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IN THE ACTIVATED SLUDGE PROCESS.

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GRADUATE COLLEGE

INVESTIGATION OF FILAMENTOUS BULKING IN THE ACTIVATED SLUDGE PROCESS

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Norman, Oklahoma

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INVESTIGATION OF FILAMENTOUS BULKING

IN THE ACTIVATED SLUDGE PROCESS

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DEDICATION

This work is dedicated
to my wife, Jackie, and
to my daughter, Dawn.

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The author wishes to express his sincere appreciation to Dr. James Robertson for his guidance and encouragement. The author is particularly grateful to Dr. Leale Streebin for his help, advice, and interest throughout this investigation. The beneficial comments of Dr. Larry Canter, Professor George Reid, Dr. Sherril Christian, and Dr. Edwin Klehr were appreciated. Thanks are due Mr. Steve Allen for photographic assistance and Mrs. Jack Price for editing this dissertation.

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ABSTRACT

The activated sludge waste treatment process provides some intellectually challenging and practically significant research problems in environmental engineering and applied microbiology. From time to time, nearly all activated sludge processes fail to provide a satisfactory degree of treatment. The majority of these process failures are due to a settling phenomenon known as filamentous activated sludge bulking. Most researchers who have investigated bulking of activated sludge agree that the sheath-forming bacterium, Sphaerotilus natans, is one of the primary organisms involved.

In this study the most common conditions associated with the bulking of activated sludge by S. natans were investigated. This study of Sphaerotilus was related to the ecology of normal activated sludge by investigating mechanisms by which the settling characteristics of activated sludge change utilizing both pure and mixed cultures of S. natans. Experiments were conducted in dual continuous-feed activated sludge systems with synthetic sewage.

Under most filamentous bulking conditions encountered in the field, improved settleability and a lower sludge volume index (SVI) were found to be a function of the following:

reduction of food stored as poly-β-hydroxybutyric acid (PHB) in bacterial cells to an optimum level; reduction of excessive cellular activity as dehydrogenase activity (TCC) to an optimum level; increase in low pH to an optimum level near neutrality. It was also concluded that PHB may serve as a binding site for flocculation, that additional capsular material may be important in flocculation, and that S. natans may contribute to the buffer capacity of the process. In addition, the increase in COD removal attributable to bulking was not high enough to suggest treatment of high strength industrial wastes under bulking conditions. The total suspended solids concentration was differentiated as to fixed inorganic and organic suspended solids, inert suspended solids, and active and inactive cell mass.

The analysis of the data as a whole showed that several variables have a tendency to vary in a pattern similar to that of the SVI. A multiple regression analysis of SVI upon PHB, TTC, cellular mass as DNA, and the pH resulted in a correlation coefficient of 0.722. This regression which accounts for over 52 per cent of the total variance of SVI, is the most reliable estimate of sludge settleability. There are, of course, other variables upon which sludge settleability and SVI are dependent. Until further studies determine other inputs and their relative importance in the model, the regression equation derived from this study should be used to estimate sludge settleability and to evaluate qualitatively the operation of activated sludge treatment systems.

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INVESTIGATION OF FILAMENTOUS BULKING IN THE ACTIVATED SLUDGE PROCESS

CHAPTER I

Introduction

The activated sludge process provides some intellectually challenging and practically significant research problems in environmental engineering and applied microbiology. The activated sludge process, one of the most popular and most effective methods of waste treatment, consists of aerating liquid wastes with preformed biological flocs. While aerating sewage in 1914, Ardern and Lockett (1914) found that more rapid purification could be achieved by collecting the solid material which accumulated during aeration and returning the sludge for aeration with the influent sewage. The solid material was given the name "activated sludge" and gravity separation following aeration became the accepted method of sludge collection. Since that time, the problem of sludge collection has been a primary concern of the activated sludge process.

Although a variety of conditions has been associated with problems in sludge collection, the most common

characteristic has been the growth of filamentous microorganisms in the sludge. This condition, which has become known as filamentous bulking, is of interest in this study. Despite the early recognition of filamentous bulking by Buswell and Long (1923), the problem has never been completely solved. Most researchers who have investigated bulking agree that the sheath-forming bacterium, Sphaerotilus natans, is one of the principal organisms involved. In spite of the numerous investigations of Sphaerotilus, very little information concerning the role of this organism has been published. In view of this, the most common conditions associated with bulking of activated sludge by S. natans were of primary concern in this investigation.

The purpose of this research was to investigate the ecology of S. natans in activated sludge and to determine the mechanism by which the settling characteristics of the activated sludge change. The most important phase of the investigation involved the quantitative evaluation of several parameters under both bulking and non-bulking conditions utilizing pure and mixed cultures of S. natans. The parameters most useful in describing the growth responses of S. natans included the settled volume of the sludge, the accumulation of the polymer, poly-β-hydroxybutyric acid, the rate of oxygen uptake, the carbon to nitrogen ratio, and the concentration of acids. The physiology of the sheath/slime layer was also investigated.

Another phase of the investigation included a comparison of the growth characteristics of other filamentous organisms when present in a mixed culture in small numbers to the growth when present in large numbers.

A final study was conducted to determine whether bulking conditions influenced the relative composition of the suspended solids concentration. The composition of the total suspended solids concentration has been evaluated quantitatively in terms of the fixed inorganic suspended solids, organic volatile suspended solids, inert suspended solids, total cell mass, inactive cell mass, and active cell mass.

It is hoped that the evaluation of the effect of each factor on the sludge settling characteristics will soon result in a solution to all filamentous bulking problems through an optimization of those parameters which favor a sludge with desirable settling characteristics.

CHAPTER II

LITERATURE REVIEW

The Activated Sludge Process

Activated sludge, trickling filters, and oxidation ponds are the major aerobic biological treatment systems used for the stabilization of organic wastes. These systems have been described as attempts to optimize the activity of different microorganisms in order to control the natural processes involved in the degradation of complex organics to their simpler, less noxious, mineral components. Through environmental control, the rates of these natural processes can be increased so that they may be accomplished in a small space and within a limited, economical period of time.

The activated sludge process is considered the most versatile of the biological treatment systems. It has been successfully applied to industrial as well as domestic waste treatment. The activated sludge process is an operation in which waste waters and flocs of microorganisms are supplied with air for the dual purpose of keeping the units aerobic and the flocs in suspension, in spite of heavy concentrations of living organisms and the absence of fixed contact media.

Although Johnson (1914) was probably the first to emphasize the importance of biology in a sewage disposal system, according to Martin (1927) the original idea of this process is attributed to Dr. Edward Ardern and Mr. William Lockett in 1913. Ardern and Lockett (1914) introduced the term "activated sludge" to identify a large mass of settleable solids formed after aerating biologically degradable wastes for a period of time.

The microorganisms which inhabit this process include bacteria, fungi, algae, protozoa, rotifers, and other higher animals. The growth of any or all types of microorganisms in the system will depend upon the chemical characteristics of the waste, the environmental limitations of the particular waste system, and the biochemical characteristics of the microorganisms. All of the organisms which grow in the system will contribute to its over-all characteristics, both good and bad. It is important to recognize that bacteria are the basic biological units in the process and, as such, will determine the characteristics of the treatment system.

Considering the importance of the microorganisms to the process, it seems reasonable that the treatment facilities would be designed and operated as a controlled environment for the living organisms instead of being operated as a hydraulic system. Since water comprises the bulk of the complex which is treated, the hydraulic aspects of the plant cannot, of course, be ignored. However, if the organisms are

to perform the function intended, it would not be reasonable to treat the living system as a purely minor part of the operation when it is indeed of primary significance.

The study of the ecology of a sewage treatment plant, the characteristics and responses of its living components, is extremely complex. Perhaps it is the very complexity of this system which has discouraged research in this direction, but the fact remains that many physical and mechanical modifications have been made to the process while biologically the process has remained the same. Although a significant amount of biological knowledge of the activated sludge process is needed, it is necessary to begin with studies involving the organisms responsible and studies of their behavior individually and collectively under specific conditions.

Activated Sludge Bulking

In activated sludge plants, under certain nutritional and environmental conditions, the sludge will develop poor settling characteristics. When this occurs, the sludge is said to be bulking. A considerable amount of attention has been paid to this phenomenon in the past; this is due to the fact that the final solids-liquid separation step is normally accomplished in a final settling tank. If the sludge fails to settle at this point, the purification process has failed.

It appears that the word bulking, as it was originally used in reference to activated sludge settling problems, was intended to suggest an increase in the volume of the sludge.

Indications of this are seen in the work of Scott (1928) who used the term bulking to describe activated sludge with poor settleability and increased volume. Donaldson (1932) described activated sludge bulking as being "a sludge condition in which the settleable solids by volume are abnormally high compared with the weight." However, Lackey and Wattie (1940) observed that the meaning of bulking had frequently been misconstrued and that the term had been used in reference to a variety of problems with settling activated sludge. Pipes (1967, a, b) said that there were many phenomena which could prevent activated sludge from settling properly and that all of them have been called bulking at one time or another.

Over thirty years ago, Heukelekian and Ingols (1940) separated bulking into two general classes: carbohydrate bulking and non-carbohydrate or sewage bulking. Carbohydrate bulking was induced by an excessive development of sludge or certain organisms comprising the sludge; this was considered due to an improper balance of food in relation to the sludge. Generally, sewage bulking was induced by a reduced amount of air producing a sufficiently limited oxygen supply. Another classification considered chronic bulking and acute bulking. Chronic bulking was a condition which was attributed to plant operations; acute bulking was a direct result of the type or quantity of wastes being discharged into the plant. A more sophisticated classification was suggested by Pipes (1967, a, b) in which three categories were recognized. They were

problems with sludge flocculation, problems with reductions in the specific gravity of the sludge, and problems with the compaction of the sludge. The term bulking was reserved for the last category. It was indicated that these problems could occur singly or in combination with one another. A brief description of each category is given below.

Problems with poor sludge flocculation are caused either by the breaking up of the floc by mechanical or physiological disturbances or by a predominance in the sludge of micro-organisms which, by nature, grow dispersed. Such problems are characterized by a very turbid supernatant above the settling sludge with the interface poorly defined, or even absent. Some material remains in suspension for a very long time.

Sludges afflicted with reductions in specific gravity usually flocculate well, but they either rise to the surface instead of settling, or they settle initially and then float to the surface after a short time. The former are those sludges with a specific gravity less than that of water, a condition caused by the presence of buoyant materials such as fats, wood particles, or gas bubbles. Sludges which settle and subsequently float do so because of gas bubbles within the floc. These bubbles are generated when the sludge is permitted to go septic. This condition is usually characterized by a clear internate with sludge at both the top and bottom of the settling sample.

Settling sludges that do not compact well are characterized by a well defined, slowly subsiding interface. The supernate is very clear and flocculation is excellent. Pipes indicated that this last category is correctly called bulking.

Two types of bulking sludge were described by a committee of the American Public Health Association (Pearse et al., 1937) as (a) a flocculated sludge with a high SVI and a low settling rate, and (b) a filamentous sludge with an extremely low settling rate. The two types of bulking have come to be known as "zoogloeal bulking" and "sphaerotilus bulking", although nonfilamentous bulking and filamentous bulking are more meaningful names.

Nonfilamentous Bulking

It has been known for some time that activated sludge bulking can occur in the absence of filamentous organisms. This condition has been called "zoogloeal bulking" and it appears to have characteristics similar to filamentous bulking. Characteristics include good flocculation, a well-developed, slowly subsiding interface, and a voluminous, settled sludge.

Clifford and Windridge (1932) reported a bulking sludge in which there were no filaments. They found that the bulking became more severe as the sludge concentration increased and as the dissolved oxygen in the mixed liquor reduced. Smit (1934) reported similar findings. Smith and Purdy (1936) encountered two types of bulking sludges and

described them as consisting on the one hand of "thin, diffuse or spongy, ragged floc" and on the other hand of "floc...heavily infested with fungal threads." Although they did not name them as such, they had described both nonfilamentous bulking and filamentous bulking respectively. One of their more important observations was that these two conditions could occur simultaneously. Genetelli and Heukelekian (1962) and Hartman (1963) reported the occurrence of nonfilamentous bulking during conditions when sludge was overloaded.

The most extensive examination of nonfilamentous bulking has been reported by Heukelekian and Weisberg (1956). They fed their laboratory activated sludge the colloidal and soluble portions of domestic sewage and found that when a sludge loading rate (SLR) between 0.5 lb/day/lb and 1.0 lb/day/lb was used, the sludge bulked but no filaments grew. They described the sludge floc as being diffuse and very lobate. The volatile portion of the sludge varied from 85% to 88% by weight. Perhaps the most significant of their results was the observation that, as the sludge bulked and the sludge volume index increased, the bound water content of the sludge increased also. In contrast to this, they found that the bound water content of filamentous bulking sludge did not increase as the bulking worsened. Therefore, they felt that this was a major difference between the two types of bulking. In contrast to earlier reports, they believed that the dissolved oxygen concentration of the mixed liquor had

very little to do with initiating nonfilamentous bulking. They also found that chlorination of the return sludge was an effective means of controlling nonfilamentous bulking.

Filamentous Bulking

In 1923 Buswell and Long (1923) reported that the activated sludge with which they had been working had become overwhelmed by filamentous microorganisms and that the sludge was settling poorly. Although they did not make the calculations, their data showed that the SVI of their samples was in some cases greater than 300. Their work is historically significant because it appears to be the first report of the association of filamentous microorganisms with poorly settling activated sludge.

At the 1927 meeting of the Yorkshire District Association of Managers of Sewage Disposal Works, Hoyle (1927) reported that for "many years" problems with settling activated sludge had been encountered and that the growth of filamentous microorganisms had been observed in many of these sludges.

Morgan and Beck (1928) and Ruchhoft and Watkins (1928) examined the occurrence of poorly settling activated sludge at the Des Plaines, Illinois Sewage Treatment Plant and found that the sludge was infested with filamentous microorganisms. The sewage received at the plant contained high concentrations of carbohydrate which had originated from illegally operated distilleries. As a result, the stills were shut down and the sludge settling problem at Des Plaines was solved. The

report is a classic not only because the settling problem was correctly attributed to the presence of filamentous microorganisms in the sludge but also because the disorder was remedied by the elimination of conditions responsible for the growth of the filaments.

In 1932, Donaldson (1932) and Haseltine (1932) observed that the reports written about the poor settleability of filamentous activated sludge were becoming more frequent. Currently, a large volume of information is available of the subject. See Table 1 for a summary of this information.

Donaldson (1932) reported that he was unable to determine whether the growth of filamentous microorganisms in activated sludge was the cause or the result of poor settleability. Ruchhoft and Kachmar (1941) said that activated sludge bulking was not caused by filaments but, rather, by some undefined condition that caused the sludge to become light and fluffy; this in turn resulted in the growth of filaments. Ford and Eckenfelder (1966) observed that their sludge had begun to bulk prior to the growth of filaments and, thus, felt that this growth was a secondary effect. However, it appears that their sludge was afflicted with zoogloeal bulking, a condition which can occur in the absence of filaments. Zoogloeal bulking has been described in an earlier section of this chapter.

Generally, most investigators share the opinion that filamentous microorganisms can cause activated sludge

Table 1

Filamentous Microorganisms Found in Activated Sludge

Investigator	Filamentous Microorganisms Described	Sludge Condition
Buswell and Long (1923)	Sphaerotilus dichotomus, Crenotrix polyspora, Beggiatoa	settling poorly
Hoyle (1927)	Sphaerotilus natans	bulking
Agersborg and Hatfield (1929)	Sphaerotilus, Beggiatoa	settling poorly
Smit (1934)	Sphaerotilus, Geotrichoides paludosus, other unidentified filamentous types	bulking
Tiegs (1939)	Thiothrix, Beggiatoa	---
Ingols and Heukelekian (1940)	Sphaerotilus, Cladothrix, unidentified fungi	bulking
Lackey and Wattie (1940)	Spaherotilus, Bacillus, unidentified Fungi Imperfecti	bulking
Okun (1949)	Sphaerotilus, Beggiatoa	bulking
Hawkes (1963)	Geotrichum candidum	bulking
Pipes and Jones (1963)	Geotrichum candidum	bulking
Pipes (1964)	Sphaerotilus, Pullularia, Geotrichum, Nocardia, Beggiatoa, Bacillus	---
Lackey et al. (1965)	Thiothrix, Beggiatoa	---
Pipes (1967, b)	Sphaerotilus, Beggiatoa, Bacillus, other fungi	frequent bulking

bulking. Smit (1932) and Heukelekian and Littman (1939) felt that the interweaving of filaments reduced the subsidence velocity of the sludge. Finstein and Heukelekian (1965) theorized that the filaments extending beyond the periphery of the floc offered resistance to the settling and compaction of the sludge. To evaluate this, they attempted to correlate SVI measurements with the total length of filaments per sludge floc. Unfortunately, their work was not particularly useful since their correlations were made between sludge from different activated sludge facilities. Their approach could have been more successful had they examined one sludge over a long period of time and correlated changes in SVI with changes in the total length of filaments per floc. In some recent work, Pipes (1967,b) has shown that there is some positive correlation between SVI and the total number of filaments per gram of sludge.

Presenting a somewhat different point of view, Dondero (1961) observed that the volume of Sphaerotilus was about three times the volume of an equal weight of Zoogloea; he concluded, therefore, that as Sphaerotilus grew in activated sludge, the density of the sludge reduced and bulking resulted.

It is apparent that, while the association of filamentous microorganisms with activated sludge bulking is well accepted, the role that these filaments play in reducing the settleability of the sludge is far from being understood. Pipes (1967, a) has offered an attractive hypothesis. He

stated that bulking sludges tend to flocculate extensively before much settling has been accomplished. In doing so, the sludge becomes a cohesive mass which resists compression and the release of water from within. The excessive growth of filamentous microorganisms in the sludge enhances the formation of the cohesive mass through bridging and intertwining.

The information presented in Table 1 lists most of the filamentous microorganisms that have been described and shows that, in general, they have been studied in conjunction with problems in activated sludge bulking. Of the genera Bacillus, Geotrichum, Beggiatoa, Cladothrix, Crenothrix, and Thiothrix listed, most were reported in association with Sphaerotilus. Although it is likely that they have contributed in some degree to the bulking condition, it is also likely that Sphaerotilus has made the major contribution to the problem.

Filamentous Bulking: Sphaerotilus natans

Since very early in the history of the process, there have been reports to indicate that a number of different filamentous microorganisms have been found in activated sludge. However, despite evidence that a variety of filamentous microorganisms could exist in activated sludge, much of the literature written about bulking referred to filamentous microorganisms collectively as Sphaerotilus, with no attempt to identify them. Thus, it has been suggested that, in some cases of activated sludge bulking, the filamentous

microorganisms referred to as Sphaerotilus may well have been something else.

The identification of the filamentous microorganisms in activated sludge is complicated by gross morphological similarities that exist between many of the species. Lackey and Wattie (1940) found that a filament forming species of Bacillus and certain species of the Fungi Imperfecti resembled Sphaerotilus very closely. Dondero et al.(1961) reported similar information and suggested that these filaments, when found in activated sludge, had probably been identified as Sphaerotilus. Such a mistake was made by Pipes and Jones (1963). They prepared and published information about the "Decomposition of Organic Wastes by Sphaerotilus"; later they found that the microorganism with which they had been working was Geotrichum condidum, a fungus.

It is apparent that progress in the ecological study of filamentous microorganisms in bulking activated sludge has been impeded by the failure of many investigators to correctly identify the filamentous organisms.

There is enough reliable information to conclude that Sphaerotilus has been a major problem in activated sludge bulking. This information has come from studies conducted on both field and laboratory activated sludge systems; it includes such classic investigations as those of Buswell and Long (1923) and Lackey and Wattie (1940), in which extensive studies were carried out to assure the identity of Sphaerotilus.

The growth of Sphaerotilus in activated sludge appears to be the result of conditions which permit it to successfully compete for substrate with the rest of the activated sludge population. These conditions may either increase the activity of Sphaerotilus or depress the activity of the rest of the population. The nature of this competition is discussed below.

Pipes (1965) found that the maximum specific growth rate (SGR) that he could demonstrate for his strains of S. natans was about 0.3 per hour. The medium on which this was achieved contained glucose, casamino acids, vitamin B_{12}, and salts. Optimum values of pH, temperature, and dissolved oxygen (DO) concentration were used and the experiments were carried out in batch culture. It was also found that the SGR of S. natans reduced substantially when peptone, ammonia, or nitrate was used as a nitrogen source in place of casamino acids, and when peptoses, polysaccharides, sugar alcohols, and Krebs' cycle intermediates replaced glucose. Deviations from optimum values of pH, temperature, and DO also reduced the SGR. Thus, it is apparent that, even under optimum conditions, S. natans grows slowly when compared to most other bacteria and should find difficulty in competing for substrate. Table 2 presents growth information for the common groups of bacteria.

However, despite unfavorably low SGR's, Sphaerotilus is able to use a wide variety of nitrogen and simple carbon

sources. Mulder and Van Veen (1963) demonstrated that Sphaerotilus required vitamin B_{12} but that this growth factor could be replaced by methionine. Lackey and Wattie (1940), Stokes (1954), and Hohnl (1955) were unaware of this growth factor requirement. Thus, the lack of growth which led them to report the unavailability of certain carbon and nitrogen sources may have been due to the lack of methionine or vitamin B_{12} in the media and not the compound being tested.

In addition to being able to metabolize many different compounds, Sphaerotilus also has the advantage of being able to grow at a wide range of temperatures and DO concentrations. Ruchhoft and Kachmar (1941), Stokes (1954), and Pipes (1965) all found that the optimum temperature for growth was 30°C but growth at 15°C was substantial. In their review Harrison and Heukelekian (1958) reported that Sphaerotilus grew well at 10°C. Results of studies suggesting that Sphaerotilus may not be affected by changes in DO as much as other organisms are given in a subsequent section of this chapter.

The nitrogen requirements of Sphaerotilus are comparatively low. Ruchhoft and Kachmar (1941) found that the mass yield per unit nitrogen assimilated by Sphaerotilus was four times higher than that for "zoogloeal bacteria" while Okrend and Dondero (1964) obtained cell nitrogen concentrations as low as 4.8% dry weight for Sphaerotilus. Thus, Sphaerotilus has the advantage over many organisms of being able to obtain large cell mass yields per unit of nitrogen consumed.

Table 2

Generation Times and Specific Growth Rates for Several Groups of Common Bacteria

Group	Normal Range of Generation Time	Corresponding Specific Growth Rate
coli-aerogenes	less than 20 minutes	2.1 $hours^{-1}$
Staphylococcus & Streptococcus	25 - 30 minutes	1.4 - 1.7 $hours^{-1}$
Pseudomonas	30 - 40 minutes	1.0 - 1.4 $hours^{-1}$
Corynebacterium	35 - 40 minutes	1.0 - 1.2 $hours^{-1}$

$$SGR = k \ln \frac{dx}{dt} = \frac{kx \text{ or } \ln x_2 - \ln x_1}{t} = k$$

when: x = number of microorganisms or total mass of microorganisms; t = time.

It is apparent from this discussion that, although Sphaerotilus is a relatively slow growing microorganism, its ability to grow at nutritional and environmental extremes makes it very versatile and increases the opportunity for its successful competition in a heterogeneous population.

There have been indications in the literature that this versatility has helped Sphaerotilus to flourish in activated sludge. Ingols and Heukelekian (1939 and 1940) found that on a synthetic waste containing a high concentration of glucose and very little nitrogen, Sphaerotilus grew more rapidly than "zoogloeal bacteria" isolated from activated sludge. Lackey and Wattie (1940) and Heukelekian and Weisberg (1956) observed that the addition of carbohydrate to sewage stimulated the growth of Sphaerotilus in activated sludge. Dondero (1961) reported that a carbon to nitrogen ratio greater than 8:1 favored the growth of Sphaerotilus over "zoogloeal bacteria" after Heukelekian (1941) observed the growth of Sphaerotilus in activated sludge having a very low DO concentration. It has also been stated that reduced temperatures have favored the growth of Sphaerotilus.

In addition to there being substrate available to Sphaerotilus under nutritional and environmental extremes, there are also indications that substrate becomes available when activated sludge is heavily loaded. Under these conditions, substrate is present in the mixed liquor for substantial periods of time; and Sphaerotilus, like the rest of

the population, is able to use it.

Ingols and Heukelekian (1940) found that poorly oxidized sludge removed substrate slowly and frequently became infested with Sphaerotilus, yet well oxidized sludge removed substrate more rapidly and was seldom overgrown with Sphaerotilus. Heukelekian (1941) observed that the addition of excessive substrate to activated sludge frequently stimulated the growth of Sphaerotilus. Okun (1949) reported that heavy loading had resulted in a bulking sludge, overgrown with Sphaerotilus.

Much has also been written about the correlation between the sludge loading rate (SLR) and the growth of Sphaerotilus in activated sludge. The indications are that, as the ratio of substrate to microorganisms as given by the SLR is increased, conditions of overloading exist and the growth of Sphaerotilus becomes more likely. The absolute value of SLR at which Sphaerotilus grows is not constant and is, to a large degree, a function of the substrate. This is shown in Table 3.

Settled Volume Studies

Theoretically, a biologically treatable waste introduced into an activated sludge process will form an optimum type of activated sludge. Whether or not the optimum sludge is developed, the formed sludge must separate from the created waste by sedimentation. This concept of settleability is a basic requirement of the process. In routine operating practice,

Table 3

Upper Limit of SLR to Prevent the Growth of *Sphaerotilus*

Author	SLR lb/day/lb	MLSS/MLVSS	Substrate
Logan and Budd (1956)	1.4	----	domestic sewage
Genetelli and Heukelekian (1962)	0.3 0.5	MLVSS MLVSS	glucose/NH_4 casein hydrolysate
Hawkes (1963)	0.5	MLSS	domestic sewage
Ford and Eckenfelder (1966)	1.0 0.9	MLSS MLSS	domestic sewage brewery waste
Pipes (1967, a)	0.3	MLVSS	domestic sewage

$$SLR = \frac{\text{lb. Biochemical Oxygen Demand (BOD) applied per day}}{\text{lb. Mixed Liquor Volatile Suspended Solids (MLVSS) under aeration}}$$

the only characteristics of the sludge itself that are measured are the concentration of solids in the aerating mixture of sludge and waste and the volume occupied by the sludge, after it has settled for a specified length of time. A few other characteristics of activated sludge have been measured during investigations of one aspect or another of the process. However, these measurements have not been adopted for operational control and very little progress has been made toward using any of them for describing the different types of activated sludge.

In his review concerning sludge density measurements, Mohlman (1934) stated that, very early in the history of the activated sludge process, it was realized that sludge volume measurements alone were not a satisfactory means of determining the rate of return sludge flow. It had been observed that the volume occupied by a fixed weight of sludge could fluctuate substantially and, therefore, concern was given to the development of sludge density measurements which would take into account both the volume and the concentration of the settled sludge. Several parameters developed independently; there was, therefore, a lack of uniformity in both the test procedures used and the units of expression. The more important of these parameters are summarized in Table 4.

Thus, to achieve uniformity, Mohlman undertook to examine the existing sludge density parameters in order to choose

Table 4

Activated Sludge Density Parameters

Originator	Year	Formula	Container	Testing Time	Name of Parameter
Theriault	1920	$\frac{\text{suspended solids (ppm)}}{\text{settled volume (cc)}}$	1000 ml graduate cylinder	15 min 30 min	sludge ratio
Donaldson	1932	$\frac{\text{suspended solids (\%)}}{\text{settled volume (\%)}}$	1000 ml graduate cylinder	30 min	sludge density index
Haseltine	1932	$\frac{\text{suspended solids (ppm)}}{\text{settled volume (\%)}}$	1000 ml graduate cylinder	30 min	sludge index
Rudolfs	1932	$\frac{\text{suspended solids (ppm)}}{\text{settled volume (\%)}}$	500 ml graduate cylinder	30 min	sludge index

the most useful of these and to recommend that the selected parameter be used by all observers. He stated that as the sludge swells up or bulks the density reduces; therefore, density measurements reduce. He felt that a more realistic measure should increase as the sludge bulks; thus, he suggested that the reciprocal of density measurements be used. He recommended as a standard the sludge volume index (SVI) expressed by

$$SVI = \frac{\text{settled volume (\%)}}{\text{suspended solids (\%)}}$$

and measured with a 1000 ml graduated cylinder using a 30 minute settling time. Although this measurement has become known as the Mohlman Index, it was actually developed previously.

Since the work of Mohlman (1934), the sludge volume index (SVI) measurement has been widely used. However, there exists some uncertainty as to the relationship between SVI measurements and conditions of bulking. Mohlman (1934) felt that sludges with an SVI near 200 were bulky; Eckenfelder and Melbinger (1957) reported that a sludge with an SVI near 50 was a normal sludge and one with an SVI near 200 was bulky. Cooke (1963) suggested that sludges with an SVI greater than 100 were bulky. This latter figure has frequently been used. It is difficult to say, however, that a sludge is bulking when it has an SVI of 101 and not bulking when its SVI is 99. Therefore, it is apparent that trends in the SVI of a sludge are more useful than single observations. The value

of a single observation is reduced even further when one considers that two different sludges which have the same SVI do not necessarily have the same settling properties, or cause the same problems.

For some time now, it has been known that sludge volume measurements, obtained by settling a sample of sludge in a graduated cylinder for a certain period of time, are subject to a high degree of fluctuation. As early as 1920, as Rudolfs and Lacy (1934) observed in their literature review, such variables as (1) vessel depth, diameter, and inclination, (2) sludge concentration, (3) sludge temperature, and (4) the degree of agitation could all affect the rate of settling and compaction of activated sludge. They reaffirmed these observations in their own laboratory.

Unfortunately, very little progress has been made in the development of models to predict the effects that the aforementioned variables have on the settling and compaction rates and hence the SVI of activated sludge. Thus, the user is left with a standard SVI measurement or some modification of it to detect conditions of activated sludge bulking.

Extracellular Physiology Studies

Experience has shown that the appearance of Sphaerotilus varies considerably with environmental conditions. In young cultures, filaments sometimes appear to be non-septate (Stokes,1954; Mulder and Van Veen,1963). Occasionally, a single sheath contains two rows of cells (Stokes, 1954).

False branching, caused by single rods slipping sideways and growing into new filaments, is common.

The sheath of Sphaerotilus natans is readily observed under the phase-contrast microscope or by negative staining with Indian Ink (Pringsheim, 1949, a). Romano (1961) and Romano and Peloquin (1963) determined the composition of the sheath of Sphaerotilus natans and found it similar to the cell walls of many bacteria with the exception that muramic acid was not detected. They described the sheath material as a protein-polysaccharide-lipid complex distinct from cell wall and slime layer materials. Upon hydrolysis with 2N HCL at 100°C, the purified sheath material liberated : reducing sugars - 36% of dry wt.; amino sugars - 11% of dry wt.; protein - 27% of dry wt.; lipid - 5.2% of dry wt. The nitrogen and phosphorus values were 7.6% and 0.5% of the dry wt. respectively.

Romano and Geason (1964), using immunological techniques, observed the pattern of sheath synthesis by means of fluorescent microscopy. They reacted Sphaerotilus natans grown for 9 hours in Stokes (1954) medium with auto-sheath antibody conjugated with fluorescein isothiocyanate, noting that the old sheath remained discretely labelled while new sheath (non-fluorescing) appeared at the ends of the filaments. Reversing the procedure resulted in fluorescence at the growing tips. From this, they concluded that sheath synthesis occurs by linear extension of existing sheath, rather

than by diffuse intercalation or intussusception. Curtis (1969) has described both a primary sheath, recognized only in young filaments, and a secondary sheath, formed by deposition of organic material.

The sheath is surrounded by a slime layer or capsule, the thickness of which varies with nutrient conditions. This capsule is polysaccharide in nature, containing fucose, glucose, galactose, and glucuronic acid in molar ratio of 1.0:0.77:0.80 (Gaudy and Wolfe, 1962). They indicate that capsule formation is a function of nitrogen concentration rather than the concentration of the carbon source: as the available organic nitrogen was increased, the production of capsule was stimulated and sheath formation inhibited. This, of course, has a direct effect on total growth, whether determined by weight or volume. Skerman (1959) suggested that capsular material may be somehow incorporated directly into the sheath. Such an occurrence seems possible because of the close relationship of capsule and cell wall and the similarities of their chemical composition.

A mechanism for the formation of the long filaments so characteristic of this organism has been suggested by Phaulp (1968). Swarming cells released from the filament are surrounded by an abundant, sticky, capsular material which may harden, forming hapteron and sheath. After lengthening from several cell divisions, the sheath is ruptured both mechanically by pressure and possibly with the assistance of

enzymes. Later, when pressure is reduced and enzymes removed, the capsule of cells present near the rupture hardens and fuses with the remnant of the original sheath. Further growth and division occur and sometimes the rupturing process is repeated several times until the long filament is produced.

In culture, this process occurs throughout the log growth phase and ceases in the period of decline and death. In a natural environment, it undoubtedly persists as long as nutrients are available and wastes removed. Cells released experimentally by mechanical disruption of filaments are also able to produce new filaments. Thus swarmers differ from other cells only in being actively motile, since all cells are identical in size, shape, and appearance.

Symons and McKinney (1958) found that in activated sludge at least a part of the polysaccharides is stored extracellularly. Washington and Symons (1962) reported an increase in biologically inert material in activated sludge supplied with glucose, acetic acid, or glycine. They did not define the chemical constituents of "biologically inert material." In the same experiment, they observed that the "biologically inert material" was depleted during endogenous respiration, while ammonia accumulated in the medium. These observations led them to conclude that the stored polymers are not true reserve compounds as long as organic nitrogen material within the cells is the true endogenous metabolite.

Many publications on substrate metabolism by either acclimated or unacclimated activated sludge show a similar conclusion. They all agree that only a part of assimilated substrate is incorporated into cellular protein, while the bulk of the remainder is accumulated as some intracellular polymerization product.

In contrast to the foregoing investigation, Wilkinson (1958) in his review on "The Extracellular Polysaccharides of Bacteria", stated that most bacteria are not likely to decompose their own extracellularly stored compounds. However, Macrae and Wilkinson (1958), Stanier et al. (1959), and Doudoroff and Stanier (1959) stated that the intracellularly accumulated polymers (starch, glycogen, lipids, PHB) are true endogenous metabolites.

Poly-β-Hydroxybutyric Acid

During investigations of the oxidative carbohydrate metabolism of various filamentous and nonfilamentous bacteria, it has been observed that many accumulated a large amount of sudanophilic granules, suggestive of poly-β-hydroxybutyric acid (PHB), an endogenous metabolite unique to certain bacteria (Lemoigne and Gerard, 1948; Doudoroff and Stanier, 1959; Forsyth et al., 1958; Rouf and Stokes, 1962; and Mulder and Van Veen, 1964). Lemoigne (1927), who isolated the polymer from cells of Bacillus megaterium, determined its empirical formula to be $(C_4H_6O_2)_n$. Examination of the PHB

granules isolated by Williamson and Wilkinson (1958) has shown that they are composed of about 90% poly-β-hydroxybutyrate and 10% of some other lipids. By isothermal distillation in chloroform, they estimated the molecular weight of the polymer to be about 5000 which suggests a chain length of about 60 residues.

The function of PHB in bacteria has been investigated by several researchers. In the oxidative assimilation of various carbon sources, from 60% to 90% of the carbon is initially accumulated within the cells as PHB. When these cells were incubated in the absence of an exogenous carbon source but in the presence of a nitrogen source and carbon dioxide, more than 90% of the polymer disappeared. Most of the polymer was redistributed into other cellular materials. Exhaustion of the nitrogen source in the presence of excess carbon and energy sources increased the accumulation of poly-β-hydroxybutyrate, when compared to carbon limited growth. Polymer formation has been inhibited by a high concentration of oxygen and no polymer synthesis will occur anaerobically. In the absence of carbon dioxide, it was found that stored polymer can serve as a carbon source and as an energy source.

The quantities of this polymer within the bacterial cell vary to a large extent with contents of up to 50% of the dry weight being reported. Studies by Macrae and Wilkinson (1958) have indicated that cells with a high content of polymer

are better able to withstand death and autolysis than those with a low polymer content.

When Crabtree et al. (1966) associated the accumulation of PHB by the floc-forming bacterium, Zoogloea ramigera, with the flocculation of this organism, the significance of polymer investigations in bacteria became more important to Sanitary Engineers. Not only did Crabtree et al. (1966) find that an accumulation of PHB preceded floc formation, but they also found that the PHB-rich cells, the isolated native polymer, and the purified polymer demonstrated an adhesive property. Floc formation was prevented by metabolic blocking of PHB synthesis and deflocculation resulted from endogenous dissimilation of PHB.

Recent work by Merrick and Doudoroff (1964) has shown that the initial stages of depolymerization are extremely complex and appear to be bound up with the structural integrity of the polymer particles. In their investigation, they found that extracts of Rhodospirillum rubrum, which degrade native polymer granules of Bacillus megaterium, consisted of three distinct fractions: a thermostable activator, a thermolabile depolymerase, and an esterase. Besides these constituents, there also appears to be a labile factor associated with the polymer granules of Bacillus megaterium. Destruction of this labile factor by various chemical or physical agents only reduces the extent of polymer digestion without affecting the rate of degradation. This suggests

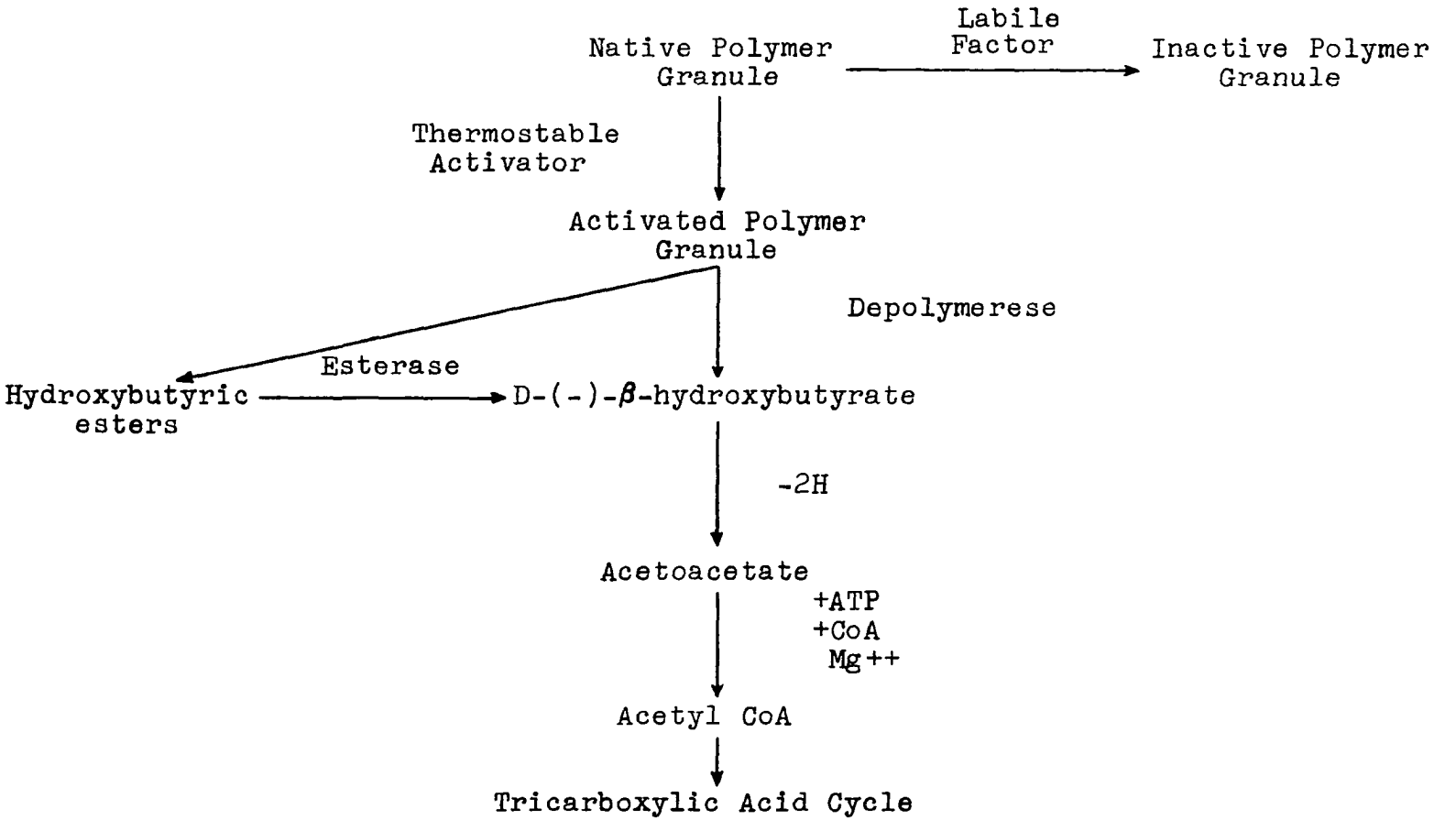

Figure 1. Pathway for Catabolism of Poly-β-Hydroxybutyric Acid.

that the hydrolysis of entire blocks of polymer molecules may be controlled by single sensitive units that are associated with them. A diagram for the pathway for polymer catabolism is shown in Figure 1. It would appear from the diagram that an organism which synthesized PHB as a storage product must have an active TCA cycle in order to utilize efficiently the acetyl CoA.

Dissolved Oxygen Studies

Reasonably early in the development of the activated sludge process, it was recognized that some dissolved oxygen concentration (DO) in the mixed liquor was required to maintain a population of aerobic microorganisms in the sludge and low DO has been suggested as a cause of filamentous bulking a number of times. Increasing aeration rate has solved bulking problems in actual process operation (Anderson, 1936), but most of the experimental work on the effect of low DO on activated sludge has been done in the laboratory.

The relationship between the mixed liquor DO and the settling characteristics of the sludge has been investigated by a number of authors. Laboratory studies indicate that Sphaerotilus natans is an aerobe (Lackey and Wattie, 1940), but its minimal requirement for oxygen may not be high. Heukelekian and Ingols (1940) were strongly convinced that bulking, produced in their laboratory activated sludge cultures fed on domestic sewage, was due to low mixed liquor DO. Later (Ingols and Heukelekian, 1940) reported that

filamentous bulking caused by feeding sugar to their cultures could be overcome by increasing the aeration rate. Okun (1949) tried aerating activated sludge cultures with pure oxygen instead of air and observed that a high DO in the mixed liquor prevented the development of filamentous growths. However, Orford et al. (1960) analyzed data from a very extensive set of laboratory activated sludge cultures and could find no statistically significant correlation between mixed liquor DO and the settling characteristics of the sludge.

Pipes (1965) found growth progressed most rapidly with at least 6.0 ppm DO at the end of the aeration period. Ruchhoft and Kachmar (1941) reported this as well. In addition they found that Sphaerotilus natans can grow and develop appreciably in substrates containing very low quantities, 0.1 - 2.0 ppm DO. It was considered significant that in a good medium the Sphaerotilus could produce up to 598 ppm solids and utilize as much as 600 - 700 ppm glucose at the low rates of aeration that were required to maintain the low DO values. These investigators also showed that Sphaerotilus natans was unable to grow in synthetic sewage medium in the absence of oxygen. Smit (1934) and Stokes (1954) both reported that Sphaerotilus could survive complete absence of dissolved oxygen for several weeks, although, of course, no growth occurred under these conditions. Stokes (1954) reported no growth in anaerobic cultures with media containing

0.2 per cent potassium nitrate. He concluded that nitrate cannot replace oxygen in the metabolism of Sphaerotilus natans. However, all of the strains of Sphaerotilus natans used by Stokes (1954) did reduce nitrate to nitrite.

The Water Pollution Research Laboratory (WPRL) in the United Kingdom (Ministry of Technology, 1966) reported that oxygen uptake of river slimes in which Sphaerotilus was dominant was recorded as between 13.5 and 71.5 mg/g dry weight of slime/hour. Pure cultures had an average oxygen uptake of 33.3 mg/g/hr at 25 C. Similar results with laboratory cultures of Sphaerotilus were obtained by Stokes (1954). Data from WPRL (Ministry of Technology, 1966) indicated that the oxygen comsumption of Sphaerotilus slimes was ten to twenty times greater per unit dry weight than that of the normally occurring aquatic macrophytes.

McKinney (1953; 1956) found that flocs of pure culture bacteria did not result until the bacteria were in an endogenous phase of metabolism where oxygen uptake is low. McKinney (1956) reported that addition of fresh substrate to recently flocculated bacteria resulted in the dispersion of the cells as a result of increased activity.

Mulder (1964) investigated the role of Sphaerotilus natans in bulking activated sludge. He suggested that the development of large flocs of Sphaerotilus natans under relatively low oxygen conditions is a primary cause of bulking and leads to even greater oxygen deficiency. In his

experiment, strains of Sphaerotilus natans and Arthrobacter globiformis, an aerobic corynebacterium isolated from activated sludge, were grown separately. A number of the cultures were heavily aerated on a vibrating shaker; the others were not agitated, which resulted in a low oxygen supply. Sludge Growth Rate (SGR) was measured by estimating cell yields after various intervals of time. The well-aerated cultures of each organism had SGR's in the same order of magnitude. However, under quiescent conditions of low DO, the SGR of Arthrobacter reduced to almost zero, while the SGR of Sphaerotilus showed only a slight reduction. Thus, changes in the concentration of DO may not affect Sphaerotilus as much as other organisms, thereby resulting in an accumulation of Sphaerotilus natans.

Composition of Suspended Solids

The mixed liquor suspended solids (MLSS) and the mixed liquor volatile suspended solids (MLVSS) are presently the most common methods of obtaining the total concentration of suspended solids and the microorganism content, respectively, in an activated sludge plant. These tests do not determine specific chemical substances, but rather they determine classes of material which have similar physical properties and similar responses to ignition. The MLVSS actually measures living and dead microorganisms and non-biological volatile solids. It is, therefore, necessary to distinguish quantitatively the living and non-living cell mass from the

inert solids.

The cell mass can be quantified by measuring the deoxyribonucleic acid (DNA) content. Although many procedures are available, the Schmidt and Tannhauser procedure followed by the Dische Diphenylamine Method is one of the most commonly used extraction procedures (Agardy and Shepherd, 1965; Schmidt, 1957; DeDeken-Grenson and DeDeken, 1959; Dische, 1955). This procedure uses cold acid extraction to precipitate high molecular weight polysaccharides, proteins, and nucleic acids. It then utilizes the resistance of DNA to alkaline digestion to separate the DNA from the ribonucleic acid (RNA). The Dische Diphenylamine reaction separates the UV- absorbing, non-nucleic acid material from the pure DNA. By employing the appropriate factors, the living and non-living cell mass can be determined.

At this point, it is necessary to distinguish the living cells from the non-living cell mass. The fraction of active cells can be evaluated by measuring dehydrogenases activity of the sludge sample. Preliminary work using the determination of dehydrogenase activity was begun by Lenhard (1956), Lenhard and Nourse (1964), and Bucksteeg and Thiele (1959). Although a better measure of activity could be obtained by determining the respiration rate of the sludge by the Warburg technique, this procedure requires specialized equipment and is not suited to serial analysis. Dehydrogenase activity, on the other hand, is both reliable and useful as a routine

method of measuring biological activity.

The various dehydrogenases are key enzymes in the biological oxidation of organic compounds. The activity of the various dehyrdogenases is, therefore, a good measurement of biological activity. This activity may be easily measured by using a tetrazolium salt, triphenyltetrazolium chloride (TCC), as the hydrogen acceptor; this couples the oxidation of the substrate to the reduction of this colorless salt to a red triphenylformazan (TF). The intensity of the red color is taken as a measure of the relative dehydrogenase activity. A transfer mechanism has been suggested by Ford et al. (1966). In this mechanism, the dehydrogenase enzymes catalyze the removal of hydrogen atoms from the organic substrate, and most of these enzymes have associated coenzymes which serve as temporary hydrogen acceptors. Each dehydrogenase is usually quite specific, not only to its organic substrate, but also to its coenzyme (Stanier et al., 1963). By applying the appropriate factors to the intensity of color development, the total active cell mass can be evaluated.

From these calculations, it is possible to determine the concentration in mg/l of the following: total suspended solids, fixed inorganic suspended solids, organic volatile suspended solids, inert suspended solids, total cell mass, inactive cell mass, and active cell mass. The flowsheet for these determinations is given in Figure 2.

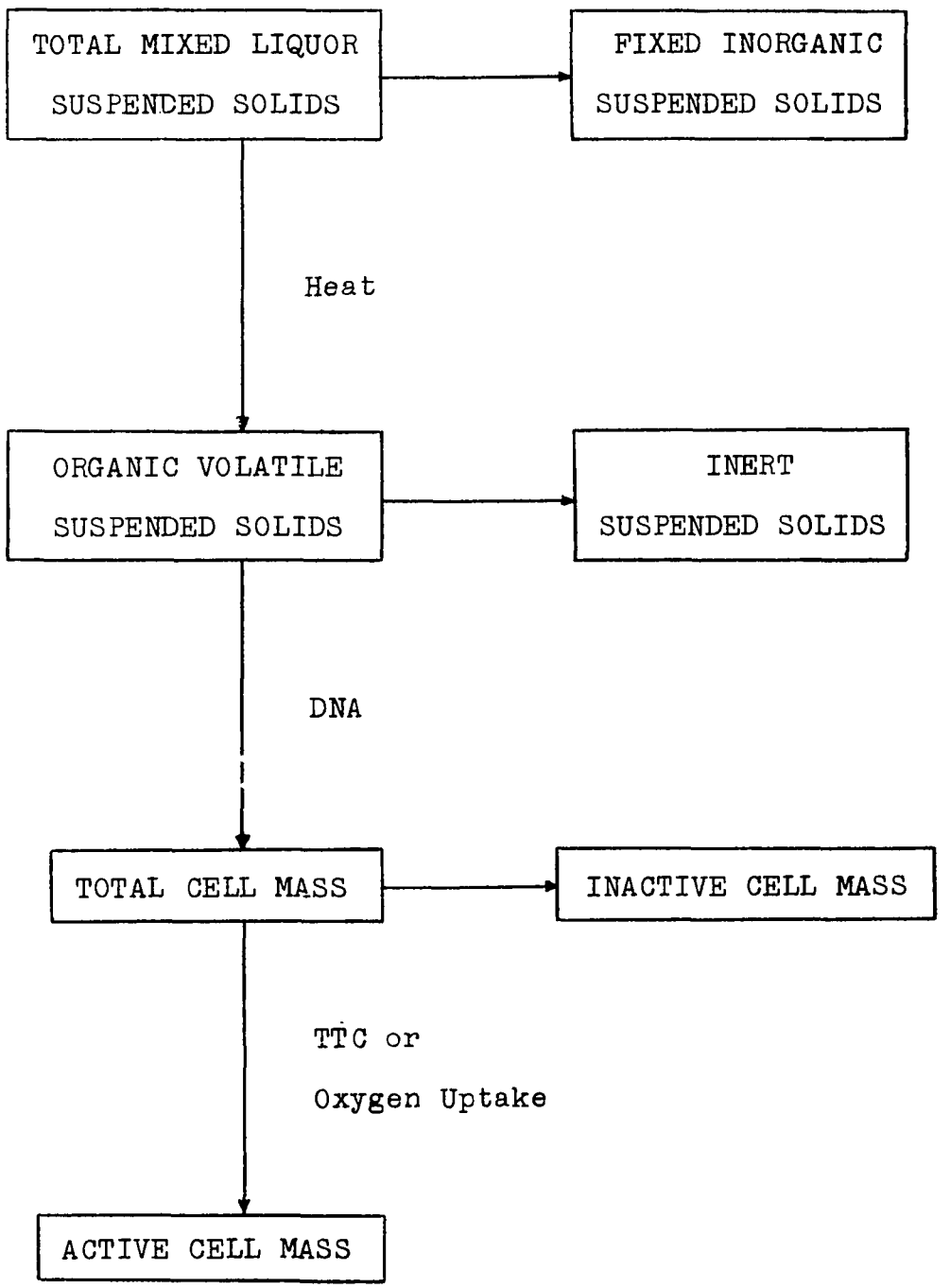

Figure 2. Flowsheet for Evaluation of Suspended Solids Composition

Causes of Filamentous Bulking

This section will be concerned primarily with those reports which were written specifically about bulking. Those reports dealing with other settling disorders, such as deflocculation and denitrification, will be neglected. Although somewhat diverse conditions are reported to cause filamentous bulking, these conditions have been grouped, in this discussion, into the two following categories: causes associated with operational conditions; causes associated with the character of raw sewage.

Operational Conditions

Among those conditions which are considered as inducements to activated sludge bulking, the sludge overloading, or addition of excessive amounts of substrate to the sludge, has been reported most frequently. Much of the information written on this subject is summarized and discussed in Table 5.

This information shows that conditions of excess substrate, which would result because of sludge overloading or reduced activity of a poorly oxidized sludge, are conducive to the growth of filamentous organisms.

The amount of aeration applied is also frequently mentioned as an aspect of the aeration tanks which causes bulking. Hoyle (1927) reported that bulking could be caused by both too little and too much aeration. Agersborg and

Table 5

The Overloading of Activated Sludge as a Cause of Bulking

Name	Summary Statement
Clifford and Windridge (1932)	Overloading with domestic sewage caused filamentous bulking.
Donaldson (1932)	Variations in organic loading caused filamentous bulking.
Haseltine (1932)	Overloading the sludge with domestic sewage of a high BOD resulted in a poorly oxidized sludge which settled poorly. It was theorized that too much absorption and not enough oxidation was reason for the growth of filaments in activated sludge.
Smit (1932)	Overloading with domestic sewage caused increases in the amount of filamentous microorganisms and reductions in the density of the settled sludge.
Ruchhoft and Smith (1939)	As the food to microorganisms ratio was increased, the density of the settled sludge reduced. Domestic sewage was used.
Pearse (1942)	Bulking is due primarily to the overloading of activated sludge.
Okun (1949)	Heavy loading of activated sludge caused filamentous bulking.
Genetelli and Heukelekian (1962)	It was found that as the sludge loading rate increased, filamentous bulking occurred. Different synthetic substrates were used and it was observed

Table 5 (continued)

	that the sludge loading rate at which bulking occurred was dependent upon the substrate.
Ford and Eckenfelder (1966)	They subjected their activated sludge to a high sludge loading rate and observed the initiation of filaments in the sludge. Domestic sewage and brewery waste were used as substrate.
Pipes (1967)	It was reported that high sludge loading rates caused a poorly oxidized sludge and a greater opportunity for the growth of filamentous organisms.

Hatfield (1929) felt that septic conditions in the aeration tanks, which were a result of faulty aeration, caused the growth of filamentous organisms in the sludge. Hatfield (1931) again made a similar observation. Donaldson (1932) noted that, short of creating septic conditions, reductions in aeration also caused filamentous organisms to grow in the sludge. Cases of filamentous bulking, apparently resulting from a dissolved oxygen concentration of less than 1.0 ppm in the aeration tanks, were cited by Ingols and Heukelekian (1940), Heukelekian (1941), and Okun (1949).

Smit (1934) and Pipes (1967, a) both felt that some controversy existed about the importance of low dissolved oxygen concentration as a cause of filamentous bulking. They stated that sludge bulking could not always be caused simply be reducing the dissolved oxygen concentration in the aeration tank. However, since overloading the sludge can reduce the dissolved oxygen concentration in the aeration tank, the possibility does exist that both sludge overload and low dissolved oxygen could be present at the same time and exert their effect together.

Another cause of activated sludge bulking, which has been considered, is that of the hydraulic regime of the aeration basin. Donaldson (1932) suggested that short circuiting through the tank could cause bulking. McKee and Fair (1942) stated that step aeration resulted in the more rapid growth of filamentous organisms than did the conventional operation.

An additional problem cited is the concentration of suspended solids carried in the aeration tank. Heukelekian and Ingols (1940) and Genetelli and Heukelekian (1964) stated that the higher the concentration of mixed liquor suspended solids, the greater the tendency toward bulking.

Characteristics of the Raw Sewage

Excessive amounts of readily useable carbonaceous material in the sewage, material such as carbohydrate or fat, has been cited frequently as a cause of filamentous bulking of activated sludge. Scot (1928) noted that the type of substrate was important in evaluating the occurence of bulking; he also observed that the wastes which contained large amounts of carbohydrates such as milk, brewery, and starch processing wastes were especially conducive to the growth of filamentous organisms in the sludge. Morgan and Beck (1928) found severe bulking at the Des Plaines, Illinois, sewage treatment plant. They reported that it had been caused by large amounts of carbohydrates discharged into the sewer from illegally operated distilleries.

Agersborg and Hatfield (1929) reported that sewage containing large amounts of carbohydrate had resulted in a bulky activated sludge. Smit (1932) felt that bulking resulted primarily from trade wastes, since he did not consider the concentration of carbohydrates in domestic sewage sufficiently high to cause bulking. Ingols and Heukelekian (1939 and 1940) as well as Sawyer (1940) reported that fats

carbohydrates, and many other materials, which would create a high useable carbon to nitrogen ratio in the sewage, were conducive to activated sludge bulking. Kraus (1945) reported another case of bulking caused by brewery wastes.

Genetelli and Heukelekian (1962) conducted a comparison between different substrates, using as a basis their effect on bulking. They observed that, while keeping the loading rate constant at various levels, those substrates containing large concentrations of carbohydrates caused the activated sludge to bulk at a lower sludge loading rate than did the substrates containing large concentrations of protein.

Another factor reported as related to bulking is that of the oxidation-reduction potential of the sewage. Morgan et al. (1936) noted that periods of low flow caused the sewage to remain in the sewers for long periods and to become septic, resulting in the growth of filamentous organisms in their plant. They found that flushing the sewers frequently during periods of low flow alleviated the condition. Pipes (1967, a) reported that filamentous bulking could be caused both by septic sewage and the addition of digester supernatant to fresh sewage. Ullrich and Smith (1952) found similar results when digester supernatant was added.

Smit (1934) and Heukelekian (1940) observed that raw sewage at either very high or very low temperature could cause bulking. However, Pipes (1967, a) felt that the effect

of raw sewage temperature on bulking, at either extreme, was not well established.

Altogether, a great variety of conditions has been reported as a cause of filamentous bulking. One common factor which links them together is that they all represent extreme conditions. The filamentous organisms, which are not normally a problem, seem to have an advantage resulting from these extremes.

Control of Filamentous Bulking

The variability in the success of measures to control activated sludge bulking is surprising. The use of return sludge chlorination as a means of controlling activated sludge bulking has had almost as many failures as successes. In fact, its success was so variable that in 1933, the Committee on Sewage Disposal of the American Public Health Association felt that its continued use as a control measure was not warranted. Explanations for this variability have not been forthcoming. However, what may be the key to the problem has been suggested in the work of Smith and Purdy (1936) and of Tapelshay (1945). They believed that many different "fungi" could be involved in bulking and that the effectiveness of a control measure was to some degree a function of the "fungus." In a previous section, it was demonstrated that different filamentous microorganisms can be found in bulking sludges. Thus, it appears necessary to apply control measures tailored to be effective against a

specific filamentous microorganism.

The following review of literature attempts to list most of the methods which have been used to control filamentous bulking and to report on the success that each has experienced.

Chlorination

As an attempt to control bulking, chlorine has been applied to both raw sewage and to the return sludge. De Laporte (1926, taken from Chamberlin, 1948) and Bell (1929, taken from Chamberlin, 1948) discovered that the addition of bleaching powder to raw sewage helped to clarify the effluent from their activated sludge plant and to improve the settleability of their sludge. Hudson (1938) stated that he had encountered a severe bulking problem which had been eliminated by the chlorination of the raw sewage. On the other hand, Morgan and Beck (1928) and Anderson (1936) reported that the filamentous bulking problem on which they had been working was not affected by the chlorination of the raw sewage.

Bell (1929) and Cascoigne (1931) noted that the settleability of bulking activated sludge was improved by the addition of bleaching powder to return sludge. Smith (1935), Purdy and Smith (1936), and Tapelshay (1945) all found that the settling characteristics of the bulking sludges improved with the chlorination of the return sludge. Adamse (1966) reported the same findings; he felt that the means by which the chlorine acted was the preferential killing of the

superficial microorganisms, the filamentous organisms included, leaving the subsurface microorganisms viable to accomplish purification. However, Morgan et al. (1936) found that chlorinating the return sludge had no effect on the sludge volume index of a sludge which was highly filamentous. Ridenour (1937) reported the same results. Heukelekian and Weisberg (1956) reported that the chlorination of the return sludge had no effect on filamentous bulking although it was a successful control against zoogloeal bulking.

Return Sludge Reaeration

Haseltine (1932) used his own theory that conditions of filamentous bulking were caused by too little oxidation and too much adsorption by the sludge; he reaerated his return sludge and found that the amount of filamentous growth reduced and the settleability of the sludge improved. However, Morgan et al. (1936) found that sludge reaeration did not control the bulking conditions which they had encountered. Kraus (1945) noted the same results.

Haseltine (1961), in a recent review, reported that the practice of sludge reaeration has, since its beginning in 1917, had variable success as a control for activated sludge bulking. On the whole he feels that, in the majority of cases, it has been successful in reducing the sludge volume index to some degree.

Regulation of Sludge Loading

One successful measure to control filamentous bulking has frequently been that of preventing activated sludge from becoming overloaded. Donaldson (1932) reported that it was helpful to eliminate high peak loads as a means of preventing bulking. Ruchhoft and Smith (1939), Logan and Budd (1956), and Hawkes (1963) reported that maintenance of the sludge loading rate at below a certain value can vastly reduce the incidence of filamentous bulking in the treatment of domestic sewage. This value ranged from 0.3 per day to 0.5 per day. Pipes (1967,b) shows, from recent work, that filamentous bulking can occur even at sludge rates below 0.3 per day.

Some cases of filamentous bulking have been successfully controlled by changing the nature of the raw sewage so as to eliminate carbonaceous material or to reduce the carbon to nitrogen ratio of the sewage. Morgan and Beck (1928) and Scott (1928) noted that the removal of a carbohydrate source from the sewage eliminated their problem of filamentous bulking. Adamse (1966) found that he was able to improve the sludge by reducing the carbon to nitrogen ratio of dairy waste, through the addition of ammonia; thus, floc-forming organisms were induced to grow in the place of filamentous organisms.

Other Methods

Kraus (1945 and 1946) developed a system in which he

added digester effluent to the return sludge reaeration unit of the plant. This, he found, reduced a problem of filamentous bulking which had developed during the treatment of a brewery waste. He found a double effect in this system, in that the digester sludge increased the specific gravity of the sludge, improving its settleability; meanwhile, the nitrogen in the digester supernate helped to reduce the high ratio of carbon to nitrogen in the waste. Kraus (1946) and Ullrich and Smith (1957) reported that, although the addition of digester effluent to the aeration tanks tended to induce bulking, the addition of the same digester effluent to the return sludge reaeration tanks worked to prevent bulking.

Donaldson (1932) has suggested that filamentous bulking could be controlled by adjustments in the amount of aeration of activated sludge. Smith (1935) reported, however, that fluctuating the amount of air supplied to his highly filamentous sludge did nothing to improve its settleability.

Roe (1957) concluded, from a review of literature concerning the preaeration of sewage, that it was generally successful in the reduction of the sludge volume index of the sludge. Kraus (1945), on the contrary, said that the preaeration of sewage was not always successful in preventing bulking.

Morgan et al. (1936) were able to eliminate a case of filamentous bulking by flushing sewers during periods of

low flow, thus preventing the sewage from becoming septic.

Ford and Eckenfelder (1966) found that they reduced the number of filaments in their sludge and improved its settleability by holding their return sludge anaerobically for short periods of time.

Operating with Bulking Sludge

Scott (1928) noted that filamentous sludge was usually very efficient in the purification of sewage. He felt, therefore, that it would be desirable to work with such a sludge, if it could be harvested after purification was completed. Pipes (1967, a) stated this idea also; he suggested that it might be possible to operate with such sludge if there were extra capacity in final sedimentation tanks and return sludge pumps.

Many times there have been attempts to use chemicals to improve the settling characteristics of bulking sludge. Unlike the measures described previously, these methods do not attempt to destroy the filamentous organisms; they attempt to provide some measure of temporary relief from settling problems. Hoyle (1927) reported the successful use of humus, a mixture of soil, lime, and water, to relieve a bulking problem. Morgan and Beck (1928) and Donaldson (1932) reported the addition of lime to a highly filamentous sludge resulted in improved settleability. Work has been done recently using polyelectrolytes to permit harvesting of bulking sludges. Singer et al. (1965) reported the greatly improved

settleability of bulking sludges as a result of cationic polyelectrolytes; they operated a batch aeration system. Jones (1966) reported the first successful continuous use of polyelectrolytes. He found that the addition of small amounts of polyelectrolyte maintained a low SVI without impairing the purification properties of his highly filamentous sludge.

CHAPTER III

MATERIALS AND METHODS

Introduction

There are a number of different physical and chemical conditions that have been associated with the bulking of activated sludge. Yet, most common of any condition associated with activated sludge bulking is the presence of Sphaerotilus natans. This bacterium is a slow growing organism compared to other organisms found in activated sludge systems. Nevertheless, it has the ability to grow at nutritional and environmental extremes which make it very versatile and increase the opportunity for its successful competition in a heterogeneous population.

The major concern of this study was to investigate the growth characteristics of S. natans in a pure culture, in a mixed culture in signfiicant numbers, and in a mixed culture in small numbers. Various nutritional and environmental extremes were imposed on the cultures and a number of parameters were used to describe the resulting growth responses. The most important parameters included the settled volume of the sludge, the accumulation of the polymer, poly-β-hydorxybutyric acid, the rate of oxygen uptake, the carbon to

nitrogen ratio, the concentration of acids, and the composition of the suspended solids. Another study will examine the sheath/slime layer for adhesive properties.

Laboratory Systems

Both continuous-feed and batch-fed aeration units were used in this study. Five pairs of batch-fed aeration systems were operated to supplement information derived from the continuous aeration systems. Although more complicated to operate, continuous systems were favored over batch systems as they better reflected the dynamic state of the natural population in its environment.

The continuous-feed aeration system selected was a New Brunswick Scientific Modular Microferm Bench Top Fermenter, Model MF-114. This apparatus is shown in Figure 3. It served as a completely mixed activated sludge system. Dual units, each with 14 liter volume, were employed throughout the test period. The 14 liter reactor was equiped with a hollow baffle heat exchanger for temperature control. This baffle assembly was also utilized for the introduction of filtered air through a single-orifice air sparger. The reacter also contained a turbine impeller, whose spped range was 100 to 800 rpm, for mixing. The head plate had provisions for inlet and outlet waste lines, sampling lines, inoculation ports, and a sludge return line. The entire reactor assembly was autoclavable.

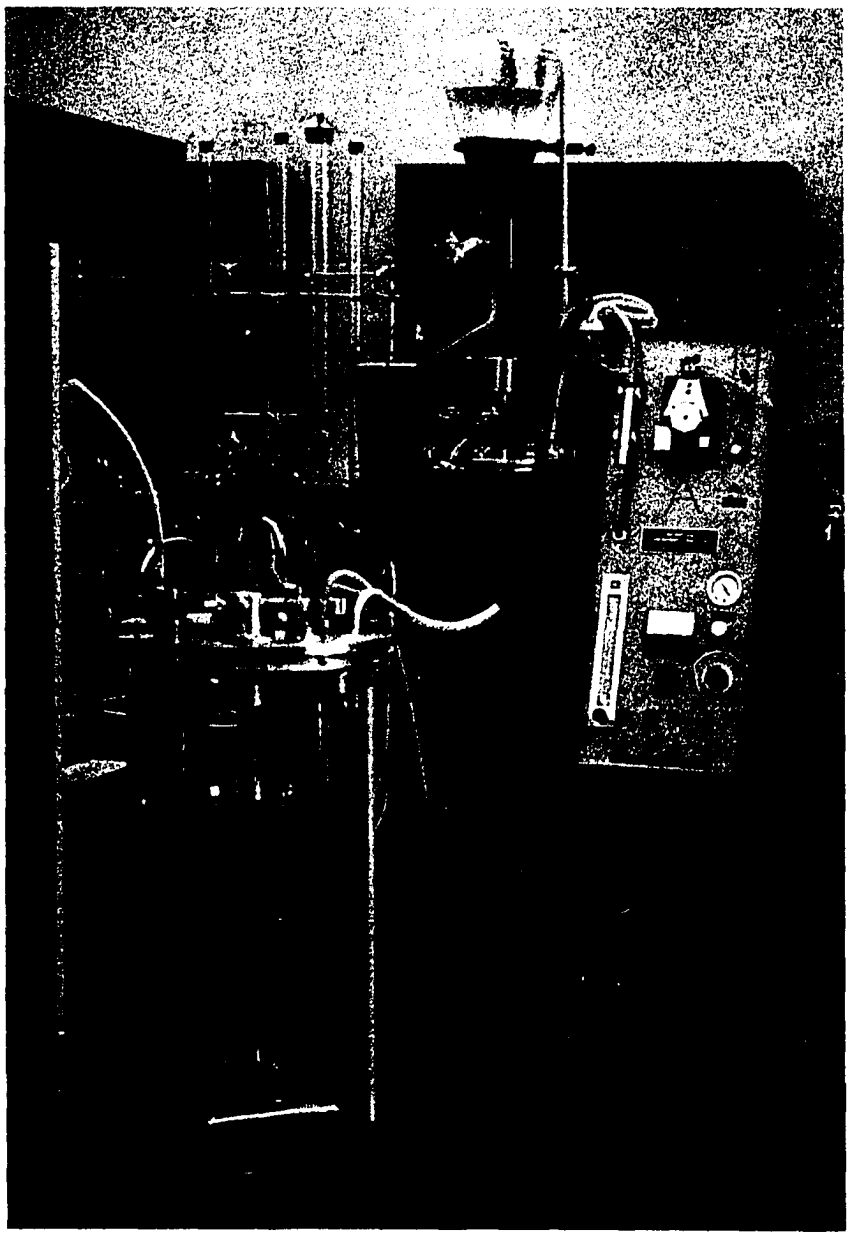

Figure 3. The activated sludge process for ecological studies of S. natans.

Synthetic wastes were pumped from 16 liter Nalgene autoclavable bottles by Sigmamotor Model T-8 peristaltic pumps through autoclavable tubing into the inlet port of the reactor. The effluent from the reactor was forced by air pressure out the waste port into a final sedimentation unit, which was located immediately above the reactor. The final sedimentation units consisted of 4 liter filtering flasks equiped with inlet and outlet lines, a sludge-return line and a sludge-removal line. Within this unit the solids separated from the liquid leaving a clarified effluent to be removed.

The dissolved oxygen (DO) concentration in the mixed liquor was the oxygen source for the sludge organisms during sedimentation and return to the aeration tank. Due to the sedimentation unit's design, it was quite likely that the settled sludge was exposed to anaerobic conditions for times long enough to alter the sludge population. Therefore, in order to avoid altering the reactor population, no settled sludge was returned.

One continuous-feed aeration system was operated under rigid asceptic controls with a pure culture of S. natans. Another aeration system, identical to the first, was operated with a mixed culture of Sphaerotilus. It was possible, then, to compare the growth characteristics of S. natans growing in isolation to its characteristics when it was forced to compete in a heterogeneous environment. By imposing similar

nutritional and environmental extremes on each system and by monitoring a number of parameters, it was possible to determine the relationships existing between the settling characteristics of the sludge and each parameter. By determining which parameters favored a good settling sludge and which favored a poor settling sludge, it follows that good plant operation and solutions to bulking should result from an optimization of those parameters which favor a good settling sludge.

The system was operated under several loading conditions. The lower limit was almost 0.2 lbs of COD applied/day/lbs MLVSS and the upper limit exceeded 1.0 lbs of COD applied/day/lbs MLVSS. Daily reactor conditions were as follows: rate of mixing varied from 100 to 120 rpm; temperature was held from 24-25°C; flow rate into the system was 4 liter/day; air flow rate ranged from 2-3 liter/min.

Generally, the reactor was sampled once before and at several time intervals after a new waste concentration was introduced. A minimum volume of sample was withdrawn from each unit at each sampling time. One thousand ml was allowed to settle in 1000 ml graduated cylinders for 60 min. Following the settled volume test, oxygen uptake and all other analytical determinations were conducted.

In the batch-fed investigation, the aeration units were similar to those described by Symons (1960). Each unit had a capacity of approximately 3 liters. Porous stone diffusers were used to introduce air or gas into each unit. The air

flow rate was approximately 1000 ml/min.

Each of five pairs of aeration systems consisted of one unit operated with a mixed culture of S. natans in which Sphaerotilus was not dominant and a second unit in which Sphaerotilus was dominant. By operating one pair at moderate pH, high oxygen tension and non-limiting nitrogen content, and the other four pairs at high pH, low pH, low oxygen tension, and limiting nitrogen content, it was possible to compare the resulting growth of the filamentous-present sludge to the filamentous-dominant sludge. Those conditions which favor the growth of filamentous organisms will also tend to result in poor plant operation and filamentous bulking.

Organisms Studied

Sphaerotilus natans ATCC 15291, acquired from the American Type Culture Collection in Washington, D. C., was used as the source of this organism throughout these studies, since it is the type species and has been widely studied.

A heterogeneous population of other organisms were acquired from area activated sludge treatment systems. Two different activated sludge systems consisting of different biological populations were used. Continuous aeration studies were conducted with mixed liquor samples from the completely-mixed aeration tank of the Moore, Oklahoma, Sewage Treatment System. The biological population consisted of small numbers of filamentous bacteria. S. natans appeared

to be the dominant filamentous form, although other filamentous organisms, including Geotrichum, were present. In the continuous investigation, one aeration system consisted of S. natans in pure culture, while the other aeration system contained a mixture of S. natans and the Moore mixed liquor sample. Nearly three grams of S. natans was added to achieve a S. natans:total bacteria ratio of approximately 1:10.

Batch aeration studies were conducted with mixed liquor samples from the conventional aeration tank of the Northside Oklahoma City, Oklahoma, Sewage Treatment System. This biological population consisted of very few filamentous organisms. In small numbers, Sphaerotilus, Geotrichum, Bacillus, and Actinomycetes were present. No one filamentous form was dominant. The batch study consisted of 10 aeration units. Five units contained the Oklahoma City mixed liquor sample and five other units consisted of a mixture of S. natans and the Oklahoma City mixed liquor sample. More than 1500 mg S. natans was added to achieve a S. natans:total bacteria ratio of approximately 1:2, and thus, assure that filamentous bacteria were the predominant bacterial type.

Culture Media

Unless otherwise stated all chemicals used were reagent grade and the amounts of all ingredients used are expressed as grams per liter (g/l) or milligrams per liter (mg/l). Where feasible, stock solutions of carbon sources were

prepared with distilled water and autoclaved separately. Dextrose, when autoclaved in the presence of other nutrients at high pH, has a tendency to partially oxidize. When required, the final pH of the medium was adjusted with autoclaved solutions of 1 N HCl or NaOH , depending on the direction of pH shift desired. All stock cultures of S. natans were maintained on the medium listed in Table 6. This medium is referred to as ASMTT medium and has a final pH of 7.4.

A starter culture of S. natans was inoculated from a three day agar slant or plate. This was accomplished aseptically by cutting out a small piece of agar containing a mass of cells and transferring the cells to 100 ml of liquid medium in a 250 ml Erlemeyer flask. This was necessary because Sphaerotilus filaments actually grow into agar and are not easily removed unless some agar is also removed. The "active" culture technique was used throughout this investigation. This method involved activation of the inoculum by periodic transfer into fresh medium. Routinely, three such transfers were made before inoculating the final culture. Final cultures to be used for experiments were harvested in the upper portion of the logarithmic growth phase. The period of growth necessary to attain this phase for S. natans 15291 was 18-24 hours.

Once the aeration systems were inoculated, they were fed continuously with a synthetic medium prepared from stock solutions as given in Table 7. The COD exerted by this 10 strength

TABLE 6

Composition of Medium Stock Cultures of S. natans

Ingredient	Composition (g/l)
Glucose (autoclaved separately)	2.5
Tryptone	2.5
K_2HPO_4	0.1
$MgSO_4$	0.1
Tris Buffer	0.3
$MgCl_2$	0.08
$CaCl_2$	0.05
NaEDTA	0.01
$FeCl_3.6H_2O$	0.01
$MnCl_2.4H_2O$	0.0014
$ZnCl_2$	0.00011
Na_2MoO_4	0.00005
$CoCl_2$	0.000005
$CuCl_2$	0.00000004
Agar	15.0
Distilled water	

TABLE 7

Composition of Synthetic Sewage Fed to Continuous Aeration Systems

Ingredient	10 Strength Composition (g/l)	100 Strength Composition (g/l)
Dextrose	75.4	454.0
Nutrient Broth	48.0	227.0
KNO_3	28.3	84.9
NaCl	28.0	84.0
K_2HPO_4	50.8	152.4
$MgSO_4$	8.0	24.0

synthetic sewage is 128,000 mg/l. For each waste loading, the appropriate number of milliliters of stock solution were diluted, resulting in 16 liters of synthetic waste with the desired COD. The desired COD was calculated from the loading velocity equation. The loading velocity is calculated as a ratio of the product of the influent COD and influent flow rate to the product of the MLVSS, the aeration tank volume, and the average detention time in the aeration tank. If a loading velocity is selected and all other parameters are known, the influent COD, and thus, the number of milliliters of stock solution required, can be easily calculated.

In batch studies, the sludge was fed once a day with a synthetic medium consisting of 565 mg dextrose, 180 mg nutrient broth, 50 mg KNO_3, 50 mg NaCl, 100 mg K_2HPO_4, and 10 mg $MgSO_4$ per liter. Other media, similar in composition and concentration to this feed, have been shown successful in promoting filamentous development and causing sludge bulking (Lackey and Wattie, 1940; Jones, 1966). This medium has a final pH of 7.5 and units involved in the low oxygen study, were operated on this feed directly. Two other pairs of aeration systems were operated on this feed, but the pH was adjusted to 4 and 10, respectively, by means described earlier. The pair of aeration systems involved in the limiting nitrogen study were fed a medium consisting of 565 mg dextrose, 50 mg NaCl, 100 mg K_2HPO_4, and 10 mg $MgSO_4$ per liter.

Settled Volume Studies

In research involving solids that will not settle, the most important parameter is one which describes sludge settleability. As listed in Table 4, there are a number of parameters that have been used to describe sludge settleability. In this study, the sludge volume index (SVI) has been used because of its simplicity and application in problems involving bulking sludges. The SVI is calculated by dividing the volume occupied by the sludge after settling 30 minutes, the sludge volume (SV), by the concentration of suspended solids in the mixture of waste and sludge, the mixed liquor suspended solids (MLSS), giving SVI in units of milliliters per gram (ml/g).

Quantitative measurements of bulking utilize fully the SVI parameter because bulking sludge usually settles to some extent. Sometimes there is a distinct demarcation between the sludge and the supernatant and sometimes the sludge line is quite hard to locate. The supernatant may have varying amounts of turbidity and it may contain suspended solids large enough to be seen as individual particles.

The assumptions made in using the SVI as a measure of sludge settleability are that the sludge settles as a mass not as individual particles, that there is a differentiation between the sludge and supernatant, and that the sludge remains settled for several hours. Under these assumptions, the SVI is a useful parameter.

Assuming that there must be a differentiation between the sludge and supernatant and realizing that this sludge line is often quite hard to locate, a maximum 30 minute settled volume of 900ml/l was selected. No restrictions were made on minimum settled volume. In order to obtain additional settling characteristics of the sludge, sludge volume was recorded at different time intervals and subsidence rates have been calculated. The settling rate, recorded in ml/min, is the subsidence rate in the 0 - 30 minute settling time; and the compaction rate is the subsidence rate in the 30 - 60 minute settling time.

Extracellular Physiology Studies

Several investigators have discussed the importance of a capsule or a bacterial slime layer to flocculation in activated sludge (Buswell and Long, 1923; Theriault and McNamee, 1936; Heukelekian and Shulhoff, 1938). It may be possible that a poorly settling sludge can be made to flocculate by increasing the amount of capsular or slime material present. It has been shown that a poorly settling sludge can be made to flocculate by addition of synthetic anionic and non-ionic polyelectrolytes (Busch and Stumm, 1968; Singer, 1968).

In view of this information, an experiment was designed to evaluate the effect of a low molecular weight and a high molecular weight polymer on the settling characteristics of two sludges, one in which filamentous organisms were present in significant numbers and another in which they were present

only in small numbers.

Polymers of dextran were used in this study. Dextran, a polysaccharide of glucose, is of biological origin. The anomeric carbon of each glucose unit is in glycosidic linkage and thus, it can be utilized for polysaccharide synthesis. The precursors of capsular polysaccharide are UDP-glucose and UDP-glucuronic acid (White et al., 1968). Gaudy and Wolf (1962) have found the molar ratios of glucose and glucuronic acid in S. natans to be 0.77 and 0.80 respectively. Therefore, it is possible for dextran to be incorporated into cells as capsular material.

Dextran is available in low molecular weight (15,000 - 20,000) and high molecular weight (5 - 40 million) quantities. It is reasonable that the low molecular weight dextran would be more available to the bacteria than the high molecular weight polymer. In addition, the high molecular dextran would be expected to form longer bridges between cells, resulting in better agglomeration than the low molecular weight polymer. It is possible, therefore, to evaluate whether flocculation of sludge mass is determined by a bridging mechanism or by a capsular mechanism, and whether filamentous organisms are important in the flocculation process.

Busch and Stumm (1968) found that bacterial aggregation occurred with some success at pH of 5 with 100 mg/l high molecular weight dextran. Therefore, this concentration of either high molecular weight or low molecular weight polymer

was added to the appropriate beakers. A Phipps Bird variable speed stirring device was used for mixing.

After polymer was added to the appropriate sample, the mixture was stirred at 100 rpm for 2 minutes; then the speed was reduced to 20 rpm for 10 minutes. At this time, a 30 minute settled volume was recorded. Next, the mixture was stirred at 20 rpm for 30 more minutes followed by another 30 minute settled volume test. The mixture was then stirred at 20 rpm for 12 hours. Following this mixing, another settled volume was recorded. At this time, a second amount of polymer, identical to that added earlier, was added and the mixture was flash mixed for 2 minutes and then stirred at 20 rpm for 2 hours. The settled volume was again recorded. After 24 hours of additional mixing at 20 rpm, a final settled volume was recorded.

The presence of the bacterial sheath/slime layer and its possible importance in bacterial aggregation was determined by means of phase microscopy and negative staining. Electron microscopy was used in an attempt to establish whether the slime layer contributes binding sites for cell aggregation.

Poly-β-Hydroxybutyric Acid

Lemoigne (1927), who first described poly-β-hydroxybutyric acid (PHB), estimated PHB by a common gravimetric method. The procedure is based on the fact that the polymer is soluble in boiling chloroform and can be separated from contaminants by extraction with other solvents. Unfortunately,

the method is not precise and it requires several milligrams of polymer.

With renewed interest in PHB in the late 1950's, a more sensitive method was devised by Williamson and Wilkinson (1958). In this procedure, the turbidity of the lipid granules was measured following complete dissolution of cells in a sodium hypochlorite solution. The method was standardized by comparison to the gravimetric method. Unfortunately, this method required the polymer to be in native lipid granules. In this study PHB was determined by the spectrophotometric method of Law and Slepecky (1961). The method involves isolation of the polymer by solvent extraction and its conversion to crotonic acid by dehydration. The principle of this method lies in the fact that the ultraviolet absorption maximum of α,β-unsaturated acids undergoes a strong bathochromic shift when concentrated sulfuric acid is employed as a solvent. The resulting absorption maximum lies within the useful range of commercial spectrophotometers and is sufficiently intense to provide a sensitive method of determination (Slepecky and Law, 1960).

For the assay of polymer using small quantities of cells, the organisms were centrifuged in polypropylene centrifuge tubes and resuspended in a volume of commercial sodium hypochlorite solution (Clorox) equal to the original volume of medium from which the cells were harvested. After 1 hour at 37°C, the lipid granules were centrifuged, washed with

water, and then washed with acetone and alcohol. A vortex mixer was used to resuspend pellets after each centrifugation.

Finally, the polymer was isolated by extraction with boiling chloroform. The chloroform extract was then passed through a sintered glass filter, and the filtrate was used for the assay of poly-β-hydroxybutyrate. An aliquot of the chloroform filtrate containing 5-50 μg of polymer was transferred to a clean test tube and the chloroform evaporated. After evaporation was complete, 10 ml. of concentrated H_2SO_4 was added; the tube was capped with a glass marble and heated for 10 minutes at 100°C in a water bath. The solution was cooled; after thorough mixing, a sample was transferred to a silica cuvette and the absorbance at 235 mμ was measured against a sulfuric acid blank. The amount of polymer was determined using a molar extinction coefficient of 1.56×10^4, calculated from a plot of polymer (crotonic acid) vs. absorbance at 235 mμ, and a molecular weight of 86 for the "depolymerized" poly-β-hydroxybutyric acid.

The spectrum of polymer in sulfuric acid was recorded from 220 to 260 mμ. This spectrum closely resembled the spectra of crotonic acid, and thus, it is reasonable to assume that interfering material was not present or present in neglibible quantities only.

The relationship between poly-β-hydroxybutyric acid concentration and absorbance at 235 mμ used in this investigation was prepared from a very pure sample of PHB. Sample purity had been previously determined by infrared spectroscopy.

Dissolved Oxygen Studies

When a large concentration of soluble organic matter is introduced into the activated sludge process, the sludge will often develop an extremely high rate of oxygen uptake. If the aeration rate is not increased, the DO concentration will fall to a very low level. This competition for dissolved oxygen at low DO has frequently been used to explain the filamentous bulking due to high organic loads (Heukelekian, 1941; Mulder, 1964; Wuhrmann, 1964). Okun (1949) suggested that high DO might also be toxic to filamentous organisms. Thus, bulking of activated sludge has been associated with extremes, both high and low, of dissolved oxygen in the aeration tank.

In view of these studies, experiments were designed to determine how effectively S. natans grows and whether it could establish a competitive advantage over other activated sludge organisms at low DO levels. The continuous laboratory system and sampling times correspond to those described in an earlier section of this chapter.

Dissolved oxygen was determined using a Weston and Stack Model 300 dissolved oxygen analyzer equiped with a Model 3 dissolved oxygen probe. The probe was fitted into the Weston and Stack BOD Agitator to obtain uniform agitation in each BOD bottle. The meter was adjusted to zero using a sodium sulfite solution. The meter was calibrated using a liquid whose DO is known. The Winkler method outlined in Standard

Methods (1965) was used for standardization. Oxygen uptake was recorded by means of this polarographic method with the help of a Rustrak Model 288 recorder. Although repeated standardizations were required throughout the study, operational characteristics were excellent.

In the later study of effects of low oxygen tension using batch cultures, low DO was obtained by bubbling compressed gas with low percentages of oxygen through the cultures in a closed system. The nitrogen:oxygen mixture used during the first 24 hours was 95:5. At the beginning of the study, the cultures had sufficient DO and a low DO uptake rate. The DO after several hours was less than 1.0 mg/l DO. From the elapsed time of 24 hours until the termination of the study, a nitrogen:oxygen mixture of 99:1 was bubbled through the cultures. After an elapsed time of 48 hours, the DO was less than 0.5 mg/l. The gas flow rates were maintained by a gas regulator at approximately 1.0 l/min.

Carbon-Nitrogen Relationship

The basic requirements of any heterotrophic organism are a carbon source for growth and energy and a nitrogen source for growth. As mentioned earlier, Ingols and Heukelekian (1939 and 1940) as well as Sawyer (1940) observed that carbohydrates would cause a high useable carbon to nitrogen ratio in sewage, leading to activated sludge bulking. Morgan and Beck (1928) and Scott (1928) reported that the removal of a carbohydrate source from the sewage eliminated their problem

of filamentous bulking. Hattingh (1963) found that bulking was due to the combined effects of high carbon to nitrogen together with high carbon to phosphorus ratios.

Dias, Dondero, and Finstein (1968) indicated that bulking by carbohydrates was an indirect effect caused by nitrogen deficiency, and that addition of increased amounts of glucose to a mixed community did not result in selection of Sphaerotilus, but usually the reverse. Adamse (1966) found that by reducing the C-N ratio of a dairy waste through the addition of ammonia, he was able to improve the settleability of the sludge.

Since a high C-N ratio has frequently been suggested as promoting filamentous bulking, the carbon and nitrogen content of the volatile organic suspended solids was studied. An F & M Model 185 Carbon, Hydrogen, Nitrogen Analyzer using a Model G Cahn Electrobalance was used for the determination. This instrument is specifically designed to provide a rapid, semi-automatic means for measuring C, H, and N content. Samples undergo complete combustion in a closed loop chamber, followed by a system where the components are separated for measurement. The analysis of an unknown sample is referred to a standard sample, cyclohexamone-2:4-Dinitrophenylhydrazone, which has C, H, and N content similar to those of the unknown samples. The peak heights are directly proportional to the amount of the reaction product and can thus be used to calculate the original sample composition.

Samples were prepared by centrifuging a 40 ml well-mixed MLSS sample from each reactor for 10 minutes at 10,000 rpm. The pellet was then washed into a porcelain dish with distilled water and placed in a drying oven, where it was evaporated to dryness at 103°C overnight. The residue was collected and ground into fine particles with a mortar and pestle. Samples were stored in glass vials. Sample size used throughout the analysis was 0.5 to 0.7 mg.

Composition of Suspended Solids

The total mixed liquor suspended solids concentration (MLSS) consists of active and inactive cells, fixed inorganic, inert, and organic volatile suspended solids. The first step in determining the composition of the suspended solids is the determination of MLVSS.

MLSS was determined by taking a 40 ml well-mixed sample from each reactor. The sample was centrifuged for 10 minutes at 10,000 rpm, and the centrate was poured off for soluble COD determination. The pellet was then washed into a tared porcelain dish with distilled water and placed in a drying oven and evaporated to dryness at 103°C overnight. The difference between the gross tare weights times the appropriate dilution factor gives the concentration of suspended solids in mg/l. The porcelain dish containing the residue was placed in a muffle furnace at 600°C for 15 to 20 minutes, cooled in a desiccator, and weighed. The difference between the two gross weights times the appropriate dilution factor

gives the concentration of mixed liquor volatile suspended solids (MLVSS) in mg/l. The difference between MLSS and MLVSS is the weight of fixed, inorganic suspended solids.

The next step in determining the composition of the suspended solids is determining the concentration of the living and dead cells in the MLVSS. Since the MLVSS consists of living and dead microorganisms and non-biological volatile solids, the difference in MLVSS and the total cell weight is the weight of inert solids. The living and non-living total cell mass can be quantitatively determined by measuring the deoxyribonucleic acid (DNA) content. The Schmidt-Tannhauser (Munro and Fleck, 1966) procedure for separation and determination of DNA was employed. A well-mixed MLSS sample containing 0.05 - 0.5 mg DNA was mixed with cold 5% trichloroacetic acid (TCA) in a centrifuge tube for 30 minutes in an ice bath. The sample was centrifuged at 10,000 rpm for 10 minutes, and the centrate was poured off. The pellet was resuspended in 5% $HClO_4$ using a vortex mixer and centrifuged. This step was repeated one more time. The final ethyl alcohol washed pellet was suspended in 2 ml of 0.3 N KOH. The tube was stoppered and incubated for 16-18 hr. at 37°C. After incubation, 0.2 ml of 3 N $HClO_4$ was added and the pH was adjusted to 2-3. The sample was centrifuged and the centrate was carefully removed. The pellet contained unhydrolyzed material, $KClO_4$ and DNA. The pellet was carefully resuspended in 1 ml of 0.01 M $HClO_4$ and

centrifuged. The pellet was resuspended in 2 ml of 0.5 N $HClO_4$ to hydrolyze DNA to DNAtides. This mixture was then heated at 100°C for 10 minutes. The sample was cooled in ice and the insoluble pellet was centrifuged off. The centrate contained the DNAtides.

Since the spectra at optical density (OD) 230, 260 and 280 mμ did not indicate pure nucleic acids, the Dische method of DNA was followed (1955). A reagent containing dephenylamine, glacial acetic acid, and concentrated sulfuric acid was mixed with the centrate containing DNAtides in a DNAtides:reagent ratio of 1:2. This mixture was boiled for 10 minutes and OD was read at 600 mμ.

The amount of DNA obtained from the standard curve (0.3 OD_{600} = approximately 30 mg DNA) times the appropriate dilution factor gave the concentration of DNA in the MLSS in the units mg/l. The total cells in mg/l is equal to a ratio of the product of the total mg/l DNA and the weight in mg of one cell to the weight in mg of DNA in one cell. An example is given below.

$$\text{Total Cells (mg/l)} = \frac{\text{mg DNA}}{\text{liter}} \times \frac{\text{one cell}}{2 \times 10^{-12} \text{ mg DNA}} \times \frac{10^{-9} \text{ mg cells}}{\text{one cell}}$$

The weight of one cell is an average figure taken from Oginsky and Umbreit (1959). The DNA content per cell apparently varies from bacteria to bacteria. The value 2×10^{-12}mg DNA/cell is an average value, based on numerous cell counts and DNA determinations.

The final step in determining the suspended solids composition is the determination of the fraction of cells that are active. The weight of active cells can be evaluated by measuring the dehydrogenase activity of the sludge sample. The difference in weight of total cells and active cells is the weight of inactive cells. The determination of dehydrogenase activity by the reduction of triphenyltetrazolium chloride (TTC) was first proposed by Lenhard and Nourse (1964) and modified by Ford et al. (1966). In the procedure used by this author, 5 ml of test activated sludge mixed liquor, added to each of four large test tubes, was mixed with 5 ml of tris-buffer. The temperature of the solution was brought to 37°C as rapidly as possible in tap water and then, the tubes were placed in a 37°C water bath. One ml of distilled water was added to the control tube and 1.0 ml of the TTC-glucose reagent was added to each of the three remaining test tubes. The tubes were incubated for exactly 15 minutes following TTC inoculation. After the 15 minute incubation, the reaction was stopped by the addition of 39 ml absolute ethyl alcohol. Each tube was shaken and centrifuged for 10 minutes at 2500 rpm. The per cent transmission of the centrate was recorded at 490 mμ, using the control solution as a blank and the average per cent transmission of the three samples as the true value. From a standard curve, the μ-moles of triphenylformazan (TF) produced was determined. This value indicated the relative dehydrogenase activity.

The amount of TF produced, obtained from the standard curve, times the appropriate dilution factor gave the total μ-moles TF produced per liter. The total active cells in mg/l is equal to a ratio of the μ-mole/l TF produced to the μ-mole TF produced per mg active cells. The value of 0.25 μ-mole TF produced per mg active cells is an average value which signifies that one cell has dehydrogenase activity relative to another cell. It is based on published values of μ-mole TF/mg MLVSS averaged over parameters such as sludge age (Ford et al., 1966). An example is given below.

$$\text{Active Cells (mg/l)} = \frac{\mu\text{-mole TF produced/liter}}{0.25\ \mu\text{-mole TF produced/active cell}}$$

From these calculations, it is possible to determine the concentration in mg/l of the following: total suspended solids, fixed inorganic suspended solids, organic volatile suspended solids, inert suspended solids, total cell mass, inactive cell mass, and active cell mass. The flowsheet for these determinations is given in Figure 2.

Analytical Procedures

Chemical Oxygen Demand (COD)

The COD test is more reliable than the BOD test as a measure of the oxidizable organic matter present in the waste. The question here is the oxidation process. In the COD determination the organic material is oxidized by a powerful oxidizing agent in the presence of a catalyst and a strong mineral acid, using heat to accelerate the process. A

process of this kind would undoubtedly assist the oxidation of organic matter which would offer considerable resistance to oxidation by a randomly mixed group of activated sludge microorganisms. The artificial conditions imposed by the COD test are far removed from the natural oxidation conditions prevailing in a mixed microbial culture. Although a constant relationship probably does not exist between COD and biodegradability, the COD is used as a measure of biodegradability because it is a simple test to perform and because under certain conditions there is statistical correlation between biodegradability and COD.

The total COD was determined from a well-mixed sample of mixed liquor and a uniform sample of effluent. Forty ml of each sample was centrifuged for 10 minutes at 10,000 rpm in a Sorval Superspeed Type SS-3 centrifuge; the centrate was used for the soluble COD determination. The procedure is outlined in Standard Methods (1965).

Acid Determinations

A photovolt Model 120 digital pH meter was used for all pH measurements. Volatile organic acids were determined by a colorimetric determination extremely useful in routine sampling. A well-mixed sample of mixed liquor was centrifuged for 10 minutes at 10,000 rpm and the centrate was used for the determination. The procedure is outlined by Montgomery et al. (1962).

CHAPTER IV

RESULTS AND DISCUSSION

Introduction

After more than 50 years of experience with the activated sludge process, most operational problems can be reduced to the question of whether or not the sludge will settle. This study has investigated filamentous organisms and sludges experiencing an overgrowth by filamentous organisms which result in populations that settle slowly and compact poorly. The filamentous organism most frequently associated with bulking is Sphaerotilus natans. The best indicator of whether the activated sludge process is operating properly, whether or not the sludge is settling satisfactorily, is the sludge volume index (SVI). Thus, S. natans has been used as the test organism and SVI has been used as a measure of settleability.

Microscopic Examination

Microscopic examination of mixed liquor has been used as an aid to operational control of biological processes for several years. Operators study the sludge from their own plant over a period of time and develop a subjective concept

of how the sludge should look when the process is operating properly. A correlation between microscopic appearance and process performance is possible. S. natans and other filamentous bacteria have been recognized in activated sludge for many years.

Figure 4 is a photomicrograph of a pure culture of S. natans. It vividly shows the physical entanglement that can result in a sludge with poor compaction characteristics. Figure 5 is a photomicrograph of a mixed culture of S. natans. The long filaments seem to reduce the density of the floc particles. If this is the case, a large percentage of filamentous organisms could result in diffuse floc particles with poor settleability. Figure 6 is a photomicrograph showing the tendency of S. natans filaments to stick together. The sheath/slime layer does appear to have definite adhesive properties. In addition, physical entanglement may be important in filament attachment. Figure 7 is a photomicrograph which shows the individual cells and sheath in more detail. In the lower right-hand corner is an empty section of sheath. In the upper region is a possible site for attachment. Figures 8-9 are electron micrographs of S. natans. Figure 8 shows a long filament and several other thin-cut sections. In the lower right-hand corner is a possible cell attachment. Here, there is an accumulation of a dark material, perhaps PHB, which may be a binding site for flocculation of S. natans. Figure 9 examines the region between associated

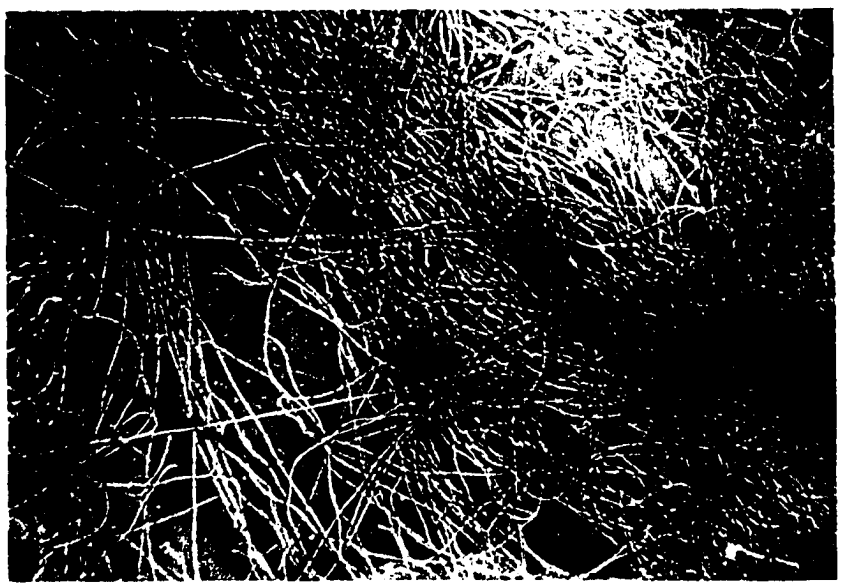

Figure 4. Photomicrograph of S. natans in pure culture x 700

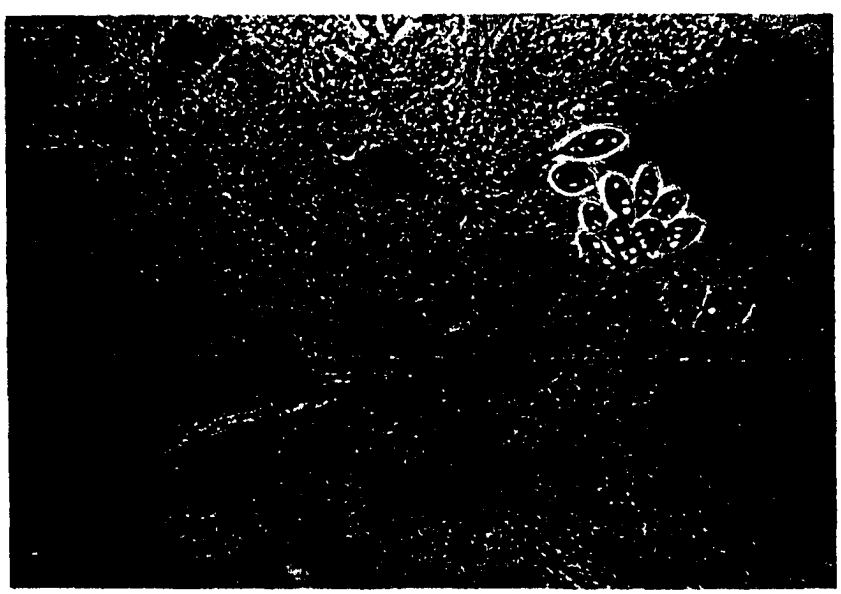

Figure 5. Photomicrograph of S. natans in mixed culture x 700

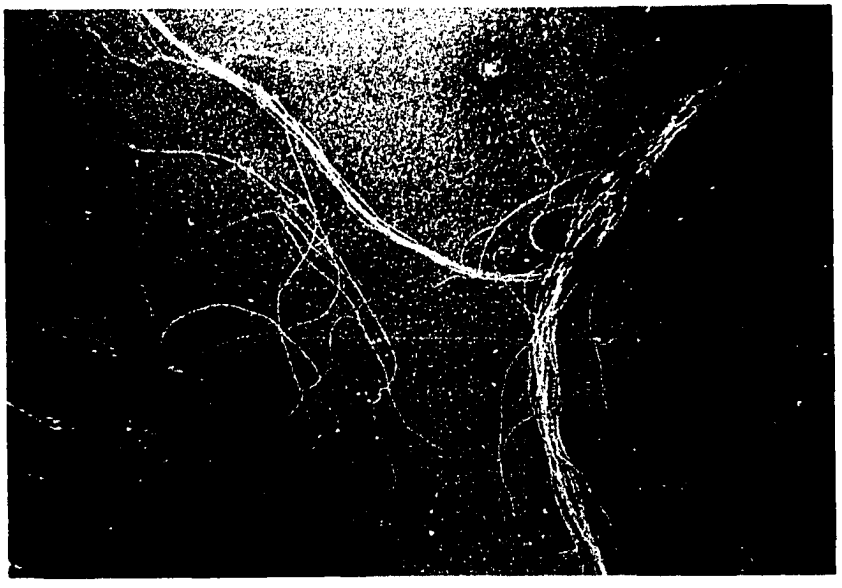

Figure 6. Photomicrograph of filaments of S. natans x 100

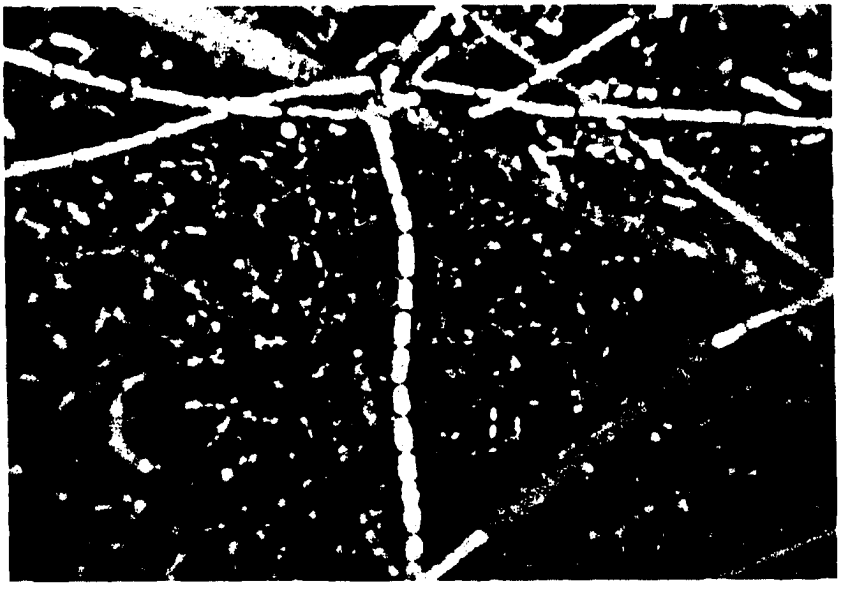

Figure 7. Photomicrograph of individual cells of S. natans x 100

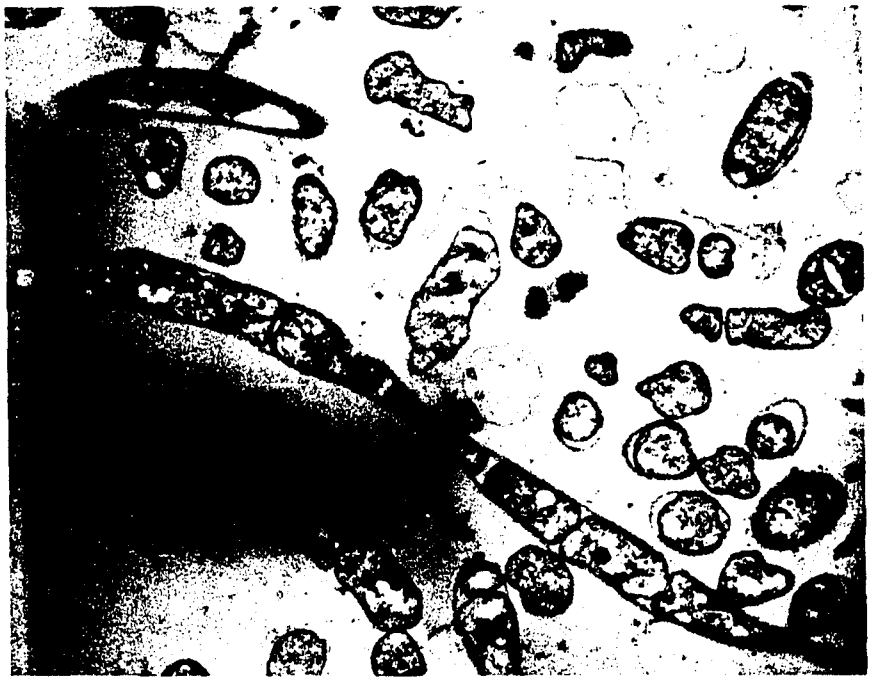

Figure 8. Electron micrograph of thin-cut section of S. natans x 7,020



Figure 9. Electron micrograph of region between cells of S. natans x 33,150

cells. There appears to be an overlapping of slime material which may result in attachment. No storage material or bridging of the cells is visible.

Sludge Volume Index

Previous experiments had shown that activated sludge when fed a high carbohydrate would bulk. Lackey and Wattie (1940) using batch culture techniques used their type "S" synthetic sewage to produce a bulking sludge from which they isolated S. natans. Pipes and Jones (1963) using the same synthetic waste, but in a continuous system, produced filamentous bulking. In each of Figures 10-12, increases in the organic loading intensity have resulted in a sludge that has poor settling and compacting characteristics. In the preliminary experiment (Figure 10) the loading intensity was increased by a factor of two, resulting in SVI's that increased by factors of five and ten.

It is also noteworthy that the initial increases in SVI for both pure and mixed S. natans cultures are significant, but that the high SVI in the pure culture experiment is of longer duration than in the mixed culture experiment. Furthermore, in neither case does the SVI return to a level as low as first experienced, and in the pure culture, there is a second peak in SVI.

In the second and third experiment (Figure 11) there are larger increases in loading intensity than observed in the preliminary experiment. The reaction of SVI to the loading

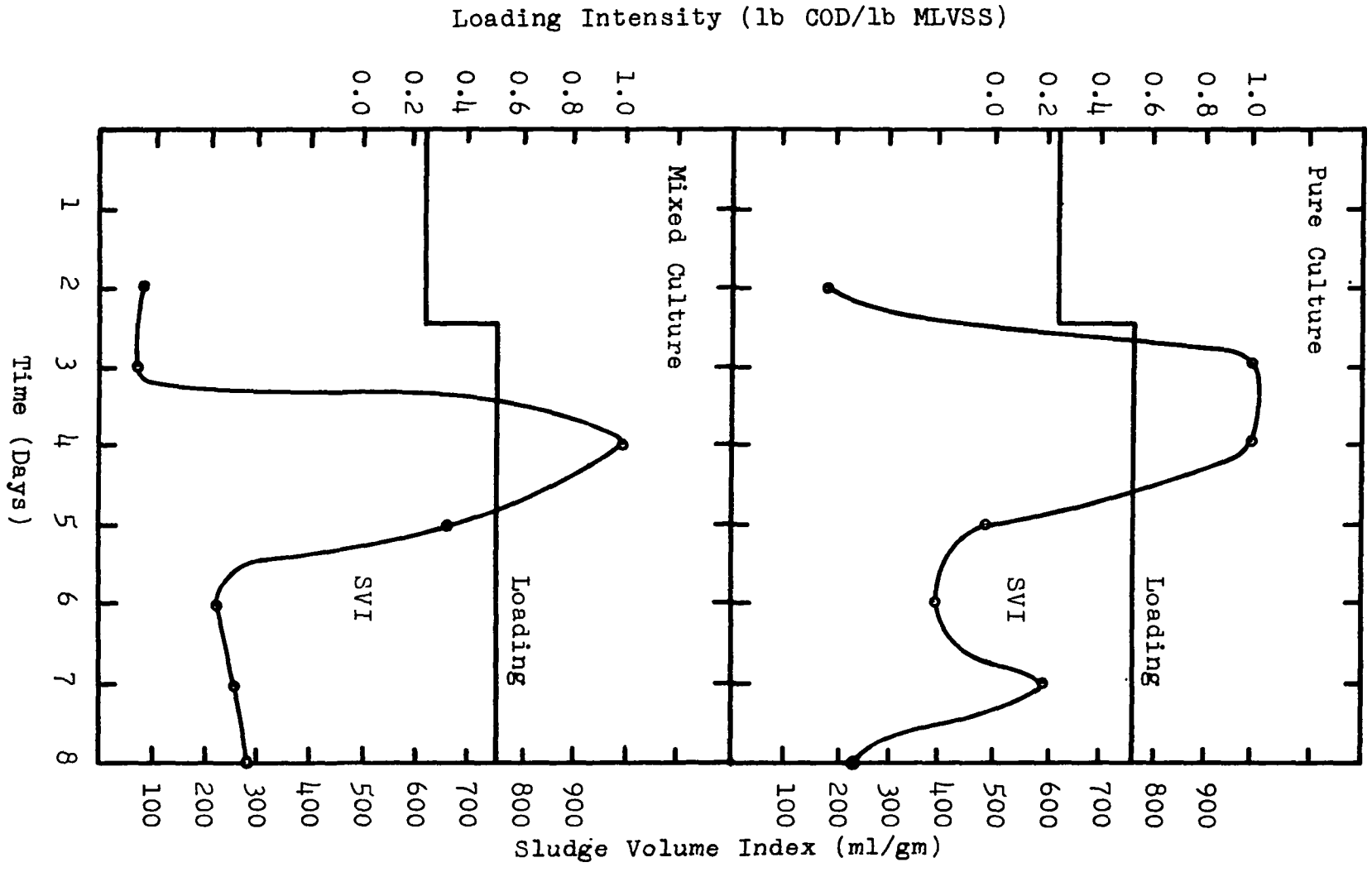

Figure 10. Preliminary Experiment
SVI and Loading Intensity for Pure and
Mixed Cultures of S. natans vs. Time

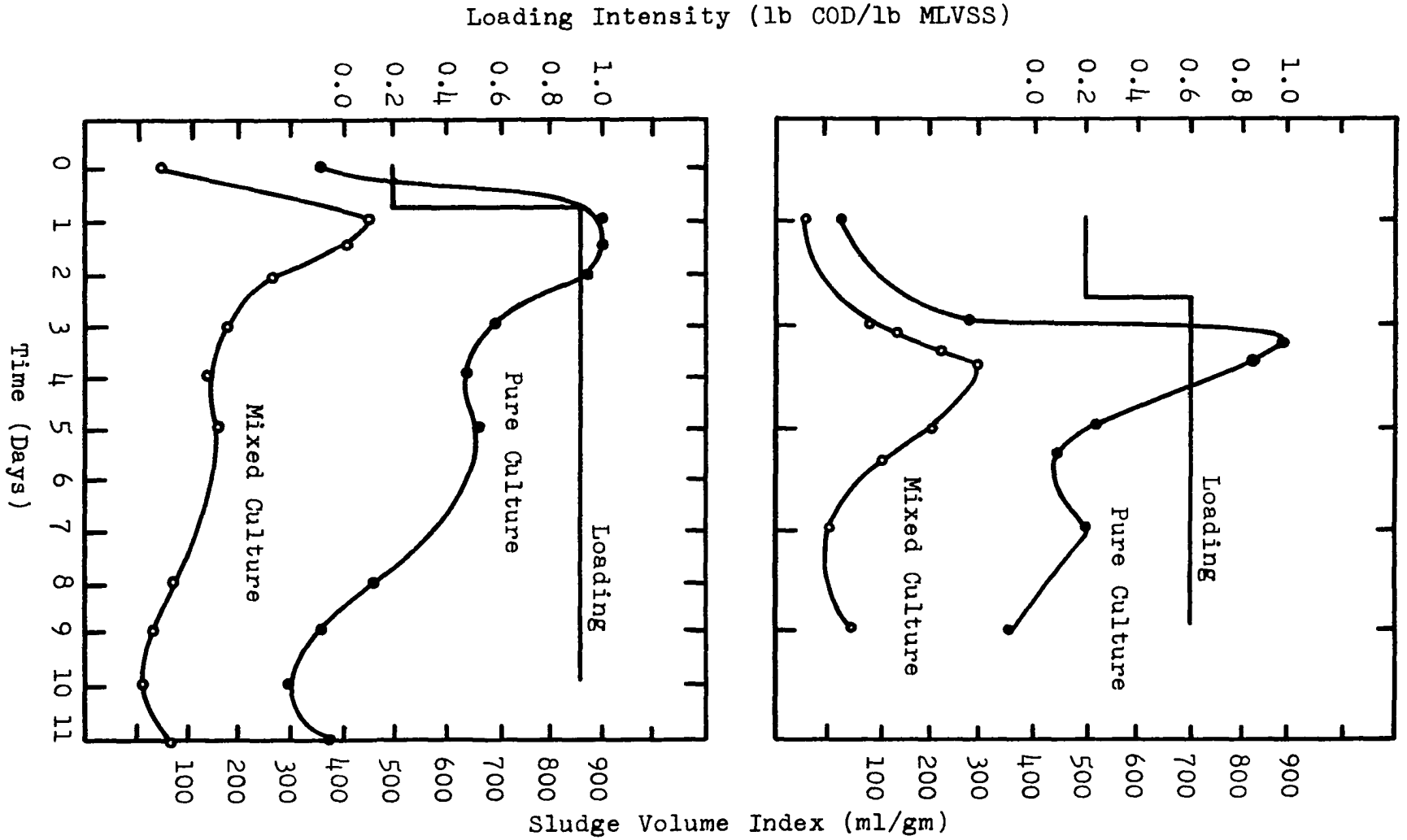

Figure 11. Second and Third Experiments: SVI and Loading Intensity for Pure and Mixed Cultures of S. natans vs. Time

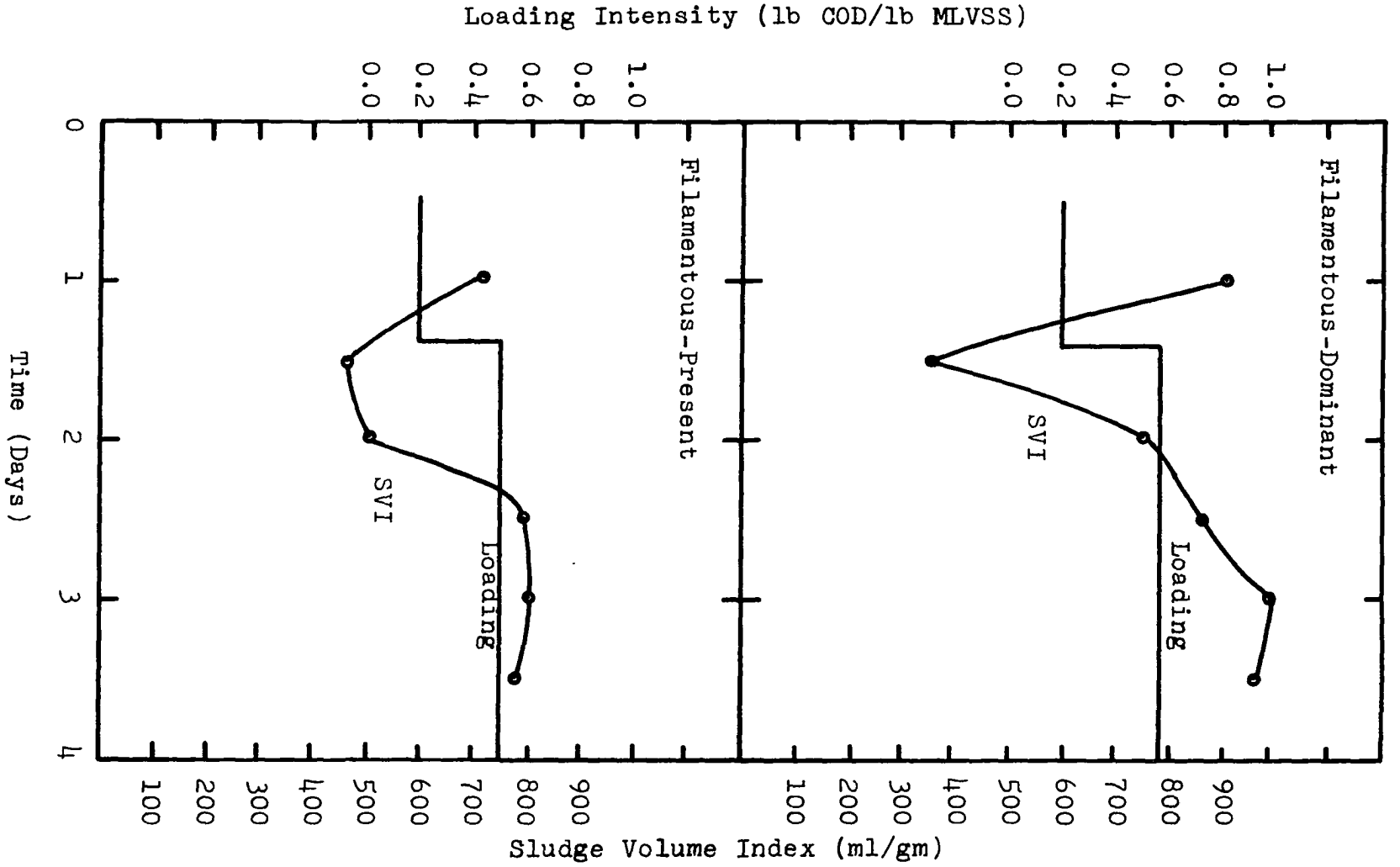

Figure 12. Fourth Experiment:
SVI and Loading Intensity for Filamentous-Dominant and Filamentous-Present Sludges vs. Time

is similar to that observed in experiment one. It should be pointed out that, in both the second and third experiments, the reaction of the pure culture of S. natans is generally more severe in duration and intensity than the reaction of the mixed activated sludge culture.

In the fourth experiment, as shown in Figure 12, a decreasing SVI reverses its trend when loading intensity is increased. In this case, the sharp increase in SVI, observed in the earlier experiments, is replaced by a gradual increase in SVI. The culture in which filamentous organisms are dominant experiences a more gradual increase in SVI than the culture in which filaments are present but not dominating. The duration of each is similarly long.

From these three figures (10-12), it is reasonable to assume that some critical increase in loading intensity, which is also a function of waste composition, must be exceeded in order to selectively develop a sludge with poor settling characteristics. Within reasonable limits, a two-fold increase in loading intensity results in approximately a two-fold increase in SVI; this is an observable change in settling characteristics. Either extended duration or increased intensity of bulking will result in poorer settling characteristics; if either duration or intensity are extreme enough, the sludge population will be lost and the activated sludge process will fail.

Preliminary Experiment

Information derived from preliminary investigations are plotted in Figures 13-15. PHB content, oxygen uptake, carbon-nitrogen ratio (C:N), and per cent cellular carbon are each compared to the SVI over the eight day test interval. As shown in Figure 13, the PHB content increases and decreases in what is apparently a direct relationship with changes in SVI. There is an obvious similarity between changes in PHB content and SVI for both the pure culture and mixed culture of S. natans. This may be one of the first reports to relate changes in PHB content to changes in sludge settling characteristics. However, a few investigators, including Crabtree et al. (1966), have observed changes in PHB content when bacterial flocculation characteristics change. The signficance of the relationship between flocculation and sludge settling characteristics in activated sludge will be discussed in a later section.

From Figure 14, there is reason to believe that oxygen uptake may be an important determinant of sludge settling characterisitcs. It appears that changes in oxygen uptake may follow changes in SVI. Therefore, increased metabolic activity and increased energy in the system will result in cellular dispersion (increased surface area) and bulking.

In the pure culture experiment (Figure 15), increased SVI is accompanied by a decrease in per cent carbon. The C:N ratio also decreases; this is a result of the decreased

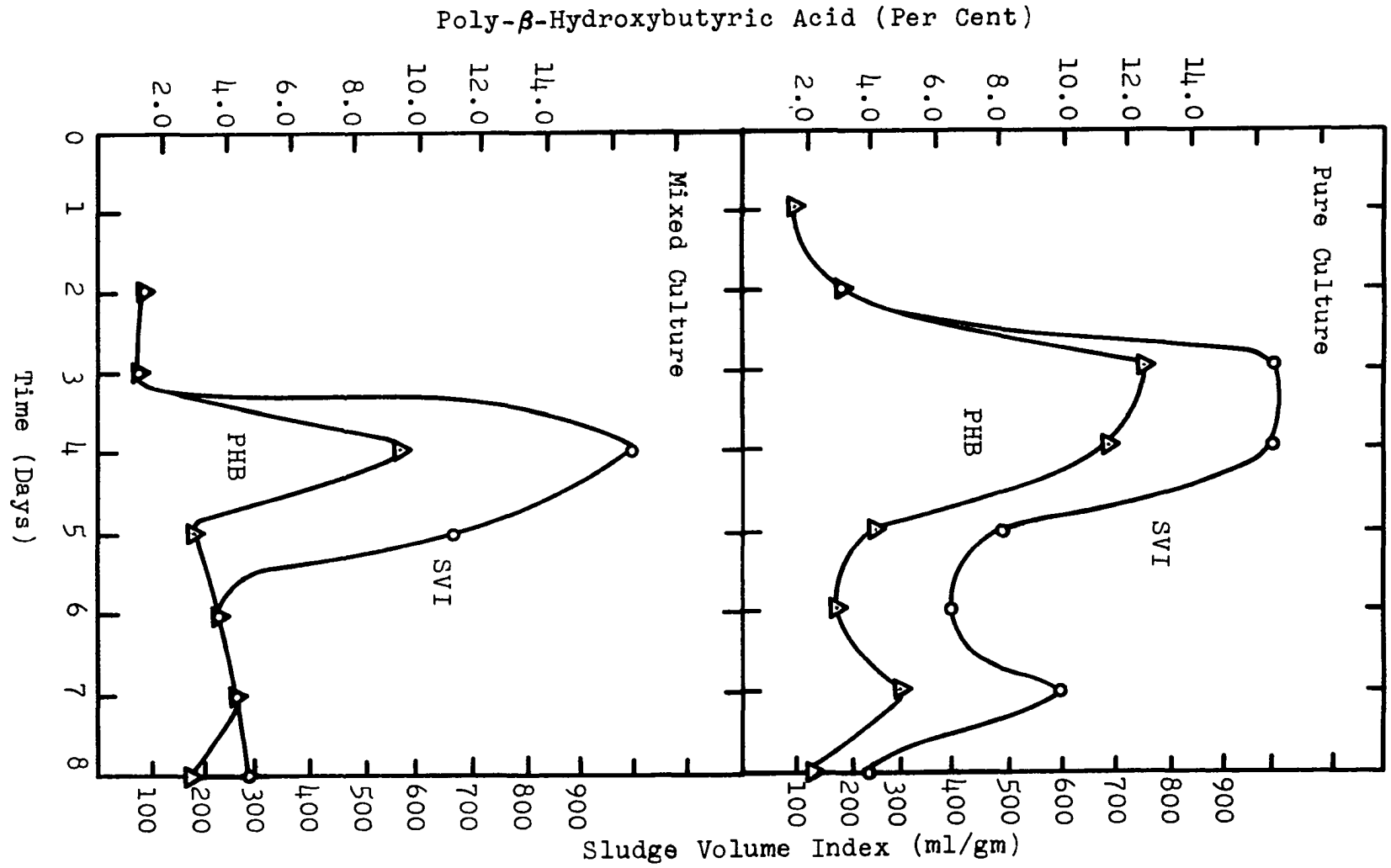

Figure 13. Preliminary Experiment:
SVI and PHB for Pure and Mixed Cultures
of S. natans vs. Time

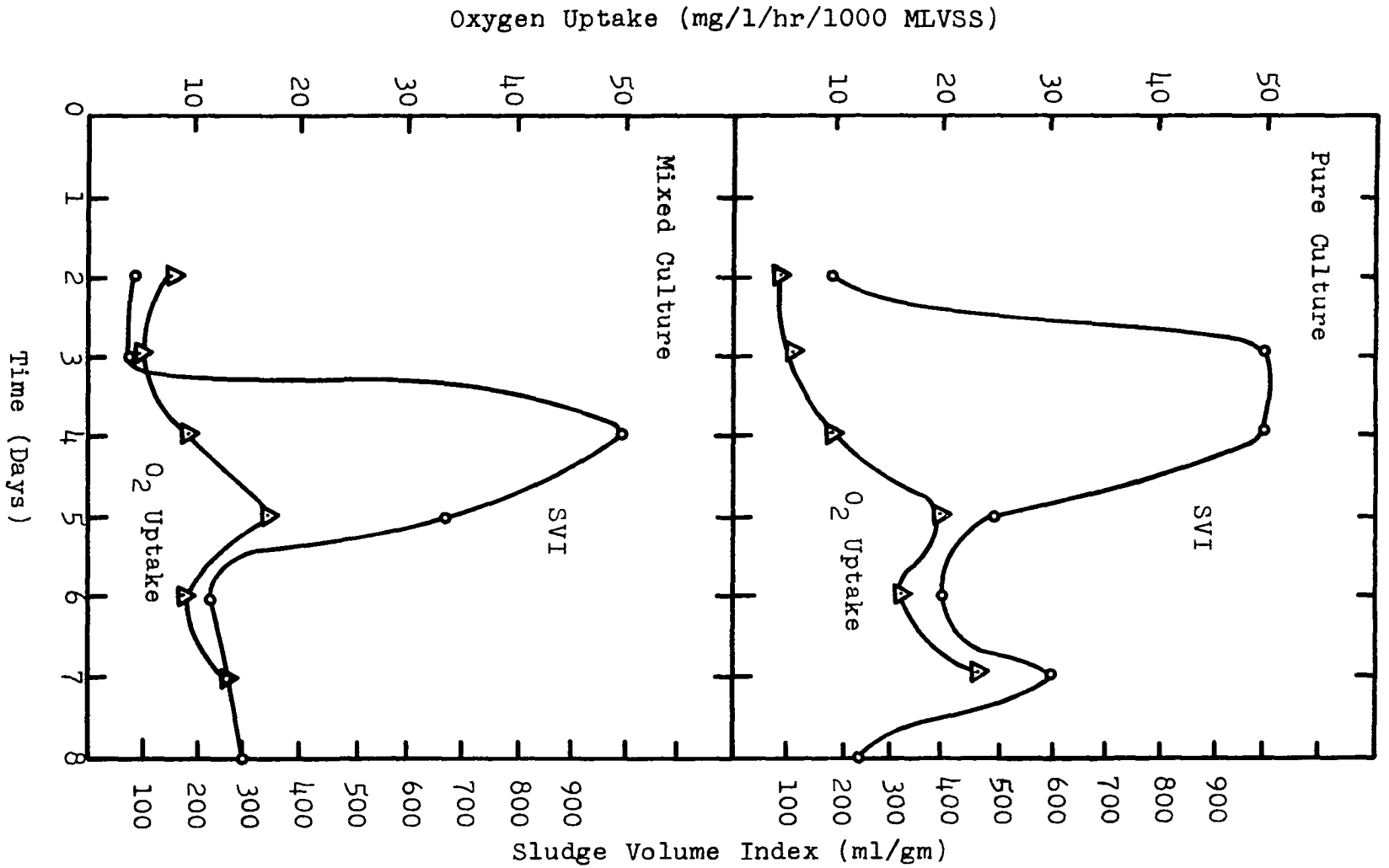

Figure 14. Preliminary Experiment: SVI and Oxygen Uptake for Pure and Mixed Cultures of S. natans vs. Time

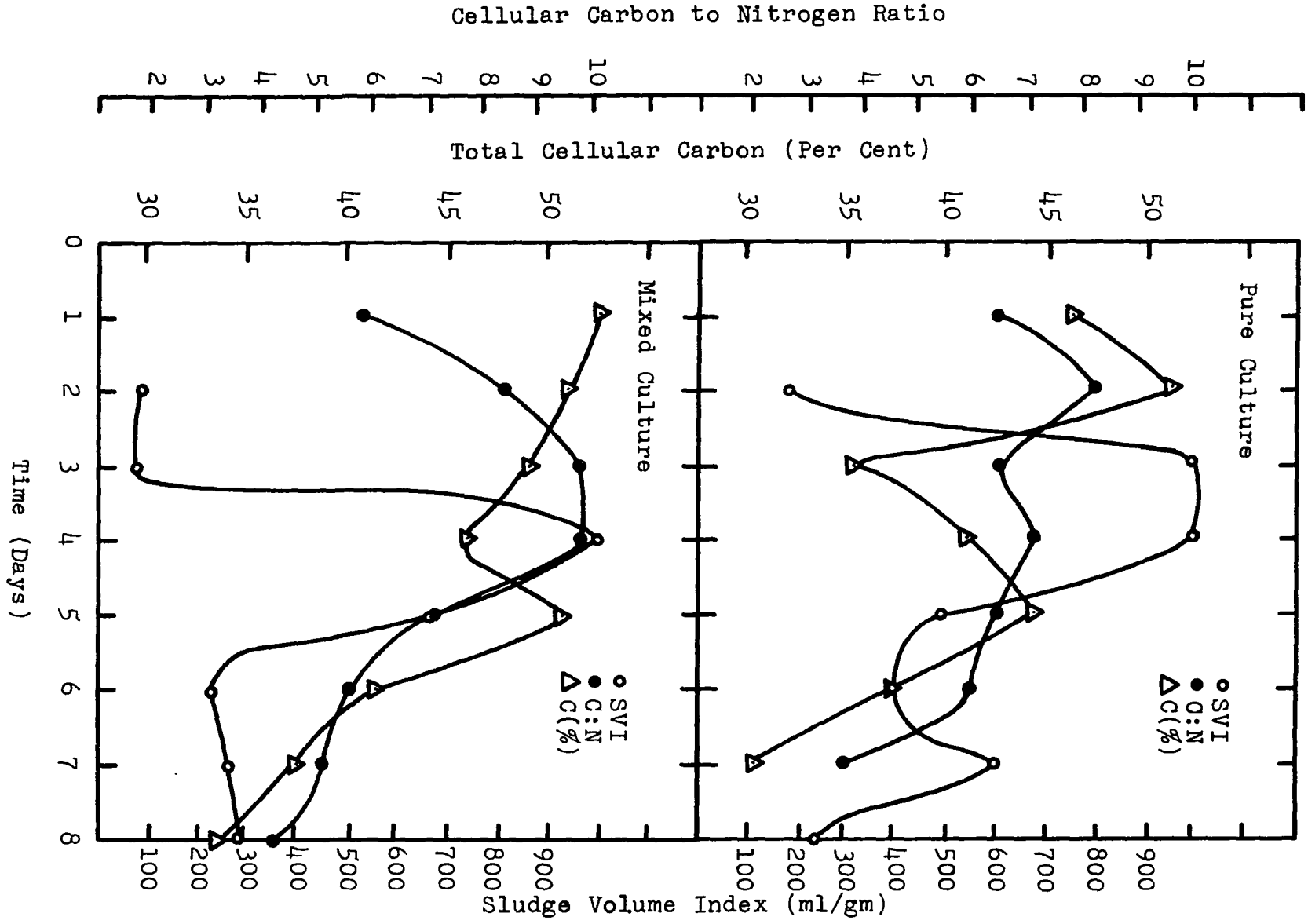

Figure 15: Preliminary Experiment:
SVI and Carbon:Nitrogen Ratio and Total Cellular Carbon for Pure and Mixed Cultures of S. natans vs. Time

per cent carbon. On the fifth day, the per cent carbon reaches a peak while the C:N ratio continues a gradual decrease. This means that the per cent nitrogen is increasing at a greater rate than per cent carbon. Since the SVI also decreases, it is possible that a low C:N ratio and a high nitrogen concentration will favor satisfactory sludge settling characteristics.

On the fifth day of the mixed culture experiment (Figure 15), a peak per cent carbon is accompanied by a decrease in C:N ratio. As in the pure culture experiment, the decreased SVI during this time indicates that low C:N and high nitrogen concentration does favor satisfactory sludge settling characteristics. At the beginning of the time period, an increase in C:N ratio is accompanied by a decrease in per cent carbon. During this time, the per cent nitrogen is also decreasing, although not as much as the per cent carbon. The severe increase in SVI at this time is probably more a reflection of the high C:N ratio than the reduced nitrogen concentration.

Comparing the pure culture data in each of the three figures (13-15), the initial increase in SVI is accompanied by a major increase in PHB content and a gradual increase in oxygen uptake; it is preceded by an increase in C:N ratio and per cent carbon. The increased SVI noted on the seventh day is accompanied by increases in PHB content, oxygen uptake, decreases in C:N ratio, and per cent cellular carbon.

In the overall comparison of the mixed culture, the increase in SVI is accompanied by an increase in PHB content, a somewhat delayed increase in oxygen uptake, an increase in C:N ratio, and a decrease in per cent cellular carbon.

Second Experiment

Figures 16-20 describe data from the four day period in the second investigation. This data is listed in the appendix in Tables 10 and 11. PHB content, cellular activity (TF produced), oxygen uptake, volatile acids concentration, pH, carbon-nitrogen ratio, and per cent cellular carbon are each compared to the SVI over this second test interval. From Figure 16, there are indications that SVI and PHB are significantly related. The pure culture experiment in Figure 16 is of major interest because it shows a rapid initial accumulation of PHB which is immediately followed by a sharp PHB decrease, then by a further accumulation of polymer. Crabtree et al. (1966) observed an initial rapid accumulation of polymer in their flocculation studies with Zoogloea. The initial effect of PHB storage was the disruption of the orderly biochemical process of cell division, resulting in incomplete cell division and uneven cell size. He found these cells, while still attached from incomplete cell division, immediately capable of synthesizing the polymer. As these cells synthesized more polymer, they also divided incompletely. This process was repeated continuously,

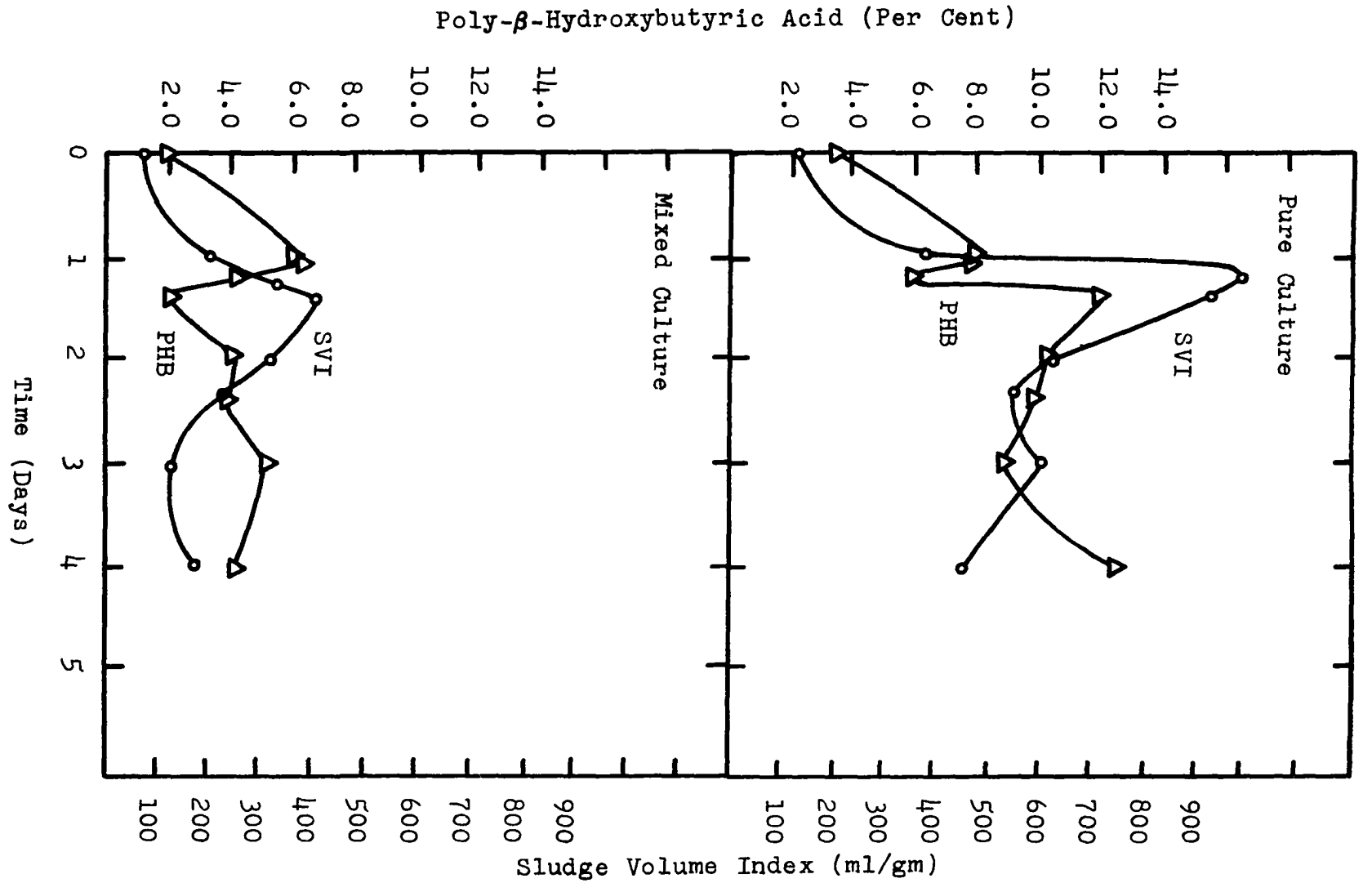

Figure 16. Second Experiment:
SVI and PHB for Pure and Mixed Cultures
of S. natans vs. Time

resulting in the formation of cellular aggregates. These aggregates then became the nucleus of larger flocs. The larger aggregates are heavier and should settle more rapidly than the lighter, smaller aggregates. What apparently has happened (Figure 16) is that the initial accumulation of the polymer was excessive, leaving many incomplete cells which were not capable of immediately synthesizing the polymer. These cells either died or, after reducing their PHB content by endogenous metabolism, were again able to accumulate PHB. When they were capable of synthesizing more PHB, the settling characteristics, SVI, improved. This improvement suggests that larger cellular aggregates may have been formed, resulting in larger heavier particles with better settling characteristics. The decrease in PHB content from day two to day three, and the PHB increase from day three to day four, is accompanied by respective increases and decreases in SVI. This suggests an inverse relationship between PHB and SVI.

In the first few hours after the loading velocity was increased in the mixed culture experiment (Figure 16), increases in PHB content were accompanied by increases in SVI. This increase in PHB is immediately followed by a decrease in polymer while the SVI continues to increase. Instead of the rapid, continued polymer accumulation observed after the initial increase and decrease in PHB content in the pure culture experiment, the mixed culture experiences only a

gradual accumulation of polymer. This suggests that the initial PHB accumulation was also excessive, and that the cells were not able to continue to accumulate PHB. It might also suggest that S. natans has a feedback repressor of PHB synthesis, while other bacteria in the mixed culture do not have such a repressor. Thus S. natans, which can rapidly accumulate a carbon reserve, could compete and survive for an important advantage over other organisms.

For about one and one-half days, until day three, PHB content decreases while SVI increases. This suggests an inverse PHB-SVI relationship similar to that suggested by the pure culture experiment during this same time interval.

Oxygen uptake, which is also a measure of biological activity, is plotted in Figure 17 with SVI. The time interval is the same. Both the pure culture and the mixed culture experiments suggest a direct relationship between oxygen uptake and SVI. In the pure culture experiment, changes in SVI seem to occur before changes in oxygen uptake. This would indicate that increased cellular activity might be a result of increased SVI, such as bulking, and not a direct cause of bulking. During the first few hours following an increase in the loading velocity, the mixed culture experiment experiences a sharp increase in activity, followed by a sharp decrease. These changes resemble those observed earlier with other parameters. From day two to day three, both parameters decrease; and from day three to day four,

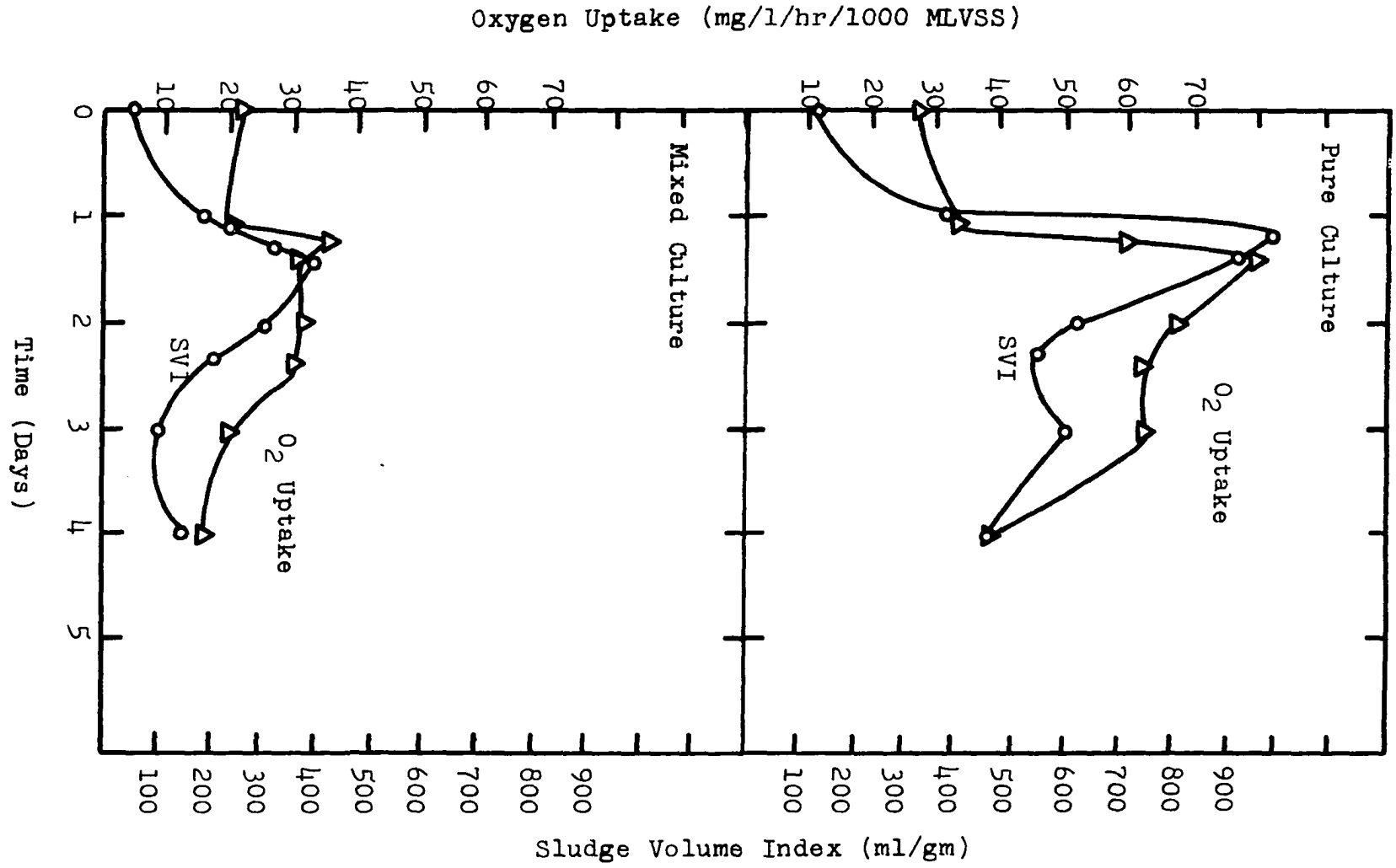

Figure 17. Second Experiment:
SVI and Oxygen Uptake for Pure and
Mixed Cultures of S. natans vs. Time

SVI increases while cellular activity decreases. This also occurred with the triphenylformazan parameter plotted in Figure 18. This figure suggests, then, that there is a direct relationship between SVI and cellular activity.

The amount of triphenylformazan produced, which is a measure of dehydrogenase or bacterial activity, is plotted in Figure 18, with SVI over the four day test period. In the pure culture experiment (Figure 18), a rapid increase in cellular activity accompanies the increase in SVI. This is followed by a gradual increase in TF, while the SVI decreases. From day two until day four, the SVI's increases and decreases are accompanied by respective increases and decreases in cellular activity. During the first few hours of the mixed culture experiment (Figure 18), SVI increases while the cellular activity increases, decreases sharply, then increases again. From this time, the two curves resemble each other, although a minor increase in SVI for day three to day four is accompanied by a decrease in cellular activity. In summary, it is likely that a direct relationship, although not an extremely important one, exists between SVI and cellular activity.

In Figure 19, the pH and volatile acids concentration are plotted with the SVI over the test period. In the pure culture, pH decreases from shortly after the organic loading is increased, until day four. However, the most rapid

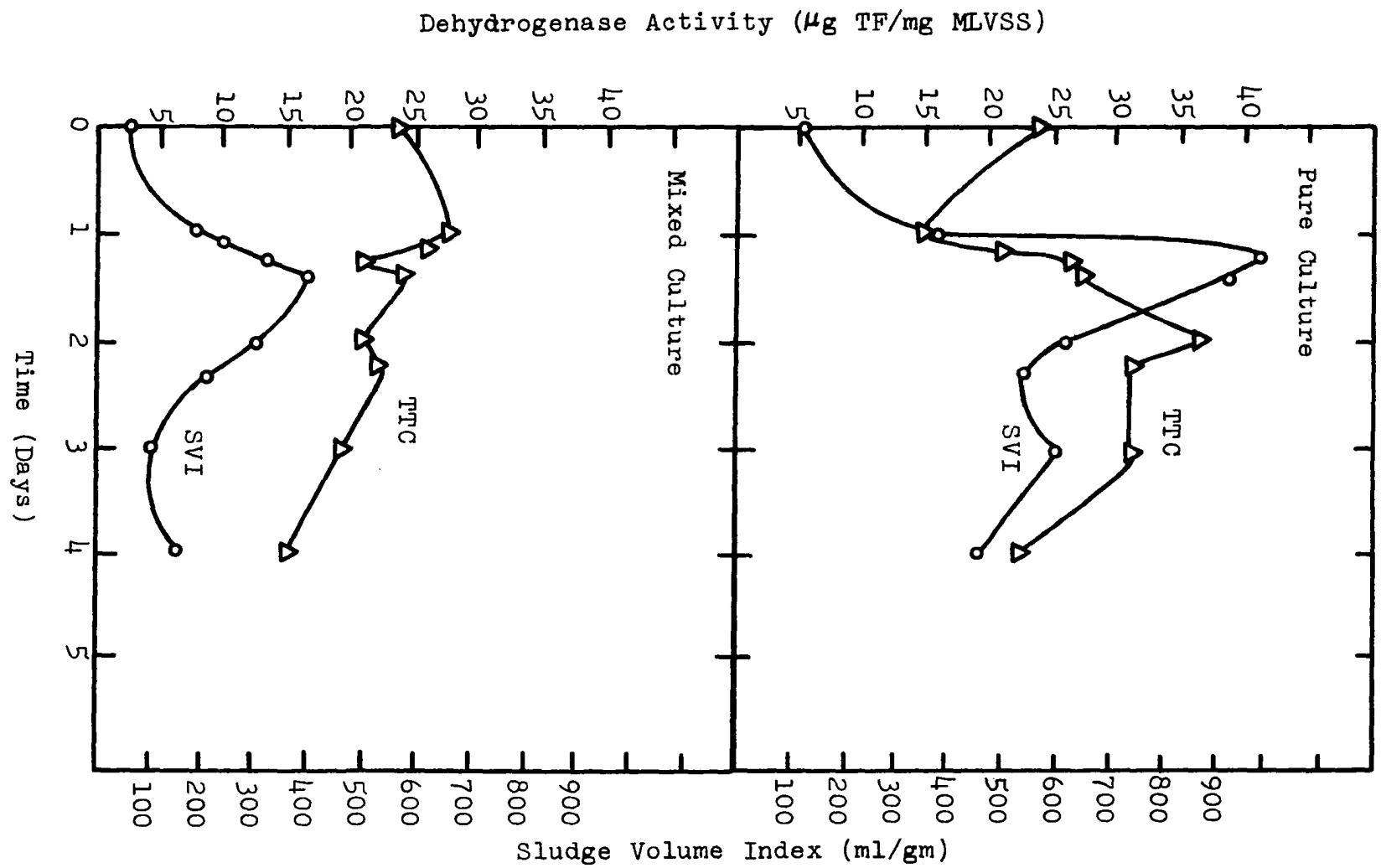

Figure 18. Second Experiment:
SVI and Dehydrogenase Activity for Pure
and Mixed Cultures of S. natans vs. Time

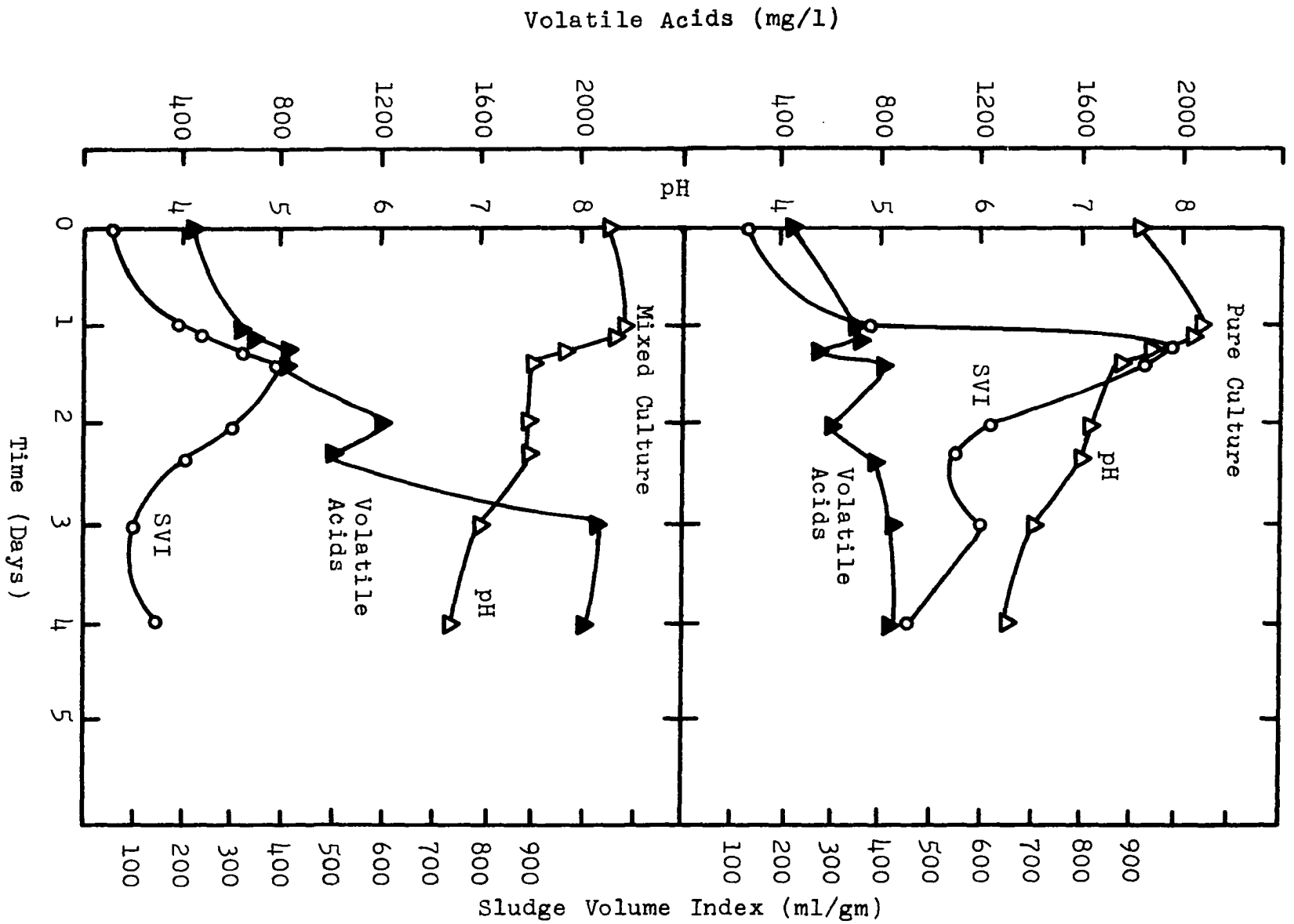

Figure 19. Second Experiment:
SVI and pH and Volatile Acids for Pure and Mixed Cultures of S. natans vs. Time

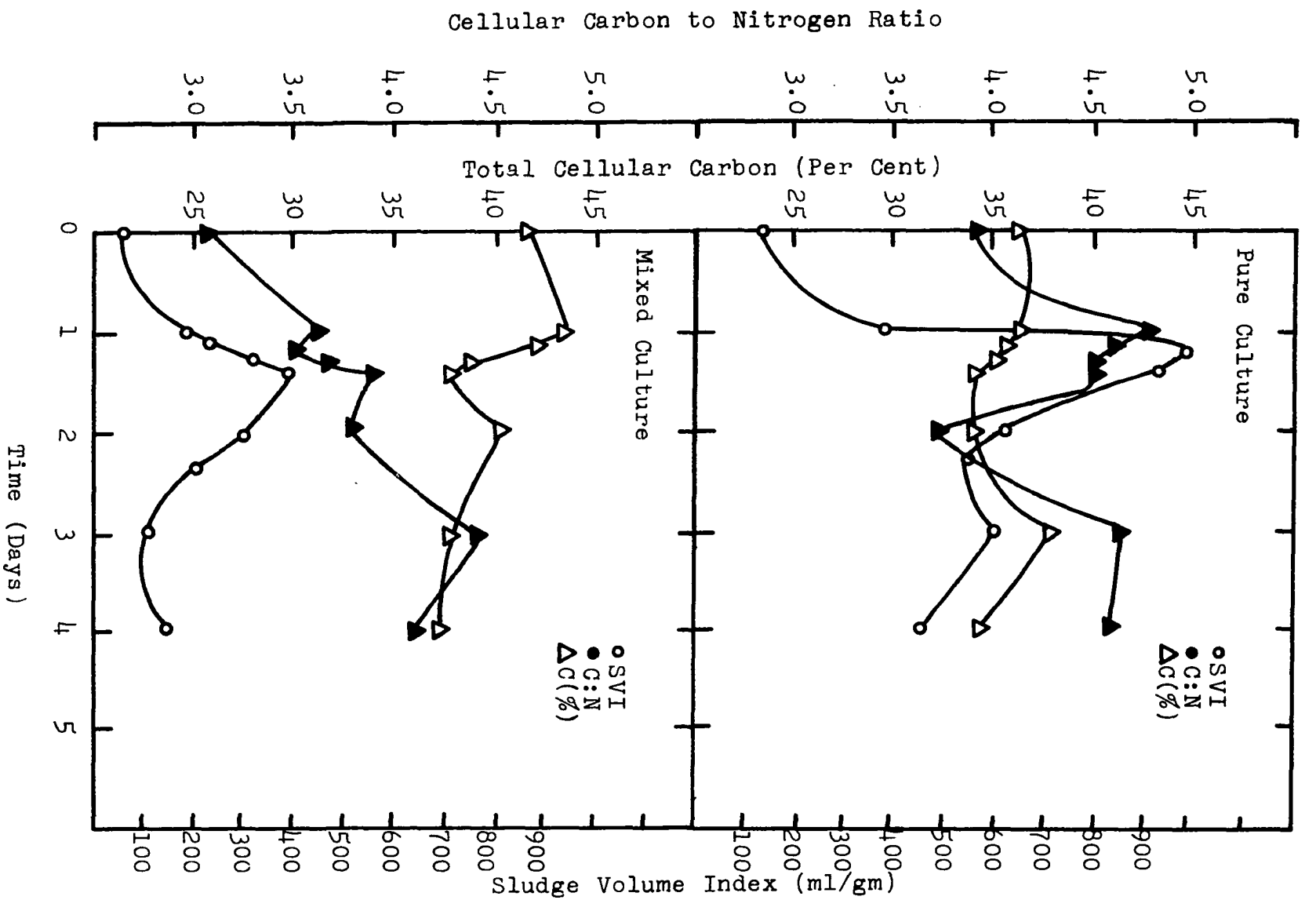

Figure 20. Second Experiment:
SVI and Carbon:Nitrogen Ratio and Total Cellular Carbon for
Pure and Mixed Cultures of S. natans vs. Time

decreases in pH occur while changes in SVI are most drastic. This might suggest that while the SVI is increasing, the cellular activity and rate of CO_2 production are also increasing. As far as the volatile acids concentration is concerned, no major increases are observed; there was an increase and decrease, and a continued increase associated with the higher organic loading velocity, which was similar to changes observed with other parameters.

In the mixed culture (Figure 19), pH again decreases throughout the time interval, with the most rapid changes occurring while the SVI increases are most severe. The volatile acids concentration increases to a serious level by day three. The decreased concentration at day two indicated that a buffering capacity existed, but it was not strong enough to neutralize the organic acids. The high increase from day three to day four may well be due to the high concentration of organic acids.

Either the buffer capacity of the pure culture was much greater than that of the mixed culture, or the mixed population produced a much higher concentration of volatile acids than S. natans. Regardless, S. natans could make an important contribution to the buffer capacity of the activated sludge process. It should also be pointed out that the very high volatile acids concentration in the mixed culture did not result in a severe case of bulking.

The carbon-nitrogen ratio and per cent cellular carbon

are plotted in Figure 20 with the SVI over the test period. In the pure culture experiment, the per cent cellular carbon increases sharply after the loading velocity is increased. Next, it decreases and levels off for several hours, followed by a decrease resulting in a minimum at day two. Meanwhile, the C:N ratio has decreased slightly, and the SVI has reached a maximum and is decreasing. During this initial period, the per cent nitrogen in the cell mass must have increased at a greater rate than the per cent carbon. Indications are that the high per cent carbon resulted in the high SVI and bulking; the increase in per cent nitrogen while per cent carbon remained constant, plus the reduction in per cent carbon, resulted in better sludge settling characteristics. At day three, per cent cellular carbon, C:N ratio, and SVI are each increasing; at day four, per cent cellular carbon, C:N ratio, and SVI are each decreasing.

In the mixed culture experiment (Figure 20), the C:N ratio increases initially and immediately drops, reaching a minimum at the same time that the SVI reaches a maximum. The per cent cellular carbon experiences an increase, decrease, and a continued increase similar to initial changes noted earlier. Apparently, the high per cent cellular carbon and not a high C:N ratio, has resulted in bulking. From day two until day four, C:N ratio decreases and SVI decreases, although at day four SVI is gradually increasing. This increase is probably a reflection of the high per cent

cellular carbon at day three. Figure 11 suggests that a high per cent of cellular carbon rather than a high C:N ratio is more significant in filamentous bulking. It is important to note that in both cultures the increase in per cent cellular carbon precedes the SVI increase. This means that an instrument that measures the total organic carbon could be used to predict bulking a few hours before it becomes severe.

In looking at the experimental period utilizing each of figures 16-20, it is possible to note similar changes in parameters. In the pure culture experiment, the increase in SVI is accompanied by increases in PHB content. Some of this increase due to the polymer is directly associated with the increase in per cent cellular carbon that preceded the increase in SVI. Following the rise in SVI, cell activity increased.

Comparing overall changes in parameters to changes in SVI, the increases in SVI are preceded by increases in per cent cellular carbon and followed by increases in cellular activity. Decreases in SVI are accompanied by decreases in cellular carbon, cell activity, and pH. The data seems to indicate that the initial rise in cellular carbon is a result of the initial increase in PHB content. It is suggested that this initial accumulation is associated with incomplete cell division, resulting in non-active cells and poor sludge settling characteristics.

It should be mentioned that, except for changes in volatile acids concentration, each parameter undergoes changes significantly more severe in the pure culture than in the mixed culture experiment. The buffer capacity in the pure culture is probably greater than that in the mixed culture.

Third Experiment

Figures 21-25 describe experimental data obtained during an eleven day test period in the third investigation. This data is listed in the appendix in Tables 14 and 15. The parameters used for evaluation are identical to those used during the second experimental test period. However, this test period is eleven days and the increase in organic loading associated with the parameter changes is somewhat greater.

Figure 21 indicates that population changes occur throughout the test period; this cyclic nature is probably a result of the unusually high organic loading. Rather than discussing each figure in isolation, both the pure and mixed cultures will be discussed with reference to changes in each of the test parameters.

The SVI of the pure culture was extremely high at day one, practically constant from day two through day six, decreasing from day six to day ten, and increasing at day eleven. The initial increase in SVI is most strikingly associated with an increase in PHB content and pH. The decrease in SVI that follows is accompanied by sharp decreases

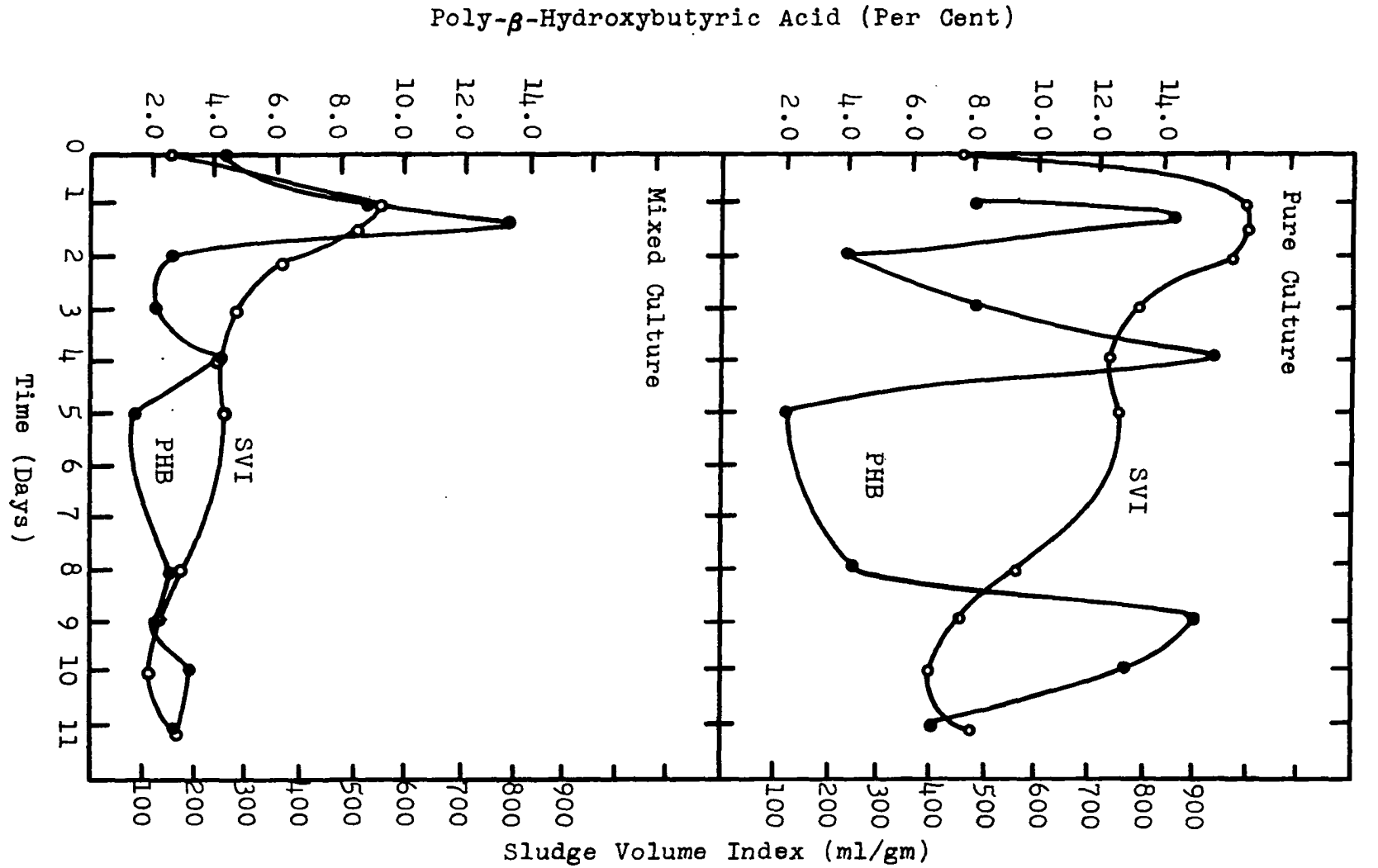

Figure 21. Third Experiment:
SVI and PHB for Pure and Mixed Cultures
of S. natans vs. Time

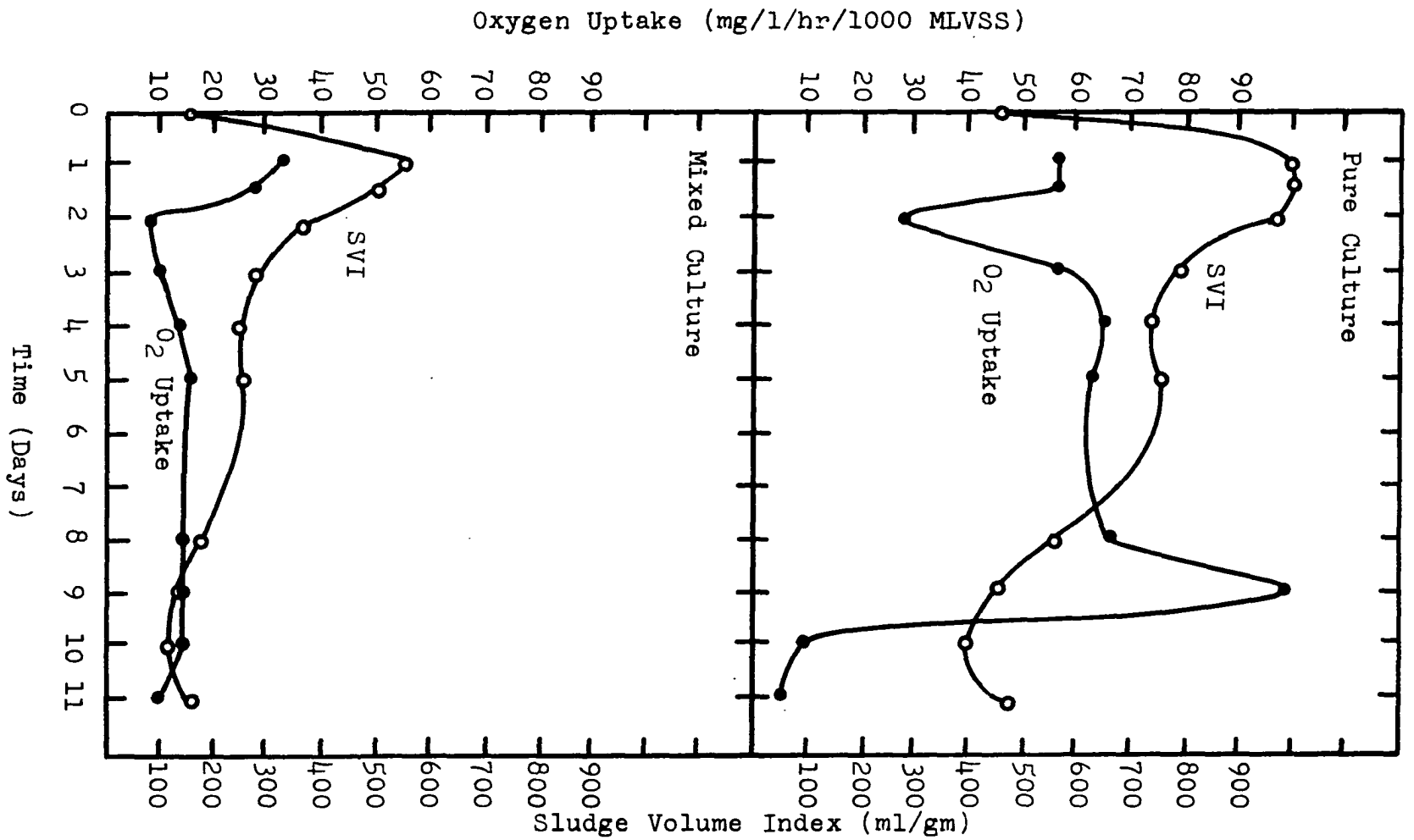

Figure 22. Third Experiment:
SVI and Oxygen Uptake for Pure and Mixed Cultures of S. natans vs. Time

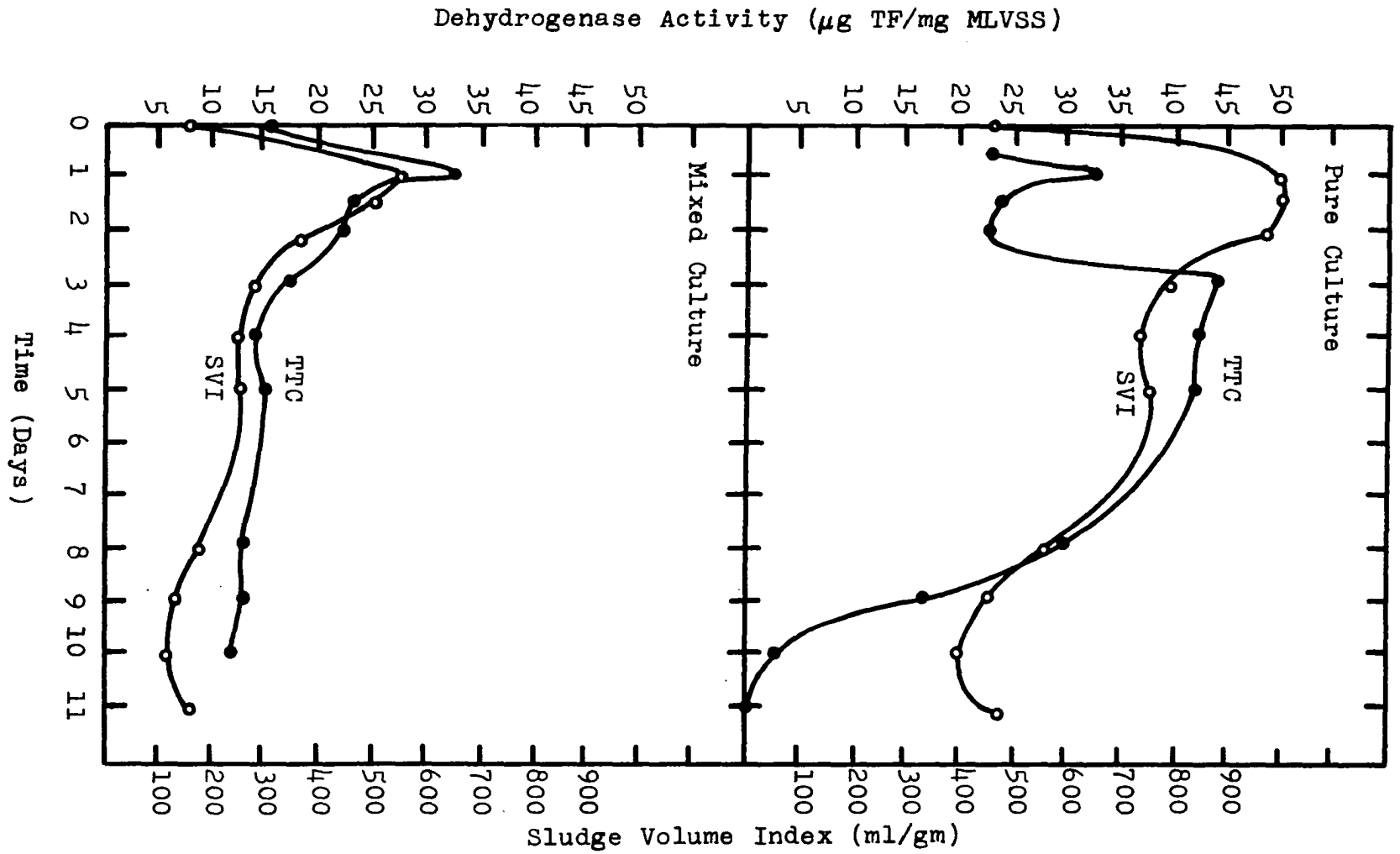

Figure 23: Third Experiment:
SVI and Dehydrogenase Activity for Pure and
Mixed Cultures of S. natans vs. Time

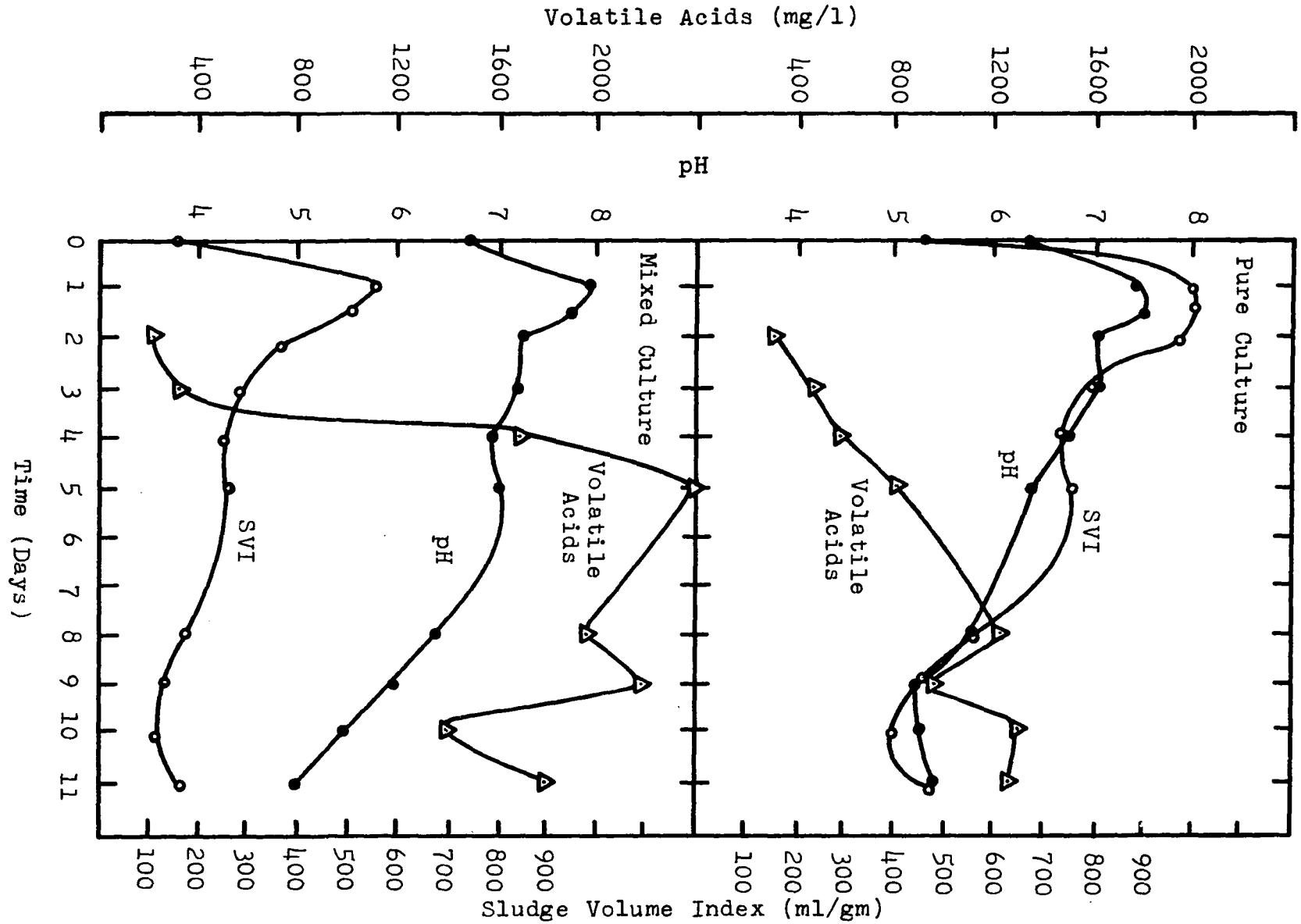

Figure 24. Third Experiment:
SVI and pH and Volatile Acids for Pure and Mixed Cultures of S. natans vs. Time

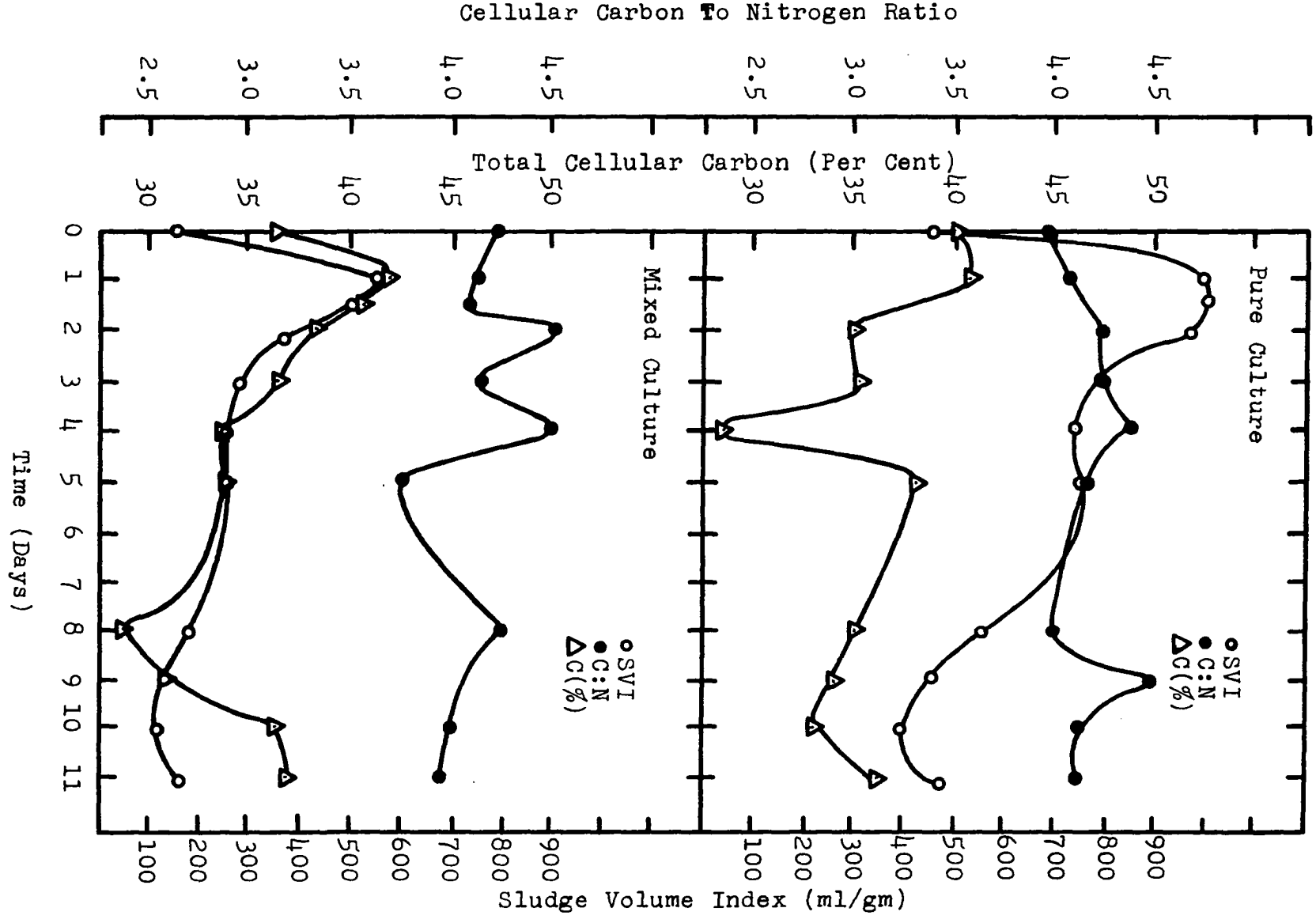

Figure 25. Third Experiment:
SVI and Carbon:Nitrogen Ratio and Total Cellular Carbon for
Pure and Mixed Cultures of S. natans vs. Time

in PHB and cellular carbon content, as well as a decrease in pH and a decrease in cell activity as shown both by decreases in oxygen uptake and triphenylformazan produced. The interruption of decreasing SVI with four days of constant SVI is caused by increases in PHB content and per cent cellular carbon. Increases in cellular activity and volatile acids concentration and decreases in C:N ratio and pH are also involved. The following period of decreasing SVI is characterized by low PHB, decreasing per cent cellular carbon, decreasing cellular activity, and decreasing pH. The final increase in SVI, noted on the eleventh day, is preceded by a major increase in PHB content, which is reflected in a high C:N ratio. The per cent cellular carbon does not increase until day ten, which means that some of the carbon may already be in the cell and for some reason is accumulated as PHB at day nine. These results cannot be clearly understood. An incongruity occurs with cell activity in that oxygen uptake records an increase in activity at the time PHB is being accumulated, while the TTC procedure does not show any increase. Also of interest is the decrease in volatile acids that occurs at this time.

The SVI in the mixed culture experiments, described in figures 21-25 is a maximum at day one, decreases until day four, and remains practically constant until day eleven. The initial SVI increase was accompanied by an increasing PHB content, cellular carbon content and cellular activity, and pH; it is followed by a continued increase in PHB content.

The decrease in SVI that followed was accompanied by decreased PHB content and decreased per cent cellular carbon content and cellular activity. A minor increase in SVI on day five is directly related to a second accumulation of PHB on day four. This PHB accumulation is reflected in an increase in cellular carbon content. Volatile acids data is interesting in that, during the last seven days, the concentration was extremely high. It should be emphasized that this did not make any obvious change in the settling characteristics of the sludge. Generally, the correlation between changes in PHB content and total cellular carbon, and between cellular activity measured by oxygen uptake and by TTC, were better for the mixed culture than for the pure culture of S. natans.

Fourth Experiment

The fourth investigation included both continuous and batch-fed aeration experiments. Results from a four day test period, utilizing the continuous-feed aeration systems used in all prior experiments, are displayed in Figures 26-27. Two cultures are included: the first contains a sludge in which filamentous organisms are the dominant type; the second contains a sludge in which filamentous organisms are present but not the dominant type. In comparing changes in SVI, there are no major differences between the filamentous-dominant and filamentous-present sludges. Microscopically, it appeared that both reactors were experiencing

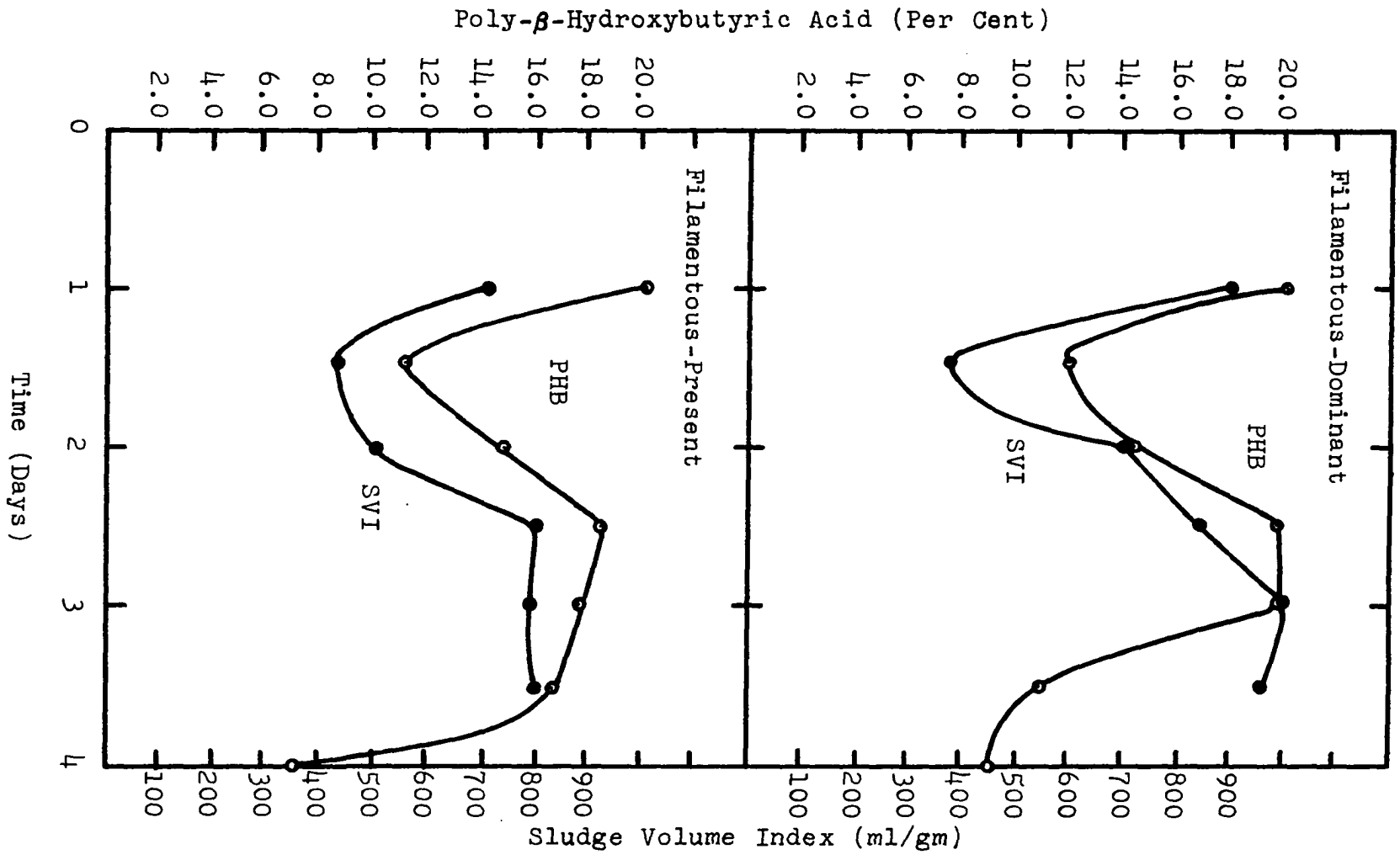

Figure 26. Fourth Experiment:
SVI and PHB for Filamentous-Dominant and
Filamentous-Present Sludges vs. Time

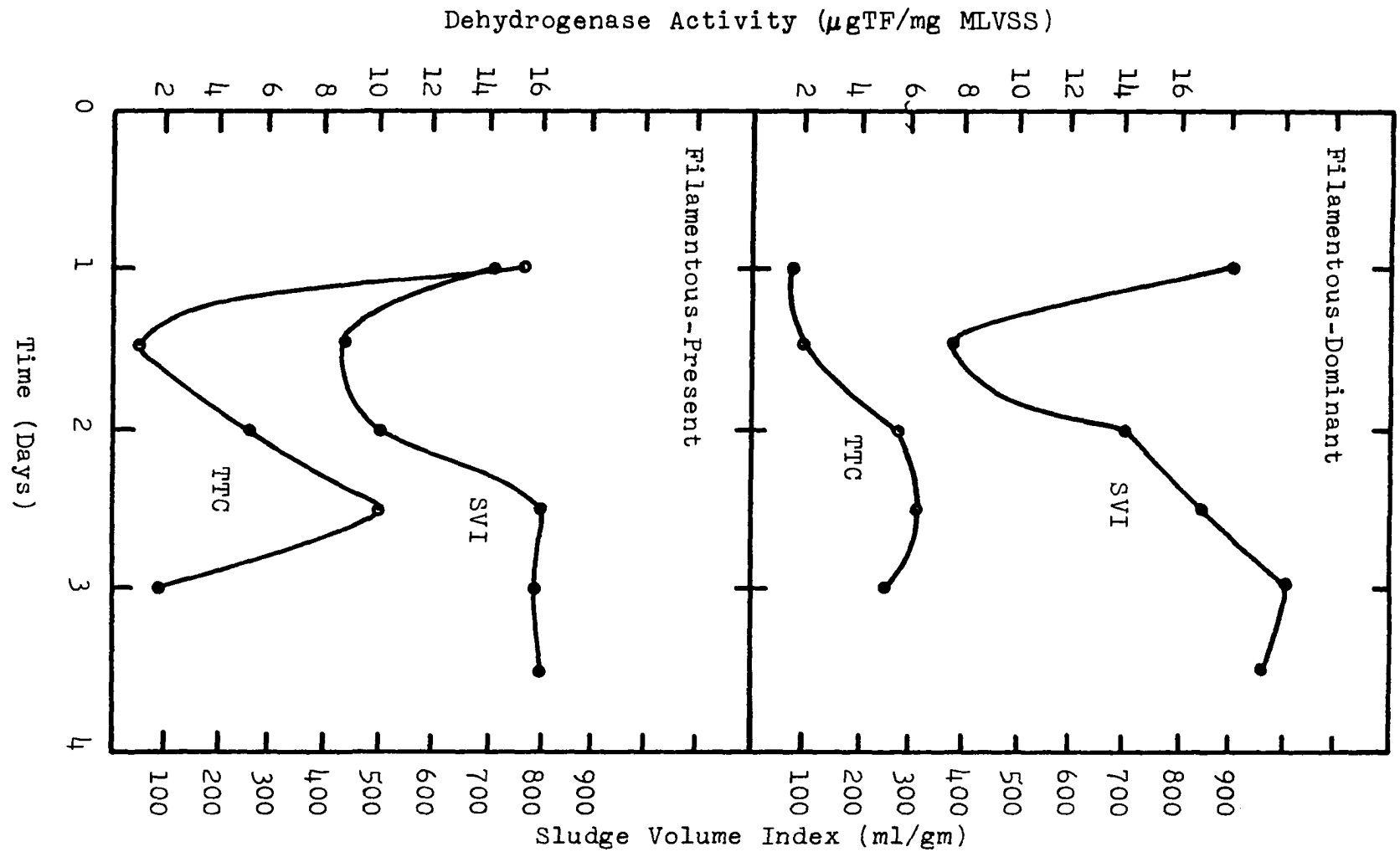

Figure 27. Fourth Experiment:
SVI and Dehydrogenase Activity for Filamentous-Dominant and Filamentous-Present Sludges vs. Time

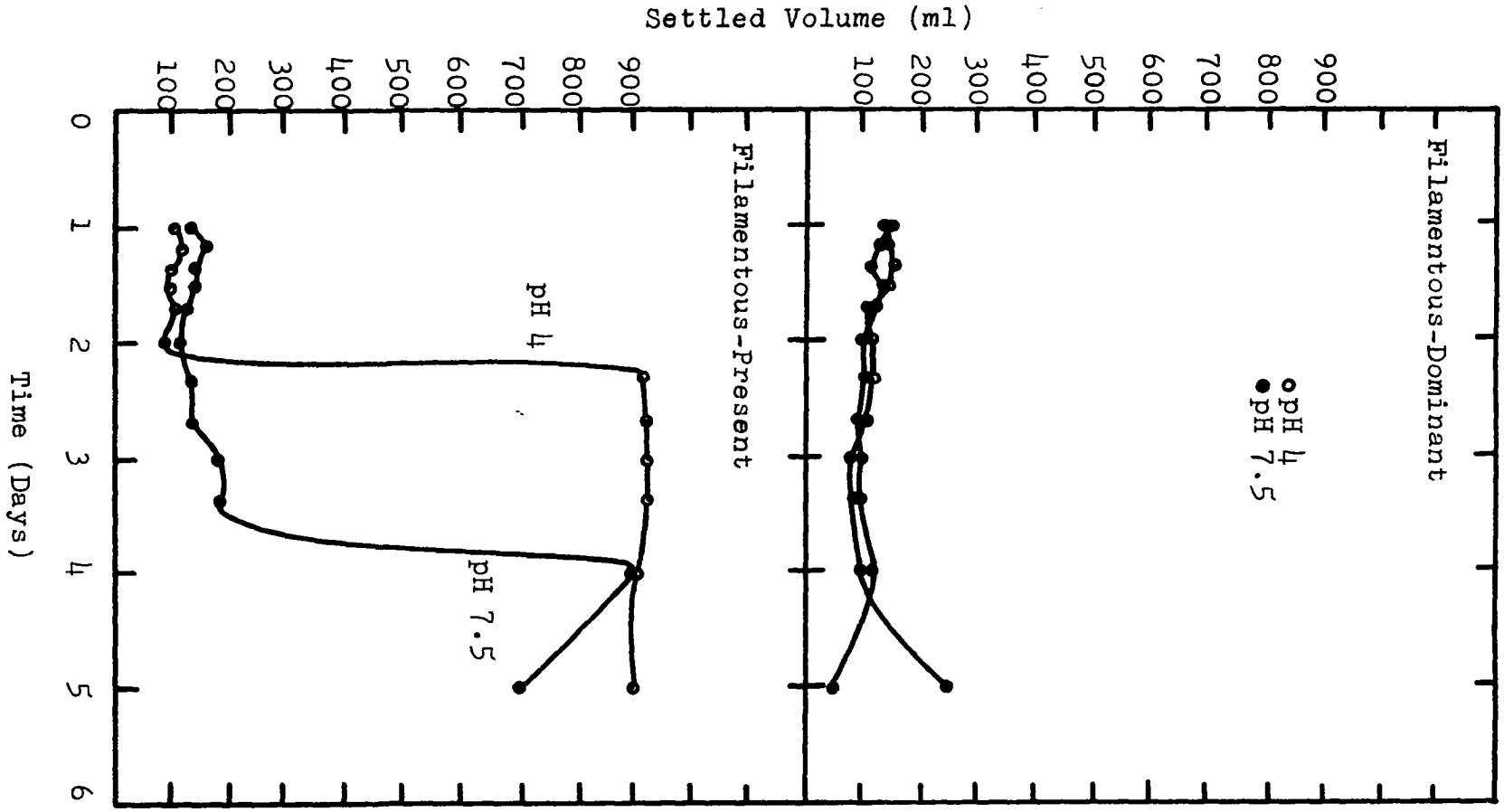

Figure 28. Batch Aeration Study:
Settled Volumes at pH 4 and pH 7.5 for Filamentous-Dominant and Filamentous-Present Sludges vs. Time.

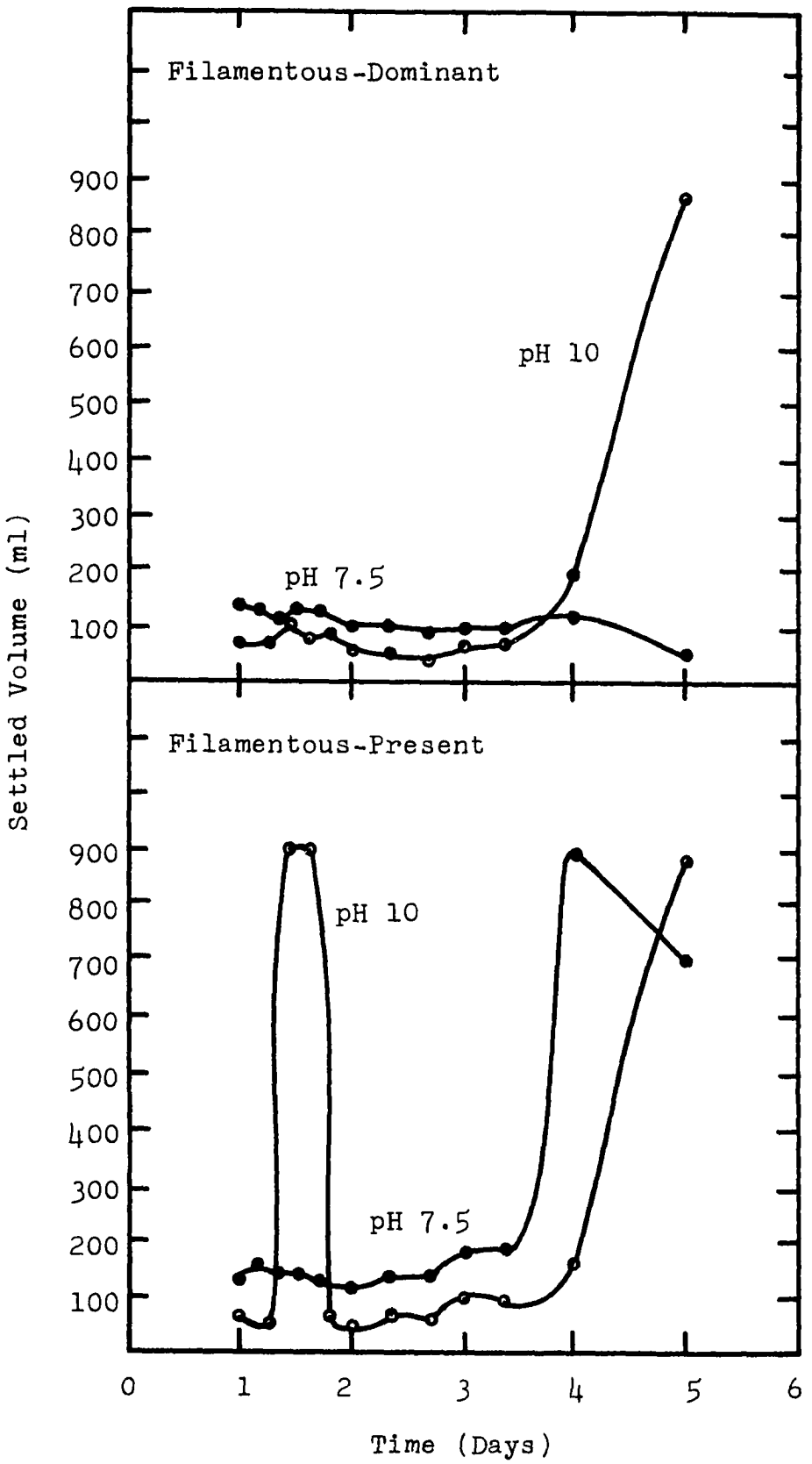

Figure 29. Batch Aeration Study: Settled Volumes at pH 10 and pH 7.5 for Filamentous-Dominant and Filamentous-Present Sludges vs. Time

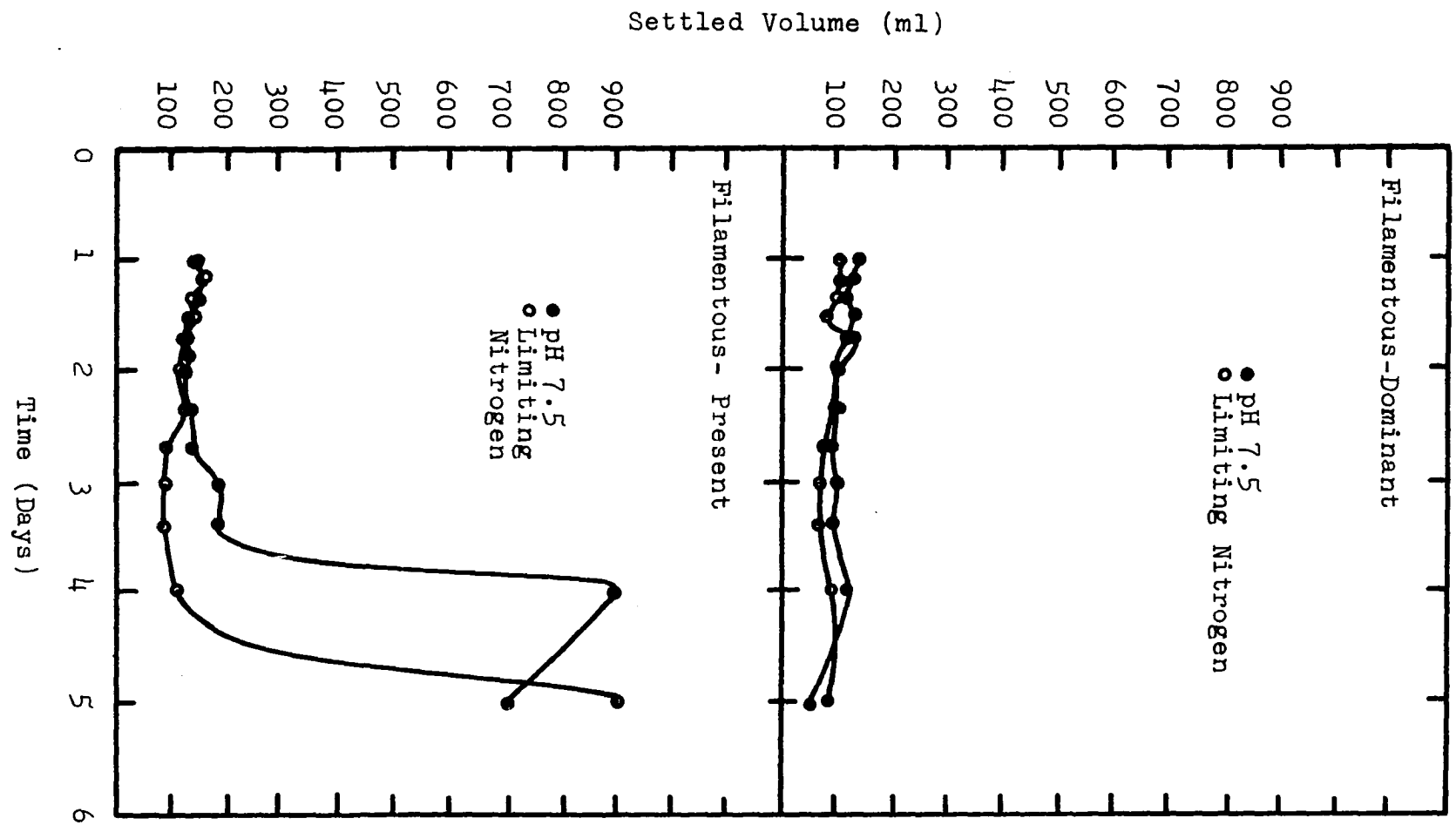

Figure 30. Batch Aeration Study:
Settled Volumes Under Condition of Limiting Nitrogen and at pH 7.5 for Filamentous-Dominant and Filamentous-Present Sludges vs. Time

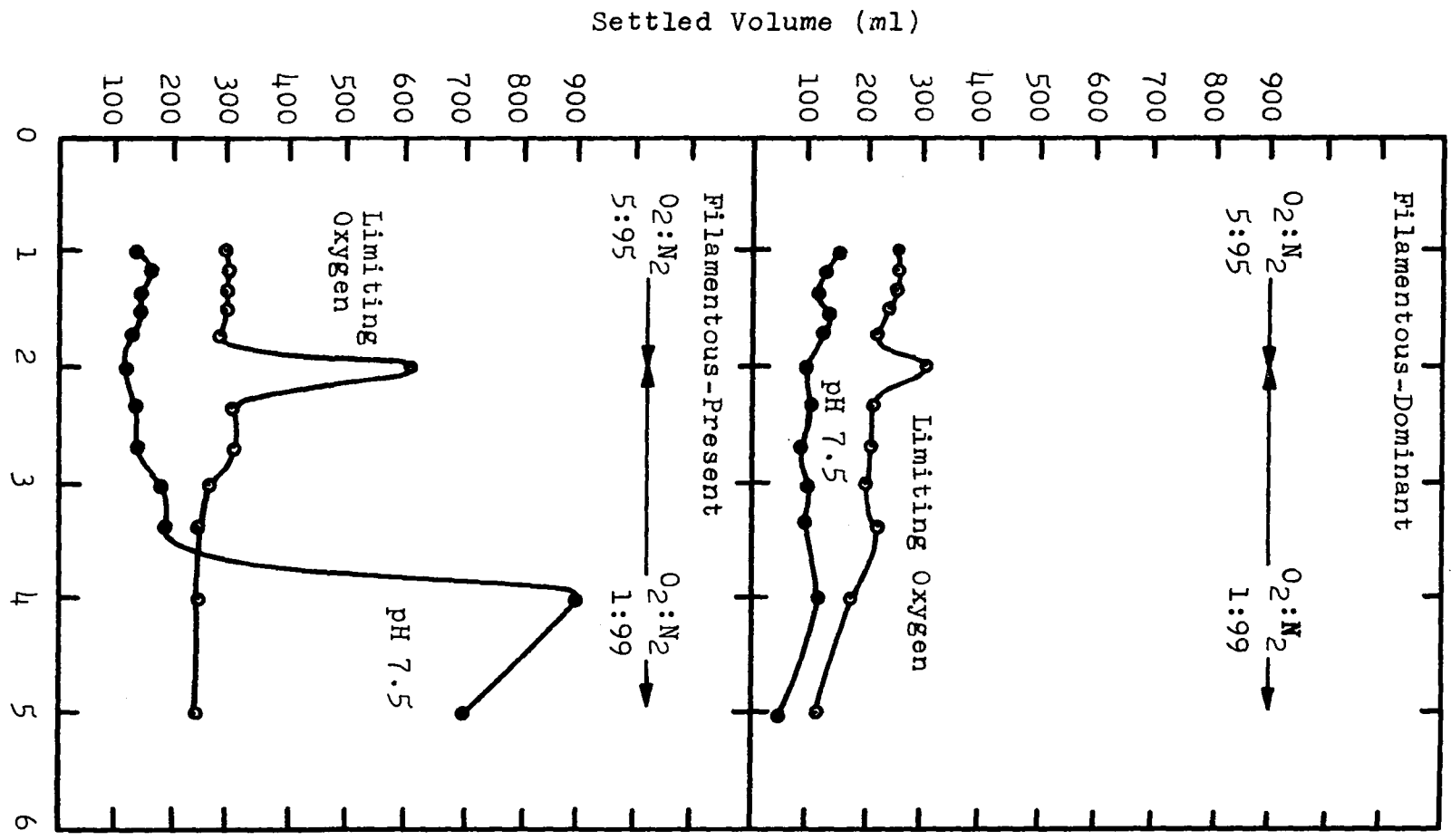

Figure 31. Batch Aeration Study:
Settled Volumes Under Condition of Limiting Oxygen and
at pH 7.5 for Filamentous-Dominant and
Filamentous-Present Sludges vs. Time

a case of filamentous bulking. This suggests that an overgrowth of filamentous organisms and bulking results from an increased loading velocity, and that additional numbers of filamentous organisms are not significantly important in causing more intense bulking, or bulking of longer duration. The changes in SVI for both cultures are quite similar to changes in PHB content. In the filamentous-dominant case (Figure 27) there is no significant similarity between SVI changes and changes in cellular activity. However, there is a similarity between these two parameters for the sludge in which the filamentous organisms are present.

Results from the batch-fed aeration experiments are illustrated in Figures 28-31. The two sludges used were filamentous-present and filamentous-dominant cultures similar to those used in the continuous-aeration experiments. The test period extended for five days.

In Figure 28, filamentous-dominant and filamentous-present sludges at pH 7.5 are compared to cultures in which the pH had been adjusted to pH 4.0. The filamentous-dominant plot shows that there are no major effects from the lowered pH. At day five, the culture at pH 4 is undergoing a significant increased settled volume compared to the culture at pH 7.5. Therefore, changes from lowered pH are more important in the filamentous-present culture than in the filamentous-dominant culture. At pH 4, the settled volume is a maximum a few hours into day two; this volume

remains high throughout the period. The pH 7.5 culture reached a maximum at day four and this volume decreased considerably at day five.

Figure 28 suggests three conclusions: first, there is only a minor tendency toward a higher settled volume (SV) in the decreased pH study involving the filamentous-dominant culture; second, there is a major tendency, both in intensity and duration, toward a high SV in the pH study involving the filamentous-present sludge; finally, there is an important tendency toward a significantly higher, more serious increased SV when the sludge has filamentous organisms present than when filaments are dominant. This suggests that, in the batch culture, filamentous organisms are able to cope with depressed pH better than nonfilamentous organisms.

Figure 29 compares two sludges at pH 7.5 to similar cultures in which the pH has been increased to pH 10. In both the filamentous-present and filamentous-dominant cases, the increased pH resulted in more serious increases in SV. In the case of the filamentous-present sludge, there is both an immediate and a delayed increase in SV. The conclusions from Figure 29 are that the increased pH results in higher SV's for both sludges and that the increased SV noted for the filamentous-present culture is more serious than for the filamentous-dominant culture.

Generally, it can be stated that both high and low pH are capable of causing increased SV and bulking, and that the bulking may be less severe in cases in which filamentous organisms are present in greater numbers. This result is such that more filamentous organisms do make a difference in the severity of bulking when the bulking results from an increased or a decreased pH. When bulking was induced by an increase in loading velocity in continuous aeration studies (Figure 12), the result was that additional filamentous growths did not affect the severity of bulking. Therefore, the effect of additional growths of filamentous organisms is sometimes, but not always, an important consideration in suggesting bulking controls. Other parameters must be taken into account.

Figure 30 shows that cultures in which the nitrogen concentration has been limited, while other factors have remained the same, do not experience more serious bulking. Although no nitrogen was found at day two (in solution), it is possible that enough cellular nitrogen remained, even after five days, to prevent any significant change in the SV. Nevertheless, the results indicate a nitrogen deficient waste will not immediately result in bulking. As found with pH changes, the limiting nitrogen content apparently has a greater effect on the SV of the filamentous-present culture than the sludge with a majority of filamentous organisms. This suggests something of a buffer capacity exerted by

filamentous organisms during environmental extremes.

Figure 31 includes the same two sludges at pH 7.5 compared to the sludges grown under conditions limiting oxygen supply. The results employing an O_2:N_2 supply of 5:95 seem more conclusive than when the supply was 1:99. The initial decreased oxygen supply, experienced by both sludges from day one to day two, resulted in increases in the SV of each sludge. The SV increase by the filamentous-present sludge was more intense than the increase by the filamentous-dominant sludge.

The batch aeration study resulted in the following conclusions: increased and decreased pH, limiting nitrogen content, and limiting oxygen supply. Each resulted in a higher SV for the filamentous-present than for the filamentous-dominant sludge. Each parameter resulted in an increased SV which became more serious as the filamentous sludge content decreased.

In order to evaluate more thoroughly the causes of increased SV noted in the batch studies, PHB content, cellular activity, and volatile acids concentration were measured at the beginning of the study and 72 hours later. Table 8 lists the changes in PHB, TTC, and volatile acids, content resulting from the 72 hour aeration period, each of the four environmental conditions, and the presence of additional filamentous organisms. The changes attributed to the environmental conditions have accounted for changes resulting from aeration

TABLE 8

Filamentous Growth Responses From
Batch Aeration Studies

Cause of Parameter Change	Resultant Parameter Change		
	PHB Content	Dehydrogenase Activity	Volatile Acids
Aeration Period (72 Hours)	- 2.91	+ 1.43	+ 970
Increased pH (pH 10)	- 1.45	+10.40	+1120
Decreased pH (pH 4)	- 0.90	0.00	+ 40
Limiting Nitrogen	+11.33	+ 9.02	- 35
Limiting Oxygen	+ 3.60	+ 6.00	+ 475
Additional Filamentous Organisms	- 1.55	+ 4.94	- 123

time and those attributable to additional filamentous organisms. The change in parameter content from the filamentous-dominant sludge to the filamentous-present sludge is designated as the change resulting from additional filamentous organisms.

From Table 8 there is apparently an important increase in volatile acids noted during the aeration period alone. This increase, together with an increased cellular activity, is important in the batch system at pH 10. There are no obvious changes in any of the recorded parameters in the pH 4 experiment. In the nitrogen limiting experiment, there are major increases in PHB content and cellular activity. Noteworthy in the oxygen limiting experiment are increases in each of the three parameters. The important change attributed to the presence of additional filamentous organisms is an increase in cellular activity without a significant increase in PHB content. This suggests that S. natans and other filamentous organisms may have the enzyme system necessary to limit their PHB content before accumulation results in cellular inactivity.

It is obvious that these three parameters do not completely explain the increased SV noted in Figures 28-31. At a given time, under a given set of environmental conditions, one factor may be of such importance that it can control the growth response. As conditions change, this factor may become less important while another factor or other factors

become important in determining the response. It is likely that there are interaction effects which make interpretations of a growth response extremely complicated.

Figures 32-33 illustrate the response of the 30-minute settled volume test to the increase in organic loading velocity. Figure 32 presents an 8-day sequence for the filamentous-present sludge, and Figure 33 presents an 8-day sequence for the sludge in which filamentous organisms were the dominant form. Table 9 lists the settling rate, compaction rate, and SVI for each sludge during the 8-day sequence. It is important to note that none of this data was useful in predicting bulking. There are only small decreases in the settling rate and small increases in the compaction rate. Parameters other than settled volume must be used to predict bulking. It is interesting to note that there is a point, when the sludge settleability is improving, at which the settling rate equals the compaction rate.

The sequence indicates that bulking usually occurs rapidly, while the recovery from bulking occurs slowly. It also indicates that the important subsidence rate is the settling rate and that it is bulking's effect on the settling rate that results in plant failure.

Figure 34 describes the responses of filamentous-present and filamentous-dominant sludges to the addition of either high molecular weight or low molecular weight dextran. It has previously been shown that it is possible for dextran

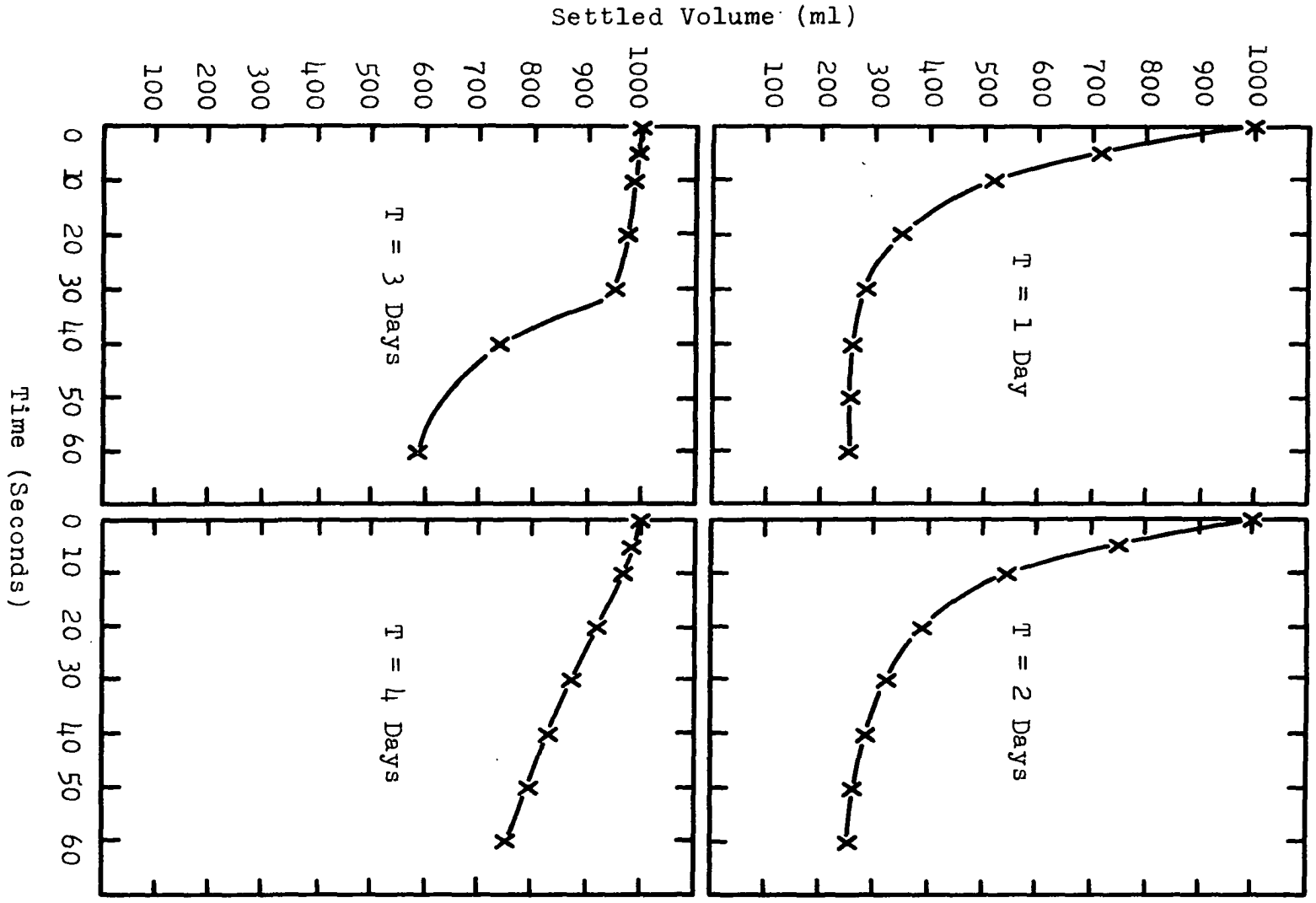

Figure 32. Fourth Experiment:
Settled Volume Sequence (8 Days) for
Filamentous-Present Sludge

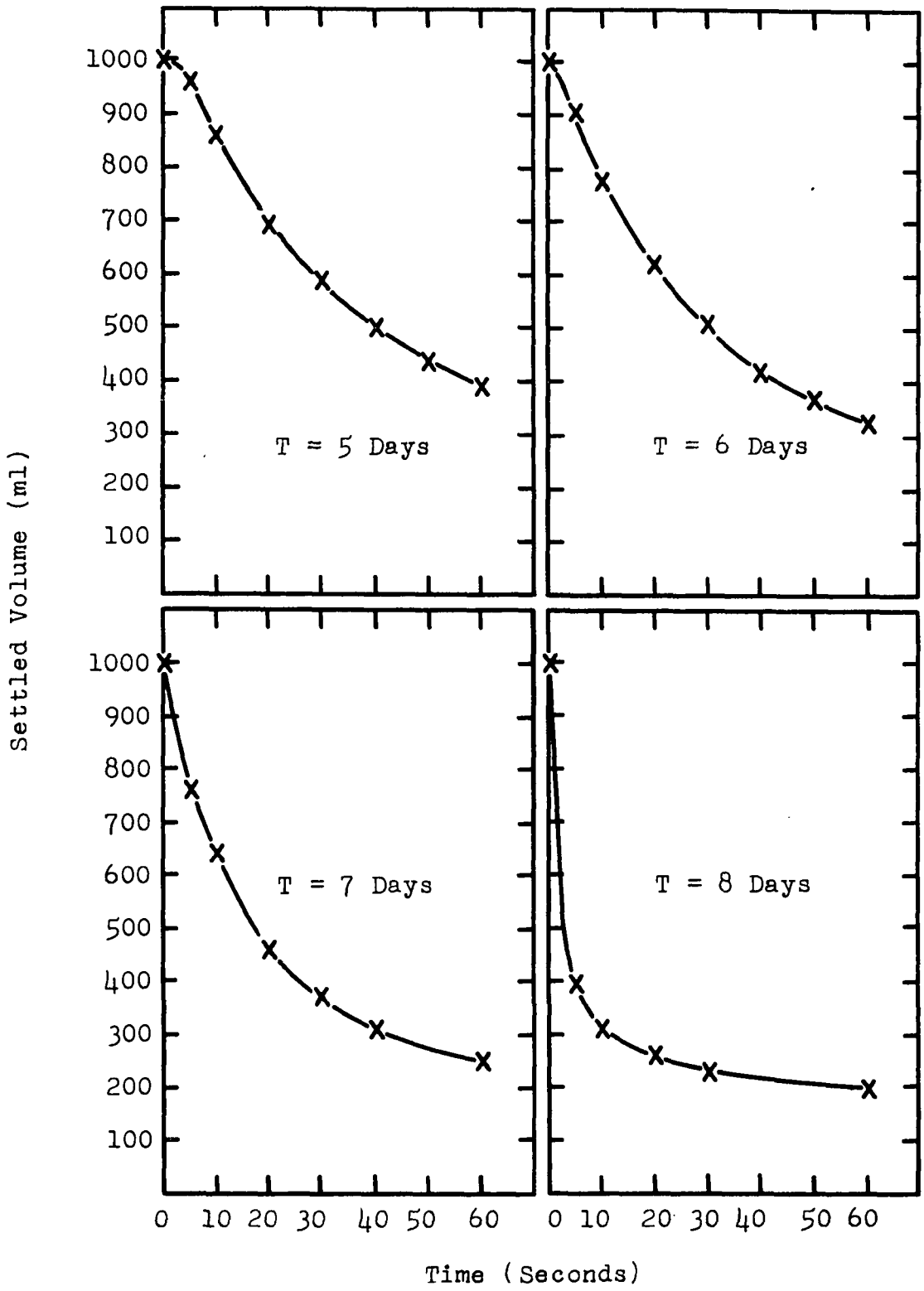

Figure 32. (Continued)

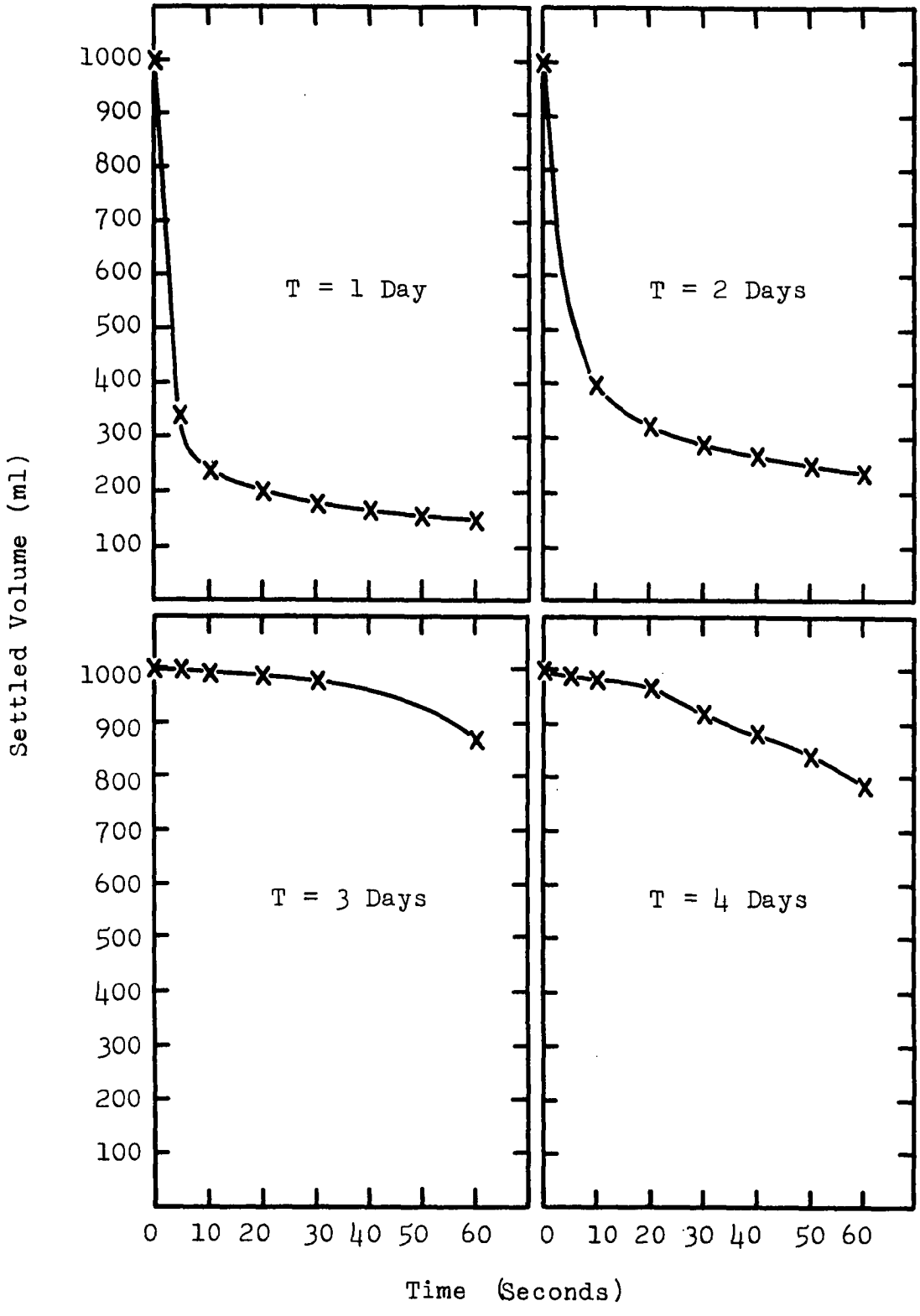

Figure 33. Fourth Experiment: Settled Volume Sequence (8 Days) for Filamentous-Dominant Sludge

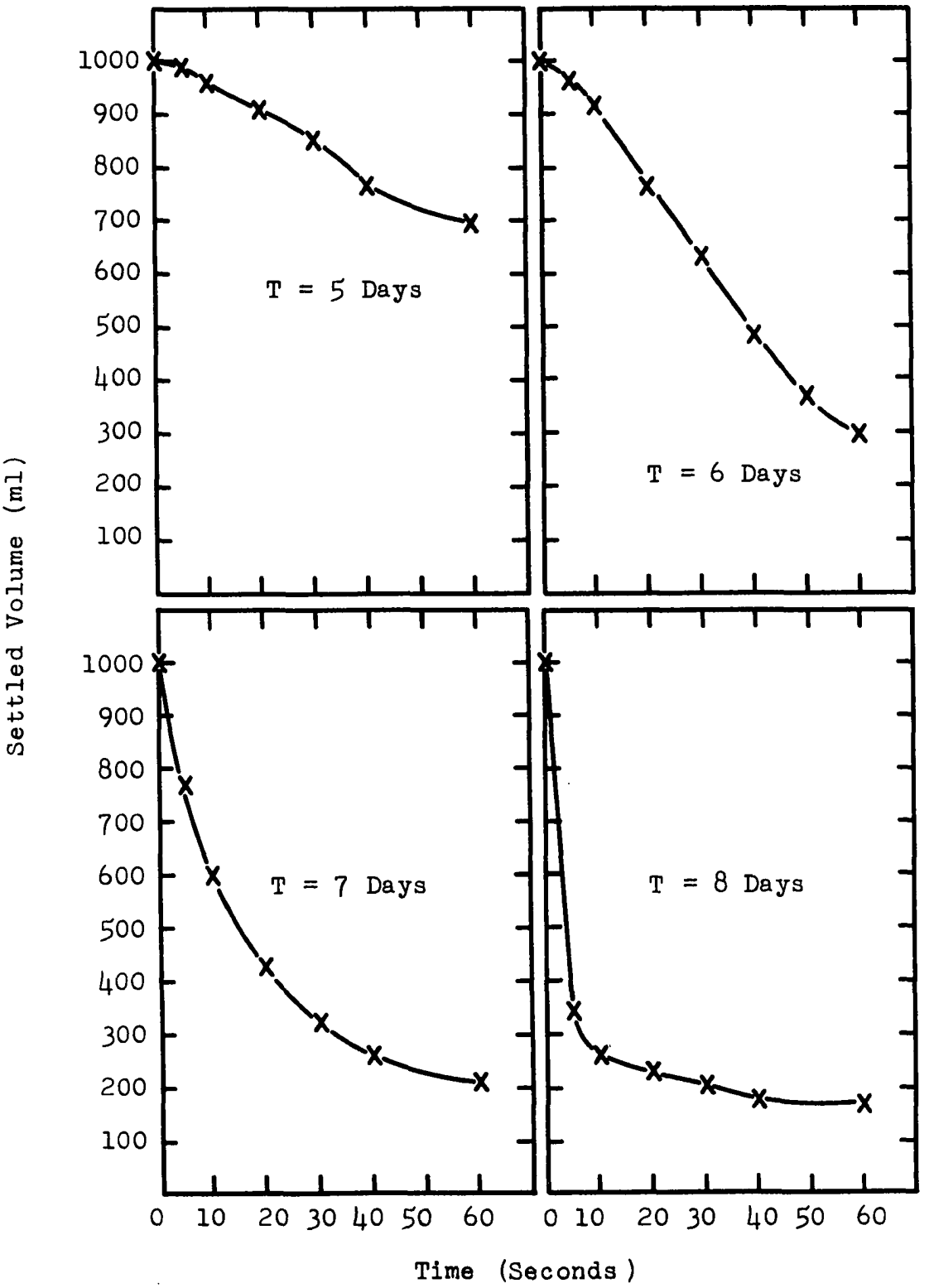

Figure 33. (Continued)

TABLE 9

Settling Characteristics During
Settled Volume Studies

	Filamentous-Present Sludge			Filamentous-Dominant Sludge		
Time (Days)	Settling Rate (ml/min)	Compaction Rate (ml/min)	SVI (ml/gr)	Settling Rate (ml/min)	Compaction Rate (ml/min)	SVI (ml/gr)
1	23.8	1.2	182	27.3	1.0	132
2	22.5	2.3	188	23.7	1.8	174
3	1.6	12.0	674	0.7	3.7	859
4	4.3	4.0	700	2.7	4.3	950
5	13.7	6.7	486	5.0	5.0	895
6	16.3	6.0	410	12.3	11.0	738
7	21.0	4.0	306	22.5	3.8	371
8	25.7	1.0	214	26.5	1.1	140

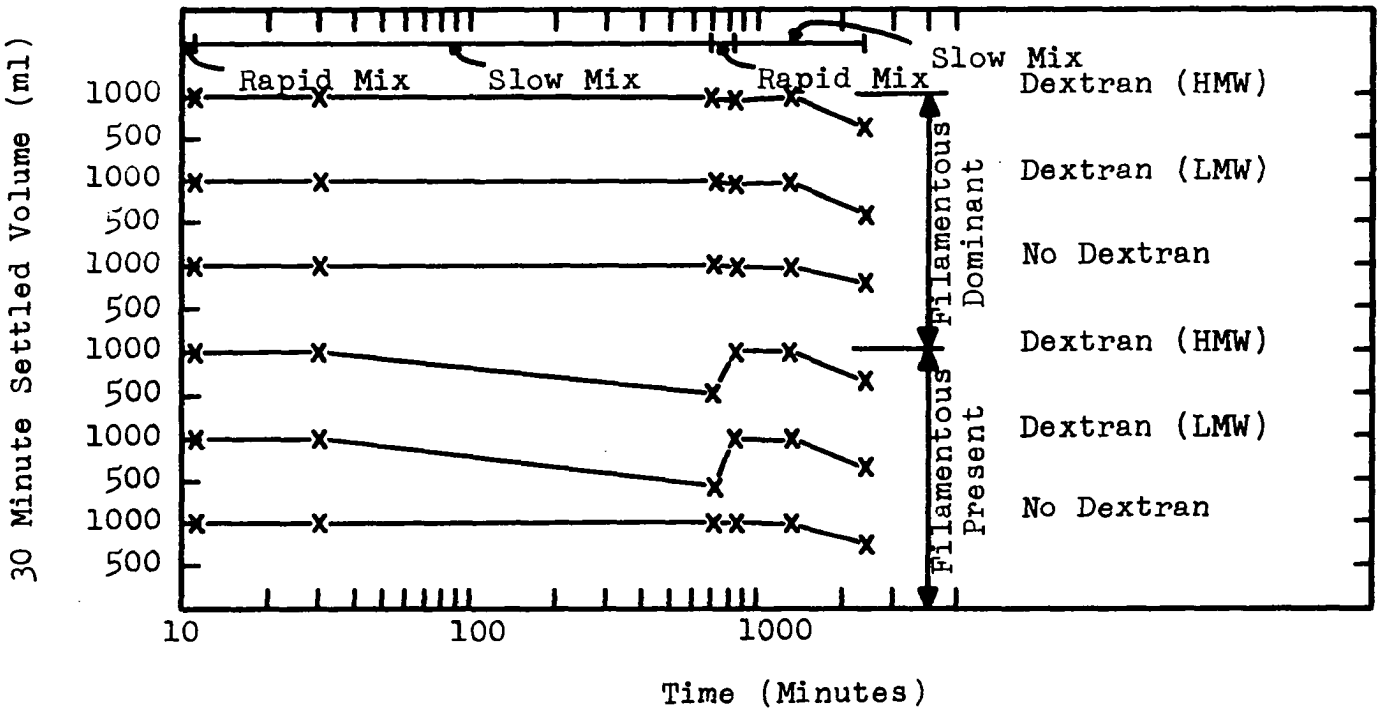

Figure 34. Dextran Study:
30-Minute Settled Volumes for Filamentous-Dominant and Filamentous-Present Sludges vs. Time

to be incorporated into bacterial cells as capsular material. The 30 minute settled volume test, which was used as the response parameter, was measured as described earlier. After the addition of dextran to the sludge samples, the results of the first two 30 minute SV tests were unchanged. In both filamentous-present and filamentous-dominant sludges there were definite particle agglomerations with low molecular weight dextran and larger particle agglomerations with the high molecular weight dextran. No clumping was visible in the sludges to which no dextran had been added. Furthermore, the amount of clumping of sludges to which dextran had been added was less noticeable for the sludge with more filamentous organisms.

After 12 hours of slow mixing, there were noticeable changes in the SV. For the filamentous-present sludge, the decrease in SV was greatest for the sample with low molecular weight dextran; the decrease in SV for the sample with high molecular weight dextran was less, but still significant. The sample to which no dextran had been added did not have a noticeable SV change. Microscopically, all three cultures had approximately the same amount of filamentous organisms. Both forms of dextran reduced the SV after 12 hours and not after one-half hour. The simpler, low molecular weight dextran resulted in a better SV reduction than the more complex, high molecular weight dextran. Although direct physical attachment of particles bridged to the polymer was possible,

no significant initial SV reduction occurred. Therefore, it was postulated that the material was incorporated into sheath or capsular material which resulted in successful flocculation. Two hours after a second amount of polymer was added to the respective samples, each sample had recorded nearly a maximum SV.

After 24 additional hours, all samples had a reduced SV. For both sludges, the samples with low and high molecular weight dextran had approximately the same SV, and an SV less than that for the sample with no dextran. Since many hours were required for a reduction in the SV, it was again believed that the polymer affected the SV through incorporation into capsular material rather than through a direct physical contact.

In the first dextran experiment (Figure 34), only minor changes occurred in the SV for the samples of filamentous-dominant sludge, while the second experiment showed significant changes resemoling those for filamentous-present culture. The additional filamentous organisms either were not able to convert the polymer into capsular material directly or were unable to rapidly accumulate enough material to offset the physical effects of the filaments. Thus it may be assumed that the nonfilamentous bacteria had a more effective role in flocculation than did the filamentous organisms.

System Dynamics

This section pertains to changes in the process--changes in the organic removal rate, changes in the adsorptive capacity of the suspended solids, and changes in the suspended solids. The changes in the relative composition of the suspended solids will also be discussed. This data (composition of suspended solids) is listed in the appendix in Tables 12 and 13 for the second investigation and in Tables 16 and 17 for the third investigation.

Figures 35-37 are plots of organic removal rates versus time for both pure and mixed cultures of S. natans. The upper graph, (influent COD - soluble effluent COD)/(influent COD), is the per cent of influent COD removed by the system, or the total removal. The lower graph, (total ML COD - soluble effluent COD)/(MLVSS), is the number of pounds of COD removed from the system per pound of VSS, or the system removal.

In the preliminary experiment (Figure 35) the removal rates for the pure culture are similar to each other and to the SVI (See Figure 10). This figure shows that the removal rates increase as the SVI increases and they decrease as the SVI decreases. It appears that the removal rate, after its initial increase attributable to bulking, is not so high as it was originally. The two removal rates for the mixed culture are not particularly similar to each other or to the SVI (See Figure 10). It is important to mention that both

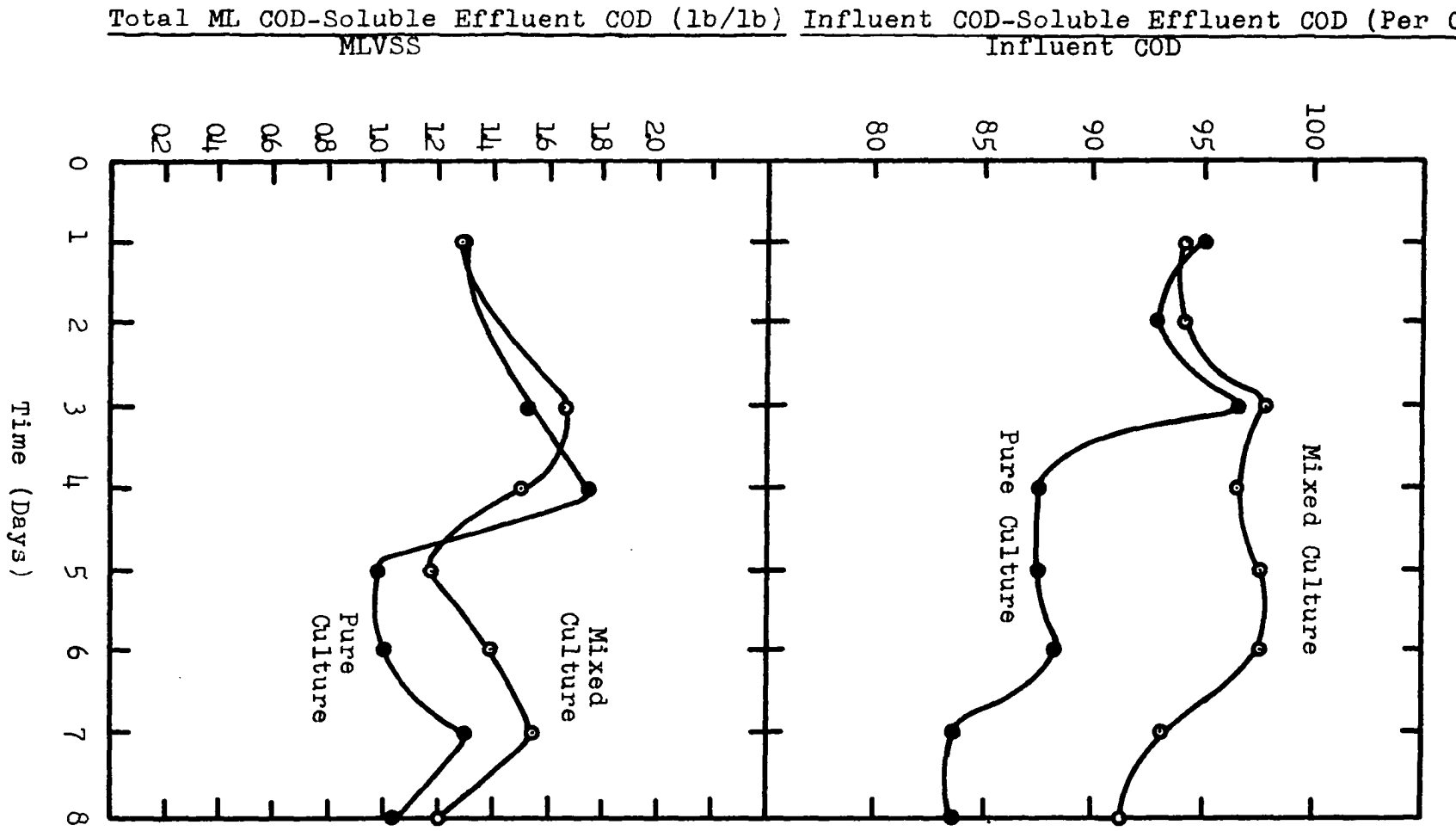

Figure 35. Preliminary Experiment:
Total Influent Removal and Removal from Process Vessel
for Pure and Mixed Cultures of S. natans vs. Time

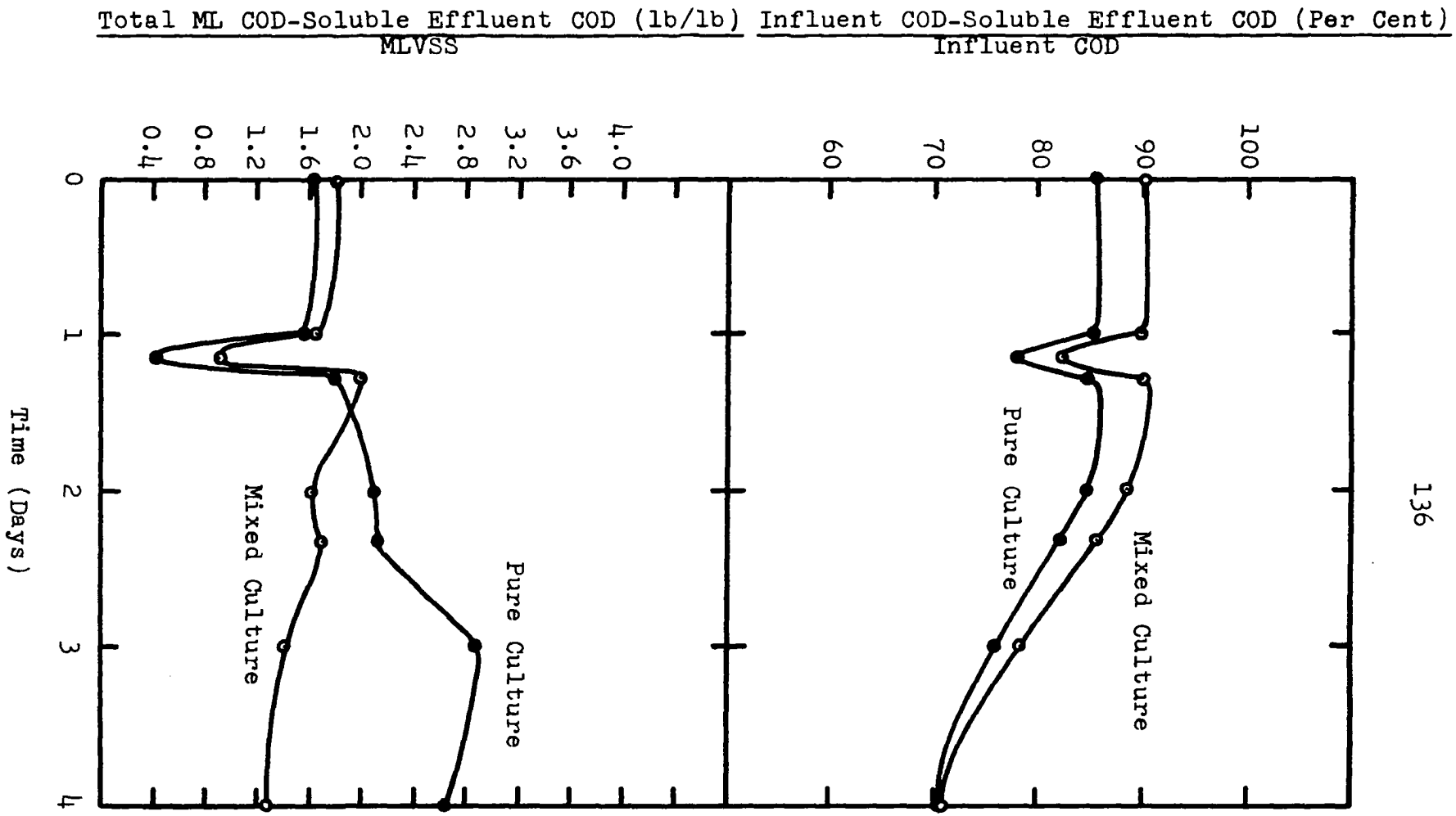

Figure 36. Second Experiment:
Total Influent Removal and Removal from Process Vessel for
Pure and Mixed Cultures of S. natans vs. Time

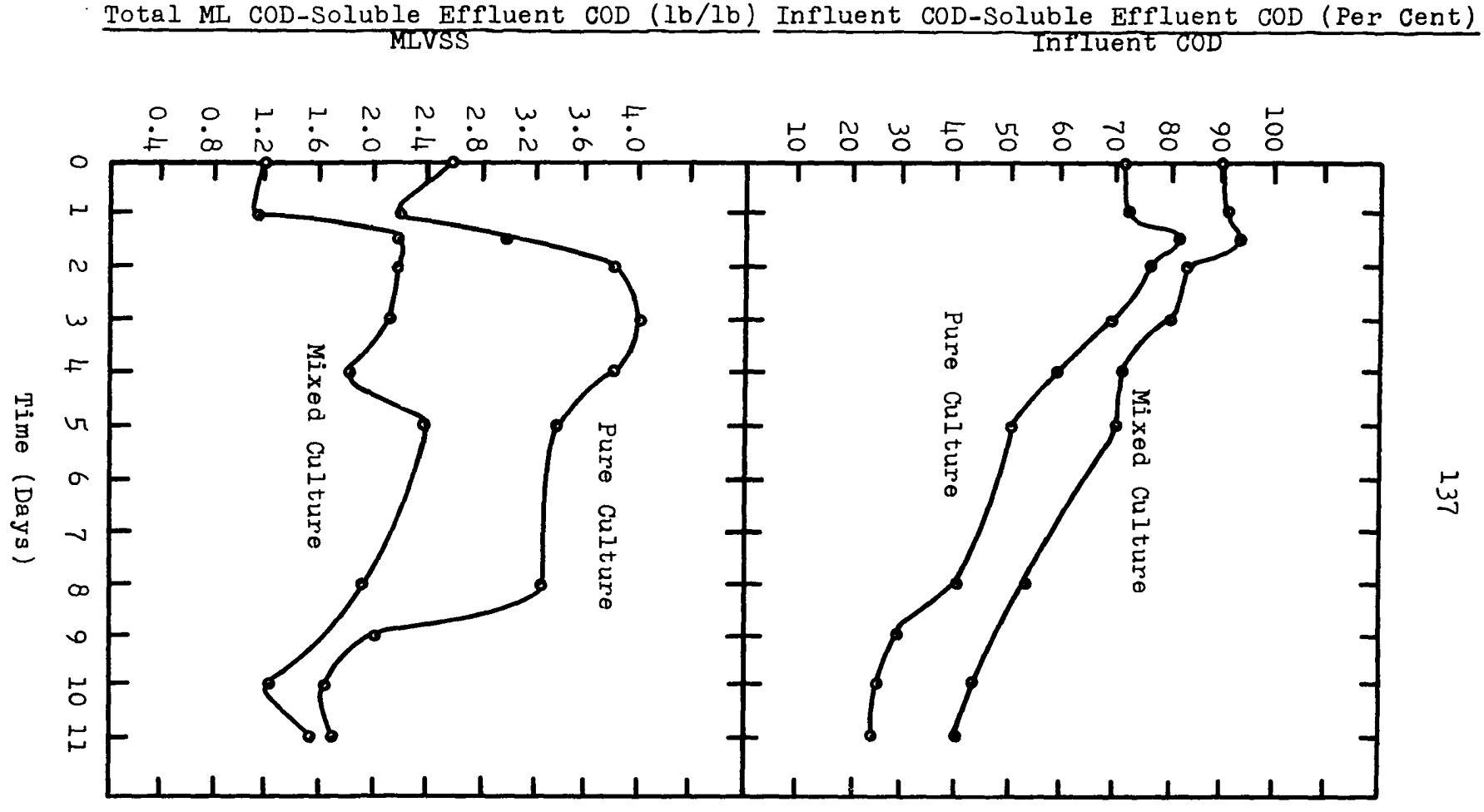

Figure 37. Third Experiment:
Total Influent Removal and Removal from Process Vessel for
Pure and Mixed Cultures of S. natans vs. Time

removal rates are higher for the mixed culture than for the pure culture.

In Figure 36, for the second experiment, the two removal rates for both pure and mixed culture are immediately depressed by the initial increase in loading velocity. After the recovery of the rate of total removal by the system, there is a decrease in the removal efficiency for both cultures throughout the remaining time period. This indicates that bulking results in severe effects in both systems. After the recovery of the system removal rate, there are decreases in the removal efficiency of the mixed culture similar to that noted from the other removal rate. However, the removal efficiency by the pure culture during the same time period remained constant and then increased to a maximum at day three and decreased from day three to day four. These differences in removal rates indicate that the VSS does affect the system and the removal efficiency. The severe bulking apparently affected the mixed culture more than the pure culture; the pure culture, after an extended lag period, increased its removal efficiency significantly.

In Figure 37, for the third experiment, the total removal by the system for both cultures undergo initial increases followed by significant decreases throughout the remainder of the time period. There are indications that bulking has resulted in decreased removal efficiences. In the system removal, there are initial increases and high

removals until the end of the time period. A comparison of these curves with Figure 11 shows a similarity with the corresponding SVI. In the earlier experiments, no relationship was indicated. It is possible that the increased loading resulted in a bulking that was not so severe as earlier. There does not seem to be a lag period or any significant effects from the bulking.

In summary, Figures 35-37 include two removal rates that describe the system differently. The total removal by the system indicates that major effects in the removal efficiency resulted from bulking and that the removal efficiency for the mixed culture was greater than for the pure culture. The system removal indicates that the degree of bulking is probably very important in determining removal efficiencies following bulking and that the removal efficiency for mixed cultures are not always greater than for the pure culture. Thus, diversity of population does not always increase effectiveness. In each figure, the changes in the system were more likely to be noted in the system removal, the removal from the system, than in the total removal by the system. By including the concentration of suspended solids in the removal function, it is more sensitive to system changes. On the basis of these three figures, there is an indication of a trend toward a higher removal efficiency at increased SVI as long as bulking is not too severe. This is reasonable in view of the higher ratio of surface area to

volume at increased SVI. This increased ratio is attributable to dispersion of flocs and growth of filamentous organisms. However, under severe bulking, efficiency does drop and there are major changes in the system activities. It appears that cellular growth characteristics are affected.

Figures 38-40 describe the adsorptive capacity of the suspended solids. The upper graph, (total ML COD - soluble ML COD)/MLSS, is the grams of COD in the suspended solids per gram of suspended solids in the mixed liquor aeration tank. The lower graph, (total effluent COD - soluble effluent COD)/MLSS, is the grams of COD in the suspended solids per gram of suspended solids in the effluent.

In the preliminary study (Figure 38), both cultures undergo changes in the ML COD attributable to the solids. Since the functions include the COD adsorbed and the COD exerted by the suspended solids, an increased function is expected following the increase in loading velocity. The changes in the effluent COD attributable to the solids are more erratic. The pure culture plot resembles the corresponding PHB content plot in Figure 13, while the mixed culture plot does not resemble any single plot. Indications are that the pure culture figure is a function of the COD exerted by the suspended solids, and that changes in the adsorptive capacity of the cells are not important. As for the mixed culture, both changes in the adsorptive capacity and changes in the characteristics of the suspended solids

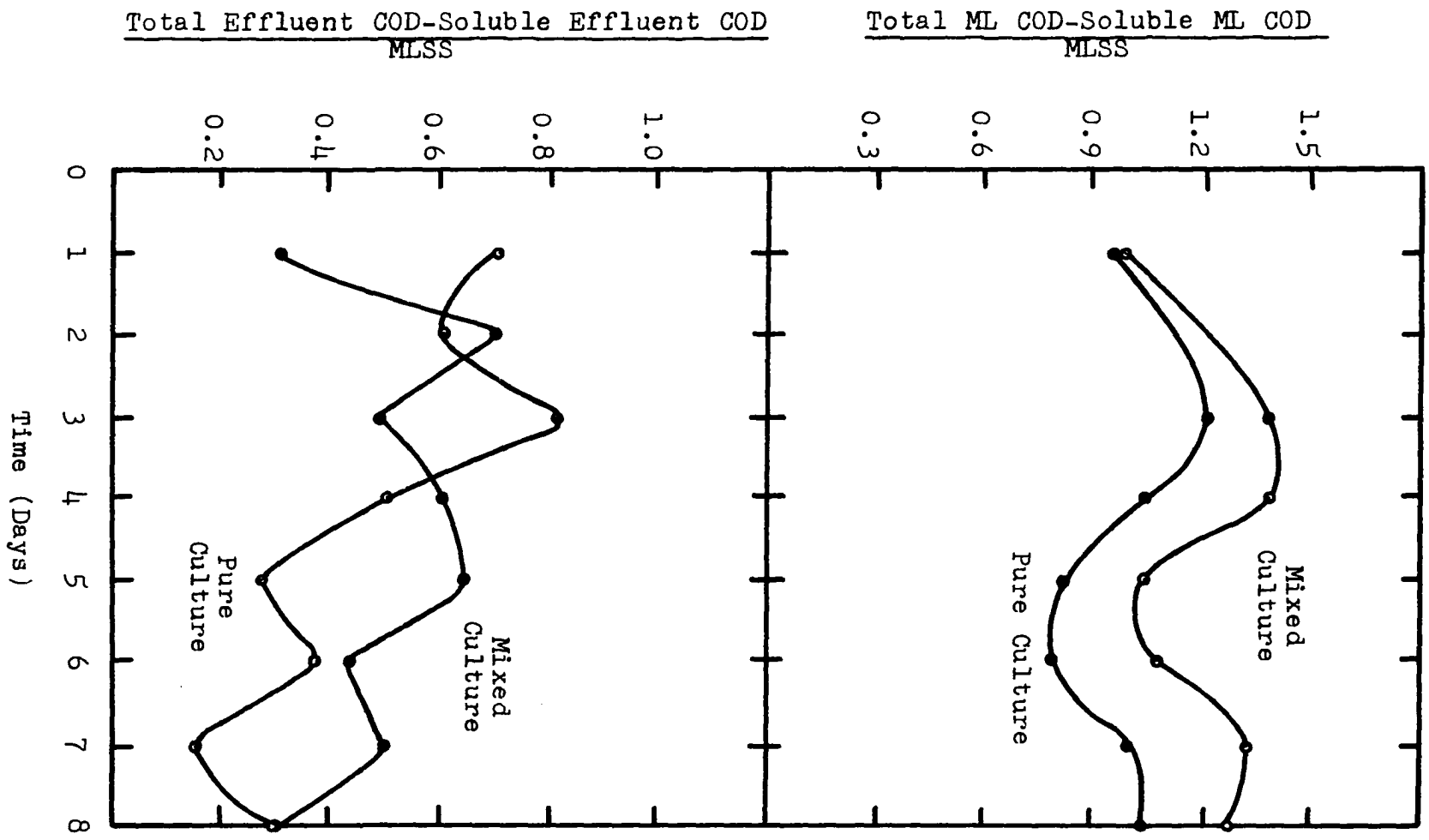

Figure 38. Preliminary Experiment:
Adsorptive Functions of Process from Mixed Liquor and Effluent for Pure and Mixed Cultures of S. natans vs. Time

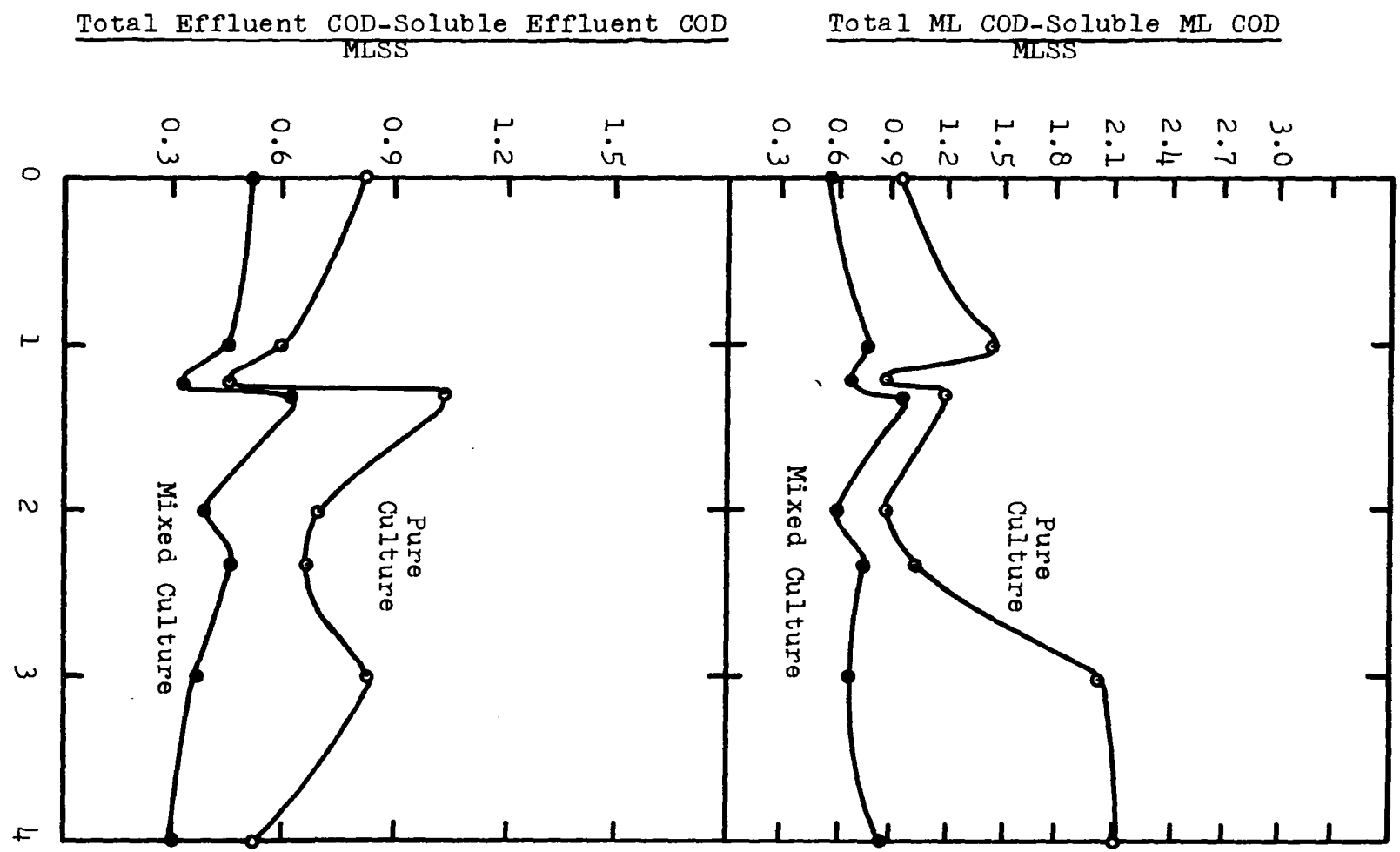

Figure 39. Second Experiment:
Adsorptive Functions of Process from Mixed Liquor and Effluent for Pure and Mixed Cultures of S. natans vs. Time

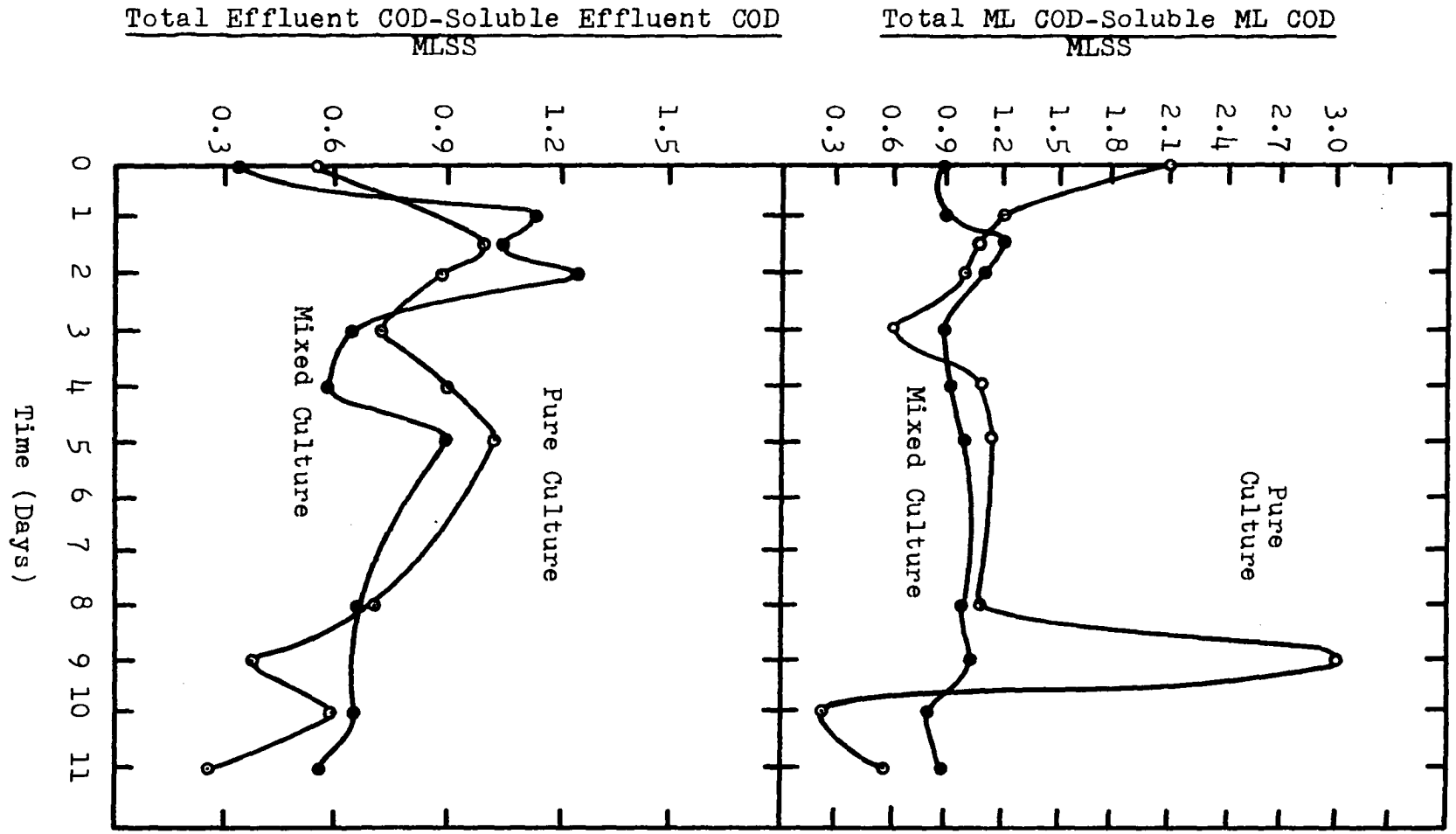

Figure 40. Third Experiment:
Adsorptive Functions of Process from Mixed Liquor and Effluent for Pure and Mixed Cultures of S. natans vs. Time

seem to be important. Interactions make the function extremely complex.

In Figures 39-40, the adsorptive functions are plotted for the second and third experiment. PHB content seems to be important in numerous changes. Since the PHB content of the pure culture is greater than that of the mixed culture (See Figures 16 and 21), it is not possible to conclude that S. natans has a higher adsorptive capacity than other microorganisms.

In Figures 41-43, the total suspended solids (TSS) and volatile suspended solids (VSS) as well as a ratio of volatile to total suspended solids are plotted versus time for both pure and mixed cultures of S. natans. These figures represent the preliminary, second, and third experiments.

In the preliminary experiment (Figure 41) the pure culture TSS and VSS apparently vary somewhat inversely with the SVI (See Figure 10). The mixed culture TSS and VSS, although somewhat erratic, increase throughout the test period. The SVI (See Figure 10) does not appear to be related to either TSS or VSS. For both cultures (Figure 41), the ratio VSS:TSS remains fairly constant. The mixed culture ratio has more variation than the pure culture, but this variation is not extremely important. The pure culture ratio seems to average just above 85 per cent, while the mixed culture ratio averages at approximately 90 per cent. This indicates that the amount of fixed inorganic suspended

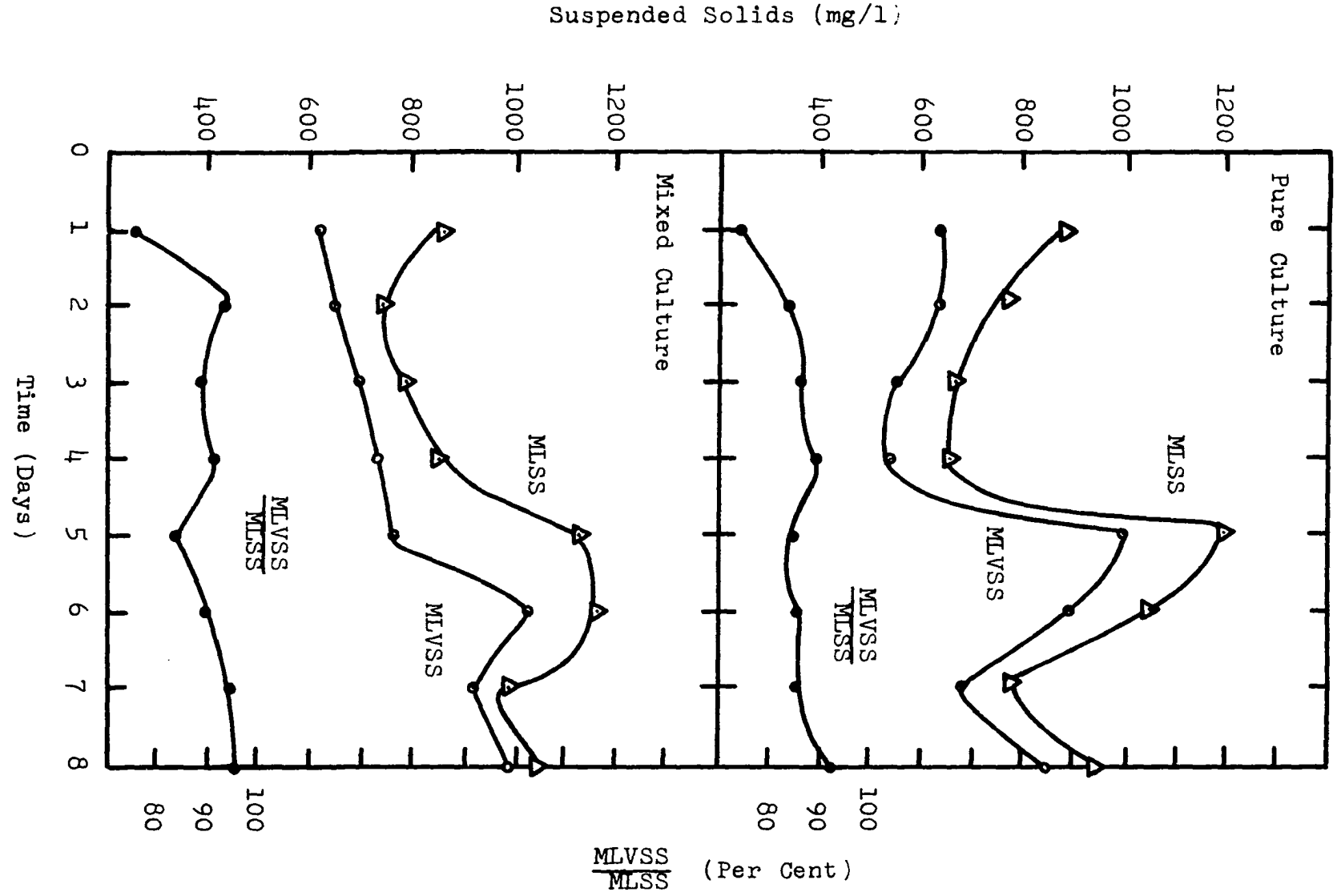

Figure 41. Preliminary Experiment:
Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) and Ratio of VSS to TSS for Pure and Mixed Cultures of
S. natans vs. Time

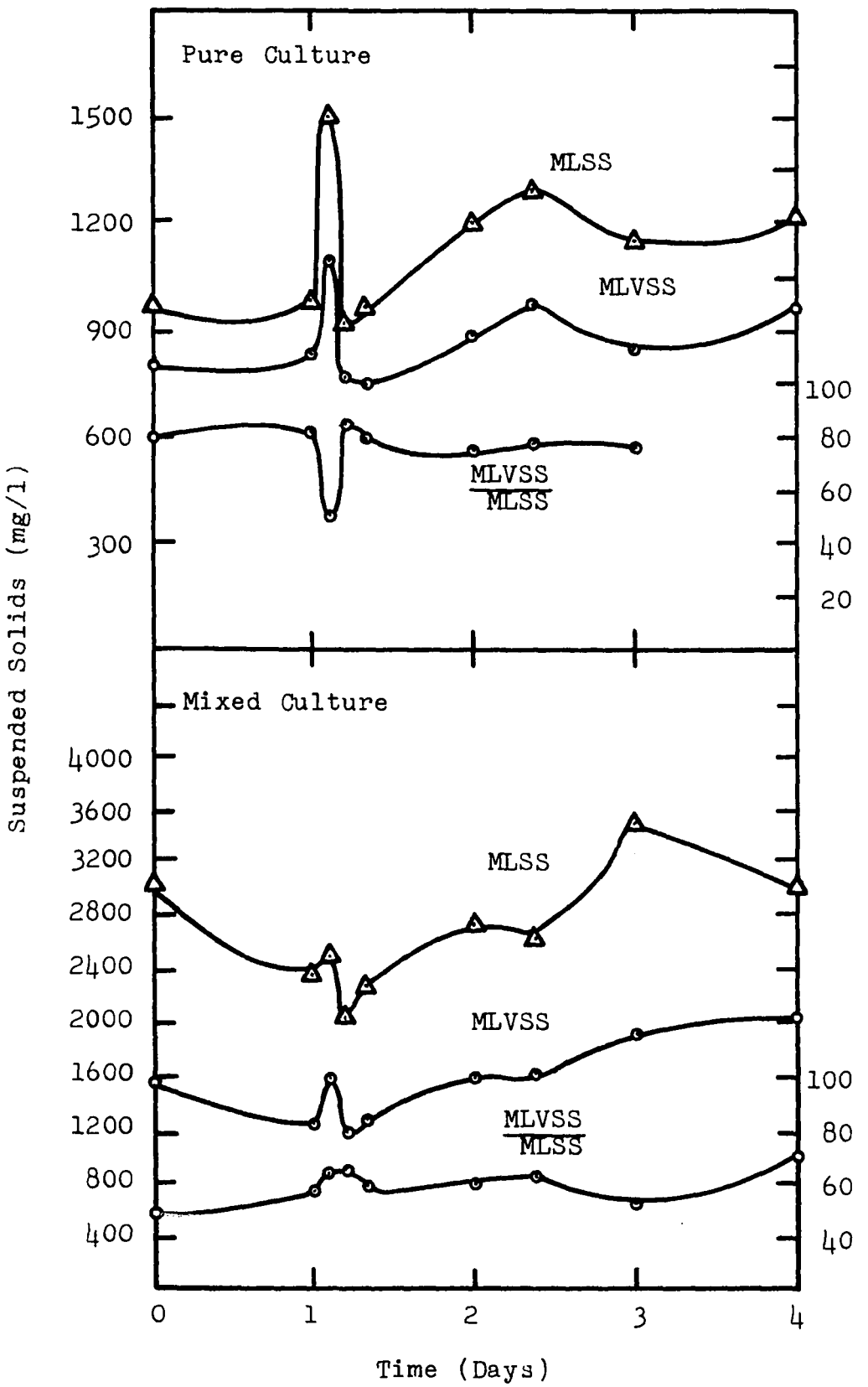

Figure 42. Second Experiment: Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) and Ratio of VSS to TSS for Pure and Mixed Cultures of S. natans vs. Time

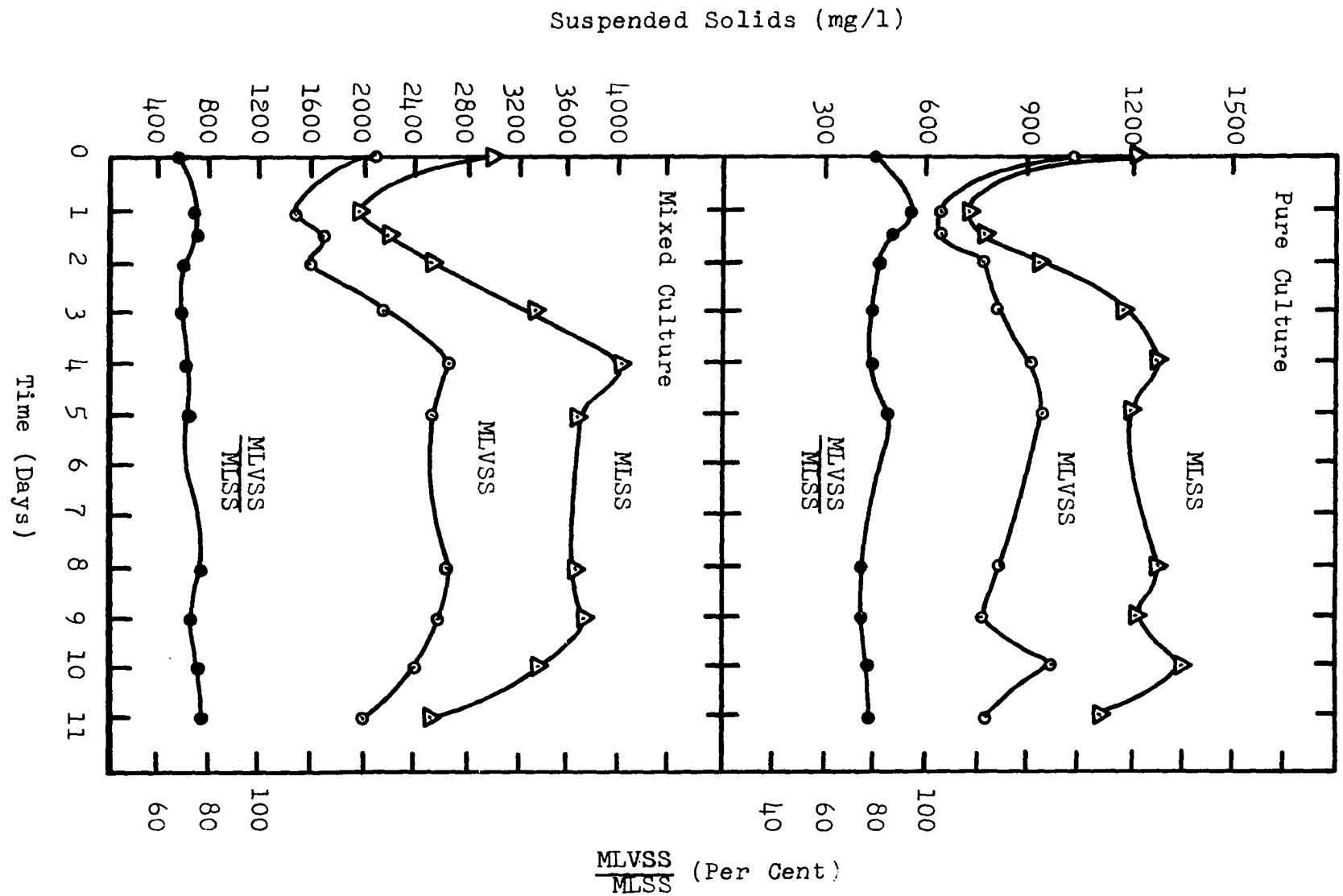

Figure 43. Third Experiment:
Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS)
and Ratio of VSS to TSS for Pure and Mixed Cultures
of S. natans vs. Time

solids is generally greater for the pure culture than for the mixed culture.

In the second experiment (Figure 42), the pure culture TSS and VSS are extremely important parameters. Immediately following the increase in loading velocity, both the TSS and VSS increase significantly within a few hours, while the ratio VSS:TSS decreases significantly. Soon all parameters return to a point resembling the initial point. From this time, both the TSS and VSS seem to vary somewhat inversely with the SVI (See Figure 11); the VSS:TSS remains fairly constant at 80 per cent. Referring to the initial changes in parameter, the decrease in VSS:TSS indicates the increase in TSS is much greater than the increase in VSS. This means that there is an important increase in fixed inorganic suspended solids.

The mixed culture TSS and VSS apparently are not significantly related to the SVI (See Figure 11); there are initial increases in TSS, VSS, and the ratio VSS:TSS following the increase in loading velocity. This indicates that the VSS increased at a greater rate than the TSS and that the amount of fixed inorganic suspended solids decreased. The ratio of VSS:TSS, although a little more erratic than for the pure culture, was fairly constant at approximately 60 per cent. This means that the VSS accounted for approximately 20 per cent more of the TSS in the pure culture than in the mixed culture.

In the third experiment (Figure 43) the pure culture and the mixed culture TSS and VSS appear to vary inversely with the SVI (See Figure 11). In both cases, there are initial drops in TSS and VSS, and corresponding increases in the ratio VSS:TSS. Although the ratio VSS:TSS is a little more erratic for the pure culture than the mixed culture, both are fairly constant at approximately 70 per cent. In the pure culture, the ratio initially is over 80 per cent; this indicates the VSS became a greater fraction of the TSS when the SVI was a maximum.

In summary, results from Figures 41-43 indicate no absolute relationship between the suspended solids and the SVI, although there is a slight tendency for the two factors to vary inversely. In addition, there seems to be no relationship between the ratio VSS:TSS and SVI even though the initial ratio tends to increase as the SVI increases.

Figures 44-47 show the relative composition of the suspended solids. In the upper figure, the volatile organic suspended solids, the total cell mass, and the active cell mass are plotted. By subtraction, the inactive, the inert suspended solids, and the fixed inorganic suspended solids are obtained. In the lower figure, the active and inactive and the total cell mass, the inert suspended solids, and the fixed inorganic suspended solids are plotted. Each parameter is given in terms of per cent of the total suspended solids concentration.

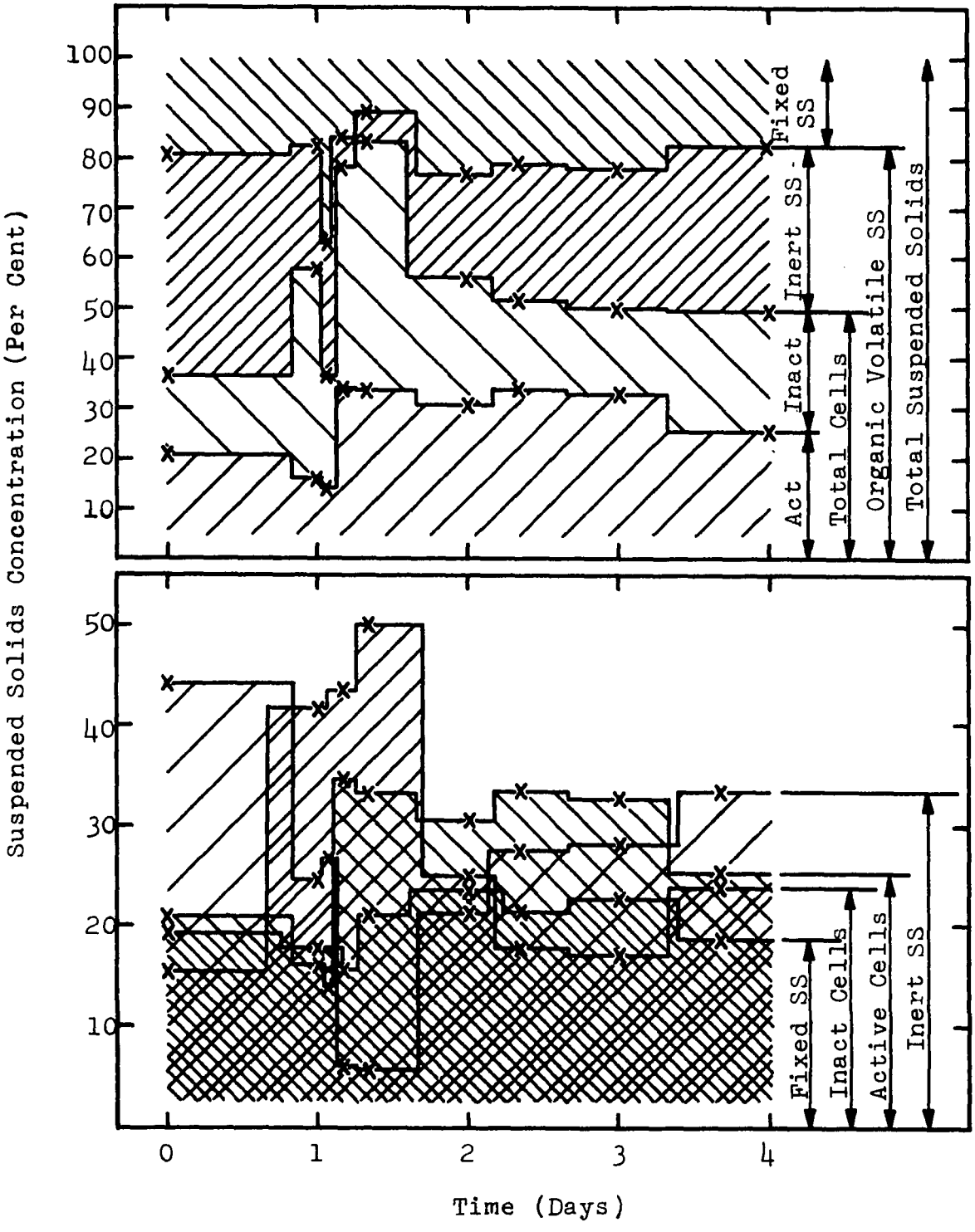

Figure 44. Second Experiment:
Relative Composition of the Suspended Solids for
Pure Culture of S. natans vs. Time

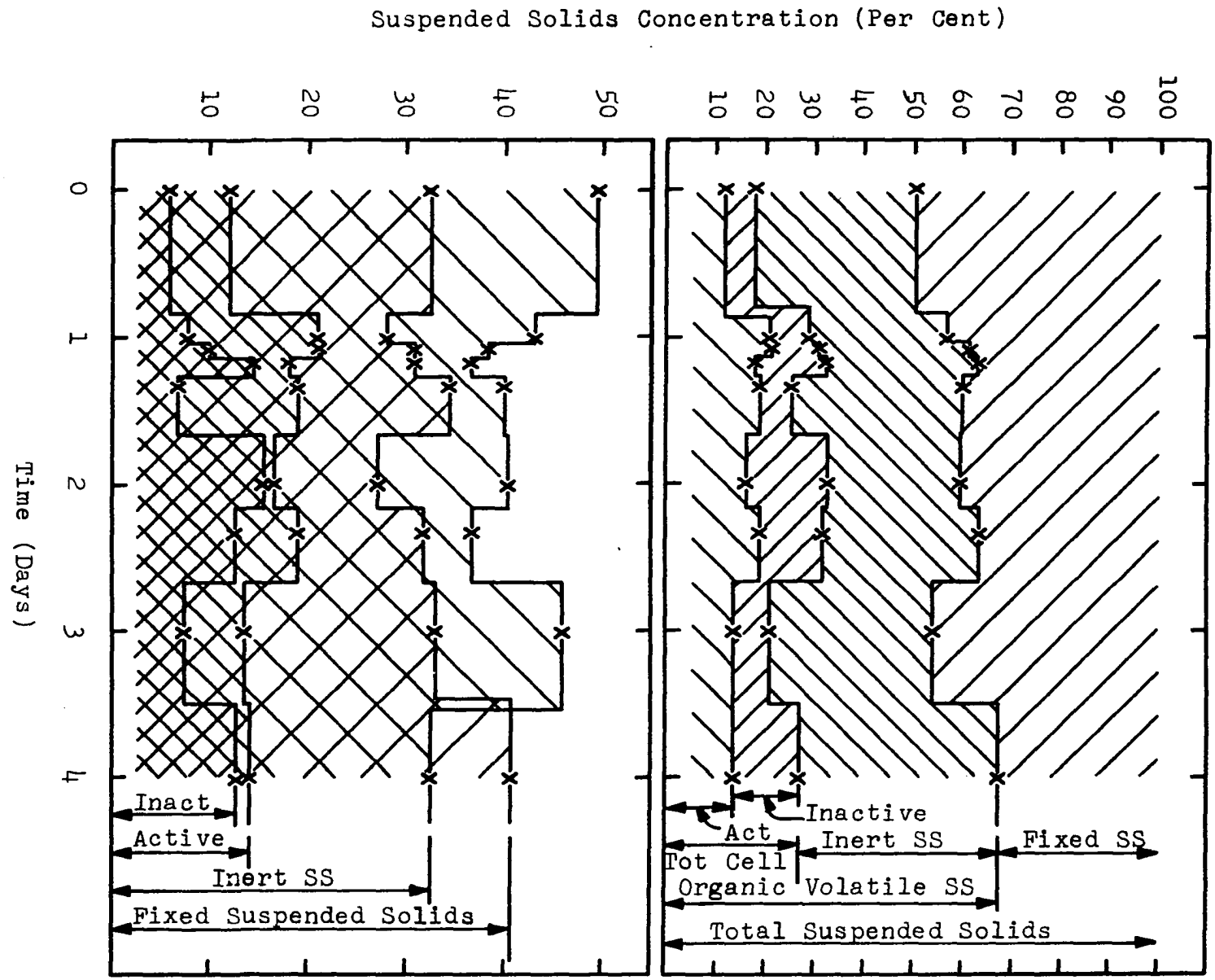

Figure 45. Second Experiment
Relative Composition of the Suspended Solids for
Mixed Culture of S. natans vs. Time

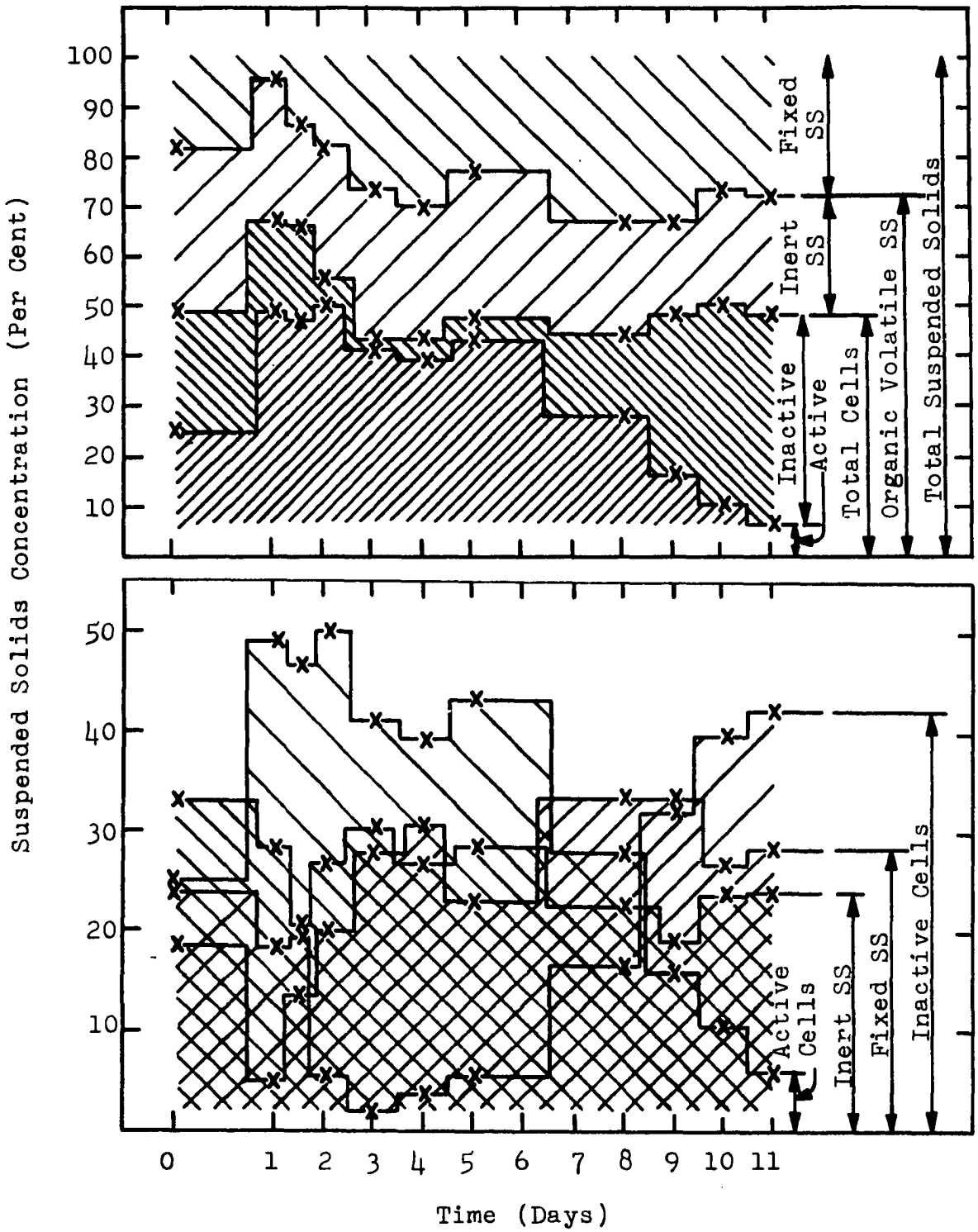

Figure 46. Third Experiment: Relative Composition of the Suspended Solids for Pure Culture of S. natans vs. Time

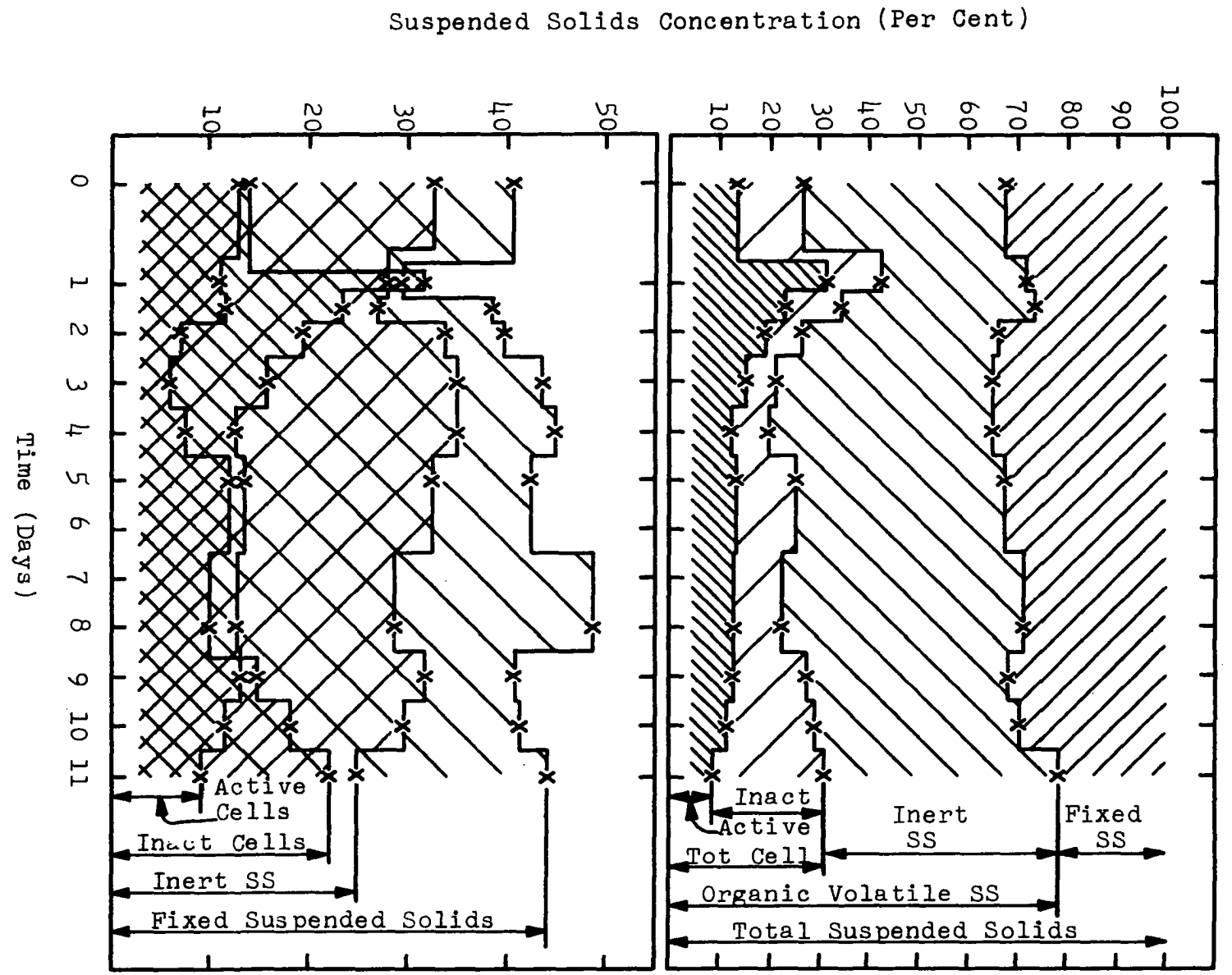

Figure 47. Third Experiment:
Relative Composition of the Suspended Solids for
Mixed Culture of S. natans vs. Time

The suspended solids composition of the pure culture in the second experiment is given in Figure 44. It is immediately obvious that very significant changes occur in the parameters following the increase in loading velocity. There is an immediate increase in the number of inactive cells and a small decrease in the active cell mass. These changes are quickly followed by increases in the active cell mass, which remains high while the inactive cell mass decreases. Accompanying these changes are a severe drop in the inert suspended solids concentration and a minor decrease in the fixed inorganic suspended solids.

The inert suspended solids increase during the last two days of the period. Changes in the inert suspended solids concentration appear to be similar, but opposite, to changes in the inactive cell mass. The changes in the fixed inorganic suspended solids are small, with the concentration remaining near 20 per cent. During the period of severe bulking, the inactive cell mass was 50 per cent of the total suspended solids. Meanwhile, the active cell concentration increased from less than 15 per cent to almost 35 per cent of the TSS.

The suspended solids composition of the mixed culture in the second experiment is given in Figure 45. After the increase in loading velocity, there are increases in the organic volatile suspended solids, the active, inactive, and total cell mass; there are decreases in the inert suspended

solids. The fixed inorganic suspended solids undergo only small changes, remaining just above 30 per cent. Following the initial decrease in the inert suspended solids concentration, it remains fairly constant until an increase at day three and a sharp decrease at day four. The increase at day three is accompanied by decreases in both the active and inactive cell mass, and the decrease at day four is accompanied by increases in the inactive cell mass and the fixed inorganic suspended solids concentration.

In comparisons of the pure and mixed cultures (Figures 44-45), there are obviously more severe changes in parameters for the pure culture. The mixed culture has a higher per cent of fixed inorganic suspended solids and inert suspended solids. Although the VSS concentrations are similar in per cent, the pure culture has over 50 per cent total cell mass compared to only 27 per cent for the mixed culture. The per cent active cells are 17 per cent for the mixed culture and 27 per cent for the pure culture. It should be emphasized that the higher active and total cell mass is not only important in this discussion, but it is important in all discussions of the biological systems.

Figures 46-47 describe the suspended composition for the pure culture and mixed culture in the third experiment. In the pure culture experiment (Figure 46), there are initial increases in organic volatile suspended solids, total and active cell mass, and decreases in the inactive cell mass,

the inert suspended solids, and the fixed inorganic suspended solids. For several days following the increase in the loading velocity, there are high active and low inactive cell masses. Toward the end of the time period, the number of active cells decreases, and the inactive cell mass has a corresponding increase. The fixed inorganic suspended solids, after an initial decrease, remains near 30 per cent. It appears that the intensity of bulking in this experiment did not result in the extreme effects observed in Figure 44. The inactive cell mass is most indicative of a less severe bulking in the third experiment. The per cent of active cells increases in the third experiment in a manner similar to the inactive cell mass in the second experiment.

In the mixed culture (Figure 47), it is immediately obvious that the active cell mass increases significantly following the increase in loading velocity. Meanwhile, there are minor decreases in the inactive cell mass. It is meaningful to note that only minor changes occur in the volatile organic suspended solids and fixed inorganic suspended solids concentrations. This is probably a result of the bulking's being less severe than in other cases. The mixed culture suspended solids is composed of 33 per cent fixed inorganic suspended solids, 41 per cent inert suspended solids, 28 per cent total cell mass, and 16 per cent active cell mass, while the pure culture composition is 24 per cent fixed inorganic suspended solids, 25 per cent inert suspended

solids, 51 per cent total cell mass, and 33 per cent active cell mass.

In summary (Figures 44-47), it appears that more drastic changes occur in the pure culture than in the mixed culture. The heterogeneous population seems to add a dimension of stability to the system responses. The average composition of the suspended solids of the mixed culture is 36 per cent fixed inorganic suspended solids and 64 per cent volatile organic suspended solids, of which 37 per cent is inert suspended solids and 27 per cent is total cell mass. The active cell mass is 17 per cent compared to 10 per cent for the inactive cell mass. The overall composition of the suspended solids of the pure culture is 23 per cent fixed inorganic suspended solids and 77 per cent volatile organic suspended solids, of which 24 per cent is inert suspended solids and 53 per cent is total cell mass. The active cell mass is 30 per cent compared to 23 per cent for the inactive cell mass. Thus, the most obvious differences in cultures is the high concentration of inactive solids in the mixed culture compared to high cellular concentrations in the pure culture. Corresponding to increases in SVI are increases in active total cell mass and organic volatile suspended solids, and decreases in inert suspended solids and fixed inorganic suspended solids. When bulking conditions are extremely severe as in Figure 44, there are major increases in inactive cell mass and decreases in active cell mass.

Statistical Analysis

Very early in this study, it became obvious that sludge settling characteristics were a function of several variables. It has been shown that several parameters have a tendency to vary in a pattern similar to that of the sludge volume index (SVI). The SVI was the dependent variable; the independent variables were oxygen uptake, Poly -β- hydroxybutyric acid (PHB), deoxyribonucleic acid (DNA), dehydrogenase activity (TTC), volatile acids concentration, the pH, and the cellular carbon-to-nitrogen ratio. Data from the second and third experiments which was used for these analyses, is listed in the appendix in Tables 10, 11, 14 and 15.

The first step in this statistical study was a factor analysis. Since one of the important assumptions in the regression model is that each of the independent variables is truly independent, the principal component analysis and the varimax rotation of the factor matrix were computed. Two conclusions resulted from this analysis. The first was that oxygen uptake and TTC were accounting for approximately the same variance in the original set of variables; the second was that the volatile acids concentration and the pH were accounting for about the same variance. Therefore, the factor analysis disclosed that either oxygen uptake or TTC should have been measured. The determination of both parameters was redundant; there was a similar redundancy between the volatile acids concentration and the pH.

While it was not as obvious as with the other parameters above, the factor analysis noted a possible interaction between PHB and the C:N ratio. The chemical composition of PHB is such that there must be a redundancy between the accumulation of PHB, the increase in cellular carbon content, and the increase in C:N ratio. The PHB content and the C:N ratio are both evaluating a similar factor and are not independent. Since PHB is dependent on a single measurable quantity, while the C:N ratio is a complex function of several quantities, PHB was used in the model and the C:N ratio was eliminated.

The second step in the statistical analysis was a multiple linear regression. Many combinations of independent variables were tested with the dependent variable. Thus, the effects of one or more independent variables on the dependent variable could be examined, with the other independent variables kept constant.

In the pure culture with a sample size of nineteen observations, the most significant results were obtained using the PHB content, DNA, TTC, and the pH as independent variables. TTC resulted in a higher multiple correlation coefficient than oxygen uptake; the pH was better than the volatile acids concentration. The multiple correlation coefficient was 0.632 and the F statistic was significant. Therefore, there was a correlation or a relationship between SVI and these four independent variables. In addition, the

hypothesis that all the true partial regression coefficients are equal to zero is rejected and the regression of SVI in each of the four independent variables accounts for a significant amount of variation in SVI.

In the mixed culture with a sample size of nineteen observations, the most significant results were obtained using the PHB content, TTC, the pH, and the cellular carbon-to-nitrogen ratio (C:N). As was the case with the pure culture, TTC and the pH were found to result in higher respective multiple correlation coefficients than oxygen uptake and the volatile acids concentration. The multiple correlation coefficient was 0.837 and the value for the F statistic was significant at the one per cent level. Therefore, the following statements can be made. There is a correlation or a relationship between SVI and these four independent variables. The hypothesis that all the true partial regression coefficients are equal to zero is rejected. The regression of SVI on each of the four independent variables accounts for a significant amount of variation in SVI.

The maximum multiple correlation coefficients were obtained when the maximum number of parameters were utilized. Using all seven variables as independent variables, the correlation coefficients were 0.702 for the pure culture and 0.856 for the mixed culture. Using five variables, PHB, DNA, TCC, pH, and C:N, as independent variables, the

correlation coefficients were 0.667 for the pure culture and 0.849 for the mixed culture.

A single multiple regression was also computed, utilizing both the pure and mixed culture data. When all seven parameters were included in the regression, the correlation coefficient was 0.741. When only PHB, DNA, TTC, pH, and C:N were included, the coefficient was 0.723. The regression of SVI upon PHB, DNA, TTC, and pH yielded a correlation coefficient of 0.722. The F statistic for this regression is significant at the 0.1 per cent level.

The high coefficients for the mixed culture and the differences between the coefficients of the two cultures are not unreasonable. The response of a heterogeneous population is usually more stable, with less distortion and fluctuation than those of a homogeneous population. Therefore, it could be reasoned that the regression of the mixed culture's SVI upon the five independent variables is a reliable and meaningful estimate of sludge settleability.

As a final statistical study, a regression analysis was computed to determine whether a correlation existed between the eight variables for the pure culture and the corresponding variables for the mixed culture. The correlation coefficient for the SVI of the mixed and pure culture was 0.054. This is obviously not significant. The correlation coefficient for the cellular carbon-to-nitrogen ratio of the two cultures was also found to be not significant. Both the pH

and the volatile acids concentration for the two cultures had correlation coefficients that were significant at the one per cent level. The oxygen uptake, the PHB content, the DNA, and the TTC for the two cultures also had correlation coefficients that were significant. Therefore, there was a correlation or a relationship between the oxygen uptake, the PHB content, the DNA, the TTC, the pH, and the volatile acids concentration for the two cultures. There is no relationship between the C:N ratio or the SVI for the two cultures.

The main purpose of this investigation has involved the quantitative evaluation of parameters which influenced the settling characteristics of the pure and mixed populations of S. natans. In general discussion, it can be said that there is some correlation between most parameters for the two sludge populations.

Furthermore, it can be said that the multiple regression of the combined cultures' SVI upon PHB, DNA, TTC, and pH is the most reliable estimate of sludge settleability. The fraction of the total variance of SVI contributed by this regression is over 52 per cent. Therefore, these four factors, while being highly significant (significant at 0.1% level) in the determination of SVI, are not the only factors involved. The multiple regression results in a correlation coefficient of 0.722. The equation of this regression is as follows:

$$SVI = 134.0 + 9.1\ (PHB) + 270.9\ (DNA) + 10.1\ (TTC) - 57.0\ (pH)$$

While the regression equation is extremely important, it is probably more reliable qualitatively than quantitatively. Qualitatively, the equation can be used to evaluate plant operation. Optimum sludge settleability should result from low PHB content, low DNA content, low cell activity, and high pH. Actually, the model is suggestive of adjustments required to obtain optimum values for each parameter. Further work will be required to determine quantitatively the optimum level of each parameter.

CHAPTER V

SUMMARY AND CONCLUSIONS

The development of adequate control and treatment systems for municipal and industrial wastes is one of our greatest engineering problems. In order to control environmental problems, the complex manner in which organisms interact must be understood. This knowledge must then be applied to the problems. In general, a more complete knowledge of the waste-organism complex is required and this should be initiated by pure culture and mixed culture studies of specific organisms found in various wastes in the activated sludge environment. This study has attempted to investigate the conditions and mechanisms which result in filamentous bulking of activated sludge by Sphaerotilus natans. This report is intended to contribute to this end.

For an organism to dominate the population in a mixed microbial population, such as the activated sludge community, it must exhibit a growth rate greater than the overall population. Under certain circumstances, which shall be described as qualitative shock loading, the activated sludge has been shown to exhibit poor settling characteristics and is said to be bulking. Under these circumstances, the sludge

consists largely of filamentous microorganisms. In the review of literature, it has been shown that Sphaerotilus is the organism most frequently associated with bulking. In order to understand how Sphaerotilus may attain the degree of dominance necessary to cause a bulking condition, it is necessary to demonstrate as thoroughly as possible which environmental conditions are associated with bulking and which of these conditions associated with bulking result from Sphaerotilus overgrowth.

This report shows that there is an important dependency of SVI on the PHB content. Three of the four figures demonstrating PHB responses indicate a direct relationship between the two variables. In Figure 16, the response by the cells to the increased loading was severe, which indicates that an intense bulking condition had occurred. Under these circumstances, excessive polymer accumulation resulted in incomplete cell divisions and an inactive cell mass. When the excess PHB was removed and the cells were again able to synthesize the polymer, sludge settleability improved. Thus, under these bulking conditions, the responses of PHB and SVI are suggestive of an inverse relationship. Nevertheless, under most bulking situations encountered in the field, better settleability and a lower SVI are a function of a reduced PHB content in the bacterial cells. It has also been shown that the relationship between SVI and PHB is similar for both the pure and mixed cultures of S. natans.

It has been suggested (Figure 16) that S. natans may have a feedback repressor of PHB synthesis, which bacteria in the mixed culture apparently do not have. The growth responses in Table 8 show that filamentous organisms are able to increase their metabolic activity without a significant increase in their PHB content. This suggests that several filamentous organisms may have a feedback repressor of PHB synthesis. It is obvious that such a repressor would give S. natans and other filamentous organisms a metabolic advantage when competing with nonfilamentous organisms.

Two parameters have shown that there is an important dependency of SVI on the metabolic activity of the cells. Results describing both oxygen uptake and dehydrogenase activity (TTC) indicate a direct relationship between metabolic activity and SVI. Increased metabolic activity and increased energy in the system have accompanied bulking conditions. The experiments suggest that improved settleability and lower SVI result from the reduction of excessive cellular activity. This should not be construed as an elimination of cellular activity, but as a reduction of excessive activity to some moderate, optimum level. It has also been found that the relationship between SVI and cellular activity is similar for both the pure and mixed cultures of S. natans.

The factor analysis indicated that both oxygen uptake and TTC were evaluating approximately the same factor. In

other words, oxygen uptake and TTC are redundant. The multiple regression analysis indicated that TTC accounted for more variation in SVI than oxygen uptake. It is therefore recommended that future studies evaluate metabolic activity by measuring the dehydrogenase activity rather than the oxygen uptake.

It has been shown that there is a dependency of SVI on the acid content in the system. While the majority of examples suggest that pH and SVI are inversely related, it does not appear that it is an extremely important relationship. At any rate, better settleability and lower SVI are a function of higher pH values. It should be understood that the higher pH values suggested are approximately pH 7-8.

Results from the statistical analysis indicated that the pH and volatile acids were similarly measuring the same factor and that pH was able to account for more variation in SVI than the volatile acids content. Therefore, it is recommended that future studies evaluate acid content by measuring the pH rather than the volatile acids concentration. In terms of the buffer capacity, the volatile acids concentration was important in that it indicated that the buffer capacity of the pure culture was probably greater than that of the mixed culture. If this was the case, S. natans could make an important contribution to the buffer capacity of the activated sludge process. It was also pointed out that extremely high concentrations of volatile acids (Figure 24)

apparently had little effect on the settling characteristics of the sludge.

It has also been shown that there is an important dependency of SVI on the number of active and inactive cells (total cell mass). The multiple regression analysis suggests that a direct relationship exists between the total cell mass and the SVI. Therefore, better settleability and lower SVI are a function of a smaller bacterial mass.

The portions of this report dealing with the batch experiments show that the filamentous organisms are better able to exist under environmental extremes. Under conditions of high and low pH , limiting nitrogen, and limiting oxygen, nonfilamentous organisms experience more intense bulking that filamentous organisms. While fewer filamentous organisms did not affect the SVI in continuous systems, fewer filamentous organisms resulted in higher SVI in batch systems.

The dextran experiment suggested that this polymer might have been incorporated into sheath or capsular material. Furthermore, it was the increase in capsular material, rather than any bridging mechanism, that apparently resulted in flocculation. It was also suggested that the filamentous organisms had a less important role in flocculation than the nonfilamentous organisms.

The sequence of sludge volumes (Figures 32-33) indicate that under these experimental conditions, sludge bulking

usually occurs very rapidly while the recovery from bulking occurs slowly. It was pointed out that the settling rate may be more important than the compacting rate in bulking, and that neither subsidence rate could be used to predict bulking.

As far as predicting bulking, most parameters either accompanied or followed the rise in SVI. However, the per cent cellular carbon apparently precedes the rise in SVI. If the increase is such that it could be measured early enough, determinations of total cellular carbon could predict a bulking situation.

Several statements can be made in regard to the results dealing with the system dynamics. The system removal rate, which is a function of the volatile suspended solids content, is extremely sensitive to system changes. It shows that there are similar removal rates for the pure and mixed cultures. In general, the experimental data indicate a higher removal efficiency at increased SVI. However, the increased removal efficiency was not high enough to realistically recommend that activated sludge processes be operated under bulking conditions. The results describing the adsorptive capacity of the suspended solids are not conclusive. There are several interaction effects of which PHB is an important one.

The composition of the suspended solids is important. While a significant relationship between either the total or

volatile suspended solids content and the SVI was not indicated, there seemed to be a tendency for the volatile suspended solids, as a fraction of the total suspended solids, to increase as the SVI increased. Increases in active cell mass and decreases in inert suspended solids and fixed inorganic suspended solids also regularly accompanied increases in SVI. Increases in inactive cell mass and decreases in active cell mass occurred when bulking conditions were extremely intense.

More severe changes in composition occurred in the pure culture than the mixed culture. The mixed culture's average suspended solids composition was 36 per cent fixed inorganic suspended solids and 64 per cent volatile organic suspended solid, of which 37 per cent was inert suspended solids and 27 per cent was total cell mass. The active cell mass was 17 per cent compared to 10 per cent for the inactive cell mass. The average composition for the pure culture was 23 per cent fixed inorganic suspended solids and 77 per cent volatile organic suspended solids, of which 24 per cent was inert suspended solids and 53 per cent total cell mass. The active cell mass was 30 per cent compared to 23 per cent for inactive cell mass. In conclusion, there were high concentrations of inactive solids in the mixed culture and high cellular concentrations in the pure culture.

The linear multiple regression analyses resulted in several correlation coefficients. With seven independent

variables, the correlation coefficients were 0.702 for the pure culture and 0.856 for the mixed culture. With PHB, DNA, TTC, pH, and C:N as independent variables, the correlation coefficients were 0.667 for the pure culture and 0.849 for the mixed culture. Therefore, the mixed culture regressions of SVI upon independent variables accounted for more variation of SVI than did the pure culture regressions upon the same variables.

Utilizing both the pure and mixed culture data, a single regression of SVI upon PHB, DNA, TTC, and pH resulted in a correlation coefficient of 0.722. The F statistic for this regression is significant at the 0.1 per cent level. This regression, which accounts for over 52 per cent of the total variance of SVI, is the most reliable estimate of sludge settleability available. The equation is:

$$SVI = 134.0 + 9.1\ (PHB) + 270.9\ (DNA) + 10.1\ (TTC) - 57.0\ (pH)$$

There are, of course, other variables upon which sludge settleability and SVI are dependent. Until further studies determine other inputs and their relative importance in the model, this regression equation should be used to estimate sludge settleability and to evaluate qualitatively the operation of activated sludge treatment systems.

Conclusions

1. Under most filamentous bulking conditions encountered in the field, improved settleability and a lower SVI are

a function of the reduction of PHB content in the bacterial cells to an optimum level.

2. Filamentous bulking conditions encountered in the field are a function of excessive activity of the bacterial cells; improved settleability and a lower SVI are a function of the reduction of cellular activity to an optimum level.
3. Improved settleability and a lower SVI are a function of the pH in the system; low pH must be increased to an optimum pH near neutrality.
4. Fifty-two per cent of the variance in SVI can be attributed to the PHB content, total cell mass (DNA), dehydrogenase activity (TTC), and the pH; the equation of the regression of SVI upon these four independent variables must be tested to determine whether it can be employed to qualitatively evaluate the operation of activated sludge treatment systems.
5. Measurements of cellular activity by dehydrogenase activity are more reliable and more significant than measurements by oxygen uptake; measurements of acid content by pH are more reliable and more significant than measurements by volatile acids concentration.
6. Sphaerotilus natans and several other filamentous organisms may have a feedback repressor of PHB synthesis which could be used to competitive advantage over other organisms.

7. Microscopic examination of S. natans indicates that PHB may serve as a binding site for flocculation.
8. Additional capsular material may be important in flocculation; filamentous organisms are less important than nonfilamentous organisms in flocculation.
9. In batch systems, the intensity of filamentous bulking becomes greater as the number of filamentous organisms increase; no changes in intensity of bulking is seen in continuous systems.
10. S. natans may contribute significantly to the buffer capacity of the activated sludge process. However, a high concentration of volatile acids may have no significant effect on the settling characteristics of the sludge.
11. Of the two subsidence rates in the sludge volume study, the settling rate may be more important than the compacting rate in sludge bulking.
12. In this system, the increase in COD removal attributable to bulking was not high enough to recommend treatment of high strength industrial waste under bulking conditions.
13. As fractions of the total suspended solids, the active cell mass and the volatile suspended solids increase and the inert suspended solids and fixed inorganic suspended solids decrease as the sludge settleability deteriorates and the SVI increases.
14. There are more severe changes in suspended solids

composition in the pure culture than in the mixed culture. The mixed culture has high concentration of inactive solids; the pure culture has a high content of cell mass. This may explain why the mixed culture regression of SVI upon independent variables accounts for more variation in SVI than does the pure culture regression upon the same variables.

15. Excessive bulking conditions should be avoided as they result in a significant replacement of active cell mass with inactive cell mass.
16. Of all measured parameters, the regular determination of total cellular carbon could be used to predict the occurrence of a bulking situation.
17. Additional research with continuous aeration systems is desirable. Research should be conducted in a manner that will minimize the complex interaction effects among parameters. Research should be conducted in which SVI is measured at several levels of each parameter in order to determine the level which results in optimum sludge settleability.

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APPENDIX

TABLE 10

Second Investigation Data: Concentration of Parameters for Pure Culture

Time Days	SVI (ml/gm)	PHB ($\frac{\mu gPHB}{mgVSS}$)	DNA ($\frac{\mu gDNA}{mgVSS}$)	TTC ($\frac{\mu gTF}{mgVSS}$)	pH (0-14)	Volatile Acids ($\frac{mg}{l}$)	Oxygen Uptake ($\frac{(mg/l/hr}{1000VSS}$)	Cellular Carbon (per cent)	Cellular C:N
0	131	3.44	1.445	23.21	7.60	425	26.2	34.55	4.13
1-1	364	7.98	2.241	14.66	8.13	675	---	42.64	4.10
1-2	330	7.95	1.520	20.78	8.10	705	35.5	41.22	4.07
1-3	982	5.96	2.974	30.92	7.67	550	60.2	40.19	4.02
1-4	930	12.04	3.607	31.51	7.37	780	90.3	40.23	3.92
2-1	621	10.50	2.307	36.28	7.15	600	76.7	31.57	3.88
2-2	549	10.12	2.085	31.94	7.05	780	63.8	----	----
3	588	8.71	2.045	31.58	6.50	825	62.8	40.86	4.22
4	455	12.50	1.928	22.95	6.23	810	40.7	39.80	3.94

TABLE 11

Second Investigation Data: Concentration of Parameters for Mixed Culture

Time Days	SVI (ml/gm)	PHB ($\frac{\mu g PHB}{mg VSS}$)	DNA ($\frac{\mu g DNA}{mg VSS}$)	TCC ($\frac{\mu g TF}{mg VSS}$)	pH (0-14)	Volatile Acids mg/l	Oxygen Uptake ($\frac{(mg/l/hr)}{1000 VSS}$)	Cellular Carbon (per cent)	Cellular C:N
0	56	2.16	1.139	24.10	8.20	425	21.6	25.36	4.69
1-1	175	6.02	1.620	27.58	8.35	660	----	30.93	4.82
1-2	236	6.55	1.598	25.72	8.30	690	21.3	30.15	4.70
1-3	358	4.15	1.637	21.32	7.85	795	34.6	31.50	4.34
1-4	393	2.12	1.366	23.73	7.67	780	30.5	23.70	4.23
2-1	312	3.77	1.742	20.51	7.50	1165	30.4	32.99	4.46
2-2	213	4.38	1.592	22.42	7.50	970	28.9	-----	----
3	116	5.12	1.239	18.70	7.05	2050	20.5	38.59	4.27
4	150	4.45	1.277	15.46	6,68	1970	15.2	36.25	4.19

TABLE 12

Second Investigation Data: Relative Composition of Mixed Liquor Suspended Solids (SS) for Pure Culture

Time day	Total SS (mg/l)	Fixed Inorganic SS (mg/l)	Volatile SS (mg/l)	Inert SS (mg/l)	Total Cell Mass (mg/l)	Inactive Cell Mass (mg/l)	Active Cell Mass (mg/l)
0	992	192	800	439	361	153	208
1-1	988	176	812	243	569	410	159
1-2	2164	1064	1100	577	523	218	305
1-3	916	144	772	55	717	399	318
1-4	968	204	764	----	861	540	321
2-1	1184	284	900	251	649	288	361
2-2	1256	268	988	344	644	224	420
3	1140	256	884	319	565	193	372
4	1220	224	996	396	600	296	304

TABLE 13

Second Investigation Data: Relative Composition of Mixed Liquor Suspended Solids (SS) for Mixed Culture

Time day	Total SS (mg/l)	Fixed Inorganic SS (mg/l)	Volatile SS (mg/l)	Inert SS (mg/l)	Total Cell Mass (mg/l)	Inactive Cell Mass (mg/l)	Active Cell Mass (mg/l)
0	3032	1504	1528	984	544	183	361
1-1	2372	1024	1348	666	682	187	495
1-2	2520	968	1552	777	775	244	531
1-3	2052	752	1300	635	665	296	369
1-4	2292	916	1376	789	587	153	434
2-1	2648	1068	1580	720	860	429	431
2-2	2540	932	1608	808	800	320	480
3	3528	1624	1904	1167	737	263	474
4	3064	996	2068	1243	825	399	426

TABLE 14

Third Investigation Data: Concentration of Parameters for Pure Culture

Time Days	SVI (ml/gm)	PHB ($\frac{\mu gPHB}{mgVSS}$)	DNA ($\frac{\mu gDNA}{mgVSS}$)	TTC ($\frac{\mu gTF}{mgVSS}$)	pH (0-14)	Volatile Acids $\frac{mg}{l}$	Oxygen Uptake $\frac{(mg/l/hr)}{1000VSS}$	Cellular Carbon (per cent)	Cellular C:N
0									
1-1	1000	8.05	2.256	32.92	7.40	---	56.3	40.62	4.04
1-2	1000	14.44	2,481	23.76	7.47	---	56.3	-----	----
2	962	3.99	2.138	22.13	7.05	300	27.9	35.19	4.16
3	763	8.15	1.821	43.83	7.02	463	56.2	35.33	4.18
4	712	15.34	1.977	42.25	6.70	550	66.5	28.52	4.41
5	727	2.18	2.022	42.02	6.30	795	63.6	37.99	4.17
8	542	4.30	2.123	31.25	5.73	1260	67.2	35.54	4.02
9	465	15.33	2.296	17.74	5.28	955	97.9	33.95	4.45
10	385	12.55	2.177	2.39	5.27	1340	9.1	33.24	4.14
11	468	7.13	2.140	0.00	5.36	1280	5.6	36.47	4.13

TABLE 15

Third Investigation Data: Concentration of Parameters for Mixed Culture

Time Days	SVI (ml/gm)	PHB ($\frac{\mu gPHB}{mgVSS}$)	DNA ($\frac{\mu gDNA}{mgVSS}$)	TTC ($\frac{\mu gTF}{mgVSS}$)	pH (0-14)	Volatile Acids $\frac{mg}{l}$	Oxygen Uptake $\frac{(mg/l/hr)}{1000VSS}$	Cellular Carbon (per cent)	Cellular C:N
0									
1-1	580	9.23	1.900	32.92	7.95	----	32.3	41.93	4.15
1-2	522	13.33	1.514	23.76	7.75	----	28.6	40.69	4.09
2	359	2.59	1.287	22.13	7.30	213	9.0	38.31	4.53
3	274	2.24	1.058	18.01	7.19	325	9.8	36.36	4.15
4	223	4.45	0.988	14.39	6.87	1665	13.1	32.99	4.47
5	234	1.53	1.192	14.88	6.90	2540	14.5	33.38	3.77
8	160	2.89	1.015	13.35	6.17	1940	12.5	28.84	4.20
9	132	2.78	1.293	14.40	5.92	2300	12.9	-----	----
10	118	3.46	1.329	12.13	5.42	1395	12.6	36.36	3.98
11	153	3.21	1.275	0.15	5.04	1810	10.6	37.07	3.95

TABLE 16

Third Investigation Data: Relative Composition of Mixed Liquor Suspended Solids (SS) for Pure Culture

Time day	Total SS (mg/l)	Fixed Inorganic SS (mg/l)	Volatile SS (mg/l)	Inert SS (mg/l)	Total Cell Mass (mg/l)	Inactive Cell Mass (mg/l)	Active Cell Mass (mg/l)
0							
1-1	672	32	640	189	451	122	329
1-2	740	100	640	144	496	151	345
2	936	184	752	249	503	----	520
3	1180	352	828	357	471	----	483
4	1264	384	880	336	544	49	495
5	1196	276	920	339	581	65	516
8	1272	424	848	286	562	209	353
9	1216	404	812	230	582	390	192
10	1352	360	992	317	675	643	32
11	1112	312	800	265	535	535	0

TABLE 17

Third Investigation Data: Relative Composition of Mixed Liquor Suspended Solids (SS) for Mixed Culture

Time day	Total SS (mg/l)	Fixed Inorganic SS (mg/l)	Volatile SS (mg/l)	Inert SS (mg/l)	Total Cell Mass (mg/l)	Inactive Cell Mass (mg/l)	Active Cell Mass (mg/l)
0							
1-1	1552	436	1116	454	662	173	489
1-2	1724	464	1260	664	596	198	398
2	2504	844	1660	993	667	178	489
3	3288	1148	2140	1433	707	194	513
4	4040	1408	2632	1820	812	308	504
5	3680	1196	2484	1559	925	433	492
8	3692	1060	2632	1797	835	367	468
9	3760	1192	2568	1531	1037	545	492
10	3384	1000	2384	1394	990	605	385
11	2524	548	1976	1189	787	783	4

Table 18

Experimental Design: Summary of Each Experiment

Experiment	Aeration System	Length (Days)	Biological Population	Parameters Studied
Preliminary	Continuous	8	S. natans in pure and mixed cultures	SVI, PHB, oxygen uptake, carbon to nitrogen ratio, total cellular carbon.
Second	Continuous	4	S. natans in pure and mixed cultures	SVI, PHB, oxygen uptake, dehydrogenase activity, pH, volatile acids, carbon to nitrogen ratio, total cellular carbon.
Third	Continuous	11	S. natans in pure and mixed cultures	SVI, PHB, oxygen uptake, dehydrogenase activity, pH, volatile acids, carbon to nitrogen ratio, total cellular carbon.
Fourth	Continuous	4	Filamentous organisms present and dominant	SVI, PHB, dehydrogenase activity.
Fourth	Batch	5	Filamentous organisms present and dominant	SV at pH 7.5, pH 4, and pH 10; SV under conditions of limiting nitrogen content and limiting oxygen supply.