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THE FUNGAL ECOLOGY OF THE ACTIVATED SLUDGE PROCESS

by

Douglas William Jaques

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Master of Arts
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THE FUNGAL ECOLOGY OF THE ACTIVATED SLUDGE PROCESS

Douglas William Jaques, M.A.

Western Michigan University, 1984

Examination of wastewater secondary influent and activated sludge reveal that bacteria and fungi may exist in a negative correlation. Activated sludge may support a resident population of microfungi. All fungi isolated from secondary influent and activated sludge belong to the form-class Deuteromycetes. The density of geofungi in activated sludge may exceed that of bacterial chemolithotrophs and rival that of bacterial heterotrophs.

ACKNOWLEDGEMENTS

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The author also acknowledges the assistance given to the present study by personnel of the Kalamazoo Wastewater Treatment facility. I am grateful to the plant supervisor, and to various foremen and operators who permitted me to have an unlimited access to the activated sludge tanks at the facility. The laboratory supervisor at the plant, Mr. Ken Leanin, provided the author with environmental data for the treatment plant, and also the particular laboratory methods employed in the collection of such data.

Douglas William Jaques

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CHAPTER I

INTRODUCTION

A bulk of literature (Adamse, 1968; Benedict et al., 1971; Burdick et al., 1982; Dias et al., 1964, etc.) documents the role which bacteria play in the purification of wastewater. The organisms, especially heterotrophic bacteria, convert organic pollutant compounds into bacterial protoplasm and slime and are recognized as forming the basis of the biological food pyramid in activated sludge.

The role of protozoans in activated sludge, especially as predators of bacteria, has also received substantial attention (Curds, 1973b, 1982; Hawkes, 1960; Jones, 1973, etc.).

The fungal element of the activated sludge community, however, has received relatively little attention, and consequently, the importance of the fungal component is not well understood.

In contradistinction to the truly aquatic fungi, the Oomycetes and Chytridiomycetes, which occur in natural waters, the fungi found in the activated sludge environment are almost all terrestrial fungi. These geofungi may act as predators on protozoans or micrometazoans, or, they may serve as primary heterotrophic competitors of the bacteria if the pH is reduced or another similar stress is placed on the bacteria.

The present paper reviews wastewater treatment, true aquatic and adventively aquatic fungi (geofungi), various components which constitute the activated sludge community, and the role which fungi

may play in that community.

This study further provides the design, the methodology, the data and the discussion, conclusions and recommendations of a scientific examination of the secondary wastewater influent and the activated sludge of the Kalamazoo Wastewater Treatment Plant, Kalamazoo, Michigan.

CHAPTER II

LITERATURE REVIEW

The Treatment of Wastewater

The removal of pollutant substances from wastewater is most effectively accomplished through a three-tiered series of processes. The first stage, called primary treatment involves four separate steps. Sewage flow entering a treatment plant must be screened in order that large, potentially damaging objects, are withdrawn from the system. This primary influent normally passes into a grit chamber, an aerated conduit in which sand and other particles of similar high relative density are induced to settle out. The wastewater, which may still contain bouyant, medium-sized solids (c. 10 cm^3), is subsequently pumped through comminutors, giant grinders which reduce solids to smaller particles. Gravity sedimentation of the finely ground organic particles usually constitutes the final step in the primary treatment of wastewater. Nemerow (1978) estimates that this process, essentially a physical treatment of wastewater, removes about 60% of the suspended solids and 35% of the biological oxygen demand (BOD) of the sewage.

Secondary treatment, the intermediate stage in the comprehensive purification of wastewater, is designed to remove suspended organic matter and soluble organic compounds from the sewage. This removal may be effected through chemical, physical or biological means or a

combination of the three methods. Biological treatment constitutes the most effective method of removal of pollutant substances from the primary effluent. Gaudy and Gaudy (1980), however, point out that the mechanisms by which microbes oxidize the wide array of organic compounds in wastewater essentially remain unelucidated.

There exists a wide variety of biological treatments for the purification of wastewater. Nemerow (1978) describes sixteen different processes for the removal of organic dissolved solids. This author includes in his review the following systems: (a) lagooning in oxidation ponds; (b) activated-sludge treatment; (c) contact stabilization; (d) wet combustion; (e) trickling filtration; and, (f) anaerobic digestion. Poole and Hobson (1979) describe several biological continuous-culture systems. A modified oxidation ditch constitutes the first system. Polluted water is propelled around a circular channel; it is aerated in the process and thus microbial degradation of organic dissolved solids is enhanced. Trickling filtration is the second biological sewage treatment which the authors consider. In this system, organically laden water is sprayed over microbially-colonized beds of crushed stone. Layers of such slime-covered stones are called bacteria beds. As wastewater trickles down through the layers of loosely packed stone, it slowly traverses the colonized substratum, and the microbes present remove dissolved organic compounds. The final system which Poole and Hobson describe is the activated sludge process. This type of secondary treatment is widespread and efficient. It is especially preferred over trickling filtration in northern climates where winter temperatures can freeze the nutrient

stream of trickling filtration and halt plant operation. In the activated sludge process, effluent from the primary treatment is pumped into aeration basins where the water is thoroughly agitated and amply oxygenated (i.e., 2.0 - 4.0 mg/L). Microorganisms in general, and bacteria in particular, oxidize the carbon compounds in the wastewater. In effecting this metabolism, the bacteria transform the dissolved organic compounds into bacterial protoplasm and (proteinaceous) slime. The slime encapsulates clumps of nonmotile bacteria. The clumps of bacteria coalesce and settle to the bottom of the aeration tanks. This phenomenon is called flocculation and the settling masses of bacteria and slime are termed floc. Substantial amounts of the floc are constantly returned to the activated sludge tanks to inoculate influent waters. Hughes and Stafford (1976), Nemerow (1978), Pike and Curds (1971), and Curds (1973a,b) all attest to the rapid removal of pollutant organic compounds through the activated sludge process. Residence time in aerated tank basins may be as low as three or four hours. After this form of secondary treatment, more than 90% of the biological oxygen demand may be removed.

In spite of the removal efficiency of the activated sludge process, effluent from this treatment may still contain concentrations of organic pollutants which threaten oxygen levels in the receiving stream. Removal of such excess amounts of carbon compounds constitutes a process which may be called tertiary treatment (Gaudy & Gaudy, 1980). This form of treatment may also be biological, chemical, physical, or a combination of these. Sand filtration is often employed, as is general drainage through appropriate soil types. The removal

of excessive amounts of phosphorus and ammonia nitrogen is sometimes referred to as tertiary treatment, but Gaudy and Gaudy (1980) prefer to call that process advanced waste treatment. In such a process, ammonia is nitrified to nitrate, and this compound can be converted through bacterial activity to nitrogen gas which can then be stripped from the liquid. Some phosphorus can be removed through bacterial metabolism, but this is often insufficient and chemical precipitation of the phosphorus is usually required.

The importance of microbial activity to the operation of a wastewater treatment plant in general, and to the functioning of the activated sludge process in particular, appears to be without question. Consequently, a knowledge of wastewater microbes and their ecology would serve to improve plant operation. More efficient oxidizing bacteria, for example, might help to reduce the residence time of wastewater in aeration tanks; knowledge of interactions between motile bacteria and predacious protozoans might permit control of turbid effluents; insights into microfungal ecology could reveal new aspects of the phenomenon of bulking (non-settling of flocculated masses); and research into the enzyme kinetics of activated sludge may facilitate the development of microbial organisms which are capable of degrading recalcitrant compounds.

Occurrence of Fungi in the Aquatic Environment

Microfungi occur throughout a wide range of freshwater habitats. Chytridiomycetes are cosmopolitan in distribution (Alexopoulos & Mims, 1979); they may act as saprophytes in littoral waters (Sparrow, 1960)

or parasitize cyanophytes in limnetic areas (Paterson, 1960). Saprolegniaceous fungi are considered omnipresent inhabitants of most lentic environments (Coker, 1923; Seymour, 1970). Sparrow (1968) describes hyphomycetous flora which grow upon decomposing leaves in stream bottoms, while Ingold (1951, 1954, 1955) reports on discomycetous fungi which inhabit the same environment and colonize the same substratum.

In spite of the relatively acidic conditions (pH 3.5 - 5.0) of sphagnum bogs, these sites are thought to harbor numerous species of zoosporic fungi (Miller, 1965). Unusual types of chytrids and the saprolegniaceous fungus Achlya treleaseana appear to be characteristic of such habitats (Sparrow, 1966).

Fungi are not only well represented in marine environments (Johnson & Sparrow, 1961), but they also occur in extremely saline bodies of water such as the Salton Sea in California (Anastasiou, 1963).

Certain lakes in Japan are directly associated with nearby volcanoes and may receive strongly acidic waters from such sources. The pH of these inorganic acidotrophic lakes may drop to 1.9. Even in such an environment, however, Suzuki (1960, 1961) isolated five species of aquatic fungi, most notably, Saprolegnia monoica var. acidamica.

Particular microfungi may be associated with polluted aquatic environments. Harvey (1952) compares the presence of microfungi in an Ohio stream which receives sewage plant effluent with nearby streams believed to be free of human pollution. Seven species of phycomycetes were isolated from the septic zone downstream from the treatment plant discharge; a single isolate, however, a saprolegniaceous fungus,

Aphanomyces sp., was the only mold found in areas of heaviest pollution.

The fungi described in the preceeding paragraphs all belong to the Division Mastigomycota. One class in this division, the Chytridiomycetes, produces motile cells (either zoospores or planogametes) which are equipped with a single, whiplash flagellum (Sparrow, 1958). The other class in this division, the Oomycetes, is characterized by the production of asexual spores which are propelled by two flagella, one of the whiplash type, the other a tinsel-like structure (Alexopoulos & Mims, 1979).

Ingold (1971) suggests that the entire evolutionary development of the Mastigomycota has taken place in the aquatic environment. These fungi, therefore, may justly be viewed as aquatic organisms. A second group of fungi is also commonly found in the aquatic environment. These fungi belong to the Ascomycetes (i.e., Saccharomycetaceae) and the Fungi Imperfecti or Deuteromycetes. These last-mentioned fungi, of course, comprise asexual forms of ascomycetous molds, or, in rare cases, Basidiomycetes whose sexual stages are unknown. Deuteromycetes commonly found in the aquatic environment include the Shpaeropsidales (i.e., Phoma), the Cryptococcales (i.e., Rhodotorula, Candida) and the Hyphomycetes (i.e., Geotrichum, Dactylaria, Alternaria, Penicillium, Aspergillus).

In spite of the near ubiquitous presence of the Ascomycetes and the Deuteromycetes in the aquatic environment, both Sparrow (1968) and Cooke (1961, 1963a) view most of these molds as geofungi. As a consequence, these authors suggest that such fungi are merely

transient inhabitants of water, usually being introduced into the aquatic environment through rainwater run-off or by precipitation from the air spora. It thus appears debatable whether these molds may be termed aquatic fungi or not. Both writers, however, agree that the Ascomycetes and the Deuteromycetes commonly found in water can usually grow and reproduce in the aquatic environment.

Cooke (1963b) lists 19 species or groups of geofungi which were consistently isolated from acid mine drainage. This same author reports Deuteromycetes which colonize environments of extremely low pH. Fungal contamination of stock solutions of chemicals illustrates this phenomenon. Penicillium janthinellum, for example, has been identified as a contaminant of one-tenth normal HCL; and the moniliaceous fungus, Geotrichum candidum, the asexual stage of the Hemiascomycete Endomyces geotrichum, has an optimum pH of 3.0.

In an earlier study, Cooke (1961) examined the incidence and the influence of geofungi in a polluted waterway. His research served to isolate more than 35 species of terrestrial fungi from the stream under investigation. Cooke suggests that fungal adventives may contribute to a reduction in biological oxygen demand through the assimilation of pollutant compounds.

Mycoflora of Wastewater Treatment Facilities

Limited numbers of hydrofungal species have been associated with raw sewage or water treatment processes.

Srivastava and Gupta (1979) report a saprolegnian in sewage of the Indian city of Kanpur. Their brief article indicates that isolation

samples were taken prior to treatment of the wastewater. Concurrent research (Vishwe & Umalkar, 1979) indicates the presence of two species of pythiaceus fungi in the sewage of a second Indian city. The report states that fungi were isolated from "activated sewage sludge." The article presents few details, and it appears that the authors may simply have tested raw sewage, before any treatment had taken place.

Cooke (1963a) records a chytrid, Rhizophidium carpophilum, which has been found as a parasite on Achlya debaryana (a saprolegnian) in waste stabilization ponds. This same author lists three other members of the Mastigomycota which occur in association with wastewater treatment. A. diffusa and Aphanomyces scaber have been isolated from waste stabilization ponds while Thraustotheca clavata has been found in the slime of trickling filters.

Hawkes (1960) noted the luxuriant growth of the oomycete, Leptomitius lacteus, in the feed channel of a bacteria bed.

The pythiaceus mold Zoophagus insidians has been isolated in samples taken from an activated sludge pilot plant (Cooke, 1963a). This mold is a documented predator on rotifers (Cooke & Ludzack, 1958).

The vast majority of fungi found in sewage treatment plants is composed of soil fungi, microbes which are considered adventives in the aquatic environment. Such a preponderance of facultative aquatic fungi may be better understood if wastewater in general and activated sludge in particular are viewed as soil and not aquatic environments (Cooke, 1963a).

While examining the aerobic heterotrophic bacteria in activated sludge, Benedict and Carlson (1971) isolated the ascomycetous yeast,

Debaromyces sp. These researchers suspect that this Hemiascomycete is instrumental in the degradation of organic wastes.

Cooke et al. (1960) investigated the yeast populations at the Dayton, Ohio sewage treatment plant. The genera Candida, Rhodotorula and Trichosporon were most frequently isolated, while Cryptococcus and Torulopsis were present but infrequently found. A total of twenty-three species of yeasts were isolated at the Dayton facility. Cooke and his coworkers also suggest that yeasts aid in sewage purification.

Jones and Schmitt (1978) maintain that the human pathogen Candida albicans is commonly found in wastewater treatment plants. These writers contend that current chlorination practices at wastewater treatment facilities are unable to prevent release of the mycologic pathogen into the environment.

Hawkes (1960) estimates that microfungi play a more significant role in bacteria beds than in activated sludge. Those fungi which the author assigns to bacteria beds include: Ascoidea rubescens, Fusarium aqueductum, Geotrichum sp., Penicillium sp., Sepedonium sp., and Sporotrichum sp. The first-mentioned fungus is a Hemiascomycete, the others are hyphomycetous molds. The same writer asserts that bacteria remain the dominant heterotrophs in the trickling filtration environment; the author elsewhere notes, however, that if acidic industrial sewage is sprayed onto bacteria beds, fungi may outcompete bacteria (Hawkes, 1957).

The presence of a predaceous Hyphomycete has been reported in activated sludge pilot plants (Pipes & Jenkins, 1965; Pipes, 1965). A moniliaceous fungus, Arthrobotrys sp. is described as a predator on

rotifers and nematodes. Later investigators (Slaka & Zahradka, 1970) have added another predaceous Hyphomycete, Dactylaria sp., to the fungi found in the activated sludge environment.

In their report on the occurrence of microfungi in the sewage of Aurangabad City, India, Vishwe and Umalkar (1979) list four Zygomycetes (Cunninghamella echinulata, Mortierella sp., Mucor sp., Rhizopus stolonifer), two Hymenoascomycetes (Chaetomium globosum, Sordaria bosen-sis), nine genera of Deuteromycetes (Aspergillus, Cladosporium, Fusarium myrothecium, Neurospora, Penicillium, Pestalotia, Phoma, Trichoderma), and the two pythiaceae fungi mentioned above.

As cited earlier, Srivastava and Gupta (1979) studied the sewage of the Indian city of Kanpur, "a big industrial and overpopulated town." Establishments which contribute wastewater to the city's sewage include "slaughter houses, dairies, tanneries, glueworks, oil mills, grease works and . . . several factories concerning textiles, dying, printing, fertilizers, paints, rubber, steel, automobiles, etc. (p.) The pH of this city's sewage was determined to lie between 1.0 and 2.0, and the temperature of the wastewater was found to vary from 18.0 to 20.0°C. The authors employed a simple test, the reduction of tensile strength of cotton, to demonstrate an apparent high degree of activity of cellulolytic microorganisms in the sewage of Kanpur. Two genera of Zygomycetes, five genera of Deuteromycetes and the single saprolegnian mentioned above were found in the wastewater of this city. Among the fungi isolated, three genera of the Deuteromycetes, Aspergillus, Penicillium and Cladosporium are known to be cellulolytic organisms (Gaudy & Gaudy, 1980); an anaerobic bacillus, Clostridium, also metabolizes

cellulose. This bacterium is commonly found in fresh water sediments and the soil. If this anaerobe is found in the sewage of Kanpur, the Indian researchers may be measuring the activity of more than one type of cellulolytic microbe.

Cooke (1963a) describes more than 350 species of microfungi which occur in polluted waters, sewage, and wastewater treatment systems. The mucoraceous fungus, Absidia corymbifera, is reported to occur in raw sewage and trickling filter slime; Mucor fragilis and M. plumbeus were isolated from all habitats sampled. M. racemosus was found in activated sludge and Rhizopus arrhizus was another Zygomycete observed in all habitats investigated. Although the familiar hemiascomycetous fungus, Saccharomyces, appeared in waste stabilization ponds and in Imhoff tank sludge, it was not isolated elsewhere in wastewater treatment systems. A single member of the Hymenoascomycetidae, Chaetomium globosum is recorded; this fungus, however, occurred in samples taken from bacteria beds, raw sewage, effluent of primary settling tanks and waste stabilization ponds. Cooke observes that the most important fungi of polluted and wastewater habitats are the moniliaceous molds. These Hyphomycetes have greater specific diversity and substantially wider occurrence in polluted aquatic environments than any other fungi. Cooke (1963a), however, does include the form-order Cryptococcales in his evaluation of the Moniliales. This is a taxonomic inclusion which later authorities may not support (Alexopoulos & Mims, 1979). Among the moniliaceous molds which Cooke isolated (the Cryptococcales excluded), Geotrichum candidum was found in all habitats surveyed; Botryotrichum piluliferum was isolated from activated sludge;

Alteraria tenuis was observed in most habitats sampled; the genus Fusarium was a ubiquitous isolate; as were the extensive genera Aspergillus and Penicillium. Other moniliaceous Hyphomycetes which Cooke lists include: Paecilomyces elegans, found in digester sludge; Trichoderma viride, isolated from all habitats sampled; Verticillium lateritium, present in most processes of wastewater treatment; Stachybotrys atra, a mold of sludges and waste stabilization ponds; and Myrothecium roridum, a common isolate of sewage treatment plants.

Community Structure and Population Dynamics in Activated Sludge

Component Populations of the Activated Sludge Community Bacteria

Curds (1982) classifies activated sludge bacteria into two categories: sludge bacteria which comprise organotrophs instrumental in the flocculation of dissolved organic compounds; and sewage bacteria, organotrophs and chemolithotrophic microbes which are suspended in the liquor and do not settle to the bottom of the activated sludge tank. Pike et al. (1972) note that less than 10% of the bacteria in activated sludge are freely dispersed. The flocculating organotrophs, therefore, are the primary agents in the transformation of organic waste into biomass. In such capacity as primary consumers, these microbes form the basic trophic level of the activated sludge community.

Organotrophic bacteria commonly associated with the efficient functioning of the activated sludge process are usually gram-negative aerobic rods and gram-negative facultatively anaerobic rods (Hawkes, 1960). Although Dias and Bhat (1964) reported activated sludges

containing as many as 42% gram-positive strains, these authors generally found that gram-negative bacteria of the genera Zoogloea and Pseudomonas predominated. Adamse (1968) studied the succession in the bacterial flora of dairy waste activated sludge. Although members of Pseudomonadaceae initially predominated, as the waste was oxidized, coryneform bacteria and large amounts of Achromobacteraceae came to be established. As early as 1935, Butterfield had noted the floc-forming characteristics of a gram-negative bacterium which was tentatively identified as a variety of Zoogloea ramigera; this author reported that Z. ramigera, or similar floc-forming organisms were able to remove more than 80% of the oxidizable material in activated sludge. Subsequent research has supported such removal efficiency of the floc-forming bacteria (Pipes & Minnigh, 1982; Burdick et al., 1982).

Protozoa

While the Sarcodina are present in the activated sludge community, their numbers and importance remains largely undetermined. Hawkes (1960), however, briefly mentions that the rhizopod Arcella, has been commonly found in high quality nitrified effluents at an English wastewater treatment plant. In the same article, this author generally associates the presence of the Sarcodina with the lowest of purification efficiencies. In a more recent publication, Curds (1982) reports that testate and naked rhizopods may rival other protozoans in numbers and biomass.

Much more research has been given to the Mastigophora and especially to the Ciliophora. The ciliates are considered to be

particularly important because they act as indicator organisms: the presence of these microorganisms and their individual specific percentage of the protozoan community, serve to indicate efficiency of an activated sludge process.

The Mastigophora are usually associated with a newly established activated sludge. Even after an inoculation of mature sludge into a new system, an ordered succession of protozoa most often occurs: flagellated protozoans appear first, followed by free-swimming ciliates, crawling ciliates and finally attached ciliates (Curds, 1973b).

The Mastigophora are also considered as indicators of inefficient activated sludge processes. These organisms are often associated with the stressed environment which various types of shock loadings (i.e., hydraulic, pH and toxic) may produce (Curds, 1982).

The Ciliophora have received the most attention of all protozoans found in activated sludge. Madoni and Ghetti (1981) studied the ciliate composition of Italian activated sludge processes; in their investigation, they found 19 holotrichs, 14 peritrichs, 8 spirotrichs and 4 suctorians. Furthermore, these authors discovered affinities between some of the ciliates and were therefore able to propose ciliate communities. A principal community, consisting of Aspidisca costata, Vorticella convallaria, Epistylis plicatilis, Trochilia minuta and V. striata octava, was generally found to obtain, while four other ciliates (including a suctorian) were listed as associated species. Five additional species or pairs of species were associated with the principal community as secondary sub-communities.

The specific composition of ciliate of activated sludge plants

in Italy is similar to that reported by Curds and Cockburn (1970) in England. Morishita (1976) reports many of the same ciliate genera in Japanese activated sludge processes. In addition, Morishita presents an efficiency index based on the numbers of peritrichs present. This evaluation, called the PC-index (Peritrichida-index), supposes that peritrichs are associated with efficient activated sludges, and that their relative numbers, frequency of occurrence, and mean number of cells per mL will provide a reasonable indication of plant performance.

Metazoa

Metazoans are more common in bacteria beds than in activated sludge. The discharge volume of supernatant waters and sludge wastage rates of the activated sludge process tend to discourage the colonization and maintenance of most metazoans. Rotifers and nematodes, however, are often present and their populations may follow the cyclic ebb and flow of bacterial populations. As a top-level predator, the presence of rotifers in the activated sludge may directly influence protozoans populations. The import of this relationship will be discussed later.

Hawkes (1960) reports that the copepod, Cyclops, the annelid, Aeolosoma and chironomid larvae have also been isolated from wastewater treatment plants.

Microfungi

As the third section of this literature examined the mycoflora of activated sludge plants, this researcher shall not return to that material here. It should be noted, however, that, as Sparrow wrote in 1968, science has not yet determined whether microfungi form structured communities as do higher plants. Microfungi are thus seen as omnipresent, opportunistic organisms which rapidly fluctuate in number according to the nutrient composition and availability of the substrate.

Environmental Conditions and Community Structure of Activated Sludge

Various selective pressures are present in the activated sludge environment. Pike and Curds (1971) provide seven types of such pressures: the sludge wastage rate; the settling of flocculated biomass; the presence of toxic material; excess or deficiency of certain nutrients; pH and the dissolved oxygen content in the activated sludge.

Under steady state conditions, the number of microbes removed in sludge wastage will equal the reproductive rate of such organisms. Microbes which have a short reproductive cycle will thus be at a competitive advantage compared to organisms with longer cycles. For this reason, most metazoans are washed out of the activated sludge process, as are some of the slower growing bacteria, such as Nitrosomonas spp.

The settling of flocculated biomass and the constant recycling of this material as a reinoculant in the activated sludge process, act as selective pressures for the settling bacteria and their attached

ciliates (viz., the peritrichs); and they serve as selective pressures against suspended bacteria and protozoans which are constantly being washed out of the system.

The presence of toxic substances in activated sludge can adversely affect the microbes in the community and their oxidative activities. Seyfried (1980) tested the resistance of 260 strains of Pseudomonas aeruginosa to varying concentrations of mercury, cadmium, arsenic and lead. P. aeruginosa is a common organotrophic bacterium of activated sludge, as are numerous other pseudomonads. Seyfried observed that all of the strains were resistant to 1000 ug/mL of arsenic and lead, while nearly all (97%) were resistant to 400 ug/mL of cadmium. On the other hand, Seyfried also discovered that more than half (54%) of the strains of P. aeruginosa were sensitive to mercury concentrations greater than 10 ug/mL.

Albright et al. (1972, 1974) reviewed the sensitivity of organotrophic bacteria to various elements. These researchers reported a hierarchy of microbial sensitivity to nine different ions: they observed that the silver ion is equal to or greater in toxicity to the cupric ion; that at the next level of toxicity, the nickel ion is greater than the barium and chromium ions; and that at the last level of toxicity, the mercuric ion is more toxic than the zinc, sodium and cadmium ions.

If toxic substances such as those mentioned above, those which figure on EPA priority pollutant lists, or toxins yet to be identified, are able to gain access to an activated sludge community, efficiency of the activated sludge process can be adversely affected. For

example, the metabolic rate of the floc-forming bacteria could be significantly reduced, or the viability of sessile ciliates could be jeopardized by the toxins. Pipes and Minnigh (1982) observed the deleterious influence of two toxic substances on two chemolithotrophic bacteria. They found that 23 mg/L of hydrazine would inhibit Nitrobacter and Nitrosomonas and that 5 mg/L of nickel would inhibit nitrification completely.

The microfungi of activated sludge communities are able to successfully compete with the organotrophic bacteria under several sets of conditions. It is important to note, however, that these fungi are effective competitors not because they favor extreme environments, but because they are able to tolerate and to grow in clearly suboptimal conditions. Hawker (1957) observes that Penicillium and Aspergillus grow on sugar saturated substrates not because they fungi prefer such a habitat, but because they have a high sugar tolerance; furthermore, for fungi that parasitize acid fruits, even though their optimum growth may occur at a pH below that of most other fungi, this optimum is nevertheless higher than the low pH of the juices of acid fruits.

The acid tolerance of fungi is rather well documented. Cooke (1963b) reported more than twenty different species of microfungi isolated from acid mine drainage with a pH as low as 4.5. In the same article, the author notes that Penicillium janthinellum has been isolated as a contaminant from .1 normal HCl, a solution which has a theoretical pH of 1.0. Benefield et al. (1975) found Geotrichum sp. would grow at pH 3.0, and that filamentous fungus would compete most successfully at such a low pH. Hawkes (1960) reports that acid flushes

of industrial waste can lower the pH of activated plants; this condition is apparently favorable to the filamentous microfungus Geotrichum, as it may then become dominant.

Microfungi are also associated with a low dissolved oxygen (DO) content which may obtain under excessive organic loading conditions (McKinney, 1957). Benefield et al. (1975) observe that when DO content in the aeration tanks drops for an extended period of time, Geotrichum sp. may become dominant. Jones (1964), for example, found that filamentous fungus was able to proliferate at levels of $.1 \text{ mg L}^{-1}$ DO.

Surprisingly, high DO content in activated sludge has also been reported to cause the growth of Geotrichum (Bhatla, 1964). This author believes that a zone of decreasing nutrient concentration develops around the periphery of each floc particle. Bhatla suggests that organic acids are released as metabolic end products from the anaerobic core of the floc particles, and that, from the diffusion of these acids, a pH gradient which radiates outward is established. Under high DO concentrations, where oxygen is able to penetrate the acid zone of the floc, and provided adequate nutrients are present, conditions may then favor the growth of filamentous organisms such as Geotrichum.

A number of researchers review the effect of various nutrients on the growth of microfungi. Benefield et al. (1975) report that excessive carbohydrates probably do not induce the growth of microfungi in activated sludge. These same scientists, however, do suggest that high carbon to nitrogen ratios may encourage the proliferation of filamentous fungi. McKinney (1957) generally supports this assessment,

and also points out that fungi generally require less nitrogen per unit mass than do bacteria, an important observation as the two types of organisms are competing with one another in the same microbial community.

Sparrow (1968) reports that hyphomycetous fungi, the geofungi which predominate in activated sludge, are much more versatile in their ability to degrade simple and polymeric carbohydrates than the more fastidious water molds such as Saprolegnia sp. Cooke (1957) also notes that the geofungi are able to reduce many carbohydrates and nitrogen sources without the addition of vitamins.

Microbial Interactions and the Activated Sludge Community

Paynter and Bungay (1971) describe two-component microbial interactions which may approximate some ecological relationships in the activated sludge community. The organisms which were studied were observed in a laboratory continuous culture, in a controlled environment and with a constant nutrient feed.

The first system contained a bacterium, Proteus vulgaris, and a yeast, Saccharomyces cerevisiae. The ascomycete produces niacin, a compound of the vitamin B complex which the enteric bacterium requires for growth. Both microbes reached a steady-state population and thus a commensal interaction was seen to obtain.

When nicotinic acid was added to the system, the bacterium no longer needed the ascomycete's metabolic by-product. S. cerevisiae was then unable to compete with P. vulgaris for the available carbon

compounds, and the yeast was subsequently washed out of the system.

Paynter and Bungay also examine the microbial interaction of predation. A cellular slime mold, Dictyostelium discoideum, was introduced into axenic cultures of either Enterobacter aerogenes, Escherichia coli, or Bacillus polymixa. In each instance, periodic oscillations of the populations ensued. Here, as in all simple predator-prey relationships, the predator is dependent on the size of the prey population. When predator populations significantly reduce the numbers of their prey, the predator populations must, after a lapse, decline as well. In nature, where a predator may prey upon a wide diversity of organisms, its numbers are not as directly tied to a single prey population, but are more closely related to the amount of available nutrients and biomass in the community as a whole.

Curds (1982) comments on the predatory role of protozoans in the activated sludge process. If bacterial populations grow unchecked, they reach self-limiting numbers; grazing ciliates (i.e., the holotrichs) prevent the bacteria from reaching such a population plateau, and thus help to ensure physiological youth of the organotrophs. Bacteria in the log phase (i.e., physiological youth) assimilate carbon compounds more efficiently than those in the decline phase (i.e., senescence).

Elsewhere, Curds (1973b) reports on the effect of ciliates, such as suctoria and some gymnostomes, which feed on other ciliates. When a gymnostome such as Hemiphrys feeds upon the stalked ciliate, Vorticella, the numbers of this latter organism may decrease precipitously. Curds believes that the fluctuations of peritrichs in

activated-sludge plants may well be associated with the predatory activity of certain gymnostomes.

The preceding author (Curds, 1973a) also reports on the decimation of bacteria-feeding ciliates when a large population of suctorians suddenly appeared. This event occurred at a full-scale activated sludge plant; removal of the free-swimming ciliates and the resulting increase in bacteria substantially reduced the quality of the plant effluent.

Yech et al. (1968) report an example of biochemical mutualism between two bacterial genera which occur in activated sludge. Proteus vulgaris and Bacillus polymixa were found to grow together on a medium which would support neither alone. The gram-positive bacterium produced nicotinic acid which the enteric organism required, while the latter released biotin, a compound necessary to the growth of B. polymixa.

Jørgensen (1956, 1962) and Edmondson (1965) refer to the antibiotic effects of some algae, notably various Chlorophytes and Chrysophytes, and Sparrow (1968) considers that such substances may have fungistatic or fungicidal properties. Hawkes (1960), however, reports that algae are usually absent from the activated sludge community. While many activated-sludge tanks today are covered, a condition which would inhibit the activity of autotrophic algae, this situation would not impede the growth of heterotrophic Cyanophytes. Sykes et al. (1979) report operational problems due to the massive growth of the blue-green alga Schizothrix calcicola in activated-sludge tanks.

Predaceous Activity of Microfungi

Fungal Predation of Freshwater Invertebrates

The ubiquitous aquatic fungi are known to accompany freshwater invertebrates in all of their numerous habitats. Research has shown that fungal obligate parasites of the freshwater invertebrates comprise an important, though poorly understood, group of predators. Amoebae are attacked by both ecto- and endo- fungal parasites of the order Zoopagales; members of the class Oomycetes and the Lagenidiales-Peronosporales Complex parasitize numerous species of rotifers; bear animalcules are hosts to endoparasites of the order Entomophthorales and nematodes are parasitized by species of the form-order Moniliales.

Although only four phyla of freshwater invertebrates are known to be preyed upon by aquatic fungi, this modest number of phyla reflects a general inattention by researchers (Duddington, 1968). Workers in the fields of fungal predation and aquatic mycology are convinced that the range of fungal attack, advancing well beyond the narrow limits already established, will be shown to encompass most if not all of the phyla of freshwater invertebrates (Duddington, 1968).

The scope of fungal predation on higher aquatic organisms was already illustrated more than forty years ago (Tiffney, 1939). This researcher reported that Saprolegnia ferax, S. parasitica, S. mixta and Achlya prolifera were observed to attack no less than forty species of fish as well as eels and lampreys.

Members of the order Zoopagales are known to parasitize Proteomyxa and Amoebina (Drechsler, 1935; Duddington, 1956). In their

random wanderings, the protozoic organisms encounter the hyphal strands of the fungi. Although the precise mechanism is not known, the amoebae which come into contact with the hyphal threads become attached to the fungus. Special penetrating hyphae, called haustoria, enter the body envelope of the amoeba and the animal's contents are absorbed by the parasite.

Some species of the Zoopagles are endozoic parasites (Drechsler, 1935). Amoebae ingest the fungal spores as they would a particle of food. Instead of being digested, however, these spores germinate and grow at the expense of the host's protoplasm. Although the infected amoeba at first shows no signs of stress, it later loses its vigor, its contractile vacuole disappears and it finally ceases all activity.

A number of freshwater fungi prey upon wheel animalcules. Recently, a new mold, Haptoglossa mirabilis, assigned to the class Oomycetes, was discovered and observed as an endoparasite attacking the Adineta rotifers (Barron, 1980). Infection is accomplished by the injection of a cylindrical sporidium into the body of the host. The infection cell forms following germination of an encysted zoospore. The growing thallus within the body of the rotifer commonly produces no more than several biflagellate zoospores.

Another oomycete, Sommerstorffia spinosa has been found parasitic on the rotifer Lepadella elliptica (Czeczuga & Proba, 1980). Seymour and Johnson (1973) report an unidentified oomycete from Iceland which is parasitic on rotifer eggs; and Sparrow (1939) describes a species of the Lagenidiales-Peronosporales Complex, subdivision Mastigomycotina, which is likewise parasitic on rotifer eggs and also on

the embryos of the wheel animalcules.

Bear animalcules are parasitized by several species of aquatic molds. Ballocephala pedicellata, a new species of Entomophthorales, which attacks Hypsibius dujardini and Diphascon pinguis has recently been described (Pohlad & Bernard, 1978). Observations were made on tardigrades which were already infected when collected; no bear animalcules were successfully infected in the laboratory. The fungus develops in the host's body and then pierces the body shell to form conidiophores. A large percentage of these fruiting bodies emerge through the legs of the tardigrades. The authors report that they were unable to ascertain whether or not the conidia are forcibly discharged from the conidiophores.

Fungal predation on the Nematoda has received substantial attention in recent years (Duddington, 1968). Most of the known fungal parasites of roundworms belong to the form-order Moniliales, of the form-class Deuteromycetes. These fungi exhibit a variety of predatory adaptations. The fungus Arthrobotrys oligospora entraps eelworms by means of an adhesive network (Drechsler, 1937). Dactylaria candida forms nonconstricting rings; if a nematode should happen into such a ring, it is unable to force its way through, and does not know enough to back out. In Dactylaria gracilis, however, fungal rings are capable of swelling through an increase in osmotic pressure to three times their non-inflated volume. The swelling action garrots the hapless nematode.

Predacious Microfungi and the Activated Sludge Community

Cooke and Ludzack (1958) report the presence of a predaceous fungus, Zoophagus insidians in several laboratory activated-sludge units. The authors had begun an experiment to determine the rate of removal of nitriles from water. During their experiment, Z. insidians appeared as a contaminant in several sludge units. Within a few days after the appearance of the fungus, quality of the effluent in affected units declined significantly: suspended solids increased by as much as tenfold and mixed liquor solids decreased by one half.

Microscopic examination revealed that the mold was preying upon rotifers of the genus Monostyla; and this wheel animalcule was the principal predator of the bacteria in the laboratory units contaminated by the fungus.

Z. insidians is a common predaceous fungus and has been reported from Europe as well as numerous locations in the United States of America.

The mycelium of each mold produces a series of short stalks. A sticky mucilaginous material is secreted and covers the tips of the hyphal stalks. When a rotifer comes into contact with such an adhering organ, it is usually held fast. Hyphae then enter the rotifer and this organism's protoplasm is subsequently transferred to the mold.

No where in the literature has this investigator found reports which indicate that rotifers are primary bacterial predators in activated-sludge plants. In the experiment associated with the present writing, however, the author suspects that Philodina may at times

have constituted the main control of bacterial populations.

If rotifers were to play the role of primary bacterial consumers, their reduction through fungal predation would alter the structure of the activated-sludge community and would consequently reduce the efficiency of the activated sludge and diminish effluent quality.

Bulking of Activated Sludge

Microbes of activated sludge transform dissolved organic compounds in the wastewater to flocculent masses of microbial protoplasm and gelatinous capsules. When the floc settles to the bottom of the aeration tanks, it may be withdrawn for disposal, or it may be used as a microbial inoculation of fresh secondary influent.

At times, the flocculent masses may not settle, but remain on the surface of the activated sludge. This phenomenon is called bulking.

Hawkes (1960) considers that bulking is a biophysical response to an alteration in the ecological balance in the activated sludge community. The author states that oxygen levels, food supply and the introduction of toxic substances appear to be the main causative factors of bulking. Pike and Curds (1971) report that bulking may also be caused by high C:N and C:P ratios or by N deficiency alone.

Numerous investigators (Hawkes, 1960; Benefield et al, 1975; Sykes et al., 1979) have associated the chemolithotrophic bacterium Sphaerotilus with the phenomenon of bulking. Sykes et al. (1979) also report that the gliding bacterium Beggiatoa, another chemolithotroph, is a bulking organism which may be present when large quantities of brewery waste are being treated. These same researchers

report that a filamentous Cyanophyte, Schizothrix calcicola was responsible for bulking in an Ohio activated-sludge plant.

Hawkes (1960) and Benefield et al (1975) assert that the fungus Geotrichum is a common bulking organism. This moniliaceous mold is found in wastewater rich in carbohydrates, and especially obtains in an acidic environment (below pH 5.5). At such a low pH, heterotrophic bacteria are at a competitive disadvantage to the mold. An activated sludge low in dissolved oxygen (i.e., $.1 \text{ mg L}^{-1}$) would also favor Geotrichum. At such low concentrations of oxygen, the activity of normal bacterial components of the activated-sludge community would be inhibited, and the Deuteromycete, although a strict aerobe, would nonetheless survive and proliferate (Jones, 1964).

CHAPTER III

STUDY DESIGN AND METHODOLOGY

Design of the Study

The experimental design of the present study encompasses three parts: determination of the environmental parameters of the activated sludge tanks at the study site (videlicet: Kalamazoo Wastewater Treatment Plant); microbiological examination of the activated sludge to ascertain composition of the microbial community; and, determination of the presence and frequency of microfungi in the activated sludge.

Determination of Environmental Parameters

The microbial populations of any habitat are a function of the environmental conditions of that site. Consequently, basic chemico-physical parameters of the activated sludge of the Kalamazoo Wastewater Treatment Plant (KWTP) were gathered as preliminary data for the present research. This information would later assist in explaining the microbial composition of the community under study.

Basic physical factors such as suspended solids per L, percentage return sludge, and flow rate of the activated sludge were all determined for the month of October 1983, the primary data collection period.

During this same time interval, BOD₅, volatile suspended solids,

DO in the mixed liquor, hydrogen ion concentration, phosphorus concentration and ammonia concentration were also determined for the activated sludge of the KWTP.

Data indicating potential concentrations of priority pollutants in the KWTP were drawn from a study prepared by the consultants, Greeley and Hansen of Chicago, Illinois. This study was conducted at the behest of the City of Kalamazoo and presented to that municipality in December 1982.

Identification of Component Populations

Preliminary inquiries had revealed the presence of bacteria, fungi, protozoans and two phyla of metazoans in the activated sludge of the KWTP. Although heterotrophic Cyanophytes were observed, the contribution of this group of organisms to the activated sludge community was considered to be outside the bounds of the present study.

As bacteria generally constitute not only the principal organotrophs in activated sludge, but also the major chemolithotrophs, an effort was made to identify the principal groups of these microbes and also to determine the relative numbers of such groups.

As primary predators of bacteria, protozoans, especially stalked ciliates of the order Peritrichida, play an important role in the activated sludge community. The significance of protozoan activity is reviewed previously, in Chapter II. Because of their crucial role in the microbial community under study, protozoans were identified to genus (and species, if possible) and their densities were also determined.

Few phyla of metazoans are reported to occur in activated sludge. The selective pressures for their removal (especially high sludge wastage rates) are too great to permit their effective colonization of the habitat. Nonetheless, members of the phyla Rotatoria and Nematoda are known to exist in activated sludge; consequently, efforts were made to record the presence and activity of such metazoan microbes.

Presence and Frequency of Microfungi

The identification of microfungi in the activated sludge community of the KWTP, and the determination of their various densities constitute the core of the present research. Except for Cooke (1956, 1958, 1959, 1960, 1963a, 1963b), few researchers have conducted systematic investigations to determine the role of fungi in the activated sludge process.

In the present experiment, classical microbiological techniques were employed to isolate and enumerate geofungi at the KWTP. Growth on selective media, association with particular bacteria, and occurrence under varying environmental conditions were all aspects of fungal activity which were closely monitored and incorporated into the present study.

Methodology

Determination of Physico-Chemical Parameters

Data on the physico-chemical parameters of the study site's environment were obtained from the Kalamazoo Wastewater Treatment Plant.

Such parameters are important to the efficient functioning of the plant and thus, are collected at least once a day and are subsequently recorded in computer files. Abbreviated descriptions of the Kalamazoo Wastewater Treatment Plant's testing procedures follow.

Temperature. The Kalamazoo Wastewater Treatment Plant reports that no temperature readings of the sewage or any part of its processing are taken at the treatment facility. During sample collection, the author did record temperature with a hand-held mercury thermometer.

Biochemical Oxygen Demand. Biochemical oxygen demand was measured through the inoculation and incubation of diluted samples of sewage. The incubation bottle was 300 mL in size; incubation temperature was $20 \pm 1^\circ\text{C}$, and the incubation period lasted for five days. Dilution factors varied according to the strength of the sample. The pH of the sample was maintained during incubation by the use of the following four buffering solutions: KH_2PO_4 , K_2HPO_4 , Na_2HPO_4 & H_2O , and NH_4Cl . The plant reports that no nitrification inhibitor was used in testing for the biochemical oxygen demand.

Volatile Suspended Solids (Volatile Nonfiltrable Residue). To determine the amount of volatile suspended solids, a sample was first dried. The drying temperature was 103 to 105°C and the drying period lasted until a constant weight was attained. The dried sample was then heated in an ignition furnace ($550 \pm 50^\circ\text{C}$) and the difference between the pre-ignition weight and the final constant weight of the sample constituted an estimate of volatile suspended solids.

Return Sludge as Percentage of Sewage Flow. The Kalamazoo Wastewater Treatment Plant recycles the sludge of the secondary treatment process. The rate of recycled sludge is not dependent on wastewater flow, but is determined by pump capacity alone. The return sludge can thus constitute more than 100% of the sewage flow.

Dissolved Oxygen in Mixed Liquor. The dissolved oxygen of the mixed liquor in the activated sludge process is determined each day by the membrane electrode method.

pH. The pH of final settling tanks is recorded at the treatment facility. A pH meter, with potentiometer and combination electrode (reference electrode and glass electrode) were utilized in the measurement of pH. The pH in the activated sludge tanks is reportedly not determined on a regular basis. The author, however, did make pH determinations when gathering activated sludge samples. Such measurements were also made with a pH meter as described above.

Phosphorus Concentration. The treatment facility reports that no phosphorus is added to the activated sludge and that the concentration of this substance is determined by a digestion method, the persulfate oxidation technique and a colorimetric test, the vanadomolybdic acid method. The plant tests for total filtrable and nonfiltrable phosphorus. The treatment plant further reports that $\text{Al}_2(\text{SO}_4)_3$ is not used to lower phosphorus concentration after the biological decomposition of wastewater organic pollutants has taken place.

Nitrogen (Ammonia) Concentration. The Kalamazoo Wastewater

Treatment Plant employs nesslerization following distillation to determine ammonia concentration. The ammonia removal is not monitored in order to establish effluent chlorination levels, but rather to meet regulatory discharge criteria.

Sampling Technique and Sampling Stations

Preparation of Sample Bottles. Wide-mouthed, 400 mL borosilicate glass bottles were employed as sampling containers. Before each collection of samples, these bottles were thoroughly washed with commercial laboratory soap and rinsed six times with deionized, glass-distilled water. Afterward, the bottles were sterilized in a dry-heat oven at 150°C for four hours. The plastic covers of the sample bottles were washed and rinsed in similar fashion, but were sterilized by means of an autoclave, heated at 120°C for 20 minutes at 15 lbs pressure.

As recommended by Booth (1982), .32 mL of 10% solution of sodium thiosulfate was added (after sterilization) to the bottles in order to dechlorinate the containers. In light of the bactericidal nature of chlorine, this procedure was felt to be of signal importance.

Sampling Stations. Two sampling stations were selected at the Kalamazoo Wastewater Treatment Plant. The first station was located at the influent conduit which leads from the primary settling tanks to the activated sludge tanks. Samples were collected by dipping the sterilized containers into a conduit detour which was routed through a composite sampler. The second station was situated over the center

of the southernmost activated sludge tank. Concrete slabs overlies these aerated basins, and cylindrical holes cut into the concrete permit direct access to the mixed liquor beneath. Long-handled dip instruments were used to retrieve a sample of the sludge which was then dispensed into the sterile sample bottles.

Microbiological Examination

As a consequence of the wide diversity of bacteria and fungi believed to be present at the two sample sites, a broad range of isolation media was employed. Specific media were used for heterotrophic bacteria, chemolithotrophic bacteria and microfungal organisms.

Nutrient agar, enriched nutrient agar and Zoogloea agar (see Appendix A) were utilized as basic media for the isolation of dominant heterotrophic bacteria. Arginine medium, a broth with high C:N ratio was employed as an aid to the identification of Zoogloea ramigera. Crabtree et al. (1965) suggest that flocculation in the arginine medium is indicative of the presence of Z. ramigera, a species of bacteria widely considered responsible for floc-formation in the activated sludge process.

The various agar media were poured into culture dishes and allowed to solidify. Within 48 hours of the pouring of the plates, .05 mL of the diluted sample water was spread over the surface of the agar with a flame-sterilized, bent-glass rod.

At 48, 72 and 96 hours after inoculation, a modified Quebec-colony counter was used to enumerate the total number of bacterial colonies on the agar surface of each culture dish.

Utilizing colony counts for each of the three nutrient media and dilution factors of inoculant water, densities of heterotrophic bacteria were estimated. In addition, all colony types were gram stained.

In similar fashion, Azotobacter medium (see Appendix A) was employed in an attempt to determine densities of bacteria of the Azotobacter group, and Thiobacillus medium (see Appendix A) was used to figure population size of sulfur oxidizing bacteria.

Because of time restrains, bacterial examination during the October 1983 study period was limited to density determinations, gram stains, fermentation testing and inspection of basic cell morphology.

During the 18 months previous to the present study, however, many bacteria had been isolated from the two sample stations and many had been identified. In the process of identification of these organisms, one or more of the following techniques were employed: (a) flagellar staining, (b) hanging drop, (c) spore stain, (d) catalase and oxidase tests, and (e) test for facultative anaerobiosis.

When examining activated sludge, bacteria were separated from the flocs by means of a homogenizing mixer. The body of the mixer was sterilized in boiling distilled water and the mixer head was sterilized with 70% ethanol. This same process was employed for all microbial inoculations taken from the activated sludge tanks.

Microfungi were treated in an analogous fashion to the bacteria. Three isolation media were utilized: Cooke's rose bengal agar, mycological agar and water agar (see Appendix A). To reduce bacterial growth, all three of the mycological media were treated with the

antibiotic streptomycin sulfate (0.05 mL/10 agar).

Microfungi were observed to grow not only in the mycological agars, but also in all the other media as well. Consequently, counts were made of fungi no matter where they occurred. By the same token, bacterial colonies which appeared on the mycological media were also recorded.

As the microfungi were generally less numerous than the bacteria, their individual colonies could be easily counted, unless there was overlap of colonies. This phenomenon became prominent in the Azoto-bacter culture plates where, because of the activity of nitrogen-fixing bacteria, nitrogen compounds became available to the microfungi.

The fungi were usually transferred to water agar where low nutrient conditions would usually prompt the mold to form sex organs. These structures are necessary for the specific identification of most microfungi as the mycelia and asexual reproductive structures of many molds are similar.

Densities of metazoans were determined through the use of a Sedgwick-Rafter counting cell. No attempt was made to determine the relative biomass of the various microinvertebrates, although it may be noted that one of the largest activated sludge metazoans (Philo-dina sp) was also the most numerous microinvertebrate.

Photographic Technique for Fungal Cultures

Representative fungal cultures were chosen to be photographed and included in the present study. The cultures were photographed with Kodak Plus-X pan, 35 mm black and white film. ASA setting was

125. A semi-automatic bellows was used with the camera, and all photos were taken on a copy stand with two 3400 K tungsten lamps. Side and back lighting were employed in the photography in an attempt to capture maximum relief of the mold bodies.

CHAPTER IV

ANALYSIS OF THE DATA

Introduction

The data collected for this study fall into two broad categories. In the first category are physioco-chemical parameters of the influent and activated sludge environments, data which were obtained from operational records of the Kalamazoo Wastewater Treatment Plant. Temperature and pH of the secondary treatment processes are not recorded at the facility and consequently the author made these determinations himself.

The second category of data relates to the microbiological examination of secondary influent and activated sludge samples. Figures and tables report densities for organotrophic and chemolithotrophic bacteria, for microfungi and also for microinvertebrates. Most fungi and microinvertebrates were identified to genus, a few to species. Several characteristics of the bacterial populations at the study sites were examined; time restraints prevented identification to family or genus.

While most physico-chemical data (temperature and pH of the activated sludge excluded) cover the entire month of October 1983, microbiological data record microbial activity for the 8th, 15th, and 22nd of the month. An additional examination of the activated sludge for microinvertebrates occurred on the 24th of October.

Physico-Chemical Data

Sewage flow for the facility for the month of October 1983 totaled 829.72 million gallons. Average daily flow was 26.77 mgal, with the maximum daily flow 34.76 mgal (3 October), and the minimum daily flow 17.91 mgal (30 October).

5-Day Biochemical Oxygen Demand: Influent and Effluent of the Activated Sludge

Figure 1 provides the secondary influent BOD_5 , while Figure 2 furnishes the activated sludge BOD_5 for the month of October 1983.

The BOD_5 of the secondary influent varies from 241 mg/L to 426 mg/L, with a monthly average of 323 mg/L. The lowest BOD_5 occurred on a Sunday, the 9th of October. The other three lowest BOD_5 values also occurred on Sundays (October 2, 16 and 30). Figure 1 thus presents a weekly cycle of biochemical oxygen demand. Sunday BOD_5 values are generally the low point in the cycle, while high points in the graph are reached during the first four working days of the week. The cycles are thought to be directly correlated with nondomestic user levels of activity, as well as general municipal sewage use.

Daily BOD_5 values for secondary effluent for the month of October 1983 varied from 7 mg/L to 98 mg/L, with an average of 26 mg/L. Although BOD_5 values were generally higher during weekdays than during weekends, the BOD_5 values for the fourth weekend of October were higher than fourteen of the period's normal working days. The peaks in the graph were not associated with any particular days of the week: Wednesday, the 5th of October, had the month's maximum value; two graph

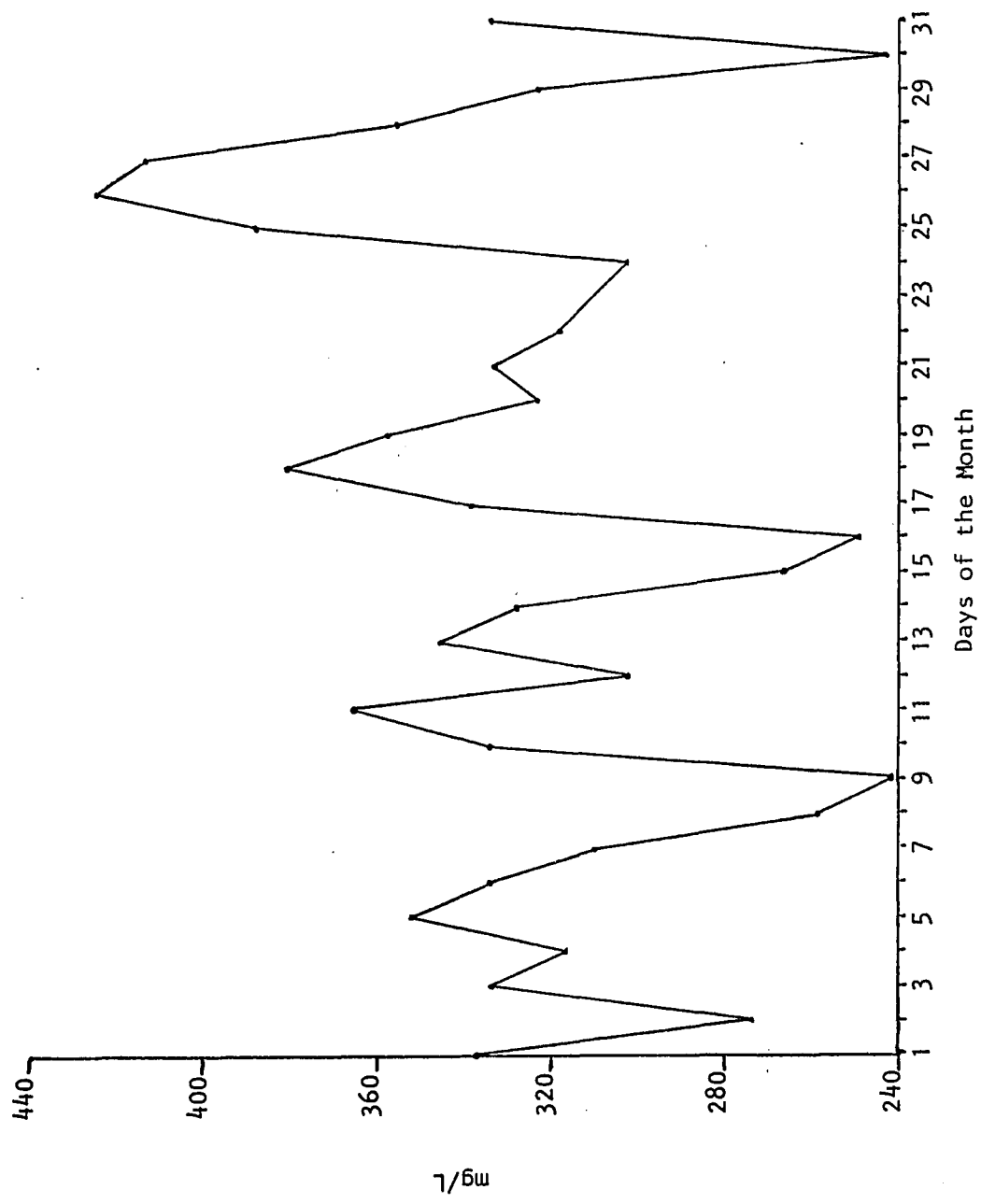


Figure 1. Secondary Treatment Data for October 1983: 5-Day Biochemical Oxygen Demand of Influent.

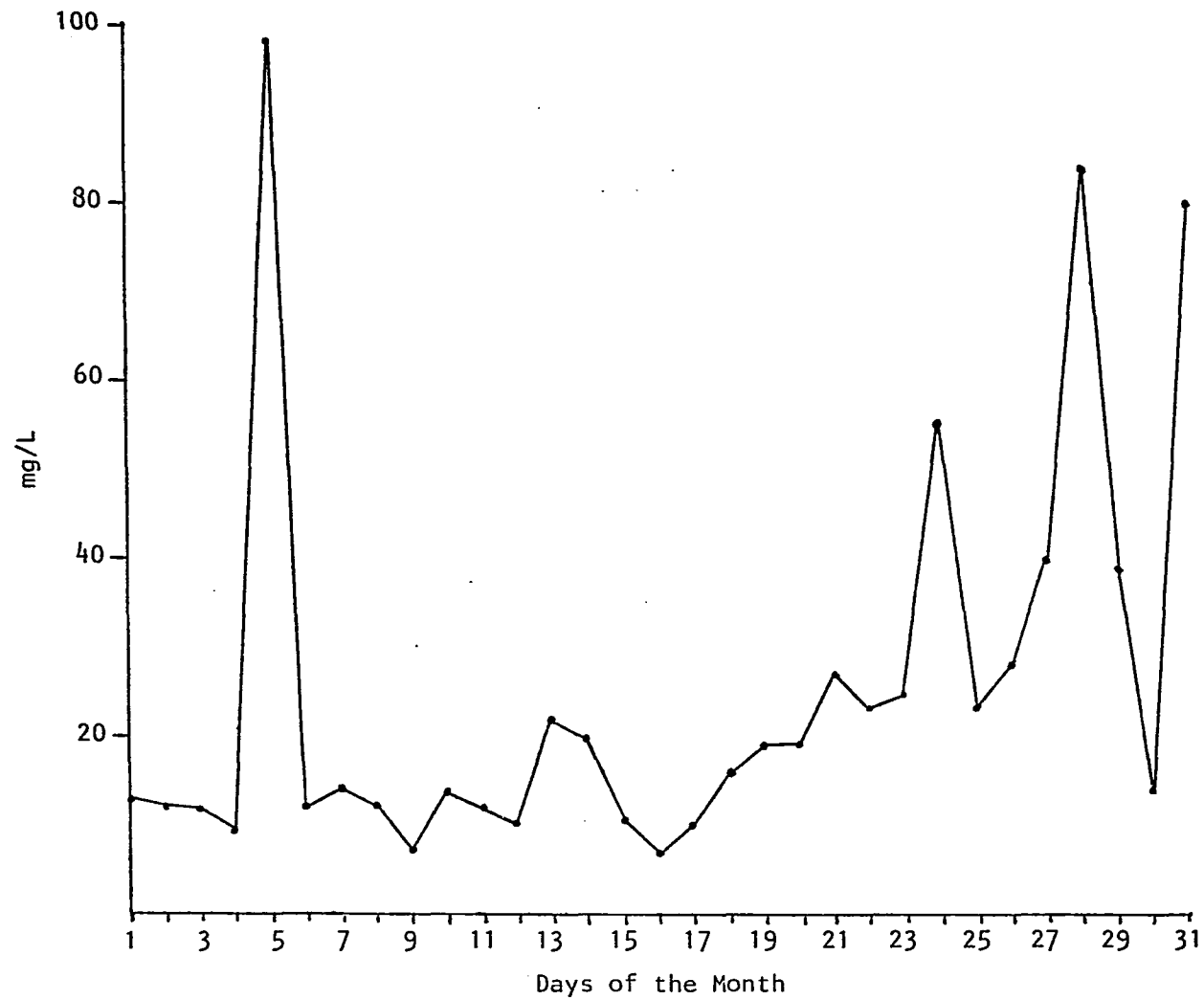


Figure 2. Secondary Treatment Data for October 1983: 5-Day Biochemical Oxygen Demand of Effluent.

peaks were reached on a Monday, one peak on a Friday.

It may be instrumental to note that, at times, the BOD_5 graph for secondary effluent exhibits similar tendencies to the BOD_5 graph for secondary influent, while at other times, little correlation seems to obtain between the two. For example, the rise in influent BOD_5 on the 5th of October is correlated with the precipitous rise in effluent BOD_5 . Likewise, the sharp drop in influent BOD_5 on the 30th of October is paralleled by the substantial decrease in effluent BOD_5 for that date. On the other hand, little correlation may appear to obtain: on the 25th of October, influent BOD_5 rose sharply while the effluent BOD_5 markedly decreased.

Volatile Suspended Solids: Influent and Effluent of the Activated Sludge

The volume of volatile suspended solids (VSS) present in secondary influent wastewater (Figure 3) does not appear to have followed a distinct, weekly cycle, although VSS values demonstrated a decline from Saturday to Sunday during the first, second and fourth weekends of the month. On the other hand, during the third weekend, a slight increase in VSS values occurred; and during the last weekend of October, the month's second highest VSS value was attained and maintained. VSS Maxima were reached on four different weekdays and considerable fluctuation generally obtained during the work week. The maximum secondary influent VSS value was 304 mg/L (25 October), the minimum value was 108 mg/L (26 October) and the month's average was 197 mg/L.

The VSS values of the secondary effluent (Figure 4) for the month of October remained at or below 40 mg/L for more than 87% of the

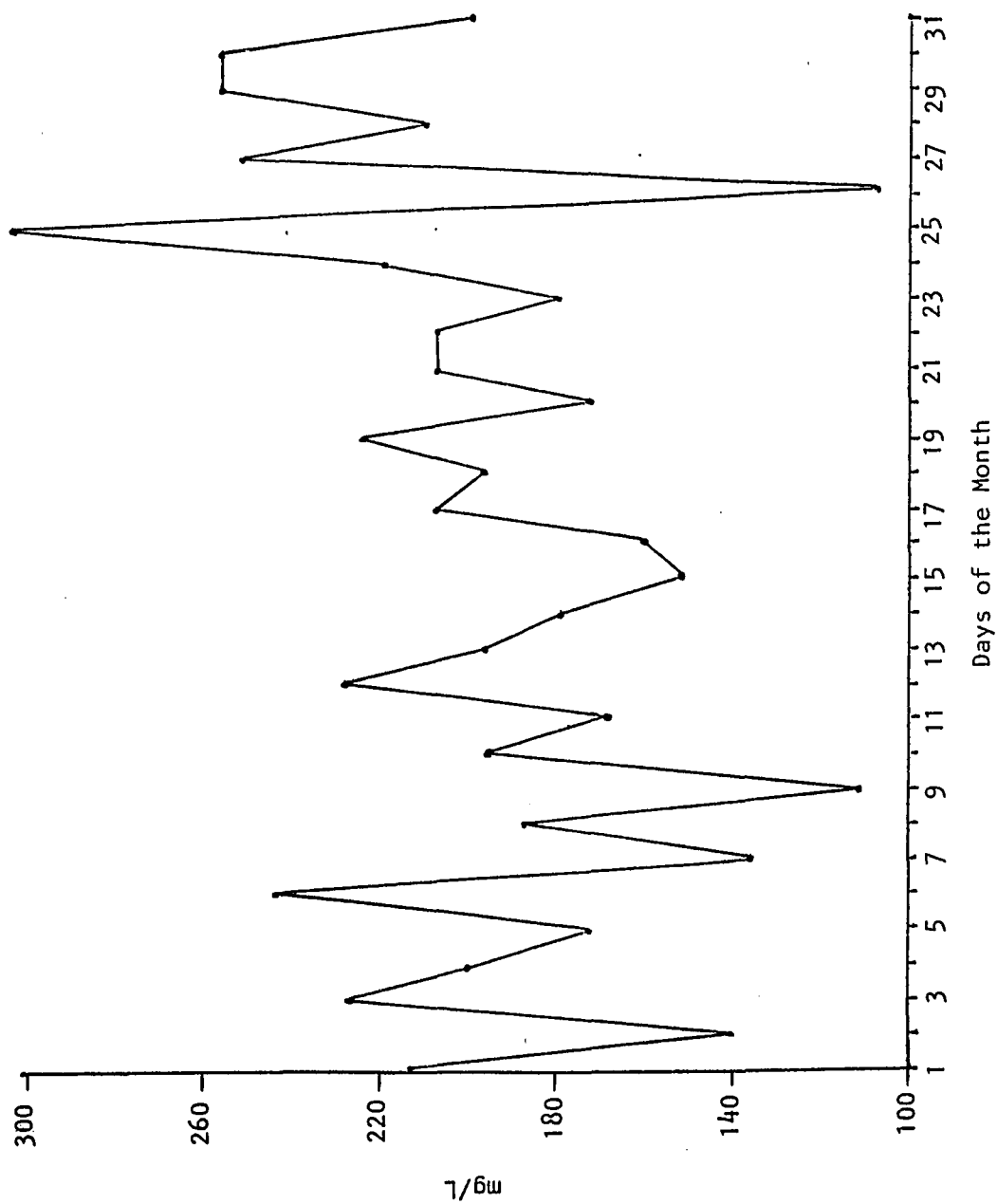


Figure 3. Secondary Treatment Data for October 1983: Volatile Suspended Solids of Influent.

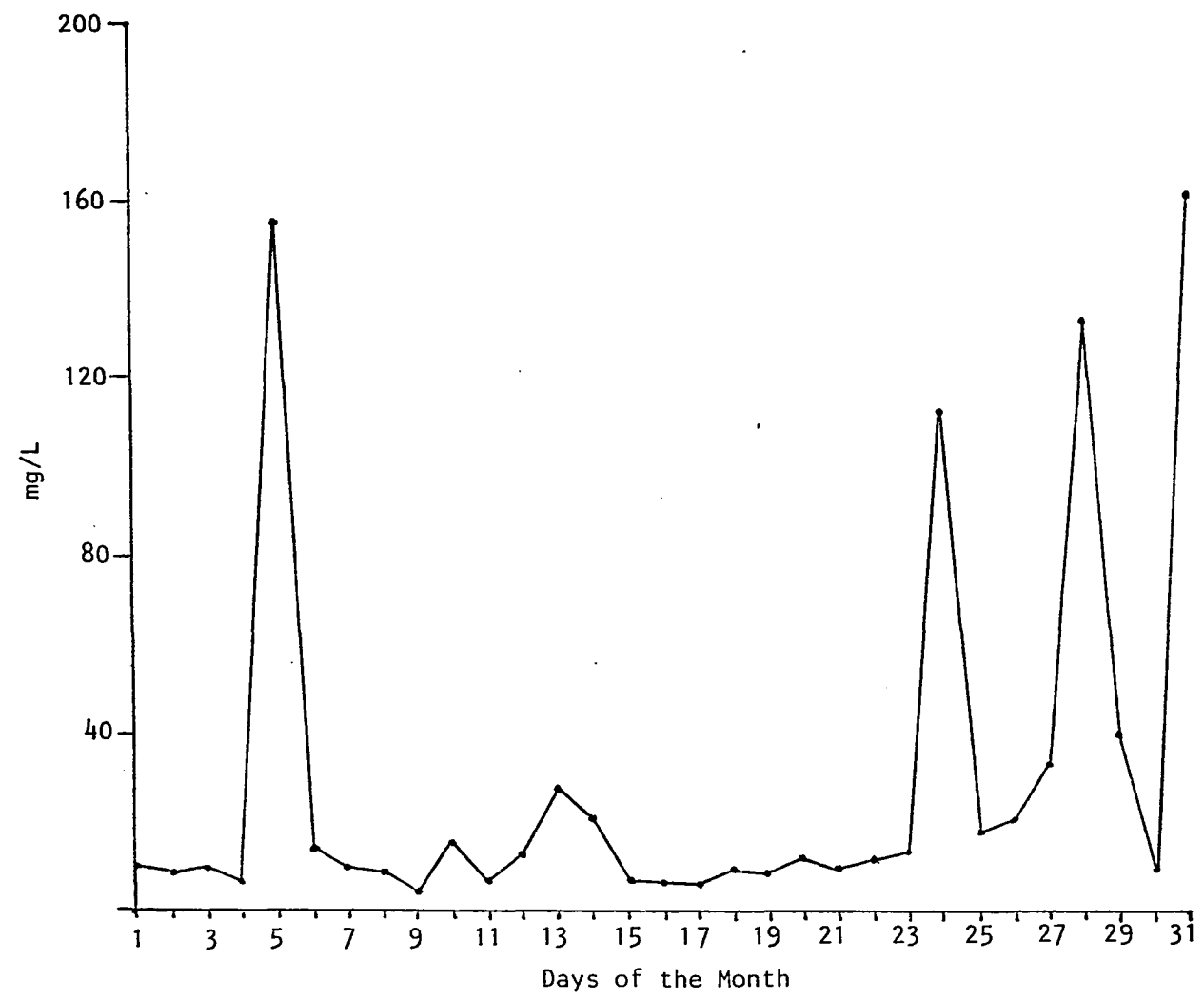


Figure 4. Secondary Treatment Data for October 1983: Volatile Suspended Solids of Effluent.

time. On four weekdays, the 5th, 24th, 28th and 31st, VSS values increased substantially. The maximum secondary effluent VSS value for October was 162 mg/L (31 October); the minimum VSS value was 4 mg/L (9 October) and the average secondary effluent VSS value for October was 30 mg/L.

Return Sludge as Percentage of Sewage Flow

Figure 5 shows the amount of return sludge (or recycled sludge) as a percentage of sewage flow. Reduced weekend sewage flow is directly correlated to a weekend rise in return sludge as a percentage of such sewage flow. The highest pairs of return sludge values for October occurred on the five weekends of the month. The maximum amount of return sludge as percentage of sewage flow was 134.0% (30 October); the minimum amount of return sludge was 71.8% (6 October) and the daily average for the month was 94.4%.

Dissolved Oxygen in Mixed Liquor

Dissolved oxygen (DO) in mixed liquor of the activated sludge tanks (Figure 6) varied from a maximum of 4.2 mg/L (9 October) to a minimum of 1.1 mg/L (27 October); the daily average for the month was 2.3 mg/L. The first half of the month (1-15 October), the daily DO average was higher (2.7 mg/L) than the average DO concentration (1.9 mg/L) for the second half of the month.

pH

The pH of the secondary treatment process is not recorded at the

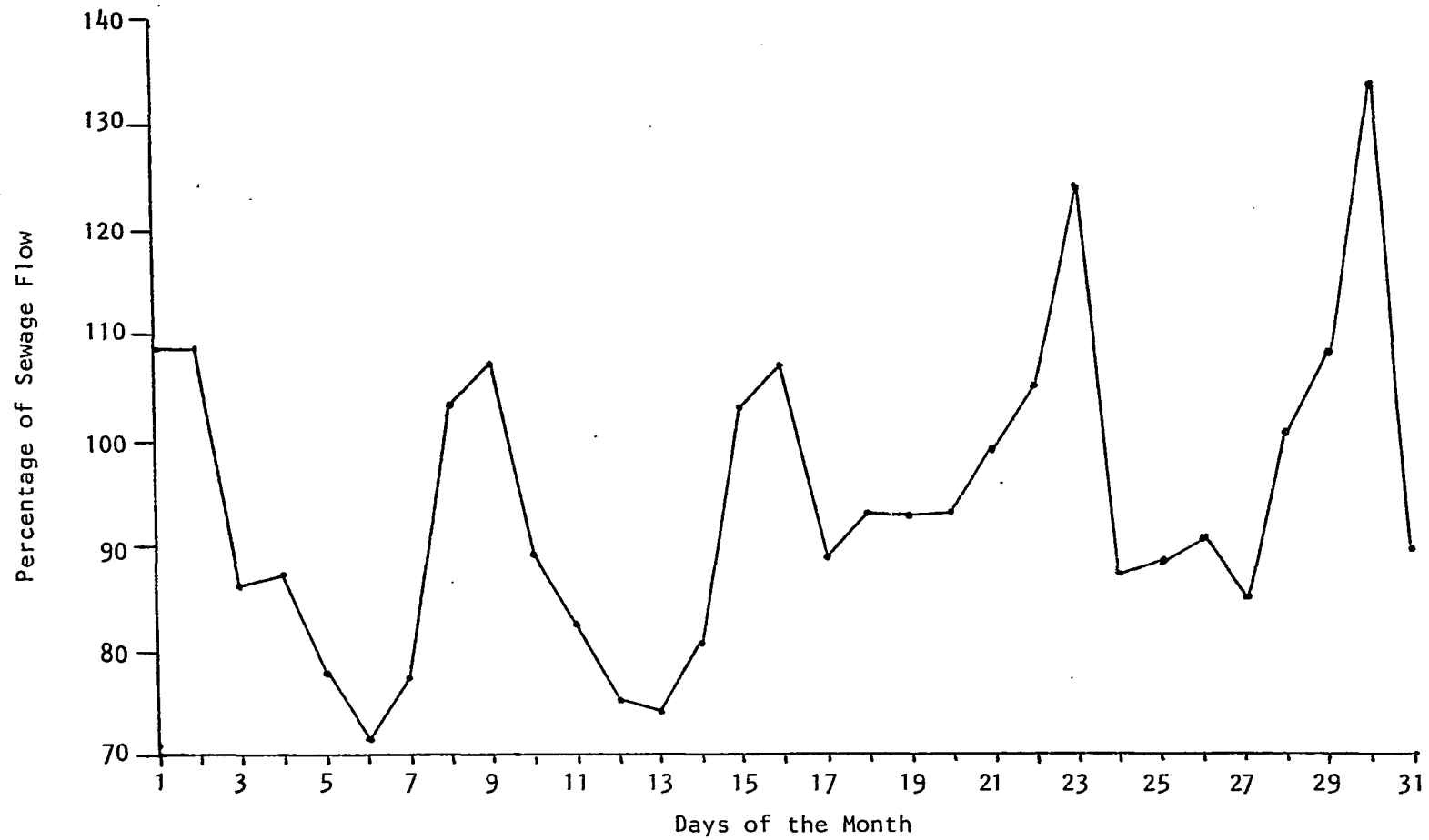


Figure 5. Secondary Treatment Data for October 1983: Return Sludge as Percentage of Sewage Flow.

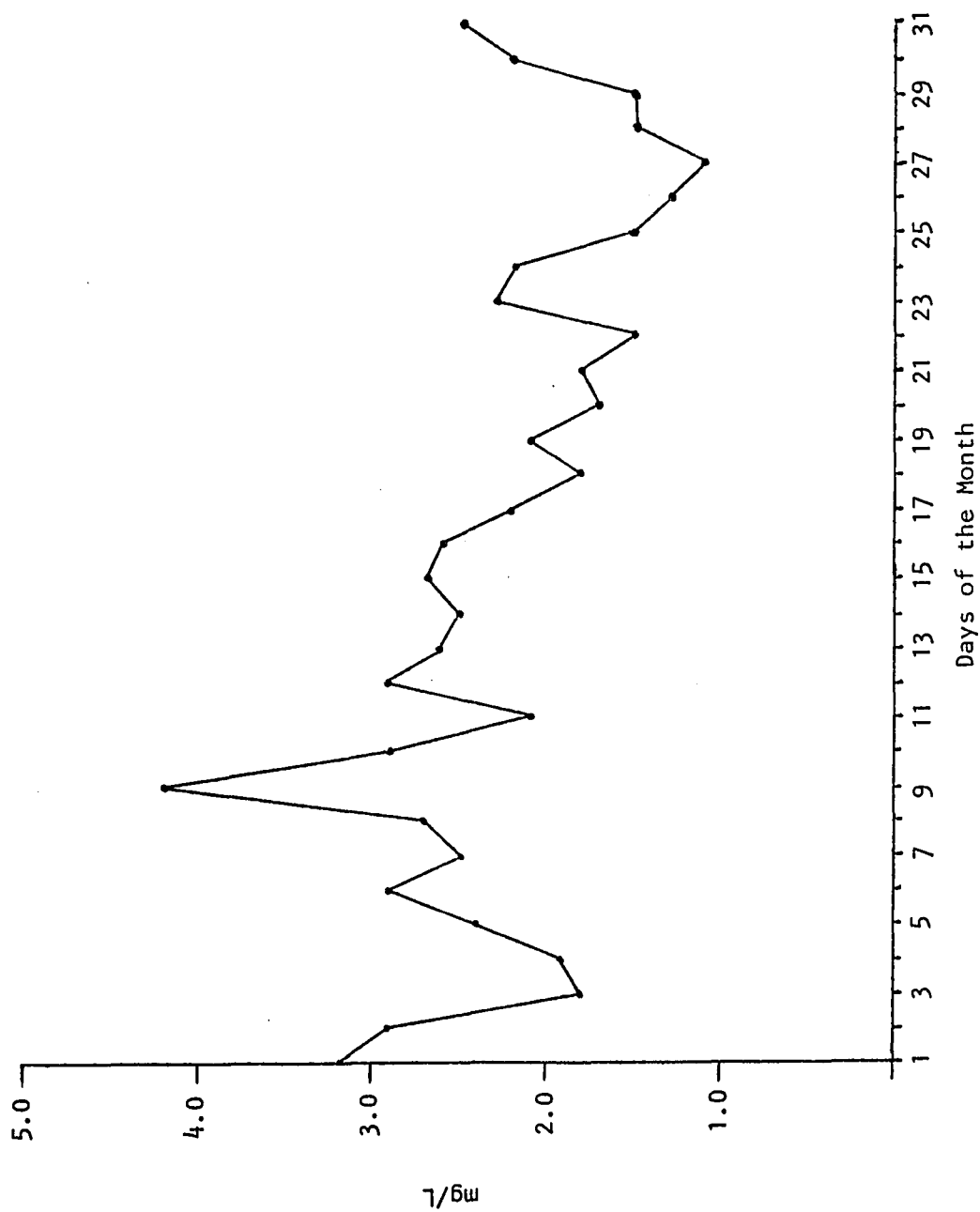


Figure 6. Secondary Treatment Data for October 1983: Dissolved Oxygen in Mixed Liquor.

Kalamazoo Wastewater Treatment Plant. The researcher, however, did obtain pH data for the secondary influent and the activated sludge process during the collection of microbiological samples. Table 1 provides such data. The average of the combined pH for both sites was 6.4. This slightly acidic pH compares with the neutral pH of the final settling tank (Figure 7). This last process had a monthly average of pH 7.1; except for a zero reading, which resulted from instrumental failure, the pH of the final settling tanks varied very little.

Table 1
Secondary Treatment: pH and Temperature

pH:	1983		
	October 8	October 15	October 22
Influent	6.6	6.2	6.2
Activated Sludge	6.6	6.5	6.4
Temperature:			
Influent	26°C	24°C	26°C
Activated Sludge	25°C	23°C	24°C

Temperature

As no temperature determinations are made at the KWTP, the author did record temperature values of secondary influent wastewater and the activated sludge. These data were also obtained when collection of microbiological samples was made. Although the three sets of

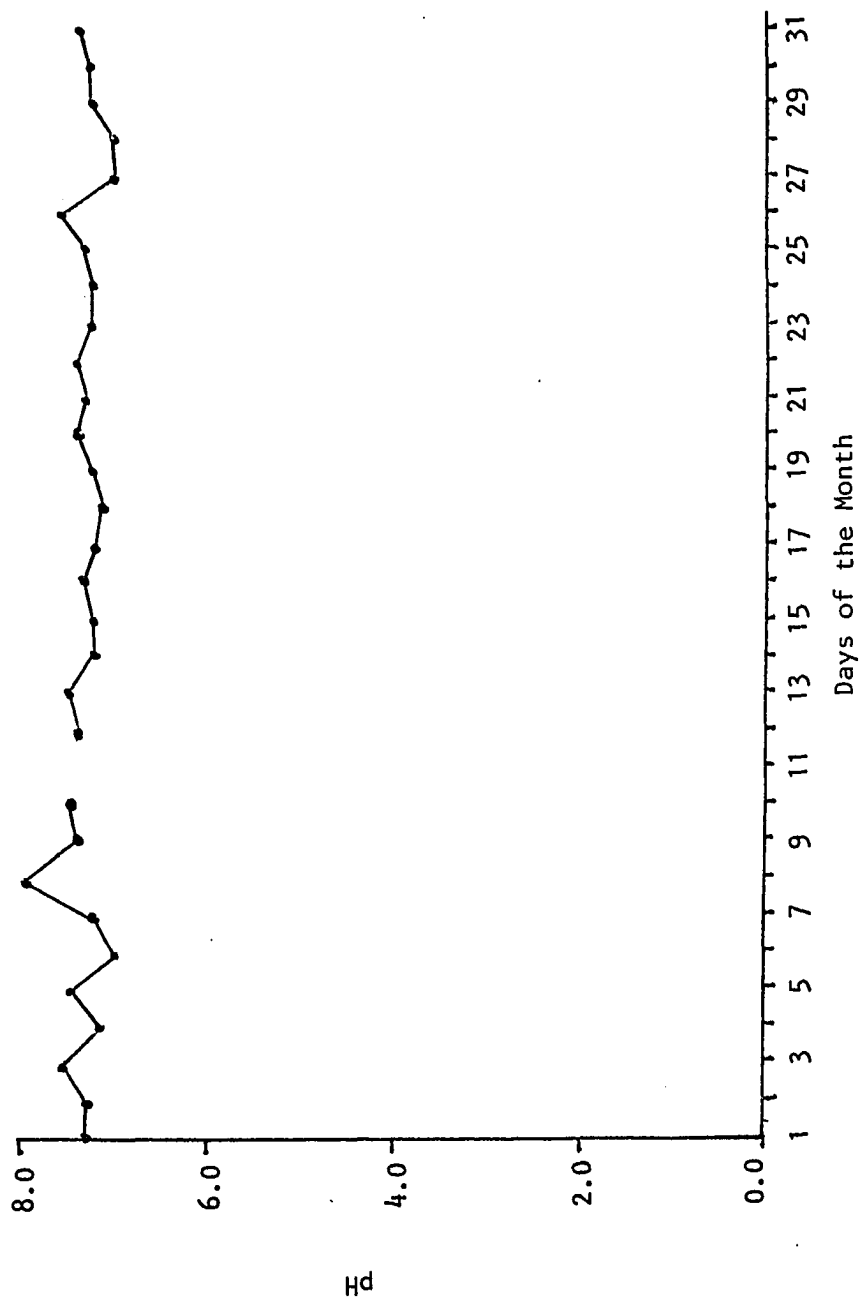


Figure 7. Secondary Treatment Data for October 1983: pH of Final Settling Tank Effluent.

temperature data are inadequate to indicate definite trends, they do tend to suggest that the activated sludge may be somewhat cooler (c. 1.0°C) than the primary wastewater.

Phosphorus Concentration of Secondary Treatment

Figure 8 provides information on the phosphorus concentration of secondary treatment influent and effluent. Removal rates of phosphorus vary considerably. Effluent concentrations of phosphorus range from a low of .7 mg/L (16 October) to a high of 8.0 mg/L (28 October). The monthly average for phosphorus concentration was 2.6 mg/L. Increased removal of phosphorus occurred on four out of five Sundays while substantial reduction in phosphorus removal took place on five different working days (5, 24, 27, 28, and 31 October). The influent concentration of phosphorus does not appear to be directly correlated with the removal rate of this substance. Decrease in influent concentrations may actually lead to an increase in the phosphorus of effluent waters (i.e., 5 and 26 October).

Ammonia Concentration of Secondary Treatment

The most remarkable aspect of the graph for the influent and effluent ammonia concentration of the KWTP secondary treatment process is the precipitous rise in effluent concentration of ammonia which occurred on the 27th of October. The entire period, however, which comprises the last two weeks of the month, exhibits a general rise in ammonia concentration in the secondary effluent. See Figure 9.

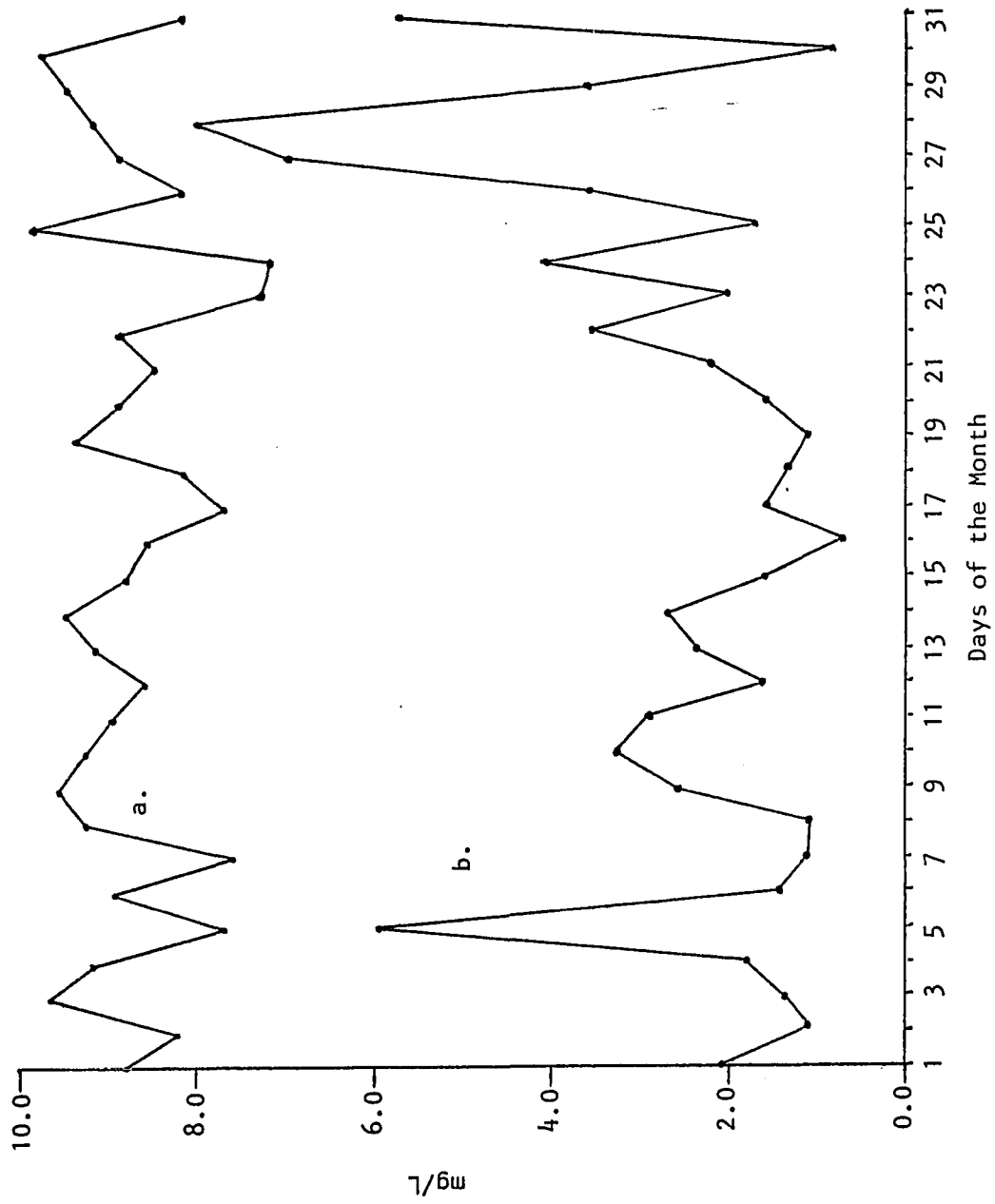


Figure 8. Secondary Treatment Data for October 1983: Phosphorus Concentration,
a. Influent, b. Effluent.

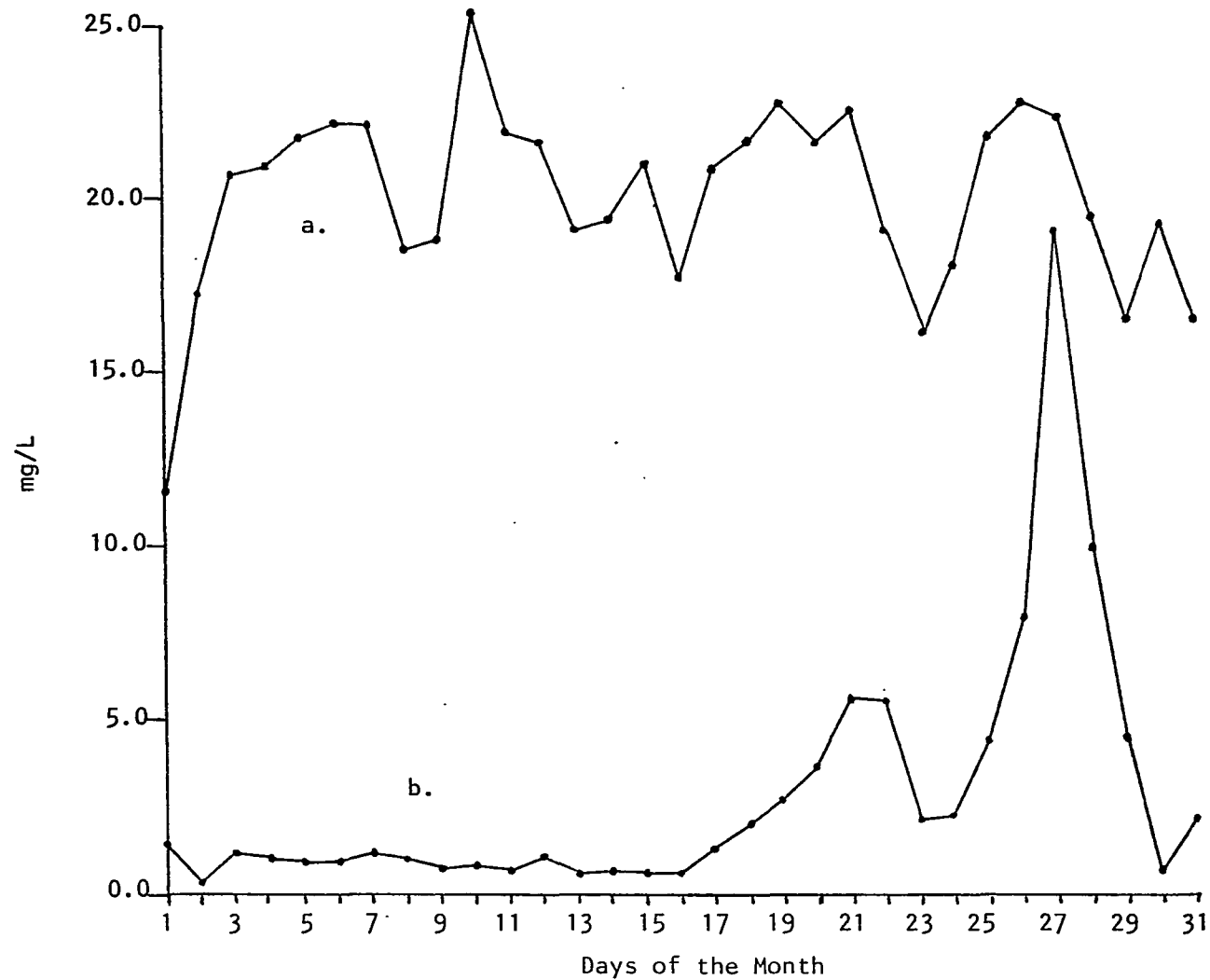


Figure 9. Secondary Treatment Data for October 1983: Ammonia Concentration, a. Influent, b. Effluent

Selected Priority Pollutants at the KWTP

Table 2 provides acute and chronic concentration values of selected toxic substances which the consultant firm Greeley and Hansen (1982) has determined are likely to be present in influent waters of the treatment facility. The consultants base their figures on a pilot plant study, on a survey of nondomestic users of the sewage system and on a review of pertinent literature.

Table 2
Selected Priority Pollutants at the KWTP

Pollutant	Influent Composition (µg/L)	
	Acute	Chronic
Cadmium	2,764	2.4
Chromium	25,799	930
Copper	2,349	349
Cyanide	700	169
Lead	5,034	171
Nickel	75,146	3,319
Silver	85.4	.9
Zinc	32,722	537
Phenol	442,700	199,800

Microbiological Data

Bacterial Density

Bacterial densities of secondary influent and activated sludge, as determined by three nutrient media (Figures 10 and 11), two mineral

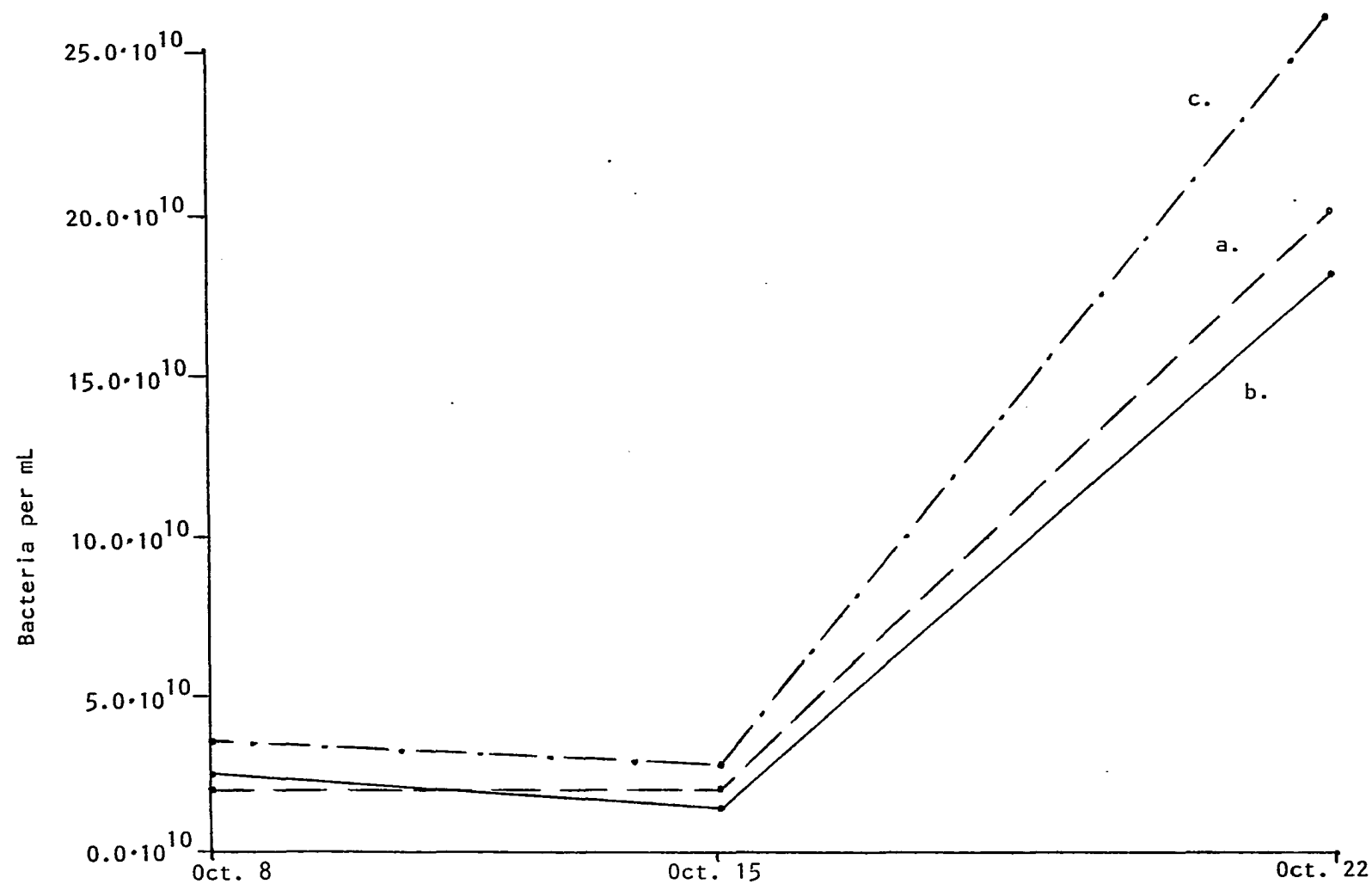


Figure 10. Bacterial Density of Secondary Influent as Determined by the Use of Three Nutrient Media: a. Nutrient Agar, b. Enriched Nutrient Agar, c. Zoogloea Agar.

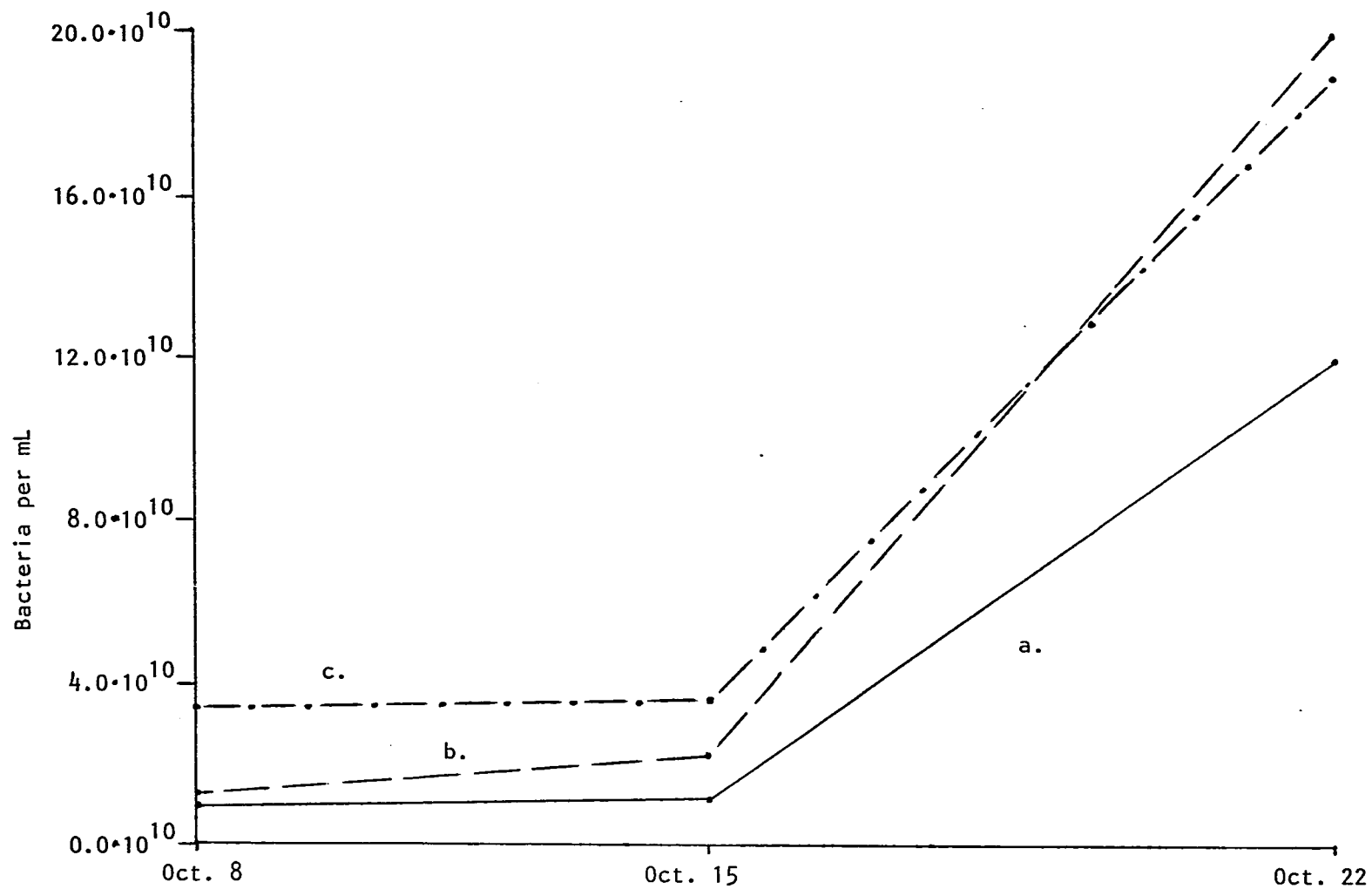


Figure 11. Bacterial Density of Activated Sludge as Determined by the Use of Three Nutrient Media: a. Nutrient Agar, b. Enriched Nutrient Agar, c. Zoogloea Agar.

agars (Figures 12 and 13), and three mycological media (Figures 14 and 15), are presented for the 8th, 15th and 22nd of October 1983.

Although all three dates are on the same day of the week, Saturday, bacterial densities are substantially higher for the third collection date. Bacterial densities for the 22nd of October increased 800% for the nutrient media, 1000% for the mycological media (inspite of the antibiotic) and more than 10,000% for the mineral agar cultures.

Bacterial densities for the secondary influent were usually higher than those for the activated sludge process. This apparent anomaly obtained in all cultures except for nutrient and mycological media inoculated on the 15th of October.

Based on nutrient cultures for the first two collection dates, bacterial densities of influent and activated sludge averaged 2.4×10^{10} and 2.1×10^{10} bacteria per milliliter, respectively.

A comparison of the relative numbers of organotrophic bacteria growing on the nutrient media with the chemolithotrophic bacteria present on the mineral agars, shows that the heterotrophs exist in a 32:1 ratio with the bacteria growing on the Azotobacter medium and in a 35:1 ratio with the sulfur-oxidizing bacteria on the Thiobacillus agar.

Basic Characteristics of Bacteria Isolated

Even though densities of bacteria rose sharply on the 22nd of October, the relative percentages of gram reaction, fermentation test types and basic cell morphology remained essentially the same (Table 3). Gram stain reactions were overwhelmingly negative; slightly less than

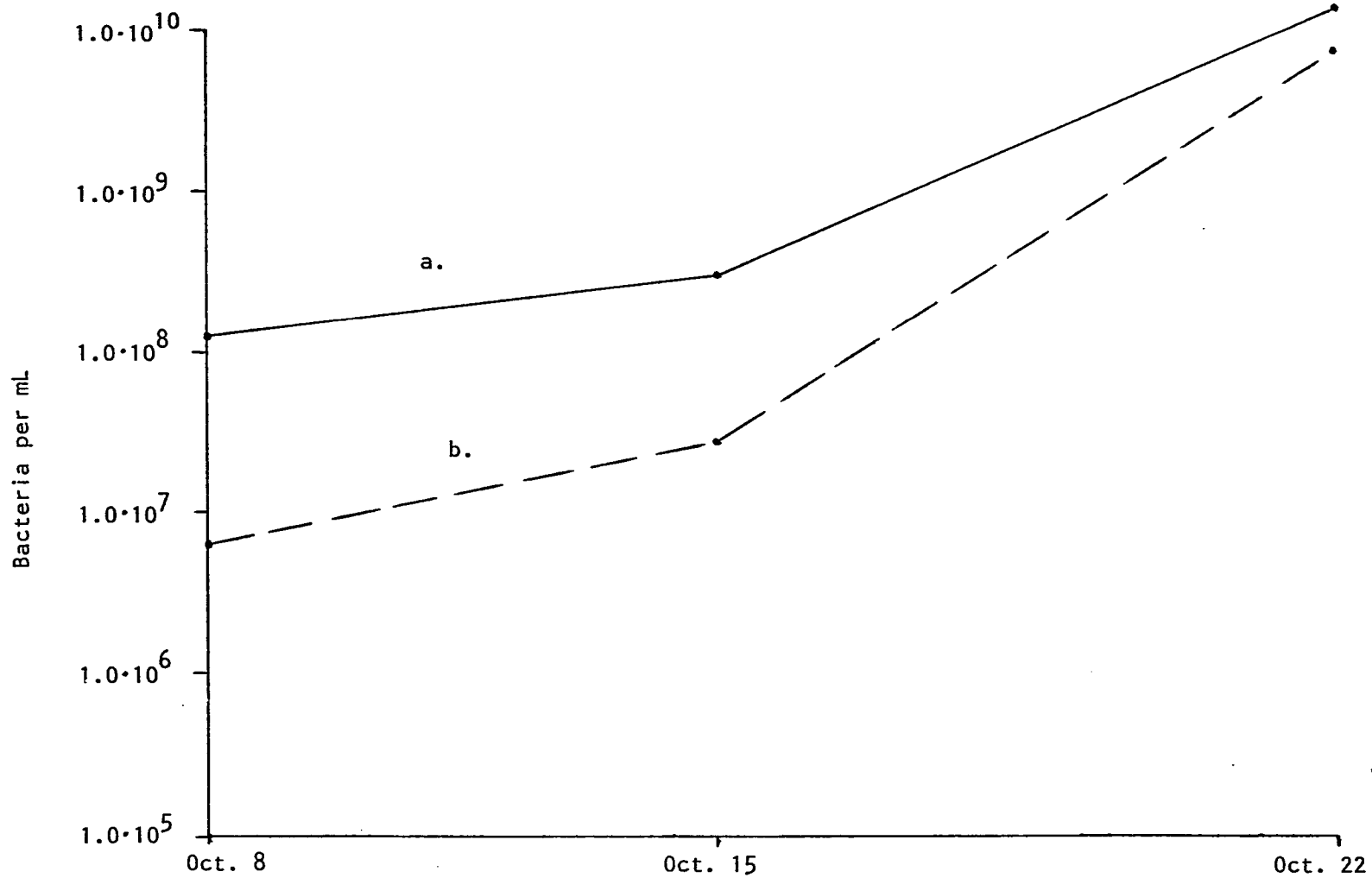


Figure 12. Density of Chemolithotrophic Bacteria in Secondary Influent as Determined by the Use of (a) Azotobacter Medium and (b) Thiobacillus Medium.

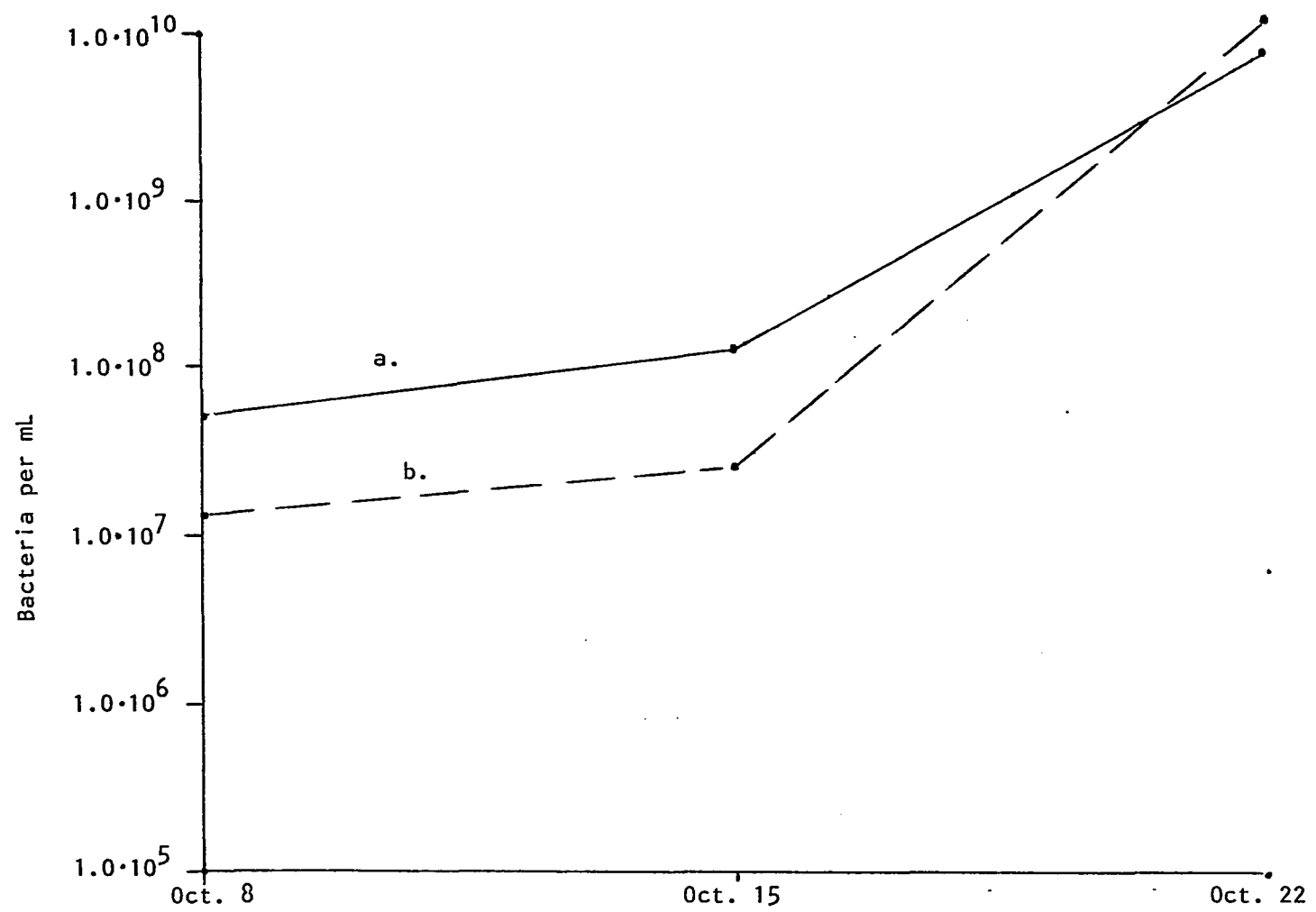


Figure 13. Density of Chemolithotrophic Bacteria in Activated Sludge as Determined by the Use of (a) Azotobacter Medium and (b) Thiobacillus Medium.

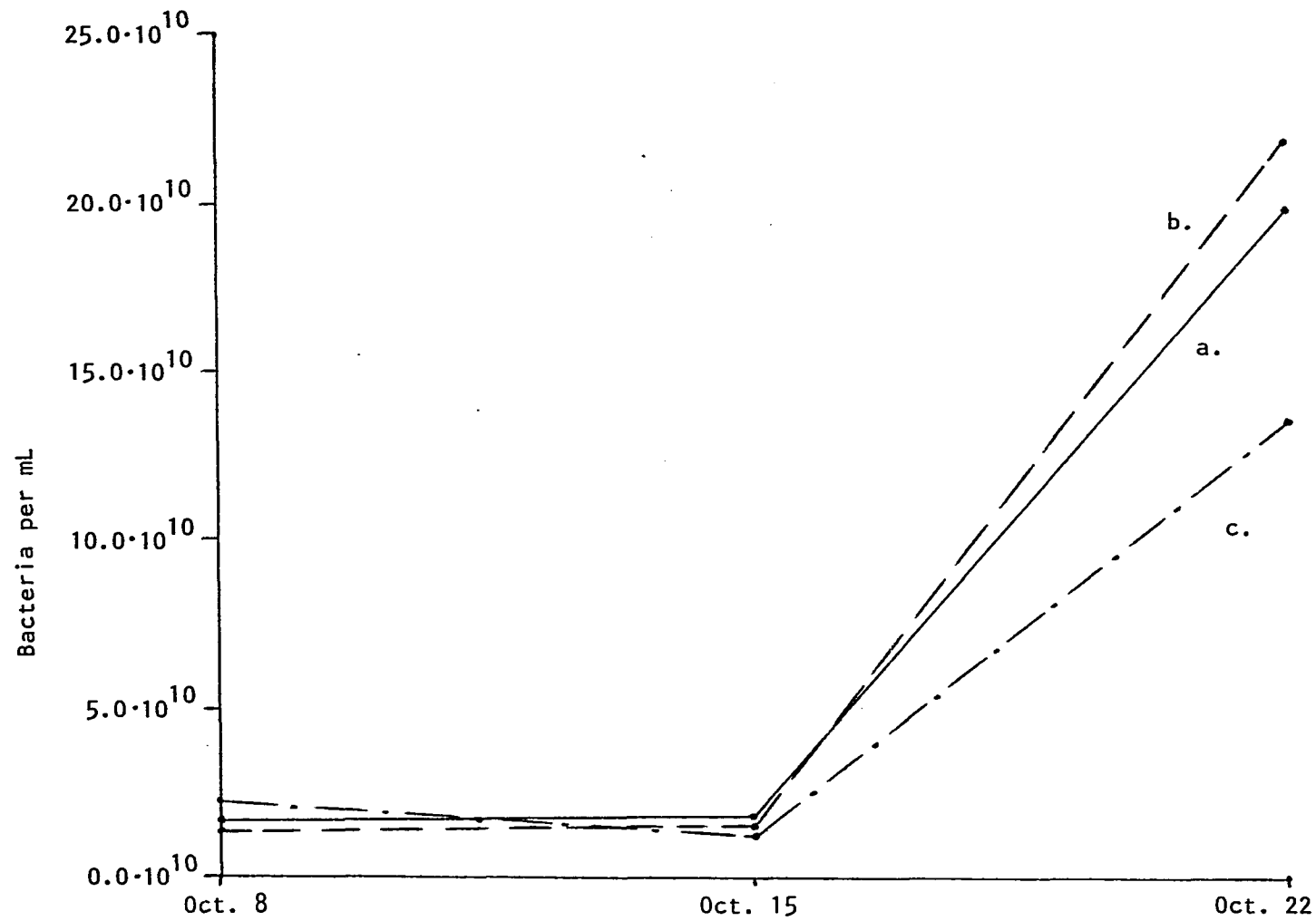


Figure 14. Bacterial Density of Secondary Influent as Determined by the Use of Three Mycological Media: a. Mycological Agar, b. Cooke's Rose Bengal Agar, c. Water Agar.

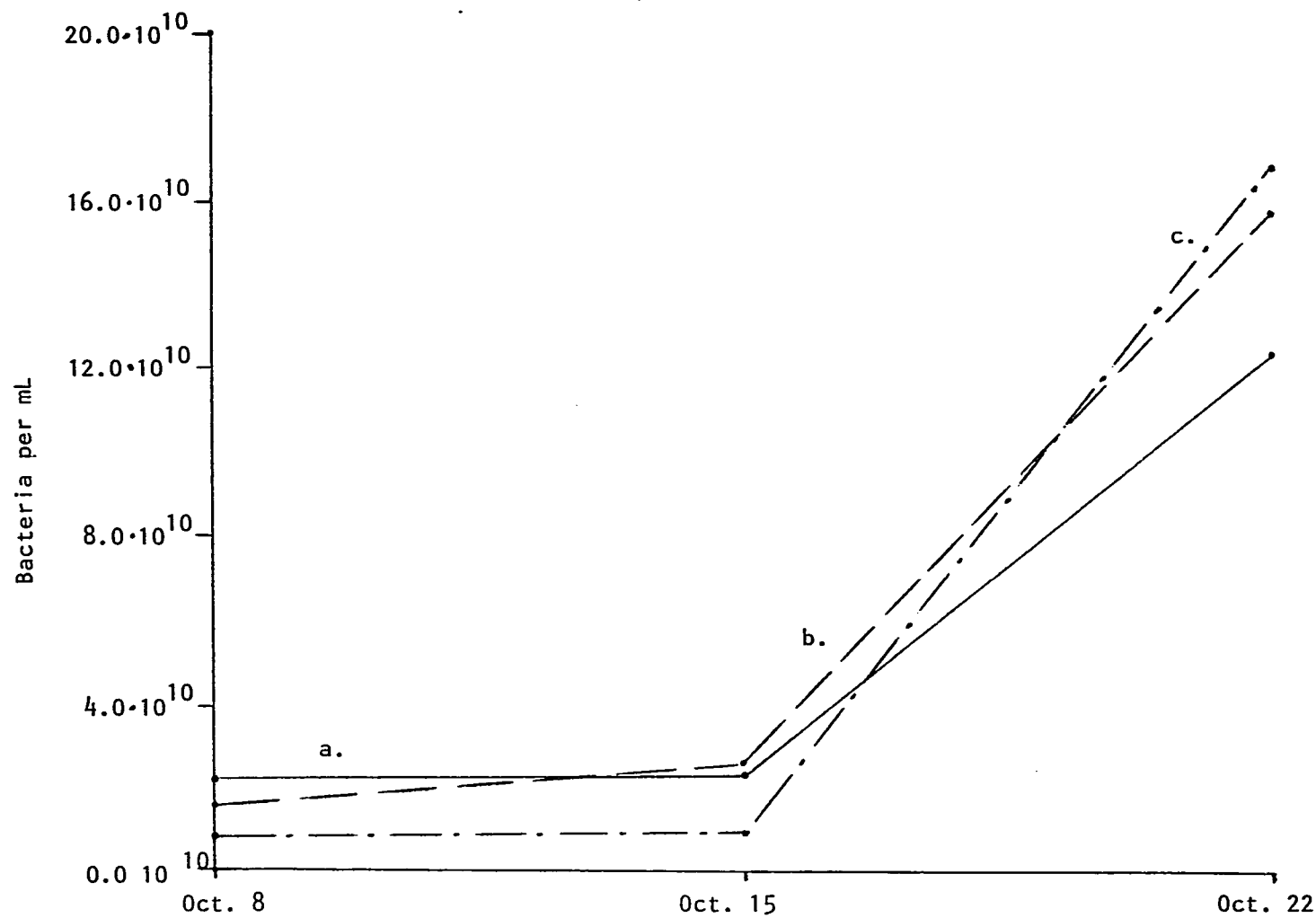


Figure 15. Bacterial Density of Activated Sludge as Determined by the Use of Three Mycological Media: a. Mycological Agar, b. Cooke's Rose Bengal Agar, c. Water Agar.

half of the bacteria were facultative anaerobes; and bacterial cells were almost exclusively rod-shaped.

Table 3
Three Basic Characteristics of Bacteria Isolated
From the KWTP

Characteristic		October 8	October 15	October 22
		40 Isolates	60 Isolates	45 Isolates
Gram Reaction:	Positive	4%	6%	3%
	Negative	96%	94%	97%
Fermentation Test:	Positive*	44%	46%	48%
	Negative+	56%	54%	52%
Cell Morphology	Coccus	4%	2%	3%
	Coryneform	2%	2%	1%
	Rod	94%	96%	96%

* indicate a facultative anaerobe

+ indicate a strict aerobe

Bacterial Genera at the KWTP

Table 4 provides a list of bacteria which the author previously isolated from the secondary treatment process at the KWTP. The pseudomonads generally appeared to be the most numerous group. Azotobacter sp. was usually found when the appropriate mineral agar was employed, as was the case for the sulfur-oxidizing bacterium, Thiobacillus sp., and the nitrifying bacterium Nitrosomonas sp. Gram-negative facultatively anaerobic rods were constantly isolated from the plant's activated sludge, although, as Table 3 tends to indicate, these organisms were not as numerous as the gram-negative aerobic rods. Gram-positive

Table 4

Bacterial Genera Isolated from Activated Sludge of the KWT
April 1982 - October 1983

Gram-Negative Aerobic Rods	Gram-Negative Chemolithotropic Bacteria
<u>Pseudomonas</u>	<u>Nitrosomonas</u>
<u>Azotobacter</u>	<u>Thiobacillus</u>
Gram-Negative Facultatively Anaerobic Rods	Gram-Positive Cocci
<u>Chromobacterium</u>	<u>Micrococcus</u>
<u>Escherichia</u>	<u>Staphylococcus</u>
<u>Flavobacterium</u>	Endospore-Forming Rods
<u>Proteus</u>	<u>Bacillus</u>
<u>Serratia</u>	

cocci were isolated on occasion, but not often encountered. The genus Bacillus was a constant but low-density isolate.

Microfungi of the Secondary Treatment Process

Tables 5 and 6 report densities for microfungi isolated from the Kalamazoo Wastewater Treatment Plant. Six different fungal genera are presented in the tables. All belong to the Fungi Imperfecti or Deuteromycetes, and all are members of the Form-Family Moniliaceae.

Botryotrichum sp. attained the highest fungal density, with 180×10^7 propagules per mL (15 October); Aspergillus niger (Figure 16) reached a density of 120×10^7 propagules per mL (8 October) and

Table 5
Density per mL of Fungal Propagules in Secondary Influent as
Determined by the Use of Selected Media

		October 8	October 15	October 22
Nutrient Agar	<u>Penicillium Lilacinum</u>	$4.0 \cdot 10^7$	$2.0 \cdot 10^7$	---
Enriched Nutrient Agar	<u>P. Lilacinum</u>	$2.0 \cdot 10^7$	$4.0 \cdot 10^7$	$20.0 \cdot 10^7$
<u>Zoogloea</u> Agar	<u>P. Lilacinum</u>	$2.0 \cdot 10^7$	$2.0 \cdot 10^7$	---
<u>Azotobacter</u> Medium	<u>Aspergillus Niger</u>	$0.06 \cdot 10^7$	---	---
	<u>Oidiodendron</u> sp.	---	$0.16 \cdot 10^7$	$10.0 \cdot 10^7$
	<u>P. Lilacinum</u>	$0.14 \cdot 10^7$	---	---
	<u>Penicillium</u> sp.	$0.06 \cdot 10^7$	---	---
<u>Thiobacillus</u> Medium	<u>Oidiodendron</u> sp.	$0.12 \cdot 10^7$	---	$4.0 \cdot 10^7$
Mycological Agar	<u>A. Niger</u>	$30.0 \cdot 10^7$	$12.0 \cdot 10^7$	---
Cooke's Rose Bengal Agar	<u>Oidiodendron</u> sp.	$16.0 \cdot 10^7$	---	$40.0 \cdot 10^7$
	<u>P. Lilacinum</u>	---	$20.0 \cdot 10^7$	$20.0 \cdot 10^7$
Water Agar	<u>Oidiodendron</u> sp.	$4.0 \cdot 10^7$	---	$80.0 \cdot 10^7$
	<u>Pencillium</u> sp	$14.0 \cdot 10^7$	$4.0 \cdot 10^7$	---
	<u>Trichoderma Viride</u>	---	$10.0 \cdot 10^7$	---

Table 6

Density per mL of Fungal Propagules in Activated Sludge as Determined by the Use of Selected Media

		October 8	October 15	October 22
Nutrient Agar	<u>Penicillium Lilacinum</u>	---	---	$20.0 \cdot 10^7$
	<u>Penicillium</u> sp.	$12.0 \cdot 10^7$	$14.0 \cdot 10^7$	---
Enriched Nutrient Agar	<u>Penicillium</u> sp.	$4.0 \cdot 10^7$	$4.0 \cdot 10^7$	---
	<u>Sporotrichum</u> sp.	---	$4.0 \cdot 10^7$	---
<u>Zooqloea</u> Agar	<u>P. Lilacinum</u>	$2.0 \cdot 10^7$	$2.0 \cdot 10^7$	---
<u>Azotobacter</u> Medium	<u>Aspergillus Niger</u>	$0.04 \cdot 10^7$	---	---
	<u>Botryotrichum</u> sp.	---	$0.10 \cdot 10^7$	$2.0 \cdot 10^7$
	<u>P. Lilacinum</u>	$0.04 \cdot 10^7$	---	$2.0 \cdot 10^7$
	<u>Sporotrichum</u> sp.	---	$0.08 \cdot 10^7$	---
<u>Thiobacilus</u> Medium	<u>Botryotrichum</u> sp.	$0.16 \cdot 10^7$	$0.02 \cdot 10^7$	---
	<u>P. Lilacinum</u>	---	$0.04 \cdot 10^7$	---
Mycological Agar	<u>A. Niger</u>	$38.0 \cdot 10^7$	$16.0 \cdot 10^7$	$120.0 \cdot 10^7$
	<u>P. Lilacinum</u>	---	$4.0 \cdot 10^7$	---
	<u>Sporotrichum</u> sp.	---	$4.0 \cdot 10^7$	---
Cooke's Rose Bengal Agar	<u>A. Niger</u>	$8.0 \cdot 10^7$	$16.0 \cdot 10^7$	---
	<u>Botryotrichum</u> sp.	---	$6.0 \cdot 10^7$	---
	<u>P. Lilacinum</u>	$24.0 \cdot 10^7$	$8.0 \cdot 10^7$	$20.0 \cdot 10^7$
Water Agar	<u>Botryotrichum</u> sp.	---	$180.0 \cdot 10^7$	---
	<u>P. Lilacinum</u>	$22.0 \cdot 10^7$	---	$40.0 \cdot 10^7$
	<u>Trichoderma Viride</u>	---	$2.0 \cdot 10^7$	$20.0 \cdot 10^7$

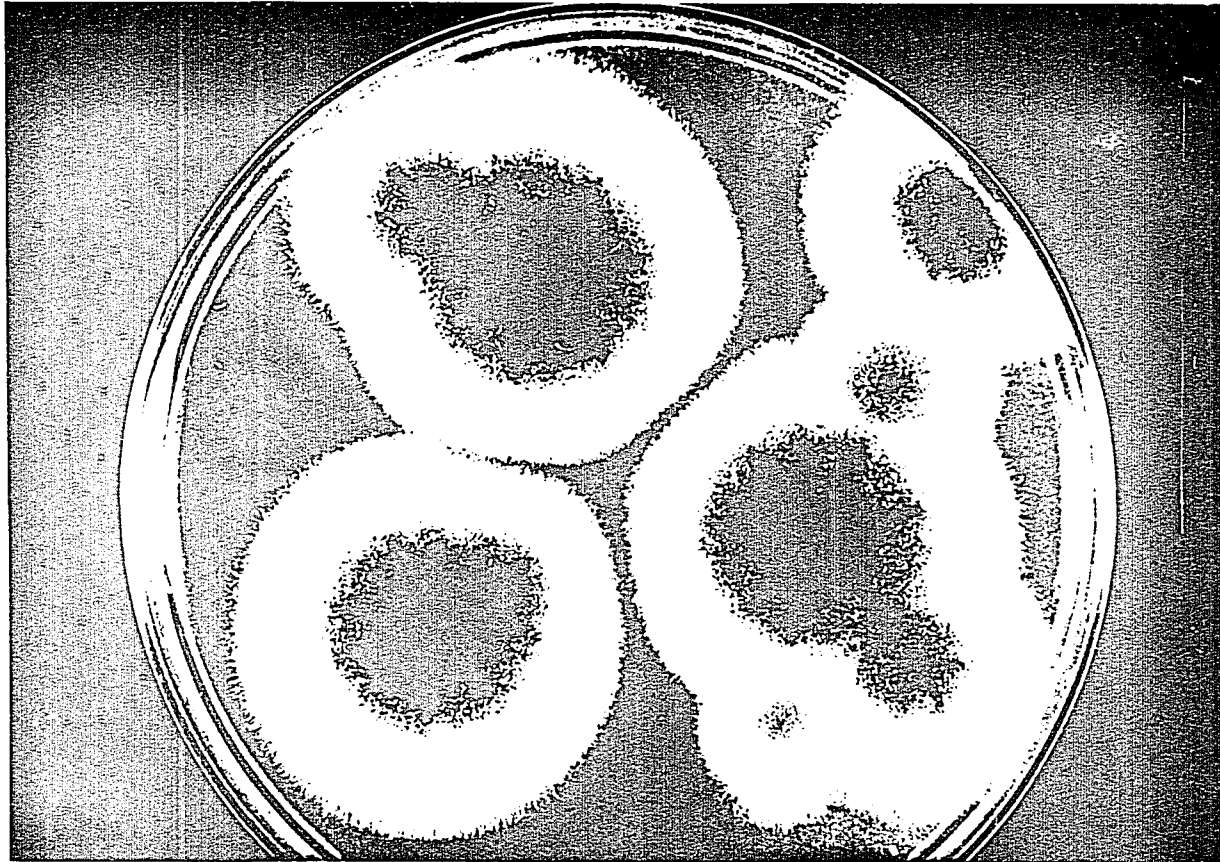


Figure 16. Secondary Treatment Mycological Isolate: Aspergillus Niger Grown on Sabouraud's Agar. This photograph was taken with back lighting x 1.35.

Oidiodendron sp. was found to have 80×10^7 fungal units per mL (22 October). The genus Penicillium (Figure 17) was also well represented and reached 40×10^7 propagules per mL (22 October).

Fungal diversity remained relatively constant during the three week sample period, although the numbers of fungal propagules taken as a sum of their determined densities rose from 182×10^7 to 314.4×10^7 to 418×10^7 propagules.

The secondary influent contained a total propagule density which was slightly more than half (53%) of the total propagule density of the activated sludge tanks. While the secondary influent had one mold (Oidiodendron sp.) not found in the activated sludge, this latter process supported two microfungi (Sporotrichum sp. and Botryotrichum sp.) which do not occur in the secondary influent.

The geofungus, Geotrichum candidum, deserves special note. This mold would not isolate on the media employed and with the dilution factors and laboratory techniques used. Upon microscopic examination of the activated sludge, however, the mycelia of G. candidum were readily found. Figure 18 provides the estimated density for this microfungus for the sample collection dates. Densities were determined by the use of a Sedgwick-Rafter counting cell. Figures 19 and 20 show a G. candidum colony under different lighting conditions. Isolation of the colony was made by needle transfer to Sabouraud's agar, with subsequent transfers of rapidly growing hyphae. This last procedure eventually eliminated bacterial contaminants.

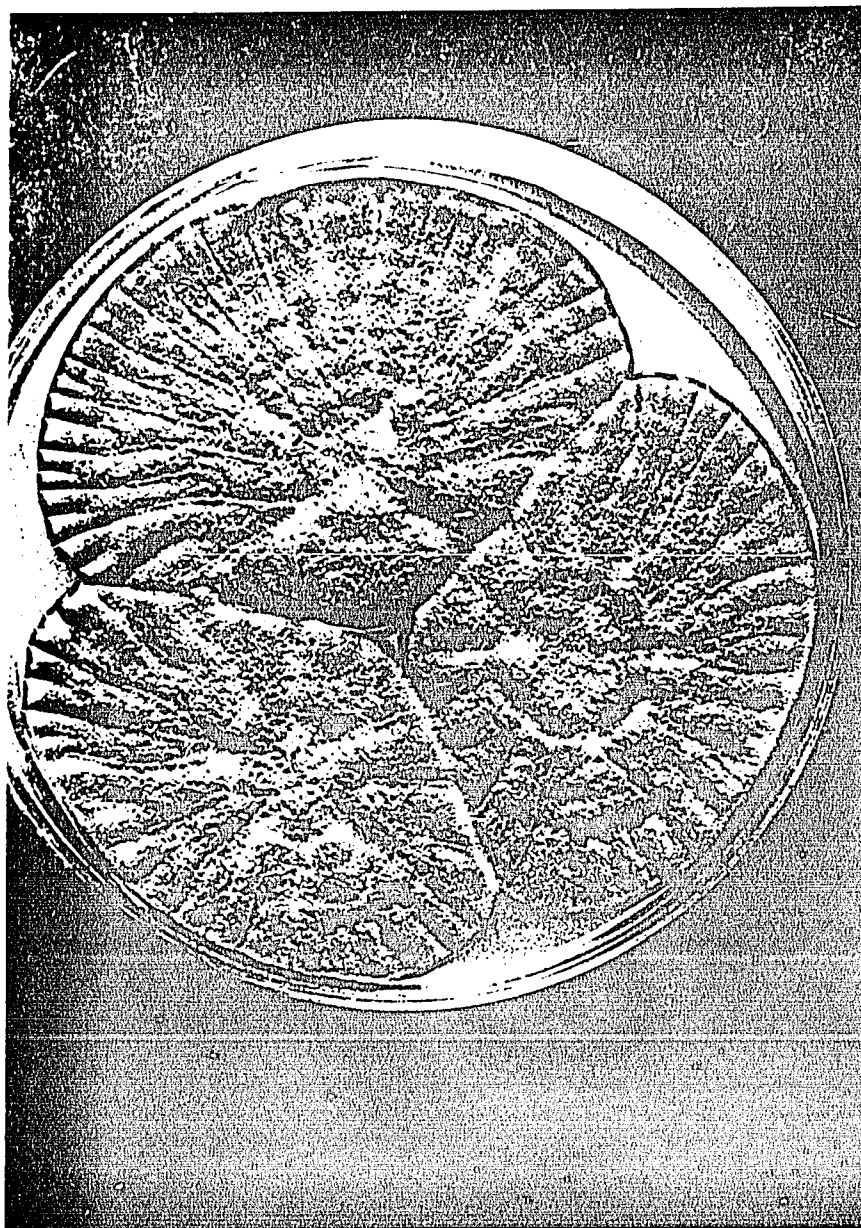


Figure 17. Secondary Treatment Mycological Isolate: *Penicillium* sp. Grown on Sabouraud's Agar. Photograph was taken with side lighting x 1.20.

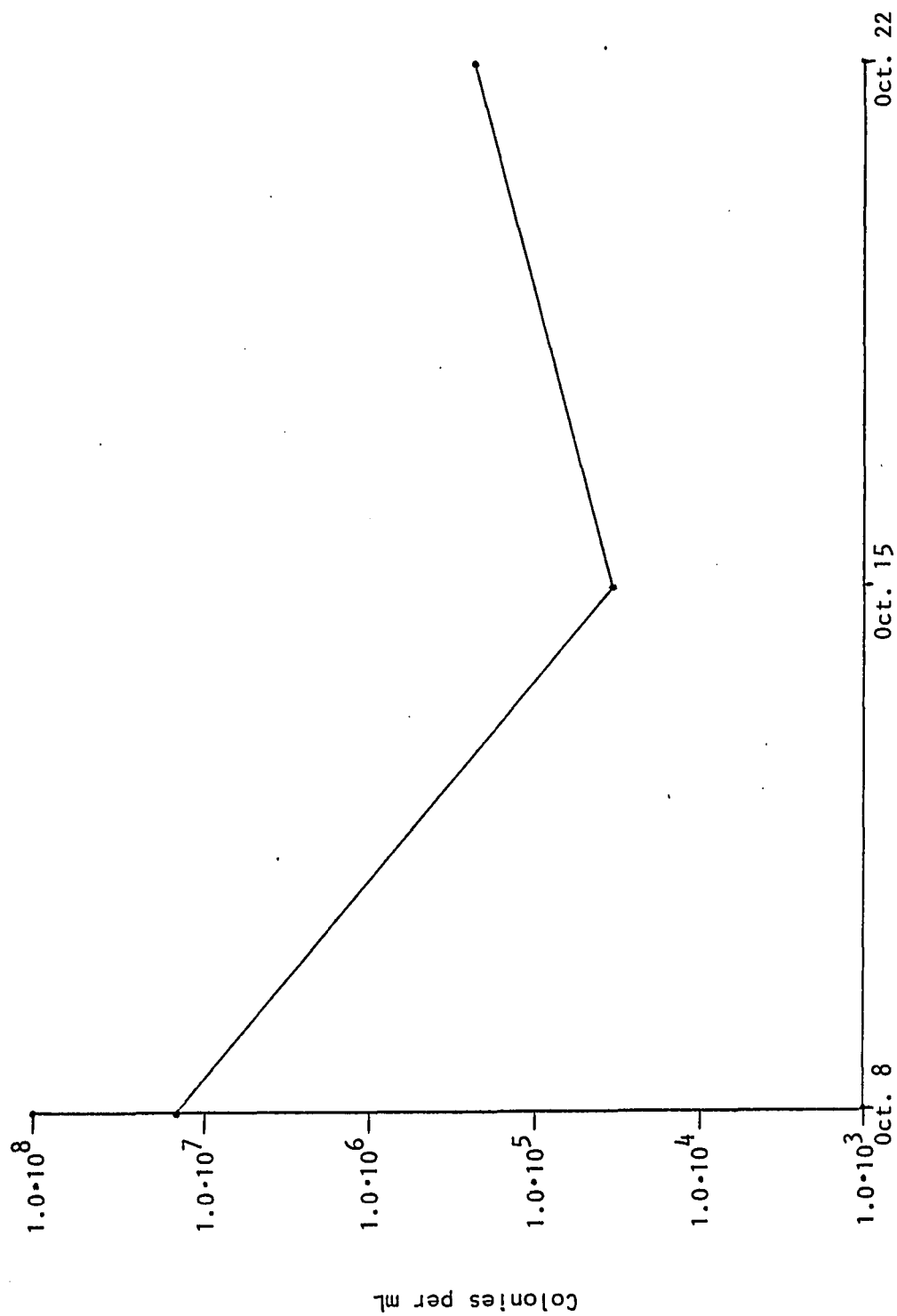


Figure 18. Density of Geotrichum Candidum Colonies in Activated Sludge

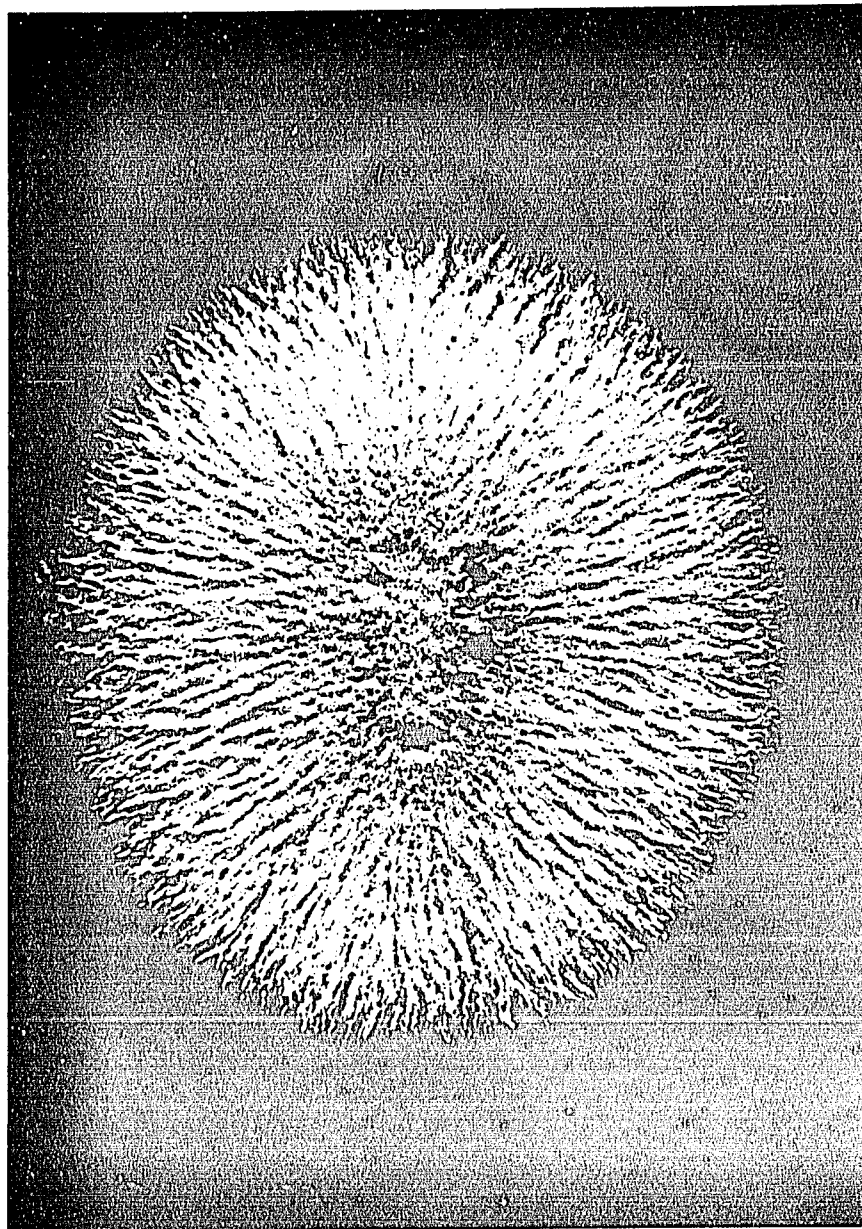


Figure 19. Secondary Treatment Isolate: Geotrichum Candidum Grown on Sabouraud's Agar. Photograph was taken with side lighting x 4.0.

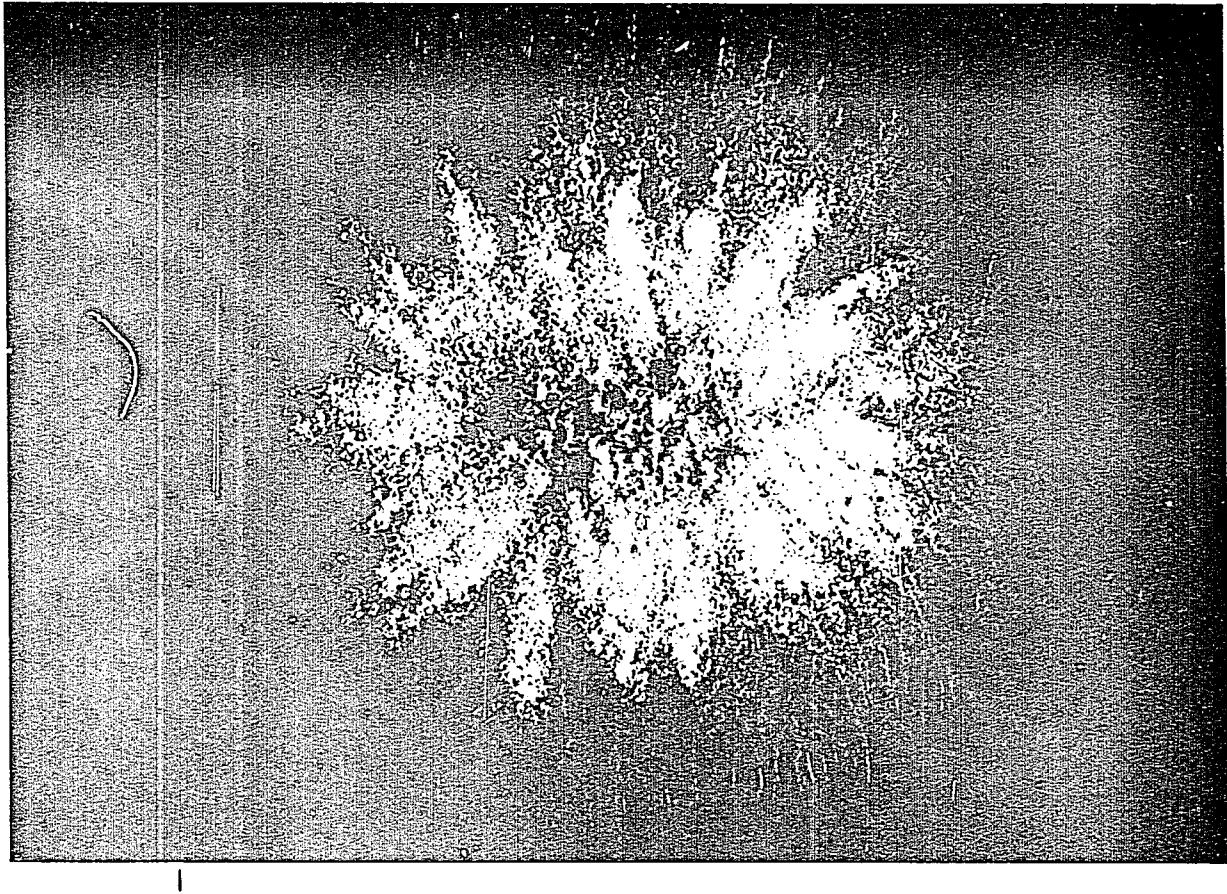


Figure 20. Secondary Treatment Mycological Isolate: Geotrichum Candidum Grown on Sabouraud's Agar. Photograph was taken with back lighting x 4.0.

Microinvertebrates of the Activated Sludge

Because of the low oxygen concentration, relatively few active microinvertebrates are to be found in the secondary influent. Consequently, the author restricted his microscopic examination for microinvertebrates to activated sludge. Table 7 and Figures 21 and 22 furnish a list of microinvertebrates found in the activated sludge habitat and provide their relative densities.

In collecting this data, an important change was made; instead of collecting data for three Saturdays, two Saturdays and a Monday were selected. The addition of the weekday exhibited how the microinvertebrate community could dramatically change.

Flagellates were present on both the 15th and 22nd of October, but were completely absent on Monday, the 24th of October.

The ciliates Euplotes sp. and Vorticella sp. were present at each sampling, but they both underwent substantial decreases on Monday, the 24th of October. As has been noted elsewhere in this study, the number of peritrichs is indicative of sludge quality, the higher the relative number, the better the activated sludge.

The rotifer Philodina sp. showed densities of 35.0×10^5 and 36.0×10^5 per L on the two Saturdays, but then dropped more than seven fold on Monday the 24th of October.

The activated sludge environment appeared to be under stress on the last sampling date. Effluent BOD had risen to one of the month's highest levels; effluent volatile suspended solids had likewise reached a peak concentration and DO of the mixed liquor had fallen to 1.5 mg/L.

Table 7
Density of Microinvertebrates Found in Activated Sludge (Organisms per L)

		October 15	October 22	October 24
Flagellates:	<u>Peranema Trichophorum</u>	$0.40 \cdot 10^5$	$0.80 \cdot 10^5$	---
	Unidentified Flagellates	---	$1.40 \cdot 10^5$	---
Ciliates:	<u>Euplotes</u> sp.	$10.00 \cdot 10^5$	$10.00 \cdot 10^5$	$2.00 \cdot 10^5$
	<u>Lionotus Fasciola</u>	---	$1.20 \cdot 10^5$	$0.20 \cdot 10^5$
	<u>Podophrya Fixa</u>	$0.80 \cdot 10^5$	---	---
	* <u>Vorticella</u> sp.	$16.00 \cdot 10^5$	$1.40 \cdot 10^5$	$0.50 \cdot 10^5$
Rotifers:	<u>Monostyla</u> sp.	$3.00 \cdot 10^5$	$0.60 \cdot 10^5$	---
	<u>Philodina</u> sp.	$35.00 \cdot 10^5$	$36.00 \cdot 10^5$	$4.80 \cdot 10^5$
	<u>Philodina</u> sp. eggs	$7.20 \cdot 10^5$	$12.00 \cdot 10^5$	$0.20 \cdot 10^5$
Aocarids +		$0.80 \cdot 10^5$	$0.40 \cdot 10^5$	$0.20 \cdot 10^5$

* Discrete units counted, whether unicellular or colonial.

+ Inactive, c. 50% with mycelial growth attached.

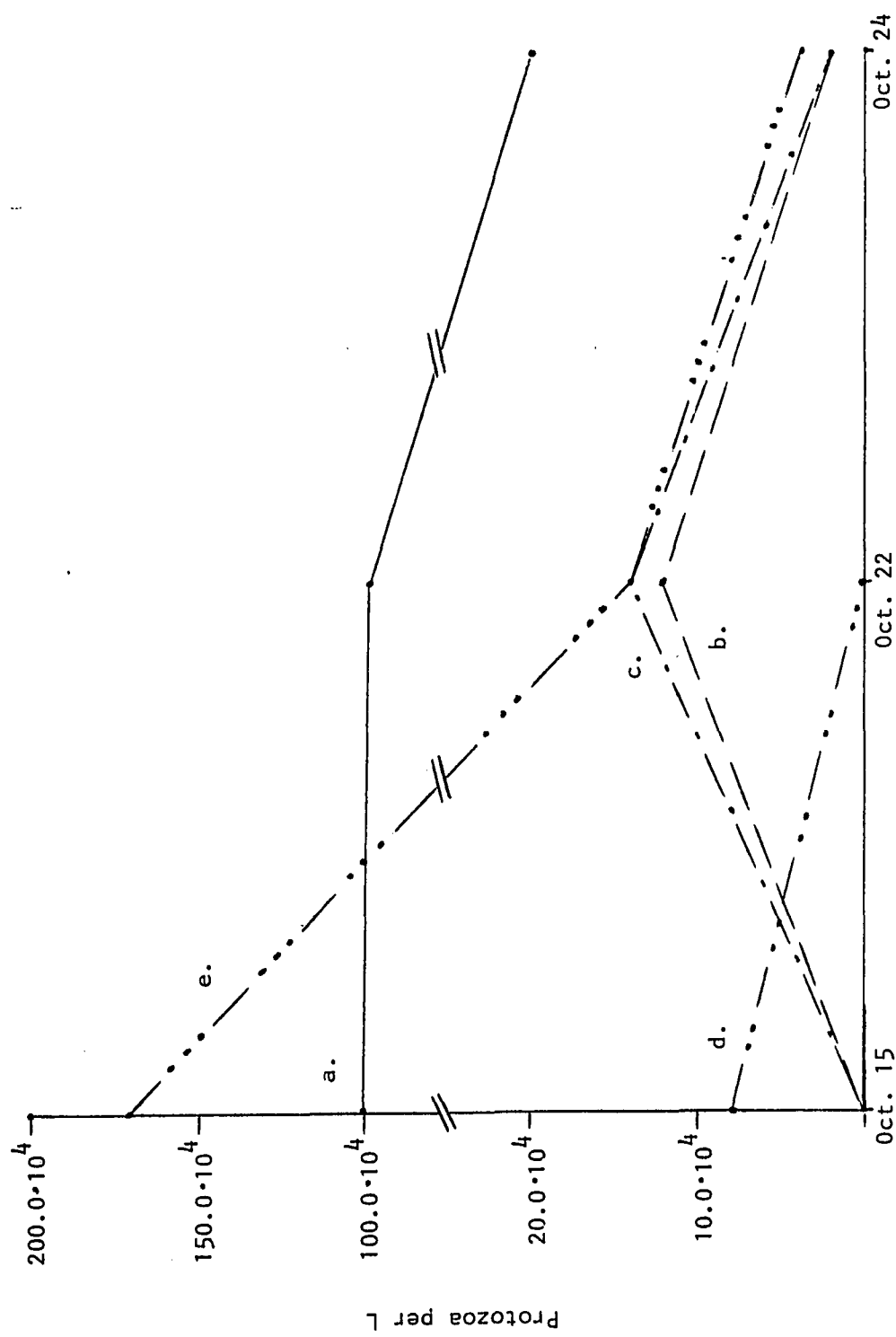


Figure 21. Density of Ciliated Protozoa in Activated Sludge: a. *Euplotes* sp.,
b. *Lionotus fasciola*, c. *Paramecium* sp., d. *Podophrya fixa*, e. *Vorticella* sp.

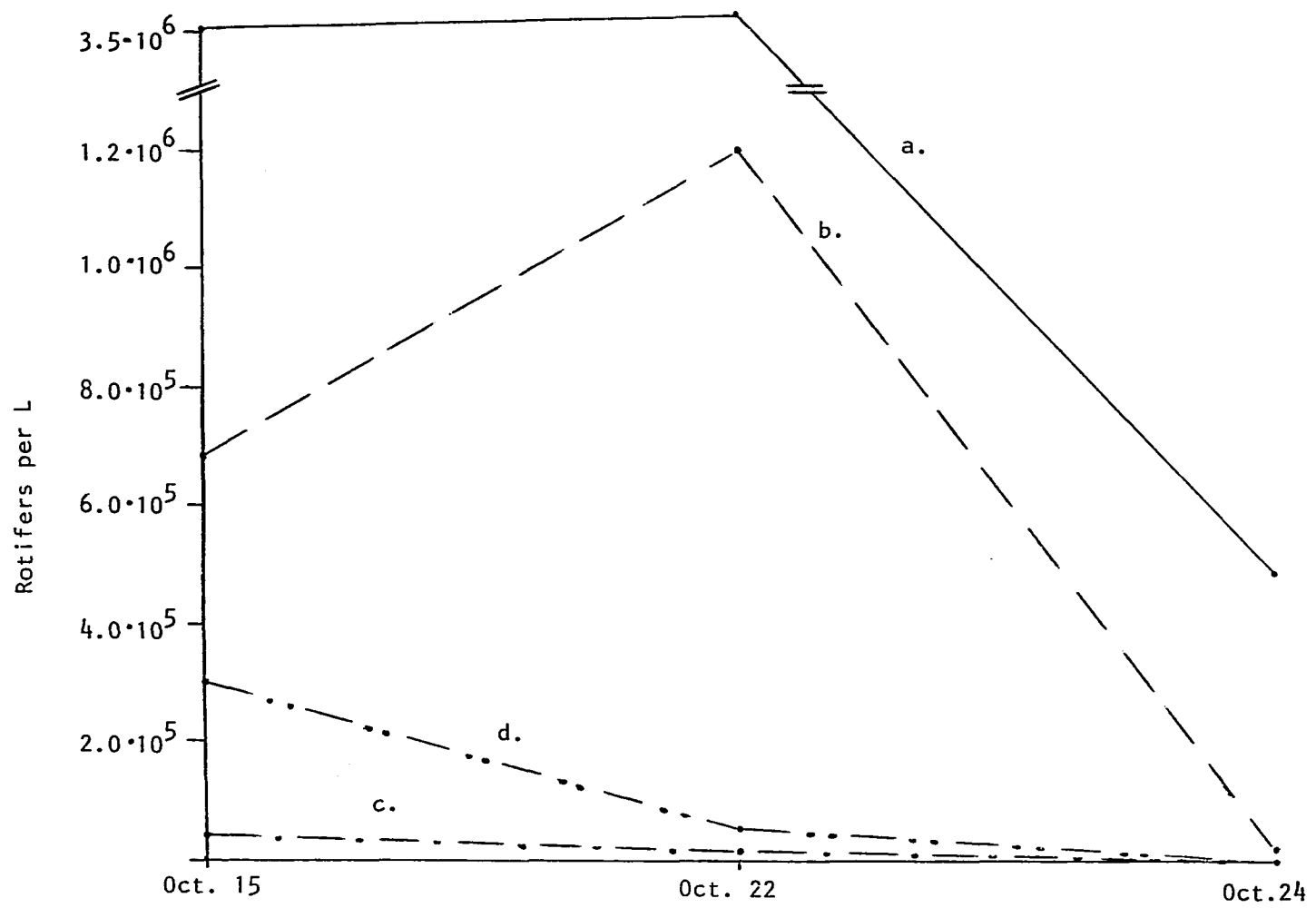


Figure 22. Density of Rotifers (and Eggs) in Activated Sludge: a. *Philobina* sp., b. *Philodina* eggs, c. Inactive *Philodina* sp. with Mycelia Attached, d. *Monostyla* sp.

CHAPTER V

DISCUSSION

Microfungi in Secondary Influent and Activated Sludge

All fungi isolated at the Kalamazoo Wastewater Treatment Plant are moniliaceous soil fungi. This finding tends to support the contention by Cooke (1963a) that the activated sludge environment is more terrestrial than aquatic. Indeed, the Oomycetes and Chytridiomycetes, the principal classes of aquatic fungi, are seldom found at any stage of secondary wastewater treatment.

The density of fungal propagules and their specific composition vary for the same sampling period between secondary influent and activated sludge. For the three test dates, total propagules of the activated sludge fungi exceeded combined numbers of influent fungi by 150%, 500% and 120%, respectively. Furthermore, one genus of fungus, Oidium sp. was found only in the secondary influent, while two genera, Botryotrichus sp. and Sporotrichum sp. were isolated from activated sludge and not the influent. The ranking of fungal densities in the two environments also varied considerably, although Penicillium lilacinum, Penicillium sp. and Aspergillus niger were commonly dominant or subdominant components in both communities.

The reasons for the fungal diversity between these two habitats may be interpreted in several ways. In particular, the turbulence caused by compressed-air aeration in the activated sludge tanks may produce stress for some fungi, while the low dissolved oxygen of the

influent may suppress the growth of other molds. The return sludge, a constant inoculum of the activated sludge tanks, may retain certain fungi more readily in the same manner that it conserves attached ciliates such as Vorticella sp.

Whatever the differences between secondary influent and activated sludge, the latter habitat supports a much greater fungal density, and an apparent greater number of fungal genera. This observation tends to indicate that fungi which enter the wastewater treatment plant may actually be in an active phase of growth and/or assimilation of nutrients. This point is of signal importance for only one fungus, Geotrichum candidum, was observed to have formed mycelia in the activated sludge. This geofungus was consistently found when activated sludge samples were observed microscopically. There was some difficulty in obtaining accurate density estimates of this fungus because most colonies were actively producing vast quantities of arthrospores (asexual spores) which likely saturated significant areas of the habitat.

Geotrichum candidum may well be a permanent member of the activated sludge community, an opportunistic organism which proliferates under conditions which may be restrictive to the bacterial component of the community. One such condition is a pH ; as the pH drops, fungi, and G. candidum in particular, are under less physiological stress than most bacteria of the activated sludge, and are able to outcompete these prokaryotes.

Immigration and Stability of Fungal Components of the
Secondary Wastewater Treatment System

Pencillium lilacinum and Penicillium sp. (Figure 17, Tables 5 and 6) together had the highest total number of propagules isolated from influent and activated sludge samples. This genus was also omnipresent, occurring in all samples of both habitats investigated.

Aspergillus niger (Figure 16, Tables 5 and 6) exhibited the second highest total number of propagules from both sources. This particular mold was present at all sample sites except the secondary influent on the 22nd of October. Even though A. niger was absent from the secondary influent on the 22nd of October, the same day, this geofungus was found to have an activated sludge density of 120×10^7 propagules per mL. Such an observation would suggest that A. niger could maintain itself in the activated sludge without a constant influent inoculation.

Botrytrichum sp. provides an additional example of a fungus which appears to be able to maintain itself in the activated sludge without a constant inoculation; indeed, substantial numbers of this geofungus were found in the aeration tanks while no examples of this Deuteromycete were isolated from any of the secondary influent samples.

On the other hand, the influent waters appear to be providing a constant inoculation of the mold Oidiodendron sp., yet this organism was not isolated from the activated sludge. It would appear that the activated sludge environment is inhospitable to this fungus.

Comparison of Bacterial and Fungal Densities in Activated Sludge

Figure 23 provides a comparison of bacterial and fungal densities observed for the activated sludge environment. This figure suggests a possible negative correlation between bacterial and fungal densities. Between the first two data collection dates, the overall density of bacteria remained relatively constant, while the density of the most numerous geofungus recorded substantially increased. Data collected on the 22nd of October (see Figures 11, 13, 15 and Table 6, as well as Figure 23) indicate a marked increase in the densities of heterotrophic and chemolithotrophic bacteria, although geofungal densities of the activated sludge diminished.

The rise in fungal densities on the second week may be explained in various ways. The diversity of immigration of fungal genera was the highest on that date, although the total numbers of inoculant organisms was lowest for the test period. The physical and chemical parameters in Figures 1-9 do not appear to vary significantly between the second collection date and the first and third dates. Two further possibilities arise: the literature states that the pH of activated sludge may be acidic to such a degree as to favor the growth and proliferation of fungi rather than bacteria; secondly, various important bacterial organotrophs such as Pseudomonas aeruginosa, may have been suppressed by the presence of toxic substances in the wastewater. Seyfried (1980), for example, demonstrates that heterotrophic bacteria can exhibit sensitivity to heavy metals.

The present author had determined the pH of the KWTP aeration

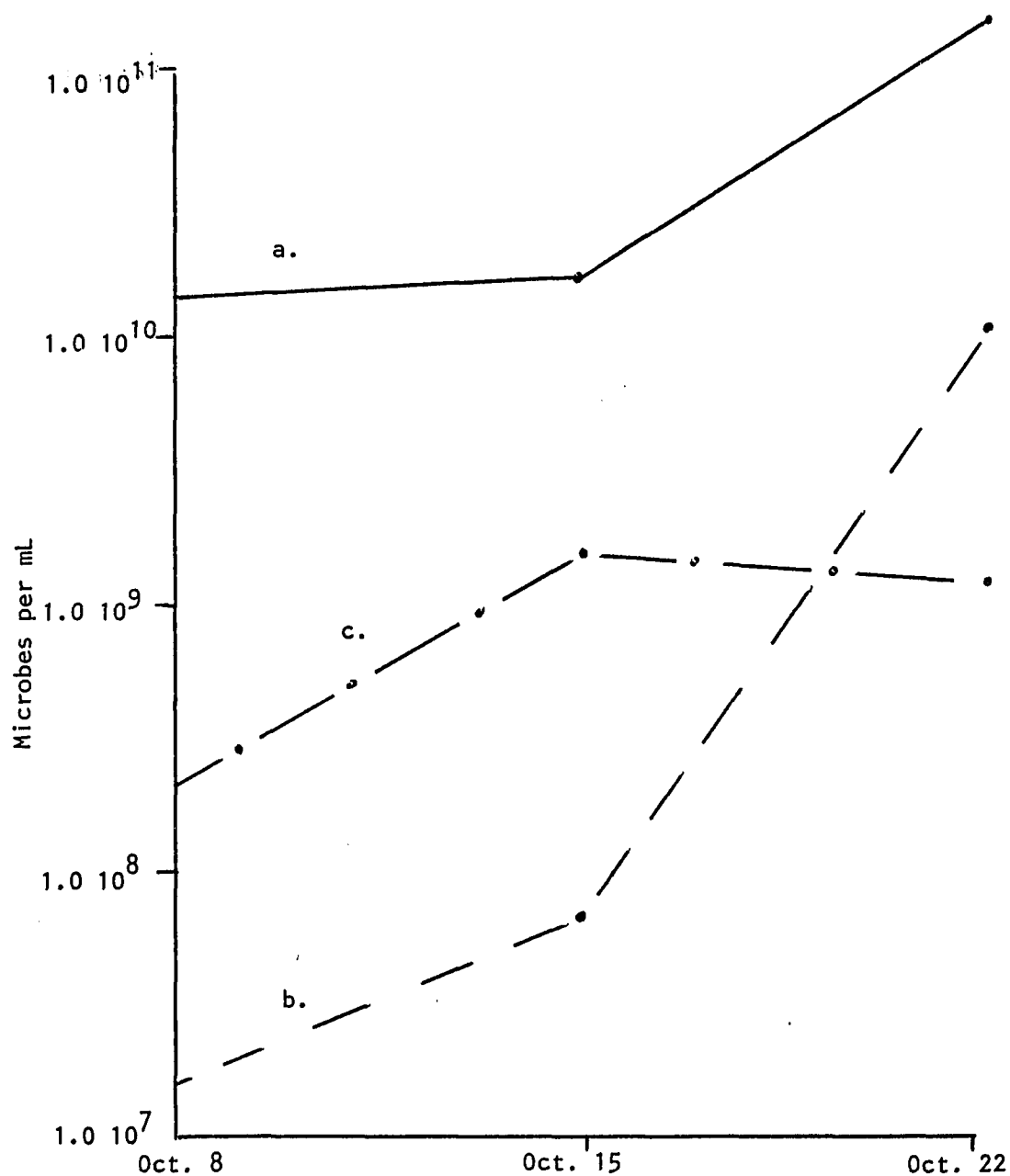


Figure 23. Comparison of Bacterial and Fungal Densities of Activated Sludge, a. Heterotrophic Bacteria, b. Chemolithotrophic Bacteria, c. Microfungi (Highest Density of a Geofungus Recorded for Each Date).

tanks at the time of sampling to be at or above 6.4. It seems unlikely that this pH reading would inhibit major heterotrophic bacteria.

On the other hand, the Greeley and Hansen report (1982) clearly indicates that numerous toxic substances, such as heavy metals, phenolic compounds and some industrial residues enter the Kalamazoo facility. However, as there exists no scheduled monitoring of such substances at the KWTP, it is impossible to know whether or not these toxins played a role in the relative densities of bacteria and fungi.

Interactions Between Microfungi and Metazoans of the Activated Sludge Community

Geofungi were found to be heterotrophs and possibly predators in the activated sludge community.

Limited though constant numbers (1.0×10^6 to 4.0×10^6 per L) of moribund nematodes were found with attached mycelia. It was impossible, however, to determine whether fungi played any role in the debilitated state of these roundworms. The culture and identification of the fungi attached to the nematodes were not in the scope of the present study.

Dead rotifers of the genus Philodina sp. were occasionally (less than 1% of the rotifers examined) found which also exhibited attached mycelial growth. The role of fungi in the demise of such rotifers was impossible to ascertain.

Cooke and Ludzack (1958) point out, however, that rotifer

populations in a bench-scale activated sludge unit were completely reduced by the predacious fungus Zoophagus insidians.

If such a role were determined for fungi in the Kalamazoo Wastewater Treatment Plant, then the fluctuation in rotifer populations in the activated sludge might be more readily explained.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

The present study has addressed the ecology of fungi in the activated sludge environment. It sought, in the main, to determine the fungal components of the system and to estimate the densities of such fungi. This research then strove to establish the significance of basic interactions between fungi and other activated sludge microbes, especially bacteria. In consideration of material presented earlier in this paper, the following conclusions appear warranted:

1. There may exist a negative correlation between the bacteria and geofungi of activated sludge. Change in the environment of the secondary wastewater process may favor fungi over bacteria.
2. Activated sludge appears to have a resident population of microfungi.
3. All fungi isolated at the Kalamazoo Wastewater Treatment Plant belong to the form-class Deuteromycetes.
4. The density of geofungi in activated sludge may exceed that of bacterial chemolithotrophs and rival that of bacterial heterotrophs.

There are two broad recommendations which the author wishes to offer. The first relates to the management of the Kalamazoo Wastewater Treatment Plant; the second suggests the orientation which further studies in the mycology of activated sludge might take.

I recommend that the physico-chemical parameters of the activated

sludge process be determined with more precision. This suggestion pertains especially to pH, temperature and the detection of toxic substances. Isolated though regular discharges by nondomestic sewage users of acidic or toxic substances could substantially alter the biological composition of the activated sludge, and hence, seriously impact upon the entire plant's performance.

The biological activity of the activated sludge could be monitored much more closely. At the minimal, one might schedule the monitoring of stalked ciliate populations and perhaps the periodic examination of activated sludge for the presence and density of the filamentous fungus Geotrichum candidum. The number of peritrichs per mL has often been used to determine plant efficiency, and knowledge of the number of G. candidum mycelia per unit volume could indicate the degree or the potential for bulking (non-settling of the sludge).

Future studies in the mycology of activated sludge will hopefully determine what relationships obtain between the fungal and bacterial components in secondary seration tanks. I would also suggest that such research be carried out over several seasons to determine whether the fungal and bacterial populations are cyclic in nature. I would also recommend that the relative impact of toxic substances on these two groups of microbes be ascertained and that serious attempts to monitor for such substances be incorporated into the study.

After such studies have been completed, the role of fungi in the purification of water should be much better understood than at

present. Although their ultimate importance remains in dispute at this time, the present study has effectively shown that geofungi are prominently found in activated sludge and that they may be an integral part of that community's biota.

APPENDIX A
CULTURE MEDIA - INGREDIENTS per L

CULTURE MEDIA

Ingredients per L

NUTRIENT AGAR

Peptone	5.0 gr
Beef Extract	3.0 gr
Agar	15.0 gr
Distilled Water	1 L

ENRICHED NUTRIENT AGAR

Peptone	5.0 gr
Beef Extract	3.0 gr
Yeast Extract	5.0 gr
Glucose	5.0 gr
Agar	15.0 gr
Distilled Water	1 L

ZOOGLOEA MEDIUM

Casitone	5.0 gr
Glycerol	5.0 gr
Na-Lactate	0.5 gr
Yeast Autolysate	1.0 gr
Agar	15.0 gr
Distilled Water	1 L

AZOTOBACTER MEDIUM

KH_2PO_4	0.2 gr
K_2HPO_4	0.8 gr
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.2 gr
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0.1 gr
FeCl_3	Trace
Na_2MoO_4	Trace
Yeast Extract	0.5 gr
Sucrose	20.0 gr
Agar	15.0 gr
Distilled Water	1 L

THIOBACILLUS MEDIUM

Na_2HPO_4	1.2 gr
KH_2PO_4	1.8 gr
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.1 gr
$(\text{NH}_4)_2\text{SO}_4$	0.1 gr

CaCl_2	0.03 gr
FeCl_3	0.02 gr
MnSO_4	0.02 gr
$\text{Na}_2\text{S}_2\text{O}_3$	10.0 gr
Agar	15.0 gr
Distilled Water	1 L

MYCOLOGICAL AGAR

Neopeptone	10.0 gr
Dextrose	40.0 gr
Agar	10.0 gr
Streptomycin Sulfate	5.0 mL
Distilled Water	1 L

COOKE'S ROSE BENGAL AGAR

Neopeptone	5.0 gr
Dextrose	10.0 gr
Rose Bengal	0.035 g
Streptomycin Sulfate	5.0 mL
Agar	20.0 gr
Distilled Water	1 L

WATER AGAR

Agar	20.0 gr
Streptomycin Sulfate	5.0 mL
Distilled Water	1 L

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