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Some observations on the role of coliphages in the number of *Escherichia coli* in oxidation ponds

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INTRODUCTION

In the literature dealing with the decrease in bacterial numbers of streams and rivers, the activity of bacteriophages is usually advanced as a factor possibly of significance in explanation of this phenomenon. When sewage is treated in oxidation ponds an attempt is made to create conditions which are similar in their effect to those existing in a river where self-purification is known to take place.

The question naturally arises whether or not bacteriophages play any significant role in reducing the numbers of faecal bacteria in such ponds. Ware & Mellon (1956) conducted an experiment on the coli/coliphage relationship in a conventional sewage works, but could find no evidence to support the hypothesis that the reduction in number of coliform bacteria during sewage treatment should be ascribed to the action of bacteriophages.

The existence of experimental oxidation ponds at the Pretoria sewage works rendered it possible to study further the probability of a coli/coliphage relationship in these oxidation ponds. Two of these ponds were connected in series, the primary one receiving raw domestic sewage at a loading of approximately 160–180 lb. Biochemical Oxidation Demand (B.O.D.) per acre per day. The retention period of each pond was approximately 20 days.

METHODS

Enumeration and isolation of Escherichia coli *in raw sewage and pond effluents*

After the ponds had been in operation for nearly a year, counts were made of the coliform bacteria in the influent of the first pond and the effluents of both ponds, using the roll tube method as described by Pretorius (1961). From each set of roll tubes, 20–30 colonies at a time were isolated and purified by streaking out on MacConkey's agar. Single colonies were then subcultured into nutrient broth. Only one commercial brand of media (Difco) was used in this study.

Enumeration of coliphages

A typical *Escherichia coli* culture agreeing in all respects with the description given by Breed, Murray & Smith (1957) and selected from strains found to be sensitive to coliphages was used for determining, by the agar layer method of Adams (1950), the coliphages present in the influent and effluents of the ponds. As

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the samples contained very high numbers of coliform bacteria, it was necessary to centrifuge all samples at 4000 rev./min. for 30 min. to remove excess bacteria. One millilitre of the supernatant fluid was then used in the quantitative determination of the coliphages.

Determination of the proportion of phage-sensitive and phage-resistant bacteria

Propagation of specific bacteriophages. The same samples used for the enumeration and isolation of *E. coli* (see above) were mixed with samples of primary and secondary oxidation pond water and centrifuged as described above in order to remove the excess of bacteria and suspended organic matter. The supernatant fluid was then stored in a refrigerator (2° C.) while the subcultures of colonies described above were being purified. For propagating the specific bacteriophage, 1 ml. of each purified bacterial culture was mixed with 25 ml. of nutrient broth and this in turn was added to 25 ml. of the cooled centrifuged supernatant fluid, after the latter had reached room temperature. This specific bacteriophage enrichment culture was thereupon incubated overnight at 37° C.

This enrichment culture was centrifuged on the following day. Centrifuging alone was found to be inadequate for removing the majority of bacteria, because after 3 hr. the plates used for detecting bacterial lysis were so overgrown with non-coliform bacteria that very few or no lytic zones could be detected. For this reason it was found necessary to resort to filtration after centrifuging in order to remove the excess bacteria. For this purpose a membrane filter (Oxoid grade A.P.) was found more suitable than the Seitz filter, which appeared either to inactivate or remove a certain percentage of the phages present.

Determination of phage relationship. For determining the proportion of phage-resistant and phage-sensitive coliform bacteria the filtered phage suspension was tested against each bacterium isolated in the influent and effluents of the different ponds. The proportion of phage-sensitive to phage-resistant bacteria was noted. All the above described procedures were repeated seven times over a period of 10 weeks.

RESULTS AND CONCLUSIONS

The results in Table 1 indicate that when sewage water was treated in two oxidation ponds in series, the number of *E. coli* was reduced at an equal rate of about 94 % in each pond. On the other hand, the number of coliphages was not only reduced at a lower rate than *E. coli* bacteria, but the rate also differed from the primary to the secondary pond. Raw sewage contained a greater proportion of phage-resistant bacteria than phage-sensitive bacteria. This ratio, however, changed during the purification process through the ponds, resulting in an increase in the proportion of phage-sensitive bacteria as indicated in Table 2.

If the hypothesis is valid that the reduction in numbers of *E. coli* were due to the lytic action of coliphages then the rate of reduction of coliphages would have been the same for both ponds. In addition to this, an increase in the proportion of phage-resistant coliform bacteria would have been expected in the effluents. Since the reverse was observed, it seems unlikely that much bacteriophage lysis of coliform

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bacteria occurred during the purification of sewage in oxidation ponds. This finding corresponds to that of Ware & Mellon (1956) concerning the coli/coliphage relationship in a conventional sewage works.

From the results in Table 1 it is clear that the number of coliphages was reduced at a lower rate than the number of *E. coli*. If the coliphages do not lyse *E. coli* present in an oxidation pond, it follows that the coliphages are not multiplying in the ponds, and therefore, the smaller die-off of the phages would seem to be due to an ability to survive longer than *E. coli* when exposed to identical environmental conditions.

Table 1. *Average number of faecal coliforms and coliphages in the influent and effluents of a primary and secondary oxidation ponds in series*

	Faecal coliforms/ml.	Reduction (%)	Coliphages /ml.	Reduction (%)
Influent of primary oxidation pond (raw sewage)	136,800	—	2,650	—
Effluent of primary oxidation pond	8,100	94	670	74·7
Effluent of secondary oxidation pond	370	95·4	290	56·7

Table 2. *Proportion of the phage-resistant and phage-sensitive bacteria in the influent and effluents of oxidation ponds in series*

	No. of *E. coli* isolated over a period of 10 weeks	No. of phage-resistant bacteria	No. of phage-sensitive bacteria	Proportion of phage-resistant:sensitive (%)
Influent of primary oxidation pond (raw sewage)	136	80	56	58·8:41·2
Effluent of primary oxidation pond	134	66	68	49·3:50·7
Effluent of secondary oxidation pond	86	41	45	47·7:52·3

SUMMARY

No evidence was found to support the view that coliphages as such play an important role in reducing the number of *E. coli* in sewage water when it is treated in oxidation ponds. Coliphages seem to have greater powers of survival than *E. coli* in oxidation ponds.

REFERENCES

ADAMS, MARK H. (1950). *Methods in Medical Research*, vol. II. Editor-in-chief, J. H. Comroe. Chicago: Year Book Publishers.

BREED, ROBERT S., MURRAY, E. G. D. & SMITH, NATHAN R. (1957). *Bergey's Manual of Determinative Bacteriology*, 7th ed. Baltimore: Williams and Wilkins.

PRETORIUS, W. A. (1961). Investigations on the use of the roll tube method for counting *Escherichia coli* I in water. *J. appl. Bact.* (in the Press).

WARE, G. E. & MELLON, M. A. (1956). Some observations on the coli/coliphage relationship in sewage. *J. Hyg., Camb.* **54**, 99.

https://doi.org/10.1017/S0022172400020374 Published online by Cambridge University Press