

Isolation and Identification of Bacteria Present in the Activated Sludge Unit, in the Treatment of Industrial Waste Water

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ABSTRACT

Activated sludge or extended aeration treatment involves a continuous system where aerobic biological growths are mixed with waste water and then separated in a gravity clarifier. Therefore, waste treatment system such as the activated sludge system depend on the activities of communities of living organism. In this study, an attempt was made on the identification of the bacterial population involved in the laboratory activated sludge unit. The results showed that gram-negative bacilli with a yellow pigment was considered as a major group of the population.

INTRODUCTION

The identification of microorganisms involves comparison of an unknown microbe with similar microbes that are already known, thus eventually the former unknown is named. Both processes depend on adequate information for characterizing the known unit. Many methods have been proposed for making such information readily available, some are based on the use of dichotomous keys, and others on diagnostic keys and tables.

The majority of bacteria in activated sludge belong to gram-negative genera. The principal genera are *Achromobacter*, *Alcaligenes*, *Bacillus*, *Flavobacterium*, *Micrococcus* and *Pseudomonas*. Others dominating in sludge treating industrial effluents, include *Thiobacillus*, *Acinetobacter*, *Achromobacter* and the nitrifying organism *Nitrosomonas* and *Nitrobacter* (3,4,5).

The population of coliform of raw sewage predominantly consisted of *Escherichia coli*, whereas in activated sludge and effluent, the proportion of this species declined with simultaneous increase in proportion of *Achromobacter* and *Escherichia* other than *Escherichia coli* (1). The aim of this study was to isolate and to identify the bacteria which may be present in the activated sludge unit.

MATERIALS AND METHODS

All the chemicals used were Analar grade, the media used was either Oxoid or Difco. Distilled water was used to prepare all solutions.

Using an aseptic technique, the standard dilution methods for recovery of bacteria from activated sludge was used. Primary isolation was effected by streaking sample on the surface of a dry nutrient agar plate. Such plates were then incubated at 30°C for 36 hours. Single colonies were removed from these plates and subcultured on new nutrient agar plates. This procedure was repeated to obtain pure cultures.

The various characterization and biochemical tests were carried out to assist in the taxonomy of isolated organisms. According to the standard method the outline of morphological and physiological characteristics such as Gram-reaction, spore stain, motility, pigment production are shown in the tables of the results section.

RESULTS

The dominant bacteria were isolated from the cultures on nutrient agar plates. The quantitative estimates of the microbial population are shown in Table 1.

The morphological and physiological characteristics of the cultures are given in Table 2; As it shown out of twenty isolates, two of them were gram-positive. Most of the strains produce pigments on nutrient agar plates, with yellow pigmented bacteria predominant.

Further examinations were carried out on the cultures (Table 3,4). The comparison of results with Bergey Manual showed that of the thirteen gram-negative bacilli isolated, ten (Nos. 1, 2, 4, 6, 7, 9, 14, 16, 18 and 20) were identified as genus of *Flavobacterium* and three (Nos. 12,17 and 11) were identified as genus of *Alcaligenes*. High percentages of similarities were found with *Flavobacterium aquatile*, more than with other species of the genus, and with *Alcaligenes faecalis* more than with other species of genus *Alcaligenes*.

The other gram-negative rods (Nos. 3, 5, 15 and 19) were identified as genus *Pseudomonas*. High percentage similarities were found with *Pseudomonas stutzeri*, more than other species of genus *Pseudomonas*.

Two strains of gram-positive cocci (Nos. 10,13), were identified as genus of *Micrococcus*. High percentage similarities were found with *Micrococcus luteus*, more than with other species of the genus.

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Table 1. Bacterial counts of samples taken from the activated sludge unit. Bacteria were isolated in pour plates on nutrient agar plates

Dilution	Viable Colony Counts	
	Sample 1	Sample 2
10 ⁻¹	>300	>300
10 ⁻²	>300	>300
10 ⁻³	286	282
10 ⁻⁴	81	76
10 ⁻⁵	10	12
10 ⁻⁶	2	4

Table 2. An outline of the morphological and physiological characteristics of bacteria isolated from the activated sludge unit

Sample No.	Morphology	Gram reaction	Spores	Growth anaerobically	Motility	Pigment	Carbohydrate (F/O/-)
1	Rod	-	-	-	-	Yellow	-
2	Rod	-	-	-	-	Yellow	-
3	Rod	-	-	-	+	Yellow	0
4	Rod	-	-	-	-	Yellow	-
5	Rod	-	-	-	+	Yellow	-
6	Rod	-	-	-	-	Yellow	-
7	Rod	-	-	-	-	Yellow	F
8	Rod	-	-	+	+	White	-
9	Rod	-	-	-	-	Yellow	-
10	Coccus	+	-	+	-	Yellow	0
11	Rod	-	-	+	+	-	-
12	Rod	-	-	+	+	-	-
13	Coccus	+	-	+	-	Yellow	0
14	Rod	-	-	+	+	Yellow	-
15	Rod	-	-	-	+	Yellow	0
16	Rod	-	-	-	-	Yellow	-
17	Rod	-	-	+	+	-	-
18	Rod	-	-	-	-	Yellow	-
19	Rod	-	-	-	+	Yellow	0
20	Rod	-	-	-	-	Yellow	-

F=fermentative; O=Oxidative; - = no action

Table 3. Characteristics of isolated bacteria in peptone water and nutrient broth sugars for acid production

Sample No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Glucose	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	-	-	+	-
Sucrose	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-
Lactose	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-
Maltose	+	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
Manniteol	-	-	+	-	-	-	-	+	+	-	-	-	+	-	+	-	-	-	+	-
Xylase	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-

Table 4. An outline of the tests used in classifying the isolated dominant bacteria from the activated sludge unit

Sample No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Oxidase	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Methyl red test	-	-	+	-	-	-	-	+	-	0	-	-	0	-	-	-	-	-	+	-
Indol formation	-	-	-	-	-	-	-	+	-	0	-	-	0	-	-	-	-	-	-	-
Voges-Proskauer test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelatin hydrolysis	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	+	-	+	-	+
Strach hydrolysis	-	+	+	+	+	-	-	-	+	-	-	+	+	-	+	-	-	-	+	-
Chitin hydrolysis	-	-	-	-	-	-	-	-	-	0	-	-	0	-	-	-	-	-	-	-
Casein hydrolysis	+	+	-	+	-	+	+	+	+	-	+	-	-	-	-	+	+	+	-	+
Aesculin hydrolysis	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Citrate utilization	-	-	+	-	+	-	-	+	-	+	-	-	-	-	+	-	-	+	+	-
H ₂ S from TSI	-	-	+	-	-	+	-	-	-	0	-	-	0	-	+	-	-	-	-	-
Urease production	-	-	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
Ammonia from peptone water	+	+	+	+	-	+	+	-	+	-	+	+	-	+	+	+	+	+	+	-
Phenylalanine	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-
Nitrate to Nitrite	-	-	+	-	+	+	-	+	-	+	+	+	+	+	+	-	+	-	+	-
Arginine decarboxylase	+	-	-	-	-	-	-	+	-	0	-	-	0	-	-	-	-	-	-	+
Lysin decarboxylase	-	-	-	-	-	-	-	+	-	0	-	-	0	-	-	-	-	-	-	-
Ornithine decarboxylase	-	-	-	-	-	-	-	+	-	0	-	-	0	-	-	-	-	-	-	-
OPNG	-	-	+	-	-	-	-	+	-	0	-	-	0	-	+	-	-	-	+	-

0 = not tested

DISCUSSION

The bacteria predominant in activated sludges are largely derived from water courses. Basically the bacteria are responsible for the degradation of organic and inorganic

compounds. They derive their nutritional requirement from the compounds presented to them in the influent waste. They are able to synthesize their enzymes, metabolic intermediates, structural proteins, lipids and nucleic acids from carbon compound in the feed, together with other elements.

The derive their energy from oxidizing either organic compounds (chemoorganotrophic metabolism), or inorganic compounds (chemolithotrophic metabolism), such as reduced

sulfur or nitrogen compounds. They use the energy for their bodily functions, reproduction and growth.

Many research reported that gram-negative bacteria constituted the most genera of bacteria isolated from activated sludge (5,2). The results of this study also indicated that gram-negative bacillus bacteria constituted the majority of bacteria in the activated sludge. In this study the majority of the isolated gram-negative bacteria belonged to the genus *Flavobacterium*, while 22% of isolated belonged to genus *Pseudomonas*. The presence of gram-positive bacteria has been reported by some worker. In this study the only gram-positive found from the activated sludge belonged to the genus *Micrococcus*.

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