The Influence of an Anaerobic Zone in Activated Sludge Systems on the Bacterial Population Structure

D F TOERIEN*, H J J VAN VUUREN**, D N SADIE* AND R P TRACEY**
[INSTITUTE FOR ENVIRONMENTAL SCIENCES* AND DEPARTMENT OF MICROBIOLOGY**,
UNIVERSITY OF THE ORANGE FREE STATE, BLOEMFONTEIN 9301,
REPUBLIC OF SOUTH AFRICA]

Abstract

Bacterial cultures isolated from activated sludge systems with or without anaerobic zones by Davelaar, Davies and Wiechers (1978) were grouped by an analysis of some phenotypic properties. On a broad basis (50% similarity) little evidence was obtained that the presence or absence of anaerobic zones resulted in selective changes in the population structure. However, clusters at higher (about 80%) similarities did show signs of selective changes but when compared to the phosphate-accumulating properties (Davelaar *et al.*, 1978) of the isolates, these selective changes appeared to be unimportant in terms of biological phosphate removal. The need for further basic microbiological studies, also on bacterial population structures, is discussed.

Introduction

The enhanced removal of phosphate during the treatment of waste waters in activated sludge systems has often been noted (Milbury, McCauley and Hawthorne, 1971; Vacker, Connell and Wells, 1967; Barnard, 1976; Fuhs and Chen, 1975; Davelaar, Davies and Wiechers, 1978). This process could be important in the control of the eutrophication of surface waters (Fuhs and Chen, 1975; Davelaar *et al.*, 1978).

Davelaar *et al.* (1978) investigated the significance of an anaerobic zone for the biological removal of phosphate in laboratory-scale denitrifying activated sludge units. They isolated 90 bacterial cultures from these units and determined their phosphate-accumulating properties in an aerated liquid medium according to Fuhs and Chen (1975). Bacteria with phosphate-accumulating properties occurred in the sludges of units with or without anaerobic zones and the authors concluded that biological phosphate removal in activated sludge treatment is not dependent on a major change (selection) in the composition of the microflora.

The question still remains whether phosphate accumulation is a property of a specific group(s) of activated sludge bacteria as suggested by Fuhs and Chen (1975). In order to answer this question we grouped the bacterial isolates of Davelaar *et al.* (1978) by a numerical analysis of some phenotypic properties and compared the results with the phosphate-accumulating properties reported by Davelaar *et al.* (1978) and the type of activated sludge systems from which the isolates were derived.

Materials and Methods

Bacterial Isolates

We obtained the cultures isolated by Davelaar *et al.* (1978) from the National Institute for Water Research. Some of these cultures were not viable and eventually 72 cultures were used in the analysis. All cultures were streaked onto nutrient agar to verify their purity and pure stock cultures were kept on nutrient agar slants at 4°C. If a culture was found to be impure, stock cultures were obtained of all constituent bacteria.

The bacterial cultures were obtained by Davelaar *et al.* (1978) from two 5ℓ laboratory activated sludge units operated simultaneously at 20°C. The units were inoculated with the sludge from a pilot activated sludge plant and were fed with pasteurized settled domestic sewage. The only difference between the units was the division of the anoxic zone in the first unit into an anoxic and anaerobic section in the second unit (Davelaar *et al.*, 1978). Later on in their experiments the sludges of the two units were interchanged in order to vary the treatment received by a specific sludge. The designations and

sources of the bacterial cultures used in this study are summarized in Table 1.

Phenotypic Properties of Isolates

The following physiological and biochemical reactions were recorded using the API 20E system (Analytab Products Inc., Plainview, N.Y.): O-nitrophenyl-β-D-galactosidase; arginine dihidrolase; lysine and ornithine decarboxylase; citrate utilization; H_2S production; urease; tryptophan deaminase; indole production; acetoin production; gelatinase; oxidase; reduction of nitrate to nitrite; and the fermentation of glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose (Smith *et al.*, 1972).

Bacterial cultures were streaked onto nutrient agar plates and incubated at 25°C for 24 h. Inoculation of the micro tubes was carried out according to the manufacturers' instructions. Results were recorded after 24 h and 48 h of incubation respectively.

Numerical Analysis of Phenotypic Properties

Results obtained with the API 20E system were recorded as positive or negative. The resemblance of each organism with every other organism was calculated using the similarity coefficient of Sokal and Michener (Sneath and Sokal, 1973). Isolates were clustered by the unweighted pair group method using an electronic computer. Redundant characters were not used for calculating coefficients of association.

Results

The isolates clustered into two main groups (I and II) at the 50% similarity level (Figure 1). Group I contained 41 isolates (57 percent of all isolates) and Group II 31 isolates (43 percent of all isolates). Examination of the origin of the isolates contained in each group indicated that 55 percent of all isolates originating from sludges with an anaerobic zone and 60 percent of all isolates originating from sludges without an anaerobic zone occurred in Group I. Similarly 45 percent of all isolates from sludges with an anaerobic zone and 40% of all isolates from sludges without an anaerobic zone occurred in Group II. There is therefore little indication that based on a broad division of the isolates, the presence or absence of an anaerobic zone led to a selection of specific groups of bacteria.

Further examination of group I showed the presence of four clusters recognizable at the 82,5% similarity level (clusters I-1, I-2, I-3 and I-4, Figure 1) as well as a diffuse group (I 5) The origin of the isolates in each cluster is evaluated in Table 2.

Bacteria isolated from activated sludge units with an anaerobic zone were present in higher proportions than for the total group (i.e. 63%) in clusters I-3, I-4 and I-5 and lower in clusters I-1 and I-2. The opposite was true for the bacteria isolated from units without an anaerobic zone. Similarly bacteria isolated from sludges with an anaerobic zone were present in a higher proportion in cluster II-1 and in a lower proportion in cluster II-2 (Table 3). The opposite was also true for bacteria isolated from sludges without an anaerobic zone.

Although little evidence was found on a broad division that selection of specific groups is induced by the presence of an anaerobic zone in activated sludge units (see earlier), the results suggest that when narrower divisions are used, signs of selective influences are detectable.

TABLE 1
THE DESIGNATIONS AND SOURCES OF BACTERIAL CULTURES USED IN THIS STUDY

Culture Numbers	Number of isolates	Type of sludge	Isolation medium
AI 1 to AI 20	16	Sludge 1 with anaerobic zone	Nutrient agar
AII 1 to AII 19 1	10	Sludge 2 without anaerobic zone	Nutrient agar
AAC 1 to AAC 10	14	Sludge 1 with anaerobic zone	Fuhs and Chen medium*
BI 1 to BI 20	17	Sludge 2 with anaerobic zone	Nutrient agar
BII 1 to BII 20 2	15	Sludge 1 without anaerobic zone	Nutrient agar

*Fuhs and Chen (1975) medium for isolation of phosphate-accumulating organisms

TABLE 2
EVALUATION OF THE ORIGIN OF ISOLATES IN THE CLUSTERS OF GROUP I

Type of sludge	Percentage of isolates from a particular sludge in clusters					
	I-1	I-2	I-3	I-4	I-5	Total Group I
With anaerobic zone	54	44	75	83	78	63
Without anaerobic zone	46	56	25	17	22	37

TABLE 3
EVALUATION OF THE ORIGIN OF ISOLATES IN THE CLUSTERS OF GROUP II

Type of sludge	Percentage of isolates from a particular sludge in clusters		
	II-1	II-2	Total Group II
With anaerobic zone	75	60	68
Without anaerobic zone	25	40	32

To determine if such selective influences could be important as far as biological phosphate removal activity is concerned, we evaluated the occurrence of phosphate-accumulating solates (as determined by Davelaar *et al.* 1978) in the various clusters, as well as the two broad groups (I and II). The results are summarized in Table 4.

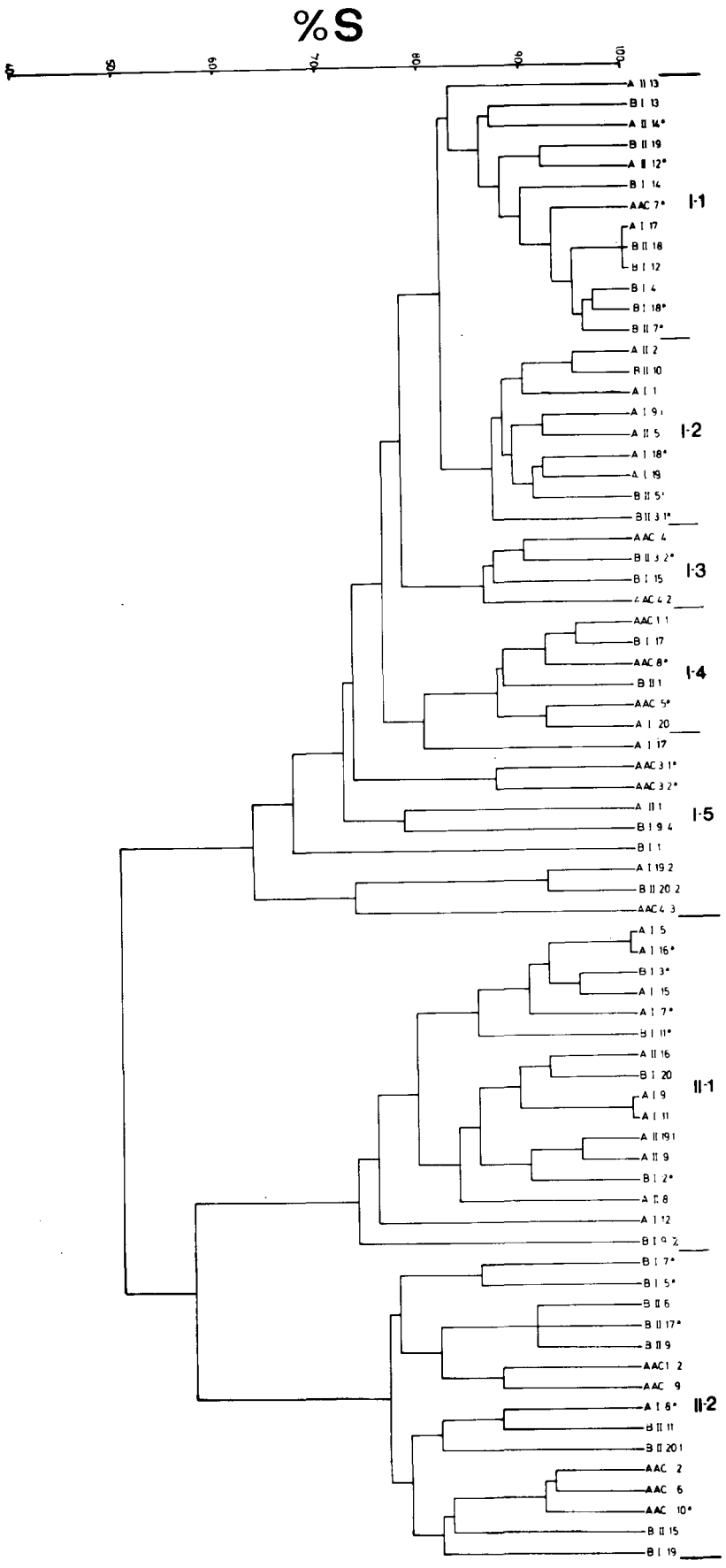

Figure 1
Dendrogram of the phenotypic similarities of the isolates.
Phosphate accumulation by an isolate denoted by a

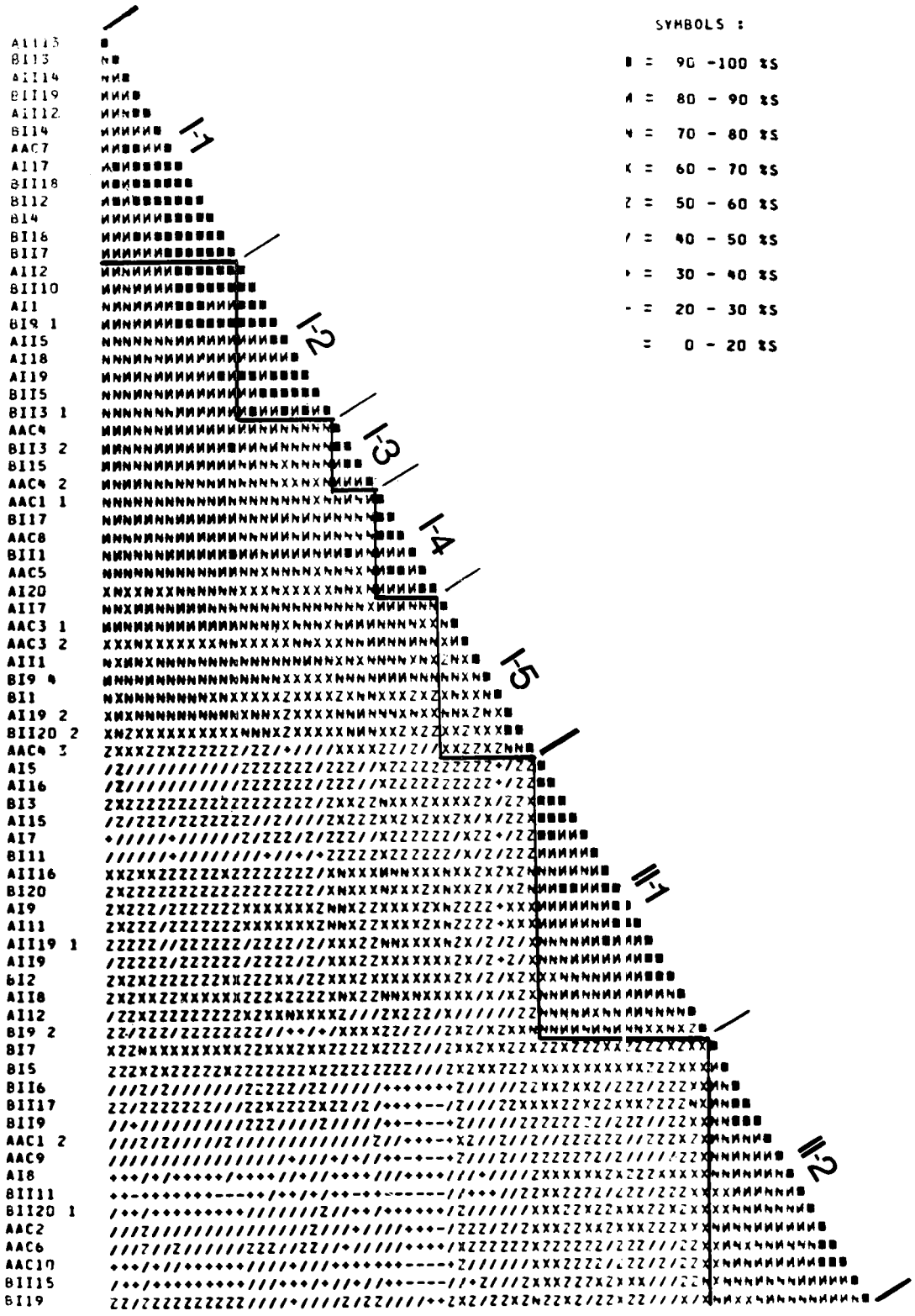

Figure 2
Shaded similarity matrix of the isolates

The percentage of phosphate-accumulating isolates in the two main groups, as well as the various clusters, was remarkably constant at about one-third of the isolates. This tends to suggest that even though signs of selective influences were detectable when narrow divisions (clusters) of the population structure were used, these influences did not affect the ratios at which phosphate-accumulating organisms occurred. As a consequence it is doubtful if such selective processes would significantly affect the potential phosphate removal capacity.

To be certain that similarities between phosphate-ac-

TABLE 4
OCCURRENCE OF PHOSPHATE-ACCUMULATING ISOLATES IN GROUPS I AND II AND IN THE VARIOUS CLUSTERS

Groups and Clusters	Phosphate-accumulating isolates as a percentage of all isolates
Group I	32
Cluster I-1	38
Cluster I-2	33
Cluster I-3	25
Cluster I-4	33
Cluster I-5	22
Group II	32
Cluster II-1	31
Cluster II-2	33
All isolates	32

cumulating isolates were not disguised by the two-dimensional nature of the dendrogram (Figure 1) the shaded similarity matrix (Figure 2) of the isolates was examined. The similarity matrix did not show similarities differing from that of the dendrogram.

Discussion

Fuhs and Chen (1975) ascribed phosphate removal in their activated sludge systems to the presence of a single (or closely related) group of micro-organisms which can store large amounts of polyphosphates intracellularly. These organisms were identified as belonging to the *Acinetobacter-Moraxella-Mima* group of bacteria. Davelaar *et al.* (1978) examined their isolates for phosphate-accumulation according to the methods employed by Fuhs and Chen (1975), and found phosphate-accumulating properties in a large number of their isolates. Our grouping of these isolates by a numerical analysis of some phenotypic properties (Figure 1, Table 4) indicated that the phosphate-accumulation was not the property of a single group only of isolates, but were distributed amongst a number of clusters. The conclusion of Fuhs and Chen (1975) or the method employed by Davelaar *et al.* (1978) to detect phosphate-accumulation may therefore be suspect. In this regard it is quite interesting that several isolates which were phenotypically similar (Figure 1) differed in their phosphate-accumulating properties. This is especially true for isolates AI 5 and AI 16 (cluster II-1, Figure 1) which were phenotypically identical but were found to have differing phosphate-accumulating properties by Davelaar *et al.* (1978). Methods to detect phosphate-accumulation in micro-organisms, and its relationship to enhanced biological phosphate removal, seem to need more extended studies.

Fuhs and Chen (1975) mentioned that an aerobic laboratory activated sludge unit which did not contain *Acinetobacter*-type bacteria could not be induced to remove phosphate biologically. Davelaar *et al.* (1978) inoculated their laboratory-scale units from a fully aerobic pilot plant unit and found enhanced biological phosphate accumulation when their laboratory-scale systems were operated with an anaerobic zone included. They concluded that the mode of operation, rather than the character of the sludge, was of critical importance in determining the extent of phosphate removal. Our results suggest that, opposite to the suggestions of Fuhs and Chen (1975), the inclusion of an anaerobic zone did not lead to significant selective or enrichment processes in the bacterial populations of Davelaar *et al.* (1978). However, the experiments of Davelaar *et al.* (1978) extended over a relatively short period of time (about three months), whilst Fuhs and Chen (1975) referred to a time lapse of five years since primary inoculation of their unit.

Davelaar *et al.* (1978) operated their systems for a period of 49 days at a sludge age of 15 days before interchanging the sludges. In the case of the unit operated without an anaerobic zone, this sludge showed enhanced phosphate removal immediately upon being subjected to an anaerobic phase. Since our results (Figure 1, Table 4) showed no indication of large-scale changes in the bacterial population structure, the postulate of Fuhs and Chen (1975) that phosphate-accumulating bacteria must be enriched in activated sludge before enhanced phosphate removal will be evident, could be in error.

The discrepancies between the findings of Fuhs and Chen (1975) and Davelaar *et al.* (1978) indicate that more basic research on the bacterial populations of activated sludge is needed. Our results suggest that analyses of the bacterial population structures could be helpful in a better understanding of the process of biological phosphate removal.

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