Short communication

Faecal clostridia and indicator bacteria levels in an eutrophic impoundment

D P Sartory*

Hydrological Research Institute, Department of Water Affairs, Private Bag X313, Pretoria 0001, South Africa.

Abstract

Faecal clostridia, faecal coliforms and faecal streptococci numbers in Roodeplaat Dam and two inflowing rivers were monitored for eight months from January to August 1985. Faecal clostridia occurred at mid-dam sampling stations and inflowing waters in greater numbers than faecal streptococci, which occurred in greater numbers at mid-dam stations than faecal coliforms, emphasising the difference in environmental persistance of the three indicator groups. Of 135 faecal clostridia isolates 105 (78%) were confirmed as *C. perfringens*. The results indicate that faecal clostridia enumeration may be a useful method in the microbiological assessment of water quality.

Introduction

Clostridium perfringens has, for many years, been advocated as an indicator of faecal contamination of water (Wilson and Blair, 1925; Bonde, 1966; Cabelli, 1977; Bisson and Cabelli, 1980; Sartory, 1986) as the organism possesses many attributes that make it suitable as a water quality indicator. *C. perfringens* is accepted to be of virtually exclusive faecal origin (Cabelli, 1977) and possesses spores that are extremely resistant to environmental stress and water treatment processes (Grabow *et al.*, 1978; Bisson and Cabelli, 1980). As the numbers of *C. perfringens* in effluents are generally greater than those of pathogenic bacteria and viruses (Burger *et al.*, 1984) the absence of *C. perfringens* may be a reliable indication of the absence of these organisms in raw water and waste-water treatment processes. One of the major drawbacks to the use of *C. perfringens*, however, has been the lack of a cheap and reliable medium and method of enumerating these obligate anaerobes.

Recent studies by Burger *et al.* (1984) and Sartory (1986) indicate that egg yolk-free tryptose-sulphite-cycloserine (TSC) agar is a cheap and efficient medium for the membrane filtration enumeration of *C. perfringens* and other sulphite reducing clostridia from water. All the isolates from both studies were sulphite reducing clostridia although the percentage contribution by *C. perfringens* was markedly different (24 to 26% in the Burger *et al.* (1984) study and 84 to 93% in the Sartory (1986) study.) The major isolate in the Burger *et al.* (1984) study was *C. ghoni* making up 60 to 66% of isolates. This species was not encountered in the Sartory (1986) study. Examining the data from the two studies Sartory (1986) pointed out that all the isolates were of predominantly faecal or urino-genital origin and so in their own right could act as indicators of faecal contamination. Sartory (1986) has suggested the employment of TSC agar and a simple confirmation procedure for the enumeration of faecal clostridia and *C. perfringens* in a manner analogous to that for faecal coliforms and *Escherichia coli*. The development of this concept, however, is dependent upon further media studies and more information on the occurrence and relative persistence of the various species of sulphite reducing clostridia of faecal origin in relation to other indicator bacteria.

This short paper presents data for an eight-month period (January – August, 1985) on the numbers of faecal clostridia, faecal coliforms and faecal streptococci recorded from Roodeplaat Dam, an eutrophic impoundment whose principal inflow, the Pienaars River, receives sewage effluent.

Materials and methods

Samples were collected from five mid-dam stations and two inflowing rivers of Roodeplaat Dam (Fig. 1), an eutrophic impoundment 25 km north-east of Pretoria which is used for recreational purposes, as a source of irrigation water and as a potable water supply (Pieterse and Bruwer, 1980). The two rivers sampled were the Pienaars River (A2M27), the major contributor to the impoundment which receives substantial sewage from the Baviaanspoort sewage works (up to 25% of the inflow of the impoundment according to Pieterse and Bruwer, 1980) and the Hartbeesspruit (A2M28) which receives urban runoff.

Samples were collected in sterile 500 mℓ bottles, stored in cool-bags, and analysed within 3 h of collection. Faecal clostridia, faecal coliforms and faecal streptococci were enumerated by the membrane filtration technique using Gelman GN6 membrane filters (Gelman Instrument Co.). Faecal clostridia were enumerated on TSC agar (Oxoid Ltd., codes CM587 and SR88) (Sartory, 1986), the plates being incubated anaerobically for 20 to 24 h at 45°C. All black sulphite reducing colonies were counted as faecal clostridia. Colonies were picked off for confirmation as *C. perfringens* according to Sartory (1986). Faecal coliforms were enumerated on m-FC medium (Difco Laboratories) without rosolic acid (Sartory, 1980), the plates being incubated for 20 to 24 h at 44,5°C. All blue colonies were counted as faecal coliforms. Faecal streptococci were enumerated on m-Enterococcus agar (Oxoid Ltd., code CM377) (Slanetz and Bartley, 1957), the plates being incubated for 44 to 48 h at 35°C. All deep red colonies were recorded as faecal streptococci. Single determinations were made for each sample. One hundred millilitres of sample were filtered for each bacterial group, except for A2M27 and A2M28 where 100 mℓ, 10 mℓ and 1 mℓ (added to 9 mℓ quarter strength Ringers solution prior to filtration) aliquots were filtered per determinand.

Results and discussion

The results of 15 samplings between January and August 1985 are

*Present address: County Analyst Laboratory, County Public Analyst, Hampshire Scientific Service, Hyde Park Road, Portsmouth, PO 5 4LL, United Kingdom.

Received 7 June 1987.

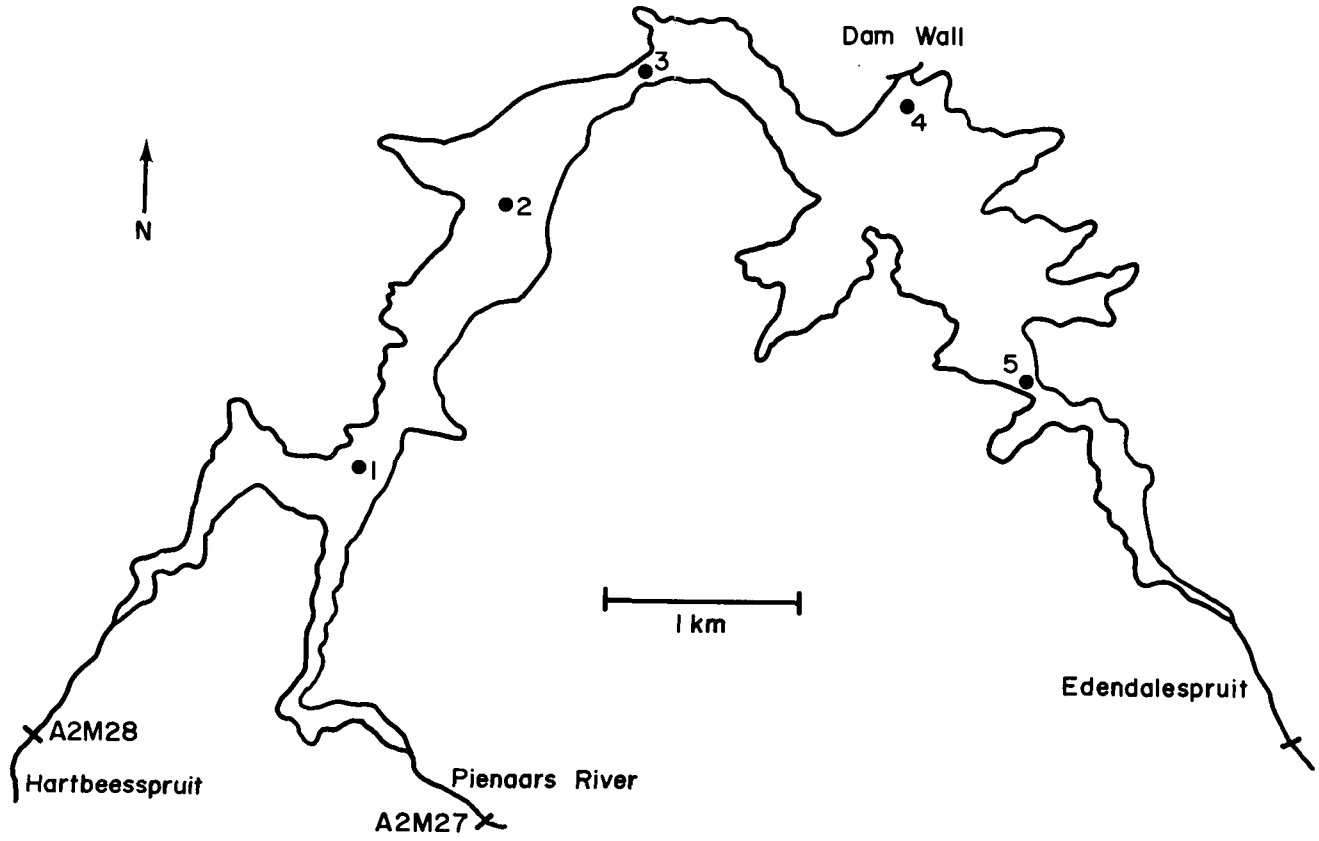

Figure 1
Map of Roodeplaat Dam showing sampling stations (1 to 5, A2M27 and A2M28).

given in Table 1. The Pienaars River (A2M27), which receives sewage effluent, carried the greatest number of faecal indicator bacteria. The numbers of faecal clostridia (FCl) ranged from 1 020 to 5 700 colony forming units (cfu) 100 $m\ell^{-1}$, while faecal coliforms (FC) ranged from 460 to 2 800 cfu 100 $m\ell^{-1}$ and faecal streptococci (FS) from 200 to 2 800 cfu 100 $m\ell^{-1}$. The numbers from the Hartbeesspruit (A2M28) are generally five to twenty-fold lower. There was, however, no consistency in the ratios of FC to FCl, ranging from 0,16 to 1,65 at A2M27 and 0,07 to 3,00 at A2M28. At A2M27 FCl outnumbered FC in 10 out of 14 samples while at A2M28 FCl outnumbered FC in 5 out of 9 samples.

At the mid-dam stations FCl were consistently isolated at all stations in greater numbers than FS, which were more frequently encountered and in greater numbers than FC. The stations in the arm of the impoundment receiving polluted waters (Stations 1, 2 and 3) recorded FCl on all thirteen samplings in numbers ranging from 1 to 150 cfu 100 $m\ell^{-1}$ while FC were recorded on only 3 to 5 occasions with a maximum count of 16 cfu 100 $m\ell^{-1}$. Station 5 in the arm of the impoundment receiving unpolluted waters recorded FCl levels of 1 to 14 cfu 100 $m\ell^{-1}$ on 11 occasions and FC counts only twice. One hundred and thirty-five isolates were picked off from plates from mid-dam stations for confirmation as *C. perfringens*. Of these, 105 (78%) were confirmed as *C. perfringens*, 22 (16%) were non-motile, nitrate-reducing, lactose-fermenting and gelatin liquefaction negative clostridia (*C. perenne, C. celatum, C. paraperfringens* and *C. rectum*) and 8 (6%) were motile clostridia (non-nitrate-reducing, non-lactose-fermenting and gelatin liquefying).

In sewage samples FC generally occur in greater numbers than FS and FCl but at A2M27 on the Pienaars River, approximately 4,5 km downstream of the Baviaanspoort sewage works, the numerical order was FCl > FC > FS while in the impoundment the order was further modified to FCl > FS > FC. These results demonstrate the relative environmental persistance of the three faecal indicator groups with FCl being considerably more persistent than FS which in turn are more persistent than FC. This extreme persistence of FCl has been cited as an argument against their use as recreational water quality indicators (Bisson and Cabelli, 1980) as they may persist in sediments and be resuspended. This does not seem to have occurred at Roodeplaat Dam, which is generally stratified during January to April and destratified during May to August when resuspension could occur. There was no obvious change in FCl levels during the eight months of this study. The situation in shallower bathing areas, however, would be different. Further studies would also need to be done to assess the relative persistence of FCl against pathogenic bacteria and, particularly, enteroviruses and helminth eggs in situations like those at Roodeplaat Dam. FCl certainly act as good indicators of the extent to which faecal contamination can have an impact. The FCl counts from Station 5 may possibly reflect the extent of agricultural runoff pollution that reaches the impoundment via the Edendalespruit.

In these studies 78% of the isolates were confirmed as *C. perfringens* while only 6% were motile clostridia. The contrasts with the egg yolk-free TSC isolates of Burger *et al.* (1984) from Windhoek maturation pond effluent where 70% of the isolates

TABLE 1

FAECAL CLOSTRIDIA (FCL), FAECAL COLIFORM (FC) AND FAECAL STREPTOCOCCI (FS) NUMBERS IN ROODEPLAAT DAM (STATIONS 1 TO 5) AND THE PIENAARS RIVER (A2M27) AND HARTBEESSPRUIT (A2M28), JANUARY TO AUGUST 1985. COUNTS IN COLONY FORMING UNITS (CFU) 100 $m\ell^{-1}$.

Sampling date	A2M27			A2M28			Station 1			Station 2			Station 3			Station 4			Station 5		
	FCL	FC	FS	FCL	FC	FS	FCL	FC	FS	FCL	FC	FS	FCL	FC	FS	FCL	FC	FS	FCL	FC	FS
17/01							70	0	14	50	0	50	12	0	7	3	3	10	3	0	21
30/01	1 500	500	100				16	0	5	55	0	36	13	0	11	5	2	1	2	0	3
13/02	2 800	460	1 700	180	40	1 100	17	3	2	26	2	53	8	0	3	9	1	0	9	0	6
27/02	1 900	1 400	2 800	85	130	1 800	150	3	8	25	0	47	50	2	7	0	1	1	0	0	3
12/03	1 700	680	1 500	700	50	1 500															
27/03	2 000	2 000	1 040	300	850	500															
24/04	3 100	2 300	300				36	0	3	44	0	19	28	0	2	14	0	0	1	1	2
08/05	1 700	2 800	600	100	70	125	26	0	0	27	0	3	50	16	0	7	0	0	8	0	1
22/05	2 000	2 000	200	600	41	130	11	0	2	10	0	1	50	0	0	25	0	0	2	0	0
05/06	5 700	2 300	400				120	0	2	50	0	4	50	0	4	11	0	0	10	0	0
19/06	2 300	660	580				46	2	7	80	1	5	37	1	6	22	0	1	14	0	7
03/07	2 200	1 830	1 400				90	1	0	19	0	2	40	0	5	13	0	1	4	0	0
17/07	1 800	820	410	70	117	30	12	0	0	20	0	0	11	0	0	2	0	0	11	0	0
31/07	1 450	600	210	90	80	80	9	1	1	3	1	1	8	1	2	8	2	0	3	2	0
14/08	1 020	1 360	300	20	60	23	1	0	0	2	0	0	1	0	0	0	0	0	0	0	0

were motile clostridia (66% *C. ghoni*, 2% *C. sphenoides* and 2% *C. novyi*). These data may reflect a difference in the environmental persistence between motile and non-motile species of faecal clostridia, an aspect which may prove useful in assessing the age of, or remoteness from, the source of pollution.

The data presented here, indicate that faecal clostridia and *C. perfringens* could prove to be more useful tools in the microbiological assessment of water quality than has been accepted in the past.

Acknowledgements

I am indebted to Mrs E Truter for her expert technical assistance. This paper is published with the permission of the Department of Water Affairs.

References

BISSON, J.W. and CABELLI, V.J. (1980) *Clostridium perfringens* as a water pollution indicator. *J. Wat. Pollut. Control Fed.* **52** 241-248.

BONDE, G.J. (1966) Bacteriological methods for estimation of water pollution. *Hlth. Lab. Sci.* **3** 124-128.

BURGER, J.S., NUPEN, E.M. and GRABOW, W.O.K. (1984) Evaluation of four growth media for the membrane filtration counting of *Clostridium perfringens* in water. *Water SA* **10** 185-188.

CABELLI, V.J. (1977) *Clostridium perfringens* as water quality indicator. In: Hoadley, A.W. and Dutka, B.J. (Editors) *Bacterial Indicators/Health Hazards Associated with Water.* Publication ASTM STP 635, American Society for Testing and Materials, Philadelphia. 65-79.

GRABOW, W.O.K. BATEMAN, B.W. and BURGER, J.S. (1978) Microbiological quality indicators for routine monitoring of wastewater reclamation systems. *Prog. Wat. Technol.* **10** 317-327.

PIETERSE, A.J.H. and BRUWER, C.A. (1980) Roodeplaat Dam. In: Walmsley, R.D. and Butty, M. (Editors) *Limnology of Some Selected South African Impoundments.* Water Research Commission, Pretoria. 119-129.

SARTORY, D.P. (1980) Membrane filtration faecal coliform determinations with unmodified and modified m-FC medium. *Water SA* **6** 113-115.

SARTORY, D.P. (1986) Membrane filtration enumeration of faecal clostridia and *Clostridium perfringens* in water. *Wat. Res.* **20** 1255-1260.

SLANETZ, L.W. and BARTLEY, C.H. (1957) Numbers of enterococci in water, sewage and faeces determined by the membrane filter technique with an improved medium. *J. Bact.* **74** 591-595.

WILSON, W.J. and BLAIR, E.M.McV. (1925) Correlations of the sulphite-reduction test with other tests in the bacteriological examination of water. *J. Hyg. Camb.* **24** 111-119.

GUIDE TO AUTHORS

AIMS AND SCOPE

This journal publishes refereed, original work in all branches of water science, technology and engineering. This includes water resources development; the hydrological cycle; surface hydrology; geohydrology and hydrometeorology; limnology; mineralisation; treatment and management of municipal and industrial water and wastewater; treatment and disposal of sewage sludge; environmental pollution control; water quality and treatment; aquaculture; agricultural water science; etc.

Contributions may take the form of a paper, a critical review or a short communication. A **paper** is a comprehensive contribution to the subject, including introduction, experimental information and discussion of results. A **review** may be prepared by invitation or authors may submit it for consideration to the Editor. A **review** is an authoritative, critical account of recent and current research in a specific field to which the author has made notable contributions. A **short communication** is a concise account of new and significant findings.

GENERAL

Submission of manuscripts

The submission of a paper will be taken to indicate that it has not, and will not, without the consent of the Editor, be submitted for publication elsewhere. Manuscripts should be submitted to: **The Editor, WATER SA, P O Box 824, Pretoria, 0001, South Africa**

Reprints

One hundred free reprints of each paper will be provided. Any additional copies or reprints must be ordered from the printer (address available on request).

Language

Papers will be accepted in English or Afrikaans. Papers written in Afrikaans should carry an extended English summary to facilitate information retrieval by international abstracting agencies.

Abstracts

Papers should be accompanied by an abstract. Abstracts have become increasingly important with the growth of electronic data storage. In preparing abstracts, authors should give brief, factual information about the objectives, methods, results and conclusions of the work. Unsubstantiated viewpoints should not be included.

Refereeing

Manuscripts will be submitted to and assessed by referees. Authors bear sole responsibility for the factual accuracy of their publications.

Correspondence

State the name and address of the author to whom correspondence should be addressed on the title page.

SCRIPT REQUIREMENTS

Lay-out of manuscripts

An original typed script in double spacing together with three copies should be submitted. Words normally italicized should be typed in italics or underlined. The **title** should be concise and followed by authors' names and complete addresses. A paper may be organized under main headings such as **Introduction**, **Experimental**, **Results**, **Discussion** (or **Results** and **Discussion**), **Conclusions**, **Acknowledgements** and **References**.

Contents of manuscripts

The International System of Units (SI) applies. Technical and familiar abbreviations may be used, but must be defined if any doubt exists.

Tables

Tables are numbered in arabic numerals (Table 1) and should bear a short but adequate descriptive caption. Their appropriate position in the text should be indicated.

Illustrations and line drawings

One set of original figures and two sets of copies should accompany each submission. Photographs should be on glossy paper (half-tone illustrations should be kept to a minimum) and enlarged sufficiently to permit clear reproduction in half-tone. All illustrations, line-drawings and photographs must be fully identified on the back, numbered consecutively and be provided with descriptive captions typed on a separate sheet. Authors are requested to use proper drawing equipment for uniform lines and lettering of a size which will be clearly legible after reduction. Freehand or typewritten lettering and lines are not acceptable. The originals should be packed carefully, with cardboard backing, to avoid damage in transit.

References

Authors are responsible for the accuracy of references. References to published literature should be quoted in the text as follows: Smith (1982) or (Smith, 1982). Where more than three authors are involved, the first author's name followed by *et al.* and the date should be used.

All references are listed alphabetically at the end of each paper and not given as footnotes. The names of all authors should be given in the list of references. Titles of journals or periodicals are abbreviated according to **Chemical Abstracts Service Source Index** (Cassi).

Two examples of the presentation of references are the following:

Grabow, W.O.K., Coubrough, P., Nupen, E.M. and Bateman, B.W. (1984) Evaluation of coliphages as indicators of the virological quality of sewage-polluted water. *Water SA* **10**(1) 7-14.

Wetzel, R.G. (1975) *Limnology*. W.B. Saunders Company, Philadelphia, p 324..