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STUDIES ON THE AEROBIC BACTERIOLOGY OF WASTE STABILIZATION PONDS

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CHAPTER I

INTRODUCTION

The need for a healthful environment is common to all peoples. It cuts across boundaries of occupation, race, class, and politics. If it differs from neighborhood to neighborhood, and from region to region, it differs not in fundamentals but only in complexity. When we speak of a healthful environment we think mainly of safe, wholesome, potable water, of a healthful diet, of clean air, and of shelter for our families where they live, work, and rest.

The relationship between health and economics is not simple. Until recent years no economic value was attached to the human resources of a country, nor were they included in natural resources surveys. However, it is now recognized that health can be made a positive economic factor rather than a neutral or even a negative one. While it may be assumed generally that every investment in health is returned many times over to the economy, it is practical to ask how to invest money in health so as to secure the greatest return per dollar. To determine if the gain from sanitation is worth the cost is not to question if it pays to save a life. The question is how, with available resources, investment in sanitation can secure a satisfactory return in the abundance and richness of human life.

One of the major items in any program for sanitation is the double problem of water supply and waste water disposal. These two are so closely interrelated that they may very appropriately be discussed together.

The World Health Organization's (1) proposed program and budget estimates for the year 1952 stated:

> The ravages of water-born insect-carried and excreta-transmitted diseases outweigh in economic and public health importance those of almost any other group of diseases. Their control is based on universally accepted principles of sanitation and hygiene. Their origins are nondebatable; their epidemiology has long been known. The cost of correction although significant, are often not insuperable if ingenuity and imagination are applied. It is evident this is work which will yield very large returns.

The installation of a public water supply system in advance of public sewers is a serious sanitary error. Sewers should always accompany public water supply and be regarded as a part of one unified system.

Water purification plants from a public health standpoint are the last line of defense in providing people with water free of contamination. These plants, as good as they are, are limited in their capacity to remove "disease organisms" from polluted water and may be unable to remove toxic or poisonous matter that may result from heavy pollution. Sometimes the water from rivers, lakes, or wells carry too much pollution; then the "organisms" and toxic materials can slip through the water treatment defenses into drinking water.

The typhoid death rate has always been considered to be an accurate barometer of sanitary status as this is the only water-borne disease which can be considered to be reported with any degree of accuracy. The typhoid death rate has dropped to less than one per million in the United States urban population. The qualitative change that has taken place

according to Okum (2) may be better appreciated if it is realized that at the turn of the century a student at a large university could expect to see several of his classmates die of typhoid during his 4-year period of study. Now the rate in the United States is so low that a death from this disease would hardly be expected to occur in a student body once in 200 years.

The situation is similar with respect to other sewage-borne diseases such as: cholera, amebic and bacillary dysentery, gastroenteritis, infectious hepatitis, poliomylitis, schistosomiasis, and tularemia.

Thusfar waste water has been considered from the standpoint of mortality only. There are other aspects which should be considered. The waterways of the country has always been one of its greatest national resources. Today most lakes, streams, bays, and rivers in populated areas of the world are being degraded more and more by man-made pollution.

Many of the shellfish areas of the United States have become subject to an increasing degree of pollution due to the practice of using rivers and harbors as vehicles for the disposal of waste water from the communities bordering thereon. Such pollution has become so great in certain areas that authorities have been obliged to prohibit the taking of shellfish for food purposes from extensive areas. In this way each year millions of dollars worth of food is lost.

Waste water is potentially and actually dangerous, a fact which has been demonstrated on numerous occasions. Its danger, however, consists chiefly in the disease-producing organisms which may be present. Since domestic drainage contains or may contain the various discharges of humans and since these discharges are vehicles of certain infections, it

must always be regarded as dangerous.

Inasmuch as waste water may at anytime contain any or all of the discharges of the animal body, and furthermore, since it may contain actively infectious materials, its safe and proper disposal is one of the first necessities of sanitary science and public health. Thus, waste treatment is one of the ways by which health protection can be maintained. Waste treatment works are now an essential element in providing the people with a safe environment.

What to do about waste water treatment facilities for a small town of from 1,000 to 10,000 population is a problem that often faces public health officials. Most towns with a population of 1,000 to 10,000 cannot afford a costly waste water treatment plant and often could not provide for adequate operation even if they could afford the plant. However, with increasing population and the resulting increase in stream pollution, the improper treatment of domestic drainage can no longer be condoned. These small towns find themselves hard pressed between the Department of Health on one side and lack of funds on the other. The need for a treatment method with both a low initial investment and a low cost of operation for these towns is a challenge which must be met.

A municipality not only considers the efficiency of a sewage disposal plant and construction cost, but the operation and maintenance cost must also be reasonable as these factors play an important part in the selection of the method of waste water disposal to be used. This selection requires consideration of many factors. Besides the conventional systems of waste water treatment, a relatively new method has recently gained widespread acceptance. This new method utilizes a device which is called a Waste Stabilization Pond (W.S.P.). Where

applicable the W.S.P. in many cases will save municipalities considerable in construction and operation costs.

Since land, especially in small towns, is relatively inexpensive compared to construction work involved in the building of a treatment plant, and since the actual construction cost of a W.S.P. is a very small figure, this type of treatment has been readily accepted by small communities.

The South Dakota State Department of Health (3) has obtained rather complete cost data on many W.S.P. installations in that state. For 15 South Dakota towns varying in population from 367 to 8,871 and averaging 1,102, land cost ranged from $950 to $12,375, averaging $3,558 per installation or $3.23 per capita. Construction cost varied from $4,276 to $31,000 averaging $14,385 per installation or $13 per capita. Total costs were from $5,276 to $42,139, averaging $17,943 per installation or $16.28 per capita. For the three largest communities (2,500 to 3,000 in population) per capita cost averaged $2.78 for land, $10.73 for construction, and $13.51 for the complete facility. For the smaller communities (below 400 in population) per capita prices averaged $11.38 for land, $7.83 for construction. Under conditions prevailing in South Dakota, W.S.P. for small communities can be provided at a per capita cost comparable to that for communities in the 2,500 range. Considering the necessary land, dike construction, sewer lines and appurtenances, the cost of a W.S.P. will commonly be 50 per cent or less than what a conventional plant would cost the community.

CHAPTER II

THE WASTE STABILIZATION POND

The terms lagoon and oxidation pond have been used interchangeably for all types of earthen ponds incorporated into waste treatment plants. The term "lagoon" has become well established in the states of North and South Dakota where they treat raw sewage. In other parts of the United States, for example in Texas and California, similar structures are referred to as "oxidation ponds". These states usually use oxidation ponds as secondary treatment plants. The Texas State Department of Health (4) defines: "Oxidation ponds are those ponds of regular and controlled shape, depth, and marginal area, specifically designed and constructed as a waste treatment devide. All other ponds incorporated into treatment plants, such as those formed by damming ravines and dry creeks, whose shape, depth, and marginal area are not controlled are classified as lagoons."

At a meeting attended by representatives of the United States Public Health Service and the states of South Dakota and California (5) it was suggested that the name "stabilization pond" be used for the sewage treatment devices heretofore called "lagoons" or "oxidation ponds". Because, from a chemical standpoint, the processes of both oxidation and reduction occur in such ponds, it is felt that stabilization pond seems a proper name for this sewage treatment device.

There seems to be two schools of thought for the application of waste stabilization ponds. One group feels that primary treatment of some approved type should precede the W.S.P. In other words, a stabilization pond should be considered only as a secondary treatment process. This view is held by the state boards of health in such states as Texas, Illinois, and California. Those who hold the other view, represented by North and South Dakota, are very outspoken in their belief that, if W.S.P. are to be used at all they should be designed to provide complete waste water treatment. The feeling is that one of the main advantages of the W.S.P., low or practically negligible maintenance, is defeated if the customary problems associated with primary plant operation must be retained.

The first prolonged attempts to treat sewage in oxidation ponds in the United States were carried out by Caldwell (6) in the southwest where sunlight and temperature were most favorable for maintaining suitable esthetic conditions. The City of San Antonio, Texas, is reported to have utilized the principle as early as 1901. In California, the satisfactory operation of a W.S.P. was first observed by Gillespie (7) at Santa Rosa in 1924. Aware of the success of this type of facility in the southwest, the community of Fessenden, North Dakota, began to discharge raw sewage into a diked slough in 1928 (8). Observation of the Fessenden pond indicated that satisfactory operation could be obtained in the northern parts of the United States regardless of severe winter conditions such as those characteristic of the Dakotas.

According to Mackenthem et al. (9), during 1955 Wisconsin became interested in W.S.P. and an initial group of four such units were constructed and placed in service during the latter part of 1956. Since

then, W.S.P. for the treatment of raw or partially pre-treated waste water have become very popular among the smaller municipalities and have sprung up in many parts of the country, especially in the Southwest, the Northern Plain states, and more recently in the Midwest.

Organic matter decomposes rapidly in water because of its ready availability to microorganisms and their enzymes. As the sewage enters the pond it is teeming with bacteria, most of which prefer aerobic conditions. When oxygen is available (oxygen being the ultimate hydrogen acceptor in the oxidation of organic substances), the saprophytic microorganisms convert complex suspended or dissolved organic compounds into simple substances such as carbon dioxide and ammonia. These substances, together with adequate light and nutrients are the principal factors involved in the development of the green algae generally found in the W.S.P.

The activity of algae that is usually cited in connection with the W.S.P. is the production of oxygen as a by-product of photosynthesis. This oxygen is then used to satisfy the Biochemical Oxygen Demand (BOD) of organic matter in waste water. BOD is defined as the quantity of oxygen required in a given time and at a given temperature to satisfy the combined biological and chemical oxidation demands of the waste water. The standard incubation period for the laboratory determination of BOD is 5 days at 20^{o} C. and indicates 68 per cent of the total ultimate oxygen demand (10). It is the standard test used for determining the strength of waste water.

Successful functioning of W.S.P. depends upon the establishment of biological populations, biotas, whose life processes will result in adequate waste treatment without production of obnoxious odors or other

undesirable features. Neel (5) offers a simplified version of the basic principles on which the W.S.P. is believed to operate. This food and energy cycle assumes the presence and the broad functions of the following general groups.

1. Bacteria subsist and grow upon decomposable organic compounds, furnishing as by-products nutrients necessary to algal growth.

2. Algae usually manufacture their own food by using the energy of light to combine hydrogen and carbon dioxide to form simple sugars. This process, photosynthesis, entracts the hydrogen from water and releases oxygen from water as a by-product. Nutrient elements (potassium, nitrogen, phosphorous, magnesium, etc.) required for algal growth are available in raw sewage.

3. Animal components feed upon bacteria, algae, each other, and waste solids. Their nutrition rests either directly or indirectly upon sewage elements.

The atmospheric oxygen is another resource which makes oxygen available for bacterial respiration. The treatment process occurring in the W.S.P. may therefore be said to result from a symbiosis of bacteria and algae.

However, the specific roles played by various members of the biota in reducing particular classes of wastes (carbonaceous, nitrogenous, phosphorous compounds, etc.) are at present incompletely understood. Many workers offer theories concerning the functioning of stabilization ponds but few of them satisfactorily explain all of the phenomena observed.

Before any further discussion can be made concerning the factors affecting purification, it may be well to consider briefly the properties

of algae.

During the breakdown of sewage organic matter by aerobic bacteria, algae grow abundantly in the presence of sunlight. Life processes of algae are the reverse of bacteria. Algae can utilize water and carbon dioxide with traces of potassium, nitrogen, and phosphorous to produce starch and free oxygen (11).

In accordance with the simplified photosynthetic equation ($2CO_2 + 2H_2O + \text{Light} \rightarrow 2(CH_2O) + O_2$) the production of cellular material is accompanied by a stoichiometric production of oxygen. The generally accepted pattern of this reaction is that oxygen is derived from the photochemical decomposition of the water molecule, rather than from a splitting of the carbon dioxide molecule. The CO_2 molecule serves as a hydrogen acceptor within the algal cell, resulting in production of a substance of the general formula (CH_2O) which may be termed "primary cell material". The oxygen is liberated outside the cell. Thus, water serves as a hydrogen donor for the reduction of CO_2 in algal photosynthesis, and the liberation of oxygen is incidental to the process.

The properties of the algal cell--that is, size, form, shape, density, color, rate of growth and photosynthesis, respiration rate, vitality, content of carbohydrate, fat, and the content of various mineral ions for two species of algae were subjected to a comprehensive study by Oswald et al. (12,13,14) at the University of California. They especially studied the properties of two important genera of algae which are usually dominant in W.S.P. in the Southwestern United States, namely Chlorella and Euglena.

Both Chlorella and Euglena were found to photosynthesize rapidly in a rich nutritional environment. Both tended to overextend their popula-

tion, and both passed through a microscopically observable series of morphological phases that remain remarkably constant when nutritional conditions were constant. Both algae entered a senescent phase that was marked by reduced photosynthetic capacity, yellow color, reduced chlorophyll content, and increased tendency toward cell enlargement with accompanying high respiration rate. These organisms (Chlorella and Euglena) were found to attain their maximum populations in continuous cultures operated at detention periods of 1 to 5 days depending on the amount of available light in the culture.

Young algae may release 20 times as much oxygen in photosynthesis as they utilize in metabolism. Quantatively, Oswald (15), reports, the growth of 1 gram of algae is usually accompanied by the production of a minimum of 1.6 grams of molecular oxygen.

Algae often play a dual role. As an example the green algae, Chlorella, metabolizes either as a phototroph in the light or as a facultative chemoorganotroph in the dark (16). Consequently, not only may the time of day affect the type of metabolism but also the depth at which the algal cell finds itself. The turbidity and the organic content of the water will jointly determine the extent to which the algae will be phototrophic or chemotrophic. However, numerous experiments have shown that neither Chlorella nor other common stabilization pond algae (Scenedesmus, Chlamydomonas, Ankistrodesmus) can grow oxidatively on sewage in the dark, so it must be concluded that the oxidation of organic matter in sewage is carried out by bacteria and that algae provide oxygen for continued bacterial oxidation (17).

Under optimum conditions the algal cells remain young, that is, they are maintained in the logarithmic phase of reproduction. Such cells

have relatively high concentrations of chlorophyll and protein, and low concentrations of carbohydrates and fats.

The effective factors in algal photosynthesis are: illumination, carbon dioxide, temperature, and nutrient. Any one of these may on occasion control the algal processes.

The importance of light upon the rate of oxygen production by an algal cell has been established. The visible portion of sunlight is the prime mover of photosynthesis (15). There is a maximum intensity of light which algal cells can utilize during a sustained period; therefore, all light energy supplied at a higher rate is partially wasted. This critical light intensity is different among species. Oswald (15) reports about 600 foot candles for Chlorella and 1200 foot candles for Euglena.

The effect of temperature on photosynthesis in the algae has not been given the critical study it deserves. Growth in Chlorella usually reaches a maximum between 25^{o} and 35^{o} C. In contrast there are several species which can grow on snow at temperatures very close to 0^{o} C. The major variation in algae of stabilization ponds, both qualitatively and quantitatively occur as seasonal variations. These seasonal variations are generally considered to be due to temperature changes (18).

The sustained photosynthesis of algae depends directly on the capacity of the culture medium to supply inorganic nutrients over an extended period of time at a rate sufficient to support the growth potential of the algae present. The rate of pick-up of CO_2 is rapid and approximately constant for the younger, rapidly growing cells, and decreases as the cells become older and grow more slowly.

Certain inorganic nutrients, specifically, Ca, Mg, K, Mn, NH_4^+, and PO_4^- are important in the nutrition of algae (19). It should be noted

that the quantities needed are different for species of algae and lie within a large range. A deficiency in any one of these substances may alter metabolism and affect the growth rate of the algae. The mere presence of these nutrients in the medium does not guarantee that they will be available to the algae.

It should be noted that algae can live in a considerable range of pH values. In W.S.P., pH values range from 8 to 10. Some investigators consider these high values to be due to the absorption of carbon dioxide from the water by the algae (20,21). Others (22) feel that this is not a satisfactory explanation believing that the pH reactions which occur in stabilization ponds are due to bacterial rather than algal activities. More complete information concerning the metabolic types of bacteria active in W.S.P. treatment is necessary before the complex action occurring in a W.S.P. can be completely understood.

Wastes entering a pond undergo reactions which simultaneously bring about their deposition in the form of sludge and their partial decomposition into carbon dioxide, ammonia, phosphate and other compounds. Once deposited, and if oxygen is available, sludge is decomposed aerobically and more of these compounds are formed. If little or no oxygen is available, the sludge may undergo partial or complete anaerobic decomposition. From the standpoint of odor, nuisance prevention and complete organic stabilization, the organic matter in the wastes being treated in W.S.P. is best decomposed by aerobic bacteria.

Historically, the bacteriology of waste treatment processes has not received the study it merits. Johnson (23), in 1914, noted the importance of bacteria in trickling filters when he observed that the filter medium in the upper layers of the filter became coated with a slimy growth of

organisms which he identified as Zoogloea ramigera. He concluded that "The zoogloea is perhaps the most characteristic and important organism of sewage treatment." His observation was not widely accepted, however, as other workers of this era tended to stress the importance of coli-aerogenes bacteria. For example, Harris et al. (24), in 1927, concluded that 61 per cent of the bacteria in activated sludge were of the Bacterium aerogenes type and 38 per cent were Proteus sp. Butterfield et al. (25), in 1937, revived the importance of the zoogloea and Wattie (26) in 1943 concluded that "The predominant bacteria of activated sludge and trickling filters have been isolated in pure culture. It would appear that the zoogloeal bacteria might be considered in one group." The uniformity of this group has since been disputed by McKinney (27). He reclassified the zoogloeal bacteria into many different genera and families and formed the opinion that a wide variety of organisms under suitable conditions could produce extracellular slime. James (28), in 1964, after a study of the bacteriology of trickling filters concluded that 90 per cent of his total counts consisted of Gram-negative rod-shaped bacteria belonging to the genera Achromobacter, Alcaligenes, Flavobacterium, Pseudomonas, and Zoogloea.

The bacteriology of stabilization ponds is made conspicuous by its absence from the literature. It would appear logical to assume that Zoogloea ramigera would not be of importance in W.S.P. treatment due to unfavorable environmental conditions encountered therein. The pH factor alone would limit the growth of this organism. Zoogloea ramigera has an optimum pH range of 7.0 to 7.4 (29) and few properly functioning W.S.P. operate at pH values below 7.5.

According to Oswald (30), the oxidation of sewage organic matter in

W.S.P. has been found experimentally to follow the reaction: $C_{11}H_{29}O_7N + 14O_2 + H^+ \rightarrow 11CO_2 + 13H_2O + NH_4^+$. If this equation is taken at face value, it strongly suggests that the responsible organisms are strictly oxidative in their metabolism. Wattie (26) found that the Zoogloea were fermentative in their metabolism of carbohydrates.

Oxidation of the ammonia formed in the equation rarely occurs in the W.S.P. In fact, it is common knowledge that the addition of nitrate to a malfunctioning pond is beneficial. This indicates that the active population has the ability to reduce nitrates, an ability the Zoogloea do not possess (29). The above discussion can be summarized by saying that: (a) The role of the Zoogloea in waste treatment has been over-estimated as indicated by James (28) or (b) It therefore appears that the environmental conditions in W.S.P. are such that Zoogloea are not a logical choice and qualitative studies should be carried out to determine the responsible organisms.

Encouragement of the process of aerobic oxidation of sewage requires that design parameters be selected which promote a large and varied bacterial population, a continued source of molecular oxygen, an ideal temperature, an optimum pH, a balanced nutrient source and adequate time for the process to become established. Although biological oxidation could result in complete oxidation of organic matter, it does not proceed to completion in W.S.P. because the sludge which reaches the pond bottom is in a zone usually void of molecular oxygen. Therefore, the deposited organic matter is no longer subject to aerobic oxidation but must be decomposed anaerobically or resuspended for aerobic oxidation.

Two types of anaerobic decomposition are involved in the W.S.P. (30). One form, sometimes known as putrefaction or acid-forming fermentation,

utilizes oxygen from organic matter itself or from the oxygen-rich anions, such as sulfates, and gives rise to hydrogen, carbon dioxide, hydrogen sulfide, and other odorous gases and to organic acids. The organic acids may accumulate in solution or serve as substrate for further decomposition. The occurrence of acid formation in sludge deposits is the primary cause of objectionable odors which may be omitted by the W.S.P.

Under ideal conditions, the organic acid products of putrefaction quickly undergo a second type of fermentation, known as alkaline or methane fermentation, which results in methane gas formation together with some carbon dioxide and hydrogen. The essential conditions for methane fermentation (30) are: an abundance of organic matter being continually converted to fermentable organic acids, an adequate population of methane bacteria, a pH level within the range of 6.5 to 7.5, alkalinity in quantities sufficient to buffer the organic acids, temperatures from 5^{o} to 60^{o} C., the lack of toxic substances, and a sustained absence of oxygen. Methane fermentation is not always established in the W.S.P. In some cases the necessary environmental requirements may not be met or sustained for a sufficient length of time. Should the required conditions exist in the sludge for a sufficient period of time, methane fermentation will become established. Once established, it will contribute significantly to odor as well as BOD removal. Even though these conditions are seldom met in shallow stabilization ponds such as those found in the Midwest, further study is merited in this area of W.S.P. treatment.

The efficiency of W.S.P. can be discussed in the light of change in (a) coliform density, (b) chemical quality, and (c) physical characteristics.

a. The W.S.P. has the proven ability to reduce coliform organisms. Bartsch et al. (8) as a result of extensive studies on five W.S.P. in the Dakotas reported: "Reductions in coliform density, that is, M.P.N. were 99 per cent during more than 50 per cent of the time, and except for one sampling period at two installations, were 95 per cent or greater at all times."

There are two generally recognized theories as to how coliform organisms are removed in the W.S.P. One theory is that a substance toxic to bacteria is released by the algae, and it is known that the algae Chlorella, a common inhabitant of W.S.P., produces such a substance (31).

Another theory is that the long storage period with subsequent settling, and the extreme competition between the organisms for food is responsible for these substantial reductions. Kabler (32) states, after a literature review and discussion on this subject: "It is probable that the fate of other enteric bacteria is similar to that of the coliforms." The removal of pathogenic organisms from wastes by W.S.P. is an area which needs investigation (18).

b. In terms of BOD, a properly loaded and designed W.S.P. affords a degree of treatment comparable to that attained with conventional secondary treatment (18). In a study at Kearney, Nebraska, BOD reductions ranged from 32 to 90 per cent with the lower figure occurring under winter conditions (33). During favorable seasons 90 per cent removal was effected with a loading of more than 1,000 people per acre. During favorable seasons the Kearney lagoon removed 127 pounds of a mean daily BOD loading of 157 pounds per acre per day, an efficiency in excess of 80 per cent.

Parker (34) as a result of a study at Melbourne, Australia reported that with a BOD loading up to 66 pounds per acre per day almost complete removal was achieved. When the BOD load was increased to 105 pounds per acre per day increased removal was obtained, but the BOD of the filtered effluent was increased.

c. Most investigators report a reduction in suspended solids of about 50 per cent, which at first may seem rather low. This high value of suspended solids is due to algal cells (16). When environmental conditions in receiving streams are unfavorable, the algae will die and be available for decomposition; consequently, their BOD will be added to the BOD of receiving streams. Thus, algae control in receiving streams is a frequently encountered problem.

CHAPTER III

PURPOSE AND SCOPE

The purpose of any waste water treatment process is to alter the characteristics of the waste water so that it may be disposed of without causing a menace to health or be an esthetic nuisance. The importance of bacterial action in the treatment of waste waters has been accepted for many years. Although this has been recognized for many years, the exact nature of the organisms responsible for treatment has not been determined with any degree of certainty.

It is extremely difficult if not impossible to formulate theories explaining the reactions known to occur in W.S.P. on the basis of what is now known about its biology. The absence of information pertaining to the bacteriology of stabilization ponds prompted this research project which had for its purpose the elucidation and enumeration of the organisms responsible for treatment obtained therein. The project was divided into two parts. The first part was concerned with the isolation and identification of the principal genera of bacteria present in the W.S.P. Observation of the effect of various loading rates on the genera and numbers of bacteria present constituted the second phase of the project.

Operational parameters such as dissolved oxygen, pH, ammonia, nitrate, organic nitrogen, and genera of algae were observed throughout

the study both on the laboratory and actual field W.S.P. This data, in conjunction with information gained in the bacteriological phase of the study, allows speculations to be made concerning the basic mechanisms of W.S.P. treatment.

CHAPTER IV

EXPERIMENTAL EQUIPMENT AND PROCEDURE

An experimental W.S.P. was constructed in the laboratory consisting of a 64 liter glass fish aquarium with a surface area of 0.19 sq. m., and a liquid depth of 32 cm. The aquarium was 30 cm in width; 60 cm in length. A baffle extending 13 cm in depth was placed 12 cm from the influent end to prevent short circuiting.

The experimental pond was fed raw unsettled domestic waste obtained from the influent of the Oklahoma City South Side sewage treatment plant by means of a Chemical Rubber Company automatic chemical solution feeder. The pond was fed continuously at a rate calculated to give the desired loading.

Light was provided by two Westinghouse (No. F40/GRO) 40 watt Plant-Gro greenhouse fluorescent light bulbs. These bulbs were located 55 cm above the liquid surface giving a light intensity of 200 ft. candles. An Intermatic model T101 timer switch was used to provide a 12-hour on, 12-hour off light cycle.

An overflow baffle was installed at the end opposite the influent although very little overflow occurred as evaporation and sampling procedures maintained the desired liquid level.

The pond was divided lengthwise into six equal parts. This arrangement gave seven sampling points beginning at the influent and proceeding

to the effluent. These sampling points were used throughout the study for obtaining liquid samples for bacteriological as well as chemical analyses.

Media

All media were sterilized for 15 minutes at 121° C. and were prepared with distilled water. The following media were used in this study.

Nutrient Agar

This medium consisted of Bacto-beef extract, 3 g; Bacto-peptone, 5 g; Bacto-agar, 15 g; water, 1000 ml; pH 6.8.

Yeast-extract Agar

This formulation was composed of Bacto-yeast extract, 3 g; Bacto-peptone, 5 g; Bacto-agar, 15 g; water, 1000 ml; pH 6.6.

Tryptone Glucose Extract Agar

This material contained Bacto-beef extract, 3 g; Bacto-tryptone, 5 g; Bacto-dextrose, 1 g; Bacto-agar, 15 g; water, 1000 ml; pH 7.0.

Nutrient Gelatin

This medium contained Bacto-beef extract, 3 g; Bacto-peptone, 5 g; Bacto-gelatin, 120 g; water, 1000 ml; pH 6.8.

MacConkey Agar

This contained gelysate, 17 g; polypeptone, 3 g; lactose, 10 g; bile salts mixture, 1.5 g; NaCl, 5 g; water, 1000 ml; agar, 13.5 g; neutral red, 0.03 g; crystal violet, 0.001 g; pH 7.1.

Lactose Broth

This was made of polypeptone, 5 g; beef extract, 3 g; lactose, 5 g; water, 1000 ml; pH 6.9.

Dextrose Broth

This consisted of Bacto-beef extract, 3 g; Bacto-tryptose, 10 g; Bacto-dextrose, 5 g; NaCl, 5 g; water, 1000 ml; pH 7.2.

Indole Nitrite Medium

The composition of this medium was trypticase, 20 g; disodium phosphate, 2 g; dextrose, 1 g; agar, 1 g; potassium nitrate, 1 g; water, 1000 ml; pH 7.2.

MR-VP Medium

This contained polypeptone, 7 g; dextrose, 5 g; potassium phosphate, 5 g; water, 1000 ml; pH 6.9.

Ringer Solution

This solution was prepared from sodium chloride, 2.15 g; potassium chloride, 0.075 g; calcium chloride, 0.12 g; sodium thiosulfate pentahydrate, 0.5 g; water, 1000 ml; pH 6.6. This solution was used at 0.25 of the original strength.

All liquid samples were obtained 4 cm below the liquid surface of the W.S.P. by means of volumetric pipets. Samples to be used for bacteriological purposes were serially diluted in Ringer solution and plated on the appropriate media. Three different types of nutrient media were compared for their ability to support the growth of the bacteria occurring in W.S.P. Of the three, nutrient agar, yeast-extract agar,

and tryptone glucose extract agar, nutrient agar consistently gave higher counts. It was, therefore, selected as the medium to be used in this study.

The following tests were performed routinely:

a) Duplicate colony counts were made with nutrient agar. The plates were incubated for 72 ± 6 hrs. at 25 ± 2° C. to obtain estimates of the numbers of saprophytes present.

b) Coli-aerogenes counts were obtained by plating on MacConkey agar. These plates were incubated at 25 ± 2° C. and read at 24 ± 2 hrs.

c) Spores of Bacillus sp. were enumerated by colony counts (nutrient agar incubated for 72 ± 6 hrs. at 30° C.) on pond water heated to 80° C. for 30 minutes.

To enable a more detailed picture of the nature of the bacterial flora to be obtained, the more commonly occurring colony types were picked off the total count plates and purified by successive transfer in MR-VP medium and streaking on nutrient agar. In the following test, cultures were incubated at 25 ± 2° C. MR-VP medium cultures were examined for morphology and Gram reaction. These same cultures were examined for motility (hanging drop) and used to inoculate the following: (a) Lactose and dextrose broth containing 0.025 g of phenol red/l which was incubated for 48 hrs. Acid and gas production were recorded. (b) Indole-Nitrite medium which was incubated for 48 hrs. These cultures were tested for the presence of indole by adding 0.2 ml of Kovacs' reagent. Nitrites were detected by a pink color on the addition of a few drops of a-naphthylamine acetate solution in 5N acetic acid. (c) Nutrient gelatin stabs were made and incubated for 5 days at 20° C. Proteolysis was detected by observing liquification of

the gelatin.

From the results obtained on the above tests, it was possible to generically identify the organisms by reference to Bergey's Manual of Determinative Bacteriology (29), and with the results of counts, to obtain a profile of the qualitative and quantitative composition of the bacterial flora of the W.S.P.

Chemical Tests

All samples for chemical analysis were centrifuged at 3,200 g for 10 minutes at 5° C. Analyses of BOD, DO, ammonia, organic nitrogen, and pH were carried out as suggested in Standard Methods for the Examination of Water and Wastewater (10).

Final reading for DO were obtained colorimetrically by means of a Bausch and Lomb Spectronic 20 spectrophotometer operated at 450 mu. Percentage transmittance was converted to mg/lDO by referring to standard tables supplied by the Hach Chemical Company.

Ammonia was determined by direct nesslerization. A wavelength of 400 mu was used for determining results on the spectrophotometer.

Organic nitrogen was determined by the Kjeldahl method. The procedure given in Standard Methods for the Examination of Water and Wastewater (10) was used without modification.

NitraVer powder pillows obtained from the Hach Chemical Company were used for the determination of nitrate and nitrite. A Bausch and Lomb Spectronic 20, operated at 400 mu, was also used in these analyses. As only minute quantities of nitrate and nitrite were found in the W.S.P. water tested, these determinations were discontinued in the early stages of the study.

Determination of pH was accomplished by means of a Beckman expanded scale pH meter.

CHAPTER V

DISCUSSION OF OBSERVATIONS

Qualitative Studies

Operation of the laboratory model W.S.P. began in October 1965 utilizing effluent from the Quail Creek addition W.S.P. as starter seed. This pond, located in Northwest Oklahoma City, was operating effectively at the time and its effluent contained a vigorous algae population composed of Chlorella and Euglena. A sludge blanket was provided by the addition of ripe digester sludge obtained from the Oklahoma City South Side sewage treatment plant.

Shortly after beginning the addition of raw domestic waste, the algae Euglena was decreased drastically in number and Chlorella occurred in almost pure culture. Initially, feeding was accomplished by adding the day's supply rapidly within a period of 4 hours; however, this system proved unsatisfactory as inconsistent bacteriological and chemical results were obtained. This situation was alleviated by the addition of an automatic solution feeder which metered raw domestic waste into the pond continuously at a steady rate. With this arrangement, the system worked satisfactorily and consistent results were obtained.

In order to demonstrate the effect of loading rate on the bacteriology of W.S.P., 15 lb/acre/day loading increments, beginning at 15 lb/acre/day, were used in this study. This represented hydrolic loadings of

0.008 MG/acre/day (7.4 $1/M^2$/day) increasing to 0.03 MG/acre/day (28.4 $1/M^2$/day). It was considered advisable to make the initial bacterial identifications at the lower loading level since the bacterial population would be less varied.

The first plate counts made were accomplished by the pour plate method as described in Standard Methods (10). An incubation temperature of 20° C. was used and the plates were read at 48 $\pm$ 3 hrs. This method quickly proved to be unsatisfactory as low inconsistent counts resulted.

The aerobic conditions encountered in properly operating W.S.P. indicate that strictly aerobic culture methods should be used in studying their bacteriology. With this in mind the "bent rod" method of plating was utilized. This method consisted of placing 0.1 ml of the appropriate dilution on solidified agar plates. A bent glass rod was then used to spread the sample evenly over the plate. The temperature of incubation was concurrently raised to 25° C. and the length of incubation changed to 72 $\pm$ 6 hrs. These changes in culture conditions were made after it was observed that the water temperature in the experimental W.S.P. was 25° C. It was further noted that higher total counts were obtained on plates incubated 72 hrs. instead of the suggested 48 hrs.

The "bent rod" method proved to be far superior to the pour plate method. The organisms were located on the surface of the agar where oxygen was available. More readily identifiable colonies as well as higher total counts were obtained under these conditions.

Selection of sampling depth was another problem encountered at this point in the investigation. The results of plate counts at various depths are shown in Figure 1. These counts were made on liquid samples obtained at the influent end of the pond. The maximum plate counts were

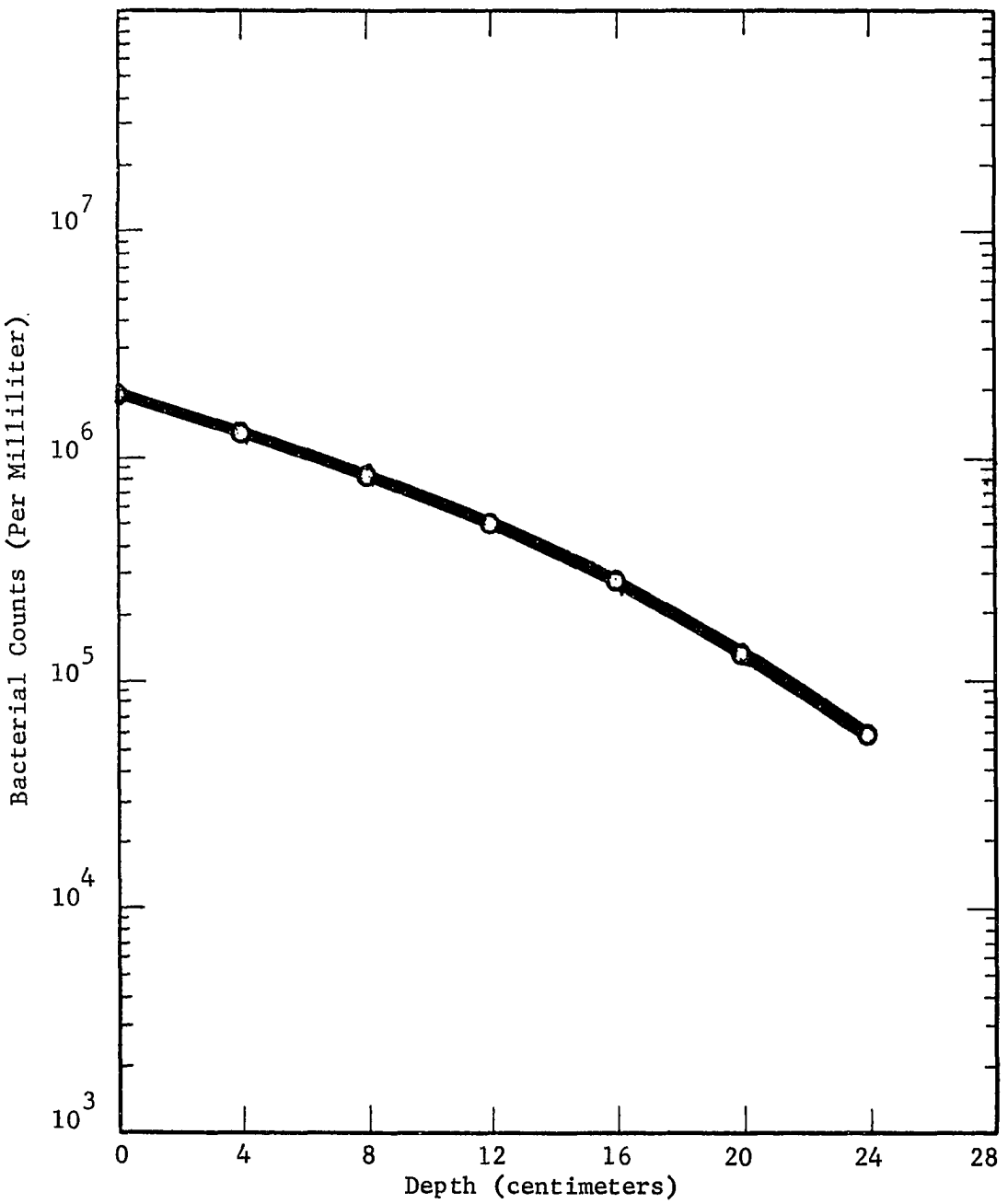

Figure 1. Variations in bacterial counts with depth.

found near the surface; therefore, a depth of 4 cm was selected for routine sampling.

Careful examination of several sets of plates revealed that four distinct colony types appeared with regularity. These four colony types made up 85 per cent or more of the total population at all times.

Well isolated colonies of each type were subcultured in MR-VP medium. This medium was chosen for enrichment as it was able to support excellent growth of the four organisms isolated.

Table 1 summarizes the characteristics of the bacteria isolated from the experimental W.S.P. The flora consisted mainly of saprophytic, Gram-negative, rod-shaped bacteria. The most frequently occurring genera were Achromobacter, Pseudomonas, and Flavobacterium.

The members of these genera together constituted over 80 per cent of the total flora in the plate counts. The remaining Gram-negative, rod-shaped bacteria present were coli-aerogenes bacteria which, although always present, never made up more than 10 per cent of the total count. The only commonly occurring Gram-positive, rod-shaped bacteria were sporeformers of the genus Bacillus but these again were never present in large numbers.

The presence of Streptococcus faecalis was noted on numerous occasions; however, no effort was made to determine its behavior in W.S.P. The "coli-aerogenes" or "coliform" group may be defined as all of the aerobic and facultative anaerobic, Gram-negative, nonsporeforming, rod-shaped bacteria which ferment lactose with gas formation within 48 hrs. at 35^{o} C. Escherichia coli, Escherichia freundii, and Aerobacter aerogenes make up the bulk of this group. These bacteria were considered as a group in this work and no attempt was made to determine the

TABLE 1

SOME CHARACTERISTICS OF BACTERIA ISOLATED FROM AN EXPERIMENTAL W.S.P.

Culture No.	Morphology	Size (μ)	Motility	Spores	Gram reaction	Indole formation	Gelatin liquefaction	Pigmentation	Acid from Glucose	Acid from Lactose	Nitrate reduction	Identification
1	Rod	1x0.5	+	-	-	-	+	None	±	-	+	Achromobacter sp.
2	Rod	2x0.5	+	-	-	-	-	Buff	-	-	+	Pseudomonas sp.
3	Rod	2.5x0.5	±	-	-	-	+	Yellow	-	-	-	Flavobacterium sp.
4	Rod	2x0.5	+	-	-	±	-	White	+	+	+	"coliform group"
5	Rod	3-4x1.5	+	+	+	-	+	White	+	-	±	Bacillus sp.

relative abundance of Escherichia and Aerobacter.

Many investigators have commented on the futility of attempting to classify the organisms of the Pseudomonas-Achromobacter-Flavobacterium group as to species. For instance, Klinge (35) wrote of the situation, that "In many instances the species characteristics now listed are so vague and inadequate that laboratory reidentification of a culture that has lost its label would be impossible." In view of these inadequate species characterizations, no attempt was made to identify the bacteria isolated in this study beyond the generic stage.

When examining the flora of natural habitats, one usually finds a high proportion of Gram-negative rods. Because of their nearly universal distribution, they are of great general importance and many members of the group are believed to play leading roles in water and soil. The function of returning protein nitrogen into circulation as ammonia under aerobic conditions is allotted to this group. Many show great versatility in metabolism, so that no matter how refractory an organic material may appear, if it is broken down by bacterial action an organism of this group will almost certainly be involved.

Those members of the Eubacteriales which were heterotrophic, had straight cells, and did not form spores were listed in the family Bacteriaceae in the early editions of Bergey's Manual. The Gram-negative saprophytes of this family were then divided according to color, e.g., Flavobacterium, yellow; Pseudomonas, green, and those forming no pigment, Achromobacter. Division was based on pigmentation and flagellation type was not regarded as important. Thus there was established a conception of the Pseudomonas-Achromobacter-Flavobacterium group as bacteria associated taxonomically as well as ecologically.

In the fifth edition of the Manual it was no longer felt possible to include peritrichous and polar organisms in one family. The genus Pseudomonas was removed from the Eubacteriales and, along with other polar flagellates, given order rank.

It is not surprising, therefore, to find these organisms closely associated in the W.S.P. They occur commonly in proteinaceous media and usually dominate the flora under these conditions. Waste which enters stabilization ponds has commonly traveled through several miles of sewer pipe. The readily digestable carbohydrates may be used by such organisms as those found in the coli-aerogenes group during the time involved in transit. Waste entering the W.S.P. would then have a proportionally high content of proteinaceous material, a situation which is favorable to the metabolic processes of the Pseudomonas-Achromobacter-Flavobacterium group.

The metabolic processes of bacteria considered dominant in W.S.P. must be compatible with operational data gathered from established field ponds. One of the most difficult observations to explain has been that of pH. The high pH encountered in stabilization pond treatment indicates that the predominant organisms in the bacterial flora must have the ability to produce these high pH values as well as the ability to actively function after conditions of high pH have been produced. The ability to produce alkaline reactions in culture media containing a minimal amount of protein has been established for the bacteria isolated during this study. Bender and Livine (36), after a study on acid production from carbohydrates by strains of Pseudomonas, concluded that in media containing protein any acid produced was completely masked by alkali production from the protein. As 85 per cent or more of the

bacterial population of W.S.P. is made up of organisms of the Pseudomonas-Achromobacter-Flavobacterium group, it is quite likely that this same masking effect accounts for the observed alkaline conditions. Organisms of this group are known to be highly resistant to alkaline conditions but are susceptible to acidic conditions (37). Their optimum pH lies between 7.2 and 7.5. They are killed at pH values below 5.5.

Oxygen tension is an operational parameter which undergoes considerable daily variation. Dissolved oxygen in W.S.P. may vary from zero after 10 to 12 hours of darkness to several times saturation after 10 to 12 hours of sunlight. This requires that the bacterial flora have the ability to withstand these variations. Pseudomonas and Achromobacter are facultative aerobes; however, the majority of the Flavobacterium strains found in this study were obligate aerobes. Klinge (35) states that none of these organisms can persist indefinitely under anaerobic conditions. He insists that they simply withstand periods of anaerobiasis by substituting such compounds as nitrate for oxygen as final electron acceptors. It is a well established fact that the addition of nitrate is beneficial to a failing W.S.P. During the course of this investigation, the opportunity to observe this phenomenon occurred. Table 2 summarizes these observations.

The data presented in Table 2 was obtained from the effluent of a 0.5 acre pond located in Northeast Oklahoma City. It receives only domestic wastes at an organic loading rate of 60 lb/acre/day. Officials responsible for its operation had been receiving sporadic odor complaints and on February 14, 1966, an effluent sample was obtained. Analysis showed that the pond was operating effectively considering the season and high loading rate; therefore no action was taken. Shortly thereafter,

the number of complaints increased and on March 21 another sample was taken for analysis. It was noted that condition in the pond had indeed deteriorated; therefore, it was decided that ammonium nitrate would be added in an amount calculated to give 10 ppm nitrate. Samples obtained on March 23 and 25 showed that a remarkable recovery was being achieved. No further nitrate additions were made until March 29 when it was observed that conditions in the pond were again deteriorating. At this point, it was decided that nitrate would be added every fourth day until the facility could be enlarged. With this arrangement, no further complaints were lodged and complete recovery was eventually effected.

TABLE 2

CHANGES IN W.S.P. EFFLUENT CHARACTERISTICS OCCURRING DURING TREATMENT WITH NITRATE

Date	Total Bacterial Counts Per Milliliter	DO (Per Cent Saturation)	BOD (Per Cent Reduction)	NH_3 (mg/l)
2-14-66	2.4×10^7	38	75	45
3-21-66	1.7×10^6	0	56	84
3-23-66	1.5×10^7	33	61	88
3-25-66	2.6×10^7	40	72	85
3-29-66	1.7×10^7	0	62	70
4-19-66	2.2×10^7	78	73	54

The point of particular interest here is the large changes which occurred in the total plate count. Anaerobiasis caused a sharp decrease in count while the addition of nitrate produced an increase. It would

appear therefore that nitrate acts as electron acceptor for W.S.P. organisms during short periods of anaerobiasis and that prolonged periods of anaerobic conditions result in a decrease in bacterial numbers and resulting pond failure.

Temperature is a parameter which shows marked seasonal variation. The bacteria isolated in this study are capable of growth between 0^{o} and 42^{o} C. with an optimum of 22^{o} - 25^{o} C. (35). This temperature range is compatible with that found in W.S.P.

From the above discussion, it can readily be seen that the physical, chemical, and metabolic requirements of the Pseudomonas-Achromobacter-Flavobacterium group are compatible with the environmental conditions found in the W.S.P. Their occurrence in dominant proportions indicates that they are the organisms primarily responsible for stabilization of waste in stabilization ponds.

Quantitative Studies

Differentiation of Pseudomonas, Achromobacter, and Flavobacterium was made possible by the distinct colony characteristics which each genus presented on nutrient agar. Pseudomonas colonies were buff colored and approximately 4 mm in diameter. Those of Achromobacter were colorless, 2 mm in diameter, and gave the appearance of a small drop of water. Flavobacterium colonies were similar to Achromobacter in size but were bright yellow in color.

The three genera also varied with respect to the length of time required for colony development. Pseudomonas colonies developed within 24 hours; Achromobacter, 48 hours; and 72 hours were required for the development of Flavobacterium colonies.

Bacto-MacConkey differential plating medium was used for the enumeration of the coliform organisms. Colonies of lactose fermenting bacteria appeared brick red in color and were surrounded by a zone of precipitated bile. The plates were counted after incubation for 18-24 hours as prolonged incubation led to confusion of results.

Figure 2 summarizes the results of total and differential plate counts taken while the experimental W.S.P. was operating at an organic loading rate of 15 lb/acre/day. At this loading rate, the total count was found to decrease by 20 per cent lengthwise across the pond. The genus Achromobacter constituted a dominant proportion of the total count. It formed 70 per cent of the total bacterial population in the area of the influent and increased to 80 per cent at the effluent. This genus decreased by 14 per cent in count across the pond.

The genus Flavobacterium was the second most numerous: influent, 14 per cent; sampling point three, 20 per cent; and effluent, 10 per cent. The effluent count represented a 40 per cent decrease from that obtained at the number three sampling point.

The genus Pseudomonas made up only 10 per cent of the total population at the influent decreasing to 7 per cent at the effluent. An overall decrease of 44 per cent was noted.

The percentage reduction of the coliforms was 99.4 per cent. This percentage was calculated from average counts observed in the raw domestic waste. An 88 per cent reduction was achieved immediately with the remainder occurring with increased retention time.

As shown in Figure 3, 88 per cent of the BOD was reduced in the immediate vicinity of the influent. Overall BOD reduction was 92 per cent at this loading.

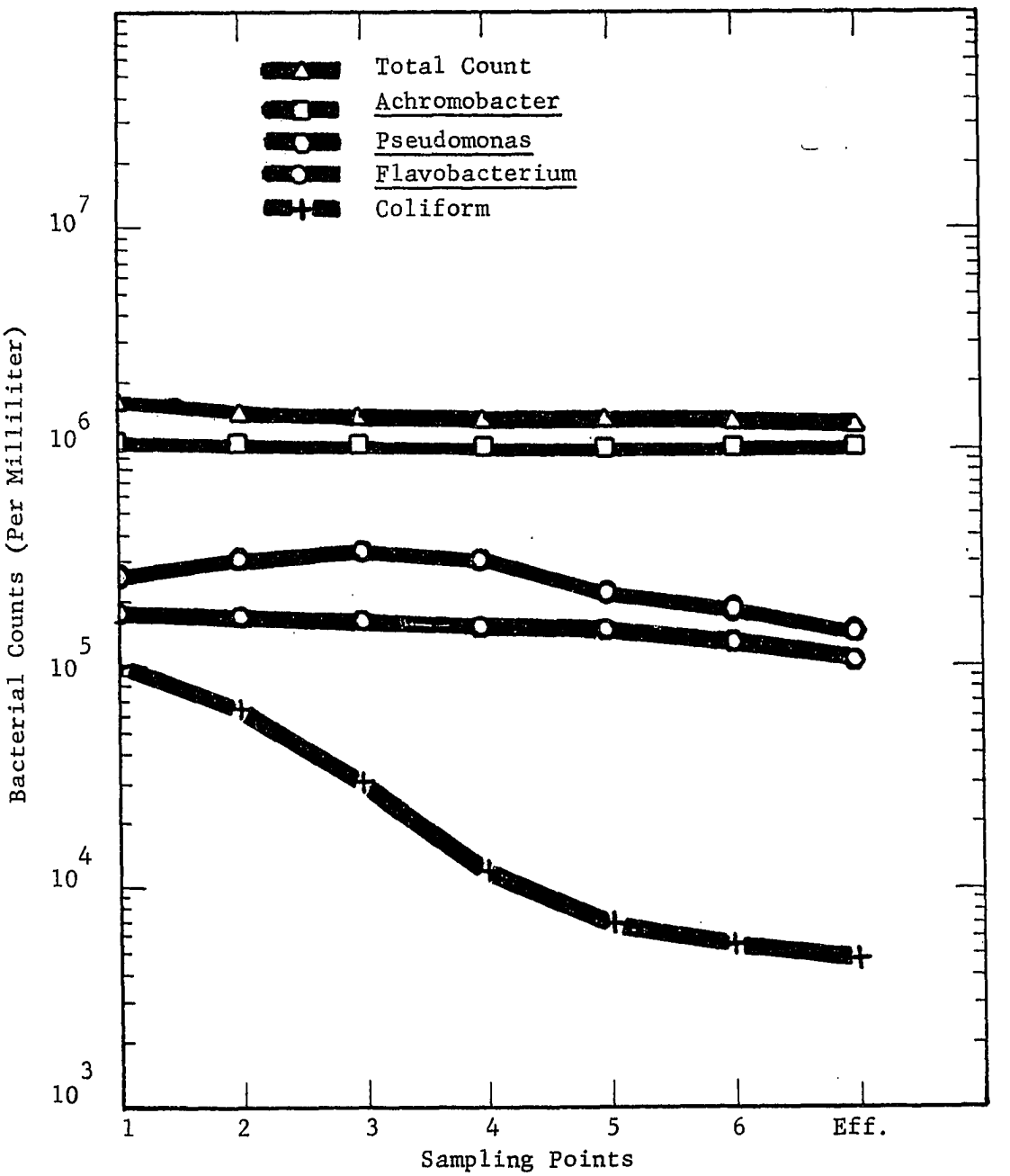

Figure 2. Bacterial counts obtained at 15 lb/acre/day loading rate. (Data compiled from Tables 3, 4, 5, 6, and 7.)

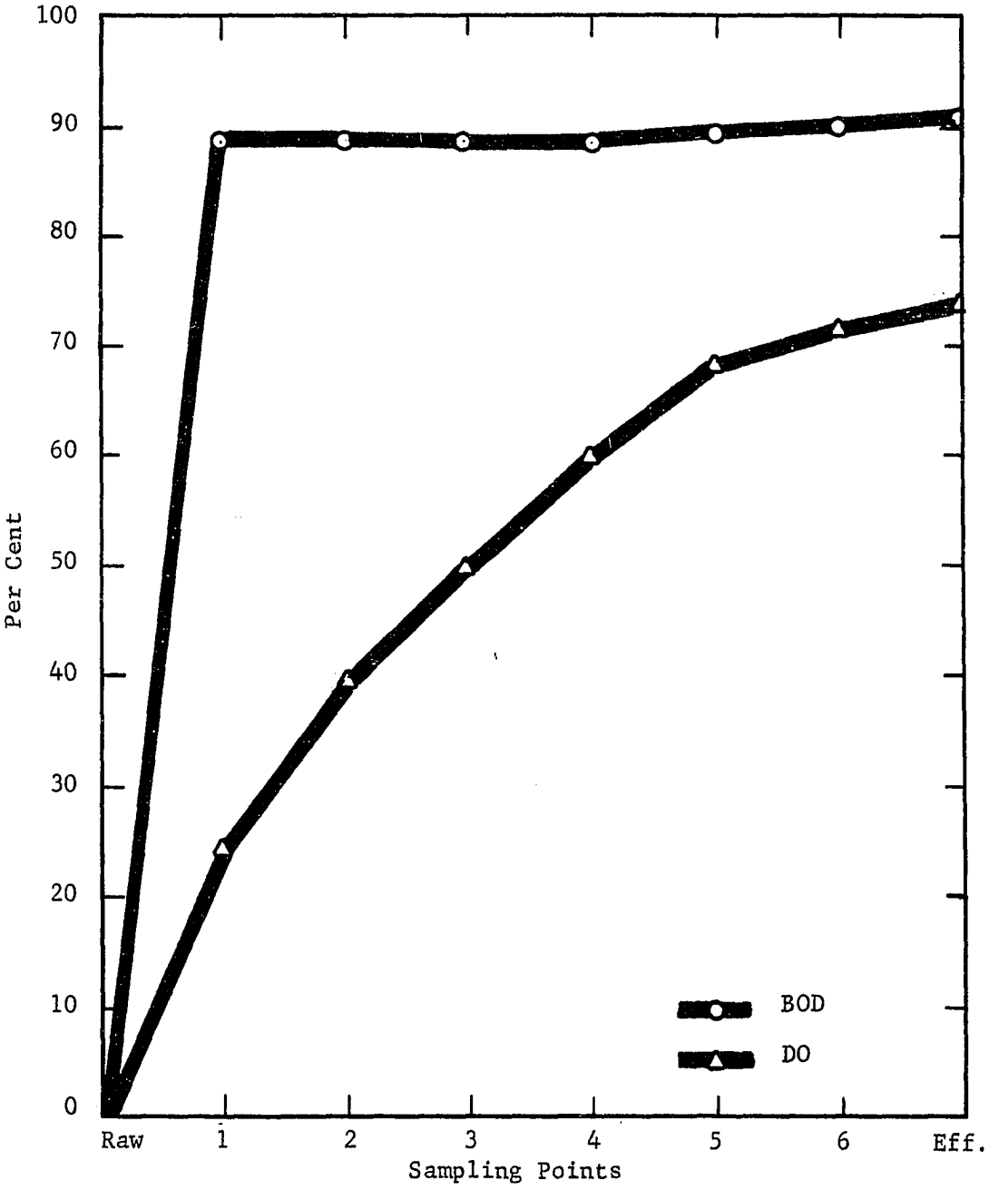

Figure 3. BOD (per cent reduction) and DO (per cent saturation) at each sampling point for the 15 lb/acre/day loading rate. (Data compiled from Tables 18, 19, 20, and 21.)

DO data is also represented in Figure 3. It can be seen that aerobic conditions prevailed throughout the pond after 8 hours of light. The DO ranged from 20 per cent of saturation at 25° C. at the influent to 70 per cent at the effluent. After 12 hours of light, a saturated DO was attained at the effluent.

The 30 lb/acre/day plate count data is presented in Figure 4. When compared to the 15 lb/acre/day count, it can be seen that a 60 per cent rise in total count has occurred. The total count also shows a higher reduction--54 per cent--from influent to effluent.

The genus Achromobacter increased substantially in number; however, percentage wise it decreased from 70 to 65 per cent of the total. Proportionally, the genus Pseudomonas demonstrated a marked increase from 10 per cent of the total at 15 lb/acre/day to 25 per cent of the total at 30 lb/acre/day. In contrast, Flavobacterium decreased from 14 to 6 per cent of the total count.

The percentage reduction of the coliforms was 99.5 per cent at this loading. This percentage was calculated from average counts observed in the raw domestic waste. A 93 per cent reduction was achieved immediately with the remainder occurring with increased retention time.

As shown in Figure 5, 92 per cent reduction in BOD was accomplished within the immediate vicinity of the influent. Concurrently, organic nitrogen was reduced 68 per cent and ammonia 34 per cent. Overall, BOD was reduced by 95 per cent; organic nitrogen, 84 per cent; and ammonia, 37 per cent. These percentages were calculated from average values observed in the raw domestic waste.

After 8 hours of light, DO values ranged from 10 per cent saturation at the influent to 45 per cent at the effluent. After 12 hours of light,

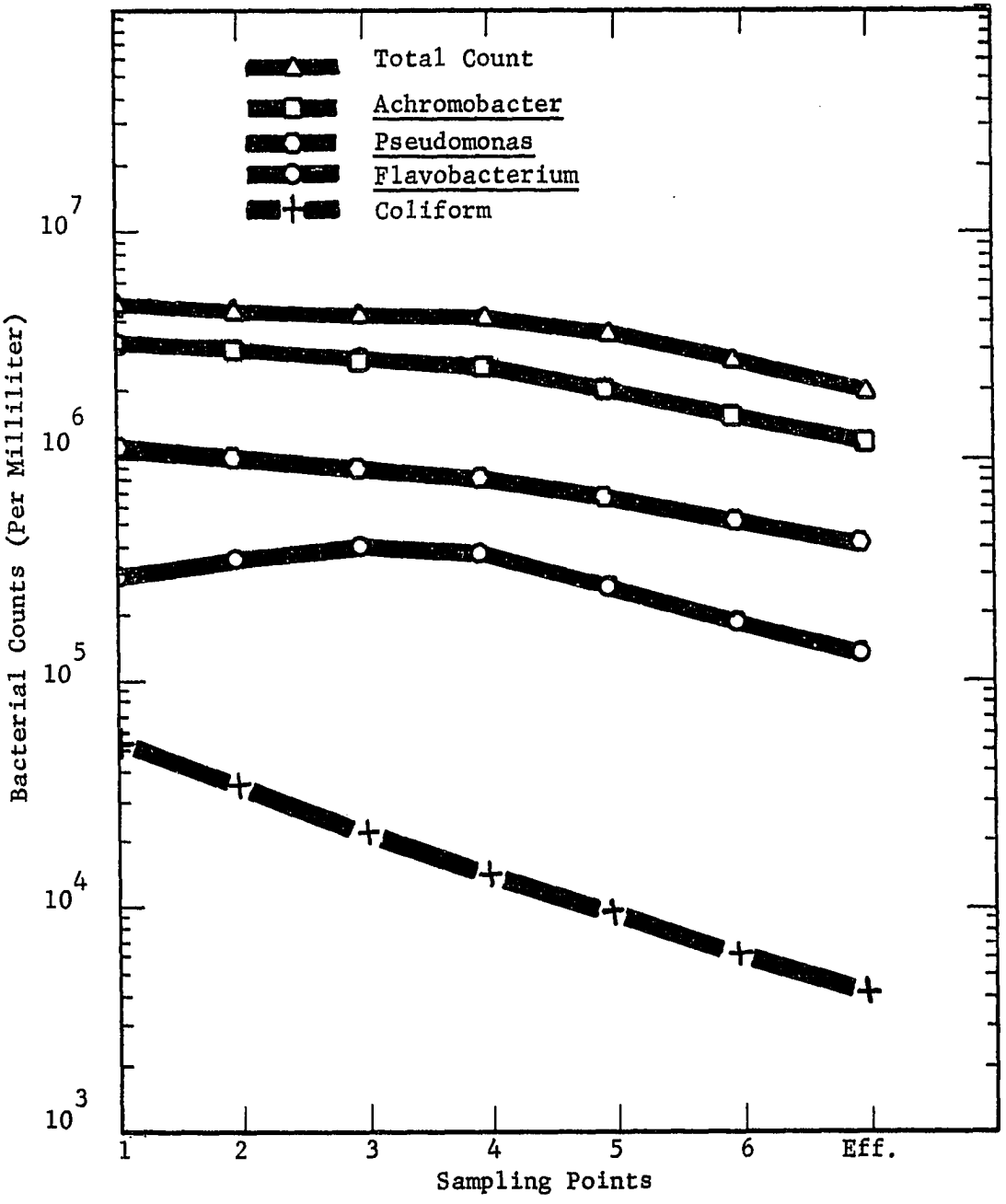

Figure 4. Bacterial counts obtained at 30 lb/acre/day loading rate. (Data compiled from Tables 8, 9, 10, 11, and 12.)

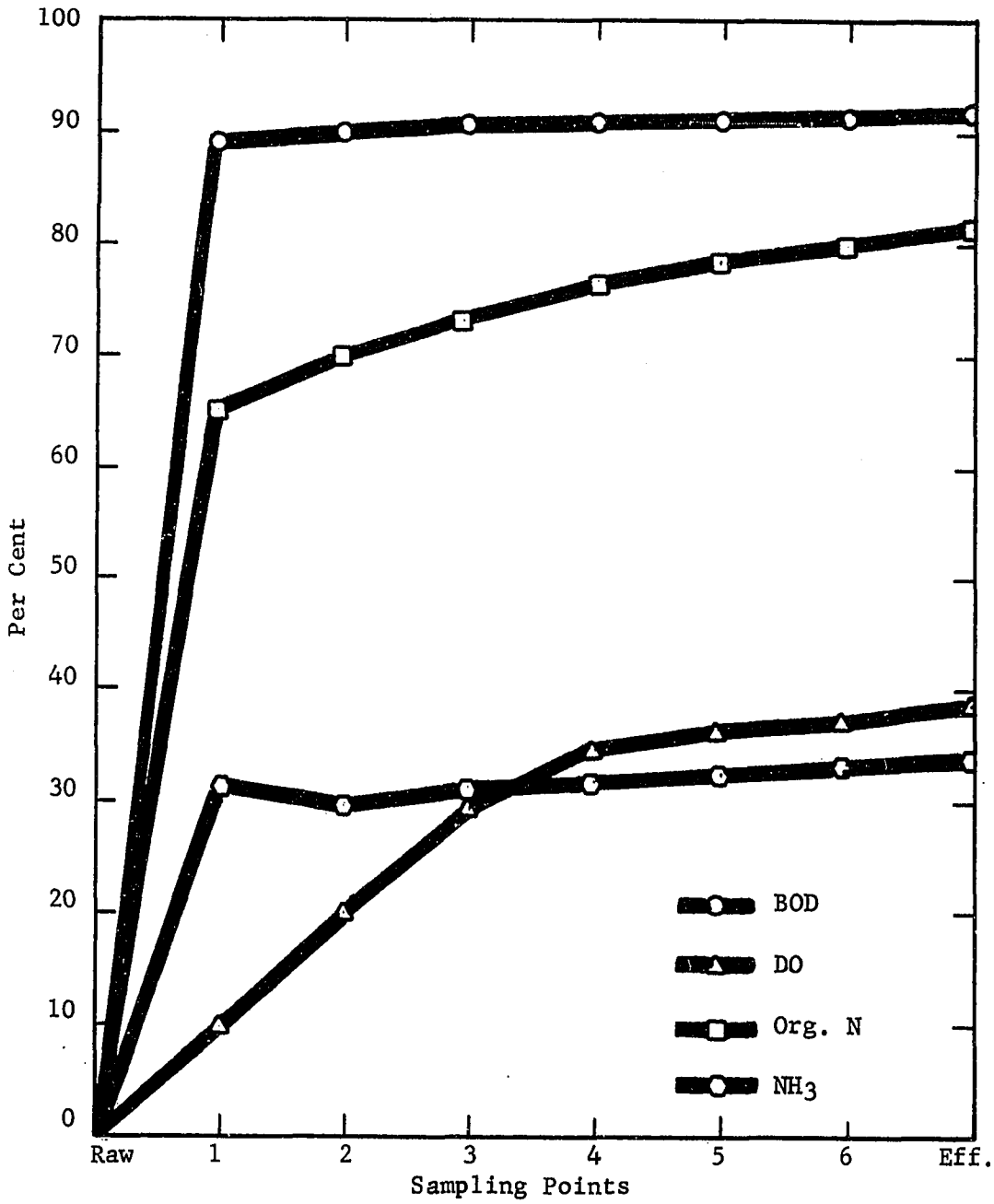

Figure 5. BOD (per cent reduction), DO (per cent saturation), NH_3 (per cent reduction), and Organic Nitrogen (per cent reduction) at each sampling point for the 30 lb/acre/day loading rate. (Data compiled from Tables 18, 19, 20, and 21.)

the effluent DO ranged from 80 to 100 per cent saturation. Anaerobic conditions were noticed in the area of the influent after 12 hours of darkness; however, this condition disappeared quickly when light was applied.

The 45 lb/acre/day plate count results are given in Figure 6. When compared to the 30 lb/acre/day count, it can be seen that a 60 per cent increase in total count has occurred in the area of the influent. At this loading, a 61 per cent reduction in total count was effected lengthwise across the pond.

Achromobacter demonstrated no significant increase in number at this loading and, therefore, could no longer be considered dominant. Percentage wise, it decreased from 65 to 31 per cent of the total count. Simultaneously, Pseudomonas increased from 25 to 43 per cent of the total. Flavobacterium was present in small numbers at the influent but increased with retention time as oxygen became more available.

Coliform reduction decreased to 96 per cent at this loading. A 90 per cent reduction was achieved immediately with the remainder occurring with increased retention.

A selection of operational data (Figure 7) indicates that the majority of activity was located in the immediate vicinity of the influent: BOD, 89 per cent reduction; organic nitrogen, 64 per cent reduction; and ammonia, 20 per cent reduction. This immediate decrease in ammonia is probably due to the high pH encountered with the resultant loss to the atmosphere. After the initial decrease, a plateau was reached. In this area, ammonia production from protein digestion equaled the amount lost due to algae activity and emission to the atmosphere.

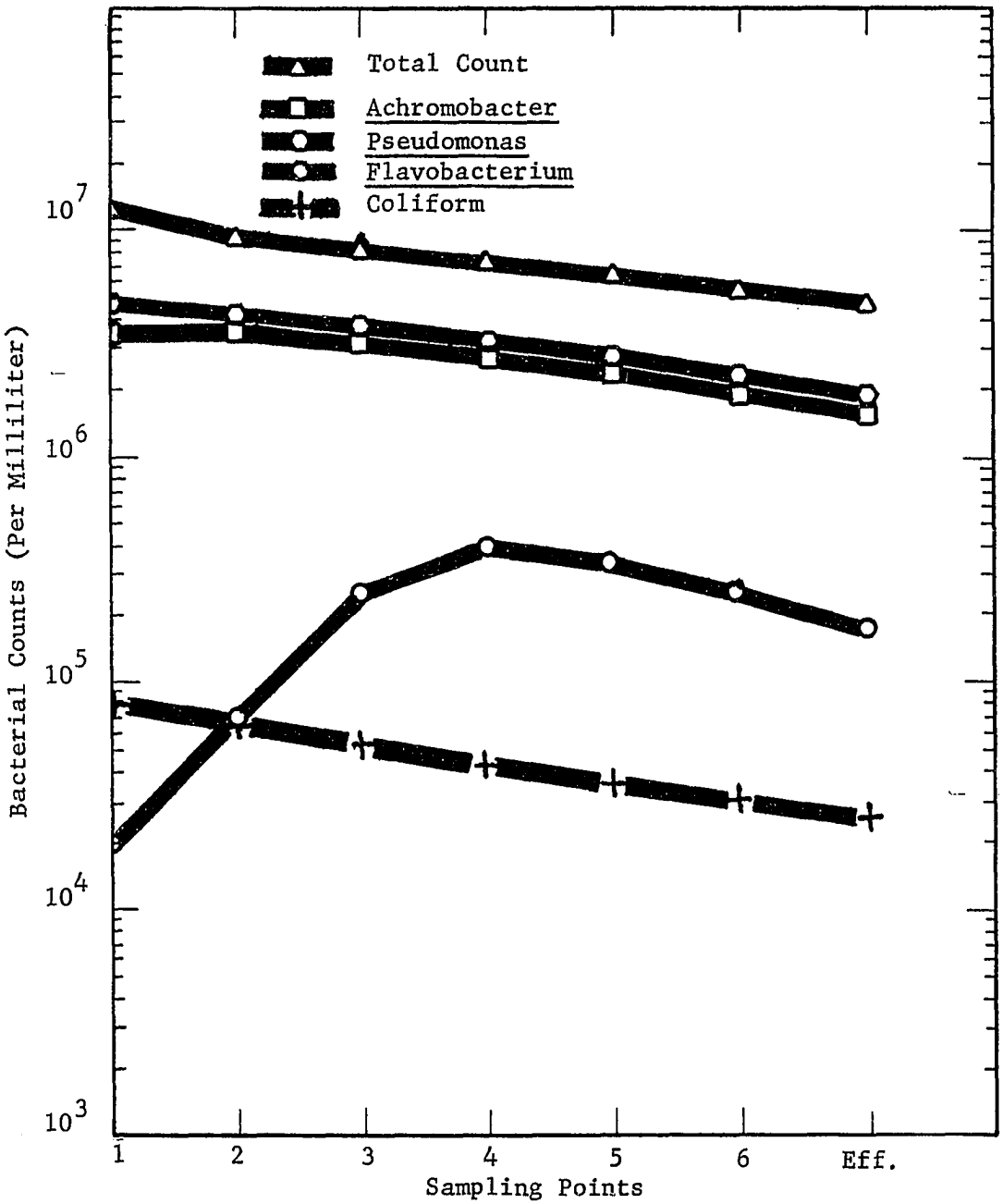

Figure 6. Bacterial counts obtained at 45 lb/acre/day loading rate. (Data compiled from Tables 13, 14, 15, 16, and 17.)

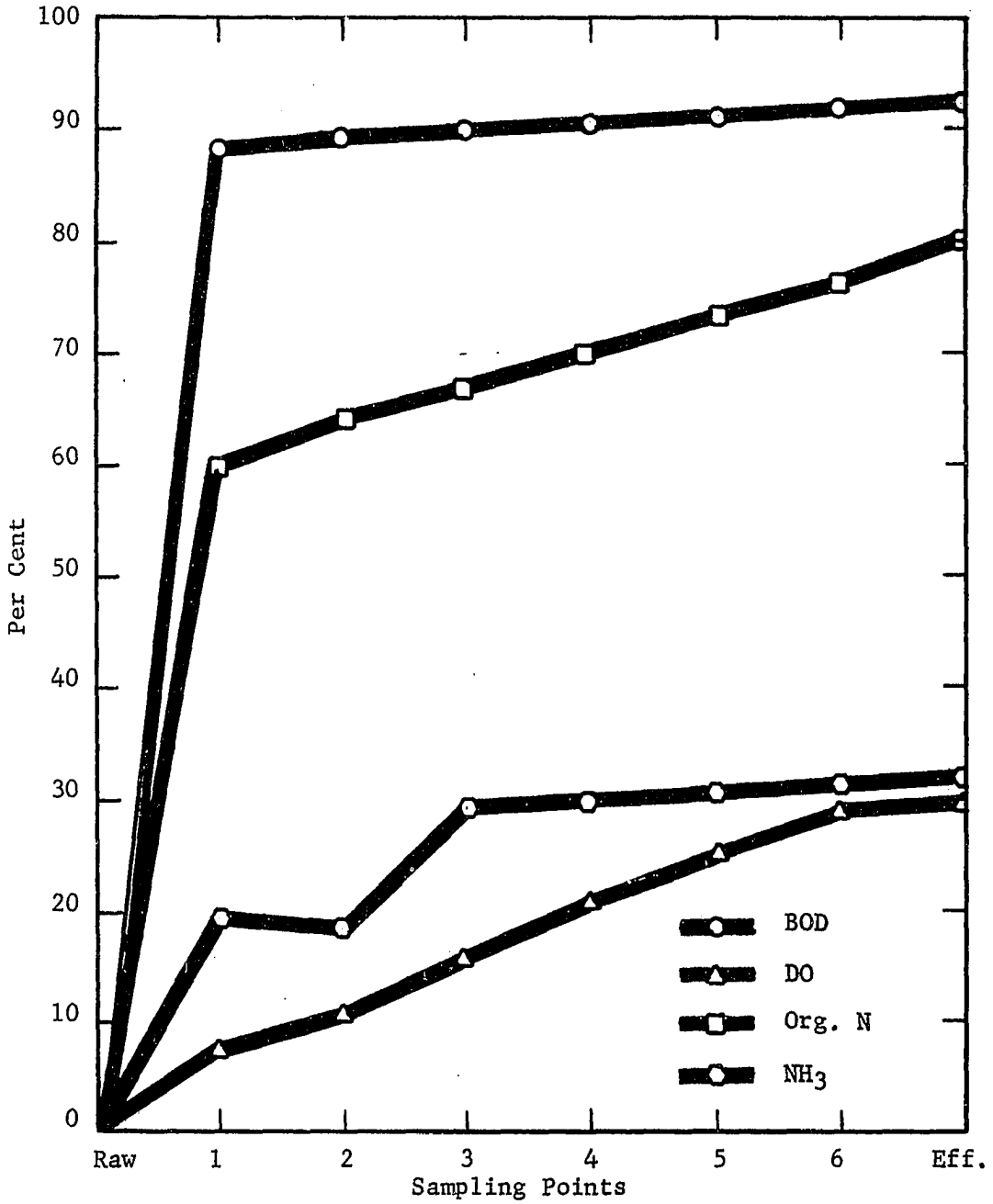

Figure 7. BOD (per cent reduction), DO (per cent saturation), NH_3 (per cent reduction), and Organic Nitrogen (per cent reduction) at each sampling point for the 45 lb/acre/day loading rate. (Data compiled from Tables 18, 19, 20, and 21.)

Dissolved oxygen was becoming a critical factor at this loading rate. Anaerobic conditions prevailed throughout the pond after 12 hours of darkness. Aerobic conditions were re-established when light was applied, however, DO values ranged from only 10 per cent saturation at the influent to 35 per cent saturation at the effluent after 8 hours of light. An increase of the organic loading to 60 lb/acre/day resulted in a further decrease in dissolved oxygen (Figure 8). Anaerobic conditions existed continually in the influent portion of the pond. After 8 hours of light, only 20 per cent saturation was attained in the effluent and the entire pond was void of oxygen after 12 hours of darkness. Figure 8 also indicates that the overall efficiency of the pond had decreased at this loading rate. BOD reduction in the area of the influent diminished to 70 per cent and an overall reduction of 85 per cent was attained. Organic nitrogen and ammonia reduction was also diminished at this loading. Organic nitrogen was reduced by 65 per cent and ammonia by only 20 per cent. The experimental pond operated under these conditions for a period of two weeks. At this time, the algae population began to disappear and the pond ultimately failed.

Examination of bacterial data (Figure 9) collected while the experimental W.S.P. was operating at a loading rate of 60 lb/acre/day reveals some explanation as to why treatment efficiency was lost. It is immediately apparent that the direct relationship which was shown to exist between loading rate and bacterial count does not apply at this loading. In fact, a decrease in number from that observed at the 45 lb/acre/day loading has taken place. This decreased bacterial population was not sufficient to stabilize the organic load being presented especially in view of the absence of dissolved oxygen. An increase in

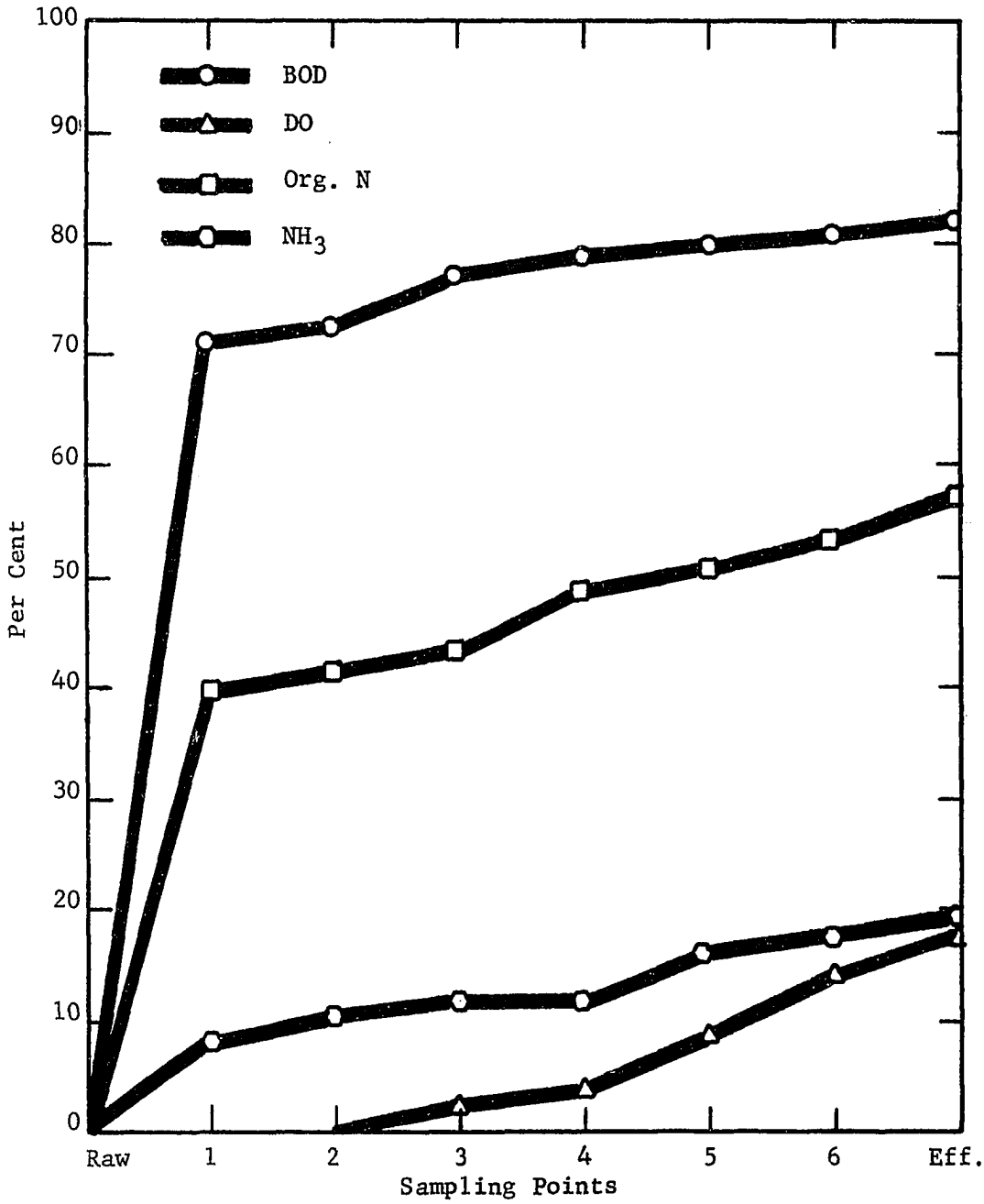

Figure 8. BOD (per cent reduction), DO (per cent saturation), NH_3 (per cent reduction), and Organic Nitrogen (per cent reduction) at each sampling point for the 60 lb/acre/day loading rate. (Data compiled from Tables 18, 19, 20, and 21.)

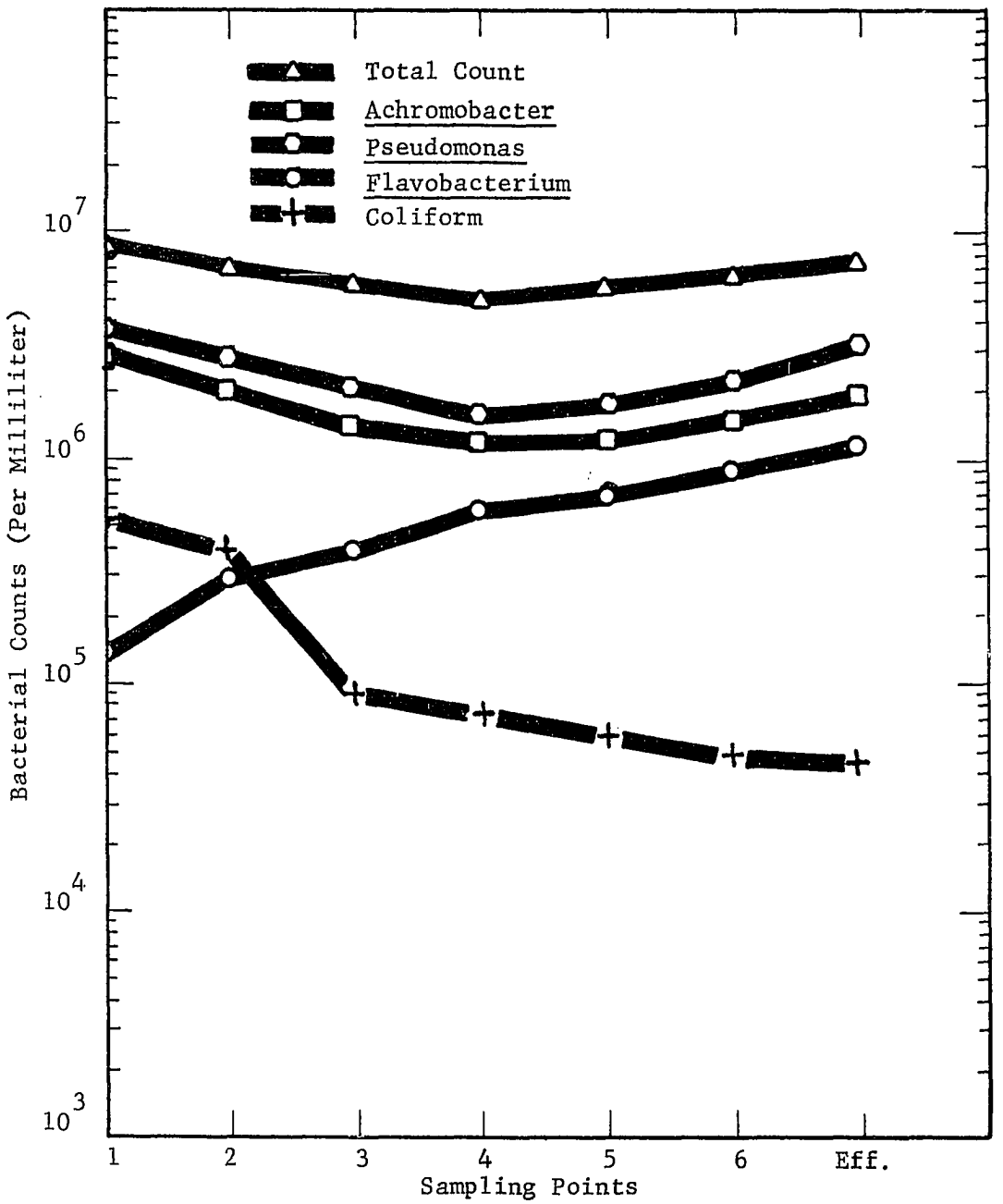

Figure 9. Bacterial counts obtained at 60 lb/acre/day loading rate.

count did ensue near the effluent where dissolved oxygen was present, however, this characteristic was lost as conditions, with regard to dissolved oxygen, deteriorated. Complete failure of treatment efficiency resulted shortly thereafter.

Proportionally, no alteration was observed in the individual components of the total count. In combination, Pseudomonas and Achromobacter made up 80 per cent of the total population. Flavobacterium was numerous in the area where dissolved oxygen was available.

Coliform reduction was 95 per cent, a slight decrease from the 96 per cent accomplished at the previous loading. Throughout this study, it was observed that coliform and BOD reduction were closely associated. These observations conform with the theory that extreme competition for nutrients is responsible for reductions in coliform bacteria in Waste Stabilization Ponds.

In order to further validate the results obtained from the experimental W.S.P., samples were obtained from effectively operating field ponds for bacteriological study. The Oklahoma City Christian College stabilization pond was chosen for this purpose. This three acre pond which serves an enrollment of some 2,000 students, receives approximately 30 lb/acre/day of organic load.

The results of this study indicated that the data obtained from the experimental W.S.P. are accurate. The Achromobacter, Pseudomonas, Flavobacterium group was found to be dominant in the field pond making up 90 per cent of the total count. As found in the experimental pond (Figure 4), Achromobacter was the most numerous organism at this loading constituting over 60 per cent of the total count. Pseudomonas made up 25 per cent and Flavobacterium, 5 per cent of the total count. Coliform

reduction was 99.7 per cent compared to 99.5 per cent in the experimental pond.

Numerically, the field operating stabilization pond counts were found to be slightly higher. This variation could be caused by experimental error as a relatively small number of field pond samples were examined.

CHAPTER VI

SUMMARY AND CONCLUSIONS

This investigation was designed to elucidate and enumerate the bacteria responsible for the domestic waste treatment obtained in Waste Stabilization Ponds. This was accomplished by employing an experimental pond constructed in the laboratory in conjunction with field studies.

The experimental pond was subjected to various loading rates, beginning at 15 lb/acre/day and increasing at multiples thereof to 60 lb/acre/day, in order to determine the effect of organic load on the bacterial flora.

Based on the results of the various bacteriological and chemical determinations made during these experiments, the following conclusions have been drawn.

1) The bacteriological procedures recommended in Standard Methods for the Examination of Water and Wastewater (10) were inadequate for the detailed examination of W.S.P. bacteriology. The "pour plate" method of preparing plates was found unsatisfactory due to the low, inconsistent counts and to the difficulty in differentiating colony types which characterized this method. The "bent rod spreader" method proved superior and was utilized in this study. It is also recommended that in future studies of this nature, incubation temperatures be the

same as the original pond water and that the period of incubation be 72 hours or longer.

2) The dominant bacterial flora, as assessed by morphological and biochemical study, consisted of Gram-negative, rod-shaped bacteria belonging to the genera Achromobacter, Pseudomonas, and Flavobacterium. Together the members of these genera constituted 90-95 per cent of the total count. The only commonly occurring Gram-positive bacteria, other than Streptococcus faecalis, were sporeformers of the genus Bacillus but these were never present in large numbers.

3) The metabolic processes of bacteria considered dominant in Waste Stabilization Ponds must be compatible with data gathered from operational field ponds. In assessing the importance of the Achromobacter-Pseudomonas-Flavobacterium group, the following observations were considered:

a) The numbers of these genera made up 90-95 per cent of the total population. The numerical importance of an organism is a guide to its true functional importance.

b) When examining the flora of natural habitats, one usually finds a high proportion of Gram-negative rods and, because of their universal distribution, they are of great general importance.

c) These organisms occur commonly in proteinaceous media and usually dominate the flora under these conditions. Domestic waste entering the Waste Stabilization Pond has a comparatively high content of protein material.

d) The ability to produce alkaline reactions in culture media containing a minimal amount of protein has been established for the bacteria isolated during this study. Organisms of this group are

known to be highly resistant to alkaline conditions after such conditions have been established.

e) Oxygen tension is an operational parameter which shows a great amount of daily variation and may vary from zero after 10 to 12 hours of darkness to several times saturation after 10 to 12 hours of sunlight. Achromobacter and Pseudomonas are capable of withstanding periods of anaerobiasis by substituting such compounds as nitrate for oxygen as final electron acceptors.

f) Temperature is a parameter which shows marked seasonal variations. The bacteria isolated during this study are capable of growth between 0^{o} and 42^{o} C., a temperature range which is compatible with that observed in Waste Stabilization Ponds.

g) A decrease in treatment efficiency was accompanied by a numerical decrease in the organisms of this group. The return of treatment efficiency was likewise accompanied by an increase in organisms of this group.

From the above discussion, it can be seen that the characteristics of the Achromobacter-Pseudomonas-Flavobacterium group are compatible with the environmental conditions found in the Waste Stabilization Pond. Their occurrence in dominant proportions indicates that they are the organisms primarily responsible for stabilization of waste in stabilization ponds.

4) The relationship between total bacterial count and loading rate was found to be direct. An increase in organic load elicited an increase in the bacterial population provided sufficient dissolved oxygen was available. This direct relationship was not followed by each genus. Achromobacter dominated the flora at the lower loading levels, however,

it was surpassed in number by the Pseudomonas at the higher levels. Although the proportions of each genus varied from loading to loading, no change in treatment efficiency was noted until dissolved oxygen became a limiting factor.

5) Coliform reduction was found to be closely associated with BOD removal, indicating that coliforms are removed because of their inability to successfully compete for nutrients in the W.S.P. environment.

6) The principal site of bacterial activity was located within the immediate vicinity of the influent. From 88 to 92 per cent of the ultimate BOD removal and 88 to 93 per cent of coliform reduction occurred in this area.

Based on the conclusions drawn from this study, a proposed theory of Waste Stabilization Pond action may be formulated. A symbiotic relationship does exist in which algae use the carbon dioxide, phosphate, and ammonia resulting from bacterial decomposition to synthesize algal cell material and in so doing, release oxygen. This oxygen, produced in the upper layers of the pond, may, through mixing, become available for the oxidation of dissolved organic matter by bacteria.

The principal area of bacterial activity was found to be located within the immediate vicinity of the influent. Bacteria in this area are maintained in a logrithmic state of growth and incoming BOD is reduced almost immediately. This would indicate that very short detention periods would suffice, however, a large oxygen demand is created in this area which cannot be satisfied by the limited algal population located there. It would appear, therefore, that the oxygen demand of this relatively small area of high bacterial activity is met by oxygen produced in outlying areas of the pond where the bacterial oxygen demand

is low. Oxygen transfer could be brought about by wind action, convection currents and diffusion. In this manner an "oxygen reserve" is maintained. It was noted that ponds can survive short periods of anaerobiasis occurring near the influent, however, when anaerobic conditions occur in the effluent failure is imminent.

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APPENDIX I

TABULATED DATA--RESULTS OF BACTERIOLOGICAL COUNTS OBTAINED AT 15 LB/ACRE/DAY LOADING RATE

TABLE 3

TOTAL BACTERIAL COUNTS PER MILLILITER OBTAINED AT 15 LB/ACRE/DAY LOADING RATE

Sampling Points						
1	2	3	4	5	6	Effluent
1.0×10^6	2.0×10^6	1.4×10^6	1.3×10^6	1.0×10^6	1.2×10^6	1.1×10^6
1.3×10^6	2.0×10^6	1.3×10^6	1.2×10^6	1.8×10^6	1.4×10^6	1.3×10^6
2.7×10^6	1.2×10^6	1.6×10^6	1.0×10^6	1.8×10^6	1.7×10^6	1.5×10^6
1.2×10^6	1.3×10^6	2.1×10^6	2.0×10^6	1.3×10^6	1.4×10^6	1.6×10^6
1.0×10^6	2.2×10^6	2.0×10^6	1.0×10^6	1.2×10^6	1.7×10^6	1.5×10^6
2.4×10^6	2.0×10^6	1.7×10^6	2.0×10^6	2.1×10^6	2.0×10^6	1.4×10^6
2.3×10^6	1.8×10^6	2.0×10^6	2.2×10^6	1.6×10^6	1.5×10^6	1.3×10^6
2.5×10^6	2.0×10^6	2.0×10^6	1.8×10^6	1.5×10^6	1.4×10^6	1.6×10^6
2.1×10^6	1.5×10^6	1.6×10^6	1.6×10^6	1.6×10^6	1.6×10^6	1.4×10^6
1.8×10^6	1.4×10^6	1.5×10^6	1.4×10^6	1.5×10^6	1.4×10^6	1.3×10^6

TABLE 4

PSEUDOMONAS COUNTS PER MILLILITER OBTAINED AT 15 LB/ACRE/DAY LOADING RATE

Sampling Points						
1	2	3	4	5	6	Effluent
2.5×10^5	1.3×10^5	1.7×10^5	1.6×10^5	1.4×10^5	1.1×10^5	8.5×10^4
1.0×10^5	1.1×10^5	1.8×10^5	1.5×10^5	1.8×10^5	2.0×10^5	9.1×10^4
1.2×10^5	2.0×10^5	2.0×10^5	1.0×10^5	2.0×10^5	1.4×10^5	1.1×10^5
1.7×10^5	2.4×10^5	1.0×10^5	1.8×10^5	1.0×10^5	1.3×10^5	1.2×10^5
2.5×10^5	2.2×10^5	1.5×10^5	2.2×10^5	1.6×10^5	1.1×10^5	1.1×10^5
1.8×10^5	1.8×10^5	2.0×10^5	1.4×10^5	1.3×10^5	1.5×10^5	7.0×10^4
1.3×10^5	1.7×10^5	1.7×10^5	2.1×10^5	1.5×10^5	1.2×10^5	1.5×10^5
2.0×10^5	2.0×10^5	1.8×10^5	1.8×10^5	1.7×10^5	1.3×10^5	1.0×10^5
2.2×10^5	2.0×10^5	1.9×10^5	1.7×10^5	1.6×10^5	1.6×10^5	1.3×10^5
1.5×10^5	1.9×10^5	1.4×10^5	2.0×10^5	1.8×10^5	1.5×10^5	8.0×10^4

TABLE 5

FLAVOBACTERIUM COUNTS PER MILLILITER OBTAINED AT 15 LB/ACRE/DAY LOADING RATE

Sampling Points						
1	2	3	4	5	6	Effluent
3.4×10^5	3.1×10^5	3.8×10^5	3.2×10^5	2.3×10^5	1.5×10^5	1.0×10^5
1.8×10^5	3.0×10^5	3.4×10^5	3.0×10^5	2.0×10^5	2.0×10^5	1.4×10^5
2.0×10^5	3.2×10^5	4.8×10^5	3.6×10^5	2.6×10^5	2.4×10^5	1.8×10^5
1.5×10^5	1.9×10^5	2.0×10^5	2.2×10^5	1.6×10^5	1.4×10^5	1.1×10^5
2.0×10^5	2.3×10^5	2.6×10^5	2.4×10^5	1.9×10^5	1.7×10^5	1.5×10^5
2.8×10^5	2.9×10^5	3.3×10^5	2.9×10^4	2.3×10^5	2.1×10^5	1.7×10^5
2.5×10^5	3.3×10^5	3.7×10^5	3.2×10^4	2.4×10^5	1.8×10^5	1.2×10^5
2.8×10^5	3.4×10^5	3.8×10^5	3.3×10^4	3.1×10^5	2.8×10^5	2.3×10^5
3.1×10^5	3.0×10^5	3.5×10^5	2.8×10^4	2.2×10^5	2.0×10^5	1.2×10^5
2.4×10^5	2.6×10^5	3.1×10^5	2.5×10^4	1.6×10^5	1.3×10^5	1.0×10^5

TABLE 6

ACHROMOBACTER COUNTS PER MILLILITER OBTAINED AT 15 LB/ACRE/DAY LOADING RATE

Sampling Points						
1	2	3	4	5	6	Effluent
1.4×10^6	1.1×10^6	1.1×10^6	1.1×10^6	1.1×10^6	1.0×10^5	1.0×10^6
1.4×10^6	1.0×10^6	1.2×10^6	1.4×10^6	1.5×10^6	1.5×10^6	1.1×10^6
1.0×10^6	1.3×10^6	1.8×10^6	1.2×10^6	1.0×10^6	1.0×10^6	9.0×10^5
1.3×10^6	1.5×10^6	1.1×10^6	1.5×10^6	1.4×10^6	1.6×10^6	1.1×10^6
1.8×10^6	1.7×10^6	1.7×10^6	1.8×10^6	1.0×10^6	1.7×10^6	1.0×10^6
9.5×10^5	1.4×10^6	1.5×10^6	1.6×10^6	1.7×10^6	1.4×10^6	1.2×10^6
1.7×10^6	1.6×10^6	1.6×10^6	1.4×10^6	1.6×10^6	1.3×10^6	1.1×10^6
1.5×10^6	1.8×10^6	1.0×10^6	1.3×10^6	1.6×10^6	1.7×10^6	1.0×10^6
1.5×10^6	1.5×10^6	1.3×10^6	1.4×10^6	1.5×10^6	1.2×10^6	1.1×10^6
1.1×10^6	1.4×10^6	1.4×10^6	1.3×10^6	1.4×10^6	1.6×10^6	1.2×10^6

TABLE 7

COLIFORM COUNTS PER MILLILITER OBTAINED AT 15 LB/ACRE/DAY LOADING RATE

Sampling Points						
1	2	3	4	5	6	Effluent
1.0×10^5	6.0×10^4	2.1×10^4	2.1×10^4	1.0×10^4	7.1×10^3	6.4×10^3
6.5×10^4	6.1×10^4	2.0×10^4	1.0×10^4	7.6×10^3	7.0×10^3	4.2×10^3
9.0×10^4	9.0×10^4	5.0×10^4	2.0×10^4	1.0×10^4	1.0×10^4	5.3×10^3
1.1×10^5	6.8×10^4	3.2×10^4	9.2×10^3	6.1×10^3	8.0×10^3	8.0×10^3
9.0×10^4	8.0×10^4	2.3×10^4	1.0×10^4	1.0×10^4	5.6×10^3	3.6×10^3
1.2×10^5	7.0×10^4	5.0×10^4	8.6×10^3	6.2×10^3	4.2×10^3	4.1×10^3
1.0×10^5	6.0×10^4	2.4×10^4	1.1×10^4	5.5×10^3	5.4×10^3	5.0×10^3
1.4×10^5	7.0×10^4	4.4×10^4	1.0×10^4	8.0×10^3	3.0×10^3	5.0×10^3
1.0×10^5	5.0×10^4	2.3×10^5	8.4×10^3	5.0×10^3	4.6×10^3	4.1×10^3
9.0×10^4	6.0×10^4	1.5×10^4	1.0×10^4	9.0×10^3	7.0×10^3	6.0×10^3

APPENDIX II

TABULATED DATA--RESULTS OF BACTERIOLOGICAL COUNTS OBTAINED AT 30 LB/ACRE/DAY LOADING RATE

TABLE 8

TOTAL BACTERIAL COUNTS PER MILLILITER OBTAINED AT 30 LB/ACRE/DAY LOADING RATE

Sampling Points						
1	2	3	4	5	6	Effluent
8.0×10^6	6.8×10^6	5.6×10^6	5.5×10^6	4.0×10^6	3.6×10^6	3.3×10^6
5.3×10^6	3.5×10^6	3.0×10^6	3.0×10^6	2.0×10^6	2.3×10^6	1.0×10^6
4.5×10^6	6.4×10^6	6.2×10^6	5.0×10^6	4.0×10^6	3.8×10^6	3.3×10^6
6.0×10^6	5.9×10^6	5.0×10^6	4.8×10^6	3.0×10^6	2.6×10^6	3.0×10^6
2.6×10^6	2.5×10^6	2.5×10^6	2.5×10^6	2.8×10^6	1.4×10^6	1.0×10^6
2.5×10^6	2.5×10^6	4.5×10^6	4.2×10^6	4.0×10^6	3.0×10^6	1.1×10^6
3.5×10^6	3.0×10^6	2.9×10^6	2.6×10^6	3.5×10^6	2.5×10^6	2.0×10^6
4.1×10^6	4.0×10^6	4.1×10^6	3.9×10^6	3.7×10^6	2.7×10^6	2.5×10^6
4.6×10^6	4.5×10^6	4.5×10^6	4.5×10^6	3.5×10^6	2.1×10^6	1.9×10^6
5.1×10^6	4.6×10^6	4.1×10^6	3.7×10^6	3.9×10^6	3.1×10^6	2.2×10^6

TABLE 9

PSEUDOMONAS COUNTS PER MILLILITER OBTAINED AT 30 LB/ACRE/DAY LOADING RATE

Sampling Points						
1	2	3	4	5	6	Effluent
7.5×10^5	7.8×10^5	6.8×10^5	5.5×10^5	4.3×10^5	3.1×10^5	2.3×10^5
1.0×10^6	8.0×10^5	8.0×10^5	7.0×10^5	5.0×10^5	5.0×10^5	3.0×10^5
1.4×10^6	1.5×10^6	1.0×10^6	1.0×10^6	8.0×10^5	8.0×10^5	6.0×10^5
8.0×10^5	1.4×10^6	8.0×10^5	8.0×10^5	5.0×10^5	4.0×10^5	4.0×10^5
1.2×10^6	5.0×10^5	6.0×10^5	8.0×10^5	8.0×10^5	5.0×10^5	3.0×10^5
1.0×10^6	1.2×10^6	1.0×10^6	1.0×10^6	5.3×10^5	4.2×10^5	4.5×10^5
7.5×10^6	8.0×10^5	1.0×10^6	1.0×10^6	9.0×10^5	8.5×10^5	8.2×10^5
1.3×10^6	1.0×10^6	7.5×10^5	6.0×10^5	6.2×10^5	4.0×10^5	4.0×10^5
1.5×10^6	1.3×10^6	9.2×10^5	6.5×10^5	6.4×10^5	6.3×10^5	3.2×10^5
1.1×10^6	1.0×10^6	8.5×10^5	6.6×10^5	5.8×10^5	4.6×10^5	2.9×10^5

TABLE 10

FLAVOBACTERIUM COUNTS PER MILLILITER OBTAINED AT 30 LB/ACRE/DAY LOADING RATE

Sampling Points						
1	2	3	4	5	6	Effluent
3.0×10^5	4.1×10^5	4.3×10^5	3.0×10^5	2.6×10^5	2.5×10^5	1.5×10^5
1.5×10^5	2.3×10^5	2.6×10^5	2.5×10^5	2.4×10^5	1.3×10^5	1.2×10^5
3.9×10^5	3.8×10^5	3.6×10^5	3.2×10^5	1.8×10^5	1.6×10^5	1.0×10^5
4.6×10^5	4.7×10^5	5.1×10^5	5.0×10^5	3.9×10^5	2.3×10^5	2.1×10^5
2.2×10^5	3.2×10^5	4.7×10^5	4.2×10^5	3.4×10^5	2.1×10^5	1.3×10^5
3.1×10^5	3.7×10^5	4.0×10^5	3.9×10^5	2.8×10^5	2.2×10^5	1.0×10^5
1.8×10^5	2.4×10^5	3.4×10^5	3.7×10^5	2.5×10^5	1.7×10^5	1.2×10^5
2.4×10^5	2.8×10^5	3.3×10^5	2.8×10^5	2.7×10^5	1.4×10^5	1.0×10^5
3.3×10^5	3.8×10^5	3.5×10^5	3.2×10^5	1.8×10^5	1.7×10^5	1.3×10^5
3.6×10^5	4.5×10^5	4.8×10^5	4.1×10^5	3.2×10^5	1.4×10^5	1.1×10^5

TABLE 11

ACHROMOBACTER COUNTS PER MILLILITER OBTAINED AT 30 LB/ACRE/DAY LOADING RATE

Sampling Points						
1	2	3	4	5	6	Effluent
4.4×10^6	4.7×10^6	4.5×10^6	4.4×10^6	3.2×10^6	3.0×10^6	1.8×10^6
3.3×10^6	1.5×10^6	1.5×10^6	1.6×10^6	1.0×10^6	1.0×10^6	1.0×10^6
1.8×10^6	4.2×10^6	4.0×10^6	3.3×10^6	2.5×10^6	2.3×10^6	2.3×10^6
4.0×10^6	4.0×10^6	3.2×10^6	3.0×10^6	2.0×10^6	1.9×10^6	1.7×10^6
3.0×10^6	3.0×10^6	2.7×10^6	1.2×10^6	1.1×10^6	8.6×10^5	6.9×10^5
2.3×10^6	2.1×10^6	2.5×10^6	2.0×10^6	1.5×10^6	2.5×10^6	1.2×10^6
2.0×10^6	2.5×10^6	3.0×10^6	4.0×10^6	2.5×10^6	1.5×10^6	1.1×10^6
2.5×10^6	2.6×10^6	2.1×10^6	2.2×10^6	2.1×10^6	1.6×10^6	1.2×10^6
2.6×10^6	2.1×10^6	2.4×10^6	2.1×10^6	2.0×10^6	1.4×10^6	1.1×10^6
3.5×10^6	2.9×10^6	2.2×10^6	1.8×10^6	1.4×10^6	1.2×10^6	9.6×10^5

TABLE 12

COLIFORM COUNTS PER MILLILITER OBTAINED AT 30 LB/ACRE/DAY LOADING RATE

Sampling Points						
1	2	3	4	5	6	Effluent
9.0×10^4	5.0×10^4	5.0×10^4	3.0×10^4	8.9×10^3	3.0×10^3	2.0×10^3
4.4×10^4	2.8×10^4	2.4×10^4	1.5×10^4	8.0×10^3	9.0×10^3	6.0×10^3
5.0×10^4	2.5×10^4	1.7×10^4	1.7×10^4	8.0×10^3	6.6×10^3	5.4×10^3
3.6×10^4	3.5×10^4	1.8×10^4	1.4×10^4	1.2×10^4	7.6×10^3	6.1×10^3
4.0×10^4	3.0×10^4	2.0×10^4	2.0×10^4	1.4×10^4	8.1×10^3	4.9×10^3
6.1×10^4	4.0×10^4	2.4×10^4	1.8×10^4	1.4×10^4	1.0×10^4	5.2×10^3
3.7×10^4	2.8×10^4	1.0×10^4	1.0×10^4	7.6×10^3	5.5×10^3	4.0×10^3
7.1×10^4	3.1×10^4	2.0×10^4	1.2×10^4	7.0×10^3	6.5×10^3	3.2×10^3
6.5×10^4	3.5×10^4	1.5×10^4	1.3×10^4	8.1×10^3	6.0×10^3	2.3×10^3
5.5×10^4	2.9×10^4	1.3×10^4	1.1×10^4	8.0×10^3	3.5×10^3	1.5×10^3

APPENDIX III

TABULATED DATA--RESULTS OF BACTERIOLOGICAL COUNTS OBTAINED AT 45 LB/ACRE/DAY LOADING RATE

TABLE 13

TOTAL BACTERIAL COUNTS PER MILLILITER OBTAINED AT 45 LB/ACRE/DAY LOADING RATE

Sampling Points						
1	2	3	4	5	6	Effluent
8.1×10^6	5.6×10^6	5.5×10^6	5.4×10^6	5.3×10^6	4.6×10^6	3.6×10^6
9.6×10^6	6.3×10^6	6.1×10^6	5.8×10^6	5.5×10^6	4.7×10^6	4.1×10^6
1.0×10^7	9.3×10^6	9.2×10^6	6.8×10^6	5.7×10^6	5.2×10^6	4.5×10^6
9.2×10^6	8.4×10^6	7.7×10^6	6.5×10^6	6.1×10^6	4.9×10^6	3.8×10^6
1.2×10^7	9.1×10^6	8.5×10^6	7.3×10^6	6.9×10^6	5.8×10^6	5.1×10^6
1.4×10^7	1.1×10^7	8.7×10^6	7.8×10^6	7.1×10^6	6.0×10^6	4.9×10^6
1.1×10^7	9.5×10^6	8.8×10^6	6.7×10^6	6.3×10^6	5.5×10^6	4.0×10^6
9.8×10^6	8.7×10^6	8.6×10^6	6.4×10^6	5.8×10^6	4.9×10^6	3.6×10^6
1.3×10^7	1.0×10^7	9.2×10^6	8.1×10^6	7.2×10^6	6.4×10^6	5.1×10^6
1.0×10^7	9.3×10^6	8.1×10^6	7.2×10^6	6.8×10^6	5.4×10^6	4.5×10^6

TABLE 14

PSEUDOMONAS COUNTS PER MILLILITER OBTAINED AT 45 LB/ACRE/DAY LOADING RATE

Sampling Points						
1	2	3	4	5	6	Effluent
4.7×10^6	2.6×10^6	2.3×10^6	2.2×10^6	2.1×10^6	1.9×10^6	1.5×10^6
5.1×10^6	3.4×10^6	2.8×10^6	2.5×10^6	2.3×10^6	2.1×10^6	1.7×10^6
4.7×10^6	4.8×10^6	4.5×10^6	4.0×10^6	3.6×10^6	2.7×10^6	2.4×10^6
4.3×10^6	4.1×10^6	3.6×10^6	3.3×10^6	2.7×10^6	2.3×10^6	1.8×10^6
5.6×10^6	5.4×10^6	4.8×10^6	4.3×10^6	3.7×10^6	2.9×10^6	2.5×10^6
4.1×10^6	3.9×10^6	3.3×10^6	2.8×10^6	2.4×10^6	2.0×10^6	1.6×10^6
4.8×10^6	4.2×10^6	3.5×10^6	3.2×10^6	2.8×10^6	2.7×10^6	2.5×10^6
4.5×10^6	4.1×10^6	3.0×10^6	2.4×10^6	2.1×10^6	1.7×10^6	1.7×10^6
5.4×10^6	4.7×10^6	3.2×10^6	2.6×10^6	2.2×10^6	2.1×10^6	2.0×10^6
4.0×10^6	3.5×10^6	2.9×10^6	2.3×10^6	1.9×10^6	1.5×10^6	1.6×10^6

TABLE 15

FLAVOBACTERIUM COUNTS PER MILLILITER OBTAINED AT 45 LB/ACRE/DAY LOADING RATE

Sampling Points						
1	2	3	4	5	6	Effluent
3.5×10^4	5.7×10^4	4.0×10^5	4.1×10^5	1.0×10^5	3.1×10^5	3.0×10^5
2.0×10^4	6.0×10^4	2.3×10^5	3.2×10^5	3.1×10^5	2.7×10^5	2.0×10^5
1.5×10^4	5.3×10^4	3.0×10^5	4.2×10^5	1.0×10^5	2.6×10^5	3.1×10^5
1.8×10^4	6.1×10^4	2.0×10^5	3.5×10^5	4.0×10^5	2.2×10^5	2.1×10^5
3.0×10^4	1.1×10^5	1.9×10^5	5.6×10^5	4.2×10^5	4.1×10^5	3.6×10^5
2.8×10^4	5.2×10^4	2.7×10^5	4.5×10^5	5.3×10^5	3.4×10^5	1.7×10^5
1.4×10^4	6.4×10^4	2.3×10^5	3.0×10^5	4.2×10^5	3.0×10^5	1.5×10^5
2.0×10^4	8.6×10^4	3.1×10^5	3.0×10^5	4.0×10^5	2.5×10^5	2.2×10^5
1.7×10^4	5.6×10^4	1.8×10^5	4.6×10^5	3.4×10^5	1.5×10^5	9.3×10^4
1.5×10^4	1.0×10^5	3.5×10^5	5.0×10^5	3.5×10^5	1.0×10^5	1.0×10^5

TABLE 16

ACHROMOBACTER COUNTS PER MILLILITER OBTAINED AT 45 LB/ACRE/DAY LOADING RATE

Sampling Points						
1	2	3	4	5	6	Effluent
2.6×10^6	2.8×10^6	2.6×10^6	2.7×10^6	2.5×10^6	1.9×10^6	1.9×10^6
3.1×10^6	3.4×10^6	3.2×10^6	2.4×10^6	2.8×10^6	1.4×10^6	2.2×10^6
4.0×10^6	3.1×10^6	4.6×10^6	2.9×10^6	2.0×10^6	2.0×10^6	1.8×10^6
3.6×10^6	3.0×10^6	3.0×10^6	2.6×10^6	2.4×10^6	1.7×10^6	1.9×10^6
4.3×10^6	3.5×10^6	4.0×10^6	3.2×10^6	3.2×10^6	2.1×10^6	2.1×10^6
3.6×10^6	4.1×10^6	3.5×10^6	3.6×10^6	2.7×10^6	2.7×10^6	1.6×10^6
2.9×10^6	3.2×10^6	2.9×10^6	2.7×10^6	2.1×10^6	2.2×10^6	1.5×10^6
3.2×10^6	4.0×10^6	3.2×10^6	2.5×10^6	2.3×10^6	2.0×10^6	1.7×10^6
3.9×10^6	3.4×10^6	3.0×10^6	3.0×10^6	2.0×10^6	2.5×10^6	1.4×10^6
2.9×10^6	2.7×10^6	2.7×10^6	2.5×10^6	2.2×10^6	2.4×10^6	1.5×10^6

TABLE 17

COLIFORM COUNTS PER MILLILITER OBTAINED AT 45 LB/ACRE/DAY LOADING RATE

Sampling Points						
1	2	3	4	5	6	Effluent
1.1×10^5	5.3×10^4	9.0×10^4	8.1×10^4	3.6×10^4	6.3×10^4	2.0×10^4
9.6×10^4	5.7×10^4	8.8×10^4	8.4×10^4	3.2×10^4	6.6×10^4	1.8×10^4
6.0×10^4	6.7×10^4	4.8×10^4	4.2×10^4	4.1×10^4	2.7×10^4	2.4×10^4
8.2×10^4	5.1×10^4	6.6×10^4	5.9×10^4	3.6×10^4	4.1×10^4	2.1×10^4
5.7×10^4	6.9×10^4	5.0×10^4	4.4×10^4	5.1×10^4	3.4×10^4	3.4×10^4
7.2×10^4	5.6×10^4	6.3×10^4	5.6×10^4	3.8×10^4	4.8×10^4	3.2×10^4
7.8×10^4	7.8×10^4	5.0×10^4	4.1×10^4	4.5×10^4	2.8×10^4	3.1×10^4
1.0×10^5	5.4×10^4	6.1×10^4	4.5×10^4	3.0×10^4	3.1×10^4	1.7×10^4
8.5×10^4	9.2×10^4	5.2×10^4	4.2×10^4	7.2×10^4	2.8×10^4	3.6×10^4
9.0×10^4	1.0×10^5	4.9×10^4	4.4×10^4	6.6×10^4	2.7×10^4	2.4×10^4

APPENDIX IV

TABULATED DATA--RESULTS OF CHEMICAL AND BIOCHEMICAL ANALYSES

TABLE 18

BOD ANALYSES--AVERAGE VALUES FOR THE VARIOUS LOADING RATES

Sampling Points	15 Lb/Acre/Day		30 Lb/Acre/Day		45 Lb/Acre/Day		60 Lb/Acre/Day	
	BOD (mg/1)	Per Cent Removal	BOD (mg/1)	Per Cent Removal	BOD (mg/1)	Per Cent Removal	BOD (mg/1)	Per Cent Removal
Raw	220	--	220	--	220	--	220	--
1	26	88	24	89	26	88	64	71
2	26	88	22	90	24	89	62	72
3	24	89	22	90	22	90	53	76
4	22	90	20	91	22	90	46	79
5	22	90	20	91	20	91	44	80
6	20	91	18	92	18	92	42	81
Eff.	18	92	15	93	15	93	40	82

TABLE 19

DO ANALYSES--AVERAGE VALUES FOR THE VARIOUS LOADING RATES

Sampling Points	15 Lb/Acre/Day		30 Lb/Acre/Day		45 Lb/Acre/Day		60 Lb/Acre/Day	
	DO (ppm)	Per Cent Saturation	DO (ppm)	Per Cent Saturation	DO (ppm)	Per Cent Saturation	DO (ppm)	Per Cent Saturation
Raw	0	0	0	0	0	0	0	0
1	1.8	23	0.8	10	0.6	7	0	0
2	3.0	38	1.6	20	0.8	10	0	0
3	3.9	49	2.3	29	1.2	15	0.2	2
4	4.6	58	2.6	32	1.7	21	0.4	5
5	5.2	68	2.8	35	2.0	25	0.6	8
6	5.7	71	3.0	37	2.3	29	1.0	12
Eff.	5.8	73	3.1	39	2.4	30	1.4	18

TABLE 20

ORGANIC NITROGEN--AVERAGE VALUES FOR THE VARIOUS LOADING RATES

Sampling Points	15 Lb/Acre/Day		30 Lb/Acre/Day		45 Lb/Acre/Day		60 Lb/Acre/Day	
	Org. N (mg/1)	Per Cent Removal	Org. N. (mg/1)	Per Cent Removal	Org. N (mg/1)	Per Cent Removal	Org. N (mg/1)	Per Cent Removal
Raw	26.6	--	26.6	--	26.6	--	26.6	--
1	--	--	9.3	65	10.6	60	16.0	40
2	--	--	8.0	70	9.8	63	15.7	41
3	--	--	7.5	72	9.3	65	15.4	42
4	--	--	6.7	75	8.0	70	13.8	48
5	--	--	6.1	77	7.5	72	13.6	49
6	--	--	5.3	80	6.4	76	13.0	51
Eff.	--	--	5.1	81	5.3	80	11.4	57

TABLE 21

AMMONIA--AVERAGE VALUES FOR THE VARIOUS LOADING RATES

Sampling Points	15 Lb/Acre/Day		30 Lb/Acre/Day		45 Lb/Acre/Day		60 Lb/Acre/Day	
	NH_3 (ppm)	Per Cent Removal	NH_3 (ppm)	Per Cent Removal	NH_3 (ppm)	Per Cent Removal	NH_3 (ppm)	Per Cent Removal
Raw	51.3	--	51.3	--	51.3	--	51.3	--
1	--	--	34.9	32	41.0	20	46.7	9
2	--	--	35.9	30	42.1	18	46.2	10
3	--	--	34.9	32	36.4	29	45.7	11
4	--	--	34.9	32	36.4	29	45.7	11
5	--	--	34.4	33	35.9	30	43.6	15
6	--	--	33.9	34	35.9	30	43.1	16
Eff.	--	--	33.3	35	35.4	31	41.6	19