

# 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, Arden 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). Floes are the basic ecological units of activated sludges. Heterotrophic bacteria form the basis of floes 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 floes, 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 floes 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 ( $\pm 1000$  ml) 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 ml (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 ml 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<sup>-1</sup> to disperse floes. Serial dilutions (10<sup>-1</sup> to 10<sup>-6</sup>) of samples were prepared. Triplicate, 0,1 ml volumes from the 10<sup>-4</sup> to the 10<sup>-6</sup> 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<sup>-1</sup> (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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