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DIURNAL VARIATION OF BACTERIA AND BACTERIOPHAGES IN SEWAGE EFFLUENT AND THE FLOW TIME OF SEWAGE THROUGH A TREATMENT PLANT

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The loading of the Viikki wastewater treatment plant was investigated over a period of one day. The following variables were assayed from the effluent at hourly intervals: discharge, temperature, viable count of bacteria, faecal coliform bacteria, coliphages and salmonellaphages. Results of both the bacteriological and phage assays showed a simultaneous load, accompanied by an increase in the effluent temperature indicating the presence of a high proportion of domestic wastewater. The flow time of sewage at the plant was measured using coliphages as tracers. The highest phage concentration in the effluent, corresponding to a discharge of 33 000 m^3 d^{-1}, was observed 10 h after adding the tracer. According to theoretical calculation the detention time of the plant was 18.2 h while according to the phage tracer method it was 16.9 h. The difference may at least partly be due to inactivation and removal of phages, which could not be taken into account in this experiment.

Index words: Bacteria, faecal coliforms, bacteriophages, wastewater treatment plant, sewage, flow time.

1. INTRODUCTION

The loading of a wastewater treatment plant, i.e. the input of wastewater and faecal material, varies daily. In addition to this daily variation other variations also occur as a result of seasonal effects.

The shape of the daily loading curve must be known before variations of loading or of treatment efficiency due to seasonal effects can be investigated. If sampling at different dates takes place at or near the peaks of loading great differences in the concentrations of the variables assayed will inevitably be observed. In long-term investigations the daily peaks of loading should therefore be avoided when sampling. If it is necessary to monitor the maximum or average loading frequent sampling is needed.

Berg (1974) and Safferman and Morris (1976) have stressed the need for temporal coordination

of sampling in viral survival studies. The flow time of sewage through the treatment plant must be measured to enable the temporal co-ordination.

In this investigation both the flow time of sewage and the amounts of bacteria and phages were determined. These data were necessary for further studies concerning the efficiency of wastewater treatment in removing bacteria and phages as well as for an investigation of the seasonal variation of effluent quality.

The flow time of sewage through the treatment plant was measured using coliphages as tracers (Niemelä and Kinnunen 1968, Wimpenny et al. 1972, Kawata and Olivieri 1974, Kinnunen 1978). The daily variation of loading was investigated by analysing the concentrations of viable bacteria, faecal coliform bacteria, coliphages and salmonellaphages from sewage effluent samples taken at hourly intervals.

2. MATERIALS AND METHODS

The Viikki wastewater treatment plant is designed to treat from 40 000 to 50 000 m^3 sewage daily. After pretreatment with sand trap, screen and primary sedimentation, the activated sludge treatment is applied. The sewage then flows to the secondary sedimentation basins. The total volume of the plant is about 25 000 m^3.

For the 24 h monitoring experiment two samples of effluent were taken at hourly intervals from 7 a.m. on November 29, 1975 until 7 a.m. on November 30, 1975. The discharge and temperature of the effluent were measured at sampling.

Flow time measurement was carried out using the method described by Kinnunen (1978). A suspension of 18 l of coliphage F 137 (isolated by Dr. K. Kinnunen) containing about 10^{16}PFU, was added to the influent entering the primary sedimentation basins at 11.30 p.m. on September 24, 1975. Sampling was started 20 minutes later. Effluent samples were subsequently taken at half-hourly intervals with an automatic sampler (North Hants Engineering Co Ltd, Mark 4). Some of the samples were missed due to faulty functioning of the sampler. Samples were brought to the laboratory at intervals of a few hours and analyses were carried out immediately.

The viable count was determined using pour plate technique and 10 % standard plate count agar. The plates were incubated at 20 °C for 14 days.

The pour plate technique was also employed for faecal coliform analysis. The agar used was mFC agar modified by the substitution of 0.25 g Water blue for 0.1 g. Aniline blue (S. Niemelä, personal communication). Plates were incubated at 44 °C for 24 hours. Only bright blue colonies were counted.

The phage assay was performed using the agar layer method (Adams 1966). The medium contained (g l^{-1}): Lab. Lemco Broth 8, NaCl 5, $MgSO_4 \cdot 7H_2O$ 0.246, and for firm media, agar 10. Before sterilization pH was adjusted to 7.1. The plates were incubated at 28 °C for 24 hours, except for phage F 137 which was incubated at 35 °C.

The host bacteria for phage assay were *Escherichia coli* 137 (isolated by Dr. K. Kinnunen), *E. coli* B M219 (Czechoslovak National Collection of Type Cultures) and *Salmonella typhimurium* SH 4247 (kindly provided by prof. P.H. Mäkelä).

3. RESULTS

Microbiological variables -viable count, faecal coliforms, coliphages, and salmonellaphages- indicated a heavy load from 5 p.m. to 5 a.m. (Fig. 1). During this time considerable changes in the concentrations of the variables were observed.

Both the amount and the temperature of the discharge varied during the experiment (Fig. 1). When the effluent temperature was high the concentrations (ml^{-1}) and amounts (h^{-1}) of bacteria and phages were also high. High effluent temperature may in this experiment be interpreted to indicate the presence of a high proportion of domestic wastewater in sewage.

The plateau in the loading curve observed during the morning represents the most suitable time for sampling when seasonal effects on the quality of effluent and on the efficiency of the treatment plant are investigated.

The addition of the phage F 137 produced a

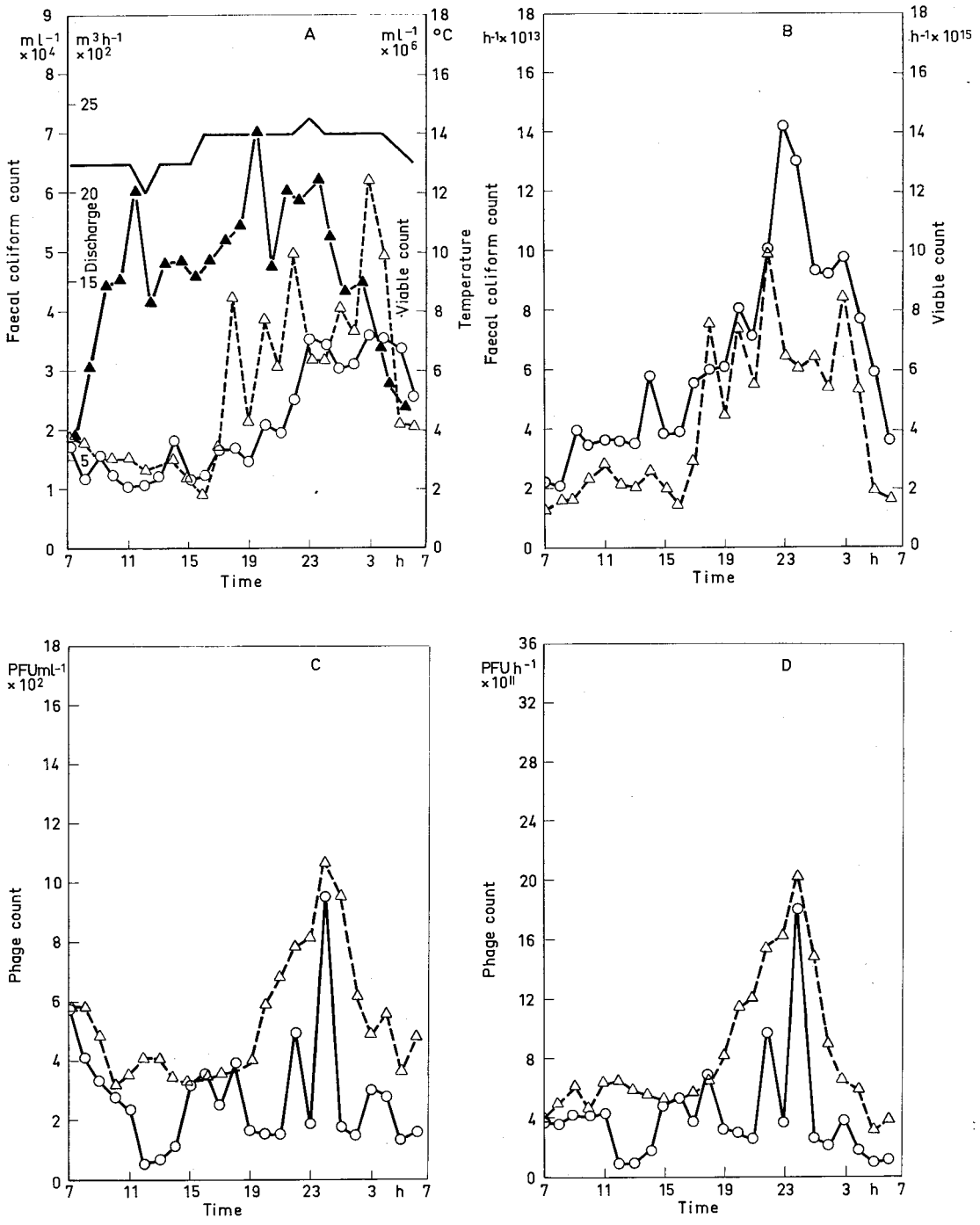

Fig. 1. 24 hour monitoring of the loading at the Viikki wastewater treatment plant (average of two samples). a. Concentrations of viable bacteria (o—o) and faecal coliform bacteria (△—△) in the effluent, temperature of the effluent (—) and discharge volume (▲—▲), b. Amounts of viable bacteria (o—o) and faecal coliform bacteria (△—△) in the effluent. c. Concentrations of phages of *E. coli* B M219 (△—△) and of *S. typhimurium* SH 4247 (o—o) in the effluent. d. Amounts of phages of *E. coli* B M219 (△—△) and of *S. typhimurium* SH 4247 (o—o) in the effluent.

distinct time versus concentration curve (Fig. 2). Before the addition of the tracer the concentration of indigenous *E. coli* 137 phages in the effluent had varied from zero to $2.5 \cdot 10^3$PFU ml^{-1}, and was 17 PFU ml^{-1} in the effluent and 8 PFU ml^{-1} in the influent at the beginning of the experiment.

The highest phage concentration in the effluent, $3 \cdot 10^5$PFU ml^{-1}, was observed 10 hours after marking the influent. The peak of the curve was sharp. The concentration of phages in the effluent decreased almost to background level within two days. The discharge during the experiment was 33 000 m^3d^{-1}. According to the calculations based on discharge and volume of the wastewater treatment plant the detention time was 18.2 h, while according to the phage method it was 16.9 h.

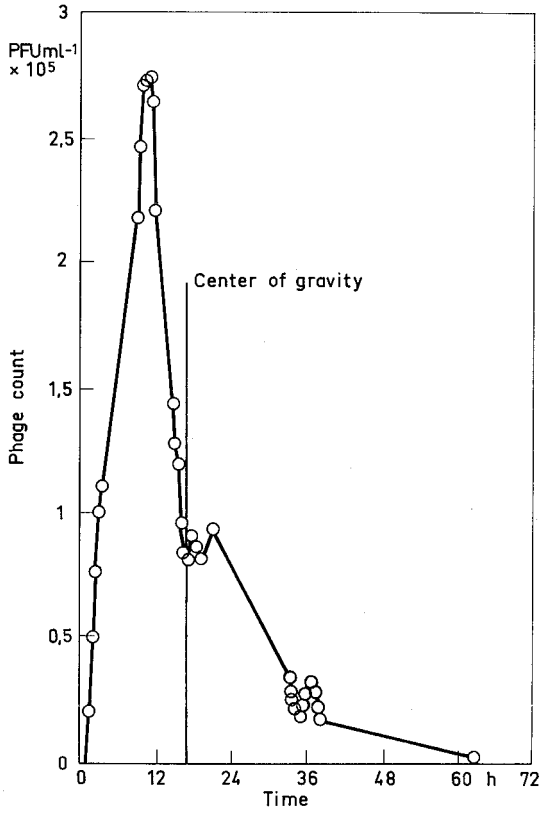

Fig. 2. Measurement of flow time of sewage at the Viikki wastewater treatment plant using coliphages as tracers. The tracer curve was drawn by joining the averages of consecutive phage counts.

4. DISCUSSION

During high discharge the concentrations of bacteria and phages tended to be high. Simultaneously with high microbial concentrations there was a rise of temperature. On the basis of these observations it can be assumed that the rise of discharge was caused by domestic wastewater. However, this observation cannot be generalized.

The great temporal differences in loading observed during one day at the Viikki wastewater treatment plant (Fig. 1) increase the risk of large sampling errors in long-term investigations of the sewage treatment process. These errors can be minimized by adjusting the sampling time to correspond to the loading plateau so that small time differences in sampling are less likely to affect significantly the concentrations of the assayed variables.

Random variation of microbiological variables of the influent, such as bacteria and coliphages, is great. The use of combined samples taken over a period of e.g. 15 minutes would help to smooth out this source of variation both in influent and effluent microbial concentrations. A combined sample gathered over a number of hours could be used for analysing the average concentrations of those variables which do not change significantly during storage. Such variables do not include concentrations of microbiological variables or variables that may change as a result of microbial activity. The analysis of combined samples yields less information than that of separate samples. The use of combined samples in microbiological wastewater studies is therefore not recommended (Bordner and Winter 1978).

Because of the differences in the daily loading, temporal coordination of influent and effluent samples is necessary when the efficiency of treatment plants is being investigated. However, as the calculated detention time may not always be reliable because of short cut currents of sewage, it is necessary to measure the flow time using tracers. The phage tracer has been found to be suitable for flow rate determinations in rivers and in some cases also in other water bodies. The method has several advantages: it is harmless to the environment, great dilution ratios can be

used, several water fractions can be marked simultaneously and the costs are relatively low (Kinnunen 1978).

The survival characteristics of phages are a problem when phages are used as tracers. Their survival is relatively good in most water types, especially if inactivation due to sunlight and high temperatures can be avoided (Niemi 1976). If the survival of phages can be monitored during the tracer experiment, as is usually the case, moderate inactivation rates are not a problem (Kinnunen 1978).

In this study the survival of phages could not be reliably estimated because of the difficulties in simulating the various treatment processes, such as primary sedimentation, activated sludge treatment and secondary sedimentation, in the laboratory. The difference between the calculated detention time, 18.2 h, and the centre of gravity of the tracer curve, 16.9 h, was 1.3 h. This difference, which can be sometimes significant, may be explained by the occurrence of short cut currents of sewage and at least partly by the inactivation of phages. The number of inactivated or lost phages rises as a function of time, with consequent alteration of the center of gravity of the tracer curve.

The peak of the phage count curve in Fig. 2 is sharp. The time corresponding to the highest phage concentration was selected to represent the flow time instead of the center of gravity of the tracer curve. The center of gravity of the tracer curve indicates the average flow time of particles while the peak value of the curve indicates the most probable flow time of a particle. The phage count curve (Fig. 2) follows the mixing of the water mass that entered the plant at the time of addition of the phage. Therefore the proportion of marked water mass in the effluent is maximal at the moment of the peak concentration of phage. Assuming a flow time of 10 h the efficiency of the wastewater treatment plant in eliminating added microbiological contaminants probably becomes lower than when the detention time is measured conventionally as the center of gravity of the tracer curve.

The single measurement of flow time corresponds only to one discharge and is, therefore, applicable only to a narrow range of situations. When temporal coordination of influent and effluent samples is required, it is advisable to time the sampling to correspond to the loading plateau.

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LOPPUTIIVISTELMÄ

Viikin jätevedenpuhdistamon kuormitusta seurattiin yhden vuorokauden ajan. Puhdistetusta jätevedestä määritettiin tunnin välein pesäkeluku sekä fekaalisten koliformisten bakteerien, kolifaagien ja salmonellafaagien pitoisuudet. Lisäksi mitattiin samanaikaisesti virtaama ja lämpötila. Kaikki mikrobiologiset muuttujat osoittivat voimakkaan kuormituksen sijoittuvan klo 17 ja klo 5 välille. Voimakkaan kuormituksen aikana esiintyi useita erillisiä kuormitushuippuja. Puhdistetun jäteveden lämpötila oli korkea samanaikaisesti suurten bakteeri- ja faagipitoisuuksien kanssa. Korkea lämpötila ilmensi tarkkailun aikana asumajäteveden suurta osuutta puhdistetussa jätevedessä.

Tämän lisäksi mitattiin jäteveden kulkeutumisnopeus puhdistamon läpi käyttäen kolifaageja merkkiaineena. Puhdistetussa jätevedessä havaittiin korkein faagikonsentraatio 10 h faagien lisäyksen jälkeen virtaaman ollessa 33 000 m^3d^{-1}. Virtaaman ja tilavuuden avulla laskettu jäteveden kulkeutumisnopeus oli 18.2 h faagimenetelmän antaessa tulokseksi 16.9 h. Viipymien ero voidaan ainakin osaksi selittää johtuvan faagien inaktivoitumisesta ja poistumista. Näitä ilmiöitä ei kuitenkaan voitu ottaa huomioon tässä kokeessa.

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