# A microbiological survey of ten activated sludge plants

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## Abstract

The activated sludge process is an aerobic biological method for organic matter reduction from waste water. The objective of this research was to conduct a survey of micro-organisms present in 10 activated sludge plants. The following groups of organisms were investigated; eubacteria, filamentous bacteria, fungi, yeasts, algae and protozoa. Eubacteria, fungi and yeasts were isolated on casitone glycerol yeast extract agar, rose bengal chloramphenicol agar and yeast malt extract agar respectively. Twenty-two different genera of eubacteria were isolated and identified as mainly gram-positive rods belonging to the genus *Bacillus*. Spore-forming bacteria predominated over non-spore formers. *Microthrix parvicella* was the most common filamentous organism detected in the activated sludge plants studied. When compared to the rest of the microflora, fungi and yeasts were not detected in large magnitude, mostly belonging to common genera. Algal types detected were the common fresh-water and polluted water algae. Protozoans were well represented with the most common types being *Paramecium* and *Euplotes* spp.

## Introduction

Prior to 1914 sewage treatment practice mainly comprised the following unit operations viz., screening, detritus removal, sedimentation or chemical precipitation, percolating filters followed by humus tanks. During 1914, Ardern and Lockett introduced the activated sludge process as a biological method of organic matter reduction from sewage. This method is presently still employed globally for the treatment of waste water (Murray, 1987).

The activated sludge process comprises 2 liquid stream processing units - the aeration basin (biological reactor) and the secondary clarifier. The aeration basin provides the environment for transformation and removal of pollutants by a mixed variable consortium of micro- and macro-organisms termed activated sludge. The micro-organisms include eubacteria, filamentous bacteria, algae, fungi, protozoa and rotifers (Jenkins et al., 1986). Rotifers, nematode worms and more rarely oligochaete worms and chironomid larvae may be found (Curds, 1982). Flocs are the basic ecological units of activated sludges. Heterotrophic bacteria form the basis of flocs by attaching to each other and to filamentous bacteria. The floc macrostructure is formed by filamentous bacteria which facilitate adhesion to floc-forming bacteria. Fungal hyphae are often associated with flocs, but rarely predominate under normal operating conditions. Protozoans contribute to the process by feeding on pathogenic bacteria and by removing dispersed bacteria which results in larger flocs and improved sludge settleability (Curds and Cockburn, 1970).

Although it is accepted that micro-organisms are directly responsible for the effectiveness and success of the activated sludge treatment process, the complexity of microbiological populations is often under-estimated during design of the latter. Full understanding of the ecological, physiological and biochemical activities of the microflora is necessary for optimal control of the process (Adamse et al., 1984).

Studies on the bacterial flora of activated sludges have been the subject of a comparatively small number of publications. The results of the investigations are divergent, due to the use of different methods and examination of different types of sludge. Although domestic activated sludge treatment systems in South Africa have been relatively widely studied, little quantitative information describing microbiological populations of sludges has been communicated. In particular, microbiological surveys of activated sludge plants in Natal have only received scant attention. Therefore the objectives of the present study were firstly to determine the microbiological populations of 10 activated sludge plants and subsequently to compare the prevalence and predominance of eubacteria, filamentous bacteria, algae, protozoa, yeasts and fungi.

## Materials and methods

### Sampling and mixed liquor suspended solids (MLSS) determination

Grab samples of return activated sludge (± 1 000 m*l*) were collected in sterile bottles from the following waste-water works in Natal, South Africa: Umlaas, Amanzimtoti, New Germany, Hammarsdale, Pietermaritzburg, Kwa Mashu, Tongaat, Northern Works, Southern Works and Phoenix (Table 1). Samples were stored at 4°C prior to use and processed within 24 h. Triplicate samples of 100 m*l* liquid sludge were dried overnight in pre-weighed porcelain dishes in an oven at 105°C and re-weighed to determine the sludge MLSS.

### Enumeration, isolation, characterisation and identification of Eubacteria

A 50 m*l* volume of each sludge sample was homogenised using a Sorvall Omni-Mixer 17106 (Du-Pont Instruments, Newton, USA) for 4 min at 16 000 r· min$^{-1}$ to disperse flocs. Serial dilutions ($10^{-1}$ to $10^{-6}$) of samples were prepared. Triplicate, 0,1 m*l* volumes from the $10^{-4}$ to the $10^{-6}$ dilutions were plated on casitone glycerol yeast-extract agar (CGYA) using the spread plate technique. Plates were incubated at 30°C for 48 h and only those plates showing between 30 and 300 colonies were enumerated. Counts were expressed as CFU·g$^{-1}$ (Table 1). Bacterial colonies were differentiated on the basis of colonial morphology (configuration, margin and elevation) and pigmentation. Colonies were coded, subcultured on CGYA plates and re-incubated at 30°C. Repeated subculturing

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TABLE 1
WASTE TYPES TREATED AND EUBACTERIAL VIABLE COUNTS* FOR 10 ACTIVATED SLUDGE PLANTS

| Plant name | Waste type | Viable count (colony forming units $g^{-1}$) |
|---|---|---|
| New Germany (NG) | 15% Industrial 85% Domestic | $7{,}10 \times 10^9 \pm 4{,}70 \times 10^8$ |
| Tongaat (T) | 40% Industrial 60% Domestic | $5{,}51 \times 10^9 \pm 6{,}33 \times 10^8$ |
| Pietermaritzburg (PMB) | 5% Industrial 95% Domestic | $5{,}10 \times 10^9 \pm 3{,}86 \times 10^8$ |
| Kwa Mashu (KM) | 100% Domestic | $1{,}04 \times 10^{10} \pm 1{,}70 \times 10^9$ |
| Phoenix (P) | 100% Domestic | $4{,}99 \times 10^9 \pm 7{,}86 \times 10^8$ |
| Amanzimtoti (A) | 100% Industrial | $2{,}48 \times 10^9 \pm 2{,}81 \times 10^8$ |
| Umlaas (U) | 100% Domestic | $6{,}27 \times 10^9 \pm 3{,}19 \times 10^8$ |
| Southern Works (SW) | 16% Industrial 84% Domestic | $3{,}28 \times 10^{10} \pm 1{,}63 \times 10^{10}$ |
| Northern Works (NW) | 8% Industrial 92% Domestic | $3{,}79 \times 10^9 \pm 2{,}41 \times 10^8$ |
| Hammarsdale (H) | 95% Industrial | $1{,}38 \times 10^9 \pm 3{,}35 \times 10^8$ |

* Values are means of and standard deviations of 3 determinations

was conducted to obtain pure cultures.

Purity was determined by Gram staining and microscopic observation. Staining procedures employed for characterisation included Gram stains and spore stains performed on 24 h cultures from CGYA plates. Determination of oxygen requirement was performed by using the anaerobic jar method. Motility was detected by examining wet-mount preparations of 24 h cultures. Measurement of bacterial cell diameters was conducted using light microscopy. Biochemical tests conducted for the purpose of characterisation were performed at room temperature (25°C). Cytochrome oxidase was determined by testing for oxidation of tetramethyl-p-phenylene diamine dihydrochloride (Frobisher et al., 1974). The oxidase test was used to differentiate Enterobacteriaceae from other gram-negative organisms. Presence of the enzyme catalase in the isolates was assayed by using a 3 % solution of hydrogen peroxide (Frobisher et al., 1974). The API 20E and 20NE identification systems were used to identify gram-negative enteric and non-enteric bacteria respectively. Gram-positive isolates were identified using appropriate taxonomic techniques. (Schleifer, 1986; Sneath, 1986; Kandler and Weiss, 1986; Jones and Collins, 1986). Bacilli were identified to the genus level.

### Characterisation and identification of filamentous bacteria

Air-dried smears of freshly collected sludge were prepared on microscope slides. The morphological characteristics and staining reactions described by Jenkins et al. (1986), were used to identify filamentous bacteria. Characteristics of filamentous bacteria determined using light microscopy included: Gram reactions, Neisser reactions, presence of sulphur granules and polyhydroxybutyrate cell inclusions, trichome diameter, length, shape and location, presence of cell septa, indentations and sheaths, epiphytic growth, and cell shape and size. Identification was achieved using appropriate taxonomic keys and tables of Eikelboom and Van Buijsen (1983) and Jenkins et al. (1986).

### Isolation, characterisation and identification of fungi

Aliquots of 0,1 m*l* homogenised sludge were plated on rose bengal chloramphenicol agar (RBCA). Plates were incubated at 30°C for 7 d to allow for development of pigment on colonies to facilitate complete differentiation of fungal types. Repeated subculturing on RBCA was necessary to obtain pure cultures. Sporulation was induced by subjecting cultures to ultraviolet light. Isolates were characterised according to morphological features, cultural characteristics such as pigmentation of the mycelium and direction of growth of the hypha, whether aerial or lateral, microscopic observation of structures involved in asexual reproduction e.g., conidia or spores, and in sexual reproduction, and the presence of fruiting bodies. Identification was accomplished using appropriate taxonomic techniques (Gilman, 1959; Smith, 1967; Hazen et al., 1973; Webster, 1978 and Alexopoulos and Mims, 1979).

### Isolation, characterisation and identification of yeasts

Aliquots of 0,1 m*l* homogenised sludge were plated on yeast malt extract agar (YMEA). Plates were incubated at 30°C for 72 h. Colonies were purified by repeated subculturing on YMEA and purity confirmed microscopically. Microscopic observation of cellular morphology was conducted. Pure cultures were identified using ATB32C identification kits along with the ATB32C data base and appropriate literature (Barnett et al., 1983).

### Characterisation and identification of algae and protozoa

Wet mounts on microscope slides of freshly collected sludge samples were viewed under the photomicroscope. Algae were identified on the basis of pigmentation and gross cellular morphology. Appropriate techniques were used to identify algae (*Standard Methods*, 1976; Bellinger, 1980). Protozoa were identified on the basis of cellular morphology e.g., shape, size, inclusions, (Buchsbaum, 1976; Van Rensburg et al. 1980; Eikelboom and Van Buijsen, 1983).

## Results

### Eubacteria

Viable bacterial numbers in homogenised MLSS samples ranged from $1{,}38 \times 10^9$ to $3{,}28 \times 10^{10}$ Cfu·$g^{-1}$ (Table 1). Analyses of variance (ANOVA) proved that all numbers except Southern Works did not differ significantly ($P < 0{,}01$).

Twenty-two different genera of eubacteria were isolated and identified from the 10 sludges (Table 2). *Bacillus* spp. was detected in all sludges investigated (Table 2, Figs. 1 to 10). The most predominant genera recorded for all sludges were, *Bacillus* spp., *Cellulomonas* spp., *Pseudomonas* spp., *Listeria* spp., *Lactobacillus* spp. and *Microbacterium* spp.detected in 100%, 80%, 50%, 40%, 40%, and 40% respectively, of plants investigated. (Table 2). Sludge obtained from New Germany, Tongaat, Pietermaritzburg and Amanzimtoti works showed highest bacterial diversity (Table 2, Figs. 1,2,3 and 6). Hammarsdale sludge showed lowest bacterial diversity (Table 2, Fig. 10). Gram-positive *Bacillus* spp. were the predominant bacterial type detected in sludges from Tongaat (Fig. 2), Pietermaritzburg (Fig. 3), Kwa Mashu (Fig. 4), Phoenix (Fig. 5), Amanzimtoti (Fig. 6), Southern Works (Fig. 8), Northern

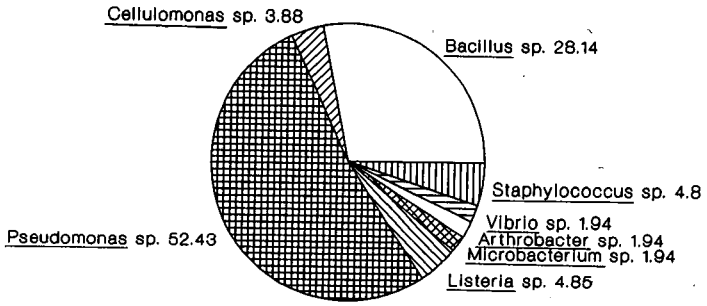


***Figure 1***
*Percentage representation of Eubacteria isolated from the New Germany activated sludge plant*

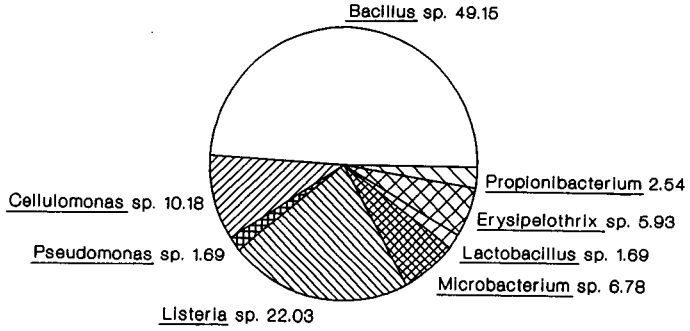


***Figure 2***
*Percentage representation of Eubacteria isolated from the Tongaat activated sludge plant*

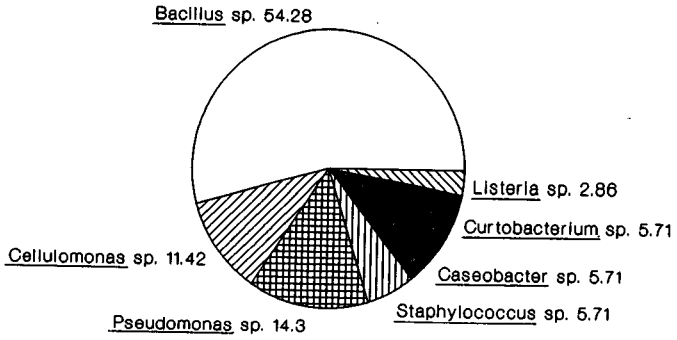


***Figure 3***
*Percentage representation of Eubacteria isolated from the Pietermaritzburg activated sludge plant*

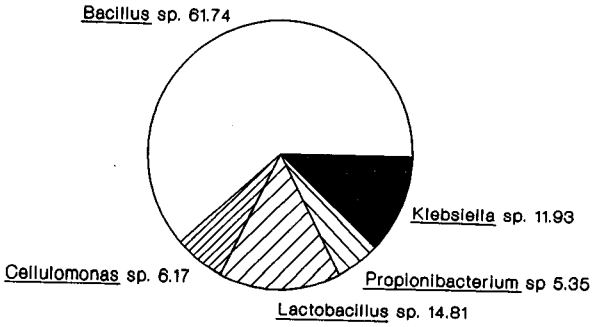


***Figure 4***
*Percentage representation of Eubacteria isolated from the Kwa Mashu activated sludge plant*

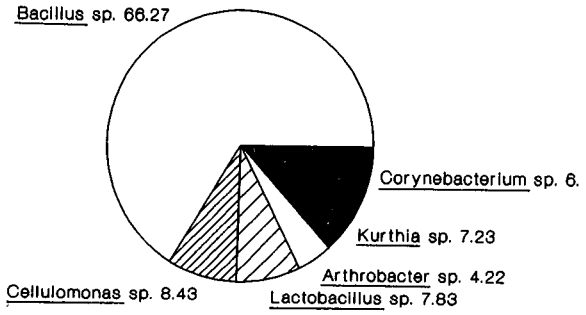


***Figure 5***
*Percentage representation of Eubacteria isolated from the Phoenix activated sludge plant*

TABLE 2
EUBACTERIA ISOLATED AND FREQUENCY OF OCCURRENCE IN 10 ACTIVATED SLUDGE PLANTS

| Organism name | NG | T | PMB | KM | P | A | U | SW | NW | H |
|---|---|---|---|---|---|---|---|---|---|---|
| *Bacillus* spp. | 28,14 | 49,15 | 54,28 | 61,74 | 66,27 | 65,34 | 37,04 | 63,61 | 65,20 | 61,34 |
| *Cellulomonas* spp. | 3,88 | 10,18 | 11,42 | 6,17 | 8,43 | | 0,93 | 13,64 | 2,61 | |
| *Pseudomonas* spp. | 52,43 | 1,69 | 14,30 | | | | | 4,54 | 11,30 | |
| *Listeria* spp. | 4,85 | 22,03 | 2,86 | | | 0,79 | | | | |
| *Lactobacillus* spp. | | 1,69 | | 14,81 | 7,83 | 8,67 | | | | |
| *Microbacterium* spp. | 1,94 | 6,78 | | | | 4,72 | | | | 19,33 |
| *Arthrobacter* spp. | 1,94 | | | | 4,22 | | 4,63 | | | |
| *Vibrio* spp. | 1,94 | | | | | 3,15 | | | 5,22 | |
| *Staphylococcus* spp. | 4,85 | | 5,71 | | | | | | | |
| *Erysipelothrix* spp. | | 5,93 | | | | | | 15,15 | | |
| *Propionibacterium* | | 2,54 | | 5,35 | | | | | | |
| *Alcaligenes* spp. | | | | | | | 53,70 | | 1,74 | |
| *Caseobacter* spp. | | | 5,71 | | | | | | 13,92 | |
| *Curtobacterium* spp. | | | 5,71 | | | | | | | |
| *Klebsiella* spp. | | | | 11,93 | | | | | | |
| *Kurthia* spp. | | | | | 7,23 | | | | | |
| *Corynebacterium* spp. | | | | | 6,02 | | | | | |
| *Flavobacterium* spp. | | | | | | 1,57 | | | | |
| *Micrococcus* spp. | | | | | | 15,74 | | | | |
| *Renibacterium* spp. | | | | | | | 3,70 | | | |
| *Agrobacterium radiobacter* | | | | | | | | 3,03 | | |
| CDC Ser IIB | | | | | | | | | | 19,33 |

KEY: NG = New Germany, T = Tongaat, PMB = Pietermaritzburg, KM = Kwa Mashu, P = Phoenix, A = Amanzimtoti, U = Umlaas, SW = Southern Works, NW = Northern Works, H = Hammarsdale

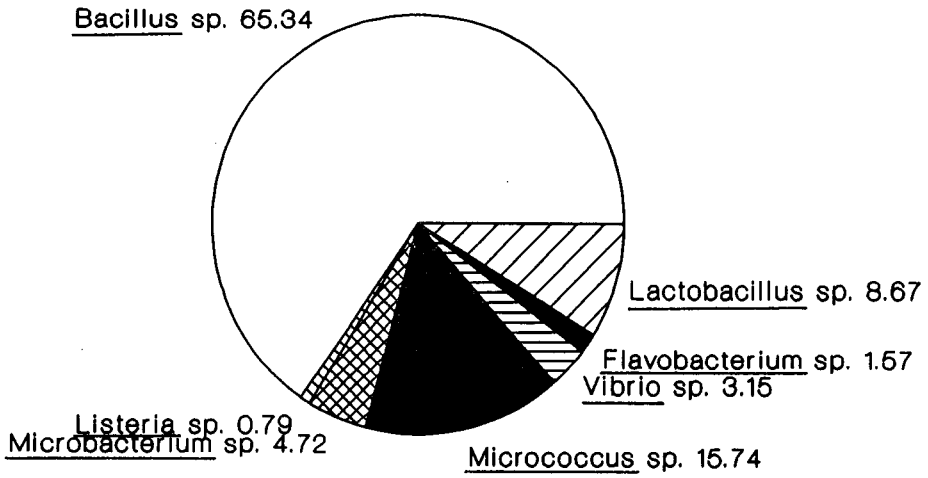


***Figure 6***
*Percentage representation of Eubacteria isolated from the Amanzimtoti activated sludge plant*

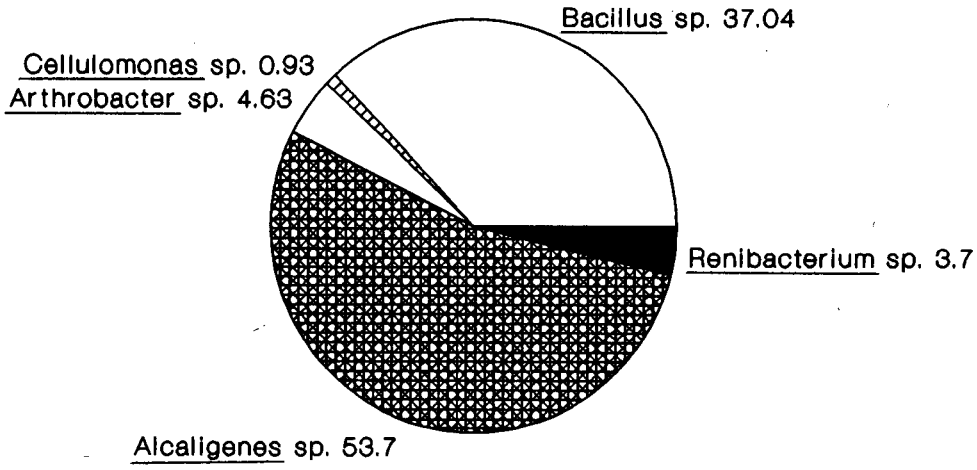


***Figure 7***
*Percentage representation of Eubacteria isolated from the Umlaas activated sludge plant*

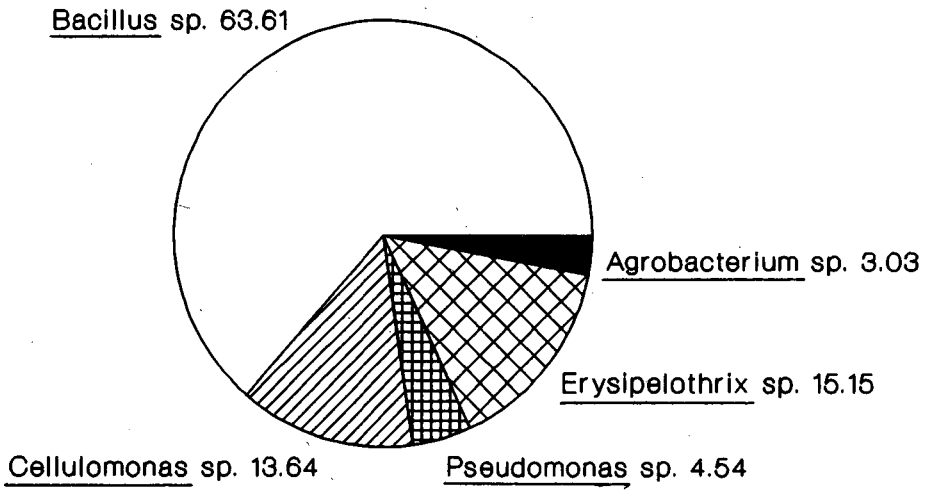


***Figure 8***
*Percentage representation of Eubacteria isolated from the Southern Works activated sludge plant*

**TABLE 3**
**FILAMENTOUS BACTERIA IDENTIFIED AND FREQUENCY OF OCCURRENCE IN 10 ACTIVATED SLUDGE PLANTS**

| Name of bacteria | NW | P | KM | SW | U | NG | H | PMB | T | A | Frequency of occurrence |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Microthrix parvicella* | * | * | * | * | * | * | * | * | * | * | 100 |
| *Type 0041* | | * | * | * | * | * | | | | * | 60 |
| *Nocardia* spp. | | | | | | * | * | * | * | * | 50 |
| *Sphaerotilus natans* | * | | | | | * | | * | | * | 40 |
| *Type 0675* | | * | | | * | * | | | | | 30 |
| *Nostocoida limicola II* | | | | | | | | | * | * | 20 |
| *Type 0092* | * | | | | * | | | | | | 20 |
| *Type 1701* | | | | * | | | * | | | | 20 |
| *Type 021N* | | | | * | | | | | * | | 20 |
| *Thiothrix I* | | | * | | | | | | | | 10 |
| *Cyanophyaceae* | | | | | * | | | | | | 10 |

KEY: NG = New Germany, T = Tongaat, PMB = Pietermaritzburg, KM = Kwa Mashu, P = Phoenix, A = Amanzimtoti, U = Umlaas, SW = Southern Works, NW = Northern Works, H = Hammarsdale, * = presence of organism

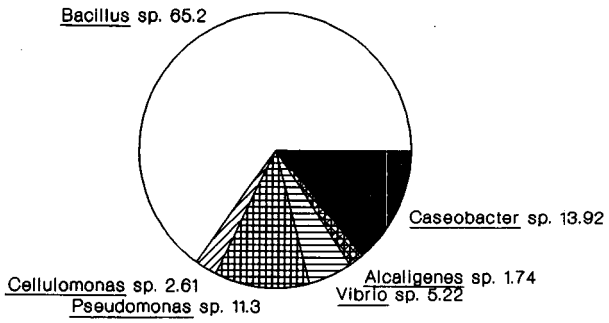


***Figure 9***
*Percentage representation of Eubacteria isolated from the Northern Works activated sludge plant*

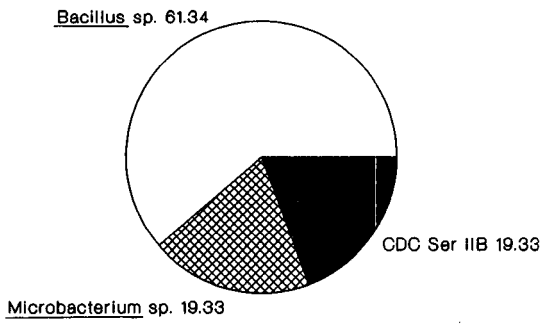


***Figure 10***
*Percentage representation of Eubacteria isolated from the Hammarsdale activated sludge plant*

**TABLE 4**
**FUNGI ISOLATED AND FREQUENCY OF OCCURRENCE IN 10 ACTIVATED SLUDGE PLANTS**

| Name of fungus | T | PMB | U | NW | A | P | SW | NG | KM | H | Frequency of occurrence |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Penicillium* spp. | * | | | | | * | * | | | | 30 |
| *Cladosporium* spp. | | | * | | | * | | | * | | 30 |
| *Aspergillus* spp. | * | | | | * | | * | | | | 30 |
| *Harpella* spp. | | * | | | | | | * | | | 20 |
| *Fusarium* spp. | | | * | | | | * | | | | 20 |
| *Trichoderma* spp. | * | | * | | | | | | | | 20 |
| *Chrysosporium* spp. | | | | * | | * | | | | | 20 |
| *Helminthosporium* spp. | | | | | * | * | | | | | 20 |
| *Geotrichum* sp. | | * | | | * | | | | | | 20 |
| *Phoma* spp. | | | | * | | | | | | | 10 |
| *Chaetomium* spp. | | | | | * | | | | | | 10 |
| *Rhizopus* spp. | * | | | | | | | | | | 10 |
| *Gonatobotrys* spp. | | | * | | | | | | | | 10 |
| *Botrytis* spp. | | | | * | | | | | | | 10 |
| *Epidermophyton* spp. | | | | | * | | | | | | 10 |
| *Coriolus* spp. | | | | | | * | | | | | 10 |
| *Zygorhyncus* spp. | | | | | | * | | | | | 10 |
| *Enterobryus* spp. | | | | | | * | | | | | 10 |
| *Pestalotia* spp. | | | | | | | | * | | | 10 |
| *Colletotrichum* spp. | | | | | | | | * | | | 10 |
| *Trichophyton* spp. | | | | | | | | | * | | 10 |
| *Nematogonum humicola* | | | | | | | | | | * | 10 |
| Deuteromycete | | * | | | | | | | | | 10 |
| Myxomycete | | | | | | * | | | | | 10 |

KEY: NG = New Germany, T = Tongaat, PMB = Pietermaritzburg, KM = Kwa Mashu, P = Phoenix, A = Amanzimtoti, U = Umlaas, SW = Southern Works, NW = Northern Works, H = Hammarsdale, * = presence of organism

TABLE 5
YEASTS ISOLATED AND FREQUENCY OF OCCURRENCE IN 10 ACTIVATED SLUDGE PLANTS

| Name of yeast | T | PMB | U | NW | A | P | SW | NG | KM | H | Frequency of occurrence |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Candida* spp. | | * | | | * | * | | | * | * | 50 |
| *Rhodotorula rubra* | | | * | * | | | | * | | * | 40 |
| *Tricho cutaneum* | * | | | | | | | * | | * | 30 |
| *Rhodotorula* spp. | | | * | | | | | | | | 10 |
| *Candida humicola* | | | | | | | | * | | | 10 |
| *Candida inconspicua* | | | | | | | | | * | | 10 |
| *Candida catenulata* | | | | | | | | | * | | 10 |
| *Candida glabrata* | * | | | | | | | | | | 10 |
| *Pichia* spp. | | | | | * | | | | | | 10 |
| *Tricho capitatum* | | | | | | | | * | | | 10 |
| *Crypto laurentii* | | | | | | | | | * | | 10 |

KEY: NG = New Germany, T = Tongaat, PMB = Pietermaritzburg, KM = Kwa Mashu, P = Phoenix, A = Amanzimtoti, U = Umlaas, SW = Southern Works, NW = Northern Works, H = Hammarsdale, * = presence of organism

Works (Fig. 9) and Hammarsdale (Fig. 10). In contrast, sludges from New Germany, (Fig. 1) and Umlaas (Fig. 7) were predominated by *Pseudomonas* spp. and *Alcaligenes* spp., respectively.

**Filamentous bacteria**

Eleven types were detected from the 10 sludges investigated. *Microthrix parvicella* was observed in all sludges (Table 3). Predominant filament types identified were *M. parvicella, Type 0041* and *Nocardia* sp. Sludges from Umlaas, New Germany and Amanzimtoti harboured higher numbers of filamentous types (Table 3).

**Fungi, algae and protozoa**

Twenty-four fungal genera were isolated from the 10 sludges investigated. *Penicillium* spp., *Cladosporium* spp. and *Aspergillus* spp. were present in each of Tongaat, Phoenix and Southern Works (Table 4). Phoenix sludge contained the greatest diversity of fungal types (Table 4). Eleven yeast types were isolated and identified from the 10 sludges (Table 5). New Germany and Kwa Mashu sludges contained the greatest diversity of yeast types comparatively. Twenty-six types of algae were observed in the 10 sludges under study. Northern Works, Phoenix, New Germany and Tongaat contained the highest diversity of algal types. *Chlorella* spp. were detected in all plants except New Germany (Table 6). *Anabaena* spp. and *Volvox* spp. also occurred at relatively high frequencies (Table 6). Sixteen types of protozoa were observed in the 10 sludges under investigation. *Paramecium* spp., *Aspidisca* spp., *Euplotes* spp. and *Amoeba* spp. were most predominant among the 10 sludges (Table 7). Northern Works sludge contained the greatest diversity of protozoan types, when compared to the other plants studied.

## Discussion

The choice of suitable culture media is essential in order to achieve the best possible representation and enumeration of microbial populations in activated sludge. To isolate a large and representative eubacterial range, the medium employed must be non-selective. Previous work by Ishwarlall (1990) showed casitone glycerol yeast agar (CGYA), when compared to several other media, to be the ideal medium supporting the growth of the greatest diversity of eubacterial types from activated sludge. The results of the present work produced high viable counts, on CGYA, in all plants sampled, ranging from $1{,}38 \times 10^9 \pm 3{,}35 \times 10^8$ to $3{,}28 \times 10^{10} \pm 1{,}63 \times 10^{10}$ (Table 1) These counts compared favourably with similar work conducted by Pike and Carrington (1972) on domestic waste-water treatment works using CGYA. Some notable differences in the viable numbers between the plants studied could be attributed to differences in operational parameters such as sludge age, environmental variables and waste-water characteristics which deter-

**TABLE 6**
**PRESENCE AND FREQUENCY OF OCCURRENCE OF ALGAE IN 10 ACTIVATED SLUDGE PLANTS.**

| Name of alga | NW | P | KM | SW | U | NG | H | PMB | T | A | Frequency of occurrence |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Chlorella* spp. | * | * | * | * | * | | * | * | * | * | 90 |
| *Anabaena* spp. | * | * | * | | * | | | | * | * | 60 |
| *Volvox* spp. | * | | | * | | * | * | | | * | 50 |
| *Chlorococcum* spp. | | * | | * | | | * | | * | | 40 |
| *Coelastrum* spp. | | * | * | | | * | * | | | | 40 |
| *Anacystis* spp. | | | * | * | | * | * | | | | 40 |
| *Botryococcus* spp. | * | | | | | | | * | | * | 30 |
| *Sphaerocystis* spp. | | * | * | | | * | | | | | 30 |
| *Ulothrix* spp. | | * | | * | | | | * | | | 30 |
| *Gomphosphaeria* spp. | | | | * | | * | * | | | | 30 |
| *Fragilaria* spp. | * | | * | | | | | | | | 20 |
| *Hydrodictyon* spp. | | | | | | | * | | | * | 20 |
| *Diatoma* spp. | | | * | | | * | | | | | 20 |
| *Chlamydomonas* spp. | * | | | | | | | | * | | 20 |
| *Nodularia* spp. | | * | | | | | | | | | 10 |
| *Lepocinclis* | * | | | | | | | | | | 10 |
| *Nitzschia* spp. | | | | | | | | | | * | 10 |
| *Navicula* spp. | | | | | * | | | | | | 10 |
| *Chromulina* spp. | | | | | | | | | * | | 10 |
| *Gonium* spp. | | | | | | | | | * | | 10 |
| *Agmenellum* spp. | | | | | | | | | * | | 10 |
| *Carteria* spp. | | | | | | | | | * | | 10 |
| *Euglena* spp. | * | | | | | | | | | | 10 |
| *Gomphonema* spp. | | * | | | | | | | | | 10 |
| *Cyclotella* spp. | | | | | | * | | | | | 10 |
| *Phytoconis* spp. | | | | | | * | | | | | 10 |

KEY: NG = New Germany, T = Tongaat, PMB = Pietermaritzburg, KM = Kwa Mashu, P = Phoenix, A = Amanzimtoti, U = Umlaas, SW = Southern Works, NW = Northern Works, H = Hammarsdale, * = presence of organism

TABLE 7
PRESENCE AND FREQUENCY OF OCCURRENCE OF PROTOZOA IN 10 ACTIVATED SLUDGE PLANTS

| Name of protozoan | NW | P | KM | SW | U | NG | H | PMB | T | A | Frequency of occurrence |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Paramecium* spp. | * | * | * | * | * | * | | * | * | | 80 |
| *Aspidisca* spp. | * | * | | * | * | * | * | * | | * | 80 |
| *Euplotes* spp. | * | * | | * | * | | * | * | | * | 70 |
| *Amoeba* spp. | * | * | | * | * | | | * | * | * | 70 |
| *Vorticella* spp. | * | * | | | * | | | * | | | 40 |
| *Blepharisma* spp. | | | * | * | | * | | | * | | 40 |
| *Poteriodendron* spp. | * | | | | | | | * | | * | 30 |
| *Colpidium colpodem* | | | | * | | * | * | | | | 30 |
| *Epistylis* spp. | * | | | | | | | * | | | 20 |
| *Spirostomum* spp. | * | | | | | * | | | | | 20 |
| *Pleuromonas* spp. | | | * | * | | | | | | | 20 |
| *Opercularia* spp. | | | | * | * | | | | | | 20 |
| *Carchesium* spp. | * | | | | | | | | | | 10 |
| *Bodo* spp. | | | * | | | | | | | | 10 |
| *Trachelophylum pusillum* | | | * | | | | | | | | 10 |
| *Chilodonella cuculatus* | | | | | | | | | * | | 10 |

KEY: NG = New Germany, T = Tongaat, PMB = Pietermaritzburg, KM = Kwa Mashu, P = Phoenix, A = Amanzimtoti, U = Umlaas, SW = Southern Works, NW = Northern Works, H = Hammarsdale, * = presence of organism

mine the microbial population in activated sludges.

Allen (1940) homogenised sludge of domestic origin and showed that most of the eubacterial population were gram-negative bacilli belonging to the genera *Pseudomonas* spp., *Flavobacterium* spp., and *Achromobacter* spp. In contrast the results of the present study showed that except for the New Germany and Umlaas activated sludges, the remaining 8 plants sampled exhibited a predominantly gram-positive eubacterial population (Table 2). Spore-forming bacteria predominated over non-spore formers. This occurrence could be attributed to environmental stress such as a drastic change in the waste water being treated, agitation within the aeration basin, facilitating the adaptation of certain types of species which are more resistant to such environments. Several workers conducting studies on activated sludges have reported that rod-shaped bacteria occur at higher frequencies than cocci (McKinney and Weichlein, 1953; Dias and Bhatt, 1964; Kasan and Baecker, 1989). The results of the present study confirm these findings (Figs. 1 to 10).

The type of waste treated ie., domestic, industrial or mixtures (Table1), dictates to a great degree the eubacterial microflora present. Water treatment plants such as New Germany and Umlaas that treated primarily domestic waste contained larger numbers of gram-negative rods such as *Pseudomonas* spp. and *Alcaligenes* spp. (Table 2), which are potential pathogens of gastrointestinal and faecal origin. These results substantiate the findings of Dias and Bhatt (1964). Plants that treated a mixed influent type ie., both domestic and industrial, displayed predominantly gram-positive bacteria. Therefore the chemical nature of wastes treated determines bacterial diversity (McKinney and Weichlein, 1953). Overall, evaluation of all 10 water treatment works showed that bacterial diversity was limited.

A diverse population of filamentous bacteria was identified in

the 10 waste-water treatment plants (Table 3). All of these have previously been identified in domestic and industrial activated sludge plants (Cyrus and Sladaka, 1970; Farquhar and Boyle, 1971; Eikelboom, 1975; Strom and Jenkins, 1984). The results of the present study confirm the findings of Blackbeard et al. (1986) who showed Type 0092 and *M. parvicella* to be the predominant filamentous types present in activated sludge plants in South Africa. The high frequency of occurrence of *M. parvicella*, Type 0041 and *Nocardia* spp. can be attributed to their ability to adapt and withstand environmental stress. Some of the common problems experienced by waste-water works are bulking and foaming. Studies have shown that such phenomena are related to increased presence of certain filamentous types such as Type 0041, *M. parvicella*, Type 0675 and *Nocardia* sp. (Jenkins et al., 1986 and Eikelboom, 1977). The present research substantiates the above-mentioned findings. The New Germany Waste Water Treatment Plant showed sludge bulking which could be related to an abundance of Type 0041, *M. parvicella* and Type 0675 whose presence was confirmed microscopically. In agreement with work done by Blackbeard and Ekama (1984), who related the presence of *Nocardia* spp. to foaming problems, the present work has demonstrated that overgrowth of *Nocardia* and *M. parvicella* could be related to the foaming problems experienced at the Tongaat Waste Water Works. The present study has also revealed no marked difference between filamentous bacterial populations of domestic and industrial sludges e.g. New Germany treating primarily domestic waste and Amanzimtoti treating industrial waste displayed 4 out of 5 common filamentous types (Table 3). This was contrary to the findings of Eikelboom (1977) which showed that a clear difference exists between the population of filamentous organisms in plants fed with domestic sewage and those treating industrial waste water.

Fungi are not normally found as dominant organisms in the activated sludge treatment process (Tomlinson and Williams, 1975). Most of the fungi recovered from the 10 plants studied belonged to common genera (Table 4). Although fungi prevailed in all plants studied, there was no degree of predominance of specific genera in any of the plants. These findings contradict those of Tomlinson and Williams (1975) who reported *Geotrichum candidum* and *Trichosporon* spp. to be present in relatively large numbers in activated sludge of industrial origin.

Studies by Kahn et al. (1990) showed yeast to be present in insignificant numbers and sometimes absent in activated sludge plants. In contrast, results of the present study have shown that yeasts were present in all plants although not predominating among the microflora. *Candida* spp. were the most common type of yeast isolated. Plants treating large domestic loads generally produce yeasts of gastrointestinal and faecal origin (Cooke, 1958) This has been confirmed by the present research which showed most of the isolates to be common clinical pathogens (Table 5).

Algae are known to be common inhabitants in most water systems, and are known to play a role in the treatment of waste e.g., sewage using stabilisation ponds (Palmer, 1980) but little is known about their contribution in the activated sludge process. The algae detected were of the common fresh and polluted water types (Table 6). The high frequency of occurrence of *Chlorella* spp., *Anabaena* spp. and *Volvox* spp. was similar to most fresh-water systems.

In most activated sludges several higher organisms such as protozoans and rotifers may be found together with the bacteria that form the flocs. They feed on bacterial cells that are free in the liquid and at the edge of flocs thus contributing to clarification and subsequently to improved effluent quality (Curds and Cockburn, 1970; Eikelboom and Van Buijsen, 1983). Microscope observations of samples from the 10 plants showed many different types of protozoans (Table 7); the most common type being *Paramecium* spp., *Aspidisca* spp., *Euplotes* spp., and *Amoeba* spp. which were mainly free-swimming and crawling over the floc surface (Gray, 1990). The predominant species identified in activated sludge belonged to the class *Ciliata* although flagellates and rhizopods were also present when considered together as a single group. Contrary to the findings of Curds (1982) that the activated sludge process selects for sedentary forms, the present work has shown that the free-swimming ciliates predominated. This could be attributed to the high degree of agitation in the aeration basin, thus preventing surface attachment of the sedentary protozoans. This type of environment proved more suitable to free-swimming ciliates. The information obtained during the present survey provided the foundation for further investigations emphasising the role of individual taxons and the entire microbiota in promulgating the activated sludge process. The organisms present could be further engineered to enhance efficient functioning of the process. The survey has enriched our understanding of the microbiology of activated sludge in the Natal region and facilitated global comparison of studies of a similar nature.

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