

RESPIRATION RATES ON VARIOUS NITROGENOUS SUBSTRATES BY
ZOOGLAEA RAMIGERA—A BACTERIUM FOUND IN AEROBIC PHASES OF SEWAGE TREATMENT

By

Lirvil Gene Rich

An ABSTRACT of Thesis Submitted to the Graduate Committee in
Partial Fulfillment of the Requirements for the Degree of
DOCTOR OF PHILOSOPHY

in

BIOCHEMISTRY

Approved:

~~_____~~
In Charge of Major Work

~~_____~~
Head of Department

~~_____~~
Dean of Applied Science

~~_____~~
Director of Graduate Studies

Virginia Polytechnic Institute
Blacksburg, Virginia

1951

The investigation herein reported is an attempt to obtain some basic facts concerning the role of bacteria in biochemical oxidation of sewage through a study of the food preferences of the bacterium, Zoogloea ramigera (Kruse). This bacterium is reported to represent the most prominent species of bacteria found in both the activated sludge tanks and the trickling filter units of conventional sewage treatment plants. Up to the present time, however, very little investigation has been made of the organism. Long confused with other members of the zoogloal population with which it is associated, it has only recently been included in Bergey's Manual of Determinative Bacteriology, where it is listed in the Class Schizomycetes of the Order Eubacteriales.

The bacterium used in the investigation was obtained from biological slime scraped from stone at a six inch depth of the trickling filter unit of the Virginia Polytechnic Institute sewage treatment plant. Isolation of the bacterium was accomplished by a dilution technique. Because the morphological and biochemical characteristics displayed by cultures of the isolated organism were very similar to those ascribed to Zoogloea ramigera by Bergey's Manual, the bacterium was identified as a variety of the same species.

The organism was cultivated in a synthetic medium having the chemical composition of an average domestic sewage fortified with a 1% yeast extract content to supply necessary growth factors. In preparation for investigation, the bacteria from a 24 hour culture was harvested by centrifugation at 4000 rpm and was resuspended and washed three times in buffer solution (pH 6.8).

The food preferences of Zoogloea ramigera were investigated by measuring the rate of oxygen consumption by the organism in the presence of a

variety of nitrogenous substrates, on the theory that, since the organism is strictly aerobic, a large proportion of the metabolic mechanisms involved in the breakdown of such organic material would be of an aerobic nature. The oxygen consumption was determined by measuring the change in the dissolved oxygen concentration of the bacterial suspension by means of the dropping mercury electrode method of analysis. Although this method, called polarography, has a wide application in the field of inorganic and organic chemical analysis, very little use of it has been made in respiration studies.

The apparatus used in polarography is composed of a continual flow of mercury drops as the cathode, a quiet mercury pool as the anode, and an electrical circuit capable of measuring the current when a potential is established between the electrodes. Since the amount of current is directly proportional to the amount of dissolved oxygen present in the solutions in which the electrodes are immersed, periodic reading of the current flow will determine the rate of oxygen respiration of whatever micro-organism may be present. In the present investigation a 5 unit dropping mercury electrode assembly was devised to minimize the time limitation imposed by using a single unit.

The respiration rates of Zoogloea ramigera were observed on glucose, buffer solution alone, and on a variety of proteins, proteoses, peptones, amino acids, and other nitrogenous substrates. From these observations, the following conclusions were made:

1. Polarography can be successfully adapted to the study of bacterial respiration rates if a multiple dropping mercury electrode assembly is employed.
2. The respiration of bacteria is independent of the oxygen

tension in their suspensions for oxygen concentrations down to at least 1.50 p.p.m.

3. Zoogloea ramigera demonstrates a marked preference for proteoses and peptones as substrates over both purified proteins and individual amino acids.
4. Zoogloea ramigera is capable of degrading purified proteins, and there is as much difference between the rates of decomposition of different proteins of animal origin as between the rates of the decomposition of animal and plant proteins.
5. The respiration of Zoogloea ramigera on both glucose and urea, if it occurs at all, is slight.
6. Ammonia in concentrations of 25 p.p.m. or more inhibits the normal respiration of Zoogloea ramigera.