SOLVING SLUDGE BULKING PROBLEMS THROUGH FILAMENTOUS ORGANISM IDENTIFICATION: CASE STUDIES IN MASSACHUSETTS

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A Master's Project

Presented by

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ABSTRACT

In the past decade, a significant amount of research effort has been directed towards understanding the nature and causes of activated sludge filamentous bulking and its control. In particular, the relationships between filament types and causative conditions have been established for a number of organisms. Filamentous organism identification and bulking control manuals have been developed and are available for use by treatment plant staff.

The goals of this research include further substantiation of the filament type-cause relationship through case studies and an evaluation of the applicability of the identification methods for use by treatment plant staff for bulking control. Six activated sludge plants in Massachusetts experiencing filamentous bulking problems were studied. The dominant filamentous organisms were identified to determine probable causative conditions. Plant operating data were also analyzed to substantiate the organism-cause relationship. Remedial actions were suggested and most of those plants which implemented the suggested control measures were successful in eliminating the bulking problem.

It was found that although long term monitoring of the activated sludge filamentous organisms and floc structure is beneficial to process control, most plants do not have the resources available to implement the procedures. For a plant to obtain the necessary equipment, supplies and training, it will cost approximately $4135. Through the case

studies, it has been shown that in most situations, one sample of the activated sludge analyzed during a bulking episode can yield the necessary information to determine the probable cause of bulking and evaluate remedial alternatives.

Mail in services for filament identification exist and are relatively inexpensive, but the turnaround time for results is 1-2 weeks. It is suggested that regional or statewide technical assistance groups such as TATS in Massachusetts incorporate filamentous organism identification and bulking control expertise in their services to provide local, cost effective assistance to individual plants.

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CHAPTER I

INTRODUCTION

The activated sludge process is the most commonly used process for the secondary treatment of domestic wastewater (Metcalf & Eddy, 1979). It is flexible, due to the ease of process modifications, reliable, and over 75 years of design and operation experience exists worldwide. The process consists of two unit operations: aeration and clarification. Aeration provides an aerobic environment for the oxidation and assimilation of organic matter. The function of clarification is to provide for the gravity separation of flocculated solids and microorganisms from the effluent and thickening of the solids for return to the aeration tank to maintain sufficient bacterial mass and sludge age.

Solids separation is the primary factor controlling the performance of the activated sludge process. Insufficient compaction and settling of the sludge is generally considered a "bulking" problem. It can be induced by an abundance of filamentous organisms, lack of filamentous organisms and a dispersed floc structure, the metabolic state of the organisms and the hydraulics of the secondary clarifier due to either design or operational problems.

As shown in Table 1, the bulking problem is quite common among plants treating domestic wastewater. Activated sludge plants treating industrial wastes such as food processing or paper mill wastes will

Table 1: The Magnitude of the Bulking Problem in Plants Treating Domestic Wastewater

Location	% A-S Plants Bulking	Source
West Germany	45	Wagner, 1982
United Kingdom	63	Tomlinson, 1982
South Africa	56	Blackbeard et al., 1986
Netherlands	40-50	Eikelboom, 1982
United States	50	Eikelboom, 1982
--Massachusetts	60	Woodworth, 1989

generally have a higher incidence of bulking.

By far, filamentous bulking is the most common cause of solids separation problems. This condition is characterized by a population shift from a predominance of floc-forming organisms in normal activated sludge to a predominance of filamentous organisms. These excess filaments reduce the density of the sludge flocs as well as their settling velocity and thus prevent the normal compaction of the sludge in the final sedimentation tanks.

Filamentous bulking can lead to the production of dilute return and waste activated sludge streams, increased sludge handling costs due to increased sludge volume and increased chemical usage, hydraulic overloading of sludge handling processes, high effluent suspended solids and biochemical oxygen demand (BOD_5) resulting from sludge blanket overflow from the secondary clarifier (Lao et al., 1984b) and reduced disinfection effectiveness due to increased solids in the effluent. In severe cases, washout of the activated sludge biomass and ultimate failure of the process may occur. Ultimately, this translates to reduced treatment efficiency and possible NPDES discharge permit violations (approximately 50% of U.S. activated sludge plants do not consistently meet their NPDES discharge standards (Richard, 1989a)).

More than 26 different filamentous organisms causing sludge bulking have been identified (Eikelboom, 1975a;b). Recent work by Strom and Jenkins (1984) and Richard et al. (1982a) has shown a correlation between the predominance of particular filamentous organisms and

specific operational conditions (low DO, low F/M, etc.) causing the bulking situation.

Although the associations of waste treatment parameters with filamentous organisms are not 100% accurate, identification of the dominant filamentous organisms in a bulking sludge aids the plant operator in determining which operational parameters to look at first so that proper corrective strategies can be implemented to eliminate the cause of the filamentous organism predominance and control their growth.

Microscopic investigation of activated sludge is a simple analysis which gives information about the form and structure of flocs, the presence of filamentous microorganisms, the numbers and types of protozoa, etc. Regular microscopic investigation of the sludge contributes to a better insight into the composition and structure of the sludge floc and through this into the functioning of the activated sludge process.

This research was undertaken with the following objectives:

1. To determine the cause(s) of filamentous bulking at several activated sludge plants in Massachusetts through filamentous organism identification.
2. To determine if plant operating data support what the organism identification suggests is the cause(s) of the problem.
3. To determine possible remedial actions based on organism identification and data analysis.
4. To evaluate the ease of implementation of the identi-

fication procedures by treatment plant staff with limited background in microbiological techniques.

5. To include this research as case studies in Activated Sludge Bulking Handbook written by the principal investigator of this research (Switzenbaum et al., 1990).

To accomplish these objectives a procedure for identifying the filamentous organisms and relating them to environmental conditions had to be chosen. This was accomplished through a thorough literature review and evaluation of methods currently and previously in use. The plants experiencing filamentous bulking were identified through a survey of all Massachusetts activated sludge plants. Those experiencing bulking at the time of this study were contacted and several agreed to participate in this study.

The research reported herein is one part of a three part study. It is meant to further substantiate the relationship between particular filamentous organisms and specific operational problems and to familiarise plants in Massachusetts with the utility of microscopic investigation. The other parts of the bulking study involve the development of a short course and teaching manual on activated sludge bulking and an engineering evaluation of the bulking problem in Massachusetts including possible remedial actions.

CHAPTER II

LITERATURE REVIEW

As early as 1922, filamentous organisms were shown to be associated with sludge bulking (Martin, 1927). Investigations by Ruchhoft and Watkins (1928) on carbohydrate rich wastes indicated Sphaerotilus sp. as the filamentous organism causing bulking. For quite some time, this organism was considered the principle cause of sludge bulking without further attempts to establish the true identity of the filamentous organisms present (Pipes, 1969; Pasveer, 1969; Eikelboom, 1975a). Thus, Sphaerotilus sp. has come to be regarded as the originator of filamentous sludge.

Not all waste streams treated by the activated sludge process are carbohydrate rich. The great variety of nutrients present in industrial and domestic wastewaters creates excellent conditions for the development of a diverse population of bacteria, both unicellular and filamentous (van Veen, 1973). The possible growth of various filamentous microorganisms in activated sludge has been proposed by numerous investigators including Buswell and Long (1923), Ruchhoft and Watkins (1928), Morgan and Beck (1928), Lackey and Wattie (1940), Englebrecht (1957), Pipes (1967), Hunerberg et al. (1970), Farquhar and Boyle (1971a;b), Cyrus and Sladka (1970) and van Veen (1973) and Eikelboom (1975b).

The filamentous bacteria are characterized by their thread-like appearance caused by repeated divisions of their component cells in one plane. Filamentous bacteria are necessary as a backbone providing structure to the flocs (See Figure 1a). However, an abundance of filamentous bacteria is characterized by filaments extending from the flocs (Figure 1b) and free floating in the mixed liquor. Depending on the type of filament involved, two forms of interference in settling and compaction exist:

(1) interfloc bridging, where filaments extend from the floc surface and hold the flocs apart, and

(2) open floc structure, where the filaments grow mostly within the floc and the floc grows attached to the filaments. Here the floc becomes large, irregularly shaped and contains substantial internal voids.

With the growing interest in sludge settlability problems, an operational parameter, the sludge volume index (SVI), was developed to describe the settling characteristics of sludge (Mohlman, 1934). It is calculated by dividing the 30-minute settled volume of activated sludge in a one liter cylinder by the mixed liquor suspended solids concentration (MLSS) (Pipes, 1967) and is reported as ml/g. Various researchers define what the SVI of a "normal" sludge should be and what the SVI of a bulking sludge is. Every wastewater as well as every treatment facility is different and the SVI cannot be used to quantitatively predict the performance of settling basins. It is, however, a useful operational tool and provides a convenient test for

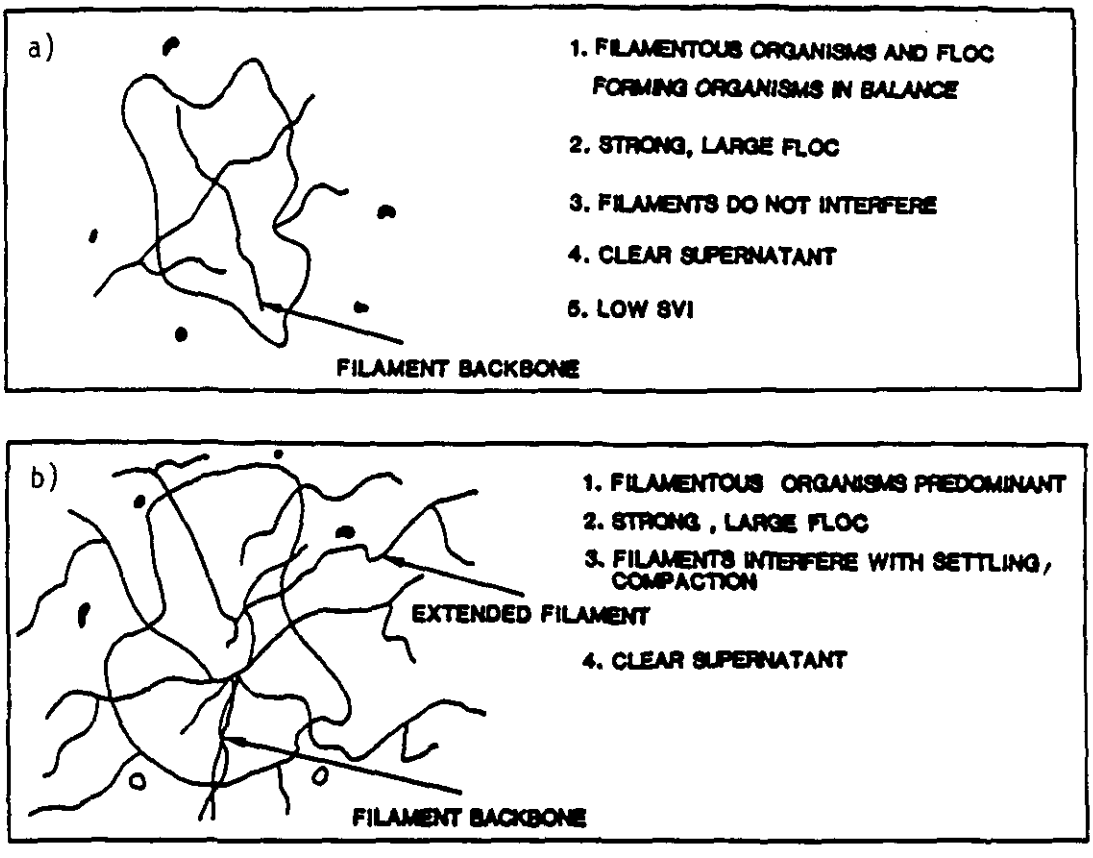


Figure 1: Filament Effect on Floc Structure (Jenkins et al., 1986)

monitoring changes in performance of a particular plant. Comparisons of values from various plants are probably meaningless because the test may measure different properties of different sludges (Dick and Vesiland, 1969). Each plant will have a specific SVI value where sludge is lost to the final effluent, which can vary from less than 100 ml/g to greater than 300 ml/g, depending on the size and performance of the final clarifiers. Thus, a bulking sludge may or may not lead to deterioration of effluent quality, depending on the specific treatment plants ability to contain the sludge within the final clarifier. In general, though, an SVI of under 150 ml/g is considered "normal" and above 150 ml/g is considered bulking (Strom and Jenkins, 1984).

As more research and experience was accumulated on the subject of sludge bulking, specific operational conditions were shown to cause bulking. Some of the more common causes of filamentous bulking are shown in Table 2. Along with the causative environmental conditions, theories on the mechanism of filamentous predominance under various conditions have been proposed (Table 3). It was also observed that associations exist between particular filamentous types and specific environmental conditions in the aeration basin (Eikelboom, 1975a, Strom and Jenkins, 1984 and Jenkins et al., 1986). Shown in Table 4 are some of the more common organism-cause relationships observed. These associations between organisms and waste treatment parameters are not specific in many cases. A specific cause and effect relationship has been established for only a few filamentous organisms (Jenkins et al.,1986). These include type 1701 (Richard et al., 1982b; Hao, 1982;

Table 2: Suggested Causes of Filamentous Bulking

Condition	Sources
Low aeration basin dissolved oxygen (DO)	Heukelekian and Ingols, 1940; Adamse, 1968; Sezgin et al., 1978; Palm et al., 1980
Low food-to-micro-organism ratio (F/M)	Logan and Budd, 1956; Ford and Eckenfelder, 1967; Pipes, 1979
Nutrient deficiency	Carter and McKinney, 1973; Wood and Tchobanoglous, 1975; Greenberg et al., 1955; Jones, 1965; Dias et al., 1968
High sulfides	Farquhar and Boyle, 1972; Voelkel et al., 1974; Merkel, 1975
Low pH	Jones, 1964
Complete mix mode	Chudoba et al., 1973a; Rensink, 1974; Houtmeyers, 1978; Tomlinson and Chambers, 1979; White et al., 1980

Chiesa and Irvine, 1985.

Table 3: Proposed Mechanisms of Filamentous Organism Predominance

Condition	Cause
Low DO	Lower Ks and μmax for filamentous organisms favors their growth at low DO (Hao, 1982; Sezgin et al., 1978) At low DO concentrations, relatively thin filaments are supplied with oxygen better than large flocs (Chudoba, 1985a)
Low F/M	Lower Ks and μmax for filamentous organisms (Chudoba et al., 1973b) Higher area/volume ratio gives filaments the metabolic advantage at low soluble organic loading (Pipes, 1967)
Nutrient deficiency	Higher A/V ratio (Pipes, 1967) Greater ability to store intracellular storage products (Stokes and Parson, 1968)
High sulfides/ Septic wastewater	Ability of some filamentous organisms to use as energy sources: inorganic, reduced sulfur compounds; low molecular weight organic acids produced by fermentation in septic sewage (Richard et al., 1983; 1984).
Complete mix mode	Induces low F/M conditions No substrate concentration gradient present (Chudoba et al., 1973a;b; Sonoda et al.,1973; Rensink, 1974; 1979; White et al., 1980; Lee et al., 1982)
Low pH	Acidic conditions (pH below 6.5) optimum for growth of certain filamentous fungi

Table 4: Dominant Filamentous Types as Indicators of Conditions Causing Sludge Bulking

Suggested Causative Conditions	Indicative Filament Types
Low DO	type 1701, S. natans H. hydrossis
Low F/M	M. parvicella, H. hydrossis, Nocardia sp., types 021N, 0041, 0675, 0092, 0581, 0961, 0803, 1851*
Septic Wastewater/ Sulfide	Thiothrix sp., Beggiatoa, type 021N
Nutrient Deficiency	Thiothrix sp., S. natans, H. hydrossis, types 0041, 0675, N. limicola II**
Low pH	fungi

Richard et al., 1982a; Strom and Jenkins, 1984.
*Lee et al., 1982.
**Richard, 1989b.

Hao et al., 1983), Sphaerotilus natans (Richard et al., 1982b; Lao et al., 1984a;b), Micothrix parvicella (Slijkhuis and Deinema, 1982; Slijkhuis 1983a;b), and type 021N (Richard et al., 1984). Part of the problem is due to the fact that organism characteristics (morphological and staining) can greatly differ outside the domestic wastewater activated sludge environment. This occurs in pure culture studies and especially when significant quantities of industrial wastes are present.

The standard investigative procedure most often used for checking the performance of the treatment process involves mainly chemical and physical analysis of influent and effluent. The results of these tests give little direct information to plant operators about the quality of the sludge floc in the aeration tank. As a result, it is often not possible to indicate the cause of a disturbance in the treatment process and initiate proper corrective action. Corrective strategies are often implemented on a trial and error basis. Currently, when a plant experiences filamentous bulking, the operator is often unclear as to the cause of the filamentous predominance or where to begin to look for operational problems. A common solution, though not always successful, is to chlorinate the return activated sludge (RAS). This may selectively kill off much of the filamentous population and temporarily alleviate the bulking condition. It has been known, however, to kill the floc-forming organisms as well. This solution is short term and costly for a recurring problem. Once the chlorination of the RAS is discontinued, the filaments will return if the operating and environmental conditions remain unchanged. Although not currently

regulated in domestic wastewater treatment, the production of total organic halides such as trihalomethanes resulting from free chlorine reactions with organic matter may be of concern in the near future. The use of filament control strategies other than RAS chlorination should be considered.

Identification of the dominant filamentous organisms in a bulking sludge aids the plant operator in determining if filamentous organisms are actually a problem and if so, which operational parameters to look at first so that proper corrective strategies can be implemented to eliminate the cause of the filamentous organism predominance and control their growth.

In order to facilitate consistent filamentous organism identification, several researchers have developed identification procedures for filamentous organisms (Farquhar and Boyle, 1971a; van Veen, 1973; Eikelboom, 1975a; Jenkins et al., 1986). Some filamentous organisms have been taxonomically classified, however, the taxonomic position of most of the types is uncertain. The taxonomic positioning into genus, species, subspecies and strains is complicated, time consuming and inappropriate for isolating filamentous microorganisms from activated sludge for the following reasons:

1. At least 30 stable features are needed for a formal recognition of a taxonomic group.
2. There is a severe lack of pure cultures of filamentous microorganisms originating from activated sludges to which isolates can be compared.

3. It is probable that many morphological, physiological and genetic changes may occur in the filamentous microorganisms when isolated in pure culture.
5. The low growth rates of most filamentous microorganisms cause the isolation and identification to take weeks or months. Thus, results obtained do not correspond to the actual state of the activated sludge environment (Wanner and Grau, 1989).

There are several advantages to identification of types rather than species. It is rapid, often requiring an hour or less to complete, it integrates all factors affecting the treatment system over a period of time, it is not subject to sampling, calculation or other errors providing the identification is correct, it serves as an independent observation for comparison with the more complex operating data and waste characteristic measurements, and it can be performed by personnel with little background in microbiology. The only major disadvantage is that it takes time and skill to learn the procedure and a good phase contrast microscope is required (Emtiazi et al., 1989).

Identification to types was first proposed by Eikelboom (1975a,b; Eikelboom and van Buijsen, 1981). The methods were immediately accepted by most of the researchers and practitioners in this field throughout the world. Eikelboom's method was further modified by Jenkins et al. (1986). The techniques are based on phase contrast microscopic observations of morphology, relationship to other organisms present and

Table 5: Selective Control Strategies

Causative Condition	Control Strategy
Low DO	Increase in aeration Use of pure O_2 Raise aeration basin MLSS
Low F/M	Reduction of MLSS Installation of a selector Change to plug flow mode
Septic Wastewater/ Sulfides	Preaeration, Prechlorination Pretreatment of industrial waste inputs
Nutrient Deficiency	Addition of nutrients (usually N, P) or anaerobic digester supernatant
Low pH	Addition of sodium hydroxide or other caustic to raise pH

staining characteristics (Gram, Neisser, observation of sulfur and PHB granules).

Stemming from the identification procedures, diagnostic manuals were developed by EPA (1977), Eikelboom and van Buijsen (1981), Jenkins et al. (1986), EPA (1987) and Hobson (1987) to aid in solving sludge bulking problems through chemical addition or process modifications. Shown in Table 5 are the most common selective strategies to eliminate a bulking problem. These manuals contain identification keys, organism descriptions, photographs of organisms in activated sludge, descriptions of types of bulking sludge, descriptions of environmental conditions favoring filamentous growth and descriptions of successful case studies.

Currently, research is being directed towards taxonomic identification and further defining the growth and nutritional requirements of filamentous microorganisms to develop further the correlation between their occurrence and bulking causes.

CHAPTER III

MATERIALS AND METHODS

3.1 Wastewater Treatment Facility Survey

Before sampling of plants could be commenced, the Massachusetts activated sludge treatment plants experiencing bulking problems had to be identified. This was accomplished through a survey conducted in a related project (Woodworth, 1990). Of the plants indicating that they were experiencing bulking at the time of this study, several were chosen for case study based on the severity of the bulking problem, availability of plant design and operating data and willingness to participate in the study. A total of 8 plants participated in this study.

3.2 Sampling

Sampling of the activated sludge mixed liquor was accomplished according to the procedures prescribed by Jenkins et al. (1986). Samples were taken at the effluent end of the aeration basin, below the surface, to exclude any foam or other floating material. Samples were collected in 1L, screw-top Nalgene containers. Containers were filled no more than half full to maintain adequate oxygenation and avoid septicity. Samples were transported to the Environmental Engineering Laboratory at the University of Massachusetts by car at ambient temperature.

Upon arrival at the lab, several air-dried smears were prepared to be used for the Gram and Neisser stains. It was especially important to prepare these immediately since prolonged storage of the mixed liquor can alter the Gram and Neisser staining reactions. If the samples could not be examined immediately, they were refrigerated at 4 degrees Centigrade. All samples were analyzed within 4 days of sampling.

3.3 Organism Identification Procedure

Eikelboom's method, as modified by Jenkins et al. (1986) was used to characterize (identify to type, rather than species) the filamentous microorganisms in activated sludge. The flocs and filaments were observed with an Olympus BHS research grade, phase contrast microscope capable of 100X to 1000X magnification. A photographic record was maintained of the samples with a Polaroid Time-Zero SX-70 Autofocus Land Camera with microscope adaptor.

Sample analysis began with low power (100X) phase contrast scanning of one or more wet mounts. Preparation of wet mounts was conducted as described by Jenkins et al. (1986). Observations at this magnification included an approximate determination of floc sizes and structural characteristics, relative amounts of protozoa and metazoa and a rating of the amount of filamentous growth present according to a previously established filament abundance ranking system as shown in Table 6. Photographs of the various abundance categories are presented in Jenkins et al. (1986).

Next, wet mounts were examined at 400X phase contrast to determine

Table 6: Subjective Scoring of Filament Abundance (Jenkins et al., 1986)

Numerical Value	Abundance	Explanation
0	none	
1	few	filaments present, but only observed in an occasional floc
2	some	filaments commonly observed, but not present in all flocs
3	common	filaments observed in all flocs, but at low density (e.g. 1-5 filaments per floc)
4	very common	filaments observed in all flocs at medium density (e.g. 5-20 per floc)
5	abundant	filaments observed in all flocs at high density (e.g. 20 per floc)
6	excessive	filaments present in all flocs - appears more filaments than floc and/or filaments growing in high abundance in bulk solution

filament effect on floc structure, number of filament types present, their lengths and relative abundance. Wet mounts were then examined at 1000X phase contrast. Characteristics of interest were the presence of branching, motility, sulfur granules or other cell inclusions, filament shape (straight, slightly bent, curled or coiled) and location (protruding from the floc or free floating, bridging flocs), color (dark or transparent), presence of a sheath, visible crosswalls or attached unicells of other bacteria, filament diameter and length, cell size and cell shape.

Staining techniques, including the Gram stain, Neisser stain, PHB stain, crystal violet sheath stain, and India ink stain, were next used to aid in identification. A sulfur oxidation test was also performed to determine if the filamentous organisms had the ability to deposit intracellular sulfur granules. Staining and sulfur test procedures are shown in Appendix II.

Results of the microscopic investigation were recorded and then compared to Jenkins' key (Figure 2), table of characteristics (Table 7), type descriptions and photographs to determine the filamentous organism type (Jenkins et al., 1986). Results were also compared with organism descriptions of other investigators (Eikelboom, 1975b; Strom and Jenkins, 1984) to account for variation of organism characteristics in wastes of different compositions.

Identified organisms were then associated with particular causative conditions as reported by Jenkins et al. (1986) and Strom and Jenkins (1984) (Table 4). Once a possible environmental condition was

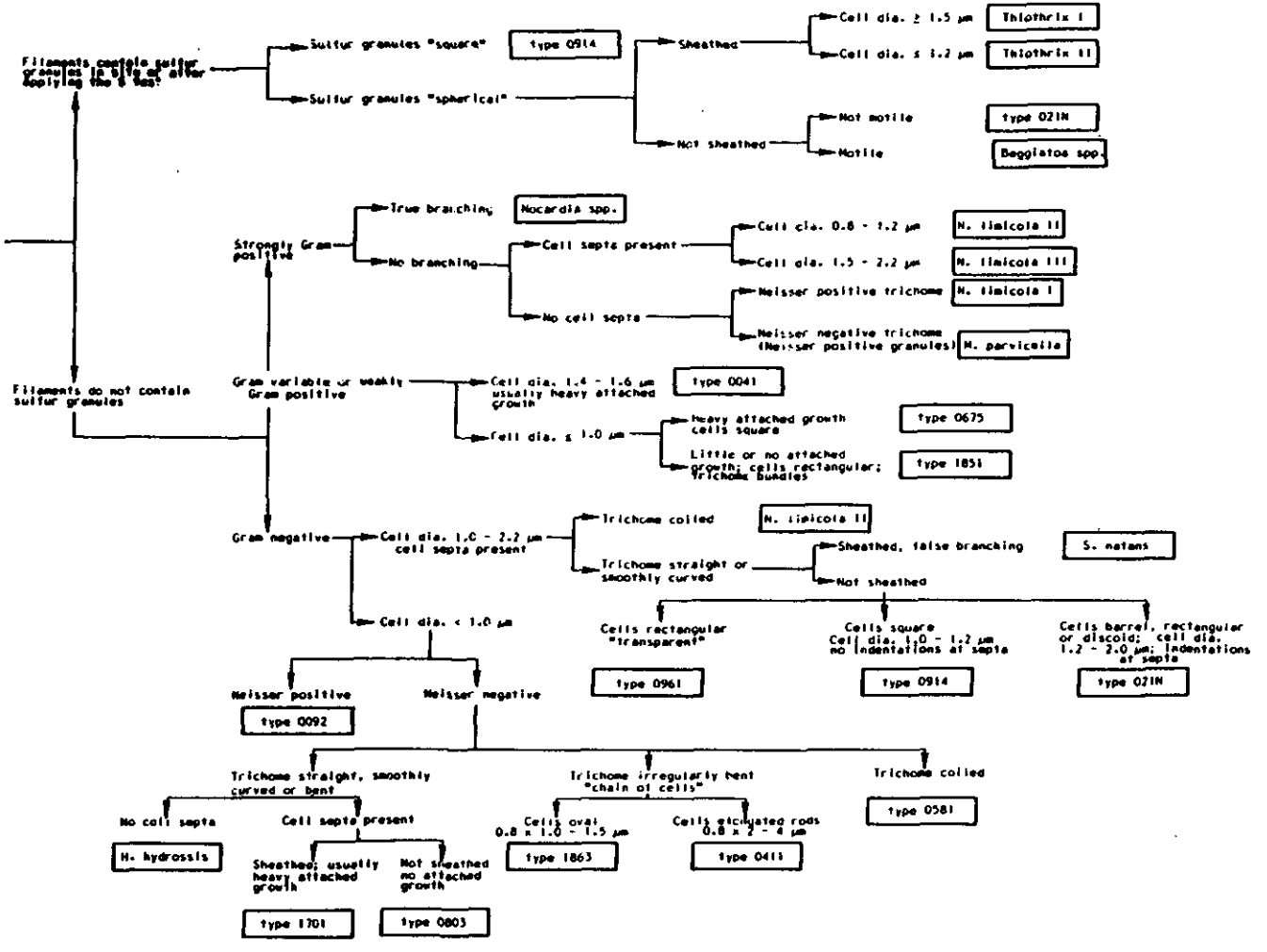


Figure 2: Dichotomous Key for Filamentous Organism "Identification" in Activated Sludge (Jenkins et al., 1986)

BRIGHT FIELD OBSERVATION				PHASE CONTRAST OBSERVATION 1000X												
FILAMENT TYPE	GRAM STAIN	NEISSER STAIN		SULFUR GRANULES		OTHER CELL INCLUSIONS	TRICHOME DIAMETER	TRICHOME LENGTH	TRICHOME SHAPE	TRICHOME LOCATION	CELL SEPTA CLEARLY OBSERVED	INDENTATIONS AT CELL SEPTA	SHEATH	ATTACHED GROWTH	CELL SHAPE AND SIZE	NOTES
		trichome	granules	in situ	S test											
S. nutans	–	–	–	–	–	PHB	1.0 - 1.4	500	St	E	+	+	+	–	round-ended rods 1.4 x 2.0	False branching
type 1701	–	–	–	–	–	PHB	0.6 - 0.8	20 - 80	St,B	I,E	+	+	+	+ +	round-ended rods 0.8 x 1.2	cell septa hard to discern
type 0041	+,V	–	–,+	–	–	–	1.4 - 1.8	100 - 500	St	I,E	+	–	+	+ +,–	squares 1.4 x 1.5 - 2.0	Neisser positive reaction occurs
type 0675	+,V	–	–,+	–	–	–	0.8 - 1.0	50 - 150	St	I	+	–	+	+ +,–	squares 1.0 x 1.0	Neisser positive reaction occurs
type 021N	–	–	–,+	–,+	+	PHB	1.0 - 2.0	50 - 500	St,SC	E	+	+	–	–	barrels, rectangles, discoid 1-2 x 1.5 - 2.0	rosettes, gonidia
Thiothrix I	–,+	–	–,+	+,–	+	PHB	1.4 - 2.5	100 - 500	St,SC	E	+	–	+	–	rectangles 2.0 x 3-6	rosettes, gonidia
Thiothrix II	–	–	–,+	+,–	+	PHB	0.8 - 1.4	50 - 200	St,SC	E	+	–	+	–	rectangles 1.0 x 1.5	rosettes, gonidia
type 0914	–,+	–	–,+	–,+	–	PHB	1.0	50 - 200	St	E,F	+	–	–	–	squares 1.0 x 1.0	sulfur granules "square"
Beggiatoa spp.	–,+	–	–,+	+,–	+	PHB	1.2 - 3.0	100 - 500	St	F	–,+	–	–	–	rectangles 2.0 x 6.0	motile: flexing and gliding
type 1851	+ weak		–	–	–	–	0.8	100 - 300	St,B	E	+,–	–	+	–,+	rectangles 0.8 x 1.5	trichome bundles
type 0803	–	–	–	–	–	–	0.8	50 - 150	St	E,F	+	–	–	–	rectangles 0.8 x 1.5	
type 0092	–	+	–	–	–	+	0.8 - 1.0	20 - 80	St,B	I	+,–	–	–	–	rectangles 0.8 x 1.5	
type 0961	–	–	–	–	–	–	0.8-1.2	40 - 80	St	E	+	–	–	–	rectangles 1.0 x 2.0	"transparent"
M. parvicella	+	–	+	–	–	PHB	0.8	100 - 400	C	I	–	–	–	–	–	large "patches"
Nocardia spp.	+,	–	+	–	–	PHB	1.0	10 - 20	I	I	+,–	–	–	–	variable 1.0 x 1 - 2	true branching
N. Limicola I	+	+	–	–	–	–	0.8	100	C	I,E	–	–	–	–	–	
N. Limicola II	–,+	+,–	–	–	–	PHB	1.2 - 1.4	100 - 200	C	I,E	+	+	–	–	discs, ovals 1.2 x 1.0	incidental branching Gram and Neisser variable
N. Limicola III	+	+	–	–	–	PHB	2.0	200 - 300	C	I,E	+	+	–	–	disc, ovals 2.0 x 1.5	
H. hydrossis	–	–	–	–	–	–	0.5	20 - 100	St,B	E,F	–	–	+	–,+	–	"rigidly straight"
type 0581	–	–	–	–	–	–	0.5 - 0.8	100 - 200	C	I	–	–	–	–	–	
type 1863	–	–	–,+	–	–	–	0.8	20 - 50	B,I	E,F	+	+	–	–	oval rods 0.8 x 1-1.5	"chain of cells"
type 0411	–	–	–	–	–	–	0.8	50 - 150	B,I	E	+	+	–	–	elongated rods 0.8 x 2-4	"chain of cells"

notation: + = positive; – = negative; V = variable; single symbol invariant; +, – or –, +, variable, the first being most observed.
Trichome shape: St = straight; B = bent; SC = smoothly curved; C = coiled; I = irregularly-shaped.
Trichome location: E = extends from floc surface; I = found mostly within the floc; F = Free in liquid between the flocs.

Table 7: Summary of Typical Morphological and Staining Characteristics (Jenkins et al., 1986)

identified, the operational data from the plant were analyzed to see if it supported the problem which the filamentous identification suggested. Once this correlation could be established (if sufficient data were available), results were discussed with the plant operator and remedial actions were suggested based on previous case studies and observations (Broderick and Sherrard, 1985; Jenkins et al., 1986) and ease of implementation at the plant. Typical effective remedial actions were presented in Table 5.

When time permitted and plants were willing to try various process modifications, the effects of the modifications on the process performance and filamentous organism populations were observed and recorded.

CHAPTER IV

RESULTS AND DISCUSSION

In this chapter, the choice of the identification technique, an evaluation of the usefulness of the techniques to treatment plant staff, a cost estimate for performing filamentous organism identification at a treatment plant will be presented. A brief summary table of the case study results will also be included. Detailed descriptions of each case study are presented in Chapter V.

The first task of this research was to conduct an intensive literature search in order to identify the techniques available for filamentous organism identification and evaluate their usefulness. Six identification techniques relating to filamentous organisms in activated sludge were found and evaluated with respect to their usefulness to treatment plant staff. Shown in Table 8 are the criteria upon which the techniques were evaluated and the results of the review. It can be seen that, compared to other techniques evaluated, the manual by Jenkins et al. (1986), Manual on the Causes and Control of Activated Sludge Bulking and Foaming, by far provides the most complete and up-to-date coverage of the filamentous organisms, their causes, case studies and bulking and foaming control measures. It should also be noted that of the plants in Massachusetts investigating filamentous bulking through organism identification, the majority use this manual. Therefore, Jenkins' manual was adopted for this study.

Table 8: Evaluation of Identification Techniques

METHOD	IDENTIFICATION KEY DEVELOPED FROM	PROCEDURE	ORGANISM DESCRIPTIONS	PHOTOGRAPHS	ORGANISM VS. CAUSE	REMEDIAL ALTERNATIVES	CASE STUDIES	RANK OF OCCURRENCE	USEFULNESS TO PLANT PERSONNEL
STANDARD	BERGEY'S MANUAL (SPECIES)	ISOLATION & TESTING	YES	NO	NO	NO	NO	NO	POOR
FARQUHAR & BOYLE 1971a, b	BERGEYS MANUAL (GENUS)	MORPHOLOGY & STAINING	YES	NO	NO	NO	NO	NO	POOR
EIKELBOOM 1975a, b	EXPERIENCE & OBSERVATIONS	MORPHOLOGY & STAINING	YES	YES	NO	NO	NO	NO	FAIR
EIKELBOOM & van BUIJSEN 1981	EXPERIENCE & OBSERVATIONS	MORPHOLOGY & STAINING	YES	YES	NO	NO	NO	NO	FAIR
JENKINS et al. 1986 ¤	EXPERIENCE & OBSERVATIONS	MORPHOLOGY & STAINING	YES	YES	YES	YES	YES	YES	EXCELLENT
EPA 1987 ‡	EXPERIENCE & OBSERVATIONS	MORPHOLOGY & STAINING	YES	YES	YES	YES	YES	YES	GOOD

¤ BASED ON PREVIOUS WORK BY EIKELBOOM
‡ CONDENSED VERSION OF JENKINS et al.

Worksheets for filamentous organism identification are provided in the manual. Through use of the techniques, these worksheets were found to be somewhat cumbersome and the order of tasks involved in organism identification does not follow the order of the worksheets. As a result, a modified identification procedure was developed. It is based on the instructions provided by Jenkins, but the worksheets are organized to follow the order of observations and also to provide space for sketches and other observations and a brief description of the treatment process and suspected problems. These worksheets are shown in Figures 3-6. Another objective of this research was to evaluate the applicability of filamentous organism identification techniques for use by treatment plant personnel. The first question which must be answered is what information can be gained through microscopic investigation of activated sludge?

It can be useful to:

(1) Establish that a settling or foam problem is indeed due to filament growth and not some other solids separation problem.

(2) Compare the causative filament(s) observed in separate bulking episodes at one plant; are the same or different filaments responsible at different times?

(3) Relate the types of filaments observed to suggested causative conditions to evaluate remedial actions.

(4) Evaluate the effect of proposed operational changes on the types and abundance of filaments present and floc

MODIFIED IDENTIFICATION PROCEDURE

I. Make Stains, Start Sulfur Test

II. A. FLOCS---See Filamentous Organism Identification Sheet

B. FILAMENTS

1. 400X, NO STAIN, WET MOUNT
   -CHECK FLOC MORPHOLOGY
   -FREE CELLS
   -FILAMENT EFFECT ON FLOC STRUCTURE
   -DOMINANT FILAMENTOUS TYPES, CHARACTERISTICS, LOCATION, MOTILITY, ETC.

2. 400X, WET MOUNT, CRYSTAL VIOLET
   -OVERALL FILAMENT ABUNDANCE
   -FILAMENT EFFECT ON FLOC STRUCTURE
   -FILAMENT LENGTHS, CHARACTERISTICS

3. 1000X, WET MOUNT, NO STAIN
   -DOMINANT FILAMENTS, CHARACTERISTICS-CELL SIZE, CROSS WALLS, SHAPE, COLOR, INCLUSIONS, ETC.

4. 1000X STAIN REACTIONS

5. RECHECK PREVIOUS STEPS IF WARRANTED BY STAINS

6. RUN THROUGH CHECKLIST

7. CHECK KEY, TABLE, DESCRIPTIONS

8. REPEAT INDIVIDUAL TESTS/OBSERVATIONS AS DEEMED NECESSARY

Figure 3: Page 1 Modified Identification Procedure

FILAMENTOUS ORGANISM IDENTIFICATION SHEET

WWTP_____

PROCESS DESCRIPTION/SVI_____

_____

REMARKS/PROBLEM_____

SAMPLE DATE_____ STORAGE_____ OBSERVATION DATE_____

****

1--100X NO STAIN

PROTOZOA, ETC._____

INORG/ORG PARTICLES_____

AMORPHOUS (INDIA INK STAIN)_____

2--100X CV DRY

FILAMENT EFFECT ON FLOC STRUCTURE (FIG 6)

INTERFLOC BRIDGING (6A,B)____

DIFFUSE FLOC STRUCTURE (6D)____

FREE FILAMENTS____

FILAMENT BACKBONE (6C)____

COMMENTS/COMPARES TO_____

FILAMENT ABUNDANCE--OVERALL (FIG 13)

_____NONE

_____FEW (FIL. IN OCCASIONAL FLOC)

_____SOME (NOT IN ALL FLOCS)

_____COMMON (1-5 PER FLOC)

_____VERY COMMON (5-20 PER FLOC)

_____ABUNDANT (20+ PER FLOC)

_____EXCESSIVE (MORE FIL. THAN FLOCS)

COMMENTS/COMPARES TO_____

3--100X CV WET

FLOC DIAMETER

	SCALE	µm
1	____	____
2	____	____
3	____	____
4	____	____
5	____	____
6	____	____
7	____	____
8	____	____
9	____	____
10	____	____
11	____	____
12	____	____
13	____	____
14	____	____
15	____	____
16	____	____
17	____	____
18	____	____
19	____	____
20	____	____

4--400X NO STAIN

FREE CELLS_____

	<150µm	150-500µm	>500µm
	SMALL	MEDIUM	LARGE

****

DESCRIPTION OF DOMINANT FILAMENTS_____

_____

_____

Figure 4: Page 2 Modified Identification Procedure
CV= crystal violet

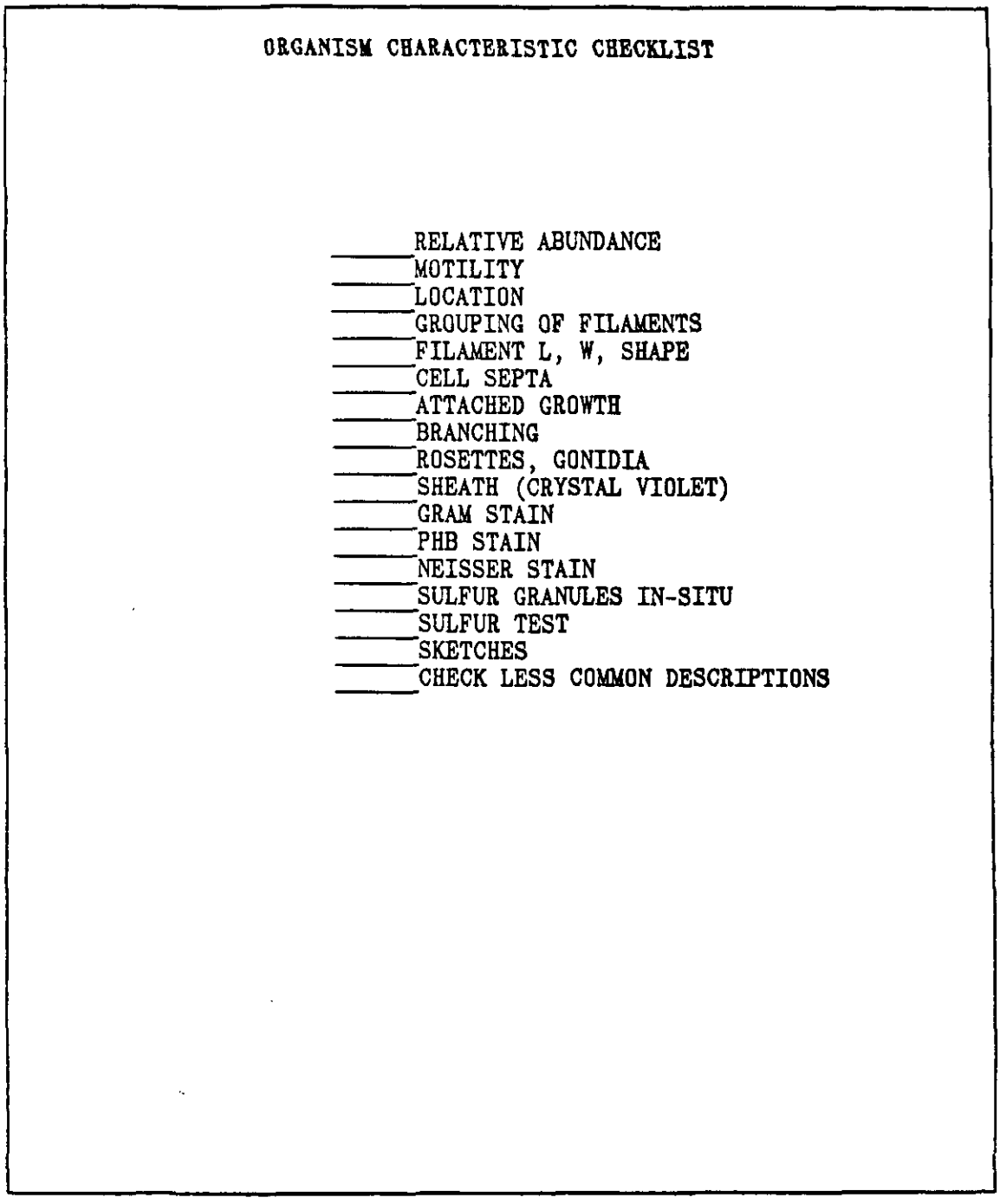


Figure 5: Page 3 Modified Identification Procedure

OBSERVATIONS AND SKETCHES

FILAMENT NUMBER_____ DOMINANT SECONDARY

FILAMENT NUMBER_____ DOMINANT SECONDARY

Figure 6: Page 4 Modified Identification Procedure

size and structure; has the appropriate remedial action been taken?

(5) Provide operators and laboratory personnel with training in activated sludge microbiology.

(6) Regular monitoring of filaments can lead to "heading off" a bulking episode before it becomes too serious.

(7) Protozoa and invertebrate groups can also be observed as indicators of solids residence time (SRT) and loading (See Figure 7 and Table 9) as there is a natural succession of different types.

Obviously, microscopic sludge investigation can be useful in terms of bulking control and long term monitoring for process control. However the techniques can be time consuming and costly to implement. Table 10 provides a rough cost estimate of equipment and supplies needed for an individual plant to become equipped to regularly monitor filamentous organisms. Overall, it would cost about $4135, not including the worker's time requirement. It may be difficult for a plant justify this expense and the manpower needed as well.

Another deterrent to looking for a solution to bulking problems is the lack of enforcement of penalties for discharge permit violations. Also, bulking only causes solids loss in severe cases. Plants with excess secondary clarifier capacity can easily handle poorly settling sludge and are content to deal with the problems as they materialize rather than prevent their occurrence.

In most cases, only one sample needs to be analyzed to determine

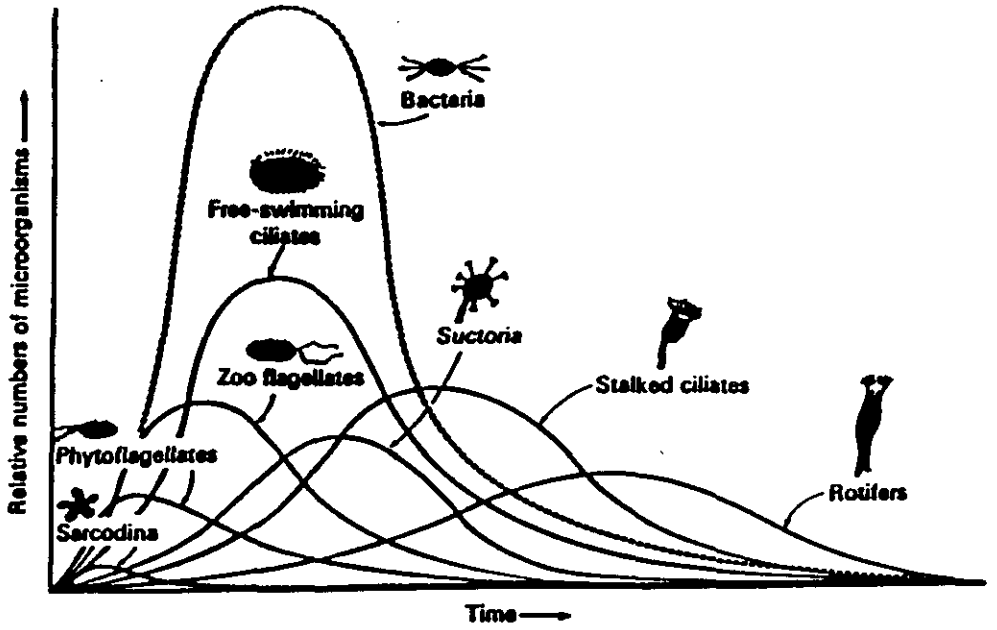


Figure 7: Relative Abundance of Protozoan Groups and Activated Sludge Process Conditions (Metcalf & Eddy, 1979)

Table 9: Organic Loading of Activated Sludge and Predominant Higher Life Forms Observed (Richard, 1989a)

Conditions	Predominant Groups
Low Organic Loading	stalked ciliates, rotifers higher invertebrates, especially nematodes
Optimum Organic Loading	good diversity of organisms, dominated by free-swimming and stalked ciliates
High Organic Loading	flagellates, amoebae, and small, free-swimming ciliates

Table 10: Cost Estimate to Implement Filamentous Organism Identification Program

Items	Cost
Equipment Costs	
Research grade, phase contrast microscope-with eyepiece micrometer, 1000x high magnification	$2500 - $3500
Camera and adaptor - for photographic record and reference	$500 (optional)
Stains, Chemicals, Lab Supplies	$400
1 Identification Confirmation	$110 (recommended)
Attendance at filament identification seminar/short course (2 day session)	$125 (recommended)
APPROXIMATE TOTAL COST	$4135*

*excludes manpower

the probable cause of a particular bulking episode (if taken during the bulking episode) and evaluate control measures. A number of researchers with extensive experience in filamentous organism identification and bulking control provide a mail in identification service with about 1-2 weeks required for results. The cost is $110 per sample. The only drawbacks of this service are the turnaround time for results and the suggested cause for bulking is based solely on organism identification. While these services are an excellent independent check on organism identification, they consider only the organisms and not the environmental and operating conditions at the treatment plant. Ideally, before remedial measures can be evaluated, plant operating conditions and data should be closely studied to determine if the organism identification correctly elucidated the problem.

Some of the larger plants in Massachusetts are currently implementing these techniques and are finding them useful, however, each of these plants has full time lab personnel devoted to process monitoring. Smaller plants do not have this luxury. What seems to be warranted is the establishment of regional or statewide assistance groups, much like the technical assistance training service (TATS) of the DEP training center in Milbury, MA. The aim of this group is to provide training to treatment plant staff and aid in operational problems. An individual trained in filamentous organism identification and activated sludge plant operation could be incorporated into this group. Not only could filamentous organism identification be done locally and quickly, but plant evaluation visits, data analysis and

operator education could also be provided as part of this service. Also, by keeping abreast of recent developments in research in this area, the TATS group (or other) could serve to keep its plant personnel informed of the aid available, where to find it, its usefulness and area plants which have benefitted.

What also needs to be studied and stressed is the long term benefits of filamentous bulking control and eradication. Most plants only consider the short term expense of money and effort in solving the problem and this in and of itself is a deterrent to action.

NPDES discharge permits and compliance enforcement can only be expected to become more stringent in the future. Long term benefits include better process control through increased understanding of the activated sludge process and microbiota, decreased operator effort, more consistent effluent quality, and in some cases, increased process capacity leading to longer plant life.

The results of the six case studies are presented in Table 11. In the choice of plants, a variety of wastewater types, flow configurations and plant capacities were included in an attempt to study plants with different bulking causes. Plants with low pH and septic wastewater problems were not encountered, however, other problems including low DO, low F/M and nutrient deficiency were studied with varying degrees of success in correcting the problem.

Table 11: Massachusetts Filamentous Bulking Case Studies

TREATMENT PLANT WASTE TREATED	PROCESS TYPE	DOMINANT FILAMENTOUS ORGANISM(S)	PROBABLE CAUSE OF BULKING	CHANGE IN OPERATION	RESULTS
1. Plant A Domestic Wastewater	Complete Mix	*Nocardia* sp. Type 0041 *M. parvicella* *S. natans*	Low F/M	None	Adequate plant capacity to control bulking and foaming
2. Plant B Domestic Wastewater Food Processing Wastes	Complete Mix	*N. limicola* II	Phosphorous Deficiency	Increased SRT Addition of Septage Increased Aeration	RAS chlorination ceased Return to nonbulking condition Reduction in filament abundance
3. Plant C Domestic Wastewater	Extended Aeration	*M. parvicella* Type 0041	Low F/M Low DO	None	None
4. Plant D Domestic Wastewater Paper Mill Wastes	Step Feed	*M. parvicella* Type 0041 Type 1851	Low F/M Nutrient Deficiency	Change to Plug Flow Short term Addition of Phosphorous	Initial success in reducing the SVI *N. limicola* II became predominant and SVI increased
5. Plant E Domestic Wastewater Paper Mill Wastes	Step Feed	*Thiothrix* II *S. natans*	Phosphorous Deficiency	Phosphorous Addition	RAS chlorination ceased Nonbulking condition achieved Reduction in filament abundance
6. Plant F Domestic Wastewater Paper Mill Wastes	Complete Mix	*Thiothrix* II *S. natans*	Nutrient Deficiency	Addition of urea and phosphoric acid	Reduction in RAS chlorination Reduction in bulking frequency and severity

CHAPTER V

CASE STUDIES

5.1 PLANT A - Low F/M

Plant A receives an average daily waste flow of 0.20 m^3/s (4.5 mgd), 63% of its average daily design flow. The plant utilizes a complete mix activated sludge system with surface aeration as shown in Figure 8. Average influent BOD_5 and total suspended solids (TSS) are 220 mg/L and 179 mg/L, respectively. Approximately 95% of the incoming wastewater is domestic, the remainder being of industrial origin. Of the total flow, 30% comes from a university campus, causing rapid flow increases in early September, late January and mid-March (after spring break), corresponding with the return of students to campus (See Figure 9).

This plant has chronic settleability problems (the SVI is usually greater than 150 ml/g). The plant operator feels this is due to rapid flow variations when students leave or return to the campus and seasonal temperature fluctuations in the wastewater. Besides settleability problems due to filamentous organisms, the plant also experiences frequent foaming problems which can cause excess solids in the final effluent due to persistent scum and foam floating over the weirs of the secondary clarifiers.

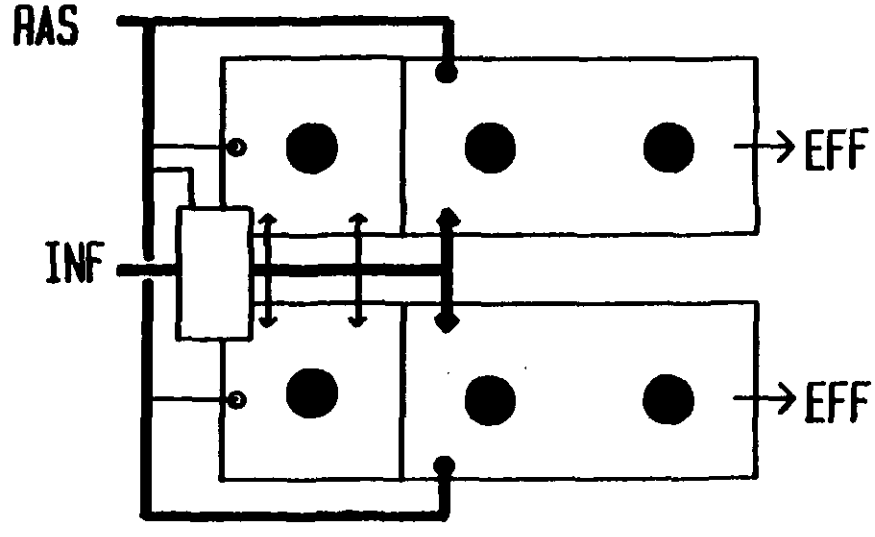


Figure 8: Plant A Flow Configuration

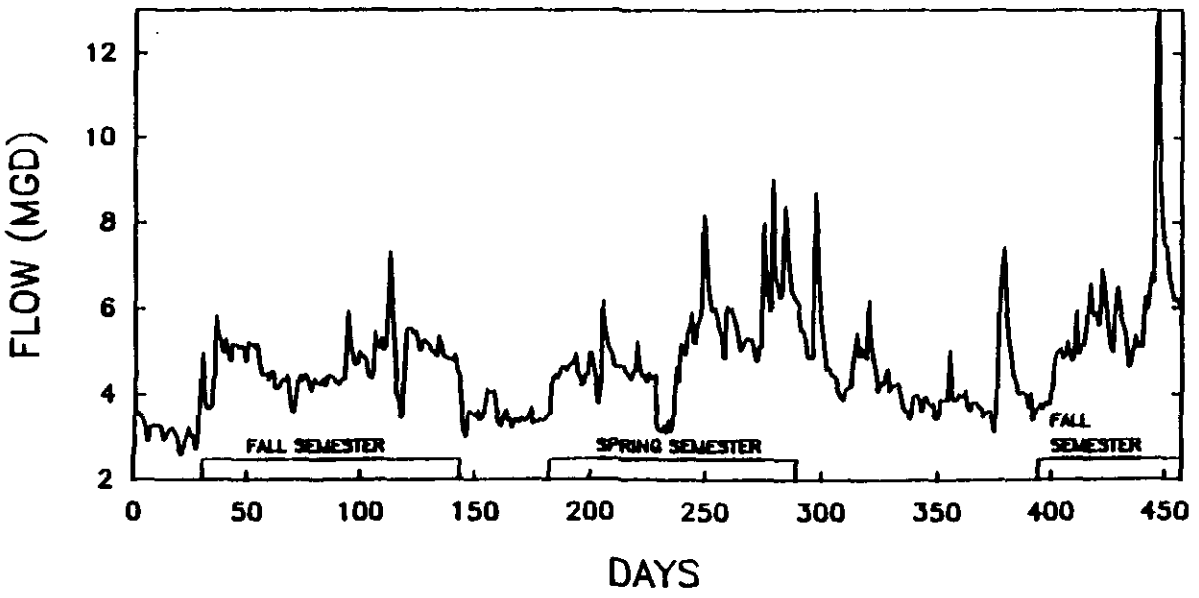


Figure 9: Plant A Influent Flow Variations

FILAMENTOUS ORGANISM IDENTIFICATION

Microscopic investigations of the activated sludge revealed "very common" filament abundance and diffuse floc structure. The dominant filamentous organisms were Nocardia sp., Type 0041, Micothrix parvicella and Sphaerotilus natans. Abundance of Nocardia and M. parvicella, causing foaming problems, are shown in Figures 10 and 11.

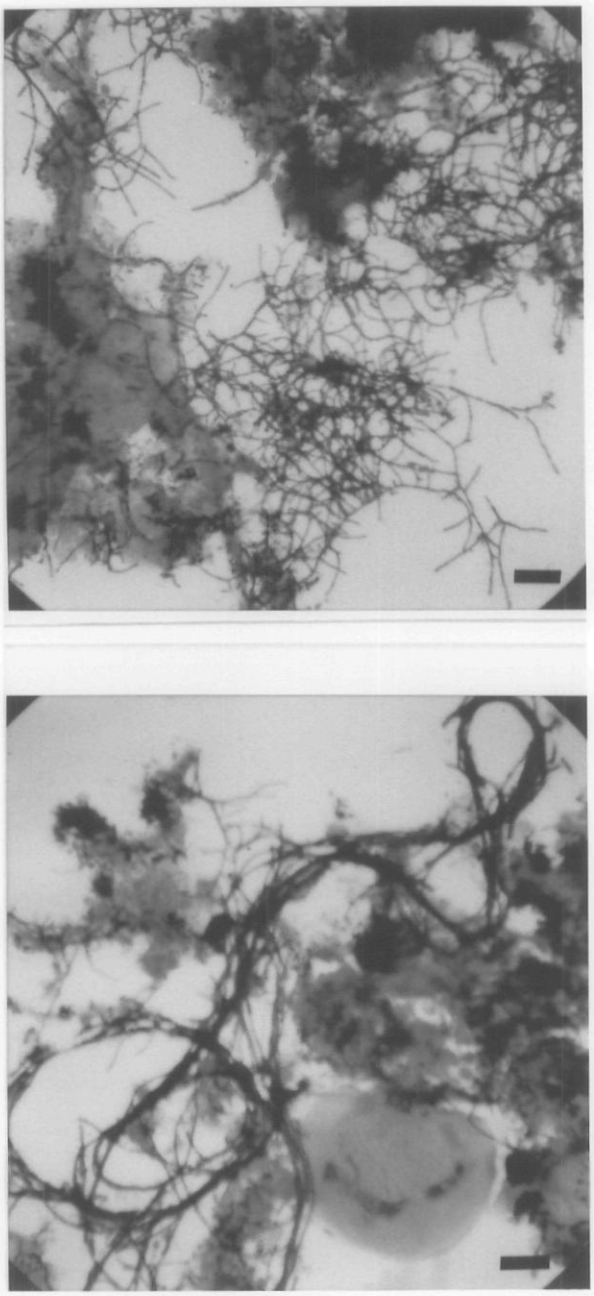
From Table 4, it can be seen that abundant growth of Nocardia sp., Type 0041 and M. parvicella are indicative of a low food-to-micro-organism ratio in the aeration basin. S. natans abundance is generally caused by a low dissolved oxygen or low nutrient condition. S. natans was abundant in some of the samples examined, but never excessive. At the time it was found to be dominant (day 220), the F/M had steadily increased from .18 to .32 in the few weeks prior (See Figure 12a). This may have induced low DO conditions for the applied F/M. Lab experiments conducted by Palm et al. (1980) showed that the higher the F/M, the greater the DO concentration required to prevent the growth of S. natans, a low DO filamentous organism.

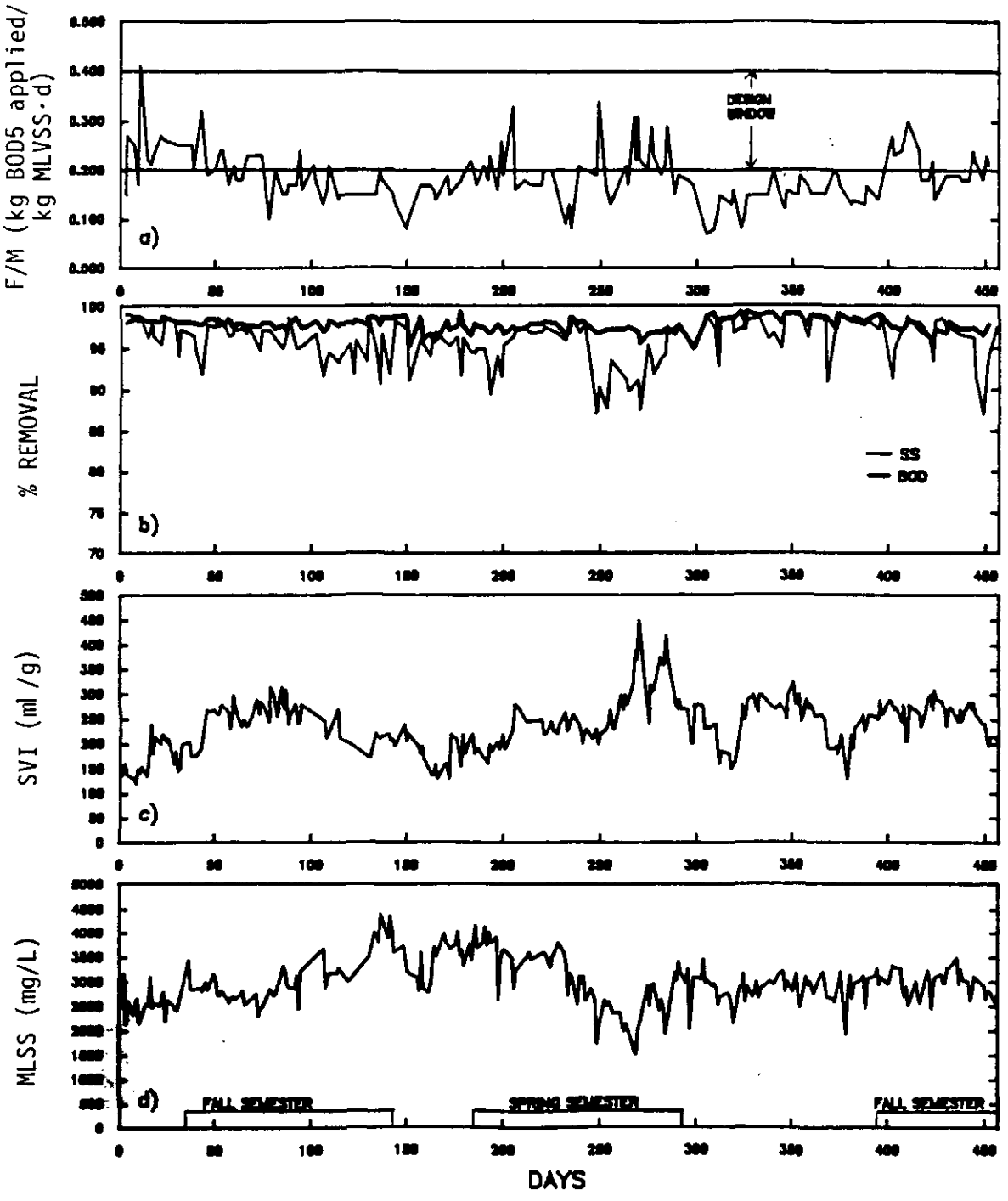
PLANT DATA ANALYSIS

Fourteen months of plant operating data were obtained for this study to support the cause of bulking as determined by the filament identification. From Figure 12a, it can be seen that the F/M is usually low compared to the typical design "operating window" for the

Figure 10: Plant A Abundance of Nocardia sp.
(1000X Gram stain, transmitted light; bar=10 μm)

Figure 11: Plant A Abundance of M. parvicella
(1000X Gram stain, transmitted light; bar=10 μm)





NOTE: MISSING DATA ARE INTERPOLATED FOR CLARITY

Figure12 : Plant A Operating Data

conventional activated sludge process (Metcalf & Eddy, 1979).

What appears to be the major cause of the low F/M conditions is the flow variations caused by the university. When the students return from a break (summer or winter), the flow suddenly increases from about 0.15 m^3/s (3.5 mgd) to 0.20 m^3/s (4.5 mgd). To handle the increased flow, more aeration tanks are brought on line. This makes it difficult to maintain a particular F/M or mixed liquor suspended solids (MLSS) until the system is equilibrated since the F/M is a function of hydraulic residence time, MLSS and BOD_5 as shown in equation 1.

$$F/M = S_0/\theta X \quad (1)$$

where S_0 = BOD_5 applied to the aeration basin, mg/L

θ = hydraulic residence time, days

X = MLSS, mg/L

CONTROL STRATEGIES

During the time of this study, the plant's discharge permit was not violated. From Figure 12b it can be seen that the effluent consistently achieved greater than 85% removal of BOD and TSS, even at times of elevated SVI (Figure 12c). Decreases in removal efficiency did not consistently correlate with increased SVI, which would be caused by increases in populations of the filamentous organisms. Decreased removal usually occurred at times of peak flows. Washout of solids may

have been due to hydraulic overloading at these times as well as Nocardia foam and scum overflowing the weirs of the secondary clarifiers.

To alleviate the filamentous problem, one or both of two strategies are employed at the plant: chlorination of the return sludge and reduction of the solids residence time (SRT). RAS chlorination is used to selectively kill filamentous organisms, reducing their abundance. This strategy is rarely used at Plant A; only when severe bulking and foaming are experienced and the effluent quality is compromised. The rationale behind lowering the SRT is twofold. First, filamentous organisms have a slower maximum growth rate (μmax) than the floc formers, so lowering the SRT may selectively inhibit the growth of the filaments. Secondly, by lowering the SRT through increased wasting of sludge (WAS), the MLSS concentration is decreased, raising the F/M (See Equation 1). This strategy is evident in Figure 12d. In late spring, days 230 to 270, the MLSS was lowered from 3500 to 1500. Within about 15 days, the SVI began to decrease steadily for a period of time. This lag time of response to process modifications has been observed by other investigators. Jenkins et al. (1986) reported that once changes have been made to discourage filamentous growth, settleability may improve only slowly since the microbial population changes at a rate proportional to the culture washout rate, SRT. Thus, greater than one SRT may be required for improvements in settleability to become apparent.

SUGGESTIONS FOR REMEDIAL ACTION

A seemingly obvious course of action would be to maintain a higher F/M ratio by lowering the SRT. Although this would probably lower the frequency of bulking and foaming problems, it would mean a consistently higher sludge wasting rate. More sludge would therefore need to be dewatered. Sludge dewatering is accomplished at the plant with dissolved air flotation (DAF) and vacuum filtration with the addition of lime, polymer and ferric chloride. Increased sludge processing means greater energy use, chemical use, increased labor and maintenance as well as more dried sludge being landfilled, and with increasing concern over landfill space limitations this is not a desirable consequence.

There has been some success in laboratory studies (Chudoba et al., 1973b; Wheeler et al., 1984; Daigger et al., 1985; Chudoba et al., 1985b; Linne and Chiesa, 1987) in the use of a selector to rectify complete-mix, low F/M bulking conditions. Ideally, a selector is a small mixing tank or series of tanks in which RAS and primary effluent are mixed prior to entering the aeration basin. This ensures a high carbonaceous substrate concentration when mixed with the RAS to select for non-filamentous organisms. The option of placing the RAS in with the primary effluent in the influent channnel (See Figure 8) is available at Plant A, but this probably does not provide enough detention time prior to the aeration basin for effective selector operation. Use of the basin area of the first aerator could be used as

a selector since sluice gates are already present. This flow configuration is shown in Figure 13.

The bulking and foaming problems at Plant A are only periodic and usually do not seriously inhibit treatment efficiency. RAS chlorination and/or temporary reduction of MLSS concentration have proven economical and effective. At this time the plant has chosen not to experiment with selector operation or maintain a consistently lower MLSS concentration because of this. The plant recognizes the cause of the settlability problems and has expressed interest in selector operation if the problems become worse or more frequent.

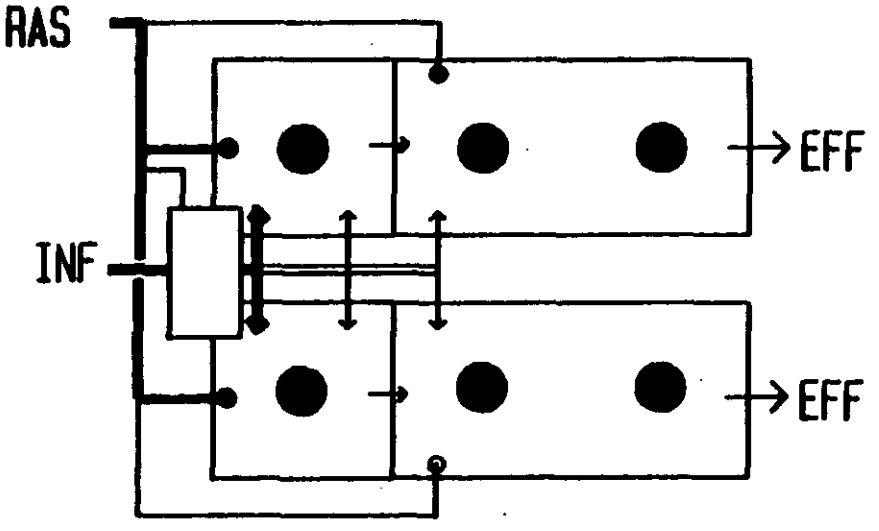


Figure 13 : Plant A Selector Configuration

5.2 PLANT B - Phosphorous Deficiency

A complete mix activated sludge process with surface aeration as shown in Figure 14 is utilized at Plant B. Currently, an average daily flow of 0.053 m^3/s (1.2 mgd) is treated, 67% of its average daily design flow of 0.078 m^3/s (1.79 mgd). Approximately 40% of the wastewater is of industrial origin including food processing and soft drink bottling. Based on design values for flow and aeration basin volume, the plant is designed to treat an average wastewater strength of 215 mg/L BOD_5, however, the current BOD_5 is 300-500 mg/L. The average influent TSS is 240 mg/L.

This plant has a history of bulking related problems including poor settling, clarifier overloading, blanket rising and overall deterioration of effluent quality during bulking episodes. The major control mechanism utilized by this plant has been chlorination of the return sludge for 2-4 days at a time. This will usually bring the bulking under control for a week or so, but filaments soon become dominant again. Excessive amounts of chlorine have also been necessary, up to 66 kg/d (145 lbs/d).

Although RAS chlorination has been able to bring bulking episodes under control, effluent quality has suffered. Shown in Table 12 are the monthly average effluent concentrations of BOD_5 and TSS for about two and one half years of operation. The plant's NPDES discharge permit specifies effluent limits of 30 mg/L for both TSS and BOD_5 on a 30-day

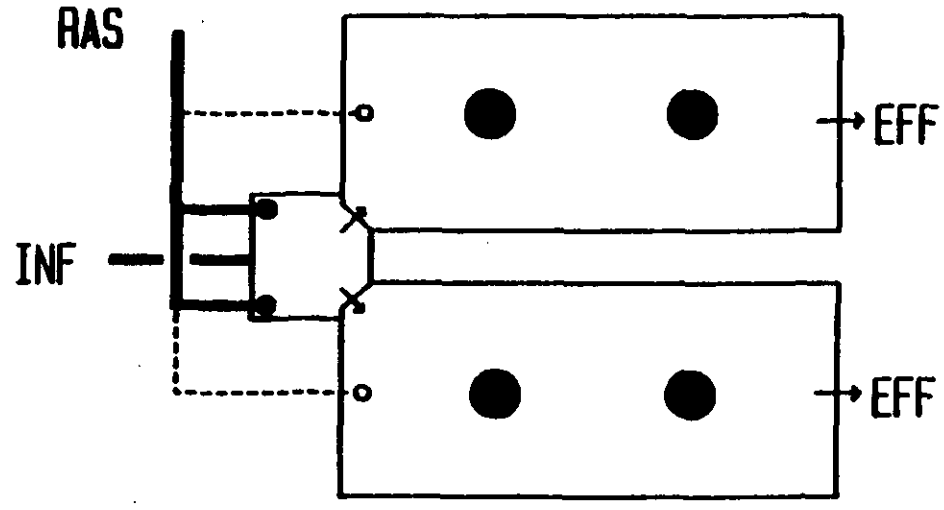


Figure 14 : Plant B Flow Configuration

Table 12: Plant B Monthly Average Discharge Concentrations

Month		Eff. BOD_5 (mg/L) 30-day average	Eff. TSS (mg/L) 30-day average	Permit Violation
January	1987	22	6	NO
February		35	46	YES
March		29	33	YES
April		20	14	NO
May		33	22	YES
June		20	23	NO
July		32	28	YES
August		72	22	YES
September		30	35	YES
October		50	12	YES
November		61	59	YES
December		62	27	YES
January	1988	53	32	YES
February		41	45	YES
March		17	8	NO
April		9	7	NO
May		8	11	NO
June		7	15	NO
July		76	26	YES
August		30	10	NO
September		22	15	NO
October		76	14	YES
November		13	9	NO
December		13	16	NO
January	1989	14	22	NO
February		16	17	NO
March		31	49	YES
April		46	46	YES
May		15	27	NO
June		31	52	YES
July		15	28	NO
August		24	17	NO
September		27	7	NO

average. Obviously, the plant has been out of compliance on many occasions.

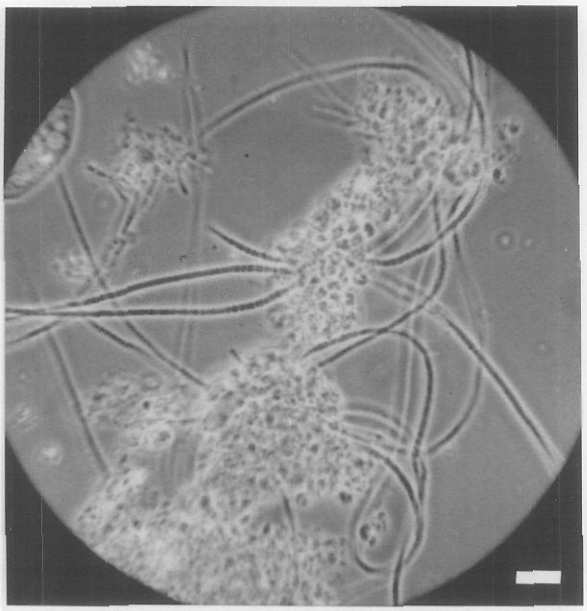
FILAMENTOUS ORGANISM IDENTIFICATION

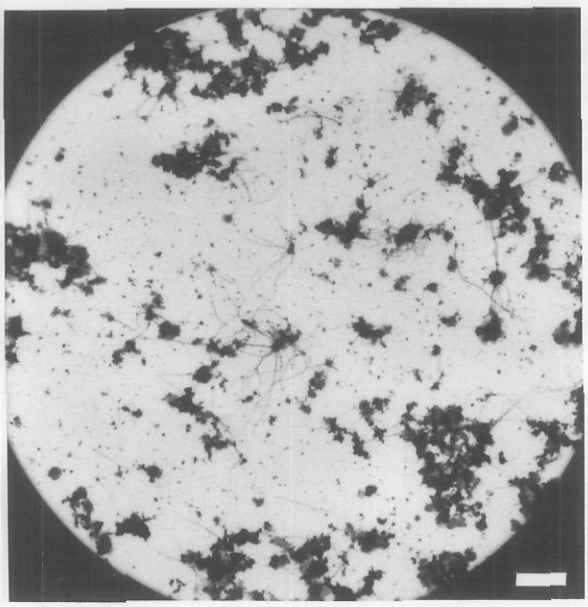
Samples obtained in May and June of 1989 (days 251 and 285) were analyzed for filamentous organisms. "Very common" growth of the filamentous organism Nostocoida limicola II was observed in both samples. This was the only filamentous organism observed. The dominance of this organism resulted in substantial bridging of flocs. A significant amount of small dispersed floc were also present (See Figures 15 and 16).

No definitive cause-effect relationship has been established for N. limicola II in activated sludge environments, although it has been observed to be associated with low F/M operation with certain organic wastes (starches), low dissolved oxygen (due to its fermentative capabilities) (Richard, 1989a) and also phosphorous deficiency, especially in industrial waste systems (Richard, 1989b). A sample of this activated sludge was sent to Dr. Michael Richard at Colorado State University for confirmation of organism identification. His analysis indicated predominance of N. limicola II exhibiting atypical staining and morphological characteristics, a common consequence of industrial waste systems. From his experience with this organism in industrial waste plants, he concluded that the probable cause of its predominance was a phosphorous deficiency (Richard, 1989b).

Figure 15: Plant B Interfloc Bridging by N. limicola II (1000X phase contrast; bar=10 μm)

Figure 16: Plant B Filament Abundance and Floc Structure (100X phase contrast, crystal violet, dried; bar=100 μm)





DATA ANALYSIS & CONTROL MEASURES

Thirteen months of plant operating data were obtained for this study. As can be seen from Figure 17a, this plant experiences frequent bulking episodes as indicated by elevated SVI values. Virtually all these episodes were brought under control by dosing the return sludge with chlorine.

A phosphorous deficiency was suggested to the plant operator as the probable cause of the bulking problems. This is fairly common with plants treating food processing wastes. The plant staff has not actually analyzed its wastewater for nutrients, but has taken several measures to control the problem and has had some success.

(1) In July (days 304+), a change was made from vacuum filtration to dissolved air flotation for dewatering of secondary sludge. About one fourth the amount of lime and ferric chloride is now used. Therefore the pH of the filtrate returned to the head of the plant is lower and there will be less precipitation of phosphorous before the aeration basin.

(2) Beginning in September of 1989 (days 366+), the plant began receiving approximately 11.4 m^3/d (3000 gal/day) of septage from home septic tanks. The reasoning behind this was to take advantage of any nutrients available from this.

(3) A fraction of the chlorinated plant effluent is now recycled to take advantage of residual chlorine available to control filaments.

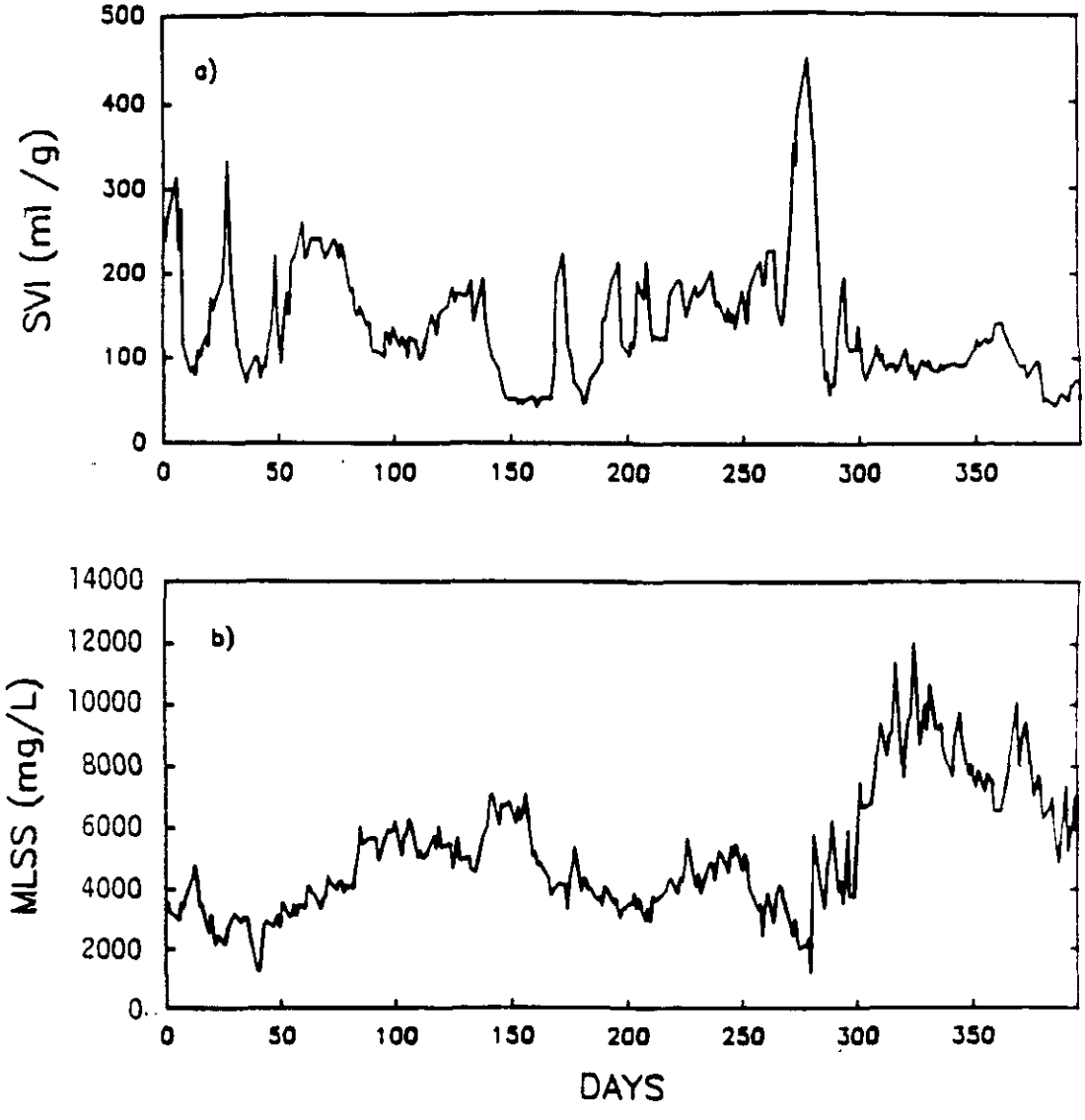


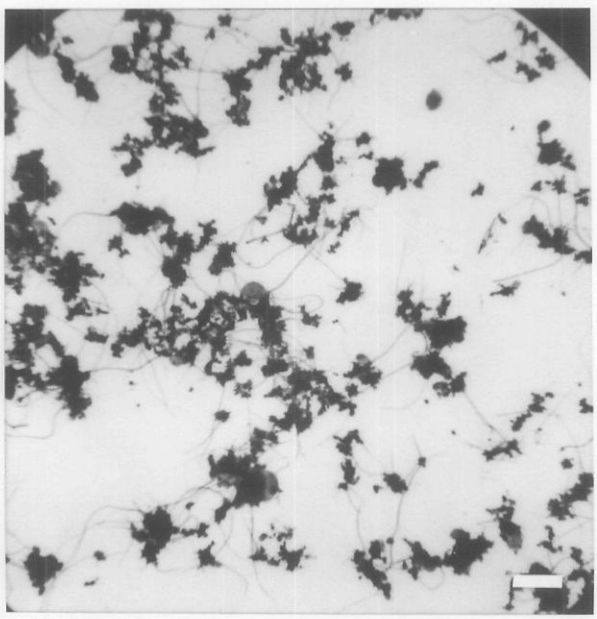
Figure 17: Plant B Operating Data

(4) The effluent weirs in the aeration basins were raised to increase O_2 transfer efficiency of the surface aerators to ensure adequate O_2 is available now that septage is being taken in.

(5) Perhaps the most significant change, however, is the increase in MLSS (See Figure 17b), which also means an increase in solids residence time. It has been demonstrated that as the SRT increases, a higher proportion of the substrate utilized by the microorganisms is used for energy and cell maintenance and less for cell reproduction (Sherrard and Shroeder, 1976). Considering a widely used elemental analysis of cells, $C_{60}H_{87}O_{23}N_{12}P$ (McCarty, 1970), it is obvious that the less new cells produced, the lower the nutrient requirements.

A sludge sample was analyzed at the end of this study. Dominant organisms present were N. limicola II and Thiothrix sp. The abundance was categorized as "common", a drop of one abundance category since implementation of control measures. Larger, more compact flocs were observed and very few dispersed flocs (See Figure 18). Since the end of this study, the plant has experienced further reduction in filament abundance (although some minor bulking episodes have occurred) and consistent attainment of < 10 mg/L BOD_5 and TSS in the effluent.

Figure 18: Plant B Filament Abundance and Floc Structure After Remedial Action (100X phase contrast, crystal violet, dried; bar=100 μm)



SUGGESTIONS FOR REMEDIAL ACTION

Since the implementation of the control measures previously mentioned, the SVI has been consistently lower (See Figure 17a, days 300+). Also there has been no need for RAS chlorination. Operating at a higher SRT may have so far alleviated the bulking problem, however, the average F/M has dropped from 0.30 to 0.14. Low F/M bulking may develop as a result. In this case the F/M should be increased and nutrient addition considered. The addition of nutrients to the aeration basin based on residual effluent concentrations of 1 mg/L inorganic nitrogen (NH_3 and NO_3-N) and 0.2 mg/L soluble PO_4-P (Richard, 1989a) will ensure adequate nutrient availability, even with the widely varying BOD_5 loading. These minimum residual concentrations in the aeration basin effluent are based solely on the concept that if there is a nutrient residual leaving the aeration basin, it is assumed that adequate nutrients were available in the aeration basin.

5.3 PLANT C - Low F/M, Low DO

Plant C is a small extended aeration plant (See Figure 19) utilizing intermittent surface aeration (1.5 hours on, 15 min. off) and without primary sedimentation. Its average daily design flow is 0.0079 m^3/s (0.18 mgd) and it is currently treating an average daily flow of 0.0048 m^3/s (0.11 mgd). The aeration basin is completely mixed and the wastewater is 100% domestic. Average influent BOD_5 and TSS are 220 mg/L and 135 mg/L, respectively.

Plant C is operated with an average mixed liquor DO of 0.2 mg/L, although the DO measurements may be artificially low. Samples are transported to the plant lab in open containers to be measured. With the high MLSS present, the DO may be depleted during transport to the lab. The intermittent aeration would also contribute to low DO conditions.

This plant has experienced high sludge blankets in the secondary clarifiers and at times severe foaming problems (See Figure 20). The foam may rise a foot or more when aerators are turned off. The plant operator feels that this is correlated to increased SRT's (> 30 days) due to inability to waste sludge to the aerobic digestor during wet weather, since digested sludge is dewatered on outdoor sludge drying beds. The higher SRT is a directly related to higher MLSS concentration and therefore a lower F/M ratio.

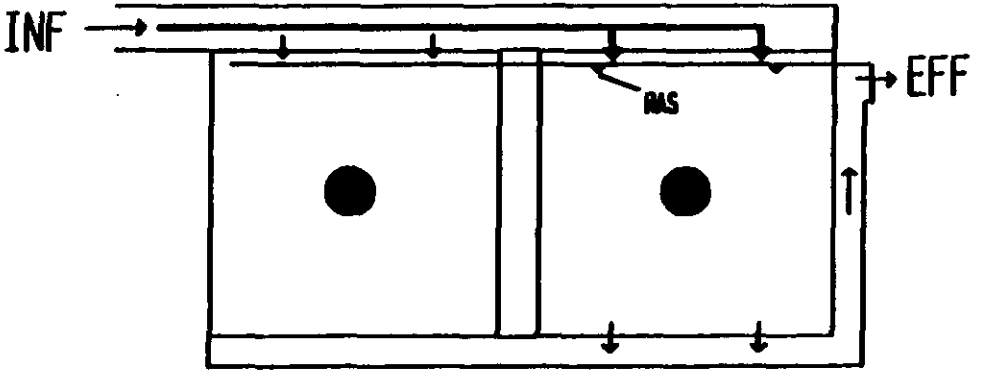
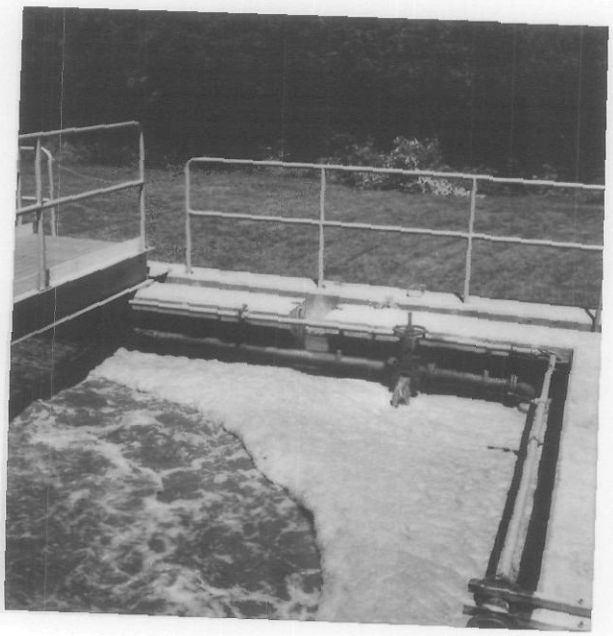


Figure 19: Plant C Flow Configuration

Figure 20: Plant C Foaming on Aeration Basin



FILAMENTOUS ORGANISM IDENTIFICATION

Foam and mixed liquor samples were examined microscopically. Micothrix parvicella and Type 0041 were found to be the dominant organisms in "abundant" to "excessive" quantities (See Figure 21). Floc formation was very weak and dispersed. Individual flocs were difficult to discern (See Figure 22).

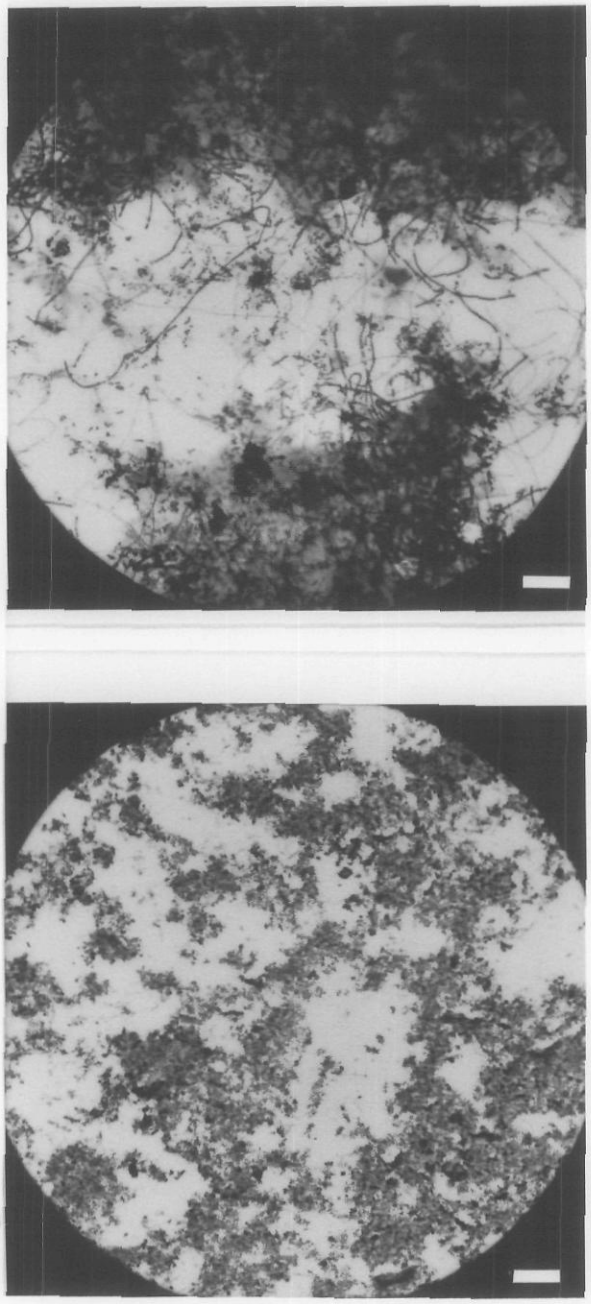
The dominant organisms present generally indicate low F/M conditions. The cause of the presence of M. parvicella foam is unclear, although it has been associated with excess grease and fats.

DATA ANALYSIS

Plant C operating data is presented in Figure 23. From Figure 23a, it can be seen that the F/M is generally on the lower end of the typical design F/M range. It also can be seen that the aeration basin DO is quite low (See Figure 23b). Although these values may be artificially low due to poor sampling technique, it is not uncommon for an extended aeration plant to have low DO problems. Throughout the study, the SVI was continually rising (See Figure 23c). Severe foaming problems were also experienced on days 22-25. The usual control measure is to add chlorine to the return sludge and hose down the foam.

Figure 21: Plant C M. parvicella and Type 0041 Abundance (1000X Gram stain, transmitted light; bar=10 μm)

Figure 22: Plant C Floc Morphology (100X phase contrast, crystal violet, dried; bar=100 μm)



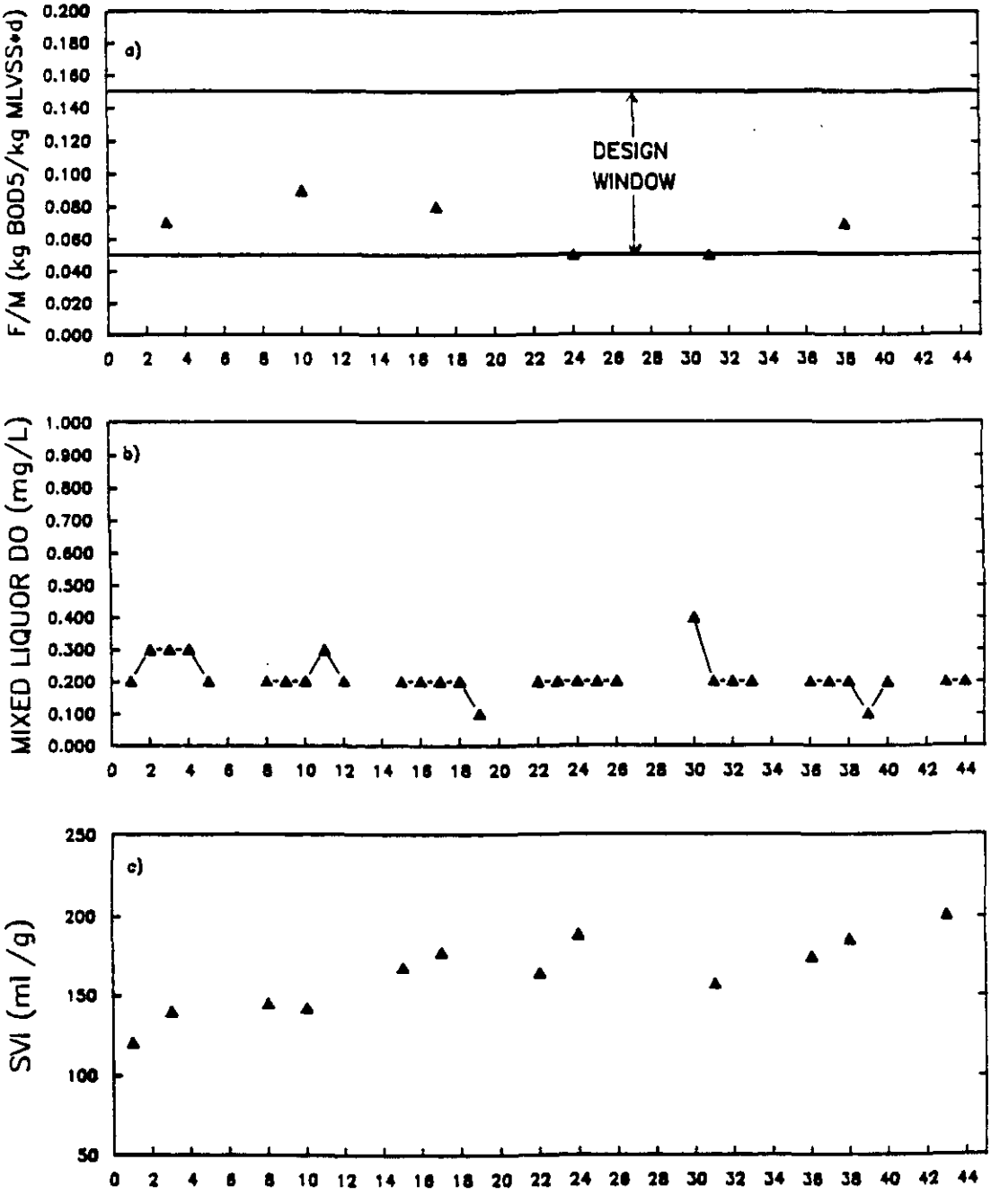


Figure 23 : Plant C Operating Data

SUGGESTIONS FOR REMEDIAL ACTION

This plant seems to be suffering from of a number of design and operational problems, most notably low DO, low F/M and inability to regularly waste sludge. With a low F/M activated sludge, the logical control measure would be to lower the MLSS by decreasing the SRT. This would affect other processes as well. Since the limited sludge storage capacity is in the aerobic digestor and its waste rate is controlled by the availability of sludge drying beds, extra sludge storage capacity would be required. This could be accomplished at the present time by using one of the aeration basins to hold digested sludge prior to drying, since only one aeration basin is presently being used. Pumps would also have to be obtained to transport digested sludge from the holding basin to the drying beds.

With an increase in F/M, the DO requirement per unit of biomass in the aeration basin would be increased. To supply additional oxygen, hydrogen peroxide (H_2O_2) could be used instead of sodium hypochlorite to destroy filaments in the RAS. This would selectively kill filamentous organisms and add oxygen to the sludge. Another alternative would be to replace the present mechanical aerators, which have inadequate oxygen transfer capacity, with more efficient and more powerful mechanical aerators or a diffused aeration system.

5.4 PLANT D - Low F/M, Nutrient Deficiency

Plant D is designed for an average daily flow of 0.63 m^3/s (14.3 mgd) and is currently operating at 80% of this, 0.50 m^3/s (11.4 mgd). A step feed activated sludge process with surface aeration, as shown in Figure 24 is utilized. About 88% of the flow is domestic wastewater, and the remainder is industrial, mostly from a paper industry. Other minor industrial contributors include a leather tannery and several circuit board manufacturers. Influent BOD_5 and TSS are 220 (range 150-700) mg/L and 220 (range 100-1800) mg/L, respectively.

In the past, Plant D has occasionally experienced bulking and foaming problems associated with Nocardia sp. proliferation. Beginning in June of 1989 (day 152+), however, the plant experienced a steady increase in filamentous organism abundance and settleability problems.

FILAMENTOUS ORGANISM IDENTIFICATION

The first sample was obtained on day 213 and the SVI at this time was 271 ml/g. It was found to contain "excessive" growth of Micothrix parvicella, Type 0041 and Type 1851 (See Figure 25). Nocardia sp. and Nostocoida limicola II were also present, but in lesser amounts. From Table 4, it can be seen that M. parvicella, Type 1851 and Nocardia sp. are generally associated with low F/M conditions. Type 0041 and N. limicola II have been associated with low F/M wastewaters as well as

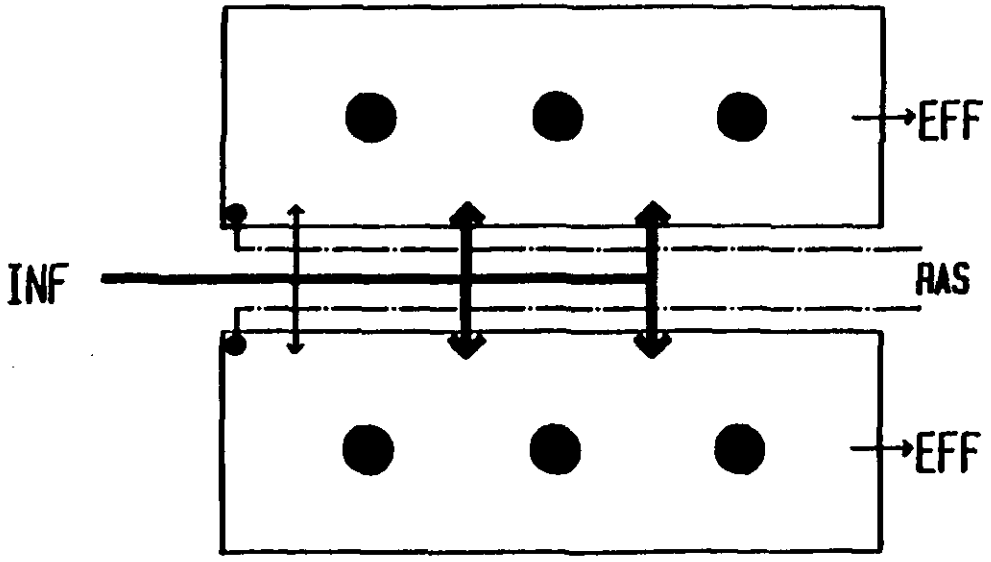
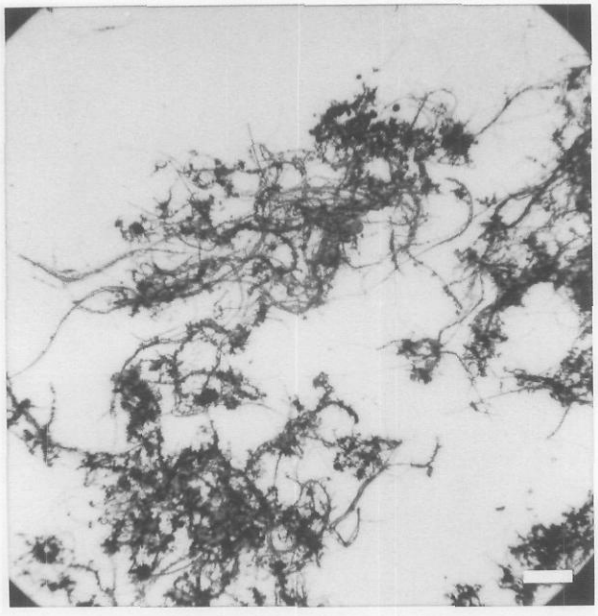
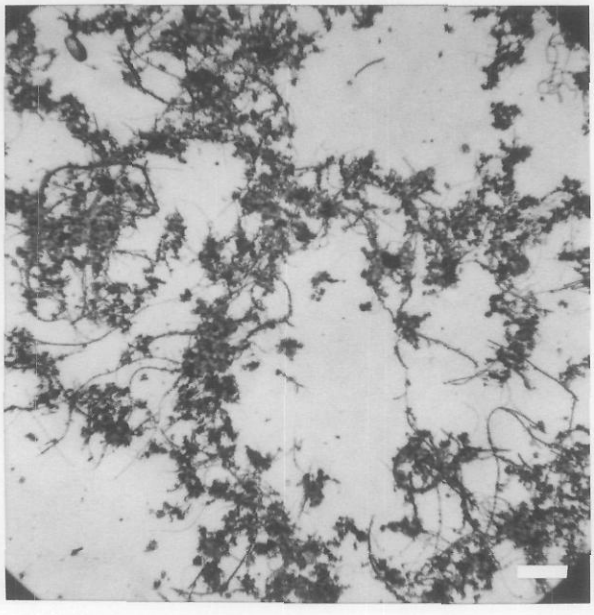


Figure 24 : Plant D Flow Configuration

Figure 25: Plant D Filament Abundance and Floc Structure-Sample 1 (100X phase contrast, crystal violet, dried; bar=100 μm)

Figure 26: Plant D Filament Abundance and Floc Structure-Sample 2 (100X phase contrast, crystal violet, dried; bar=100 μm)





nutrient deficient wastewaters. In industrial wastewaters, the predominance of N. limicola II is usually associated with a phosphorous limitation (Richard, 1989b).

A second activated sludge sample was obtained on day 268. The SVI at this time was 258 ml/g and the filament abundance was characterized as "abundant" (See Figure 26), a slight improvement from the first sample which was characterized as "excessive". More dense floc formation was also observed. The dominant filamentous organisms in this sample were Nocardia sp., Type 0041 and Type 1851. Secondary filamentous abundance of N. limicola II and Type 0961 were also observed. This sample also indicated low F/M conditions and possibly a nutrient deficiency.

DATA ANALYSIS AND CONTROL MEASURES

In late May 1989 (day 150+), the plant personnel noticed the beginnings of what was to be a steady increase in the SVI. Filament abundance increased and substantial interfloc bridging was observed. Prior to this time, RAS chlorination was generally used to control filament abundance. In June, however, it became apparent that the chlorine was causing extensive damage to the floc-forming organisms and less damage to the filamentous organisms. As a result, RAS chlorination was reduced from an average of 52.6 kg/d (116 lbs/day) to 28.6 kg/d (63 lbs/day). Corresponding with this decrease in RAS chlorination, the SVI increased (See Figure 27a).

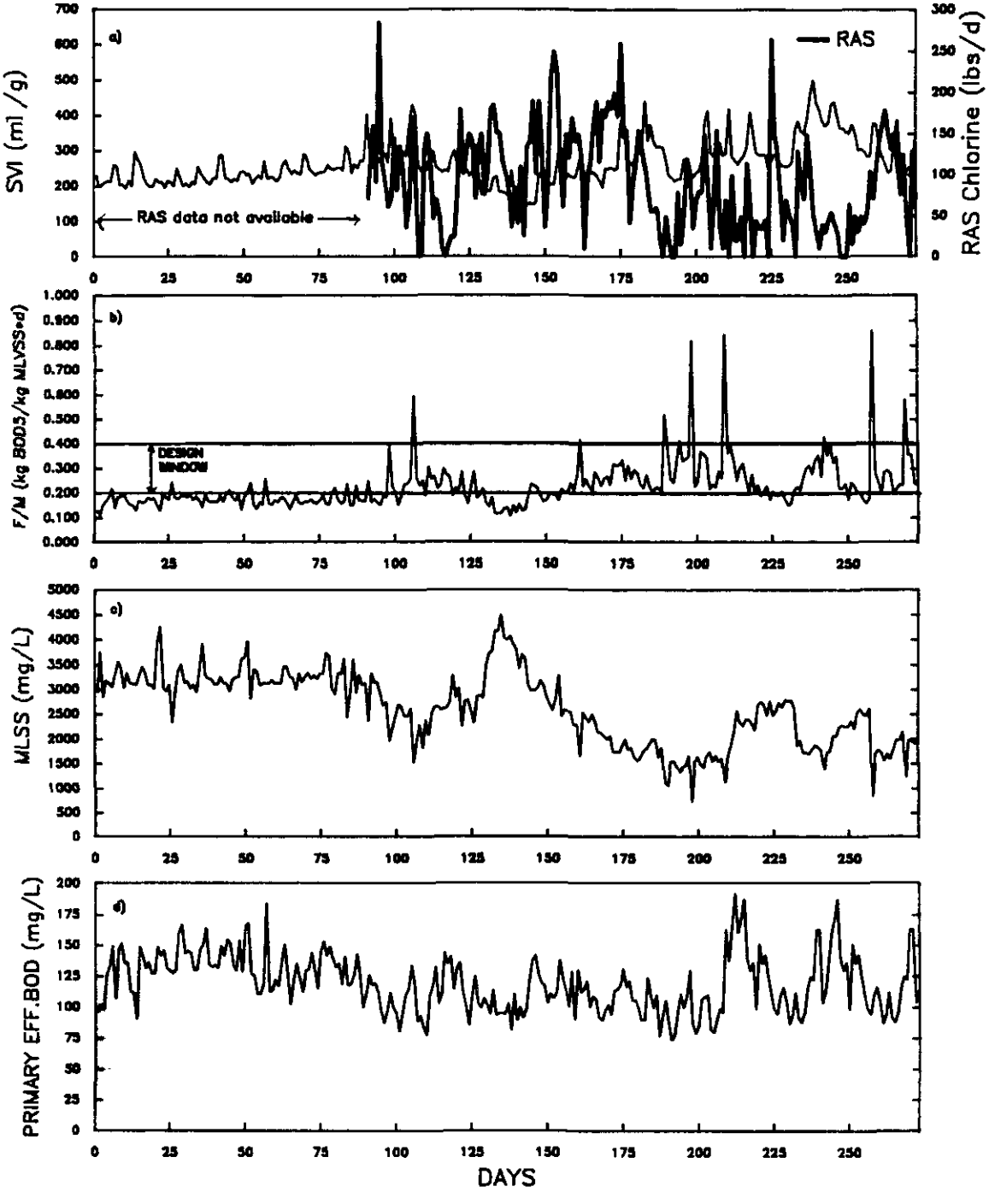


Figure 27: Plant D Operating Data

From Figure 27b, it can be seen that the F/M is generally low compared to the typical design parameter of 0.2 - 0.4 d^{-1} (Metcalf & Eddy, 1979). From Figure 27c, it can be seen that the MLSS concentration varied widely during the study. This was due to a ruptured seal in one of the secondary clarifiers which consequently caused the production of a very dilute return sludge. This caused the MLSS and subsequently the F/M to be difficult to control. Early 1989 data (days 0-90) indicates that although the F/M was low, RAS chlorination seemed to keep the SVI fairly stable. Considering the generally low F/M and the correlation with low F/M filaments being dominant, it was concluded that low F/M was the major cause of bulking along with the reduction in RAS chlorination.

It was suggested to the plant to change to a plug flow configuration (Figure 28) to produce a substrate gradient in the aeration tank which would select for non-filamentous organisms by maintaining a high F/M at the head end of the tank. It was also suggested that the plant check nutrient levels in the effluent (prior to chlorination) to determine if a nutrient deficiency was indeed present.

Several years ago the plant had changed to a plug flow configuration with little success. They found that the system was very vulnerable to hydraulic surges and toxic shock loads. They did not feel trying this again would benefit them. Towards the end of August (≈ day 236), they analyzed their secondary effluent (prior to chlorination) and found NH_3-N = 0.83 mg/L, NO_3-N = 1.0 mg/L and total PO_4-P = 0.0025 mg/L. Generally, to avoid nutrient deficiency, a residual of about 1

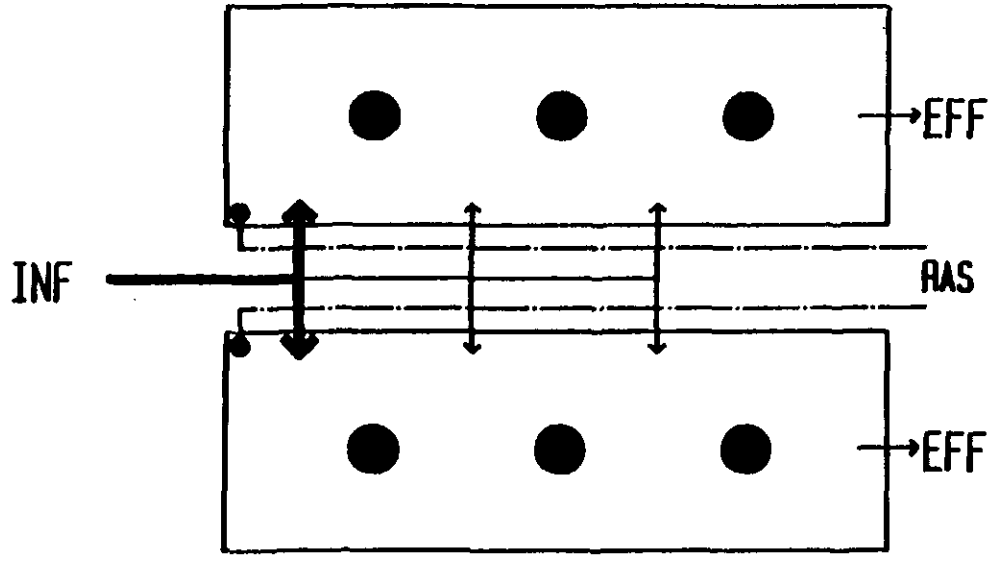


Figure 28: Plant D Plug Flow Configuration

mg/L inorganic nitrogen (NH_3 and NO_3-N) and 0.2 mg/L soluble PO_4-P should be maintained in the effluent (Richard, 1989a). Obviously, this plant appears to be deficient in phosphorous, which may also have been indicated by the presence of N. limicola II.

At the end of August (day 240+), the plant operator began adding 45.4 kg/d (100 lbs/day) of trisodium phosphate to the aeration basins. Seven days later the effluent total PO_4-P was 0.044 mg/L, a significant increase, yet still deficient. The phosphorous addition was continued for ten days and then ceased due to the high cost (about $500/week). During this ten day period, a downward trend in the SVI was noticed, but nutrient addition was not continued long enough to see if there was any real benefit due to the addition of phosphorous (See Figure 29).

On September 18th (day 261), the plant switched to a plug flow configuration (See Figure 28) (this is actually similar to CSTR's in series). They decided to use a spare aeration tank as a flow equalization basin for the primary effluent in case of a toxic or hydraulic surge. The SVI continued on a generally downward trend for about two weeks. By mid-October, excessive growth of N. limicola II was observed. From Figure 27a and 27d it can be seen that during some instances of high primary effluent BOD, the SVI was also elevated. This may support the case for a nutrient deficient wastewater.

Although the plant has experienced high SVI's and settleability problems, it has adequate capacity and has been able to maintain greater than 90% removal efficiency of BOD_5 and TSS. When bulking gets severe, polymer is added to the aeration basin effluent to enhance settling.

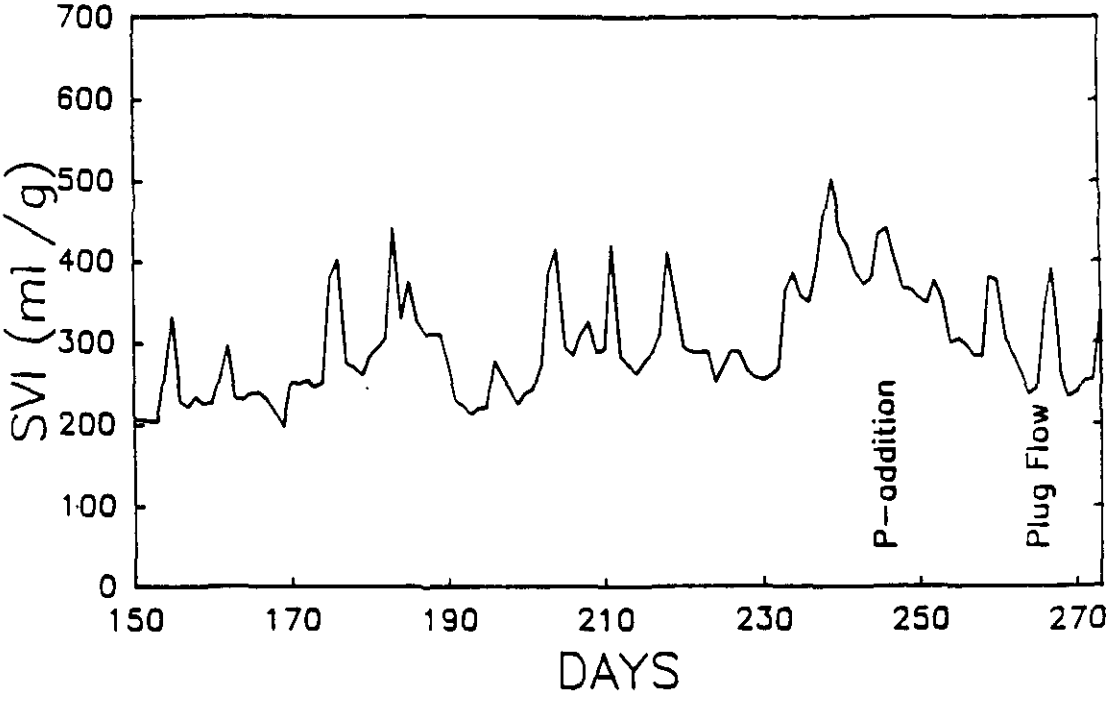


Figure 29: Plant D SVI Data

SUGGESTIONS FOR REMEDIAL ACTION

It appears that Plant D is suffering from a combination of low F/M and phosphorous limited conditions. A similar case study was reported by Jenkins et al. (1986). A plant with a mixture of domestic and pulp and paper wastwaters (among other industry) was experiencing bulking caused by Type 1851, N. limicola II, Type 0675 and Type 0041. The plant installed a selector, filament abundance subsequently decreased and a nonbulking condition was achieved. In that case, a selector provided a high F/M ratio in the mixture of RAS and primary effluent prior to aeration. A selector, in providing a higher initial F/M, may also provide a higher initial nutrient loading and so select for nonfilamentous organisms.

In the case of Plant D, a selector would be the best alternative from an operational perspective, although costs for pipe and channel rerouting and basin construction may be prohibitively expensive. The proposed selector flow configuration is shown in Figure 30. Before considering installation of a selector, nutrient addition should be evaluated once again for a longer period of time. A minimum residual PO_4-P concentration of about 0.2 mg/L in the aeration basin effluent should be maintained to ensure the availability of nutrients in the aeration basin. If nutrient addition is not shown to be beneficial, its use can be ruled out and a selector should be considered. If it is successful, either phosphorous addition alone or a combination of selector and nutrient addition may be necessary.

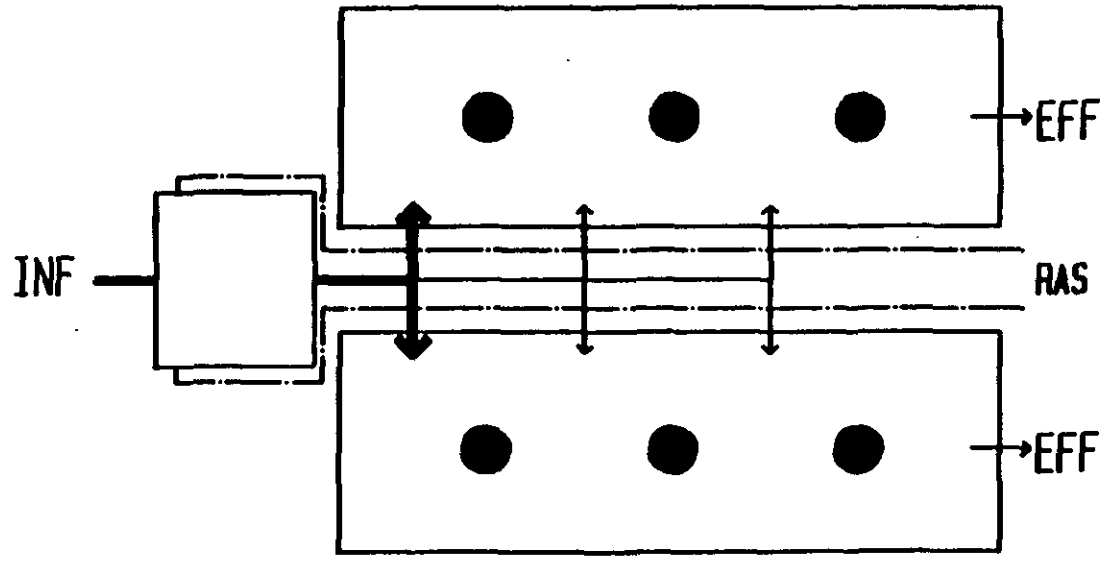


Figure 30 : Plant D Selector Configuration

In the meantime, RAS chlorination could be increased for short periods of time. The plant has found that continouus addition ofchlorine can be maintained for about 7 days before significantly affecting the floc structure.

5.5 PLANT E - Phosphorous Deficiency

Plant E utilizes a plug flow, step feed activated sludge process with diffused aeration (See Figure 31). Currently, an average daily flow of 0.044 m^3/s (1.0 mgd) which is 56% of the design average daily flow of 0.079 m^3/s (1.8 mgd) is treated. Approximately 80% of the wastewater flow is domestic, the remainder originating from a pulp and paper mill where its wastewater is pretreated with alum. The paper mill also contributes about 95% of the plants solids loading. The average influent BOD_5 and TSS are 442 mg/L and 727 mg/L, respectively.

This plant has had a history of filamentous bulking problems, the most severe of which occur during the spring and early summer. The main control measure utilized has been the addition of chlorine to the return sludge. This measure was successful for a time, but after several months it was noticed that the floc formers were being destroyed more than the filaments.

FILAMENTOUS ORGANISM IDENTIFICATION

In April of 1989, during a period of bulking, an activated sludge sample was examined. Abundant growth of Thiothrix II and somewhat less growth of Sphaerotilus natans was observed as well as significant interfloc bridging and free filaments.

As shown in Table 4, The presence of these organisms is indicative

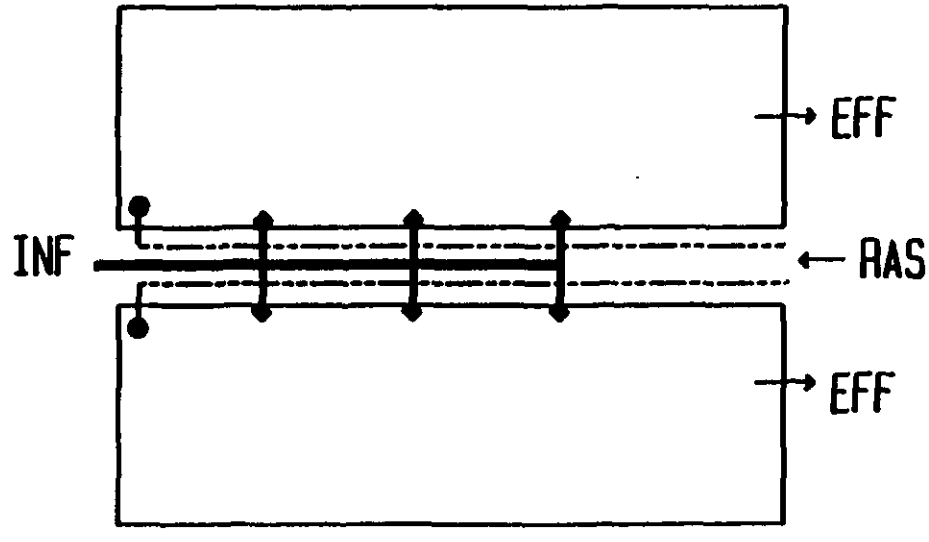


Figure 31 : Plant E Flow Configuration

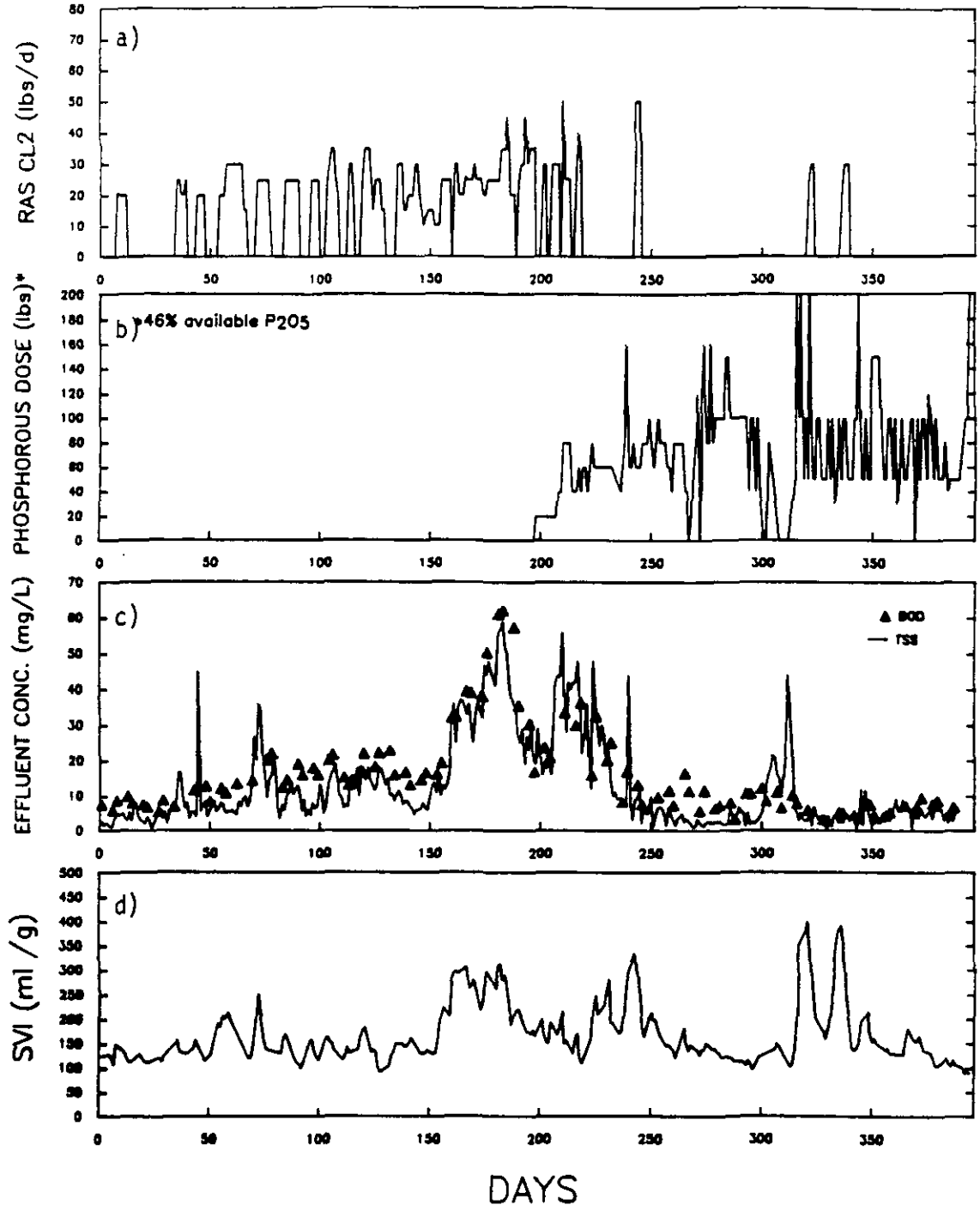
of a nutrient deficient wastewater, a common problem with mixed domestic and paper wastes.

DATA ANALYSIS AND CONTROL MEASURES

In late March of 1989, total phosphorous and nitrogen of the primary effluent was analyzed and found to be deficient in phosphorous. A sample of secondary effluent prior to chlorination was also analyzed and was found to contain 0.2 mg/L total phosphorous. In general, a minimum of 0.2 mg/L soluble PO_4-P should be maintained in the aeration basin effluent to ensure adequate nutrients have been available in the aeration basin. Thus it was determined that the plant was suffering from a phosphorous deficiency.

The SVI was initially reduced by RAS chlorination, then addition of phosphorous commenced. RAS chlorination was also discontinued at this time (Figure 32a). Primary effluent is pumped by screw pumps to the aeration basins. The phosphorous mixture (40-50% available P_2O_5, 20.4% calcium, 11.9% sulfur) was obtained in powdered form from a lawn care company. An average of 18.1-36.3 kg/d (40-80 lbs/day) (See Figure 32b) of the phosphorous mixture is added to the screw pump wet wells. Dosages are based on maintaining an aeration basin effluent orthophosphate residual of 1-2 mg/L in the secondary effluent to ensure adequate nutrients were available in the aeration basin.

Since the inception of phosphorous addition, RAS chlorination at the plant has ceased except in severe bulking cases (Figure 32a).



NOTE: MISSING DATA ARE INTERPOLATED FOR CLARITY

Figure 32 : Plant E Operating Data

Bulking frequency has decreased and the duration of bulking episodes has so far been drastically reduced. Plant performance has also been enhanced. As shown in Figure 32c, effluent BOD_5 and TSS are generally lower and more consistent. A second sample was analyzed after several months of phosphorous addition. Filament abundance had decreased. The dominant organisms present were Thiothrix sp., S. natans and Type 0041.

SUGGESTIONS FOR REMEDIAL ACTION

The addition of phosphorous was demonstrated to be effective for bulking control at this plant, drastically reducing RAS chlorine demand. It currently costs the plant about three times as much to add phosphorous as it does to add chlorine. The addition of phosphorous is a means of preventing bulking, whereas RAS chlorination alleviates the symptoms of the problem, although not always successfully.

In order to reduce costs for phosphorous addition, a form with more available phosphorous should be used. With the phosphorous mixture, only 46% by weight is actually available phosphorous. Liquid phosphoric acid could be used instead and dosed continuously instead of once a day which may provide better control of bulking. This would reduce the chemical volume needed as well as the labor involved. The dosing rate could be adjusted as necessary. There would be an initial capital outlay required for dosing and storage equipment, however. The plant is currently considering this type of phosphorous addition. Prior to implementing this approach, the plant operator wants to obtain a full

year of data with phosphorous addition to evaluate the advantages over chlorination in terms of overall plant performance and long term costs.

5.6 PLANT F - Nutrient Deficiency

Plant F is currently treating an average wastewater flow of 0.10 m^3/s (2.3 mgd), about 72% of its design capacity for average daily flow. The wastewater is 45% industrial (from a paper manufacturer) and 55% domestic. A complete mix activated sludge system is utilized with surface aeration (See Figure 33). Average influent BOD_5 and TSS are 195 mg/L and 330 mg/L, respectively.

Approximately two years prior to this study, the design engineer of the plant determined that the cause of the frequent bulking episodes was nutrient deficiency, as evidenced by the presence of the filamentous organisms Sphaerotilus natans and Thiothrix sp. Control measures implemented were the addition of nutrients: 45.4 kg/d (100 lbs/day) urea as a nitrogen source and 7.6 L/d (2 gal/day) 85% phosphoric acid. This controlled the bulking adequately, with bulking occurring less frequently.

In early September 1989, the feed pump for the urea failed and replacement parts were unavailable for about four weeks. During this time, the SVI rose to a peak of 539 ml/g (See Figure 34a) and then decreased. The decline in SVI is attributable to an increased RAS chlorination rate.

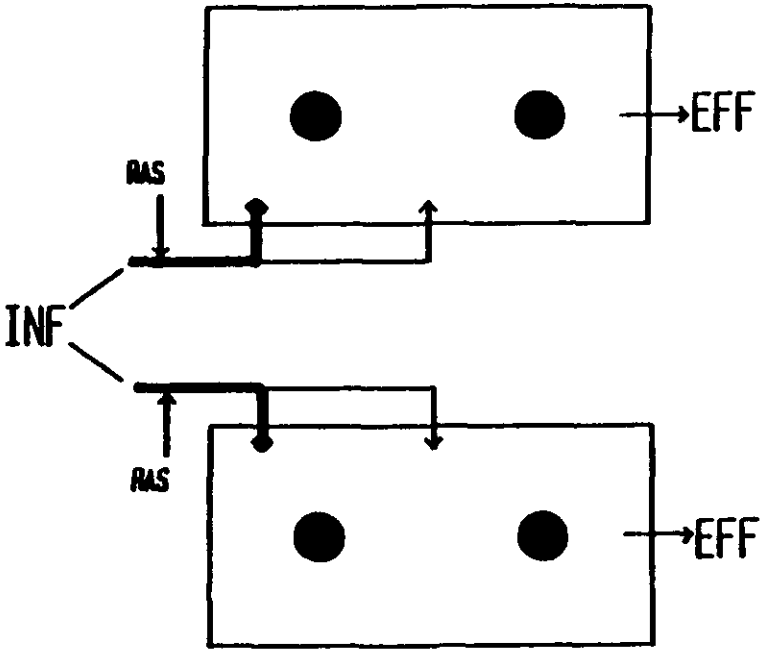


Figure 33 : Plant F Flow Configuration

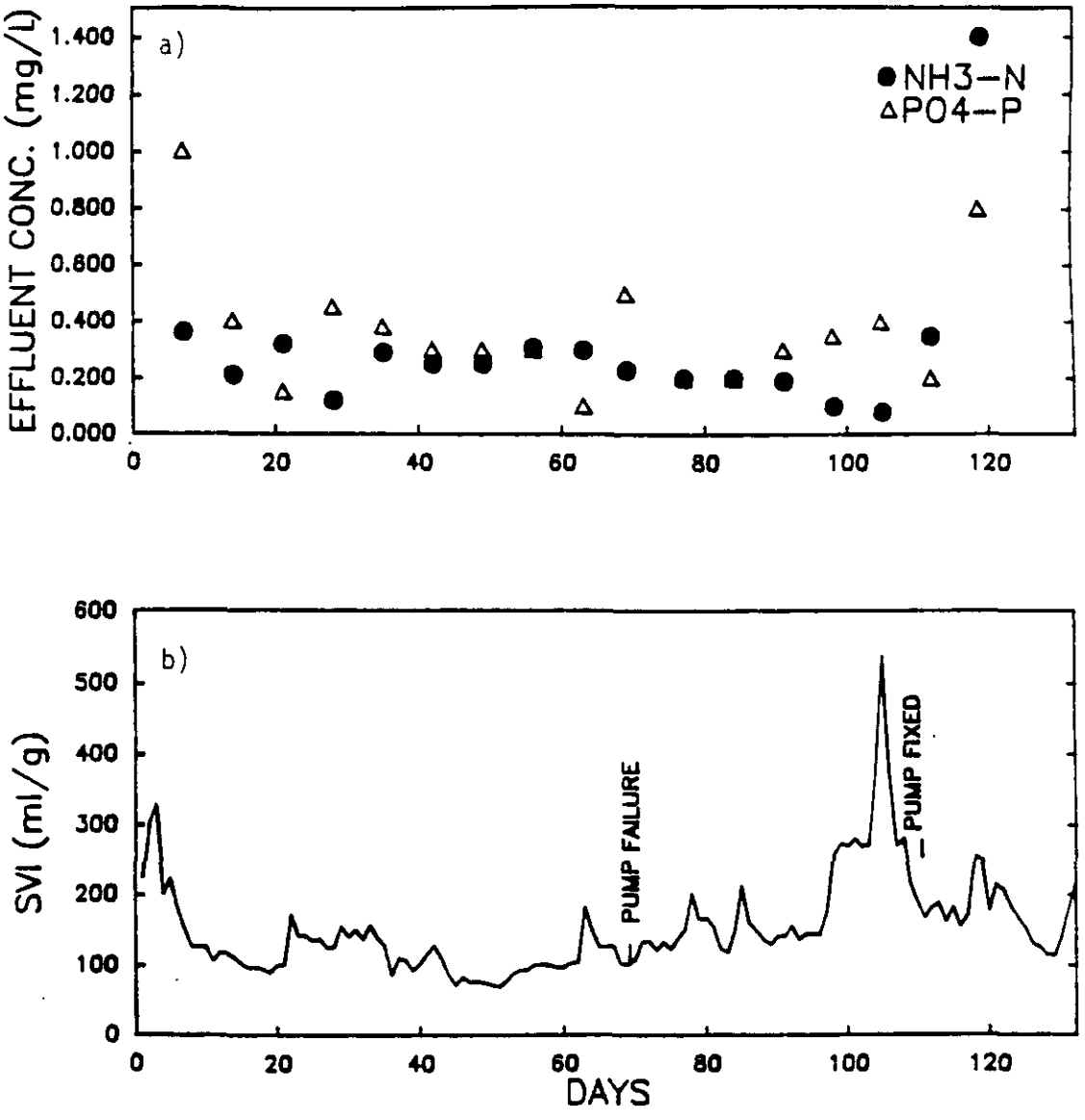


Figure 34 : Plant F Operating Data

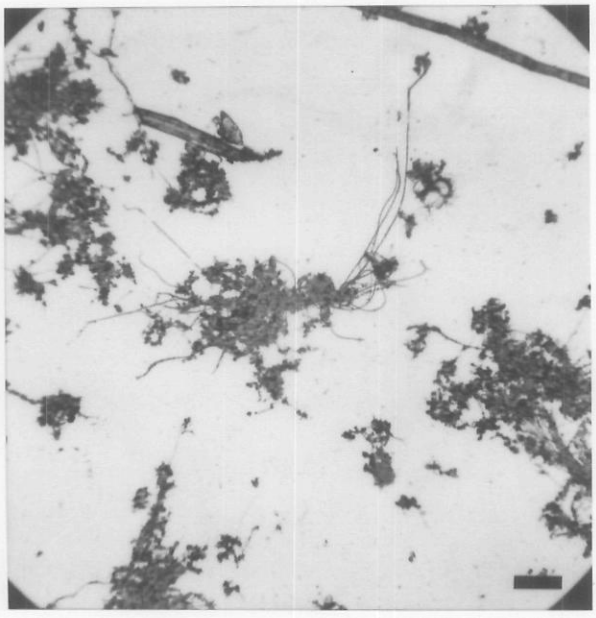
FILAMENTOUS ORGANISM IDENTIFICATION

An activated sludge sample was obtained 5 days after the peak SVI occurred. Microscopic examination revealed dominant growth of Sphaerotilus natans and Thiothrix sp. (See Figure 35). At the time of sampling, the SVI had decreased to 193 ml/g, so the overall abundance was not too serious. A slime coating as well as incidental attached growth was observed associated with S. natans, indicating a nutrient deficient wastewater. Trichomes of S. natans were unhealthy, observed to be broken, bent and with numerous gaps where the individual cells had lysed. This is a typical consequence of heavy RAS chlorination. The presence of Thiothrix sp. also can be indicative of nutrient deficiency, usually nitrogen. Thiothrix sp. also exhibited damage from chlorination. Very few rosette formations (Trichomes radiating from a common origin) were present and mostly single and broken filaments were observed.

DATA ANALYSIS

Plant operating data were obtained for three months prior to the bulking episode and are shown in Figure 34. A nutrient deficient waste is also suggested by the data. In Figure 34b, it can be seen that the SVI began to steadily increase around the second week of September, corresponding to the failure of the urea pump. At this same time, the effluent nitrogen (NH_3-N) concentration steadily decreased due to the

Figure 35: Plant F Abundance of S. natans and Thiothrix sp. (100X phase contrast, crystal violet, dried; bar=100 μm)



lack of nutrient addition (Figure 34a).

About October 13th, the SVI reached a peak and RAS chlorination was increased, leading to a rapid drop in filament abundance and SVI. On October 18th, the urea pump was placed back on line and RAS chlorination was gradually decreased over the next several days. The SVI declined for several days then increased, probably due to inadequate RAS chlorination.

SUGGESTIONS FOR REMEDIAL ACTION

A common method to avoid nutrient deficiency is to add a source of nitrogen (ammonia, $(NH_4)_2SO_4$, NH_4Cl, NH_4NO_3) and/or phosphorous (H_3PO_4, Na_3PO_4, $(NH_4)_2PO_4$) in such amounts to ensure that a residual of about 1.0 mg/L inorganic nitrogen (NH_3 and NO_3-N) and 0.2 mg/L soluble PO_4-P remains in the aeration basin effluent (Richard, 1989a). An adequate residual of phosphorous is maintained at Plant F, generally above 0.2 mg/L soluble PO_4-P. With the addition of urea at 45.4 kg/d (100 lbs/day), they are able to maintain a NH_3-N residual of about 0.25 mg/L. In conjunction with RAS chlorination, this controls the bulking fairly well, although the SVI will rise above 200 occasionally, probably when the influent BOD is high. It would be to the plant's benefit to maintain a higher residual NH_3-N (up to 1 mg/L) at all times or try to match the urea dosing to the strength of the wastewater. This would ensure adequate nutrients at times of high BOD and could reduce the need for RAS chlorination.

The problem encountered during the most recent bulking episode was due to the failure of a pump. To prevent a repeat of this situation, a backup pump should be kept on hand to be used when repair or maintenance is required on the main pump.

CHAPTER VI

CONCLUSIONS AND RECOMENDATIONS

Filamentous bulking is a significant problem affecting more than 50% of all activated sludge plants. Filamentous organism identification is a valuable aid in determining the probable cause(s) of bulking episodes and can be performed easily by a trained individual in 1-2 hours. Through this research and much previous work, case studies have established effective remedial alternatives for various bulking situations and substantiated the relationship between organism type and cause of bulking.

Several conclusions and recommendations have been reached as a result of this research. They are as follows:

(1) The probable cause(s) of filamentous bulking was determined through filamentous organism identification at the plants studied.

(2) Analysis of the operating data of the plants studied supported the cause(s) of bulking indicated by the filamentous organisms present.

(3) Remedial actions were suggested based on filamentous organism identification and plant data analysis. Of the plants which implemented remedial actions, most were successful in eliminating the bulking problem.

(4) Of the procedures evaluated, the Manual on the Causes and Control of Activated Sludge Bulking and Foaming by Jenkins et al. (1986) was found to be the most useful for filamentous organism identification and bulking control. It provides the most complete and up-to-date coverage of filamentous organisms in activated sludge, their causes, case studies and bulking and foaming control measures. It is also easily utilized by treatment plant staff.

(5) Currently in Massachusetts, it is not economically feasible for most plants to implement filamentous organism monitoring programs. Most of the plants are small and thus do not have the manpower and financial resources available.

(6) Statewide or regional assistance and training programs on activated sludge filamentous bulking should be established. These could provide cost effective and timely evaluations of local bulking problems and assistance in implementing appropriate remedial actions.

Further research should be directed towards:

(1) determining the causes of predominance of less common filamentous organisms,

(2) further defining the growth requirements of filamentous organism types,

(3) determining the characteristics of filamentous organisms in industrial waste systems, and

(4) ensuring that operator training includes information on the role of filamentous organisms in normal and bulking sludges.

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APPENDIX I
LIST OF SYMBOLS

BOD_5	5-day Biochemical Oxygen Demand (mg/L)
DO	Dissolved Oxygen (mg/L)
F/M	Food-to-Microorganism Ratio (kg BOD_5 applied/kg MLVSS*d)
MLSS	Mixed Liquor Suspended Solids (mg/L)
MLVSS	Mixed Liqour Volatile Suspended Solids (mg/L)
RAS	Return Activated Sludge
S_0	BOD_5 Applied to Aeration Basin (mg/L)
SRT	Solids Residence Time (days)
SVI	Sludge Volume Index (ml/g)
θ	Hydraulic Residence Time (days)
TSS	Total Suspended Solids (mg/L)
X	MLSS (mg/L)

APPENDIX II
STAINING PROCEDURES

Table II-1: Gram Stain Procedure

REAGENTS:

FISHER Diagnostics Gram Stain Kit SG100

Gentian Violet Solution
- Crystal Violet Stain.....0.5% w/v
- Methanol.....20% w/v
- Ammonium Oxalate.....0.8% w/v

Gram's Iodine Solution
- Iodine.....0.33% w/v
- Potassium Iodide.....0.66% w/v

Decolorizer
- Acetone.....25% v/v
- Isopropyl Alcohol.....75% v/v

Safranin Solution
- Safranin O.....1% w/v

PROCEDURE:

1. Prepare thin smears on microscope slides and thoroughly air dry (do not heat fix).
2. Stain 1 minute with Gentian Violet Solution; rinse 1 second with water.
3. Stain 1 minute with Gram's Iodine Solution; rinse well with water.
4. Hold slide at an angle and decolorize with Decolorizer added drop by drop to the smear for 25 seconds. Do not over decolorize. Blot dry.
5. Stain with Safranin Solution for 1 minute; rinse well with water and blot dry.
6. Examine under oil immersion at 1000X magnification with direct illumination (not phase contrast): Blue-violet is positive; pink to red is negative.

Table II-2: Neisser Stain Procedure

PREPARATION:

Solution 1*:
Separately prepare and store the following:

A		B	
Methylene Blue	0.1 g	Crystal Violet (10% w/v in 95% ethanol)	3.3 ml
Ethanol, 95%	5 ml	Ethanol, 95%	6.7 ml
Acetic acid, glacial	5 ml	Distilled water	100 ml
Distilled water	100 ml		

Mix 2 parts by volume of A with 1 part by volume of B; prepare fresh monthly.

Solution 2*:

Bismark Brown (1% w/v aqueous)	33.3 ml
Distilled water	66.7 ml

*Reagent grade chemicals from FISHER SCIENTIFIC

PROCEDURE:

1. Prepare thin smears on microscope slides and thoroughly air dry.
2. Stain 1 minute with Solution 1; rinse 1 second with water.
3. Stain 1 minute with solution 2; rinse well with water; blot dry.
4. Examine under oil immersion at 1000X magnification with direct illumination (not phase contrast): blue-violet is positive (either entire cell or intracellular granules; yellow-brown is negative.

Table II-3: Sulfur Oxidation Test Procedure

TEST A

SOLUTION: Sodium sulfide solution ($Na_2S \cdot 9H_20$)* 1.0 g/l (prepare weekly)

*Reagent grade from FISHER SCIENTIFIC

PROCEDURE:

1. On a microscope slide mix 1 drop of activated sludge sample and 1 drop sodium sulfide solution.
2. Allow to stand open to the air 10-20 minutes.
3. Place a coverslip on the preparation and gently press to exclude excess solution; remove expelled solution with a tissue.
4. Observe at 1000X using phase contrast. A positive S test is the observation of highly refractive, yellow-colored intracellular granules (sulfur granules).

This test, at times, gives variable results. This is due to methodological problems involving the relative concentrations of sulfide and oxygen present (sulfide oxidation is an aerobic process). An alternative sulfur oxidation test, developed by Farquhar and Boyle (1971a) may be used:

TEST B

PROCEDURE:

1. To an Erlenmeyer flask containing 100 ml of sample, add 10 mg of $Na_2S \cdot 9H_20$.
2. Gently agitate the flask and contents.
3. After 5 minutes of agitation, examine the contents of the flask under phase contrast illumination at 1000X magnification to determine if refractile bodies have begun to deposit within the microorganisms. Continue observation until deposition appears to be complete. This usually requires less than 30 minutes.

Table II-4: India Ink Reverse Stain Procedure

SOLUTION: HIGGINS (or other) waterproof Black India drawing ink

PROCEDURE:

1. Mix one drop of India ink and one drop of activated sludge on a microscope slide.
2. Place the cover slip on and observe at 1000X phase contrast.
3. In "normal" activated sludge, the India ink particles penetrate the flocs almost completely, at most leaving a clear center.
4. In activated sludge containing large amounts of exocellular polymeric material, there will be large, clear areas which contain a low density of cells.

Table II-5: Polyhydroxybutyrate (PHB) Stain Procedure

PREPARATION:

SOLUTION 1*: Sudan Black B, 0.3% w/v in 60% ethanol.

SOLUTION 2*: Safranin O, 0.5% w/v aqueous.
*Reagent grade chemicals from FISHER SCIENTIFIC

PROCEDURE:

1. Prepare a thin smear on a microscope slide and thoroughly air dry.
2. Stain 10 minutes with Solution 1; add more stain if the slide starts to dry out.
3. Rinse 1 second with water.
4. Stain 10 seconds with Solution 2; rinse well with water; blot dry.
5. Examine under oil immersion at 1000X magnification with transmitted light: PHB granules will appear as intracellular, blue-black granules while cytoplasm will be pink or clear.

Table II-6: Crystal Violet Sheath Stain Procedure

SOLUTION: Crystal Violet*, 0.1% w/v aqueous solution.
*Reagent grade from FISHER SCIENTIFIC

PROCEDURE:

1. Mix one drop of activated sludge sample with one drop Crystal Violet solution on a microscope slide, cover and examine at 1000X magnification phase contrast. Cells stain deep violet while the sheaths are clear to pink.
