Bacteria in the aerobic biodegradation of wool scouring effluent

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Abstract

The bacterial flora in the aerobic biodegradation of wool scouring effluent was studied. Phase I of the biodegradation of the effluent, which was carried out in the absence of algae and sunlight, extended over a period of 16 weeks, and Phase II in the presence of algae and sunlight extended over a period of 9 weeks. Partial clarification occurred during Phase I as indicated by the reduction in chemical oxygen demand, surfactant breakdown and lighter colour. Flocculation occurred during the period of 8 to 12 weeks and a steady state developed after 14 weeks. Although algae largely contributed to the clarification during Phase II, the bacterial population also showed a change resulting in relatively clear water. Bacteria were isolated at fortnightly intervals during the entire experimental period and their general characteristics were described. The total viable counts increased from $8 \times 10^4.m\ell^{-1}$ to $>2 \times 10^8.m\ell^{-1}$ after 16 weeks. The slight apparent reduction in numbers between 8 and 10 weeks could be explained by floc formation resulting in aggregates being counted as single cells. Chemical oxygen demand was determined during Phase I. After 14 weeks it was reduced by 72% of the initial value of approximately 6 000 $mg.\ell^{-1}$. Surfactant was rapidly degraded from approximately 60 $\mu\ell$. 5 $m\ell^{-1}$ sample after 6 weeks with practically no further detectable breakdown up to 16 weeks. Biodegradation and clarification of the wool scouring effluent was achieved in this laboratory simulated natural ecosystem.

Introduction

Effluent from the wool scouring industry, a dark and slimy slurry with an obnoxious odour, is of complex composition and poses a considerable pollution problem. According to Mozes *et al.* (1981), approximately 52,5 x 10^6 kg of raw wool, Karakul fibre and mohair was scoured in South Africa during the 1979/80 season. The pollution produced was estimated at about 7 x 10^6 kg grease, 4,7 x 10^6 kg suint, 10,5 x 10^6 kg sand and clay, and 1,2 x 10^6 kg of other contaminants which collectively represent the major impurities in the effluent. Particular problems are posed by wool grease, which is not readily biodegradable (Chao and Yang, 1981), and the detergents, which produce relatively stable emulsions (Christoe, 1977; McCracken and Chaikin, 1978; McLachlan *et al.*, 1980a). Effluent pretreatment to achieve sufficient reduction in the major impurities to meet respective local legislation requirements has been achieved by various physicochemical and biological processes (Chao and Yang, 1981; Chao *et al.*, 1980; Christoe *et al.*, 1976; McCracken and Chaikin, 1978; McLachlan *et al.*, 1980a,b; Mozes *et al.*, 1981; Pearson *et al.*, 1976; Randall and King, 1980) and their application has largely been determined by economic factors (Anderson *et al.*, 1975; Chao and Yang, 1981; Chao *et al.*, 1980).

The aerobic biological treatment systems are based on similar, fundamental biochemical principles and the importance of microorganisms in the biological treatment of wool scouring effluent has been implicated by McCracken *et al.* (1975). The oxidation and reduction of pollutants in the wool scouring effluent are represented by the combined effects of essentially bacteria and algae. In addition, these effluent treatment systems will include fungi, protozoa, rotifers and higher animals (McKinney, 1957).

Wool scouring liquors have been investigated in our laboratory for several years. A number of experiments under various conditions were conducted to achieve clarification and reduction in chemical oxygen demand (COD) as well as to obtain an indication of the bacterial flora, cyanobacteria and algae involved in the biodegradation process (Bhiman and Roth, 1984; Gokool *et al.*, 1982; Odhav *et al.*, 1986; Roth *et al.*, 1981). Values in the range of 3 000 to 150 000 $mg.\ell^{-1}$ COD have been reported for raw effluent (Chao and Yang, 1981; Christoe *et al.*, 1976; McCracken and Chaikin, 1978; Pearson *et al.*, 1976). A significant step in the biodegradation appears to be floc formation which is enhanced by exocellular mucopolysaccharides and Tago and Aida (1977) have described a strain of *Pseudomonas* which may contribute to this process. Very little other work has apparently been done on the bacteria involved in the biodegradation process of wool scouring effluent. In this laboratory study the objectives were to investigate the biodegradation of wool scouring effluent and to determine the microbial ecology of such a phenomenon.

Materials and methods

Culture media

1/3 Dextrose agar was prepared as described previously (Roth, 1958). For enriched 1/3 dextrose agar medium, effluent was centrifuged at 3 000 x g for 15 min to remove suspended solids. After filter-sterilisation of the supernatant, effluent was added at a concentration of 1% (v/v).

Minimal medium was prepared according to Phillips *et al.* (1981). For the detection of surfactant-degrading bacteria, 5 $m\ell$ non-ionic surfactant.ℓ^{-1} minimal medium was added. The composition of the surfactant was unknown and was obtained from Isipingo Textiles (Natal).

Source of effluent

Raw effluent was obtained from Isipingo Textiles (Natal). To obtain a representative sample of effluent, 100 ℓ of effluent were taken from 3 different ponds, pooled, mixed and stored in sealed containers at room temperature for not longer than 17 d before use. The effluent was dark, almost black, with an offensive odour, and contained considerable amounts of solid sludge.

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Received 6 September 1988

Biodegradation of effluent

Phase I. To assess the biodegradation of effluent by the indigenous organisms, stored effluent was thoroughly mixed and 4 ℓ raw effluent was diluted with 16 ℓ of sterile distilled water in 25 ℓ aspirator bottles. This dilution was made to obtain a watery effluent containing less sludge than in the relatively thick raw effluent collected from the ponds. The aspirator bottles were kept under constant aeration, using filtered air (sterile cotton wool), and the temperature was maintained at 28° ± 1°C in a waterbath. Phase I extended over a period of 16 weeks.

Phase II. After 4 months a steady state had apparently been reached and some flocculation had occurred. Some clarification was observed and the offensive odour was practically absent, but the effluent still had a very dark colour. Since the effluent ponds contained algae, it was considered that their presence would result in complete clarification. Algae were therefore isolated from these effluent ponds and cultured (Odhav *et al.*, 1986) using Bristol's salt agar (James, 1969) and 10 g (wet mass) of algae were added to each aspirator bottle. Since algae require sunlight for growth, the flasks were placed on a covered verandah exposed to direct sunlight with normal day/night temperature fluctuations until clarification, shown by algal biomass contraction and clear water, was obtained. This phase extended over a period of 9 weeks. The range of daily minimum and maximum temperatures during this phase was 15°C to 21°C and 24°C to 28°C respectively with an average sunlight period of 6,7 $h.d^{-1}$.

To illustrate the stages of biodegradation, a series of test tubes were prepared and used for photography.

Effluent sampling for bacterial population and analytical assays

After thorough mixing of the contents in the aspirator bottles, samples of 200 mℓ were collected at fortnightly intervals. A 1 mℓ sample was retained for viable bacterial counts and the remainder kept frozen in sterile plastic bottles for analysis of COD and surfactant.

Viable bacterial counts

Serial 10-fold dilutions of the 1 mℓ samples were made in sterile physiological saline on the day of sampling. Aliquots of 0,1 mℓ of each of the dilutions in the range from 10^{-2} to 10^{-8} were spread aseptically onto enriched 1/3 dextrose agar plates in duplicate, and incubated for 1 week at 28° ± 1°C. The numbers of bacterial colonies were determined using a colony counter at a magnification of 10x. The results were expressed as a mean of the duplicate counts. Plates were retained at 28° ± 1°C for the isolation of representative bacteria.

Bacterial isolation

To establish the nature of the bacterial flora as effluent biodegradation progressed, visual assessment of the colony morphology of the bacteria observed at different dilutions of samples taken at timed intervals was carried out. As a result of the considerable heterogeneity in the bacteria observed, it became apparent that this was possibly one of the best criteria to use for obtaining an overall picture of the bacterial flora involved in the biodegradation process.

Primary isolations of predominant bacteria, selected by virtue of the numbers of colonies with apparently similar characteristics, were made from the plates inoculated with effluent dilutions giving 200 to 400 colonies per plate. These characteristics were colonial morphology and size, pigmentation and Gram reaction. Such selected representative colonies were picked and subcultured by streaking onto enriched 1/3 dextrose agar plates. Once pure cultures were obtained they were stored on 1/3 dextrose agar slants at 4°C.

For the identification of surfactant degrading organisms, each of the purified bacteria were plated onto minimal medium and minimal medium containing surfactant, as described, and assessed for growth after incubation for 1 week at 28° ± 1°C.

Chemical oxygen demand (COD)

The COD assay was carried out according to method 508 outlined in *Standard Methods for the Examination of Water and Wastewater* (Rand *et al.*, 1979).

Determination of surfactant

The ammonium cobaltothiocyanate method (Longman, 1978) was used with slight modification. Five mℓ of chloroform was added to 5 mℓ of effluent together with 5 mℓ of ammonium cobaltothiocyanate reagent in a separating funnel. After vigorous shaking the chloroform phase, containing the blue complex of surfactant, was collected and diluted to 25 mℓ with chloroform. The optical density of the solution was measured at 320 nm using a Beckman DU-8 spectrophotometer.

The composition of the ammonium cobaltothiocyanate reagent was as follows:

522 g ammonium thiocyanate
28 g cobalt nitrate
made to 1 ℓ with distilled water.

A standard calibration curve for the surfactant was prepared using 10 to 150 μℓ of the non-ionic detergent, used by the textile industry, per 5mℓ sample. Concentration of surfactant in the effluent samples was then determined by extrapolation from the standard curve and the results expressed as a mean of triplicate determinations.

Results

The different stages of the biodegradation process of the wool scouring effluent observed are illustrated in Fig. 1 (a-e). Some clarification was achieved during Phase I as is evident particularly in the change of the intensity of the colour (cf. Fig. 1(a) and 1(b). In the absence of sunlight, flocculation occurred during the period of 8 to 12 weeks. It appeared as if a steady state had been reached and no further clarification was observed at 14 to 16 weeks. The addition of algae at this stage and exposure to sunlight resulted in an efficient clarification during Phase II producing a clear water with contracted algal biomass and containing no solid sludge materials. The clarification during the final four weeks is illustrated in Fig. 1(c-e). Fig. 1(d) shows, in particular, the formation of a membranous peripheral pellicle which becomes detached from the internal wall of the test tube. Fig. 1(e) shows the contraction of the biomass during the final stage of clarification.

Viable counts of bacteria

As shown in Fig. 2, the total aerobic viable bacterial counts increased during the first 6 to 8 weeks of the experiment. This was

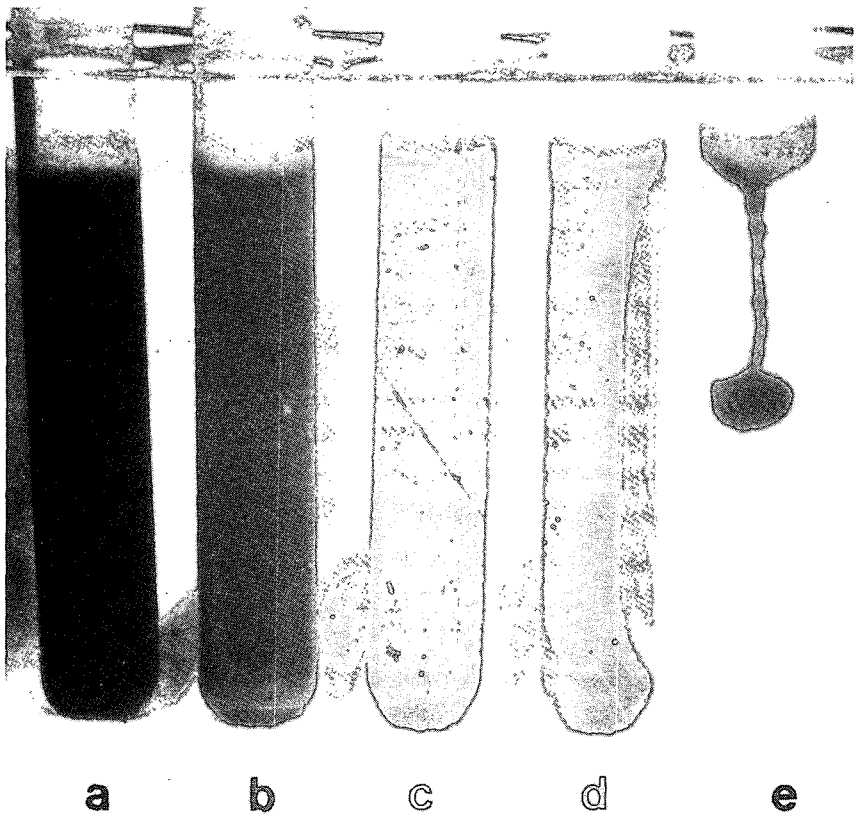

Figure 1
Photograph showing the different stages of biodegradation of the wool scouring effluent. Fig. 1(a) shows the raw effluent at the start of the experiments; 1(b) the flocculation stage after 10 weeks; 1(c) the formation of a pellicle; 1(d) the detachment of the peripheral pellicle from the wall of the test tube after 22-24 weeks; and 1(e) shows the retraction of biomass during the final stage of clarification.

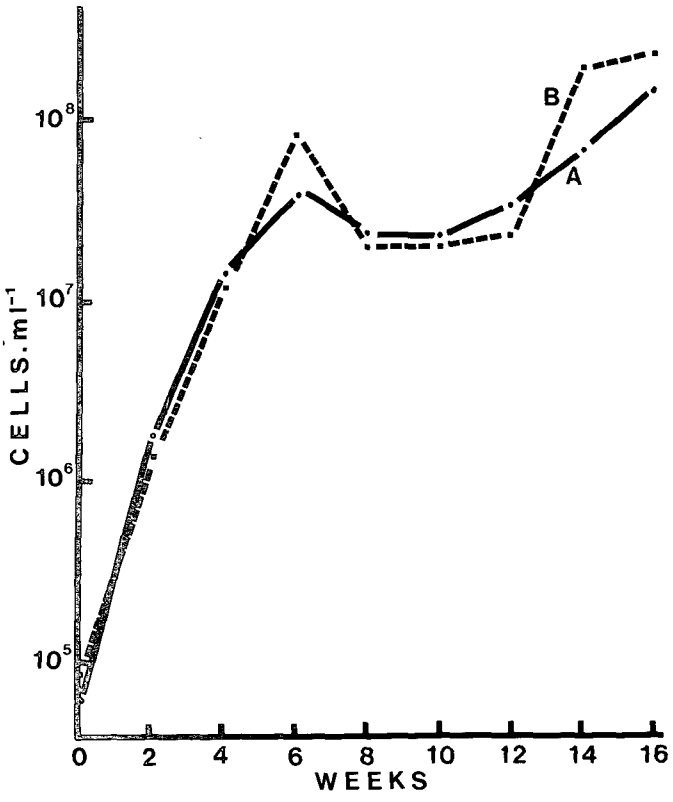

Figure 2
Total viable bacterial counts during Phase I of the experiment for vessels A and B.

followed by an apparent reduction in total numbers during the next 2 weeks. This reduction corresponded to the period of flocculation and aggregates of bacteria could possibly have been determined as single cells. After an increase during the period 12 to 14 weeks, total counts tended to plateau at approximately 2 x 10^8 cells.ml^{-1} after 14 to 16 weeks, indicating that a steady had been reached. Both vessels A and B followed a similar pattern during the entire experimental period.

Bacteria isolated from Phase I

The bacteria isolated from the effluent during the initial stages of biodegradation in the absence of algal action and sunlight are described in Table 1. The small number of isolates representing the predominant bacteria active at the start of the experiment (3 in vessel A and 4 in vessel B) indicated a relative dormancy in the bacterial flora at this stage. These numbers increased to 9 in vessel A and 8 in vessel B after 2 weeks showing a rapid increase in diverse bacterial activity upon aeration. A similar pattern was maintained for the ensuing weeks but the numbers of predominant bacterial isolates tended to be lower during the period of 10 to 14 weeks.

TABLE 1
COLONY MORPHOLOGY AND GENERAL DESCRIPTION OF BACTERIA ISOLATED AT DIFFERENT STAGES OF BIODEGRADATION OF THE WOOL SCOURING EFFLUENT DURING PHASE 1

Isolate	Colony size (mm)*	Pigmentation	Form	Margin	Elevation	Gram reaction and morphology
0A1**	3-4	Cream	Circular	Entire	Convex	+ve short rods
0A2	3-6	White	Circular	Entire	Raised margin	+ve long rods endospores
0A3	5-6	White	Irregular	Lobate	Umbonate	+ve medium rods
0B1	3-4	Cream	Circular	Entire	Convex	+ve short rods
0B2	3	Cream	Circular	Entire	Convex	-ve medium rods
0B3	1-2	Orange	Circular	Entire	Convex	-ve medium rods
0B4	5-8	White	Circular	Entire	Raised	var long rods, endospores
2A1	2-3	Cream	Circular	Entire	Convex	-ve long rods
2A2	1-2	Cream	Circular	Entire	Raised	-ve short rods
2A3	4	Green	Circular	Entire	Convex	-ve medium rods
2A4	2-3	Orange	Circular	Entire	Convex	-ve medium rods
2A5	1-2	White	Circular	Entire	Convex	-ve short rods
2A6	1-2	White	Irregular	Erose	Raised	-ve short rods
2A7	5-7	White	Irregular	Lobate	Raised	+ve medium rods, endospores
2A8	4-9	Yellow	Rhizoid	Curled	Raised	+ve medium rods, chains, spores
2A9	6-7	Yellow	Rhizoid	Lobate	Raised	+ve medium rods
2B1	2-3	Cream	Circular	Entire	Convex	-ve long rods
2B2	3-4	Cream	Circular	Entire	Raised	-ve medium rods
2B3	2-3	Cream	Circular	Entire	Raised	-ve short rods
2B4	4	Green	Circular	Entire	Convex	-ve short rods
2B5	3	Orange	Circular	Entire	Convex	-ve medium rods
2B6	1-2	White	Circular	Entire	Convex	-ve medium rods
2B7	1-2	White	Circular	Entire	Convex	-ve short rods
2B8	6-7	Yellow	Rhizoid	Lobate	Raised	+ve medium rods, chains
4A1	2	Cream	Circular	Entire	Convex	+ve short rods
4A2	4	Cream	Circular	Entire	Flat	-ve medium rods
4A3	6-8	Green	Circular	Entire	Convex	-ve medium rods
4A4	1	White	Circular	Entire	Flat	+ve short rods
4A5	2-3	White	Circular	Entire	Umbonate	+ve short rods
4A6	4-5	White	Filamentous	Filamentous	Flat	+ve medium rods, spores
4A7	< 1	White	Punctiform	Entire	Flat	-ve medium rods
4B1	2	Cream	Circular	Entire	Convex	+ve short rods
4B2	2-3	Cream	Circular	Entire	Flat	-ve medium rods
4B3	2-3	Cream	Irregular	Lobate	Wrinkled	+ve long rods
4B4	Oval	Cream	Rhizoid	Lobate	Convex	+ve medium rods, terminal spores
4B5	4	Orange	Circular	Entire	Convex	-ve medium rods

Isolate	Colony size (mm*)	Pigmentation	Form	Margin	Elevation	Gram reaction and morphology
4B6	2-3	White	Circular	Entire	Umbonate	var short rods
4B7	4-5	White	Filamentous	Filamentous	Raised	+ve medium rods, spores
4B8	< 1	White	Punctiform	Entire	Flat	-ve medium rods
6A1	3-4	Cream	Circular	Erose	Flat	+ve medium rods
6A2	2-3	Orange	Circular	Entire	Convex	-ve medium rods
6A3	1-2	White	Circular	Entire	Flat	-ve medium rods
6A4	1-2	White	Circular	Entire	Umbonate	-ve short rods
6A5	2x5	White	Oval	Entire	Flat	-ve medium rods
6B1	2-3	Cream	Circular	Entire	Convex	-ve medium rods
6B2	2	Cream	Circular	Entire	Convex	-ve medium rods
6B3	3-4	Cream	Circular	Erose	Flat	+ve medium rods
6B4	2-3	Orange	Circular	Entire	Convex	-ve medium rods
6B5	3-4	White	Circular	Entire	Flat	-ve cocci
6B6	1-2	White	Circular	Entire	Flat	-ve medium rods
6B7	2x5	White	Oval	Entire	Flat	-ve medium rods
8A1	2-3	Cream	Circular	Entire	Convex	+ve medium rods
8A2	1-2	Cream	Ovoid	Erose	Flat	+ve medium rods
8A3	2-3	Orange	Circular	Entire	Convex	var short rods
8A4	1	White	Circular	Entire	Convex	-ve long rods, spores
8A5	1-2	White	Circular	Entire	Flat	+ve medium rods
8A6	< 1	White	Punctiform	Entire	Convex	+ve medium rods
8B1	2-3	Cream	Circular	Entire	Convex	+ve medium rods
8B2	1-2	Cream	Circular	Entire	Convex	var coccobacilli
8B3	1-2	Cream	Circular	Entire	Raised	-ve medium rods
8B4	2-3	Orange	Circular	Entire	Convex	var short rods
8B5	2	White	Circular	Entire	Flat	+ve short rods
8B6	2-3	White	Ovoid	Erose	Flat	+ve medium rods
8B7	2	White	Ovoid	Erose	Flat	-ve medium rods
8B8	< 1	White	Punctiform	Entire	Convex	-ve long rods, spores
10A1	2-3	Cream	Circular	Entire	Flat	-ve long rods
10A2	2	Cream	Circular	Entire	Flat	-ve medium rods
10A3	3-4	Cream	Irregular	Undulate	Flat	+ve short rods
10A4	3-4	Green	Circular	Entire	Flat	-ve medium rods
10A5	3	White	Circular	Entire	Convex	+ve rods, chains, mucoid
10A6	1	White	Circular	Entire	Convex	-ve medium rods
10B1	1-2	Cream	Circular	Entire	Convex	+ve coccobacilli
10B2	1	Cream	Circular	Entire	Convex	-ve medium rods
10B3	3-4	Cream	Circular	Entire	Flat	-ve long rods
10B4	3-4	Cream	Irregular	Undulate	Flat	+ve short rods
10B5	1	Orange	Circular	Entire	Convex	+ve curved rods
12A1	1-2	Cream	Circular	Entire	Raised	+ve medium rods
12A2	1-2	Cream	Circular	Entire	Raised	-ve medium rods
12A3	2-3	Cream	Circular	Erose	Flat	-ve short rods
12A4	1	White	Circular	Entire	Convex	+ve medium rods
12B1	1-2	Cream	Circular	Entire	Convex	+ve coccobacilli
12B2	1	Cream	Circular	Entire	Flat	-ve medium rods
12B3	1-2	Cream	Circular	Entire	Raised	-ve medium rods
12B4	2-3	Cream	Circular	Erose	Flat	-ve short rods
12B5	< 1	Cream	Punctiform	Entire	Convex	+ve medium rods
14A1	1	Cream	Circular	Entire	Raised	+ve medium rods
14A2	2-3	Cream	Circular	Entire	Raised	+ve medium rods
14A3	3	Green	Circular	Entire	Convex	-ve medium rods
14A4	4	White	Circular	Entire	Convex	var, pleomorphic, mucoid
14A5	2-3	White	Circular	Entire	Raised	+ve cocci
14B1	1	Cream	Circular	Entire	Raised	+ve medium rods
14B2	1-2	Cream	Circular	Entire	Raised	+ve pleomorphic
14B3	1	Cream	Circular	Entire	Raised	-ve medium rods
14B4	1-2	Creen	Circular	Entire	Convex	-ve medium rods
14B5	1-2	White	Circular	Entire	Raised	+ve cocci
16A1	3	Cream	Irregular	Undulate	Raised	+ve cocci
16A2	2	Orange	Circular	Entire	Convex	+ve short rods

Isolate	Colony size (mm)*	Pigmentation	Form	Margin	Elevation	Gram reaction and morphology
16A3	1	Pink	Circular	Entire	Convex	var medium rods
16A4	2-3	Red	Circular	Entire	Convex	+ve medium rods
15A5	1	White	Circular	Entire	Convex	+ve cocci
16A6	3	White	Circular	Entire	Convex	-ve medium rods
16A7	1-2	Yellow	Circular	Entire	Pulvinate	+ve cocci
16A8	2-3	Yellow	Circular	Entire	Raised	-ve medium rods
16B1	1	Orange	Circular	Entire	Convex	+ve short rods
16B2	1	White	Circular	Entire	Convex	+ve medium rods
16B3	2-3	White	Circular	Entire	Raised	-ve medium rods
16B4	2-3	Yellow	Circular	Entire	Raised	+ve cocci
16B5	2-3	Yellow	Circular	Entire	Raised	-ve medium rods

*Colony size was determined after incubation for 7 d at 28°C

**The first number of the code refers to the weeks of incubation, A or B to the experimental vessel used and the last number enables the unequivocal identification of a particular bacterial isolate

Bacteria isolated from Phase II

Phase II, representing the final stages of the biodegradation, was carried out in the presence of sunlight and algae. The bacteria isolated from these stages are described in Table 2 and yellow, red and pink pigmented bacterial colonies became more abundant. Although not tabulated, the total viable bacterial counts were reduced rapidly from $>10^8.m\ell^{-1}$ at 16 weeks to $<10^4.m\ell^{-1}$ after 26 weeks. This was probably due to the scavenging effect of the algae and the depletion of nutrients.

TABLE 2
COLONY MORPHOLOGY AND GENERAL DESCRIPTION OF BACTERIA ISOLATED DURING PHASE II BIODEGRADATION OF THE WOOL SCOURING EFFLUENT

Isolate	Colony size (mm)*	Pigmentation	Form	Margin	Elevation	Gram reaction and morphology
18A1*	2-3	Cream	Circular	Entire	Convex	-ve medium rods
18A2	1	Orange	Circular	Entire	Convex	+ve cocci
18A3	2-3	Pink	Circular	Entire	Convex	-ve short rods
18A4	1	White	Circular	Entire	Flat	-ve long rods
18A5	2-3	Yellow	Circular	Entire	Convex	-ve long rods, mucoid
18B1	2-3	Cream	Circular	Entire	Raised	-ve rods
18B2	1-2	Orange	Circular	Entire	Convex	+ve cocci
18B3	2-3	Pink	Circular	Entire	Convex	-ve short rods
18B4	<1	Pink	Punctiform	Entire	Convex	+ve medium rods
18B5	1-2	White	Circular	Entire	Flat	-ve long rods
18B6	2-3	Yellow	Circular	Entire	Convex	-ve medium rods
20A1	1-2	Cream	Circular	Entire	Umbonate	+ve medium rods, endospores
20A2	2-3	Cream	Circular	Entire	Convex	+ve long thin rods
20A3	1-2	Cream	Circular	Entire	Convex	-ve medium rods
20A4	1-2	Orange	Circular	Entire	Convex	-ve medium rods
20A5	2-3	Pink	Circular	Entire	Convex	-ve short rods
20A6	1-2	Red	Circular	Entire	Convex	-ve long strands
20B1	2-3	Cream	Circular	Entire	Convex	+ve long rods
20B2	2-3	Cream	Circular	Entire	Convex	-ve long rods
20B3	1-2	Pink	Circular	Entire	Convex	+ve medium rods
20B4	2-3	Pink	Circular	Entire	Convex	-ve short rods

Isolate	Colony size (mm)*	Pigmentation	Form	Margin	Elevation	Gram reaction and morphology
20B5	1-2	Red	Circular	Entire	Convex	-ve long strands
20B6	< 1	White	Circular	Entire	Convex	-ve medium rods
22A1	1-2	Cream	Circular	Entire	Convex	-ve medium rods
22A2	3-4	Cream	Circular	Entire	Convex	-ve short rods, mucoid
22A3	1-2	Orange	Circular	Entire	Convex	-ve rods, chains
22A4	1-2	Pink	Circular	Entire	Convex	+ve cocci
22A5	1-2	Yellow	Circular	Entire	Convex	-ve short rods
22B1	3	Cream	Circular	Entire	Convex	-ve medium rods
22B2	1	Cream	Circular	Entire	Convex	-ve short rods
22B3	3-5	Cream	Circular	Entire	Convex	+ve short rods, mucoid
22B4	1	Pink	Circular	Entire	Convex	+ve cocci
22B5	< 1	White	Punctiform	Entire	Convex	-ve rods
24A1	2-4	Cream	Circular	Entire	Convex	+ve rods, mucoid
24A2	1-2	Orange	Circular	Entire	Convex	+ve rods
24A3	1-2	Pink	Circular	Entire	Convex	-ve pleomorphic
24A4	1	Red	Circular	Entire	Convex	+ve cocci
24A5	1-2	Yellow	Circular	Entire	Raised margin	-ve rods rods
24B1	3-4	Cream	Circular	Entire	Convex	+ve rods, mucoid
24B2	1-2	Cream	Circular	Entire	Flat	-ve pleomorphic
24B3	1-2	Cream	Circular	Entire	Raised margin	-ve rods
24B4	1-2	Pink	Circular	Entire	Convex	-ve pleomorphic
24B5	1-2	Yellow	Circular	Entire	Convex	-ve short rods
26A1	2-3	Cream	Circular	Entire	Convex	-ve long rods, mucoid
26A2	2-3	Cream	Circular	Entire	Flat	-ve short rods
26A3	1	Orange	Circular	Entire	Convex	+ ve short rods
26A4	2-3	Pink	Circular	Entire	Raised	+ve cocci
26A5	2-3	White	Circular	Entire	Raised	-ve pleomorphic
26A6	2-3	Yellow	Circular	Entire	Raised margin	-ve short rods
26A7	2-3	Yellow	Circular	Entire	Convex	-ve strands
26B1	2-3	Cream	Circular	Entire	Convex	-ve long rods, mucoid
26B2	2-3	Cream	Circular	Entire	Raised	-ve pleomorphic
26B3	3-4	Pink	Circular	Entire	Convex	+ve cocci
26B4	1-2	Pink	Circular	Entire	Convex	+ve short rods
26B5	1-2	White	Circular	Entire	Flat	-ve rods
26B6	2-3	Yellow	Circular	Entire	Convex	-ve strands

* See Table 1.

Reduction in chemical oxygen demand (COD)

COD was monitored at fortnightly intervals during Phase I and the results are presented in Fig. 3. The initial values were 5 850 mg.ℓ^{-1} COD for vessel A and 6 500 mg.ℓ^{-1} COD for vessel B. The pattern of reduction of COD was similar for both vessels A and B. The rate of reduction was highest during the initial 2 weeks of the experiment during which the COD level was reduced to approximately 75% of the initial value. This was followed by a more gradual reduction during the period up to 12 weeks after which it tailed off to reach a level of approximately 28% after 16 weeks.

Biodegradation of surfactant

The initial levels of detergent in the experimental vessels A and B were approximately 60 $\mu\ell$.5 mℓ^{-1} of sample. The biodegradation of surfactant, illustrated in Fig. 4, was rapid during the first 4 weeks. Most detergent had been biodegraded at this stage and reached a minimum of approximately 12 $\mu\ell$.5 mℓ^{-1} of effluent sample after 10 weeks. The results in both experimental vessels showed a similar trend in the reduction of the detergent used in the scouring process.

Light microscopic observations

Light microscopic studies on samples of effluent shown in Fig. 1(a-e) were undertaken with the sole intention of obtaining an overview of the types of bacteria present at the different stages of biodegradation. Their characteristics were compared with those described in reference texts for groups of microorganisms based on Prescott (1970) for the algae and Krieg and Holt (1984), Sneath *et al.* (1986) and Starr *et al.* (1981) for the bacteria and cyanobacteria.

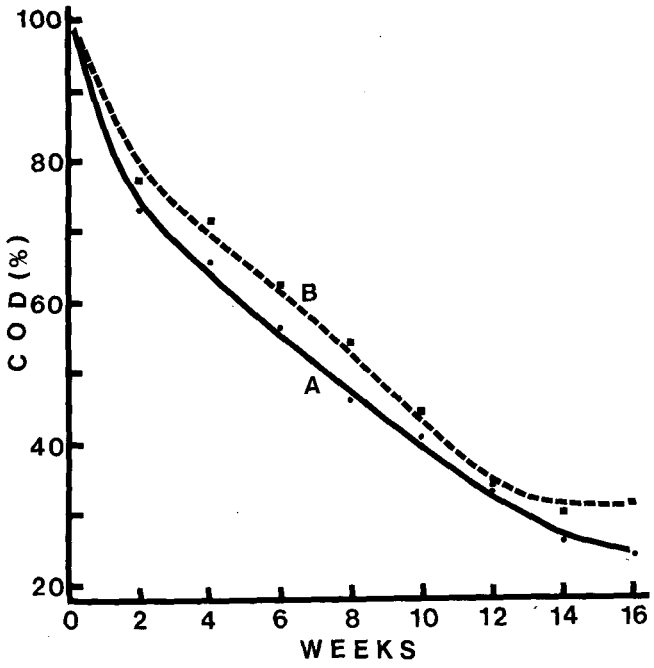

Figure 3

The reduction of COD during Phase I of the experiment. The values of 5 880 mg.ℓ$^{-1}$ COD for vessels A and 6 500 mg.ℓ$^{-1}$ COD for vessel B were taken as 100% respectively at the start of the experiment.

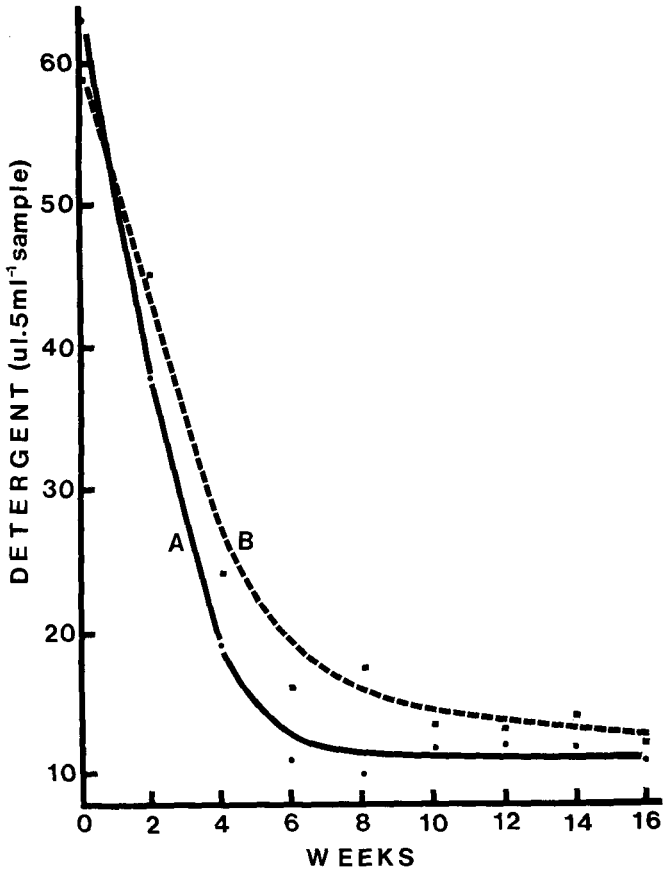

Figure 4

The biodegration of surfactant, used in the wool scouring process, in vessels A and B during Phase I.

It is, however, not practical to show all light micrographs obtained and only a few of these are presented in Figs. 5 and 6. Microscopic observation of the flocs formed in Fig. 1(b) revealed dendritic outgrowth of biological aggregates with pleomorphic rods having round, blunt or pointed ends and being actively motile by a single flagellum. This group of organisms, although not confirmed, was similar to *Zoogloea ramigera*, producing a reddish tinge in the effluent. Fig. 5(a) shows the appearance of a characteristic aggregate of *Zoogloea* after about 8 weeks. The typical growing and spreading pattern of these organisms is evident, and Fig. 5(b), during the latter stages of floc formation, shows the well-developed aggregates of *Zoogloea*. Soon after the addition of algae to the effluent, rapid changes were observed and Fig. 5(c) shows algae embedded in an apparently mucilaginous layer which was difficult to disperse. Within a period of two to four weeks the colour of the effluent rapidly cleared and Fig. 5(d), taken during these stages, shows the development of chloroplasts. The rounded and oval algal cells occurred singly or in close association with other unicellular algae. Fig. 5(e) shows algae with oogonium-like structures and the central reddish to rust-coloured haematochrome. This is typical of algal development in direct sunlight. In Fig. 5(f) a small section of a compact colony showing spreading growth on 1/3 dextrose agar is shown. This bacterium, which was isolated during the latter stages of the clarification, shows the growth pattern typical of the *Cytophaga* spp.

During the final stages of clarification, Fig. 6(a) shows, by comparison with Castenholz (1984), *Chloroflexus*-like, filamentous, gliding photosynthetic bacteria in close association with coccoid cyanobacteria, some of which have reproduced by binary fission. *Chloroflexus*-like filaments were also observed in association with microscopic algae and other microorganisms apparently held together by a mucilaginous matrix (Fig. 6b) and rod-shaped *Pseudomonas*-like and other bacteria (Fig. 6c). The typical loops in the vegetative filaments are clearly seen. Vegetative filaments with distinct short trichomes and gas-vacuolated hormogonia, similar to the oscillatorian cyanobacteria, are evident. Finally, in Fig. 6(d) an individual trichome sheath of organisms, similar to *Microchaete* spp., with basal and intercalary heterocysts, is shown. The typical tapering of the heterocysts is evident, and septal furrows and septa at various stages of completion are clearly indicated. Completion of the septa results in the formation of new cells extending the trichome length.

Discussion

Aspirator vessels A and B were replicates of the experiment conducted. This was supported by the similar patterns of viable bacterial counts, reduction in COD and the breakdown of surfactant observed in vessels A and B. When the predominant bacteria which had been isolated after two weeks were compared, it was evident that isolates 2A2 and 2B3, 2A3 and 2B4, 2A4 and 2B5, 2A5 and 2B7, and 2A8 and 2B8 appeared to be the same and only isolates 2A1, 2A6 and 2A9 did not have a counterpart in vessel B. After four weeks, isolates 4A1 and 4B1, 4A5 and 4B6, 4A6 and 4B7, and 4A7 and 4B8 appeared to be the same. Similarly, the majority of isolates from vessel A were shown to have counterparts in vessel B when a comparison was made at all successive stages of isolation during Phase I and Phase II.

From the clarification achieved in Phase I it was seen that biodegradation, in the presence of bacteria, could proceed only up to a point when a stationary stage was reached. This was supported by the colour of the effluent, total viable counts and COD,

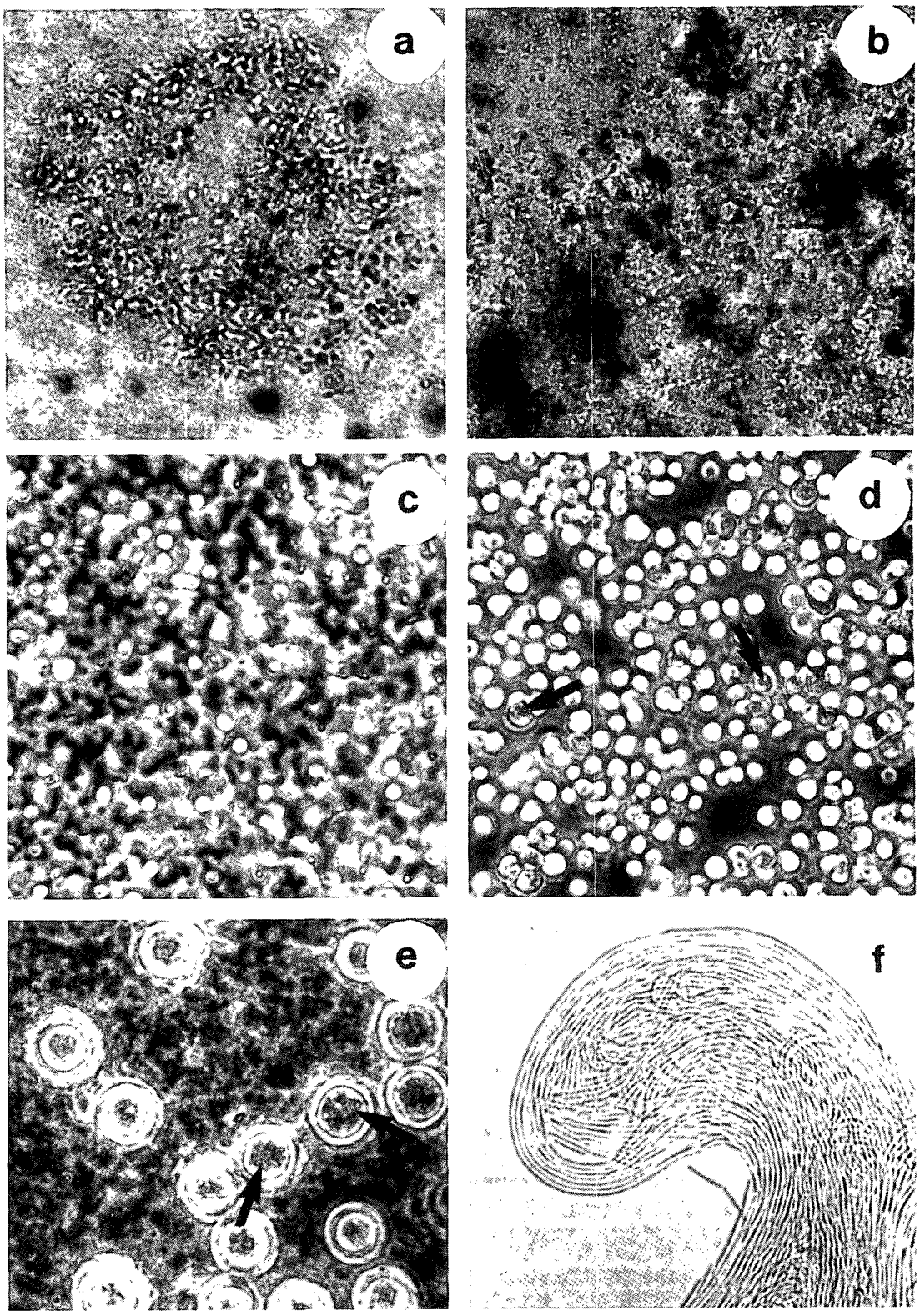

Figure 5

Light micrographs showing (a) the appearance of aggregates of Zoogloea *during early floc formation (brightfield, x850); (b) advanced floc formation and* Zoogloea *(phase contrast, x650); (c) algae embedded in a mucilaginous layer of partially degraded effluent (phase contrast, x850); (d) development of chloroplasts (arrows) when the effluent is exposed to sunlight. Note the free-floating round and oval algal cells intermingled with other unicellular algae (phase contrast, x1 000); (e) algae with oogonium-like structures and central haematochrome (arrows) which is reddish to rustbrown in colour (phase contrast, x1 200); and (f) section of part of an unidentified bacterial colony cultured on 1/3 dextrose agar but typical of* Cytophaga *spp. (brightfield, x700). A Zeiss photomicroscope was used.*

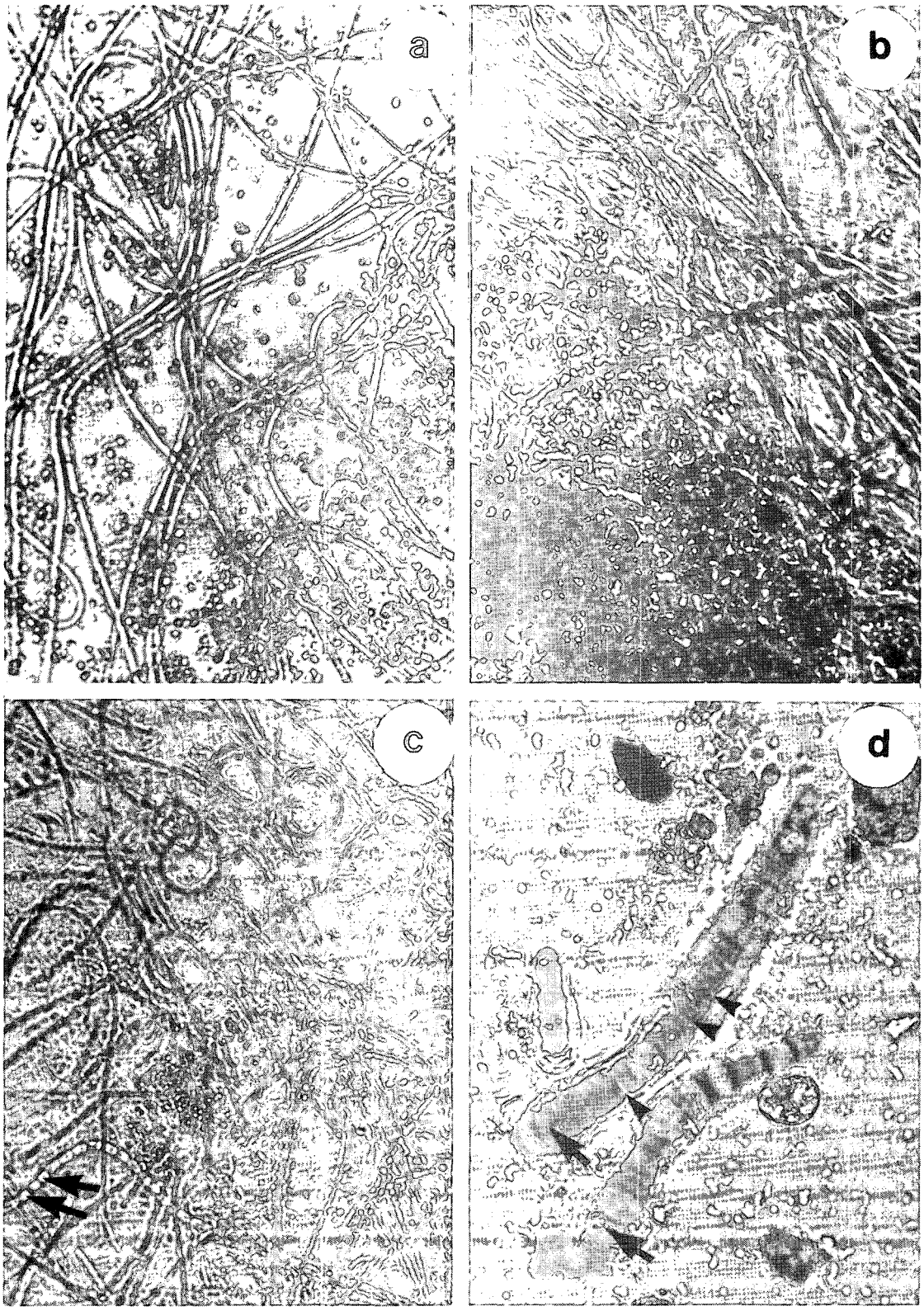

Figure 6

Light micrographs of organisms during the final stages of clarification showing Chloroflexus*-like, filamentous, gliding photosynthetic bacteria (a) in association with coccoid cyanobacteria (brightfield, x1 000); (b) in association with microscopic algae and other filamentous microorganisms within a mucilaginous matrix (phase contrast, x1 200); (c) in association with coccoid algae and rod-shaped* Pseudomonas*-like bacteria. Note the gas-vacuolated hormogonia (arrows) within the long vegetative filaments with their characteristic loops (phase contrast, x1 000); and (d) showing an individual trichome sheath of organisms similar to* Microchaete *spp. with basal and intercalary cysts. Note the typical tapering of the trichome, the heterocyts (arrows), septal furrows and septa at various states of completion (arrowheads) (phase contrast, x550). A Zeiss photomicroscope was used.*

all of which reflected very little change during the last two to four weeks of Phase I. This indicated the need for other micro-organisms to assist in the completion of the biodegradation process. In fact, algae had been present in the industrial ponds from which the original effluent was obtained. The addition of isolated algae to the experimental system, kept under natural light conditions during Phase II, resulted in successful clarification producing a clear water containing the contracted algal biomass. No solid sludge components remained. Although the algae appeared to be the major contributor to this final clarification process, a change in the bacterial flora during this phase was observed, as indicted by the bacteria isolated from and light microscopic observations on samples taken during these stages. Efficient biodegradation and clarification of the wool scouring effluent had been achieved in this simulated natural ecosystem. This was clearly indicated by the marked reduction in COD to levels approximately 28% of the initial value. Surfactant was efficiently and rapidly biodegraded from approximately 60 $\mu\ell$ to almost 12 $\mu\ell$ per 5 mℓ sample analysed.

Of the isolates from Phase I, 2A5, 2B1, 2B7, 4A4, 4A5, 4B6, 6B5, 8A5, 8A6, 8B5, 10A6, 10B4, 12A3, 12B2, 12B5, 14A5, 14B5 and 16A6 showed growth on surfactant-enriched minimal medium while no growth was observed on minimal medium. These isolates therefore represent putative surfactant degrading bacteria and are currently under investigation.

From preliminary experiments (Gokool *et al.*, 1982) it was observed that fungi and other small higher animals played only a minor role in the clarification of the effluent. This supports an earlier report by McKinney (1957). Bacteria and algae are therefore the major contributors towards the biodegradation process. It is evident that considerable bacterial diversity exists in the microflora found in the wool scouring effluent during biodegradation. With the exception of the work on floc-forming bacteria by Tago and Aida (1977), who described a *Pseudomonas* species, and preliminary work in our laboratory (Bhiman and Roth, 1984; Odhav *et al.*, 1986; Roth *et al.*, 1983), these bacteria have not received much attention and their characterisation and specific roles in the clarification process remain unresolved. Work on the taxonomic status of and the immunological relatedness between some of the many bacteria isolated at the different stages of effluent clarification is currently in progress. This should contribute towards a better understanding of the complex process of biodegradation of the wool scouring effluent.

Acknowledgement

We would like to thank Mrs L van Hooff, University of Durban Westville, for assistance with the preparation of the photographic plates.

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