EFFECT OF SUPERSONIC VIBRATIONS ON ESCHERICHIA COLI

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By

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TABLE OF CONTENTS

INTRODUCTION

The study of supersonics is attracting more and more interest from a wide range of applications. It includes applications of supersonics in various fields of work, such as;- material testing, ultrasonic agitation, television, signaling, photographing, cathode-ray screens, and medicine. The application of supersonics to sanitary work although still in its infancy, according to several authors, offers great possibilities to the experimenters. Items being studied, concerning application of supersonics to sanitary treatments are as followings;-

1, Coagulative effects - Effect of supersonic waves on coagulation is not certain as yet. Some experimenters claimed that supersonic waves do not appear to have any coagulating action,[1] but some stated that they do. The proponents of its effectiveness in coagulation said that there is a possibility of radical change in filter plant methods, and practice. It could mean either the total elimination or a great decrease in size of sand filter beds used and the use of a small electronic flocculation unit through which the raw water would flow at a relatively higher velocity.[2] The primary weakness in this hypothesis is that it was drawn after experimenting with only a particular

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[1] H. Beuthe, E. Furbaeh and C. Sorensen, "Water Purification by Supersonics," Journal of the American Water Works Association, XXXIII (1941), 373.

[2] D. M. Nielsen, "Electronics in Water Supply and Sewage Treatment," Water Works and Sewerage, 92 (September, 1945), 271-274.

specified suspension. The suspension contained only particles with specific gravity slightly more than that of the liquid in which it was suspended and with a size above a certain minimum. In ordinary raw water this condition is not true. The latest information about the coagulative effect of supersonics states that coagulative effects come about under certain conditions. Experimentally the effect of ultrasonics on certain suspensions have shown that the particles seem to attract each other, become fastened to each other, and fall to the bottom of the container. It is possible by special methods to photograph the details of this action. It also stated, concerning supersonics in general, that without the presence of gas many actions do not occur, whatever changes appeared are unstable, and chemicals shortly revert to their original states.[3]

2. Bactericidal effect - As early as 1930, bactericidal effect of supersonics was studied. Two 8800 cycles-250 watts oscillators were used at that time. It was found that the number of survivors of bacteria treated had a straight line relationship with the time of exposure. The experimenters stated that the effect was possibly due to violent agitation probably within the cells and also possibably due to comminution by the suspending medium.[4]

In 1934 the effect of supersonics on bacteria was studied

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[3] Benson Carlin, Ultrasonics, pp. 215-216.

[4] O. B. Williams and N. Gaines, "The Bactericidal Effects of High Frequency Sound Waves," Journal of Infectious Diseases, XXXXVII (1930) 485.

again. With exposure of bacteria to supersonics for ninty minutes, a definite high percentage of killing was obtained.[5] By experimenting with and without the presence of gases in the liquid it was found that the killing and dissolution of bacteria by supersonic waves was due to cavitation of the dissolved gases.[6]

In 1940 explaination of the killing due to cavitation was attempted. Cavitations were produced by supersonic waves in liquid and it was followed by the compression wave forcing the liquid around the cavities to collapse with such a force that the pressure developed is in the order of magnitude of thousands of pounds to the square inch. It has been found that this powerful mechanical action of alternate low pressure followed by extremely high pressure at rate of 8900 times per second has a killing action on bacteria. The relationship between the number of survivor bacteria vs time of exposure was studied again. It was also straight line relationship. Sterilization cannot be obtained and probably more powerful oscillators are required to attempt sterilization, as stated.[7]

Latest information, published in 1949, didn't say much

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[5] S. C. Liu and A. C. H. Yen, "Effect of Supersonic Waves on Bacteria," Proc. Soc. Expt. Biol. and Med., XXXI (1934) 1250-1252.

[6] S. C. Liu and A. C. H. Yen, "Further Studies on the Effect of Supersonic Waves on Bacteria," Proc. Soc. Expt. Biol. and Med., XXXII (1934), 485-488.

[7] D. P. Legally and G. W. Patterson, "The Action of High Frequency Sound Waves on Bacteria," Am. J. Pharm., 112 (1940), 373-377.

about bactericidal effect of supersonics, but it was mentioned that supersonic waves do have the power of disintegrating bacteria to release enzymes.[8]

3, Other effects of supersonic waves on water treatment - Carbonate hardness of tap water was reduced by waves, but non-carbonate hardness changed only slightly. If a 75 percent solution of gypsum is treated with supersonic waves, transparency of solution is decreased. The effect is increased if the solution has previously been saturated with oxygen.[9] Supersonic waves fasten chemical reactions.[10]

It was stated by Legally and Patterson in 1940 that sterilization cannot be produced, until a more powerful oscillator can be secured. The supersonic generator available now has a maximum intensity of 900 watts and a frequency of 450 kc. Comparing these value to 250 watts and 8.9 kc. that were used in 1940, the present generator is by far more powerful than the old one. Since disinfection is one of the most important steps in sanitary treatment, it may offer great possibilities to see if sterilization can be attained now. It also may mean better treatment, and also may achieve more economical procedure in sanitary works. That is why the following experiments were conducted.

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[8] Carlin, op. cit., p.218.

[9] Beuthe, Furbach and Sorensen, loc. cit.

[10] Carlin, op. cit., p.217.

EXPERIMENTS

Source of supersonic energy;-

"Ultrason," model U-300,

Televiso Product Co., Chicago.

Descriptions;-

Model U-300 May be used for either research, where high power is requisite, or for production applications. This instrument produces a maximum of 300 accoustic watts in oil bath with a small variable frequency range. A unique developement of the crystal holder is that it may be used to treat solids, powders, or may be a continual flow of chemicals that are conductive or non-conductive to electric currents. It produces a frequency of 450 kilocycles per second and an electrical output of 900 watts.

It has a range of frequencies, but 450 kc. is the optimum for the crystal. If the equipment is in good operating order, the maximum power input should be about 1200 watts. It operates at approximately 85 percent efficiency and therefore is applying up to 900 watts of energy to the crystal.

EXPERIMENTS I & II

Date;- March 9th, and 11th, 1949.

Purpose;- To find out the total bacteria count of Boomer Lake water which is the raw water of the Stillwater Water Plant.

Procedure;- Prepare three plates, each with 1 ml. of sample and agar and another three plates, each with 1/10 diluted sample and agar culture. Culture the plates for twenty four hours and make the counts.

Result;- The 1 ml. plates had too many colonies of bacteria to be counted.

Total counts for 1/10 ml. plates -

Experiment I	107
Experiment II	76

EXPERIMENT III

Date;- March 18th, 1949.

Purpose;- To find out the percentage of kill of bacteria in Boomer Lake Water after subjection to supersonic treatment.

Procedure;- Prepare the untreated control plates as in Exp. I. Prepare five glass test tubes with fifty ml. of sample water each. The test tubes are of 0.8" diameter and are sterilized. Subject the tubes to supersonic treatments with different time of exposure of 1 min., 2 min., 3 min., 4 min., and 5 min. Prepare plates with differently treated water as in Exp. I. Culture the plates for twenty four hours and make the counts. Compare results of treated and untreated water to find out percentage of kill.

Result;-

Treatments	1	2	3	4	5
Frequency in kilocycles	450	450	450	450	450
Voltage in volts	2500	2500	2500	2500	2500
Amperage in milliamperes	250	250	250	250	250
Area of exposure in inch^2	0.5	0.5	0.5	0.5	0.5
Time of exposure in min.	1	2	3	4	5
Amount treated in ml.	50	50	50	50	50
Control counts per ml.	285	285	410	410	365
Treated counts per ml.	71	120	121	108	123
Percent killed in %	75	57.5	70.5	73.7	66.3

EXPERIMENT IV

Date;- April 4th, 1949.

purpose;- From the result of last experiment, it was found that time of exposure had little effect on the percentage of killing. It may be explained by the reason that there were a definite percentage of bacteria which will not be killed by the supersonic waves no matter how long it is exposed to the waves. Thinking that one minute of exposure is enough to kill all bacteria that are to be killed, the time of exposure in this experiment will be decreased to a slightly lower range to see if the decreased time of exposure will lessen the percentage of kill. From other literature and according to Mr. Viernstein, it is known that there is 75% loss of energy when the supersonic waves are transmitted through glass. Tubes with a thin metal sheet as bottoms were tried. In general, the purpose of this experiment is still to find out the percentage of kill of bacteria in Boomer Lake Water after subjection to supersonic treatment.

Procedure;- The untreated control plates were prepared as in Exp. I. Three glass tubes were prepared as in Exp. III, and treated with different times of exposure of ½ min., 1 min. and 2 min. respectively. The plates were prepared for the treated samples as in Exp. III. Six metal test tubes with 50 ml. of sample water each were prepared. The tubes are to be o.8" in diam., and with a brass sheet of

(Exp. IV, continued)

0.006" at the bottoms. They were treated the same as the glass tubes, with two tubes for each time of exposure.

Result;- Frequency and intensity of the waves used are the same as in Exp. III.

Treatment	Type of tube	Time of exposure	Average % killed
1	glass	2 min.	37.5%
2	glass	1 min.	31.5%
3	glass	½ min.	51.2%
4	metal	2 min.	78.5%
5	metal	2 min.	
6	metal	1 min.	80.6%
7	metal	1 min.	
8	metal	½ min.	86.6%
9	metal	½ min.	

The result still can not tell us anything about the effect of different time of exposure, but it is certain that the metal-sheet bottomed tubes are more effective than the glass tubes.

EXPERIMENT V

Date;- April 11th, 1949.

Purpose;- Preparation of supersonic treatment on a special type of bacteris, namely E. Coli. To determine the E. Coli density in a pure E. Coli culture so as to find out the proper dilution of the culture to be treated.

Procedure;- Dilute the pure E. Coli broth to 1/100,000 and 1/1,000,000 dilutions. Make three agar plates of each dilution. Count the plates.

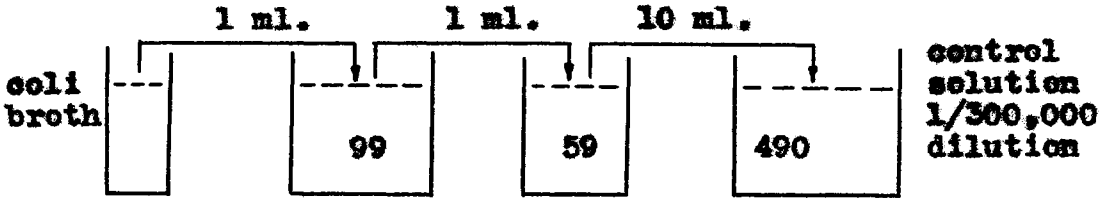
Result;- Counts on the 1/100,000 dilutions are too numerous to be counted. Counts on 1/1,000,000 (mil.) dilution averaged 300.

EXPERIMENT VI

Date;- April 25th, 1949.

Purpose;- To find out the effect of supersonic waves on a pure twenty-four-hour culture of E. Coli bacteria.

Procedure;- Dilute the pure E. Coli broth to one in three hundred thousandth with sterilized water to form control solution. According to Exp. V, the count of this diluted solution will be approximately 1,000.



Determine the counts in the control solution by making plates with a 1/10 dilution and a 1/100 dilution. Prepare the same six metal tubes as used in Exp. IV with 50 ml. of the control solution each. Treat the solution in the tubes under supersonic waves with different time of exposure;- namely 1 min., 20 sec., 10 sec. and 5 sec. Prepare plates with 1 ml. of treated solution straight and 1/10 dilution and 1/100 dilution each, and count.

Result;- Frequency and intensity of the supersonic waves used are the same as used in Exp. III.

(Exp. VI, continued)

Treatments	Time of exposure	Average % killed
1	5 sec.	96.0%
2	10 sec.	98.5%
3	10 sec.	
4	20 sec.	99.3%
5	20 sec.	
6	1 min.	99.8%

Apparently through out the treatments no sterilization can be obtained, by varying the exposure.

EXPERIMENT VII - XII

Date;- Oct. 4th, - Nov. 15th, 1949.

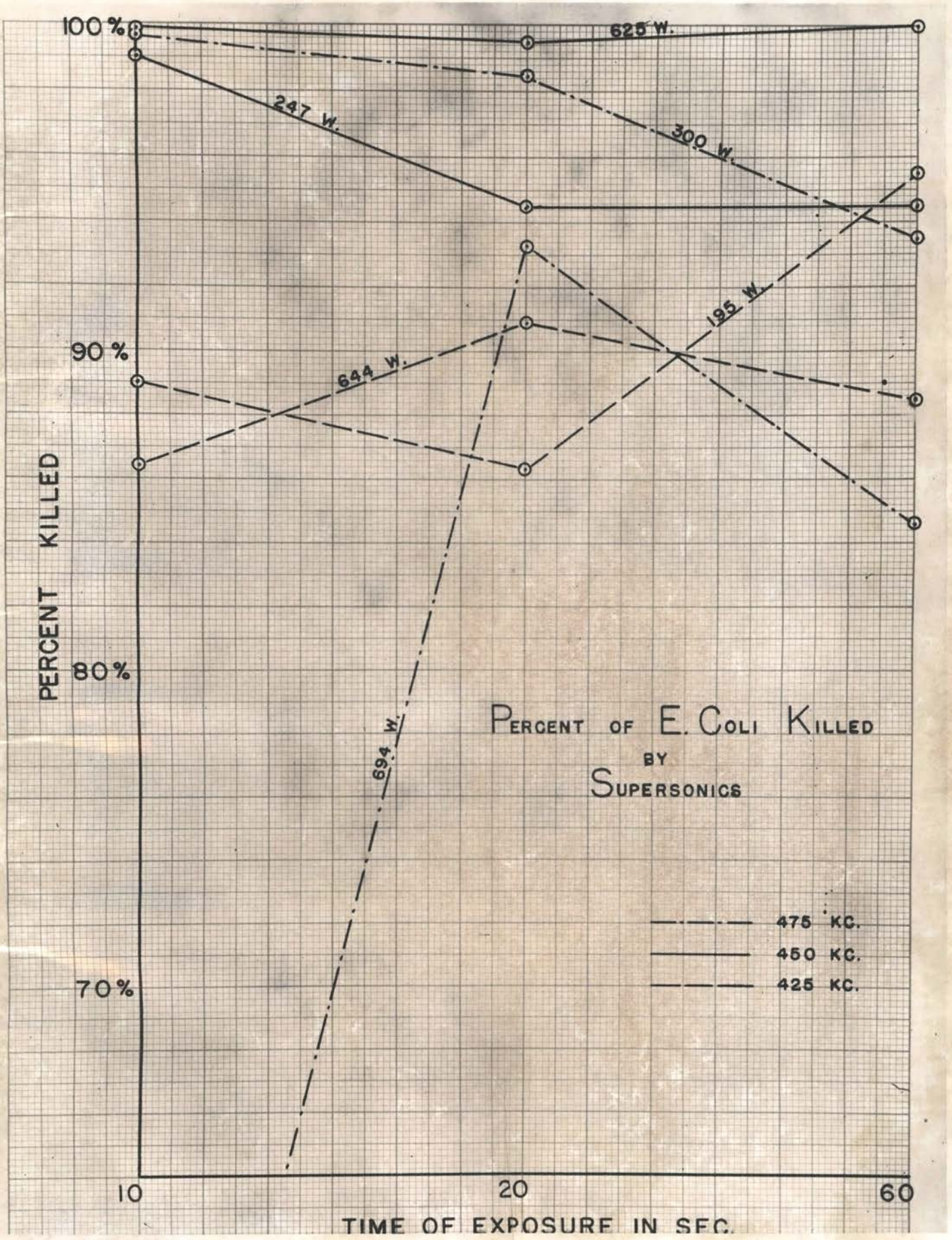
Purpose;- To find out the effect of supersonic waves on a pure twenty-four-hour culture of E. Coli under the condition of various combinations of times of exposure, frequencies, and intensities.

Procedure;- Dilute a twenty-four-hour broth culture of pure E. Coli to one in three hundred thousandth as in previous experiments. prepare the metal tubes with 50 ml. of this dilution as in previous experiments. Subject the tubes to supersonic waves under the combinations of times of exposure of 10 sec., 20 sec. and 1 min., frequencies of 425, 450 and 475 kilocycles per sec. and intensities of from 195 watts to 694 watts.

Result;- Percent of E. Coli killed by supersonic treatments.

Time of	425 kc.		450 kc.		475 kc.	
exposure	195 w.	644 w.	247 w.	625 w.	300 w.	694 w.
10 sec.	89.00%	86.43%	99.00%	99.86%	99.64%	47.10%
20 sec.	86.36%	90.91%	94.50%	99.45%	98.42%	93.32%
1 min.	95.45%	88.50%	94.50%	100.00%	93.45%	84.60%

Note;- By using the same metal tubes, used in supersonic treatments, as a container for control, it was found that the metals, themselves will kill about 20 to 25% of bacteria in about one hour of time, which is needed in the process of treatments.


100%
90%
80%
70%
PERCENT KILLED
625 W.
247 W.
300 W.
195 W.
644 W.
694 W.
PERCENT OF E. COLI KILLED
BY
SUPERSONICS
475 KC.
450 KC.
425 KC.
10
20
60
TIME OF EXPOSURE IN SEC.

DISCUSSION

In the experiments performes, it seems that sterilization with supersonic waves is still not certain. Although the combination of 450 kc. frequency, 625 watts and one min. of exposure gives 100% kill, there is the factor of Oligodynamic effect of the metal tubes. Since metal tubes with thin copper plate bottoms are the best containers available to insure the least absorption of the supersonic waves, there is no way to find out if the above combination has sterilized the pure Coli solution alone.

According to earlier experimenters, the cause of killing action of supersonic waves on bacteria is probably due to violent agitation of the bacteria cell, and the comminution by suspending medium.

In order to keep the Coli culture pure, all dilutions were made with distilled water which contained no solid particles. If in case of actual water treatment, raw water is likely to contain all kinds of impurities which will certainly help to comminute the bacteria when treated with supersonic vibrations. As mentioned in the introduction, more recent experimenters thought that supersonic waves kill bacteria by means of cavitation. Forming cavities requires the presence of dissolved gases. The same kind of difficulties can be seen here in the fact that the distilled water obtained in the laboratory contains very little or no dissolved gases while ordinary raw water will contain substantial quantities of

various gases.

The difficulty of relative absence of dissolves gases in sterilized water for dilution may be overcome by a special procedure. If a setup by means of which air can bubble up through a strong solution of chlorine, is available, we can always bubble the air again through the sterilized water to give dissolved gas in the water. Such air may carry a large enough amount of chlorine vapor into the water to effect kill of bacteria. Then it should be allowed to pass through a sodium thiosulphate solution before it reaches the water. Sodium thiosulphate will react with chlorine to take the chlorine out of the air. By such means, it is possible to produce cavitation in the water by supersonic waves and thus a better kill of bacteria may be the result.

Another improvement may be made on the experiments. In the first few experiments the containers to hold the bacteria to be treated were glass tubes. Although they are the flat bottom type of tubes they still prevented too much of the supersonic power to go through them. It was very obvious, as the percentage of kill increased when metal tubes of thin bottoms were used. Nevertheless what the optimum thickness to permit maximum transmission is yet unknown. The thickness of 0.006" of the copper plate used is only arbitrary. If only a suitable reciever[11] to measure the energy of the supersonic waves was available, it would be easy to find out the actual

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[11] General Electric Company puts out such recievers.

wave length used. With the thickness of the bottom of the metal tubes equal to the minimum thickness possible and yet equal to an integral number of the half-wave lengths, maximum amount of energy is transmitted through the bottoms.[12] If 0.006" is not an integral number of the half-wave lengths, it would be very easy to improve the result of the experiments by using a more suitable thickness for the bottoms of the tubes.

The economy of using supersonics in water works will probably depend upon the field of the supersonic machine. Usually the absorption of power from supersonic waves is small in water. Deep sea sounding work was done with supersonic waves. It would have been impossible if water absorbes too much of the wave energy. So it is quite possible that supersonic waves will penetrate a greater depth of water than that used in the experiments. Besides, the time of exposure of one minute is much less than what was used by previous experimenters.[13] Therefore if the capacity of the field of the supersonic machine can be assumed as two cubic feet which is not very much, it will be much cheaper to treat water with supersonic vibrations than with chlorine.[14]

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[12] D. Cochran and R. W. Samsel, "Ultrasonics," General Electric Review, XXXXVII (August, 1944).

[13] 90 minutes of exposure were used by Yen and Liu, Loc. cit. "At least 10 minutes of exposure to the supersonics, being able to show result." stated by Williams and Gaines, loc. cit.

[14] Based upon:- 1 ppm chlorine dosage, 10 ¢ per pound of chlorine and 2 ¢ per kilowatt hour of electricity.

CONCLUSION

The results of these experiments show definitely that there is a nearly 100% kill of E. Coli by means of supersonic treatment under the conditions of the experiments. Whether or not supersonics can be used for disinfection in sanitary treatment, remains to be seen with further experiment.

Dr. Bergmann stated that supersonic effects on one species of bacteria may be just the opposite to the effect on another species. Therefore it is improper to say that supersonic treatment can be used for disinfection, unless experiments have been performed with all water borne pathogenic bacteria. Nevertheless it is generally recognized that E. Coli is an indicator of fecal pollution. If 100% kill can be obtained on E. Coli, possibly with proper regulation of power input, frequency and other physical factors, satisfactory kill of pathogens may be obtained. Therefore the result of these experiments indicated that there is a good possibility of using supersonics, efficiently for disinfection in sanitary work.

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