The influence of altered anticorrosion treatment on the microflora of activated sludge in petrochemical plant effluent

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Abstract

Representative samples from the fully aerated activated sludge basins of the water reclamation system of a zero-effluent coal-gasification petrochemical plant were monitored before and after substitution of zinc acrylate for zinc chromate as anticorrosion agent. Fluctuations in the magnitude and metabolic activity of the microbiological population of the activated sludge during this period were quantified. The use of selective and enrichment media showed that fungi, yeasts and green algae were present in insignificant numbers. Generalised counting media and a niche-simulating medium showed that the population of the activated sludge largely comprised heterotrophic bacteria representing a narrow range of genera, two filamentous microorganisms and one genus representing the cyanobacteria. The genus diversity of heterotrophic bacteria increased after substitution of zinc acrylate for zinc chromate and glucose dehydrogenase activity of the sludge increased. Six weeks later genus diversity and dehydrogenase activity had returned to their initial status and it was suggested that the relatively simple population of microorganisms was susceptible to influence by changed anticorrosion agents but was also resilient; it acclimatised and returned to its initial status.

Introduction

Many largely qualitative studies have shown that the microbiological populations of activated sludge treatment (AST) plants are generally diverse. The microorganisms in a given sludge basin constitute a community adapted to the particular waste water treated (Verstraete and van Vaerenbergh, 1986). Such communities do not change provided the chemical composition of the waste water remains relatively constant (Cyrus and Sladka, 1970; Lewandowski, 1987). However, imbalances in environmental or chemical parameters of an activated sludge treatment plant may lead to bulking or excessive foaming in basins (Van Veen, 1973; Blackbeard and Marais, 1986; Jenkins *et al.*, 1986). Full understanding of the ecological, physiological and biochemical activities of the important microorganisms involved in the treatment system is therefore necessary for optimal control of the process (Adamse *et al.*, 1984).

The principle of the activated sludge process is that a community of microorganisms is constantly supplied with organic matter and oxygen. However, the waste-water system is usually dosed with biocides to prevent biofouling, and with anticorrosion agents which may also be biocidal or biostatic. Since the success of the activated sludge treatment depends on the activities of living microorganisms, operational parameters must be carefully controlled to avoid adverse effects of additives on the AST population.

Although municipal AST systems have been widely studied, little quantitative data describing microbiological populations of activated sludge have been published. In particular, the microbiology of effluent treatment at petrochemical plants has received only scant attention. The work reported here describes successional changes which occurred in the AST population of a petrochemical plant when anticorrosion treatment by zinc chromate was terminated and substituted by treatment with zinc acrylate.

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Materials and methods

Sampling

Five AST basins operating in parallel and each treating coal-gasification effluent were examined. Briefly, this effluent contained phenols, volatile fatty acids, hydantoins, several bases (Kasan and Baecker, 1989a), and various heavy metal ions (Kasan and Baecker, 1989b). It was of neutral pH and the system's potential to reduce COD has previously been described (Kasan, 1989). The mode of operation was that of a fully mixed system.

Shortly before the present study commenced a comprehensive pilot study included sampling 11 equidistant points from each of the 5 basins on each of 5 weekdays. Examination of 5 replicates of each of the 275 samples showed that the microbiological population throughout the AST system was uniform and constant, probably as a consequence of the fully mixed mode of operation of the system. The pilot study therefore confirmed that samples obtained from basin pumps were representative of those taken from the centres of basins. During the work reported here samples (2ℓ) of activated sludge mixed liquor were taken from each of two pumps on each basin bi-weekly over 9 weeks. Samples taken during the first 2 weeks of study confirmed that the composition of the AST population had not changed since the pilot study. Anticorrosion agents were substituted at week 2,5 and samples obtained from then until the ninth week of the study were used to monitor successive changes produced in the AST population.

All samples of mixed liquor suspended solids (MLSS) were transported to the laboratory and analysed immediately.

Quantitative analyses

Enumeration

The fresh MLSS samples were homogenised using a Sorvall Omni-Mixer (Du Pont Instruments, Conn., USA) for 4 min at 16 000 r min^{-1} to disperse flocs. Triplicate samples of unstained suspensions were placed in counting chambers (Fuchs-Rosenthal,

West Germany) and equilibrated in a humidity chamber at 100% RH for 20 min. Total cell counts were made under reduced illumination using a Nikon Alphaphot YS (Nikon, Japan) microscope. Filamentous bacteria were counted using the simplified filament counting technique (Jenkins *et al.*, 1986).

Triplicate samples of homogenised MLSS were serially diluted in physiological saline and replicate 0,1 mℓ aliquots of each dilution were spread-plated on nutrient agar (Difco) and activated sludge agar to determine total viable counts. The latter medium comprised 0,1 g peptone (Biolab), 0,1 g yeast extract (Biolab), 0,25 g glucose (Biolab), 50 mℓ supernatant from centrifuged MLSS (6 000 g for 20 min), 1,5 g Agar (Biolab), 7,5 mℓ of each of salts solutions A and B and distilled water to 100 mℓ. Salts solution A comprised 6 g NaCl, 3 g KH_2PO_4, 6 g $(NH_4)_2SO_4$, 1,2 g $MgSO_4$, 0,6 g $CaCl_2$ and distilled water to 500 mℓ. Salts solution B was a 0,6% (w/v) aqueous solution of K_2HPO_4. The final pH of each medium was 7,0. All plates were incubated at 30°C for 6 d. Distribution of bacterial and occasional yeast colonies was recorded, and isolates were purified by repeated subculture on the same media. They were stored at 4°C. Genera were identified using the criteria of Skerman (1967), Starr *et al.* (1981) and Brenner (1984), and the percentage frequency of isolation of each genus was calculated. Filamentous fungi and yeasts were isolated, but not enumerated. Potato dextrose agar (pH 4,5) plates (Difco) were inoculated similarly to select for filamentous fungi and yeasts.

Chlorophyll extraction

Algae could only be isolated in enrichment culture (Nichols, 1973) and quantification of phototrophic autotrophs was conducted by chlorophyll extraction (Sartory, 1982).

Dehydrogenase assay

The metabolic activity of activated sludge provides an indication of the biological capacity of the waste-waster treatment plant and is one of the most reliable methods for characterising the sludge (Olah and Princz, 1986). Quantification of sludge biomass by metabolic activity was therefore conducted by measuring glucose dehydrogenase activity. All such measurements were conducted using duplicate aliquots from triplicate homogenised MLSS samples, by the method of Katayama (1984).

Results and discussion

Activated sludge population

The use of nutrient agar as a generalised counting medium and activated sludge agar as a niche-simulating medium demonstrated the presence of several groups of microorganisms in the activated sludge treating the coal-gasification effluent (Table 1). Total viable counts of bacteria remained relatively constant throughout the study and no significant differences ($P < 0,05$) between populations of the five activated sludge basins, or between total viable counts of bacteria obtained from either medium, were recorded. Yeasts and filamentous fungi were seldom isolated on any of the media, and yeasts were only enumerated on the counting medium twice, in each case comprising less than 1% of the total viable count. Chlorophyll extractions suggested stable, but small populations of green algae present and these were not considered to represent predominant groups of the AST population. Standard statistical analyses (SAS/Graph Statistical Analyses Systems, SAS Inc., Cary, N.C., 1985) and microscopic examination (Fig. 1) showed that only the prokaryotes and filamentous microorganisms were prominent in the sludge. These comprised a comparatively narrow range of two filamentous (Eikelboom and Van Buijsen, 1981) microorganisms (a third filamentous microorganisms has also been recorded in other studies of this system), a cyanobacterium and 41 colony types of heterotrophic bacteria identified as *Acinetobacter* sp., *Alcaligenes* spp., *Bacillus* spp.,

TABLE 1
MICROORGANISMS ISOLATED QUALITATIVELY AND QUANTITATIVELY FROM ACTIVATED SLUDGE TREATING COAL-GASIFICATION EFFLUENT

Microorganism		Isolation
Filamentous fungi and yeasts	(Unidentified)	Potato dextrose agar
		Enumeration
Green algae	ORDER CHLOROPHYCEAE	Chlorophyll assay of sludge
Cyanobacteria	ORDER CHROOCOCCALES	Chlorophyll assay of sludge
Filamentous microorganisms	*Nostocoida limicola* Type 2 Type 0041	Filament counts of wet mounts
Heterotrophic bacteria and yeast	*Acinetobacter* sp. *Alcaligenes* spp. *Bacillus* spp. Micrococcaceae *Moraxella* spp. *Psuedomonas* spp. *Vibrio* spp. *Saccharomyces cerevisiae*	a) Total viable counts by dilution plate counting on Nutrient agar and activated sludge agar; b) Direct total cell counts by counting chamber

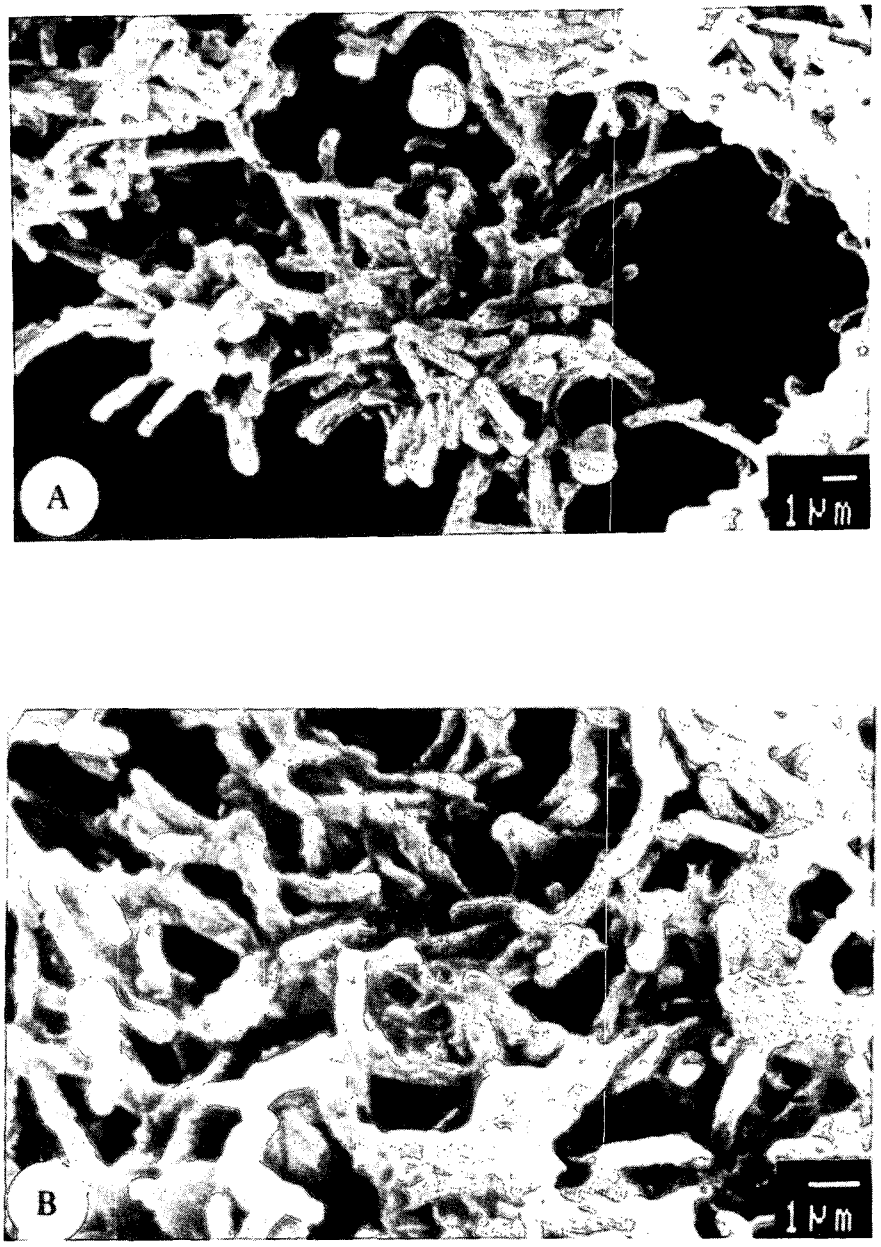

Figure 1
Representative floc (A) of activated sludge comprised largely of bacteria (B).

Micrococcaceae, *Moraxella* spp., *Pseudomonas* spp., and *Vibrio* spp. This comparatively narrow range of prominent genera of microorganisms represented those isolated from other effluent streams of petrochemical plants by Goud *et al.* (1985), whereas the ranges of prominent microorganisms in activated sludges treating domestic waste are broad (Allen, 1944; Cyrus and Sladka, 1970; Van Veen, 1973; Adamse *et al.*, 1984; Jenkins *et al.*, 1986). Kasan and Baecker (1989a) have postulated that microorganisms are not diverse in petrochemical plants because high levels of toxic metals and hydrocarbons in such effluents exert selective pressure which prevents development of many organisms which normally flourish in domestic wastes.

These results emphasise the distribution of the statistically predominant microorganisms and therefore contrast with similar studies where all organisms were evaluated qualitatively (Allen, 1944; Cyrus and Sladka, 1970; Van Veen, 1973). This is important in the present study since the predominant microorganisms probably play a major role in the treatment process.

Succession patterns

Overall no significant changes in direct total counts of eukaryotes, cyanobacteria or filamentous microorganisms in the activated sludge were recorded during the 6-week period following substitution of zinc chromate for zinc acrylate in the water reticulation system. Analysis of variance confirmed that no significant differences ($P < 0,05$) occurred between total viable counts on nutrient agar and activated sludge agar. However, while the direct total counts of heterotrophic bacteria (10^{10} cells $m\ell^{-1}$) did not fluctuate significantly ($P < 0,05$) the total viable counts of this group decreased to approximately 10^8 c.f.u. $m\ell^{-1}$ after 4,5 weeks before returning to the original levels of approximately 10^9 c.f.u.

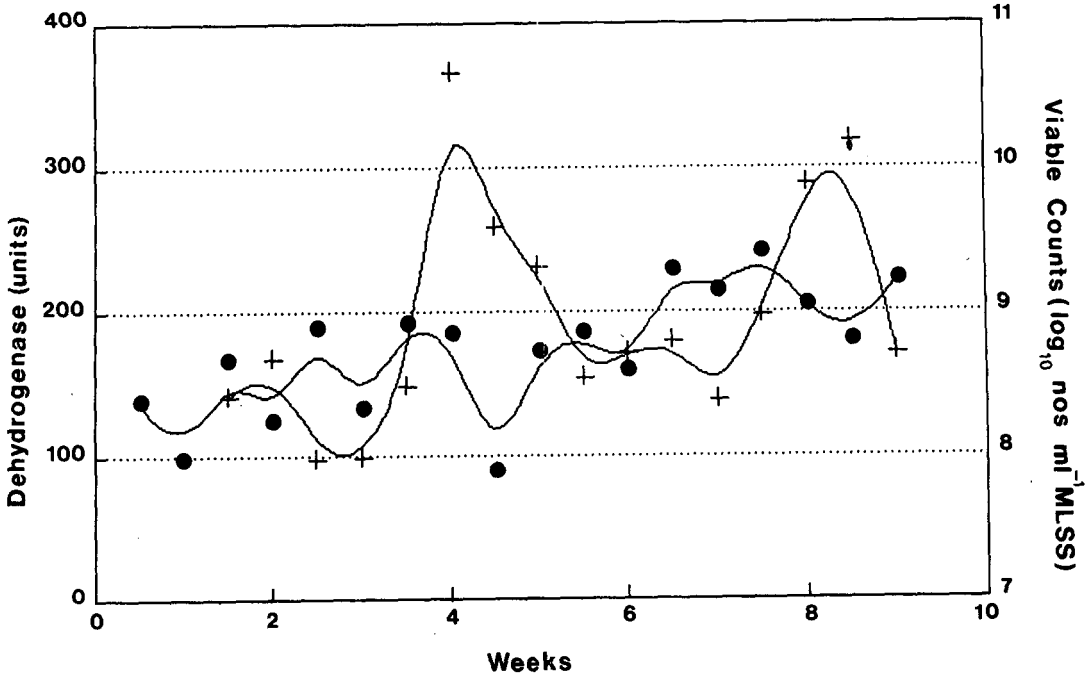

Figure 2
Mean total viable counts (●) of bacteria and dehydrogenase activity (+) of activated sludge during the 9-week study period. Week 2,5 coincides with initiated depletion of zinc chromate and addition of zinc acrylate, and week 7 coincides with shock dosage of biocide. Each point represents the mean of 60 determinations of 10 samples from five parallel activated sludge basins.

$m\ell^{-1}$. However these fluctuations showed that viable counts did not vary substantially during the period monitored. Opposite fluctuations in dehydrogenase assays of sludge followed this trend (Fig. 2), suggesting that numbers of organisms decreased with metabolic shock, and that metabolism increased during adaptations of the mixed bacterial population to the changed conditions of the effluent. Similar fluctuations in total viable counts and dehydrogenase assays coincided with shock biocide dosing at week 7 (Fig. 2).

In conjunction with these fluctuations, the diversity of predominant genera within the group of heterotrophic bacteria increased after shock. The diversity of genera then decreased during the following weeks. The 3-dimensional histograms (Figs. 3A-E) illustrate these trends in succession patterns and the values presented in the associated pi-charts quantify the proportional magnitudes ($P < 0,05$) of the bacterial populations. The percentages, given in Fig. 3 to quantify the proportional magnitudes of bacterial populations at weekly intervals should be compared to the data of Fig. 2 which provides the population magnitude as total viable counts at each interval.

Petrochemical effluent contains compounds which may inhibit microbial growth and the biological process. The system studied contained heavy metal anticorrosion agents and some heavy metals themselves are antimicrobial. However, bacteria present in petrochemical effluents may tolerate high concentrations of metals and may even accumulate these (Goud *et al.*, 1985; Kasan and Stegmann, 1987a, b; Kasan *et al.*, 1987; Kasan and Baecker, 1989b, c). Chromium-dependent organisms have been isolated from the system studied (Kasan, unpublished data) and these probably developed owing to sustained dosing of zinc chromate. When such dosing was terminated any organisms dependant on chromium compounds may have diminished in numbers and other heterotrophs could have competed for the available niche. Fluctuations such as those seen in Figs. 3A to E could therefore reflect transitory competition between heterotrophic groups during the period in which zinc acrylate was added to the system. After sustained dosage of zinc acrylate it seemed that the microbial population had stabilised.

These results add to the growing body of knowledge regarding petrochemical activated sludge treatment. The bacterial population was comparatively simple whether determined on the generalised counting medium (NA) or on the niche-simulating medium (ASA). It appeared to be a resistant, dynamic population sensitive to the addition of potentially toxic compounds, but with the capacity to recover rapidly. Rapid recovery may be attributable to the metabolic resourcefulness of a population evolved under the selective pressure exerted by continuous presence of potentially toxic compounds in this unique effluent. Alternatively, such recovery may be attributable to the possible utilisation of acrylate by the microorganisms. These questions should be addressed in future research of this nature.

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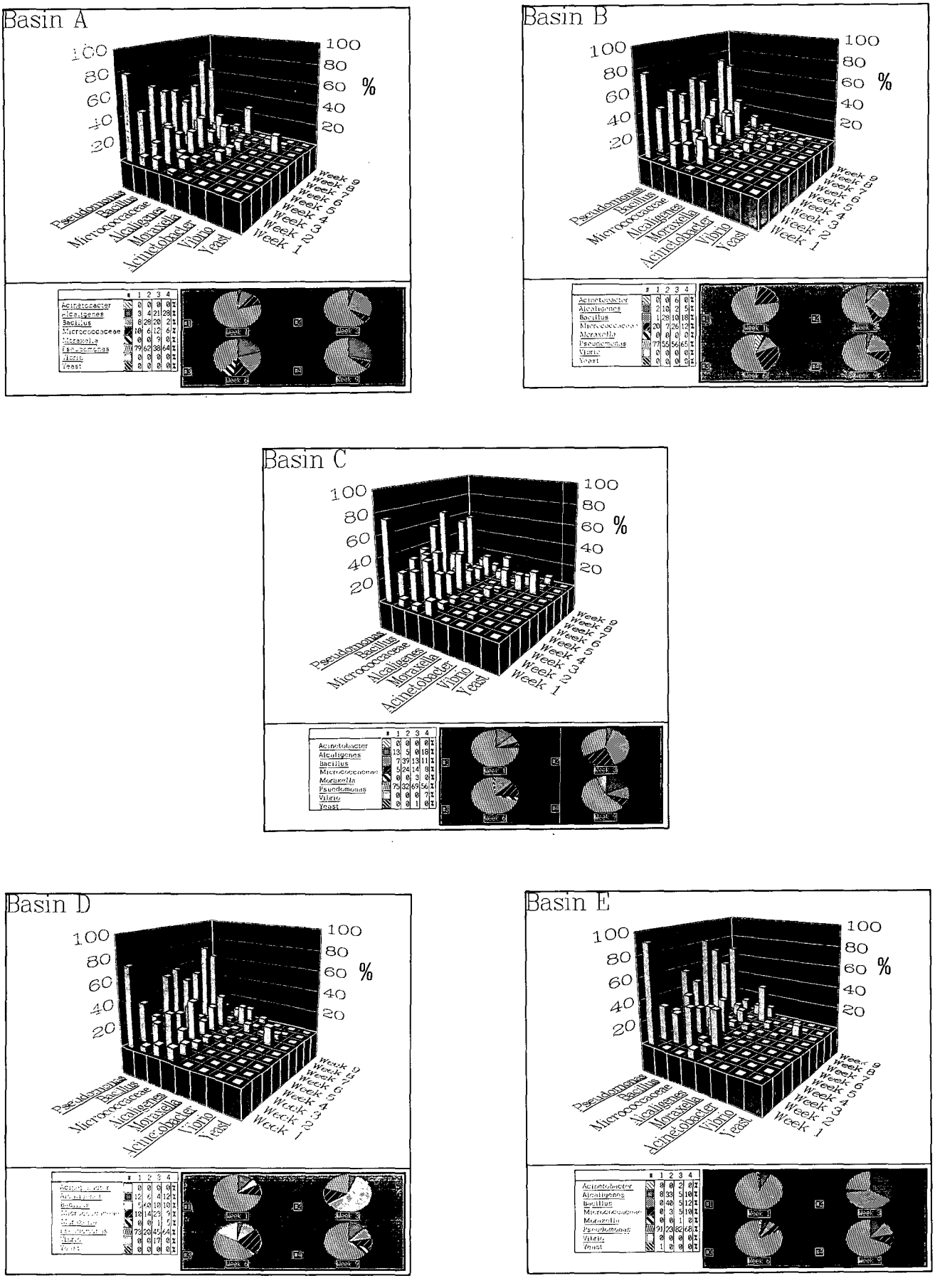

Figure 3A-E

Percentage frequencies of isolation of genera of bacteria from five parallel activated sludge basins during the 9-week study period. Mean total viable counts each week are given in Fig. 2.

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