Letter to the Editor

Letter by **D.F. Toerien and A. Gerber**, *National Institute for Water Research, P.O. Box 395, Pretoria 0001*, in connection with:

Bacterial population structure of activated sludge systems
as discussed in
The identification of heterotrophic bacteria in an activated sludge plant with particular reference to polyphosphate accumulation
by Laurraine H Lötter and Margaret Murphy published in *Water SA* **11**(4) 179 - 184 (1985).

The bacterial population structure of activated sludge systems which remove phosphate biologically, has been deduced by a number of authors (e.g. Brodisch and Joyner, 1983; Buchan, 1983; Cloete *et al.*, 1985) through the isolation of bacteria cultured on specific growth media. However, it is well known (e.g. Morita, 1982) that in many aquatic ecosystems, bacterial enumeration by way of colony forming units (CFU) represents less than 10% and often less than 1% of the number of cells which can be enumerated by direct microscopic counts. This is probably caused by phenomena such as dormancy (Stevenson, 1978), starvation (Amy and Morita, 1983) and the presence of nutritional types such as oligotrophs (Poindexter, 1981) and copiotrophs (Kjelleberg, 1984). Therefore, the linking of physiological activities (e.g. biological phosphate uptake and release) to specific groups of bacteria (e.g. *Acinetobacter* spp.) isolated on growth media from an aquatic ecosystem (activated sludge) could be misleading because this group might represent but a small proportion of the total bacterial flora. A number of authors, including Lötter and Murphy (1985) and Murphy and Lötter (1986), seem to have made such an assumption without critical evaluation.

Cloete (1984) examined the ratio of *Acinetobacter* cells enumerated microscopically directly by a fluorescent antibody technique and the total bacterial cell numbers (also determined by microscopy) of activated sludge systems. He showed that *Acinetobacter* formed less than 5% of the total cell numbers. The statement of Lötter and Murphy (1985) that *Acinetobacter* is numerically dominant in the anaerobic, anoxic and aerobic zones of activated sludge systems is thus still open to doubt until more detailed investigations are done. One possible way of addressing this question would be to compare the *Acinetobacter* numbers (determined by a fluorescent antibody technique) with the estimates of living cells (determined by a method such as the naladixic acid technique of Kogure *et al.*, 1984). Only if *Acinetobacter* forms a significant portion of all living cells, can its importance in physiological phenomena be adequately quantified.

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Reply by **Laurraine H. Lötter**, *City Health Department Laboratory, P.O. Box 1477, Johannesburg 2000*:

While the points raised in the letter concerning the problems of bacterial enumeration by viable plate count, are not disputed, it should be pointed out that in Lötter and Murphy (1985), the widely used isolation technique of serial dilution plating was used to *compare* populations under different conditions; actual enumeration was not attempted.

The results confirmed what had already been observed microscopically, namely that cells of a specific morphology containing polyphosphate granules, and appearing in large clusters dominated the activated sludge bacterial flora (Hart and Melmed, 1982; Buchan, 1983). Microscopic evaluation of sludge has revealed that large clusters of metachromatically stained cells are present during periods of good phosphorus removal.

The absence of these metachromatically stained clusters correlates well with poor phosphorus removal. Fuhs and Chen (1975) identified these 'clustering' bacteria as *Acinetobacter* spp. and considered them to play a major role in sewage purification.

Buchan (1983) further concluded that enhanced biological phosphorus removal by activated sludge is dependent on the enrichment of certain *Acinetobacter* spp. in the system.

Subsequently, using density gradient centrifugation and enumeration by a fluorescent antibody technique Cloete (1984) confirmed that over 90% of the cells containing polyphosphate inclusions in a mixed liquor sample from an activated sludge system were *Acinetobacter* spp.

Physiological characteristics of *Acinetobacter* spp. have been studied by a number of researchers (Deinema *et al.*, 1980, Lawson and Tonhazy, 1980 and Suresh *et al.*, 1985) in an attempt to assess their relevance in the activated sludge process. Application of these findings to understanding full-scale activated sludge operation has proved successful (Nicholls *et al.*, 1986).

Until another bacterial species is identified as playing a more dominant role in enhanced biological phosphorus removal, it seems useful to at least use these bacteria to study various metabolic features under various activated sludge process conditions, as was attempted in Murphy and Lötter (1986).

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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.

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