ADVANCES IN

Applied Microbiology

Edited by WAYNE W. UMBREIT

VOLUME 2

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Department of Bacteriology
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PREFACE

The response to Volume I having been favorable, we have expanded Volume II to cover a larger area of interest to the applied microbiologist. We find that interest in this area of knowledge is expanding rapidly and that *Advances* is serving as a means of communication between diverse groups.

We have also included in this volume a recent symposium on engineering practice. To facilitate the publication of this section, we have left the papers in the style in which they were presented, rather than insisting that they conform to our more usual rules. We think that this is further evidence that *Advances* can be, even more than it is at present, a flexible tool to serve the needs of the applied microbiologist for modern, sound, and basic information.

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March 15, 1960

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Newer Aspects of Waste Treatment

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I. Introduction

Waste treatment is a means of maintaining or recovering man's most precious and most abused natural resource, fresh water. Fresh water supplies were all-important in the establishment and growth of civilizations. Much of man's bitterest fighting has been incited by altercations over water rights, and the course of history may well be written around the theme of primitive and modern man's need for water.

[1] Dr. Nandor Porges, until his sudden death in April 1959, headed the U. S. Department of Agriculture's research on dairy wastes at the Eastern Utilization Research and Development Division of the Agricultural Research Service, in Philadelphia, Pennsylvania. A native of Hungary, Dr. Porges was a graduate of the University of Massachusetts, and received his master of science and doctor of philosophy degrees at Rutgers University. He is best known for his work in developing a simple, inexpensive process for the disposal of dairy wastes. For this achievement the Department of Agriculture honored his unit with its Superior Service Award. Other honors bestowed on Dr. Porges include the Chilean Nitrate of Soda Fellowships 1927–1929, Sigma Xi honor for research 1931, and membership in Phi Tau Sigma the honor society of food science 1936.

[2] Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

Dependence on rivers and streams increased as civilization progressed. Waterways became extremely important as sources of potable water as well as highways for travel. Streams became the center of domestic activities such as bathing, washing, animal watering, and waste disposal. Quite naturally, then, the abuse of this resource with disregard to fellowman began early in history. As stream pollution led to the spread of disease, the necessity of water purification and of sewage treatment began to be realized.

Modern industrialized and concentrated centers of civilization require enormous quantities of water and produce prodigious amounts of waste water. Intelligent maintenance of this water supply is a duty and a necessity. In this respect, man has been criminal against himself. Indeed, as science and industry grew, so did neglect and defilement of this essential commodity. Only recently, late in the history of our industrial expansion, has attention been turned to the conservation of water as a natural resource. In many cases, however, legislation and the threat of fines have been necessary to force correction of conditions contributing to stream pollution.

II. Aftermath of Dumping Waste into a Body of Water

The most disastrous and immediate consequence of dumping wastes into a stream is the threat to public health. Communities located down stream from where raw sewage and wastes enter are menaced by possible outbreaks of water-borne diseases that could reach epidemic proportions.

The health of the stream itself, as indicated by its aquatic life, is also affected by indiscriminate waste practices. Biologically speaking, a normal stream supports a teeming population of microorganisms, plants and animals dependent upon each other for food and upon the stream for oxygen. An adequate, dissolved oxygen content is usually maintained by natural physical reaeration of the surface waters. Under normal conditions, this process is able to replace all the oxygen lost to microbial respiration. When an extra load of organic impurities such as sewage and industrial wastes stimulates microbial growth, the supply of dissolved oxygen is quickly exhausted and cannot be replaced rapidly enough. Every stream is thus limited in its capacity to assimilate organic wastes. In many cases, organic pollution leads to a temporarily imbalanced stream condition within a localized area, with eventual recovery effected by natural reaeration. In extreme cases, recovery does not take place, vegetation and fish are destroyed, and the polluted stream becomes an open sewer with its concomitant stenches and disagreeable appearance spoiling the economic and esthetic value of the stream and its environs.

The detrimental effect of industrial organic pollutants on a body of water can be illustrated by examining the effects of the waste waters of a small dairy. An average daily waste load may contain the equivalent of 100 pounds of dried skim milk, a well-balanced food readily utilized by microorganisms. Complete combustion of this amount of milk requires about 105 pounds of oxygen. The quantity of aerated water necessary to satisfy this ultimate oxygen demand depends upon the temperature of the water. At 25° C., 8.4 parts of oxygen are dissolved in a million parts of water; hence, 12.5 million pounds of water, or practically 1.5 million gallons, will contain 105 pounds of oxygen. This relatively small amount of organic matter would require all the oxygen in a circular pond 6 feet deep with a diameter of 206 feet, or a pond of the same depth, 100 feet wide and 334 feet long. When sufficient oxygen is not available, disagreeable anaerobic conditions set in and lead to gross pollution harmful to life associated with clean streams.

III. A Glance at Waste-Treatment Procedures

Details of waste treatment are available in specific texts such as "Sewage Treatment" (Imhoff and Fair, 1947), "Stream Sanitation" (Phelps, 1944), "Industrial Wastes" (Rudolfs, 1953), "Bio-Oxidation of Organic Wastes; Theory and Design" (Eckenfelder and O'Connor, 1958), and others. There are also excellent reviews of literature published yearly on sewage, waste treatment, and water pollution by the Federation of Sewage and Industrial Wastes Association Committee on Research in *Sewage and Industrial Wastes* (R. E. Fuhrman, ed.), now in its thirty-first volume. Since many of us in the field of applied microbiology do not have the need or inclination to make a detailed study, a brief glance at waste treatment follows.

Offensive and potentially dangerous wastes are transported from household and industry by the simple and economical water-carriage system. The next step, the removal of these wastes from the water before passing into natural waterways, is accomplished in sewage-treatment plants (Imhoff and Fair, 1947). Strange as it seems, this dirty-looking water that may contain color, and suspended and soluble material, is usually over 99.9% pure water. Very few wastes entering treatment plants exceed solids concentrations of 1,000 mg. per liter, or 0.1%. Removal or stabilization of the waste is done by various methods or combinations of methods but depends mostly on the aerobic and anaerobic activity of microorganisms.

The principles, applications, and design of aerobic oxidation are discussed in a bound series of 33 contributed papers (McCabe and Eckenfelder, 1956). A similar volume of 28 papers covers anaerobic digestion and solids-liquid separation (McCabe and Eckenfelder, 1958). As the carriage-water and its load enters the treatment plant, the floating matter and coarse suspended material are removed by racks and screens. After being shredded and ground, the comminuted matter may be returned to the flowing sewage which passes through a grit chamber, if necessary, to allow sand, grit, and heavy mineral solids to settle. Otherwise, the collected material is buried, incinerated, or digested.

The sewage or carriage-water with its load of finely suspended and soluble matter enters a primary settling tank and is detained for a short while to permit sedimentation of settleable solids. The settled solids are pumped to a sludge-digestion chamber to undergo anaerobic or aerobic digestion. The residue is filtered, dried, and incinerated, or used otherwise. Conversely, the entire sewage may be treated anaerobically.

The carriage-water leaving the primary sedimentation basin usually undergoes aerobic biological treatment for stabilization of the organic matter still in suspension and in solution. This is done in various ways. If there is a limited quantity, it can undergo land treatment by irrigation or by filtration through sandy soil. Aerobic conditions are maintained by intermittent filling and emptying. Another means of removal and stabilization is by passing the liquid through filters consisting of stone or other material. The slimes of living microorganisms covering the contact material remove the organic matter as the liquid trickles through the bed. Aerobic conditions are maintained by passing air through the bed or by intermittent flow of the liquid.

A method extensively used for stabilization is the activated-sludge process in which a sludge floc is maintained in suspension by air diffused into a flowing mixture of sewage and floc. Mechanical agitation may also be used. Activated sludge is the accumulation of floc produced by the growth of zoogleal bacteria and other organisms in the presence of dissolved oxygen. Removal of soluble and suspended matter is achieved by the living mass of microorganisms maintained under aerobic conditions.

The sludge floc which has oxidized or removed the waste material is removed from the carriage-water in a secondary settling tank where the stabilized sludge settles. Various engineering designs are in use to assure settling and thickening of the sludge. A calculated amount of the settled sludge is returned to the aeration tank for re-use. The remaining or excess sludge is mixed with the solids from the primary settling tank and pumped to the digestion tank.

The carriage-water, now free of its load, leaves the final settling tank as a clear liquid. It may be chlorinated before passing into the receiving stream. An extra step may be desirable in which the clear treated effluent is aerated again to reduce the demand on the oxygen of the stream.

The excess sludge, consisting primarily of microbial cells, is conditioned further by anaerobic or aerobic digestion. (In some cases, digestion is replaced by drying and spreading on soil, by hauling to sea, or by disposal in some other manner.) During digestion, the tank contents are kept at

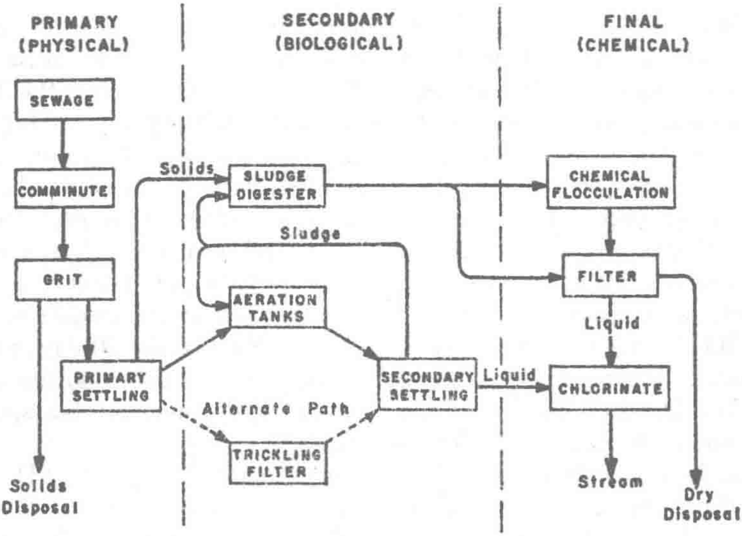

FIG. 1. Major steps in sewage treatment.

a favorable temperature. Methane gas formed in the digestion process is often burned to supply heat for this purpose. The solids are reduced and the dewatering characteristics of the sludge greatly improved by this step, sometimes by the addition of chemicals. The digested sludge is removed from the water by filtration. After this dewatering, the sludge may be air- or heat-dried and prepared for use as a fertilizer, or it may be incinerated. The separated liquid is returned through the process with the entering sewage.

Thus, treatment may be completely aerobic or completely anaerobic but usually consists of a combination of both types plus intricate mechanical and engineering features. Each treatment plant is tailored for its specific service according to the plans of the sanitary engineer, who should be cognizant of the biological processes involved. The flow diagram of Fig. 1 is a composite of essentials of the process, showing the primary or mechanical treatment, the secondary or biological treatment, and the tertiary or chemical treatment. The goal of treatment is a clear effluent, low in offensive solids and in oxygen demand and harmless to aquatic life.

IV. Problem of Concentrated Industrial Wastes

The more or less elaborate processes touched upon in the previous section on waste-treatment procedures work well with simple municipal wastes that are predominantly of household origin or with wastes of similar strength. Such wastes are further diluted by the carriage-water so that they enter the treatment plant with low concentrations of organic matter. The average strength of a municipal waste may be about 180 parts per million, or 180 mg. per liter of 5-day B.O.D. (The B.O.D. is the biochemical oxygen demand, or the quantity of oxygen utilized by the microorganisms in the biochemical oxidation of the organic matter in the waste, as determined under standard conditions at 20° C. The 5-day B.O.D. is the oxygen utilized in 5 days' incubation. The ultimate oxygen demand is usually considered as the 20-day B.O.D. and in a few wastes such as dairy wastes may be approximated by the C.O.D., or chemical oxygen demand, as determined by various methods of chromate oxidation. The 5-day B.O.D. has been generally accepted as 68.5% of the 20-day B.O.D. for sewage (Phelps, 1944), although this may vary. Thus, 1.46 times the 5-day B.O.D. will approximate the ultimate oxygen demand, but specific values should be determined for each waste.)

It has been calculated that the average amount of 5-day B.O.D. contributed per capita per day is 0.167 pound or about 75 to 76 gm. (Imhoff and Fair, 1947). The ultimate oxygen demand per capita is then about 0.244 pound or 110 gm. It is possible to determine "population equivalents" of any industrial or concentrated waste. The amount of organic matter to be treated may also be estimated from the C.O.D. if it is realized that the C.O.D. varies with the organic substance. Thus, a unit weight of sugar has a C.O.D. of 1.07 and a unit weight of protein, a C.O.D. of 1.44 (Porges *et al.*, 1950). From the average of these values, the 110 gm. of C.O.D. is equivalent to 88 gm. of dry organic matter. When this is diluted in 419 liters of water, the concentration will be 180 mg. per liter of 5-day B.O.D. More often, the waste is more dilute, allowing treatment to be accomplished easily and yielding clear effluents low in oxygen demand that may be discharged into a receiving stream.

As industries grew, the wastes received by many municipalities did not respond to treatment, and the plants were unable to cope with the extra load. Collections of data on sources of pollution have been made, and strengths of many industrial wastes were calculated in terms of their population equivalents (U. S. Public Health Service, 1944; Phelps, 1944). In many communities the industrial wastes impose a greater pollution load than that of the population itself. Selected values are shown in Table I. The daily population equivalent of the more important oxygen-demanding wastes of this country was estimated to be 134,300,000 in 1949. This does not include the added load supplied by small industries such as dairies and laundries (Rudolfs, 1953). Not only is the population equivalent of industrial wastes greater than that of municipal wastes, but the actual concentration is greater. Dairies average about 1,200 mg. per liter B.O.D.,

TABLE I
POLLUTING EFFECT OF INDUSTRIAL WASTES

Population equivalents	Source of waste	Amount handled or made
8	Dairy plant	100 lb. of milk
14	Brewery	Barrel of beer
21	Abattoir	One animal
24	Laundry	100 lb. of clothes
1,690	Straw board	Ton of paper
4,600	Sulfite pulp	Ton of paper

canneries have slightly less, but wastes from antibiotic-producing plants may exceed 13,000 mg. per liter B.O.D.

We can imagine the difficulties that sewage plant operators have when a treatment plant designed to handle wastes with a concentration of 200 mg. per liter B.O.D. suddenly receives a large volume of industrial waste with a B.O.D. of 1,000 mg. per liter. The plant becomes overloaded, incoming sewage is not treated properly, odors develop, the carriage-water is not purified, and a breakdown of the whole process may occur. Reestablishment of the proper activities in the aeration tanks may require weeks and may be almost impossible if strong wastes continue to be received.

V. Laboratory Approach to Problem

Lack of understanding of the biochemistry and microbiology involved in the stabilization of the suspended and soluble waste often leads to difficulties in waste treatment. Treatment plants were often constructed on empirical information that disregarded the effect of waste concentration and toxicity of influents. Aerobic treatment of waste waters has been practiced for about a half a century. Fortunately, domestic waste is a well-balanced biological mixture, and little or no difficulty was encountered in its treatment. The increase in volume and types of industrial wastes, treated with municipal wastes or separately, called for an application of knowledge concerning the biochemistry of treatment.

The action of microorganisms is primarily responsible for the purification of the carriage-water. Rapid purification depends on the unrestricted activities and reproduction of these organisms. The best growth and purification occur when the organic waste is nutritionally balanced. Extensive studies have been made on this phase by various workers and have been reviewed (Sawyer, 1956). The importance of oxygen has been stressed and detailed (Eckenfelder and Weston, 1956; Porges *et al.*, 1953).

Waste treatment involves the handling of relatively large quantities of dilute material. The microorganisms are usually present in higher concentrations than the waste itself. Sludge concentrations in an aeration chamber may be about 2,000 mg. per liter, while that of the organic matter may be only 200 mg. per liter.

In this discussion, the importance of selected laboratory studies in devising successful treatment of various wastes is emphasized. The biochemical oxidation of this waste is a study of the problem of propagation of microorganisms in a dilute solution. Laboratory data obtained on dairy wastes (Porges, 1956, 1958a, b) were translated to studies for a pilot plant treating 10,000 gallons of waste daily (Kountz, 1953). The results are being applied in developing satisfactory designs for the aerobic treatment of various industrial wastes (Kountz, 1954; Eckenfelder and O'Connor, 1958).

VI. Nutrient Requirements

Stabilization of a liquid waste entails the conversion of the soluble material to a removable insoluble substance, gas, and water, and depends upon the nutrition and growth of microorganisms that have the ability to gather the food supply and minerals to produce new generations of cells. Carbon, nitrogen, and phosphorus are required and occur in most wastes. The general change involving the use of the organic matter for cell synthesis and for energy may be shown as:

$$\text{Organic waste} + \text{oxygen} + \text{bacteria} \rightarrow \text{more bacteria} + CO_2 + H_2O \qquad (1)$$

The deficiency of nitrogen and of phosphorus in many industrial wastes such as those obtained from cotton, rope-making and paper-making plants, breweries, and other factories, required supplementation with these elements (Sawyer, 1956). Wastes from slaughter houses and tanneries contain nitrogen in excess of that required for stabilization by activated sludge. In such cases, other problems may arise due to nitrification, which may prevent sludge from settling and interfere with its removal.

The carbon to nitrogen ratio and the carbon to phosphorus ratio become of importance. Waste treatment workers express this as the 5-day B.O.D. to N ratio. The relationship between carbon, C.O.D., and 5-day B.O.D. is 12, 32, and 21.9 for ordinary wastes. When necessary, available nitrogen may be supplied from inorganic sources, or domestic sewage may be admixed with the industrial waste. The sludge itself may serve as a nitrogen source under certain conditions (Porges *et al.*, 1955).

Various studies showed that a B.O.D. to N ratio of 17 to 1 was optimum for stabilization of low nitrogen wastes in the presence of sewage (Helmers *et al.*, 1952). In terms of ultimate or chemical oxygen demand, the C.O.D.