decisive inactivation pathway or an ultimate disinfectant which can classically destroy all pathogens. Thus there is still a tremendously active research effort taking place to solve many of the unanswered questions regarding viral inactivation.

OBJECTIVE

Determine the effect of sulfur dioxide treatment of secondary sewage containing 1) reovirus and 2) poliovirus.

METHODS AND PROCEDURES

The first set of experiments was conducted using reovirus. Nine beakers each containing 1 liter of secondary sewage were divided into three groups with each set of conditions being monitored in triplicate. The secondary sewage was collected (prior to chlorination) from the Hyrum wastewater treatment plant, Hyrum, Utah. Three sets of conditions were established.

One set of three samples termed "control" received no further treatment. Another set of three samples labeled "pH control" were brought to pH 2.5 by the addition of HCl. The "SO_2" group of three samples received SO_2 to the extent of 500 mg/l which resulted in pH of 2.5.

One ml of reovirus type II stock (previously assayed at 2 x 10^9 virus per ml) was added to each sample. The addition of virus to the sample marked time zero. The samples were mixed at 20 rpm throughout the experiment using a standard jar test apparatus. One ml samples were taken from each of the nine flasks at various time intervals. Each 1.0 ml sample was added to 1.0 ml of 0.15 M phosphate buffer pH 7.2 in a wet ice bath and maintained at approximately 4°C until assayed later that day.

The assay for reovirus was the immunofluorescent cell count procedure utilizing Madin Darby Bovine Kidney cells (Spendlove 1967). Preliminary experiments were run to determine satisfactory buffers and dilutions that would allow assay of the treated sewage on mammalian cell cultures. The reproducibility of this assay is $\pm$ 20 percent (Barnett 1975) when sufficient numbers of infected cells are counted. A second set of experiments was also run. The study was designed to evaluate concentrations of 500 mg/l SO_2 and 1000 mg/l SO_2 in secondary wastewater spiked with reovirus. The response of both infectious reovirus (IV) and potentially infectious reovirus (PIV) were monitored. The treatment and assay procedures were essentially the same as above. For this experiment the infectious reovirus was prepared by treating potentially infectious reovirus Type II with trypsin (Sigma type IX) at 100 mg/ml for 1 hour at 37°C. A third set of experiments was run using poliovirus. Similar conditions and experimental set up as for the previous experiments were used.

The secondary sewage was spiked with poliovirus to yield a final concentration of about 10^7 poliovirus per liter, which is 100 fold higher than what is usually found in raw sewage from large metropolitan

areas. The pH of the sewage was 7.3, the pH after addition of sulfurous acid was 2.6.

RESULTS AND DISCUSSION

The data from the first experimental run are given in Table 2.

The data indicate a 90 percent reduction in reovirus by SO_2 treatment for 40 minutes. This is somewhat less inactivation than was observed following pH adjustment to 2.5 with HCl. A non-spiked sewage sample was assayed in parallel with these reovirus spiked samples. The non-spiked sample did not contain reovirus at levels sufficient to be detected by our assay procedure. The data for the second experimental run are presented in Table 3.

These data are in approximate agreement with the data of previous experiments and they do not indicate any significant difference between the response of the two forms of reovirus to SO_2 treatment.

After 40 minutes of treatment with SO_2 at 500 mg/l, 13 percent of the PIV remained and 27 percent of the IV remained. Using 1,000 mg SO_2 per liter left 10 percent of the PIV and 17 percent of the IV. The results of the SO_2 treatment of poliovirus in secondary wastewater are shown in Table 4.

The SO_2 treatment clearly inactivated the poliovirus beyond the degree observed following simple pH adjustment. The inactivation was rapid, having plateaued at the first time point (i.e. 5 min.) and resulted in 97 percent of the infectious virus being removed.

In summary, the third set of experiments using the poliovirus was the most encouraging. Disinfection conditions could possibly be optimized to achieve even a better inactivation of the poliovirus.

Table 2. Effect of SO_2 treatment on reovirus viability in secondary sewage effluent.

Sample Treatment	Viable Reovirus Present in Sample at Selected Times After Addition of Reovirus. (Time in Minutes)				
	1	5	15	30	40
Control (no adjustments)	2.5×10^6	-	-	-	2.1×10^6
pH Control (pH to 2.5 with HCl)	4×10^4	-	-	-	7×10^4
SO_2 (500 mg/l)	-	7×10^5	4×10^5	4×10^5	2×10^5

Table 3. Effect of SO_2 treatment on reovirus viability in secondary sewage effluent.

Sample Treatment		Viable Reovirus Present in Sample After Addition of SO_2 (Time in minutes).				
		1	5	15	30	40
Control	PIV[a]	7.7×10^7				8.6×10^7
Control	IV[b]	4.0×10^7				4.0×10^7
pH 2.4 Control	PIV	7.7×10^7				2.2×10^7
pH 2.4 Control	IV	3.9×10^7				2.2×10^7
SO_2 500 mg/l; pH 2.4	PIV		4.1×10^7	3.3×10^7	2.0×10^7	1.0×10^7
SO_2 500 mg/l; pH 2.4	IV		2.5×10^7	1.4×10^7	1.5×10^7	1.1×10^7
pH 2.1 Control	PIV	9.8×10^7				4.1×10^7
pH 2.1 Control	IV	3.0×10^7				1.0×10^7
SO_2 1,000 mg/l pH 2.4	PIV		3.4×10^7	2.6×10^7	1.4×10^7	0.8×10^7
SO_2 1,000 mg/l pH 2.4	IV		1.6×10^7	1.2×10^7	1.1×10^7	0.7×10^7

[a]PIV--potentially infectious virus, double shelled form which must be enzyme activated just before assay.

[b]IV--infectious virus, single shelled form.

Table 4. Effect of SO_2 treatment on poliovirus viability in secondary sewage.

Sample Treatment	Viable Poliovirus[a,b,c]/ml at Selected Treatment Times				
	1 min	5 min	15 min	30 min	40 min
Control (no adjustments pH 7.3)	102±12	-	-	-	114±22
pH Control (pH 2.6 with HCl)	48±20	-	-	-	38±14
SO_2 at 500 mg/l (pH 2.6)	-	1.8±0.6	3.2±1.4	2.6±1.6	2.6±1.2

[a]Plaque forming units $x10^{-2}$.

[b]The assay system for poliovirus was a plaque assay using a methyl cellulose overlay on monkey kidney cells.

[c]The standard deviation on the triplicate runs is indicated as plus/minus.

CONCLUSIONS

Conclusions reached from this study are:

1) Reovirus (PIV or IV) was inactivated with SO_2 treatment by up to 90 percent under the conditions used.
2) Poliovirus was inactivated by 97 percent with 500 mg/l SO_2 treatment of wastewater spiked with poliovirus.
3) There is a definite inactivation effect of the SO_2 on poliovirus when complared to the pH control.

FUTURE STUDIES REQUIRED

It is important for future studies to:

1) Optimize SO_2 inactivation conditions of viral pathogens.
2) Design and carry out more in-depth study which will give a statistical significance to viral inactivation by SO_2 treatment of wastewater.
3) Investigate the effects of SO_2 on other selected health hazard related pathogens.
4) Determine the inactivation mechanism of viruses by SO_2.

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