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Draft Version
Activated Sludge Bulking Handbook
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DRAFT VERSION
ACTIVATED SLUDGE BULKING HANDBOOK
CHAPTER 1.

1. BACKGROUND

1.a. The Nature of Wastewater Treatment

1.a.1. Water Pollution Control

Water pollution control is a very important topic and we should be proud to be part of this profession. We protect receiving waters (streams, rivers and lakes) by practicing water pollution control, or wastewater treatment, for two general reasons - public health control (prevention of water borne diseases) and aesthetics (protecting the environment).

1.a.2. BOD and Suspended Solids Removal

Wastewater is a mixture of many different components. The important contaminants of wastewater include suspended solids, biodegradable organics, pathogens, nutrients, refractory organics, heavy metals, and dissolved inorganic salts. Table 1 summarizes the important contaminants, the reason for their importance, and the treatment processes (technology) available for removing these contaminants from wastewaters.

In general, we are usually most concerned about the first three contaminants listed in Table 1 - suspended solids, biodegradable organics and pathogens.

The challenge of wastewater engineering is to design, build, and properly operate treatment plants to protect receiving waters (for health and aesthetic purposes) in an economical manner. This is no small task when one considers the enormous amount of wastewater generated (>100 gallons per person per day). One can see from Table 1 that there are a large variety of processes available to get the job done. In fact the technology exists to convert the most contaminated water imaginable to high quality drinking water (or even better!). However, the costs would be much too high.

To meet this challenge, we basically rely on two relatively inexpensive phenomena - gravity settling (sedimentation) and biological oxidation (activated sludge). While many possibilities exist, a typical flow sheet for a wastewater plant is shown in Figure 1.

The major elements of this typical plant are well known to all of us, but it is important to remember that at the heart of this plant are the two basic phenomena - settling and biological oxidation.

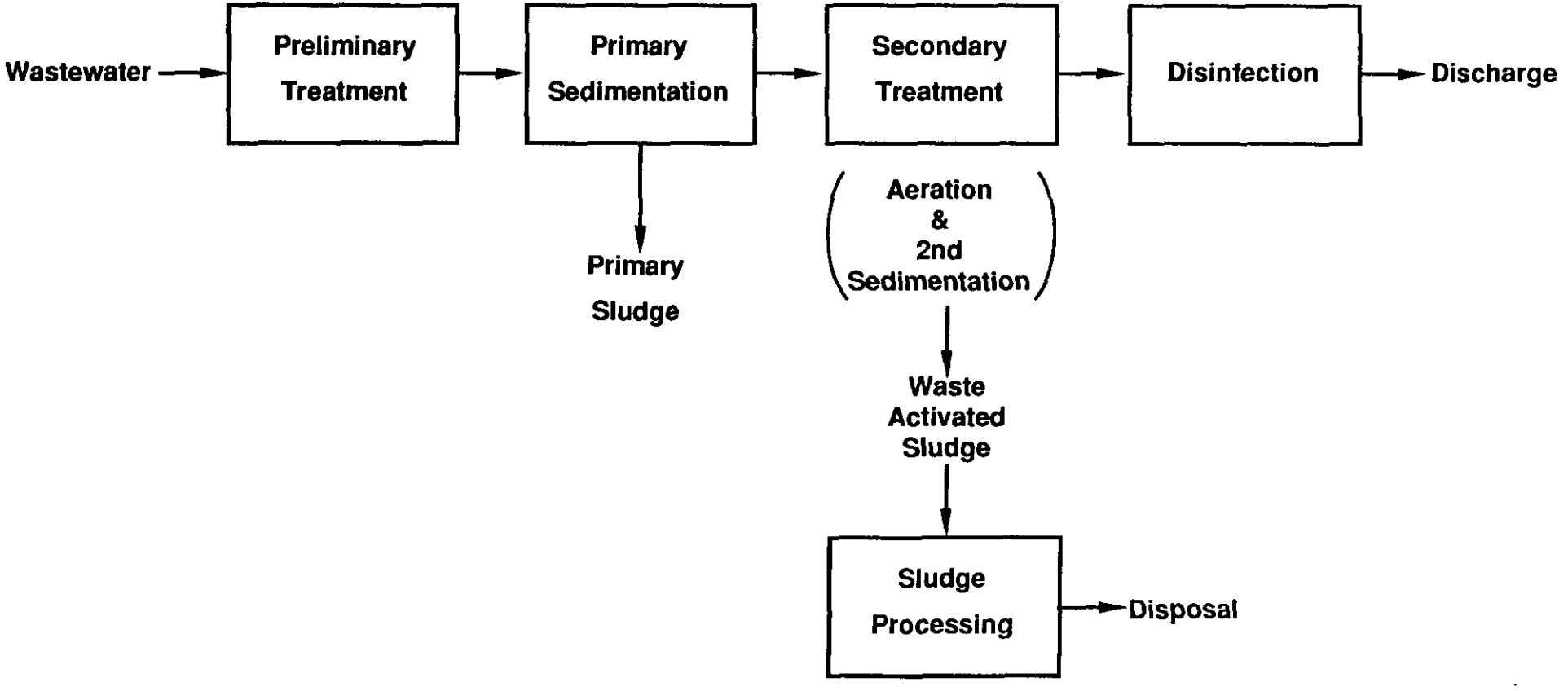
If there is such a thing, typical wastewater would have approximately 220 mg/l of suspended solids (SS) and 220 mg/l 5 day biochemical oxygen demand (BOD_5). Since preliminary treatment is designed to remove large objects, virtually no removal of BOD and SS occurs in this step. During primary sedimentation approximate 50% of the SS are removed by settling. In addition, since some of the BOD is associated with particles which can

Table 1. Important Contaminants in Wastewater and the Unit Operations, Processes, and Treatment Systems Used for Their Removal. (Reference 5)

Contaminants	Reason for importance	Unit operation, unit process, or treatment system
Suspended solids	Suspended solids can lead to development of sludge deposits and anaerobic conditions when untreated wastewater is discharged in the aquatic environment.	Sedimentation Screening and comminution Filtration variations Flotation Chemical-polymer addition/ sedimentation Coagulation/sedimentation Land-treatment systems
Biodegradable organics	Composed principally of proteins, carbohydrates and fats, biodegradable organics are measured most commonly in terms of BOD (biochemical oxygen demand) and COD (chemical oxygen demand). If discharged untreated to the environment, their biological stabilization can lead to the depletion of natural oxygen resources and to the development of septic conditions.	Activated-sludge variations Fixed-film: trickling filters Fixed-film: rotating biological contactors Lagoon variations Intermittent sand filtration Land-treatment systems Physical-chemical systems
Pathogens	Communicable diseases can be transmitted by the pathogenic organisms in wastewater.	Chlorination Hypochlorination Ozonation Land-treatment systems
Nutrients	Both nitrogen and phosphorus, along with carbon, are essential nutrients for growth. When discharged to the aquatic environment, these nutrients can lead to the growth of undesirable aquatic life. When discharged in excessive amounts on land, they can also lead to the pollution of groundwater.	<u>Nitrogen</u> Suspended-growth nitrifications and denitrification variations Fixed-film nitrification and denitrification variations Ammonia stripping Ion exchange Breakpoint chlorination Land-treatment systems <u>Phosphorus</u> Metal salt addition/sedimentation Lime coagulation/sedimentation Biological-chemical phosphorus removal Land-treatment systems

Refractory organics	These organics tend to resist conventional methods of wastewater treatment. Typical examples include surfactants, phenols, and agricultural pesticides.	Carbon adsorption Tertiary ozonation Land-treatment systems
Heavy metals	Heavy metals are usually added to wastewater from commercial and industrial activities and may have to be removed if the wastewater is to be reused.	Chemical precipitation Ion exchange Land-treatment systems
Dissolved inorganic salts	Inorganic constituents such as calcium, sodium, and sulfate are added to the original domestic water supply as a result of water use and may have to be removed if the wastewater is to be reused.	Ion exchange Reverse osmosis Electrodialysis

Figure 1. Typical Flowsheet of a Wastewater Treatment Plant



settle out, approximately 35% of the BOD is removed. This leaves 110 mg/l SS and 140 mg/l BOD after primary treatment of our typical wastewater. This is seldom sufficient effluent quality for water pollution control. In the Clean Water Act of 1972 (Public Law 92-500) in fact, minimum effluent standards were set at 30 mg/l BOD_5 and 30 mg/l SS (30 day averages) for municipal wastewater treatment plants. Therefore, additional treatment is needed after primary sedimentation. This is accomplished in secondary treatment.

The BOD remaining after primary sedimentation is composed of soluble and particulate organic matter. The soluble organics are proteins, carbohydrates and lipids. The particulates (SS) are mostly organic in nature and become biodegradable after they are hydrolyzed (or dissolved). Thus the BOD and SS may be considered to be food for microorganisms such as bacteria, fungi and protozoa. This is the problem and "cure" of water pollution control. The problem is that when untreated wastewater is discharged into receiving water, the microorganisms degrade the organics by natural processes which take many days to occur. In doing so, the water becomes depleted of oxygen which then causes unpleasant odors and sights. The "cure" is that we can use these very same natural processes (which take days) in carefully engineered and operated reactors to carry out microbial reactions in hours, by pumping oxygen into the reactors. For engineers the question is how to design these processes. For operators, the question is how to operate and control these processes.

1.b. Basic Microbiology of Activated Sludge

1.b.1. Organisms Involved

Activated sludge is a very complex ecosystem. Many different types of organisms are present and many interactions occur among the organisms. These organisms are mostly bacteria but also fungi, protozoa and rotifers. The organisms must be able to grow on the "food" (substrate) available in wastewater at ambient temperatures in a mixed, aerated environment. They must also have the ability to form flocs and settle out in secondary sedimentation

The organisms can be divided into four major classes: floc formers, saprophytes, predators, and nuisance organisms. The floc forming organisms are mostly bacteria. They form natural polymers which cause individual bacteria to clump together and become large enough to form flocs which can then settle out by gravity forces. The saprophytes are mostly bacteria. They degrade the organic matter (BOD). Some saprophytes are also floc formers. The predators are mainly protozoa and to a lesser degree rotifers. Their prey are the dispersed bacteria (those not in flocs). Note that individual bacteria do not settle well. This is why flocs are so important for the activated sludge process to work. The predators are important in producing clear effluents since dispersed bacteria make effluents look cloudy. Finally, the nuisance organisms are filamentous fungi and filamentous bacteria. These are the organisms which cause bulking problems. While most bacteria grow as spheres, rods or spirals, filamentous organisms grow in the form of very long rods, many times longer than wide. Thus the filamentous organisms do not form good settling flocs or flocs which compact very well.

1.b.2. Growth and Respiration

Most biological organisms use organic compounds in their growth and respiration processes. Organic compounds are complex materials consisting of a carbon skelton - carbon atoms linked together - to which other atoms are attached - usually hydrogen, often oxygen and sometimes others such as nitrogen, sulfur and phosphorus.

When organisms use organic compounds as food for growth the carbon atoms of the compound are used as a foundation on which to build new cellular organic material. This process can be represented by the following equation:

$$\text{Food Organics} \xrightarrow{\text{Organisms}} \text{Cellular Organics} \qquad (1)$$

This process requires energy because the organisms are building a new material, cellular organics. The energy is obtained by degrading, or burning, other food organics to carbon dioxide and water as follows:

$$\text{Food Organics} \xrightarrow{\text{Organisms}} CO_2 + HOH + \text{Energy} \qquad (2)$$

The energy released is used in the growth process of equation (1). The burning process is referred to as respiration.

Organisms also require other materials, or nutrients, in their growth and respiration processes. A source of nitrogen is required in the growth process because this element is incorporated into the new cellular organic material. A source of oxygen is required in the respiration process to burn the organic material, the carbon, to carbon dioxide. Other nutrients which are also required include phosphorus and trace elements such as magnesium, cobalt, iron and manganese.

Because the energy released by respiration is used in the growth process, these two cellular functions are not independent, but are, in fact, closely integrated and controlled within the cell. The cell can thus be considered as a factory - taking in nutrients and discharging products - as shown in Figure 2.

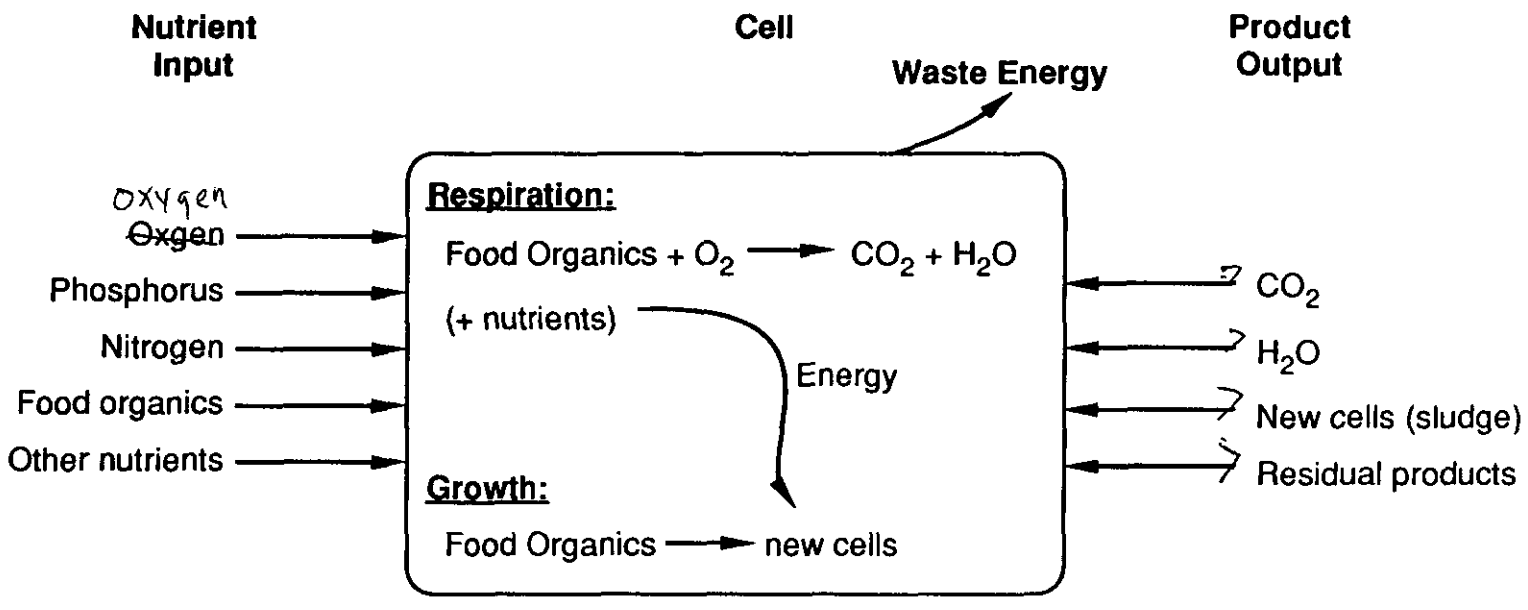
The growth and respiration processes discussed above are carried out by most living organisms. The result is a biodegradation of food organics to waste products - CO_2, H_2O, residuals at a lower energy level - and to new cells which can then perpetuate the process.

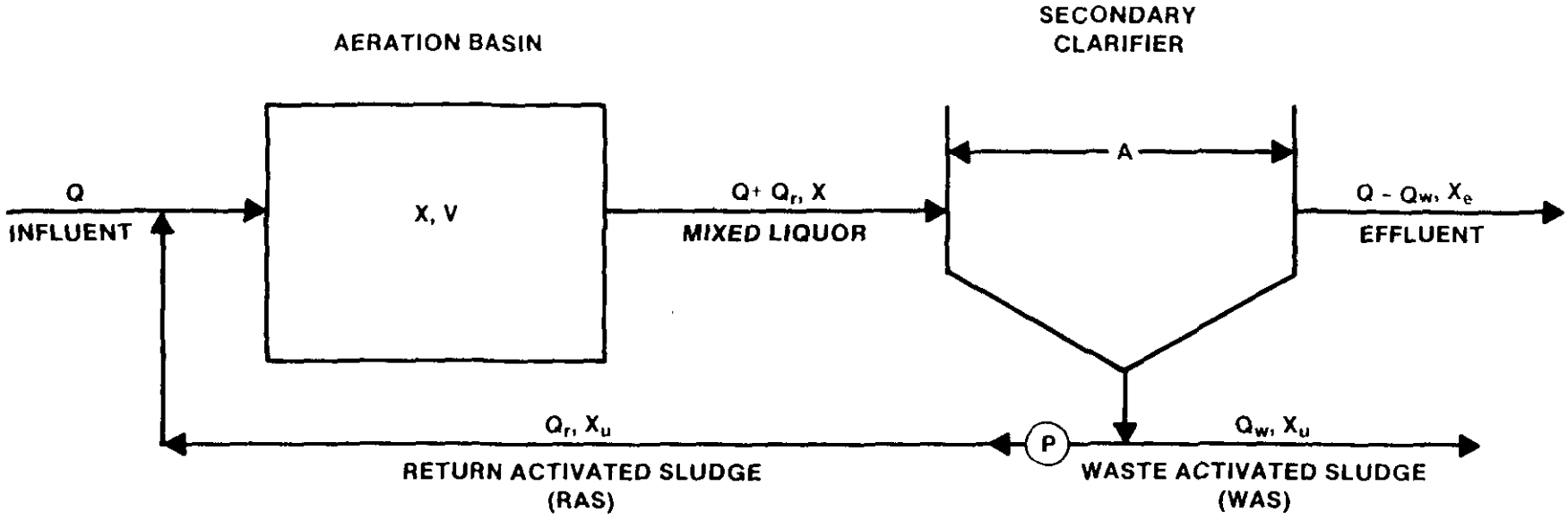
1.c. The Activated Sludge Process - Principles

1.c.1. Description

The reactions described in the previous section can be carried out in the unit process called activated sludge. In this process wastewater and microorganisms are mixed and aerated. The biological solids are then separated from the treated wastewater and returned to the aeration process as needed. A schmatic of the activated sludge process is shown in Figure 3. The major feature of activated sludge is that by recycling biological

Figure 2. Schematic of Biological Growth and Respiration





DEFINITIONS:

A = CLARIFIER SURFACE AREA
V = AERATION BASIN VOLUME
Q = INFLUENT FLOWRATE
Q_r = RAS FLOWRATE
Q_w = WAS FLOWRATE
X = AERATION BASIN MLSS CONCENTRATION
X_u = RAS SUSPENDED SOLIDS CONCENTRATION
X_e = EFFLUENT SUSPENDED SOLIDS CONCENTRATION

Figure 3. Activated Sludge Process Schematic. (Reference 10)

solids, the concentration of microorganisms can be greatly increased and maintained independently of the liquid (or wastewater) residence time. We can call the residence time of microorganisms the Solids Residence Time (SRT) and the liquid residence time the Hydraulic Residence Time (HRT).

The success of the activated sludge process (or any biological process) depends upon brings the wastewater and the microorganixms (MLSS) into contact for a sufficient period of time to allow the reactions to occur. This is governed by SRT. So activated sludge allows a high SRT for system efficiency with a low HRT for system economy. (i.e. a small tank).

1.c.2. Modifications

In wastewater engineering, flexibility is a must. The activated sludge process is quite flexible as there are many process modifications. Several of these modifications are shown in Figure 4. Several of these modifications will be discussed later as they are important in controlling activated sludge bulking (such as plug flow, oxygen aeration, and contract stabilization).

1.d. Design and Operation of the Activated Sludge Process

Several parameters are discussed in this section. Later we shall see how they are related to certain types of bulking problems.

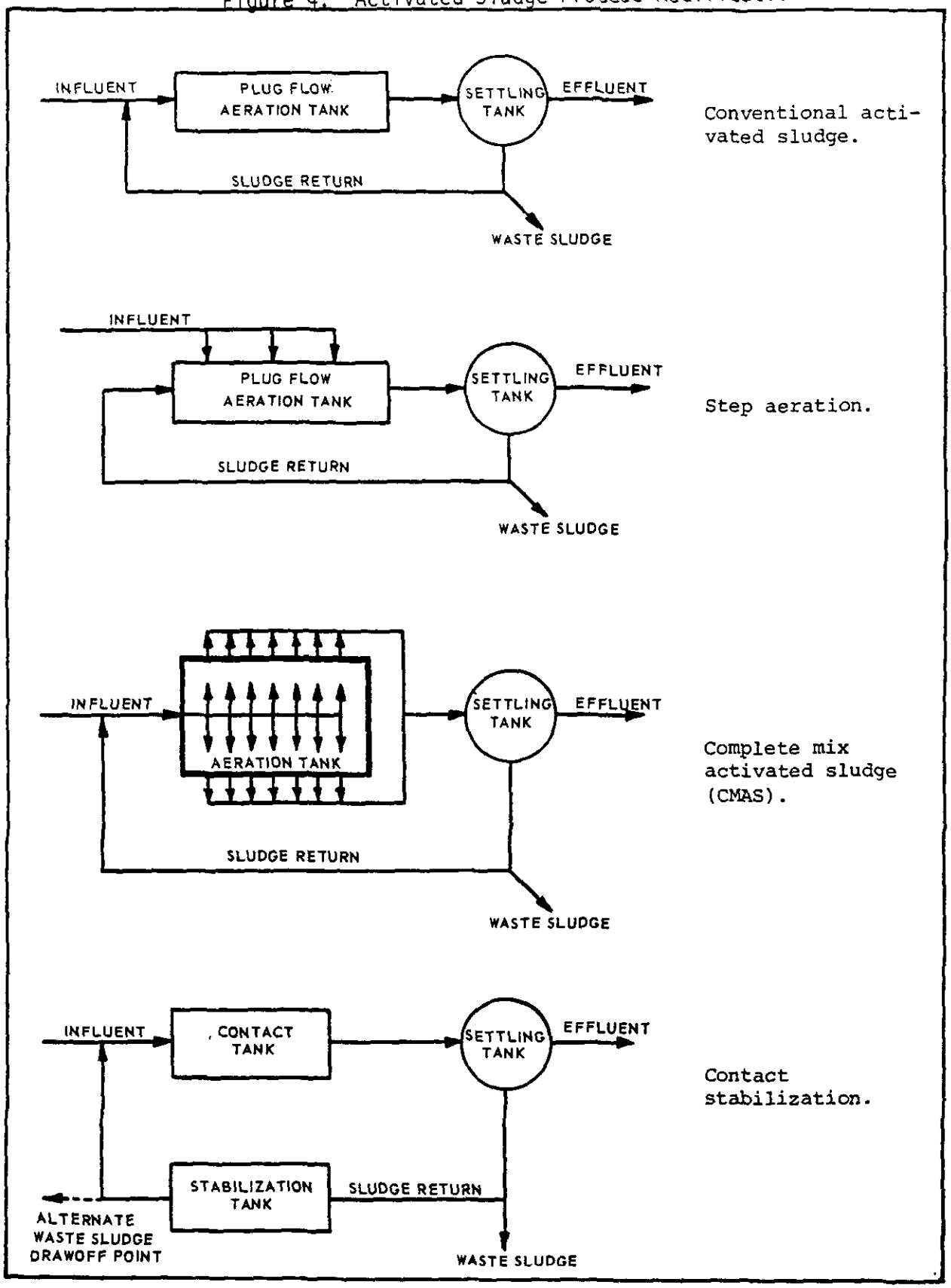
1 d.1. Definitions

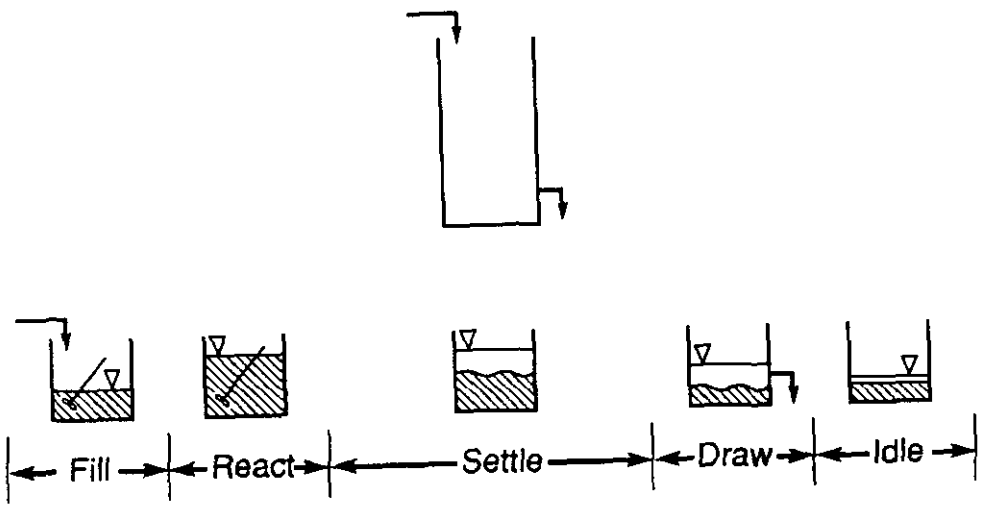
1.d.1.a. Solids retention time (SRT) or mean cell residence time (MCRT). The SRT is the average length of time that the activated sludge solids (microorganisms) are kept in the system (solids inventory/solids removed per day). It is also called the mean cell residence time (MCRT). The SRT is used to control the solids inventory and is adjusted by changing the amount of activated sludge wasted from the system. Each treatment plant will have its best SRT but, in general, the SRT will be in the range of 5-15 days. Control decisions should be based on 5 or 7 day moving averages rather than instantaneous day to day measurements.

Many people believe that the SRT concept is the best one for design, and operation and control of the activated sludge process. Indeed it recognizes the importance of the microorganism in the process. It also relates to sludge generation as lower SRTs produce higher amounts of waste sludge and higher SRTs (such as extended aeration produce lower amounts of sludge).

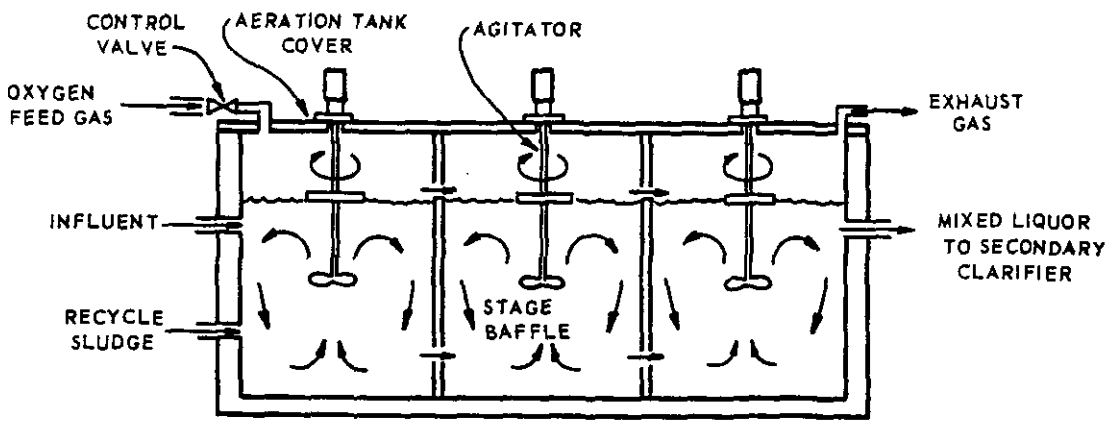
The choice of SRT value also influences the amount of oxygen consumed, the amount (if any) of nutrients which need to be added, and the final effluent quality. Perhaps most appropriate for this handbook, the choice of operating SRT influences settling of the sludge. This can be seen in Figure 5, which shows how the sludge volume index (SVI) is influenced by the operation SRT. The SVI is a measure of sludge settability and is defined as the volume occupied by 1 gram of activated sludge after 30 minutes of settling. As can be seen high SVI valves (indicative of poor settling) occur at both high (dispersed floc) and low (dispersed growth) SRT values, and thus the best settling occurs in the middle range values. As will be discussed, this is related to the microstructure of flocs.

Figure 4. Activated Sludge Process Modifications





Sequencing Batch Reactor (SBR)



Schematic diagram of multistage oxygen aeration system.

1.d.1.b. Food to Microorganism ratio (F/M). The food to mass ratio (F/M) is the organic loading divided by the solids inventory, or the pounds of BOD applied per day per pound of MLSS in the system. F/M control attempts to balance the incoming food load and mass of biological solids (microorganisms) in the plant. Control is achieved by wasting activated sludge solids. Increased wasting lowers the mass of biological solids and thus increases the F/M ratio. Similar to SRT, each plant would have an optimal value, and control decisions should not be based on instantaneous measurements. The range of F/M ratios is 0.2 to 1.0 lbs. BOD/lbs. MLVSS/day.

The F/M ratio is actually closely related to SRT. It may be a critical control value for certain types of filamentous bulking in that certain filamentous organisms thrive at low F/M values and can become more abundant than nonfilamentous organisms.

1.2.1.c. Aerator loading (lbs. BOD/1000 ft^3/day). Expresses the total loading of organics to the volume of the aeration basin. This is usually expressed as pounds of BODs applied per 1000 $ft.^3$ of aeration volume. This parameter is useful for knowing the BOD loading to a treatment plant but does not provide any information concerning the microorganisms (or solids inventory) in a treatment plant.

1.d.1.d. Mixed liquor suspended solids (MLSS). This is an estimation of the concentration of organisms in the aeration basin. It is not an exact measurement of microorganisms, but it does provide a useful estimation. Sometimes we use the volatile fraction of the MLSS to get a better approximation (MLVSS).

1.d.1.e. Detention time (or hydraulic rention time (HRT)). The average length of time that the wastewater is kept in suspension in the aeration basin. It is equal to the volume of the aeration basin divided by the flowrate (V/Q).

1.d.1.f. Recirculation ratio (Q_r/Q). The ratio of return sludge flow rate to the influent flow rate.

1.d.1.g. Zone settling velocity (ZSV). This is a measurement of the rate of which biological solids are transmitted to the bottom of the clarifier. Because of the nature of biological suspended slurriers, the particles do not settle independently, and actually hinder each other. Thus the type of settling occurring in a secondary clarifier is sometime called hindered settling.

1.d.1.h. Sludge volume index (SVI). SVI is a measurement of sludge settability and is defined as the volume occupied by 1 gram of activated sludge and 30 minutes of settling.

1.d.2. Typical Activated Sludge Design Parameters

Typical values for the design parameters for various activated sludge process modification are shown in Table 2.

Table 2. Typical Activated Sludge Design Parameters

Process Modification	Flow regime	Sludge retention time (days)	Food to microorganism ratio-#BOD_5/MLVSS/day	Aerator loading #BOD_5/1,000 ft^3 tank volume	Mixed liquor suspended solids(mg/l)	Detention time (hr)	Recirculation ratio
Conventional	Plug	5-15	0.2-0.4	20-40	1500-3000	4-8	0.25-0.5
Complete mix	Complete mix	5-15	0.2-0.6	50-120	3000-6000	3-5	0.25-1.0
Step aeration	Plug	5-15	0.2-0.4	40-60	2000-3500	3-5	0.25-0.75
Contact stabilization	Plug	5-15	0.2-0.6	30-75	1000-4000* 4000-10000+	0.5-1.5*	0.5-1.5
Extended aeration	Complete mix	20-30	0.05-0.15	10-15	2000-6000	24	0.5-2.0
Pure oxygen systems	Complete mix reactors in series	8-20	0.25-1.0	100-250	4000-8000	2-5	0.25-0.5

*Contact unit

+Stabilization tank

These design values are imiportant in providing a stable and reliable process. Later we shall see how non-optimal values can result in certain bulking problems. For further information on activated sludge design, see reference (6).

1.d.3. Control Considerations

There are various control parameters or strategies available to an activated sludge treatment plant operator such as SRT, F/M, and sludge quality. In this section various control considerations are listed.

1.d.3.a. Dissolved oxygen in aeration tank (DO). It is generally desirable to maintain DO levels of 1 to 3 mg/l. Higher levels are unnecessary and could actually cause breaking of flocs due to high shear. Lower levels may cause a type of filamentous bulking.

1.d.3.b. Return activated sludge flow rate (RAS). The return sludge from the secondary clarifier to the aeration basin is the key feature of the activated sludge process. Through return sludge the microorganisms are able to remain in the aeration basin longer than the wastewater (thus SRT can be higher than HRT). A proper RAS rate must be found for each plant. If the rate is too low, the sludge remains too long in the secondary clarifier and a condition known as rising sludge, due to denitrification, can occur if nitrification is performed.. On the other hand, if the rate is too fast, a lower HRT is imposed on the aeration basin and the loading rate to the secondary clarifier is increased which may cause a loss of solids.

1.d.3.c. Waste activated sludge flow rate (WAS). The WAS rate controls the SRT of the activated sludge process by settling the amount of activated sludge wasted. This is obviously very important as the choice of SRT controls the amount of oxygen consumption, the amount of sludge generated, the final effluent quality and influences the settleability of the mixed liquor solids (dispersed growth and dispersed floc).

1.d.3.d. Sludge blanket depth in secondary clarifier. This is the most direct method of determining the RAS rate. Various devices are used to measure the depth of the sludge blanket and then appropriate calculations can be made. Many operators favor core type tubes so that not only the height but also the degree of compaction of the blanket, or zone of transition can be seen.

1.d.3.e. Observations (color, odor, foaming, turbulence). While most of the parameters and considerations listed in the last two sections can be quantified, (that be given a number such as SRT = 5 days), not everything can be quantified. Long term conditions can be followed by direct observations of the aeration basin and clarifieer by observing the color, odor, foaming and turbulence of each. This is discussed by Hobson (see reference 3).

1.d.3.f. Microscopic evaluation. The microscope can be a significant tool in the evaluation of the activated sludge process. Using a microscope, one can examine 1)the form and structure of a floc; 2)the presence and relative numbers of filamentous organisms, and; 3) the types and relative numbers of protozoa present. This latter use is well documented in the EPA Process Control Manual For Aerobic Biological Wastewater Treatment

Facilities (see reference 9). The first two uses will be fully described later in this handbook.

CHAPTER 2

2. FILAMENTOUS BULKING

2.a. Solids Separation Problems

As has been noted, the activated sludge process is able to achieve a high SRT for system stability and efficiency and a low HRT for system economy by recycling biological solids. In this way the microorganisms can be built up to a high concentration and be maintained somewhat independently from the liquid detention time. Of course, this is only possible if good solids separation and settling occurs.

One class of problems is related to physical problems with the design of the secondary clarifier. These include such things as improper inlet design, weir placement and differential settling (that is uneven settling of the entire basin). Similarly hydraulic overloading could cause a washout of biological solids. These are not discussed in this handbook, but are of obvious importance. One may identify these types of problems with lab settling cylinder tests. See Appendix A case 1.

There are numerous solids separation problems in the activated sludge process which are related to the microorganisms. These have been summarized by Jenkins et al.(4) and are shown in Table 3.

2.a.1. Dispersed Floc and Dispersed Growth (microstructure)

As was discussed in Section 1.d.1.a. (SRT as a control parameter), the choice of SRT value influences the settling characteristics of the biological solids. This was shown in Figure 5. Jenkins (4) refers to this situation as the microstructure of the floc or its basic structure due to bacterial aggregation and flocculation. The condition known as dispersed growth occurs at low SRTs. Here the microorganisms do not form flocs, but are dispersed as individual organisms or small clumps. This is due to the physiological state of the microorganisms in that at low SRTs, the microbes are growing at a very fast rate and at these high growth rates, the organisms do not form polymers which cause the flocs to form. Thus good settling does not occur

At high SRTs a condition known as dispersed floc can occur. This is a function of the physiological state of the microorganisms in that at high SRTs the microbes are in a low growth rate and actually start to break up the good flocs previously formed. This results in poor floc structure, and poor settling.

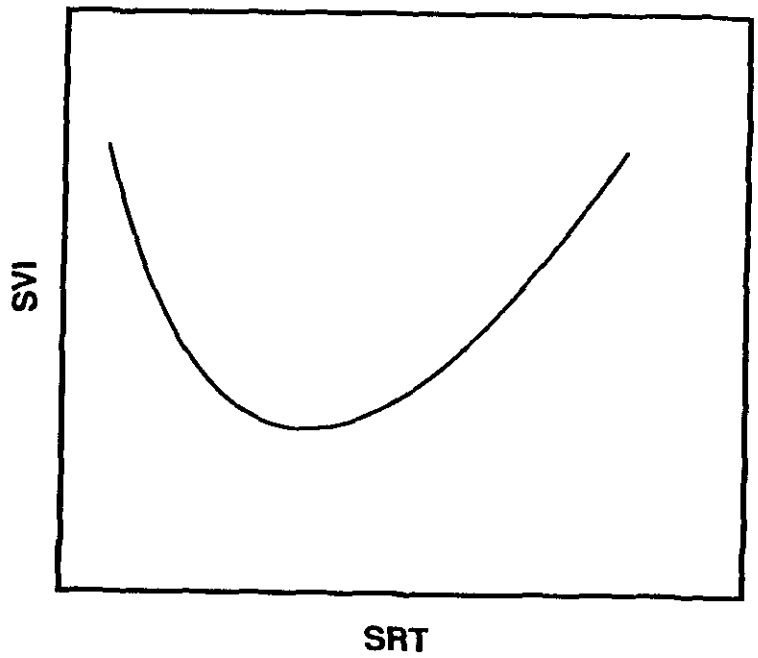
2.a.2. Other Problems (slime, blanket rising, foaming)

In this section, we will look at problems not caused by filamentous organisms or related to the microstructure characteristics (dispersed growth and dispersed floc). Slime occurs when excessive amounts of exocellular slime (polymer) are secreted and the solids become jelly-like, or slimy. This causes poor settling. This often occurs during periods of nutrient deficiency and may be corrected by adding nutrients.

Table 3. Causes and Effects of Activated Sludge Separation Problems (Reference 10)

Problem	Cause	Effect
Dispersed growth	Microorganisms do not form flocs but are dispersed, forming only clumps or single cells.	Turbid effluent. No zone settling of sludge.
Slime (jelly) Viscous bulking; (also possibly has been referred to as Non-filamentous bulking)	Microorganisms are present in large amounts of exocellular slime. In severe cases the slime imparts a jelly-like consistency to the activated sludge.	Reduced settling and compaction rates. Virtually no solids separation in severe cases resulting in overflow of sludge blanket from secondary clarifier. In less severe cases a viscous foam often is present.
Pin floc or Pinpoint floc	Small, compact, weak, roughly spherical flocs are formed, the larger of which settle rapidly. Smaller aggregates settle slowly.	Low sludge volume index (SVI) and a cloudy, turbid effluent
Bulking	Filamentous organisms extend from flocs into the bulk solution and interfere with compaction and settling of activated sludge.	High SVI-very clear superatant. Low RAS and WAS solids concentration. In severe cases overflow of sludge blanket occurs. Solids handling processes become hydraulically overloaded.
Blanket rising	Denitrification in secondary clarifier releases poorly soluble N_2 gas which attaches to activated sludge flocs and floats them to the secondary clarifier surface.	A scum of activated sludge forms on surface of secondary clarifier.
Foaming/Scum formation	Caused by (i) non-degradable surfactants and by (ii) by the presence of Nocardia spp. and sometimes by the presence of Microthrix parvicella.	Foams float large amount of activated sludge solids to the surface of treatment units. Nocardia and Microthrix foams are persistent and difficult to break mechanically. Foams accumulate and can putrify. Solids can overflow into secondary effluent or overflow tank freeboard onto walkways.

Figure 5. The Influence of SRT on SVI



Pin floc is the result of weak small flocs which have low filament levels. These flocs are small and compact. The larger one will settle but the smaller ones don't, leaving behind a cloudy turbid effluent.

Blanket rising is a condition caused by denitrification in the secondary clarifier. The nitrogen gas bubbles formed attach to the sludge flocs and cause them to float. This usually the results of sludge remaining too long in the clarifier. This can only occur in a nitrifing plant since nitrate must first be formed. Thus the SRT and dissolved oxygen concentration are related to this problem.

Foaming is caused by non-degradable surfactants. These surface active agents pass through the aeration basin undegraded and then cause foaming which floats large amounts of solids to the surface. Another type of foaming is caused by the filamentous organisms Nocardia and Microthrix. These organisms release non-degradable exocellular lipids and surfactants which cause the flocs to float. It may be due to high SRT, high MLSS concentration, high rates of aeration, or high concentrations of hydrocarbons in the influent. These foams are quite persistent and difficult to break mechanically. Foaming is further discussed in Chapter 5.

Toxic substances may cause a situation known as curdled floc according to Gerardi (2). This is caused by the interaction of positively charged heavy metals with negatively charged surface of bacterial cells. This is obviously related to the presence of toxic heavy metals in the wastewater.

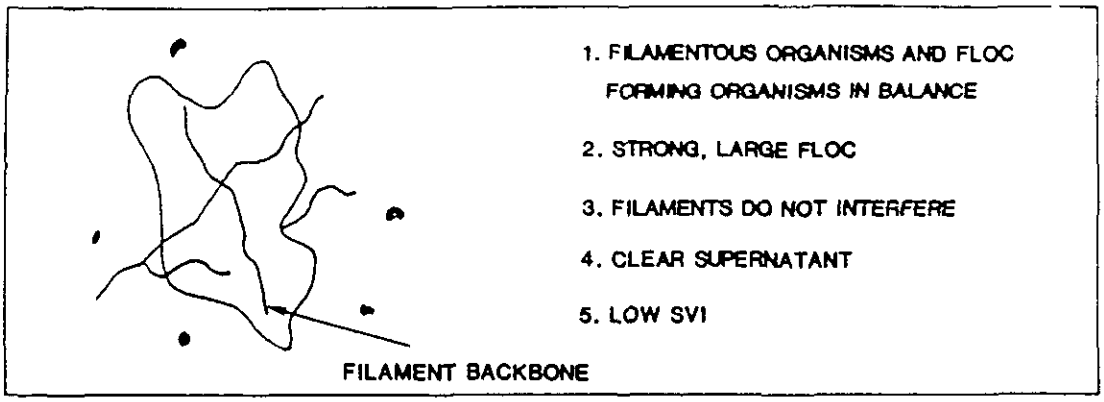
Gerardi (2) also points out that excessive shearing action by mechanical mixing can cause a "pin-point" type of floc.

2.a.3. Filamentous Bulking

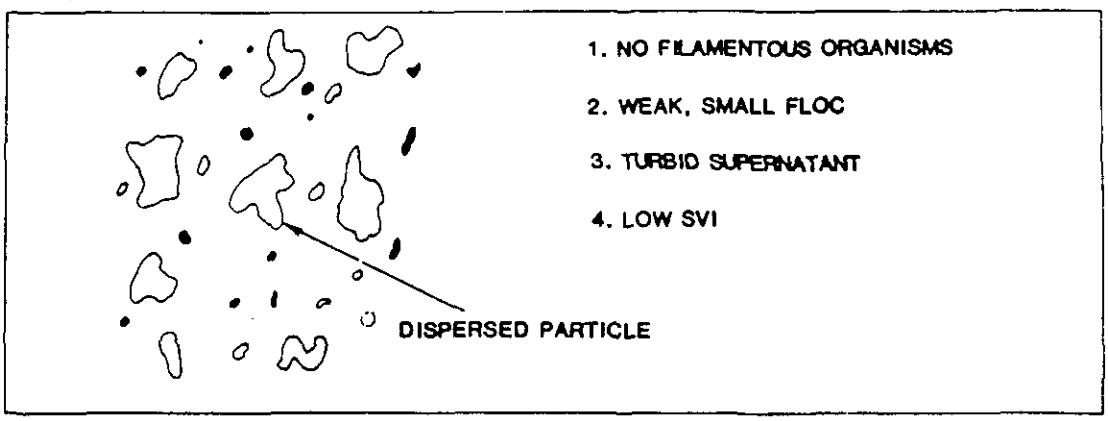
Filamentous organisms are those which grow in the form of very long rods, many times longer than wide. They may be effective degraders of BOD, but they form flocs which do not settle well or compact well.

Jenkins et al.(4) explains the relationship between filamentous and nonfilamentous organisms in terms of the macrostructure of a floc. This is shown in Figure 6. The macrostructure is due to the network of filamentous organisms within the floc. The filamentous network provides a structural frame for the floc-forming bacteria and provides strength for the floc. Three cases are shown in Figure 6. In the first case, filamentous bulking is shown to be caused by an excessive predominance of filamentous organisms, while in the third case, poor settling, pin-point floc, (as described above) is caused by a lack of filamentous organisms. The second case, nonbulking, is shown as a situation where there is a good balance between filamentous and non-filamentous organisms. This approach suggests that some filamentous organisms are necessary to form a good backbone for a strong, large floc. (Case 2). Excessive filaments, however, interfere with settling and compaction. (Case 1). Excessive filaments produce a diffuse floc structure or may lead to briding between flocs since filaments often grow beyond the boundaries of an individual floc.

A. Ideal, Non-bulking Activated Sludge Floc.



B. Pin-point Floc.



C. Filamentous Bulking Activated Sludge.

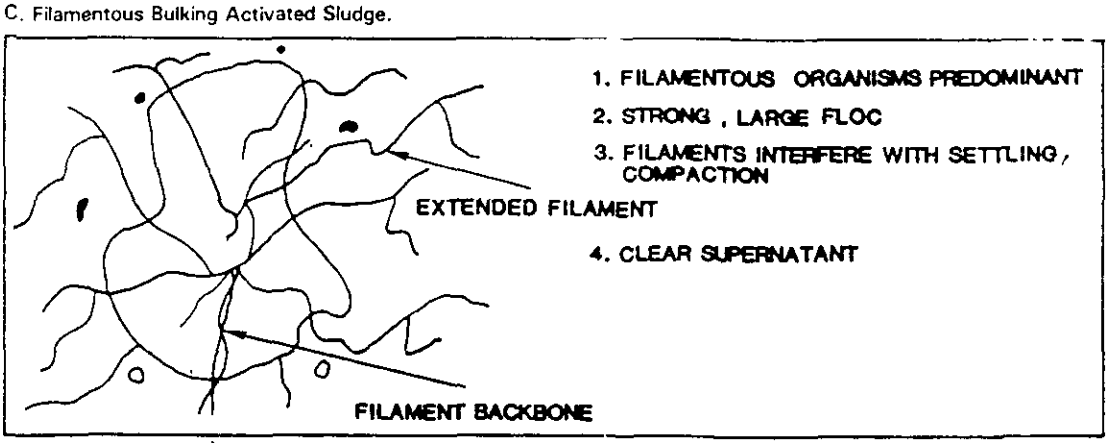


Figure 6. Effect of Filamentous Microorganisms on Activated Sludge Floc Structure. (Reference 10)

2.a.4. Summary of Solids Separation Problems

As can be seen, there are numerous solids separation problems in the activated sludge process which are related to the microorganisms (rather than to physical aspects such as hydraulics and basin design). Gerardi (2) has related these problems to the type of floc formed in the aeration basin.

In summary, according to Gerardi (2), there are nine basic types of flocs including:

1) an ideal floc containing floc-forming bacteria and filamentous bacteria that grow in balance,

2) a filamentous floc in which the predominant filamentous bacteria cause inter-floc bridging,

3) a filamentous floc in which the predominant filamentous bacteria produce a diffused floc,

4) a viscous floc containing excessive amounts of exocellular slime that is produced by the floc-forming bacteria during periods of nutrient deficiency,

5) a floating floc produced through the release of non-degradable surfactants and lipids by Nocardia,

6) a gas-entrained floc resulting from the entrapment of insoluble nitrogen gas released during denitrification.

7) a curdled floc formed by the interaction of floc-forming bacteria and relatively high concentrations of toxic substances such as multivalent cations.

8) a pin-point floc in which filamentous bacteria are absent, and

9) a dispersed floc containing small clusters of bacterial cells but lacking true floc particles.

The type of floc formed is determined by the environment in the activated sludge aeration basin. Various stimulatory conditions can bause deliterious situations which results in poor floc structure, which do not settle or compact well and then subsequest loss of biological solids. These include:

1. Environmental changes which promote the rapid and excessive growth of filamentous bacteria

2. Environmental changes which promote the production of excessive amounts of exocellular lipids, surfactants or slime

3. Operational conditions which permit denitrification

4. The presence of relatively high concentrations of toxic substances, and

5. The presence of excessive shearing action within the aeration basin

The first seven types of flocs are illustrated by line-drawings in Figure 7. Again the reader is referred to Appendix A to show how column settling tests may be used to diagnose these problems.

2.b. Causes of filamentous bulking

As has been discussed, filamentous organisms are not only usually present in activated sludge systems but are actually helpful in forming good floc structure. The problem of filamentous bulking occurs when excessive filamentous organisms are present. The predominance of filamentous organisms causes diffuse flocs, which do not settle well, and bridging of flocs, which do not compact well.

Over the recent past much progress has been made in understanding the causes of filamentous bulking. Conditions which cause a predominance of filamentous organisms over nonfilamentous organisms include:

1. Low dissolved oxygen levels in the aeration basin.

2. Low F/M loading.

3. Nutrient deficiency in wastewater (nitrogen or phosphorus.

4. Septic wastewater (sulfide), and

5. Low pH.

There are other less frequently occuring conditions which may also cause filamentous bulking. We shall later see how the identification of certain dominant filamentous organism types can be useful in pinpointing the cause or causes of bulking upsets.

2.c. Microbial Examination for Floc Characteristics and Identification of Filamentous Organisms

Microscopic examination of activated sludge is an important tool available to a wastewater treatment plant operator. As noted previously, one control strategy is based on observing the relative numbers of different protozoa and rotifers in the aeration basin. (see reference 9). In terms of bulking problems, microscopes can be used to characterize flocs and for the identification of both the abundance and types of filamentous organisms.

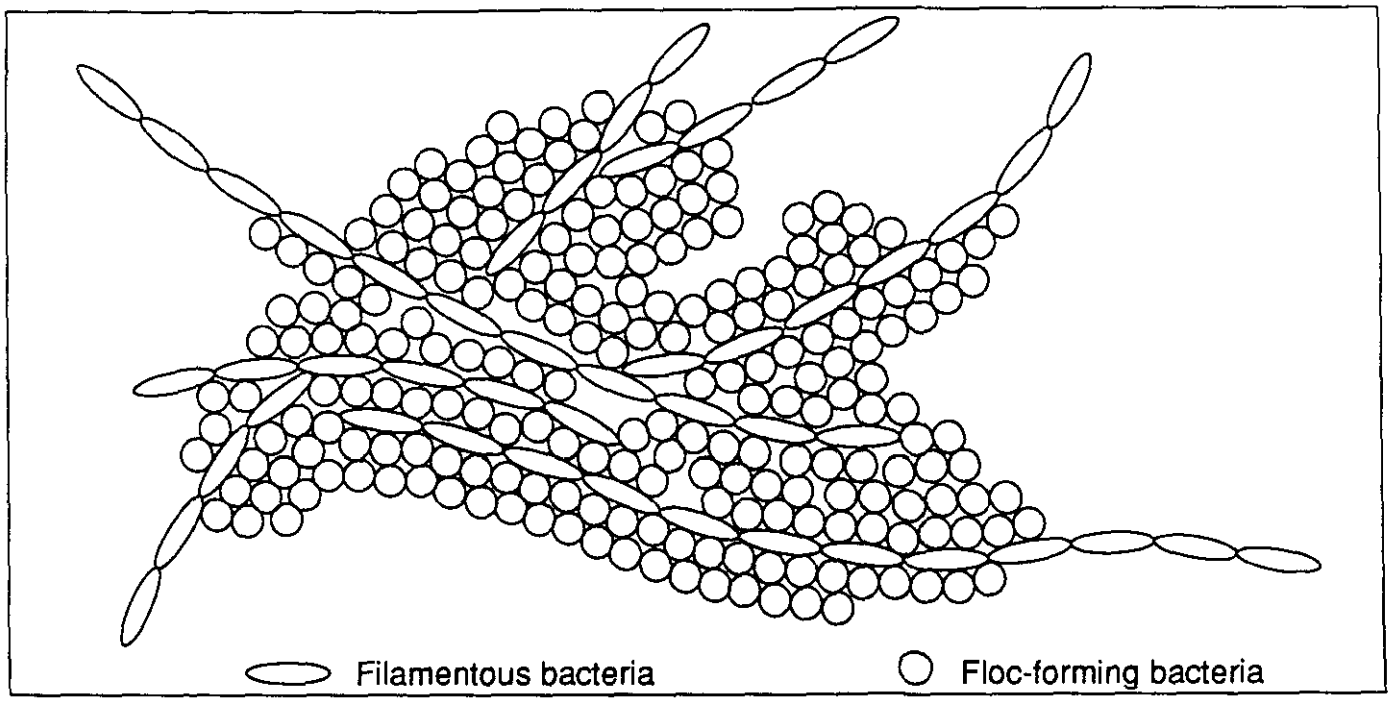
2.c.1. Sampling

Sampling is described in Section 5.1 of the EPA Manual on the Causes and Control of Activated Sludge Bulking and Foaming. (reference 10).See pages 33-34.

In general, samples are taken of the effluent end of the aeration basin or in the mixed liquor channel between the aeration basin and the secondary clarifier. Samples should be taken below the surface excluding foam and other floating materials. (Foam should be examined separately.) Samples

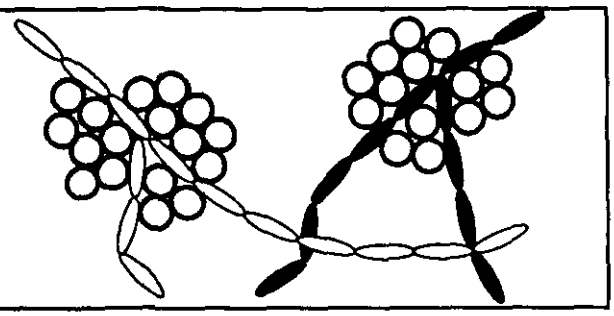
Figure 7. Line drawings of Activated Sludge Flocs (Reference 2)

1. Ideal floc (balance of filamentous and floc-forming bacteria)



2. Excessive filamentous growth

A. Inter-floc bridging

INTER-FLOC BRIDGING: Filamentous bacteria are predominant resulting in bridging of filaments between floc particles.	

B. Diffused floc

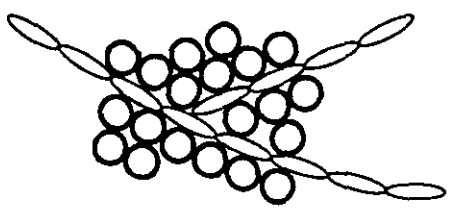
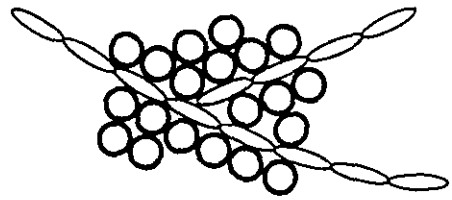
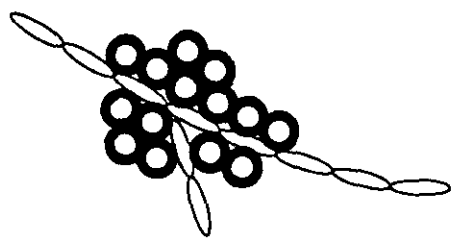
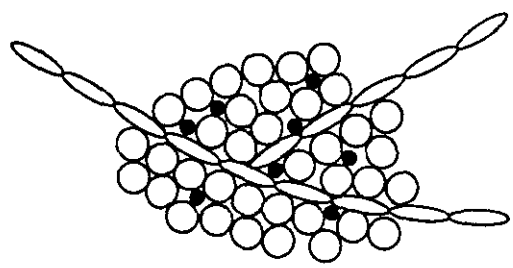
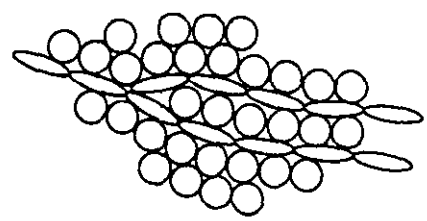
DIFFUSED FLOC: Filamentous bacteria are predominant resulting in scattered clusters of floc-forming bacteria along the length of the filaments.	

Figure 7. 3. Other problems

VISCOUS FLOC: Contains excessive amounts of exocelluar slime produced by floc-forming bacteria during periods of nutrient deficiency.	
FLOATING FLOC: Contains excessive amounts of non-degradable surfactants and lipids produced by Nocardia.	
GAS-ENTRAINED FLOC: Contains insoluble nitrogen gas released through denitrification.	
CURDLED FLOC: A dense or congealed floc formed through the interacion of floc-forming bacteria and relatively high concentrations of toxic substances.	

should not be diluted. Depending on the modification of the activated sludge process being used, different tanks may need to be sampled.

If samples are being examined on site, 5-10 ml portions are needed. They should be examined as soon as possible or stored at 4°C. If samples are being shipped, the liquid is put into containers which have airspaces equal to the sample volumes to ensure aerobic conditions. These samples should be shipped overnight.

Note that samples should be neither chemically preserved nor frozen since these procedures can alter the characteristics of the flocs and filamentous microorganisms.

2.c.2. Use of microscope

As was previously noted, the microscope can be a significant tool in the evaluation of the activated sludge process. Its use and care are explained in Appendix B of this handbook.

2.c.3. Staining.

Since there is very little contrast between microorganisms and water, stains are often used to examine microorganisms (as well as phase microscopy). In addition, stains can reveal important characteristics of an organism which are key points in the identification of a microorganism.

In examining activated sludge flocs and organism, two stains are commonly used - the Gram stain and the Neisser stain. Gram stain procedures are used to characterize the cell wall of a bacterium, either as Gram positive or negative. Neisser stain test for cellular phosphate granules - some organisms are able to store phosphate internally in granules. Other stains used include the "S test"(intracellular sulfur granules), India Ink reverse stain (to detect large amounts of extracellular polymers (slime) around the cells), PHB stain (to detect intracellular polyhydroxybrate granules), and the crystal violet stain to observe sheaths. The reagents and techniques for each of these stains are presented in the EPA Manual (reference 10) on pages 35-38.

2.c.4. Examination Procedure.

The procedure for using a microscope is described in detail in Appendix B. To prepare a sample of activated sludge for microscopic examination, it is necessary to prepare a wet mount using a cover slip. The procedure for such is described in Section 5.3 or page 38 of the EPA Manual (reference 10). For a wet mount, one drop of sample is placed in the center of a 25 x 75 mm. microscope slide. A 22 mm. No.1 cover slip is placed over the drop. (For photomicroscopic work use No. 1 1/2 .) Next press down the coverslip with a blunt object. Finally remove the liquid that is expelled from the sides of the cover slip with a tissue.

2.c.5. Floc Characterization.

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Usually 10-20 flocs are observed for floc characterization. In examining the activated sludge samples under phase microscopy, the following characteristics should be noted:

1. The shape of the floc (rounded and compact or irregular and diffused, see Figure 13a and 13b in reference (10)).

2. The size of the floc (small,less than 150 μm diameter), (medium,150-500 μm diameter), large, greater than 500 μm diameter).

3. The presence of protozoa and other particles. (See Figure 13c and 13d in referernce (10))

4. The presence of dispersed cells in the bulk solution. (See Figure 13b, reference (10)).

5. The presence and effect of filamentous organisms on floc structure (none, bridging, open floc structure). See Figure 14, reference (10)).

6. The abundance of filamentous organisms. This may be done on a subjective scoring or from 0 to 6 (see Table 4, Table 5 in the EPA manual reference (10)), or by measurement of filament length (described in the next section). Note that filamentous bacteria are considered dominant and most likely responsible for bulking if they score "very common" or greater on the subjective scale. Detailed information on floc characterization can be found in the EPA Manual (reference 10) on pages 38-43.

2.c.6. Quantification of Organism Level.

Jenkins et al. (4) present several methods to quantify the general organism level in activated sludge. One such method is shown in Table 5. This is the total extended filament length (TEFL) method of Sezgin et al. (8). Good correlations have been developed between TEFL and various conventional parameters of activated sludge settling such as SVI and zone settling velocity. In general, the SVI increased rapidly above 100 ml/g when TEFL values increased beyond 10^7 μm/ml.

2.c.7. Identification of filamentous organisms.

The identification of filamentous organisms may be useful. In this section the characteristics and identification of filamentous organisms are presented.

2.c.7.a. Characteristics. There are special features of filamentous organisms which can be used as clues in identifying the particular type of filamentous microorganism. These include: the presence of absence of branching; the type of motility; the filament shape; filament color; location of filament in floc; the presence or absence of attached growth of epiphytic unicellular bacteria; the presence or absence of cross-walls; filament diameter; filament length; cell shape; size (length and width);

Table 4. Subjective Scoring of Filament Abundance[a]. (Reference 10)

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.

[a]This scale from 0 to 6 represents a 100 to 1,000-fold range of total extended filament length.

Table 5. Filament Measurement Technique of Sezgin et al. (8)

1. Transfer 2 ml of a well-mixed activated sludge sample of known suspended solids concentration using a wide-mouth pipette (0.8 mm diameter tip) to 1 liter of distilled water in a 1.5-liter beaker and stir at 95 rpm on a jar test apparatus ($G = 85\ sec^{-1}$) for 1 min.

2. Using the same pipette, transfer 1.0 ml diluted sample to a microscopic counting chamber calibrated to contain 1.0 ml and cover with a glass cover slip.

3. Using a binocular microscope at 100 x magnification with an ocular micrometer scale, count the number of filaments present in the whole chamber or a known portion of it and place these in the following size classifications: 0 to 10 μm, 10 to 25 μm, 25 to 50 μm, 50 to 100 μm, 100 to 200 μm, 200 to 400 μm, 400 to 800 μm, and greater than 800 μm. Measure filaments of length greater than 800 μm individually.

4. Express results as the total μm of filament length per g or ml of MLSS:

   total extended filament length (TEFL) μm/gMLSS = total filament length, μm, in the 1.0 ml diluted sample x the dilution factor (x 500 in the above example) ÷ MLSS concentration, g/l

   total extended filament length (TEFL) μm/ml MLSS = total filament length, M, in the 1.0 ml diluted sample x the dilution factor (x 500 in the above example).

presence or absence of sulfur deposits; presence or absence of other granules such as phosphate and PHB; staining factors (Gram and Neisser); and several additional observations.

Detailed information of filamentous microorganism characteristics are found in Section 6.1 of the EPA manual (pages 45-61) (reference 10).

2.c.7.b. Identification. Once the filamentous microorganism characterization has been made and recorded, the organism can be identified using a dichotomous key shown in Figure 8. (Figure 28 in the EPA Manual (reference 10). Jenkins et al. (4) note that this key is not without risk as some filamentous characteristics vary and the key cannot always address all of these variables. Therefore, the filament type arrived at after using the key should be checked against the typical microorganism characteristics listed in Table 6 (Table 7 in the EPA Manual) and also against the pictures and short descriptions of each organism presented in Section 6.3 of the EPA Manual (10) (pages 61-75).

The key in Table 6 contains 22 filamentous bacteria commonly found in activated sludge plants. There are other types of filamentous organisms not listed in this key. Some were omitted to simplify the key but are described in Section 6.3 (10). Others are only rarely observed so they are not listed. Jenkins et al.(4) note that occasionally a filamentous organism is observed that is not represented by a type or genus description. Such an organism should be reported as not identified,and should not be "force-filted" into existing filament types.

While there are many different types of filamentous bacteria, approximately 10 types account for at least 90% of all bulking episodes. The relative frequency of various types of filamentous microorganisms observed in activated sludge is shown in Table 7.

2.c.8. Modified Indentification Procedure

References (4) and (10) are the primary sources of information for the examination of flocs and identification of filamentous organisms in the activated sludge process. These references are based on many years of work by Professor Jenkins and his students and other research laboratories and represent an excellent contribution to our understanding of filamentous bulking.

We have used these methods in our laboratory and based on our experience, we have modified the procedure. This is shown in Appendix C of this handbook. Along with the modified procedure are some notes based on our observations. These are not meant to contradict the primary references but only to supplement them.

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. natans	−	−	−	−	−	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 - 1.6	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 3-5	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 - 60	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 6. Summary Of Typical Morphological and Staining Characteristics of Filamentous Microorganisms Commonly Observed in Activated Sludge. (Reference 10)

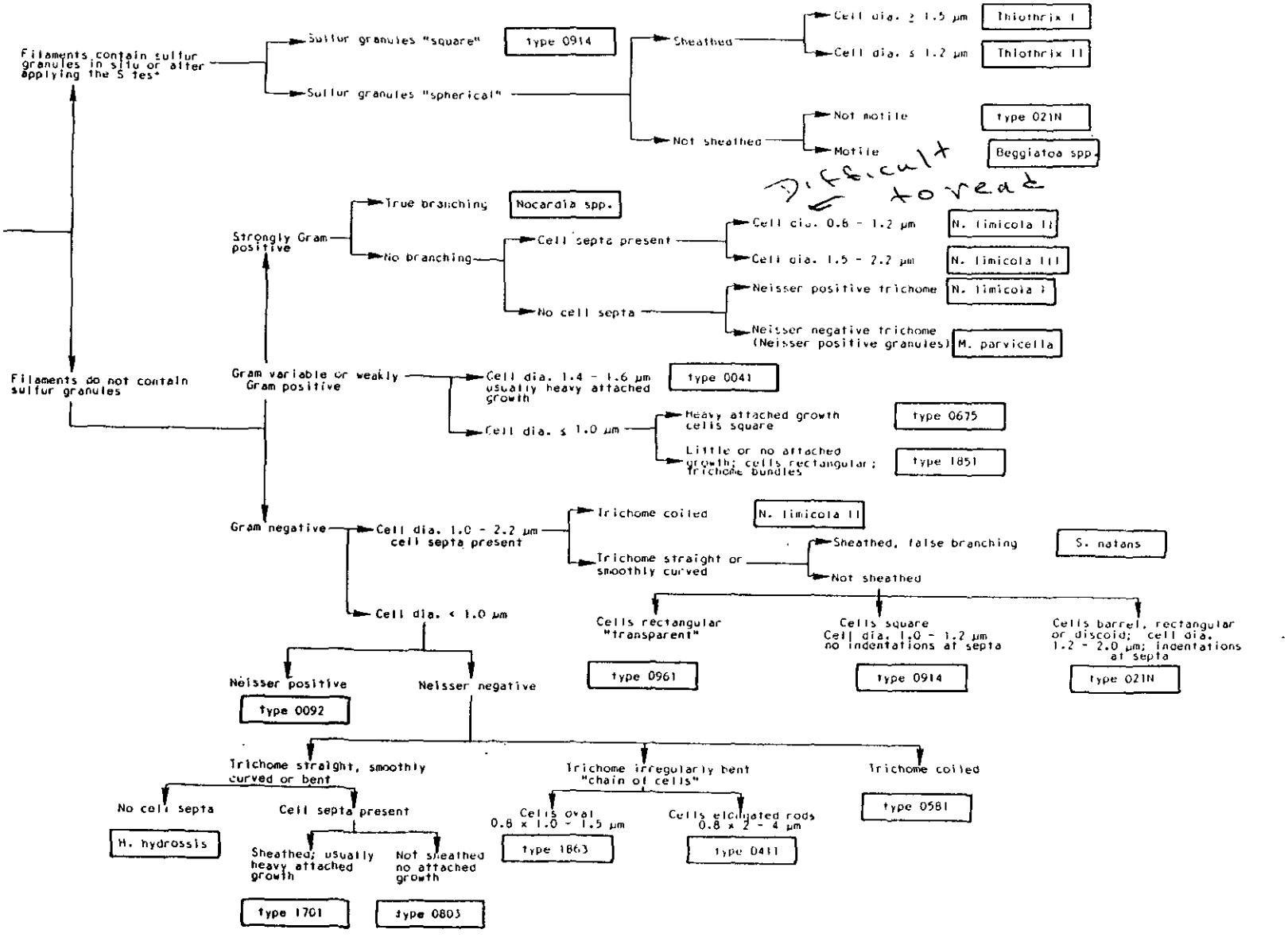

Figure 8. Dichotomous Key For Filamentous Microorganism "Identification" in Activated Sludge. (Reference 10)

Table 7. Filament Abundance in USA Bulking Activated Sludge (Reference 4)

Rank	Filamentous Organism	Percentage of Treatment Plants with Bulking Sludge where Filament was Observed to be:	
		Dominant	Secondary
1	Nocardia sp	31	17
2	type 1701	29	24
3	type 021N	19	15
4	type 0041	16	47
5	Thiothrix sp	12	20
6	Sphaerotilus natans	12	19
7	Microthrix parvicella	10	3
8	type 0092	9	4
9	Haliscomenobacter hydrossis	9	45
10	type 0675	7	16
11	type 0803	6	9
12	Nostocoida limicola	6	18
13	type 1851	6	2
14	type 0961	4	6
15	type 0581	3	1
16	Beggiatoa sp	1	4
17	fungi	1	2
18	type 0914	1	1
-	all others	1	-

CHAPTER 3

3. SELECTIVE CONTROL (Mitigation Strategies and Trouble Shooting)

3.a. Dominant Filamentous Types As Indicators of Conditions Causing Activated Sludge Bulking

Much knowledge has been gained over the recent past in the area of filamentous bulking. The keys and descriptions described in the previous section can be used to identify specific filamentous organisms in bulking activated sludge cases. Often the presence of certain organisms is indicative of certain upset conditions and thus their identification can be used to determine the cause of bulking. This relationship is shown in Table 8. (Table 2 in EPA Manual, reference 10).

3.b. Operational Changes Suggested by Filament Identification

The usefulness of filamentous organism identification in the diagnosis of the bulking problem and subsequent fixing of the problem is illustrated in Table 9. In other words, if one can identify the specific major organism or organisms occuring in a bulking episode, the cause of bulking might be known and then specific operational or design changes can be made to reduce the level of the filamentous organisms and thus control the bulking problem.

Gerardi (2) has summarized methods for controlling activated sludge bulking as slow (specific) methods or rapid (non-specific) methods. These are shown in Table 10. The methods listed in Table 9 are the slow specific type of control. The nonspecific methods will be discussed in Section 4 of the manual. The rest of this section will discuss specific, or selective procedures.

3.b.1. Increase in Aeration

Filamentous microorganisms such as Sphaerotilus natans and Type 1701,among others, have been associated with low dissolved oxygen levels in the aeration basin. Thus, an increase in the oxygen feed may overcome bulking problems associated with these organisms. Generally, this type of bulking is found in continuously fed completely mixed systems. It is also associated with high F/M values (indirectly) since the higher the F/M, the greater the required DO concentration needed to prevent bulking. Thus low DO bulking may be prevented by manipulating the F/M ratio (lowering) or manipulating the DO conentration (increasing).

There are several precautions one must consider in manipulating F/M. Lower F/M may result in nitrification, increasing the MLSS to a value which exceeds the capacity of the secondary clarifier, and finally if the F/M ratio is lowered too much, another type of bulking could result. In addition, raising the DO concentration will increase power consumption costs. For these reasons it might be less costly to operate at lower DO concentrations and control bulking by RAS chlorination.

3.b.2. Addition of Nutrients

Table 8. Dominant Filament Types as Indicators of Conditions Causing Activated Activated Sludge Bulking (Reference 10).

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
Septic Wastewater/Sulfide	Thiothrix sp., Beggiatoa and type 021N
Nutrient Deficiency	Thiothrix sp., S. natans, type 021N, and possibly H. hydrossis and types 0041 and 0675
Low pH	fungi

Table 9. Examples of "Curing" Bulking by a Change in Operation Suggested by Filament Identification (Reference 4).

Treatment Plant Waste Treated	Filamentous Organism(s) Causing Bulking	Change in Operation	Results
1. Plant A	Thiothrix sp type 1701	a) increase NH_3 supplementation	Thiothrix sp eliminated type 1701 remained
domestic wastewater and fruit processing wastes		b) increase aeration; use of liquid O_2	type 1701 reduced; return to non-bulking condition
2. Plant B	H. hydrossis	a) increase aeration	H. hydrossis eliminated
papermill wastes		b) increase sludge wastage	type 0803 eliminated; return to non-bulking condition
3. Plant C			
domestic			
a) winter operation	type 1701	a) increase aeration	type 1701 eliminated; return to non-bulking condition
b) summer operation (lower F/M for nitrification)	M. parvicella	b) increase sludge wastage	M. parvicella eliminated; return to non-bulking condition
4. Plant D			
brewery wastes	type 021N	a) increase NH_3 supplementation	type 021N eliminated; return to non-bulking condition
5. Plant E			
domestic and soluble industrial	type 0675 type 0041	a) change from step feeding to "plug flow"	types 0675 and 0041 reduced; return to non-bulking condition

6. Plant F

domestic + industrial. Oxygen activated sludge (first stage DO uptake rate 200 mgO_2/gVSS-hr	type 1701	a) increase DO concentration from 10-12 mg/l to 16-20 mg/l in first stage of aeration basin	type 1701 eliminated; return to non-bulking condition

7. Plant G

domestic + pulp and paper and other industry	type 1851 and N. limicola II 0675 and 0041	a) install selector	filament abundance decreased; non-bulking condition achieved; dominant filamentous organisms changed to types 0675 and 0041

Table 10. Control Measures for Activated Sludge Bulking.(Reference 2)

I. Slow, specific methods for controlling activated sludge bulking

- A. Identify the causative filamentous microorganism (s)
- B. Determine the probable cause (s) of the bulking, i.e., the environmental factor(s) which stimulate the growth of the filamentous microorganism(s)
- C. Make the required operational changes, for example
  1. If septic waste is the causative factor: prechlorinate
  2. If nutrient deficiency exists:
     a. Determine deficient nutrient(s): N, P, S or Fe
     b. Add nutrient(s) to the system
  3. If dissolved oxygen deficiency exists: increase aeration

II. Rapid, non-specific methods for controlling activated sludge bulking

- A. Manipulation of RAS flow rates
- B. Manipulation of waste feed points to the aeration basins
- C. Addition of chemicals to enhance the settling rate of the activated sludge
- D. Addition of toxicants to the activated sludge to kill the extended filamentous microorganisms that cause bulking.

Domestic wastewater usually contains adequate supplies of all necesssary nutrients for proper bacterial metabolism. However, there are various industrial waste which may be deficient in nutrients - especially nitrogen and/or phosphorus. Often these industrial wastes may makeup a substantial portion of the domestic wastewater. This can lead to predominance of certain filamentous bacteria, since some of these organisms have a lower N/P requirements and can compete more efficiently for available nutrients due to their large surface area. Thus they are able to outcompete floc formers under these conditions. Examples of filamentous organism which predominate under phosphorus or nitrogen deficiencies include: Microthrix parvicella, Sphaerotilus natans, Type 021N, type 1701, and the fungus, Geotrichum.

N and P requirements can be calculated based on the elemental composition of N and P in bacteria. On the basis of mixed liquor volatile suspended solids, approximately 15% of the MLVSS produced each day is composed of N, and approximately 3% of the MLVSS produced each day is P. These percentages are based on a dry weight basis. Some operators use a ratio of BOD: N:P of 100:5:1 to establish N and P requirements, but this is only an approximation since bacterial populations change in their requirements due to predominance of different forms, the operating SRT, wastewater composition, and mode of activated sludge operation. Gerardi (2) suggests target nutrient concentrations in the aeration basin effluent to assure proper N and P levels of 1.0 mg/l (ammonia and nitrate, combined) and 0.5 mg/l (orthophosphate). These are for filtrate samples which assure bioavailability. Example calculations for determining nutrient additions to correct a nutrient deficiency are shown in Appendix A of the WPCF Manual of Practice (11).

Nutrient addition may be in the form of anaerobic digester supernatant, or chemical compounds such as an hydrous ammonia or phosphoric acid. They are usually added directly to the aeration basin.

3.b.3. Prechlorination

As was pointed out previously, treatment of septic wastes can lead to the growth of the filamentous microorganisms Thiothrix and type 021N. It is believed that this results from the ability of these organisms to grow on inorganic reduced sulfur compounds and organic acids which are produced by fermentation in septic sewage. Prechlorination and preaeration are helpful in controlling this type of builking.

3.b.4. Caustic Addition

A low pH in the aeration tank can promote the growth of fungi. While an activated sludge system can remove organic matter and consume oxygen over a wide pH range (6.0 to 9.0) the best performance is obtained in the range of 7.0 to 7.5. Thus an abundance of fungi usually indicate strong acid discharges and an aeration tank pH value below 6.0. The pH can be raised by addition of caustics such as sodium hydroxide.

3.b.5. Increase in Sludge Wastage (WAS)

For low F/M bulking an increase in the rate of WAS will increase the F/M and this might eleviate the problem.

3.b.6. Installation of Selector or Process Modification

In general, activated sludge systems which are operated as continuously fed, competely mixed and at low F/M values, produce poorer settling sludges than systems which are fed intermittently or have aeration tanks where there is a relatively high local concentration of wastewater at the point where the RAS and influent enter the tank. Organisms such as M. parvicella and types 0041, 0675, 0581, 0961, 0803, and 0092 are often seen at low F/M loadings, but the specific causes for the growth of at these organisms that appear in low F/M, continuous fed, completely mixed systems, are not well understood.

Several possible means exist to control this type of bulking by process modification. Batch fed reactor, such as SBR, or changing the flow pattern from completely mixed to plug flow would help control this type of bulking. In general, any type of measure which would produce a carbonaecous substrate concentration gradient in the aeration basin, or a high substrate concentration at the point where return sludge enters the aeration basin system should control this type of bulking.

One technique which has worked well in laboratory studies (but less so in full scale systems) is the selector system. A selector is a small mixing tank or tanks where RAS and influent wastes mix prior to the aeration basin. A selector system configuration is illustrated in Figure 9.

Since the mechanisms involved in the functioning of selectors and in the growth of low F/M filamentous organisms are not known, it is not possible to be too specific about the design of selectors. Use of selectors at a treatment plant must be tested in pilot studies.

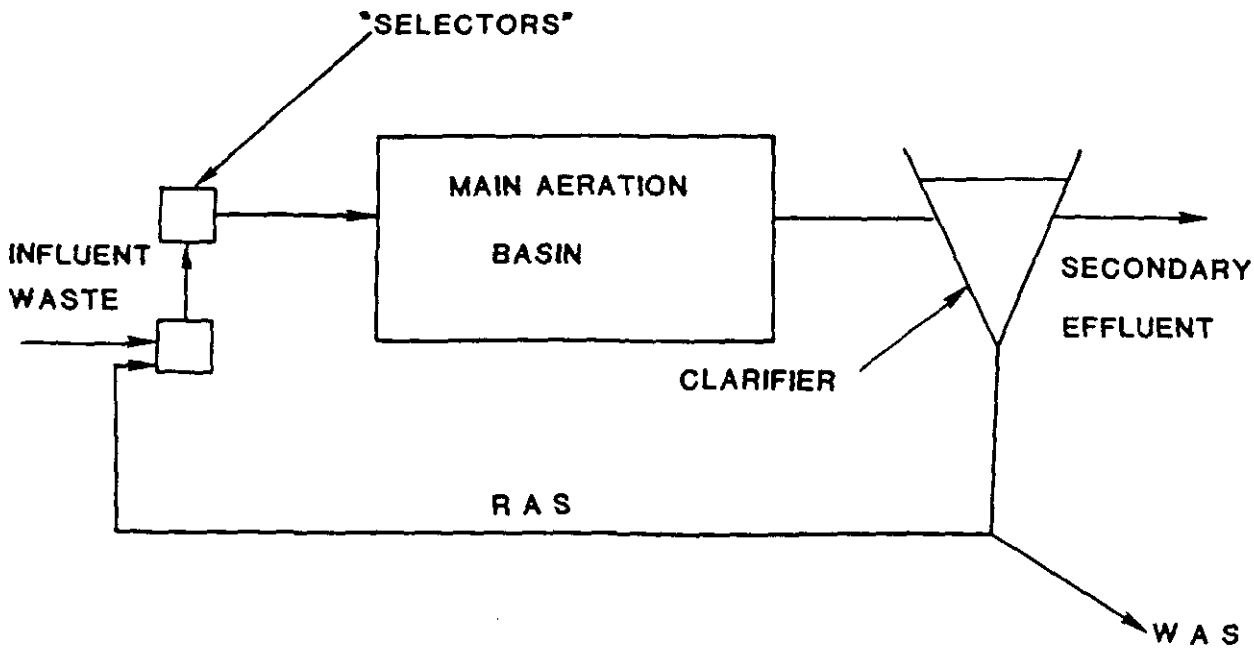

Figure 9. "Selector" Reactor System. (Reference 4)

CHAPTER 4

4. NONSELECTIVE CONTROL

In this section, we will discuss general strategies to control activated sludge bulking. These are nonselective in that they do not relate to any specific type of bulking which might be able to be controlled by a specific action, such as adding nitrogen to contol the growth of a known filamentous organisms. such as S. natans.

4.a. Chemicals To Enhance Settling

Polymers or inorganic chemicals may be added to the mixed liquor to promote better floc formation and thus improve sludge settleability without destroying or eliminating filamentous organisms.

There are a large variety of polymer types available to enhance settling by overcoming bridging of excessive filamentous organisms or diffused floc structure. Most of these polymers are high molecular weight polymers with high cationic charges. They are used either alone or in combination with other types of polymers. The best type of polymers to use and the appropriate dosages to add can be determined by using jar tests. The use of jar tests for this application is described in Appendix E of reference (11) (WPCF MOP#0M-9 Activated Sludge). Note that overdosing can degrade system performance and is quite wasteful since the use of polymers is very expensive.

Polymers are generally added to the mixed liquor as it leaves the aeration basin. Polymers have also been added to the center well of secondary clarifiers.

Lime and ferric chloride have also been used to enhance the settling rate of activated sludge flocs. Usually, they are added directly to the mixed liquor. Again, the type and dosage of chemical is best determined by jar testing. The main mechanism with inorganic chemicals is precipitation which adds mass to sludge flocs and allows better settling.

4.b. Chlorination

Chlorine may be added as a non-specific control measure to kill filamentous microorganisms. Chlorine is a well known disinfectant which attacks the enzyme systems of bacteria. It may be added as a chlorine solution produced from a gas chlorinator or as calcium or sodium hypochlorite.

Chlorine is the toxicant of choice for controlling bulking as it is economical and since most municipal treatment plants disinfect secondary effluent with chlorine, it is available already at the treatment plant. (Here, one must use caution as much smaller amounts of chlorine are required for bulking control than disinfection.) The theory behind the use of chlorine for bulking control is selective destruction. You want to kill the filamentous organism with minimal damage to the floc forming organism. This assumes that the filamentous organisms are more susceptible to chlorine than the floc formers.

The most common and preferred point of chlorine addition for filamentous bulking control is into the RAS line. It is also possible to add chlorine directly into the aeration basin or into an installed sidestream in which mixed liquor is pumped from and returned to the aeration basin. (i.e. a "loop").

The following criteria should be followed when chlorinating for bulking control to ensure effective operation:

1. A target value of the SVI (or other activated sludge settling parameter) must be established. This target value provides a boundary, a value below which the plant can be operated satisfactorily without any of the problems associated with poorly settling activated sludge.

2. Chlorination should be used only when the target SVI (or other settling parameter) is significantly and consistently exceeded.

3. Daily trend plots of SVI values should be maintained to anticipate approaching the SVI target value. This also assists in making adjustments to chlorine doses.

4. Chlorine must be added in known and controlled doses to all of the activated sludge at a point of excellent mixing. This prevents overdosing of a stream of solids. Turbid effluent results from localized over-chlorination and a reduction in treatment efficiency occurs due to excessive killing of floc-forming microorganisms. This also prevents a large part of a stream of solids from not being chlorinated.

5. Chlorine should be added where chlorine demand is minimum. The purpose of chlorination here is to kill filamentous microorganisms and not satisfy the chlorine demand. Chlorine reacts with many components of the wastewater (such as ammonia, nitrates, and sulfides) which may modify or reduce the dosed chlorine strength (or concentration) and influence the way in which the dosed chlorine kills filamentous microorganisms.

6. Chlorine doses are measured on the basis of the sludge inventory in the plant. When adding chlorine, the following must be considered: Chlorine concentration (mg/L) at the dosing point, local chlorine mass dosage, and frequency of exposure of solids inventory to the chlorine. Typical values for these parameters are found in reference (4) and are shown in Appendix D of this handbook.

7. Since adding chlorine for filamentous bulking is a tricky operation, monitoring is necessary. While chlorinating, monitor the following: sludge settling characteristics; effluent quality; and sludge 'health', including floc appearance, filamentous microorganisms (deformed cells, cells detached from the sheath, broken filaments, etc.) and Protozoan microlife.

8. Chlorine overdosing is apparent when total elimination of filamentous microorganisms occurs or small, broken-up flocs and fine particles appear together. This must be avoided.

The EPA Manual (10) lists several examples of filamentous organisms controlled by chlorination (Table 3, page 25), and presents several case histories of effective use of chlorine for filamentous bulking control. (These are listed in Chapter 7 (10)).

4.c. Hydrogen Peroxide

Another toxicant which can be used to control filamentous bulking is hydrogen peroxide. It is typically added as a 50% by volume solution in both continuous and batch dosing. The mechanism of action for H_2O_2 is attack of the sheath or cell wall of filamentous organisms. Hydrogen peroxide is added in similar fashion as chlorine. It has been noted that higher dosages and longer contact times may be necessary.

The EPA Manual (10) lists several examples of hydrogen peroxide for bulking control (in Table 4, p.26), and one case history is listed in Chapter 7 (10).

It is also possible that adding hydrogen peroxide provides additional oxygen for supplementing the DO concentration. This would be helpful if low dissolved oxygen is the cause for sludge bulking.

4.d. Manipulation of RAS Flowrate and Aeration Basin Feed Point

As was pointed out in the first part of this handout (Section 1.c.) the activated sludge process is quite flexible and can be designed and constructed in a manner to allow easy process modifications. Because of this, in many cases the adverse effects of bulking may be minimized by proper management of the system. Sludge bulking will result in poor compaction and/or settling in the secondary clarifier which will cause a decrease in the RAS solids concentration. To maintain stable operation of the aeration basin, high values of organisms (mixed liquid suspended solids) must be maintained to allow the degradation of BOD to continue. Therefore, since the RAS solids concentration has decreased, the flowrate of RAS must increase in order to maintain a high microorganism concentration in the aeration basin.

In addition to manipulating the RAS flowrate, the effects of bulking sludge can be minimized by reducing the MLSS concentration in the clarifier feed. The MLSS in the clarifier feed can be reduced by lowering the MLSS inventory in the plant. This is done by increasing the amount of solids wasted from the system (WAS rate increase). Of course, one must be careful in manipulating WAS rates since if would not be feasible to increase WAS rate if the sludge handling capacity were not sufficient to handle the increased amount of sludge. Also, since increasing WAS rate lowers the SRT (or raises F/M), other constraints may override this option. For instance a low F/M may be required to achieve nitrification.

The MLSS concentration to the clarifier feed may also be reduced by changing the operational mode of the activated sludge process. The idea

here is to reduce the MLSS concentration in the clarifier without reducing the MLSS inventory,(that is more solids in the aeration basin). The step feed (or step aeration) and contact stabilization modifications are means of achieving this objective. These process modifications were described earlier in this handbook (Section 1.c.2.). Of course these modifications are only possible when the plant is designed with the flexibility to operate in these configurations.

It should be noted that these methods do not treat the cause of bulking, but treat the symptoms. The plant continues to operate with an unstable biomass.

CHAPTER 5

5. Foaming

Foam and scum problems may be caused by filamentous organisms - mainly Nocardia. The causes of Nocardia growth are not well understood but high counts have been found at high SRTS, high MLSS levels, and warm temperature oil and grease in the wastewater. Foam and scum problems occur mainly in the aeration basin and secondary clarifier but may be carried over to sludge thickeners and digesters. These organisms release foaming agents which reduces the surface tension of the wastewater. They also release lipid materials which collect on the surface of air bubble and interferes with oxygen exchange. This results in a viscous, stable often chocolate colored sludge. It also may result in a floating sludge due to insoluble lipid materials (fat floats) and entrapped air bubbles. Gerardi (2) notes that although the organisms causing this problem are filamentous, they are short and generally present largely within the floc, rather than extending from the floc surface, so it is not a regular type of filamentous bulking.

Gerardi (1) suggests various control measures for filamentous foaming and scum problems. These are listed below:

BAFFLE. A baffle can be placed across the surface of the chlorine detention tank to prevent scum from being discharged with the secondary effluent. However, depending on the amount of scum accumulated behind the baffle, the chlorine detention tank may need to be drained and cleaned frequently.

BIOAUGMENTATION. Foaming can be controlled by the addition of selected nonionic detergent bacteria or by the addition of specialized bacteria which contain lipase enzymes that attack lipid (foam) materials, but the actinomycetes are not attacked.

CHLORINATION OF RETURNED SOLIDS. Returned mixed liquor solids can be chlorinated to destroy actinomycetes. However, actinomycetes are quite resistant to chlorination and extreme caution and daily microscopic examination of the mixed liquor microlife should be used to prevent serious damage to the microlife or decreased effluent water quality. A target value of 10 mg/L in the returned solids is suggested.

CHLORINATION OF THICKENER OVERFLOW. To prevent reseeding of the mixed liquor through actinomycetic-containing solids and supernatant that pass over the thickener effluent weirs, the effluent (overflow) from the thickener can be chlorinated. A target value of 10 mg/L in the thickener overflow is suggested.

DEFOAMING AGENTS. Defoaming agents can be used to alter surface tension within the mixed liquor and reduce foam production.

HYDROCARBONS. Many actinomycetes are able to utilize volatile organic compounds, short-chained petroleum fuels, petroleum fuel derivatives, and cyclic hydrocarbons, as food sources. Therefore, the discharge of such compounds from industrial sources should be regulated to acceptable levels.

MODE OF OPERATION. Contact stabilization should not be used during actinomycetic blooms, since this mode of operation generates a high SRT which favors the growth of very slow growing actinomycetes.

pH. Lime (calcium carbonate) addition to the mixed liquor to improved the settleability of solids or to maintain a near-neutral pH should not be practiced during actinomycetic blooms, since the growth of most actinomycetes is inhibited at pH levels lower than 5.0 and enhanced at pH levels greater than 5.8.

RECIRCULATE SLUDGE AT HIGH RATES. By recirculating sludge at a high rate the sludge blanket in the secondary clarifiers is kept low. There is some factor(s), as yet unknown, which promotes abundant growth of actinomycetes in sludges held too long in secondary clarifiers.

REDUCED AERATION RATES. By lowering dissolved oxygen levels within the mixed liquor, actinomycetic foam production decreases. However, caution should be exercised to prevent any significant lowering of treatment efficiency.

REDUCED MIXED LIQUOR SUSPENDED SOLIDS CONCENTRATION. By lowering the mixed liquor suspended solids concentration, the SRT is also reduced. Actinomycetes grow very slowly in comparison with mixed liquor bacteria and therefore, their population density and effects can be reduced by promoting a competitive edge for mixed liquor bacteria.

RETURN UNTREATED ANAEROBIC DIGESTER SUPERNATANT OR SLUDGE. There appear to be some biological factors (actinophages, nocardiolytic enzymes or lipases, or toxic metabolic waste) in anaerobic digester supernatant and untreated sludge that prevent the proliferation of actinomycetes when digester supernatant of sludge is introduced into the mixed liquor.

WATER SPRAYERS. Coarse, bib or shower type water sprayer can be used to dilute and reduce the accumulation of foam and scum. The foam produced by actinomycetes cannot usually are collapsed by conventional impact water sprayers.

As can be seen there are many possible control mechanisms to control Nocardia foaming. Since the mechanisms causing this problem are not well understood, there is no single proven method to control it.

The most important consideration in controlling Nocardia regardless of the method chosen is the prevention of foam recycling. Recycling reseeds the sludge and this must be avoided.

CHAPTER 6

6. CASE STUDIES IN MASSACHUSETTS

(to be added)

CHAPTER 7

7. ENGINEERING EVALUATION OF THE BULKING PROBLEM IN MASSACHUSETTS

(to be added)

CHAPTER 8

8. ANNOTATED BIBLIOGRAPHY

(to be added)

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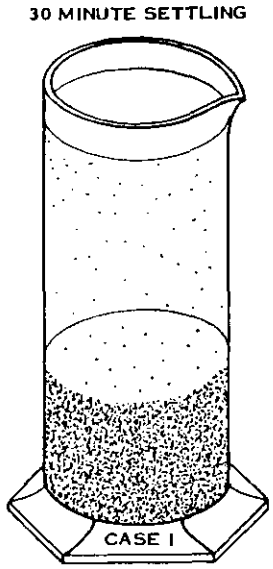
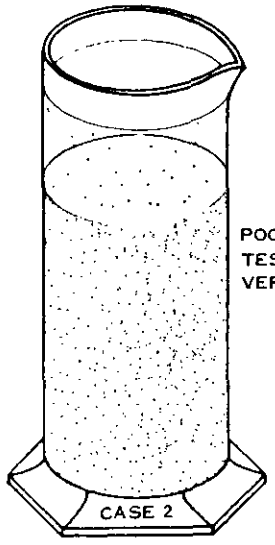
Appendix A. Column Settling Tests



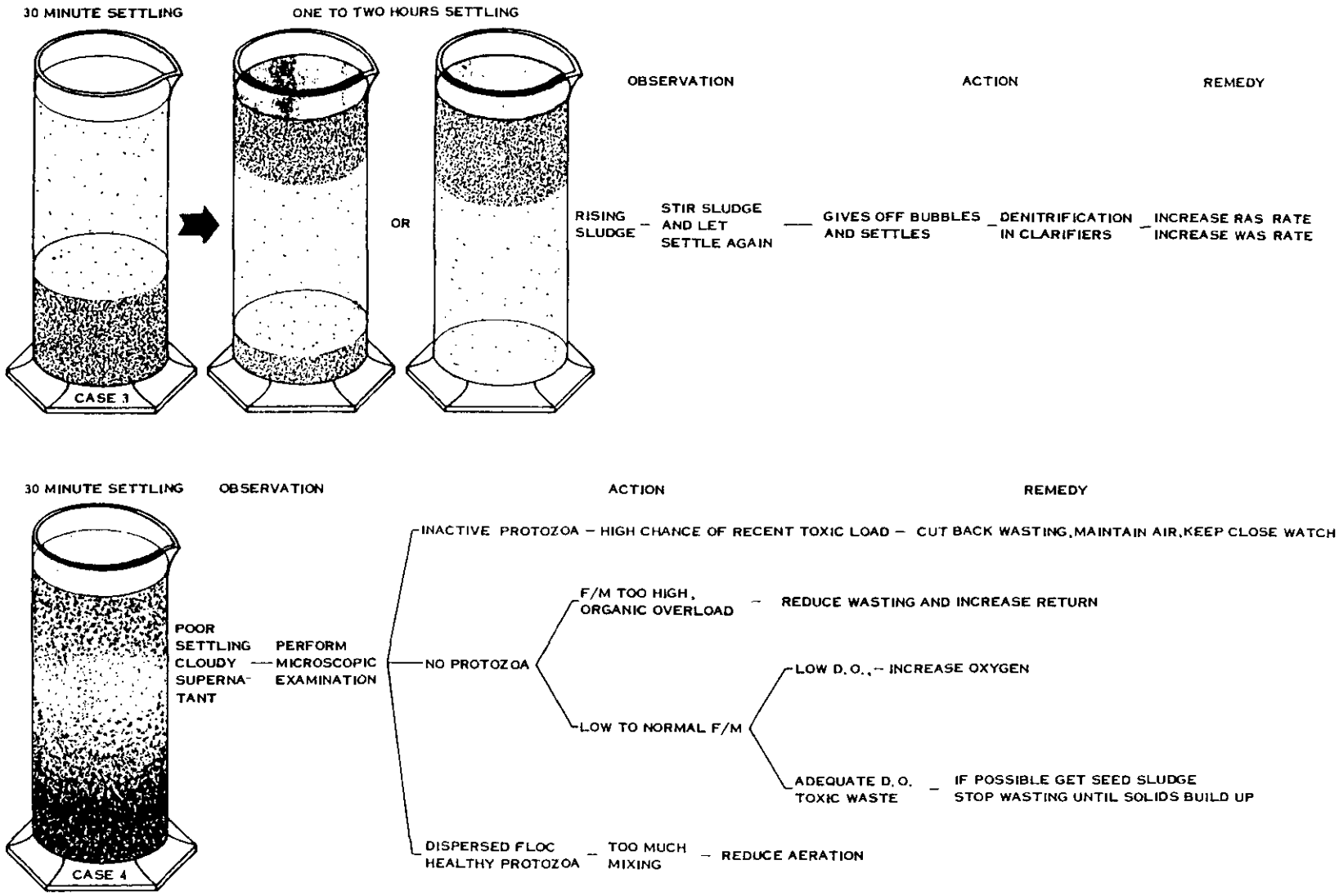
OBSERVATION	ACTION	REMEDY
GOOD SETTLING IN TEST BILLOWING IN CLARIFIER	CHECK FOR EQUIPMENT MALFUNCTIONS	REPAIR EQUIPMENT
	CHECK HYDRAULIC LOADING	IMPROVE INLET/OUTLET BAFFLING – REDUCE RAS
	MEASURE TEMPERATURE PROFILES	USE ADDITIONAL AERATION TANKS AND/OR INSTALL BAFFLES



OBSERVATION	ACTION	REMEDY		
POOR SETTLING IN TEST, SUPERNATANT VERY CLEAR	PERFORM MICROSCOPIC EXAMINATION	FILAMENTS	CHECK D.O.	IF D.O. IS LOW – INCREASE AIR TO ACHIEVE DO OF 1 TO 2 MG/L
				IF D.O. LEVELS UNEVEN – ADJUST AIR DISTRIBUTION AND/OR CLEAN DIFFUSERS
			CHECK N, P, AND FE	BOD/N > 100/5 – TRY ADDING N
				BOD/P > 100/1 – TRY ADDING P
				BOD/FE > 100/0.5 – TRY ADDING FE
			CHECK $_{P}$H – IF $_{P}$H < 6.5 – TRY RAISING pH	
			CHLORINATE RAS AT 2–3 LBS/ 1000 LBS MLVSS/DAY	
		NO FILAMENTS DISPERSED FLOC	CHECK F/M RATIO – IF HIGHER THAN USUAL, DECREASE WASTING	
			CHECK D.O. – IF ABOVE 3.0 MG/1, REDUCE AERATION	

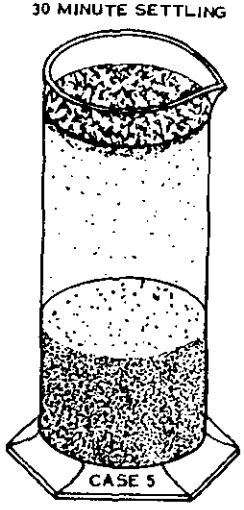
CASE 1 AND CASE 2

Figure 10. (Reference 9)

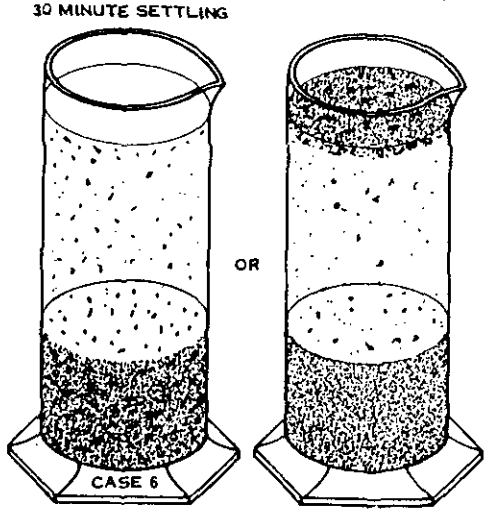


CASE 3 AND CASE 4

Figure 10. (Reference 9)



OBSERVATION	ACTION	REMEDY
ASH ON SURFACE – STIR FLOATING FLOC	RELEASES BUBBLES AND SETTLES – BEGINNING OF DENITRIFICATION	INCREASE RETURN OR INCREASE WASTING
	DOES NOT RELEASE BUBBLES NOR SETTLE – PROBABLY EXCESSIVE GREASE CHECK IF GREASE ABOVE 15% OF MLVSS BY WEIGHT	TRY TO IMPROVE GREASE CAPTURE UPSTREAM GREASE TRAP CLEANING



OBSERVATION	ACTION	REMEDY
PINPOINT FLOC OR STRAGGLERS OBSERVED IN SUPERNATANT	DENSE, COMPACT FLOC	EXTENDED AERATION ZONE–INCREASE WASTING TO MUCH SHEAR–REDUCE AERATION IF POSSIBLE
	LIGHT, FLUFFY FLOC – F/M TOO HIGH, ORGANIC OVERLOAD	REDUCE WASTING

CASE 5 AND CASE 6

Figure 10. (Reference 9)

Appendix B. Care and Use of the Microscope

Appendix B

Microbiology is the science concerned with living organisms too small to be seen with the naked eye; thus the advent of microbiology dates from the invention of the microscope. A simple microscope is little more than a biconvex lens, but a compound microscope employs two separate lens systems, thereby achieving greater magnification.

From the standpoint of construction and operational details, there are many differenct types of compound microscopes, but the principles underlying all these instruments are the same. The microscope is basically an optical system (for magnification) and an illumination system (for rendering the specimen properly visible). To comprehend the operation of the optical and illumination systems you must thoroughly understand the principles of and the relationship between magnification, resolving power, and illumination.

B.1. Magnification.

Magnification in the compound microscope is obtained by a series of two lens systems. The lens system nearest the specimen, called the objective, magnifies the specimen and produces a real image. The ocular or eyepiece lens system magnifies the real image, yielding a virtual image that is seen by the eye. The total magnification is equal to the product of the ocular magnification and the objective magnification.

The objective lens system is a combination of convex and concave lenses of different types of glass that correct for various chromatic and spherical aberrations inherent in a simple convex lens. Microscopes in most bacteriological laboratories are equipped with three objectives: the low-power objective (16 mm), the high-dry objective (4 mm), and the oil-immersion objective (1.8 mm). (The desired objective is rotated into place by means of the revolving nosepieces.) The 16 mm, 4 mm, and 1.8 mm designate the focal length of each objective. As shown in Figure 11, the shorter the focal length of the objective, the shorter is the working distance of the lens, that is, the distance between the specimen and the objective.

The objective lens focuses the light rays from the specimen to form a real image within the body tube. The real image is further magnified by the ocular lens system, which is situated at the top of the draw tube and which comprises two lenses. The lower or field lens places the real image in the focal plane of the upper or eye lens,which serves as a simiple magnifying lens, enabling the eye to focus on the virtual image of the specimen.

The total magnification obtained with a compound microscope is found by multiplying the initial magnification of the objective being employed by the magnification of the ocular. The initial magnification of an objective is engraved on the objective mount, and the magnification of the ocular is usually marked on the top of the ey-lens mount or on the side of eyepiece. The total magnification obtained with the objectives listed above is as follows: the 16 mm objective with a 10x eyepiece gives a total magnification of 100 diameters; the 4 mm objective with a 10x eyepiece gives a total magnification of 440 diameter; the 1.8 mm objective with a 10x eyepiece gives a total magnification of 950 diameters.

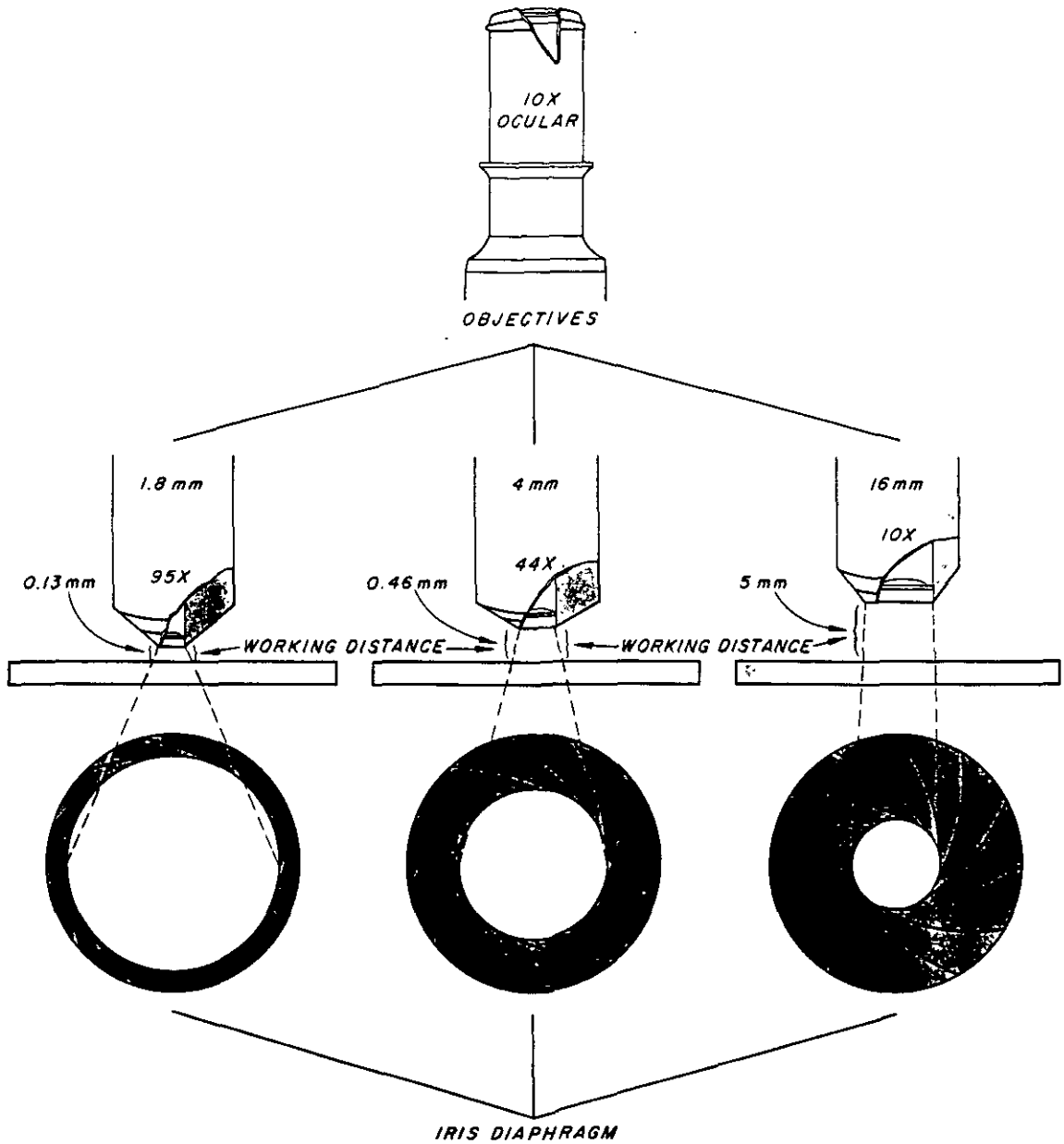


Figure 11. Relationship Between Working Distance of Objective Lens and Adjustment of Iris Diaphragm. The Shorter the Working Distance, the More Open the Disphragm. (Reference 7)

Two adjustment wheels focus the lens systems on the specimen. A coarse adjustdment moves the body tube (or the stage on some models) over a greater vertical distance and brings the specimen into approximate focus, and the fine adjustment moves the body tube more slowly for precise final focusing.

B.2. Resolving Power.

Since the total magnification of the compound microscope is the product of the magnification of two lens systems, we might expect that the total magnification would be indefinitely increased by the use of additional lenses. However, this is not so, owing to a property of lenses called resolving power. The resolving power of a lens is its ability to show two closely adjacent points as distinct and separate. This characteristic of a microscope is a function of the wavelength of the light used and a characteristic of the lens system known as its numerical aperture:

$$\text{Resolving power} = \text{diameter of smallest structure visible} = \frac{\text{wavelength}}{\text{numerical aperture}}$$

Thus, the shorter the wavelength of light used, the smaller the structure visible; for example, blue light will give a greater resolution than will red light. However, since the spectrum of visible light is relatively narrow, increasing the resolution by decreasing the wavlength of the light used is of limited value. The greatest increase in resolution of a light microscope is brought about by increasing the numerical aperture. The numerical aperture is a function of the effective diameter of the objective in relation to its focal length and the light-bending power or refractive index of the medium between the specimen and the objective. Optical factors limit the degree to which the objective may be altered to increase its numerical aperture.

Because the refractive index of air is less than that of glass, light rays are refracted or bent as they pass from the microscope slide into the air. Thus many of the light rays reflected from the specimen are refracted at so great an angle that they completely miss the objective. By interposing immersion oil, which has essentially the same refractive index as glass, betwen the slide and the objective lens, we greatly decrease refraction, and a far greater percentage of the light rays from the specimen pass directly into the objective, resulting in greater resolution and a clear image (Figure 12).

The relationship between the wavelength of light used and the numerical aperture in determining resolving power holds only for parallel light rays. When the specimen is illuminated with oblique rays in addition to direct light rays the relationship becomes

$$\text{Resolving power} = \frac{\text{wavelength}}{2\text{x numerical aperture}}$$

The substage condenser provides both oblique and direct illumination and thus further increases the resolution of a light microscope.

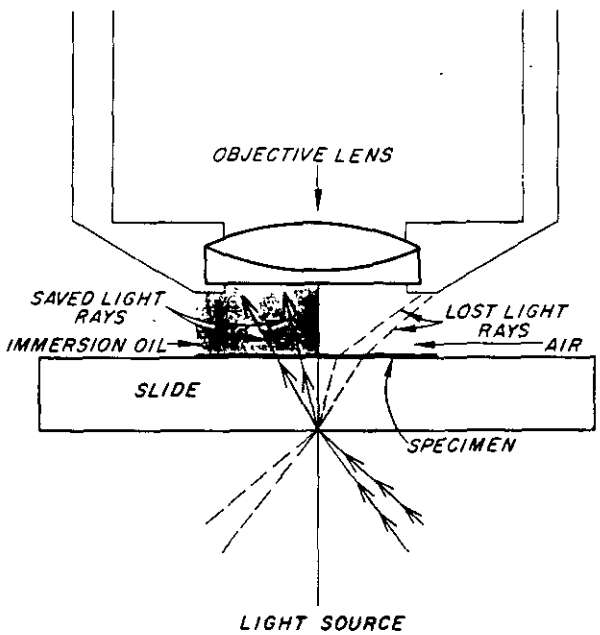


Figure 12. How the Oil-Imersion Objective Increases the Amount of Light Passing From the Specimen Into the Objective Lens. (Reference 7)

B.3. Illumination.

Just as darkness or the glare of direct sunlight can blind vision, poor illumination can obscure the field of view of a microscope. Proper illumination is essential for the efficient utilization of the magnification and resolution of a microscope.

The readiest available source of illumination is ordinary daylight, but since the intensity of daylight varies greatly, artificial light sources (generally a tungsten lamp) are most often used. The most precise of such light sources control the intensity, color, and size of the light beam.

As shown later, the light from the illuminating source passes into the substage condenser. The size of the cone of light passing into a microscope differs with each objective. As the magnification of the objective lens increases, the working distance decreases (Figure 10) and the angle of aperture of the objective increases. Therefore, with increasing magnification a larger cone of light must enter the objective. The size of the light cone is controlled by the iris diaphragm, located just above the substage condenser. When the low-power and and high-dry objectives are used, which magnify 10 and 44 times, respectively, the iris diaphragm is not opened fully since at these magnifications definition and detail is most clear when the light is not too intense. When the oil-immersion objective, which magnifies 95 times, is used, the working distance is the least and the iris diaphragm is opened more (Figure 11).

In phase-contrast microscopy the systems of illumination differs from that just described. This will be described in section 2.c.2.a.5.

B.4. Special Precautions.

To keep the microscope and lens system clean:

1. Never touch the lens. If the lenses become dirty, wipe them gently with lens paper.

2. Never leave a slide on the microscope when it is not in use.

3. Always remove oil from the oil-immersion objective after its use. If by accident oil should get on either of the lower power-objectives, wipe it off immediately with lens paper. If oil becomes dried or hardened on a lens you may remove it with lens paper lightly moistened with xylol. Caution: Too much xylol will dissolve the cement holding the lens.

4. Keep the stage of the microscope clean and dry. If any liquids are spilled, dry the stage with a piece of cheesecloth. If oil should get on the stage of the microscope, moisten a piece of cheesecloth with xylol and clean the stage, and then wipe it dry.

5. Do not tilt the microscope when working with the oil-immersion system. The oil may flow under the mechanical stage system where it will be difficult to remove or it may drip on the substage condenser and harden there.

6. When the microscope is not in use, keep it covered and in the microscope compartment.

To avoid breaking the microscope:

1. Never force the microscope. All adjustments should work freely and easily. If anything does not work correctly, do not attempt to fix it yourself, but call your equipment vendor.

2. Never allow an objective lens to touch the cover glass or the slide.

3. Never lower the body tube with the coarse adjustment while you are looking through the microscope.

4. Never exchange the objectives or oculars of different microscopes, and never under any circumstances remove the front lenses from ojectives.

5. Store your microscope when not in use in its cabinet. Put the low-power objective in the focusing position and be sure the mechanical stage does not extend beyond the edge of the microscope stage.

B.5. The Phase Microscope.

Living bacterial cells are relatively difficult to detect using the common light microscope because of the small amount of contrast between the cells and their aqueous environment. With the conventional microscope staining provides contrast between cells and their environment. A modified light microscope can create contrast without staining, however, and the most commonly used technique for this is phase microscopy.

Although the optical properties of most bacterial cells and their aqueous environment are very similar, slight differences in the light absorbance and optical path of a bacterial cell and its surroundings retard and deviate light waves passing through the cell. These retarded waves are out of phase with the remainder of the light rays but the differences are not large enough to be detectable with the ordinary light microscope. The phase microscope amplifies these phase differences and converts them into differences in light intensity, thereby increasing the contrast between cells and environment.

The phase microscope differs from the conventional light microscope in having an annular diaphragm in the substage condenser and a diffraction plate at the rear focal plane of the objective lens system. As shown in Figure 13 the annular diaphragm, as opposed to the ordinary diaphragm of the light microscope, permits only a ring of light to pass upward through the condenser and the object. As light rays pass through the object (for example, a bacterial cell), some rays are retarded or thrown out of phase with the waves passing through the area surrounding the object. Both the deviated and the undeviated light waves pass as a hollow cone of light into the objective lens system, where they pass through the diffraction plate. This plate is a disc of optical glass upon which has been coated a thin metallic layer to absorb light as well as a layer of a dielectric material

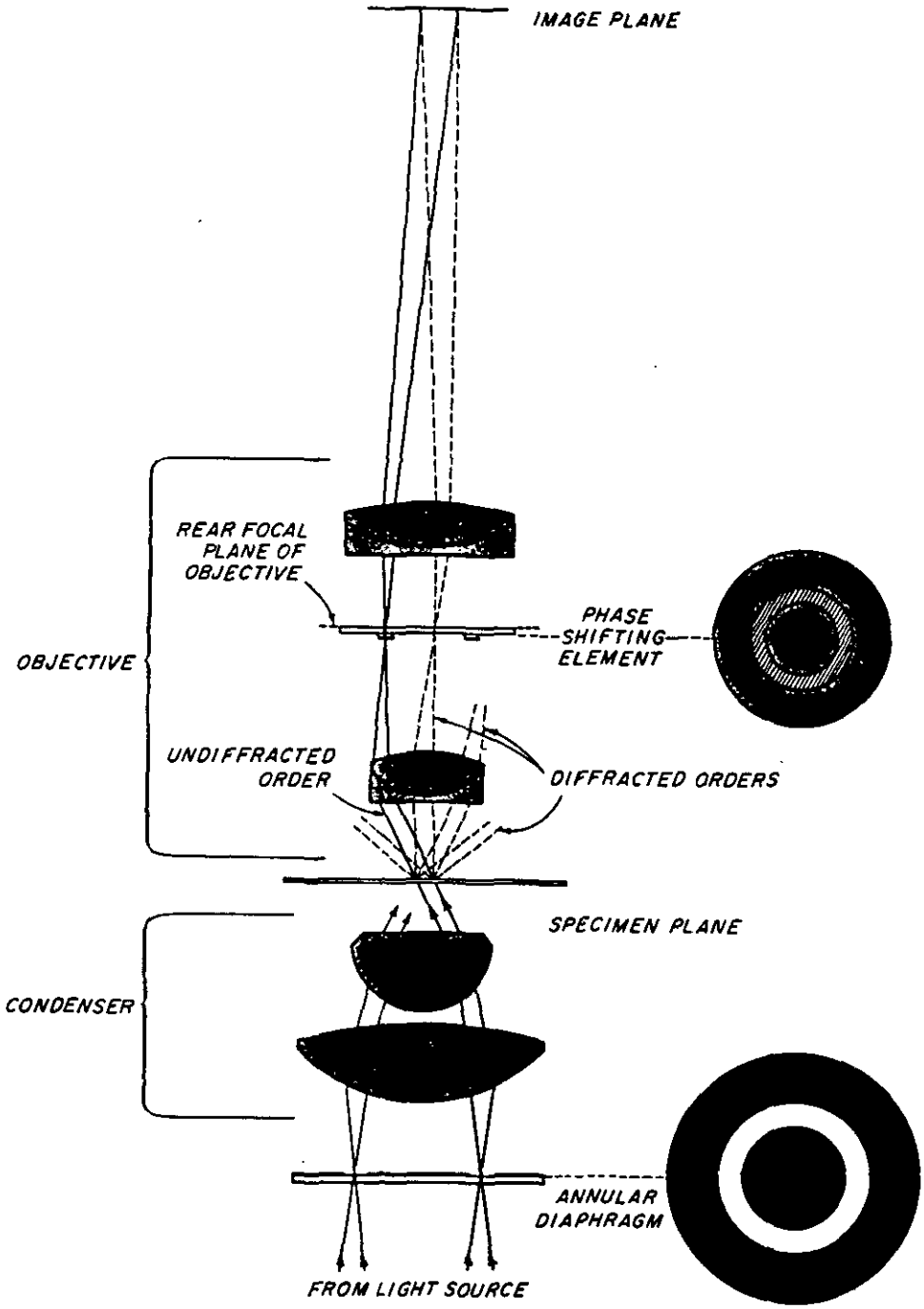


Figure 13. Image Formation By Phase Contrast. (Reference 7)

to retard light. There are two general methods of phase microscopy, bright contrast, in which the object appears as a bright image against a darkened background, and dark contrast, in which the object appears dark in contrast to its lighter surroundings. The location and combinations of light-retarding and light-absorbing material on the diffraction plate determine the type and degree of contrast.

For bright-contrast phase microscopy the light-retarding and light-absorbing material on the diffraction plate is ring-shaped and of proper size to cover the image of the annular diaphragm formed in the objective. In this system undeviated light rays from the object and the background traverse this retarding and absorbing material, whereas deviated light rays pass unimpeded through the uncoated areas of the plate. When the undeviated waves from the object are retarded by the diffraction plate they reach the focal plane of the eye-piece in the same phase as deviated rays from the object. Because these deviated and undeviated waves are "in-phase," they are additive, resulting in an increased light intensity and a bright image. In contrast the light rays of the surroundings are absorbed and retarded, resulting in a dark background.

With dark-contrast phase microscopy, the location of the retarding and absorbing material on the diffraction plate is such that the deviated light rays from the object are retarded. Thus, the phase difference between the deviated and undeviated wave interference at the focal plane of the image is subtractive, and image of the object is dark in contrast to its environment.

B.6. Parts of the compound microscope

In this section the parts of a compound microscope are described. A compound microscope is illustrated in Figure 14.

1. The BASE provides firm support for the microscope.

2. The STAGE supports the slide over a hold that admits light from the mirror or light source. The stage clips hold the slide firmly in the desired position.

3. The ARM supports the body tube and adjustment knobs.

4. The BODY TUBE holds the lenses of the ocular and those of the objectives at the proper working distance from each other.

5. The MIRROR is used to reflect light through the hole in the stage in order to illuminate the specimen. Most microscopes have a built in LIGHT SOURCE eliminating the need for a mirror.

6. The CONDENSER. On most microscopes you will notice a system of lenses directly beneath the opening of the stage. This is the condenser which is used to focus the light from below on to the specimen.

7. The IRIS DIAPHRAGM is attached below the condenser. Using the iris diaphragm control arm, the size of the opening in the iris diaphragm may be increased or decreased to regular the amount of light illuminating the specimen.

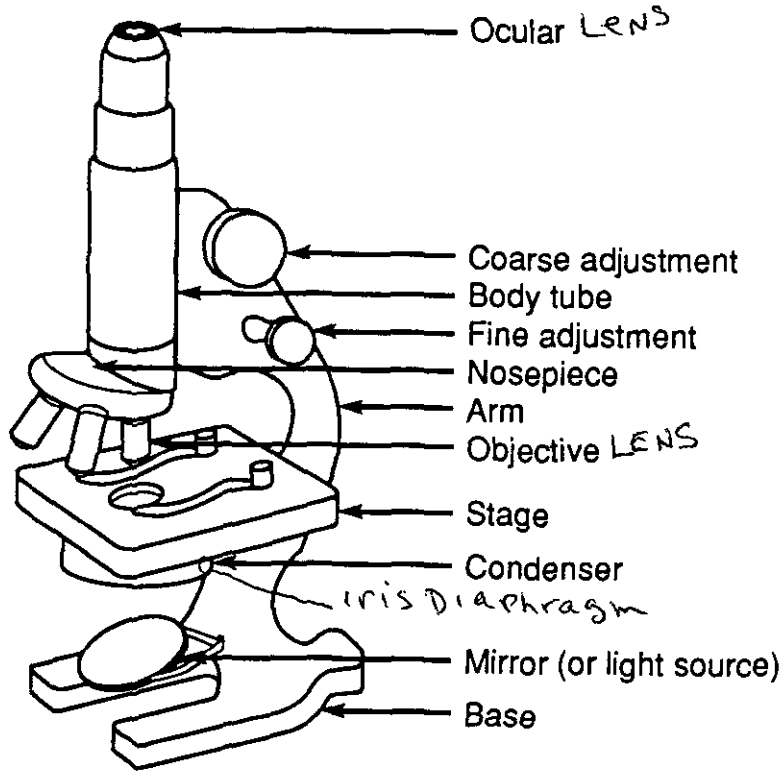


Figure 14. Compound microscope

8. The OBJECTIVES (high and low power) contain lenses of different magnifications. They are attached to a revolving nosepiece which permits the interchange of low- and high-power objectives. Note that the objectives differ in length; the low-power objective is the smaller of the two.

9. The OCULAR is the upper set of lenses which magnify the image formed by the objectives. As you look through the ocular, you may notice a black line extending from an edge to the center of the field. This is used as a pointer and is a hair glued to the inside of the ocular.

10. FOCUSING KNOBS. If you have used a hand magnifying glass, you are aware that an object is in focus only when the lens is held at a specific distance from it. When using a hand lens, this is usually accomplished by moving the lens closer to or farther from the object. On a microscope, the adjustment knobs are used to move the lenses up and down. The coarse adjustment knob is used to move the objective to approximately the desired distance while the fine adjustment knob raises or lowers the objective very slightly permitting exact focusing.

B.7. Procedure for using microscope

a. Illumination. In most cases, a built-in light source is available, so all one needs to do is turn on the light source. For microscopes without such, onemust use the mirror. Illumination by artificial light is superior to daylight for studying fine details. Place the lamp about 1 ft. from the microscope and direct the light beam to strike the mirror. Adjust the light source so that a bright spot is focused on the mirror. Adjust the plane side of the mirror to illuminate the specimen. A piece of ground glass is provided to interpose between the filament and the mirror to control the intensity of illumination.

b. Focusing. Raise the objectives using the coarse adjustment knob. Select a well-stained specimen and place on the stage and center it over the condenser.

   1. Low-Power Objective (x 10): Move the low power objective into position and lower it over the specimen. Focus by raising the objectives with the coarse control and further define the image using the fine controls. Adjust the microscope condenser to its proper vertical position by moving the condenser so that the ground glass is in focus with the specimen and lower it slightly. Control the intensity of illumination using the iris diaphragm. After completing the above steps remove an eyepiece and look down the draw tube. A uniform circle of light will be seen when the light source, mirror, and condenser have been adjusted properly

      The iris diaphragm, when manipulated, should be sharply defined. If unevenly illuminated, adjust the mirror for full illumination. Replace the eyepiece.

Binocular eyepieces are adjustable to fit the observer's eyespacing. Persons wearing lasses should be careful to prevent their lenses or the top lenses of the eyepiece from becoming scratched.

2. Medium-Power (High-Dry) Objective (x 43): Unless the objectives are parfocal (i.e., when the nosepiece is rotated to the next object the specimen remains in focus), raise the objectives about 1 cm. using the coarse focus and rotate the nosepiece until the objective clicks into place. Even with parfocal microscopes, thick specimen slides and individual differences in observers may require that the objectives be raised upon changing them.

   Next place the eye on a level with the top of the specimen slide and lower the objective slowly using the coarse adjustment until it is only just clear of the slide. Do not touch the slide with the objective. Looking through the eyepiece, focus upwards slowly until the objective is in focus. Complete the focusing with the fine adjustment. If the specimen is not in focus (the upward movement was too rapid) repeat the initial steps. Do not ever try to bring the specimen into focus by focusing downwards, when you are looking through the eyepiece.

3. Oil-Immersion Objective (x 97): The procedures in focusing a specimen using the oil immersion objective are similar to those employed with the Ihg-Dry objective except that a drop of oil is placed between the top of the specimen and bottom lens to increase the resolution. First, raise the objective until it is about 1 cm. above the slide. Rotate the nosepiece to bring the oil immersion objective into position. Add a small drop of immersion oil to the center of the specimen slide. Place your eye level with the top of the specimen slide and lower the objective carefully until it makes contact with the oil. The working distance of oil immersion objectives is extremely small so that initial attempts at focusing may be difficult. Lower the objective further without touching the slide. Looking through the eyepiece, focus upwards with the fine adjustment. If the oil film breaks or if the objective is raised more than 2-3 mm. above the slide without finding the specimen, repeat the initial steps. Adjust the mirror and iris diaphragm for optimum lighting when the specimen has been located and focused.

B.8. Calibration of the Microscope.

Measurement of microscopic objects can be performed using an ocular micrometer. The ocular micrometer is a glass disc marked by a scale of etched lines. It is inserted into the eyepiece after unscrewing the top lens. Eyepieces permanently provided with a scale for measurement can be obtained and used also for this purpose. These are, however, undesirable, as the scale in an eyepiece interferes with routine observations.

Calibrate the ocular scale as follows: Place a stage micrometer on the microscope and focus on it, starting with the low power objective. Calibrate each objective separately. The scale of the stage micrometer is generally 2 mm. long. This distance is divided into 20 parts and the distance between these lines is 100 μm. The first two divisions on the stage micrometer are divided into 20 segments each being 10 μm. To calibrate the ocular micrometer, find the number of ocular micrometer lines which correspond most closely to a number of lines on the stage micrometer; for example, it may be found that 19 divisions of the ocular micrometer correspondto 3 divisions of the stage micrometer. Each division on this part of the stage micrometer represents 100 μm; so 24 ocular divisions make 300 μm and each ocular division must equal 12.5 μm. With the high magnification objectives, the etched stage micrometer lines appear so thick that it is essential to use only one edge of these lines in setting the ocular micrometer scale.

In calibrating each lens combination, use the average of three independent measurements and carry out the calibration to the first decimal place.

An ocular scale so calibrated can be used directly in measurement. An organism on the stage may, for example, cover 10 ocular micrometer lines. Using the calibration described above, the distance between two lines is 12.5 μm and the length of the specimen would be 125 μm.

B.9. Cleaning.

Lens paper is to be used for cleaning the eyepiece and objective lens. Other than removing the eyepiece for the initial illumination adjustment, no dismantling of the microscope body and lenses is needed.

At the beginning of each time in which the microscope is to be used, wipe the lenses with lens paper. The best procedure is initially to softly brush the lens surface with the paper to remove any large particles which might scratch the lens. Next wipe off any accumulated oil. After each period of use wipe off the lenses in the same way. Xylene may be used to remove dried immersion oil from the 97x objective as well as any spills on the micxroscope stage. However, xylene should be used only sparingly because it may dissolve adhesives used to hold objective lens in place.

B.10. Storage.

After cleaning the microscope, swing the low power objective into position and lower the objectives. Cover the microscope and return it to a proper storage compartment.

B.11. Maintenance.

It will be necessary to have your microscope professionally serviced as recommended by the manufacturer in order to keep it in good operating condition. This should be performed at least once per year.

Appendix C. Modified Identification Procedure and Notes

1. MODIFIED IDENTIFICATION PROCEDURE

I. Make Stains, Start S-Test

II. A. **FLOCS---SEE PAGE C-2**

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, SHAPE, COLOR, INCLUSIONS

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

FILAMENTOUS ORGANISM IDENTIFICATION SHEET

WWTP_____

PROCESS DESCRIPTION/SVI_____

_____

REMARKS/PROBLEM_____

STORAGE

SAMPLE DATE_____ _____ OBSERVATION DATE_____

****

1--100X NO STAIN

PROTOZOA, ETC._____

INORG/ORG PARTICLES_____

AMORPHOUS?_____ INDIA INK

FLOC MORPHOLOGY(FIG 12, 6)

ROUND, COMPACT____

IRREG, DIFFUSE____

FIRM____WEAK____

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 FILAMENTS THAN FLOCS)

COMMENTS/COMPARES TO_____

3--100X CV WET

FLOC DIAMETER

SCALE	um	
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_____ <150um 150-500um >500um

SMALL MEDIUM LARGE

****

DESCRIPTION OF DOMINANT FILAMENTS

_____

_____

_____

_____

SKETCHES AND OBSERVATIONS

FILAMENT NUMBER_____

FILAMENT NUMBER_____

ORGANISM CHARACTERISTIC CHECKLIST

_____ RELATIVE ABUNANCE
_____ MOTILITY
_____ LOCATION
_____ GROUPING OF FILAMENTS
_____ FILAMENT L, W, SHAPE
_____ CELL SEPTA
_____ ATTACHED GROWTH
_____ BRANCHING
_____ ROSETTES, GONIDIA
_____ SHEATH (CRYSTAL VIOLET)
_____ GRAM STAIN
_____ PHB STAIN
_____ NEISSER STAIN
_____ SULFUR GRANULES, IN SITU?
_____ SULFUR TEST
_____ SKETCHES
_____ CHECK LESS COMMON DESCRIPTIONS

2. NOTES ON JENKINS ET AL. (REFERENCE 4) I.D. PROCEDURE

A. STAINING PROCEDURES

1. Neisser Stain

- Better results staining 1-minute rather than 30-seconds with solution 1.

2. Suffler Oxidation Test (tested on Thiothrix and Type 021N)

- Test A does not seem to work. We used hydrated sodium sulfide (Na_2S 9 H_2O).

- 10-20 minute time may be too short

- Test B negative results, floc structure broken up after overnight shaking, some larger flocs. Rovettes broken up. Overall, floc morphology significantly different than initial sample.

- Test C Farquhar & Boyle, identification of filamentous microorganisms,in activated sludge

Sulfur Deposition Test

- good results in about 1 hr (check periodically, may be bluish granules, use 1000x phase)

3. India Ink Reverse Stain

- Can't use suspension of pulverized carbon black; use commercial, water proof "India Ink" pen ink by Higgins or other.

4. Polyhydroxybutyrate (PHB) Stain

- Better to examine under 1000x phase contrast than transmitted light.

- PHB granules may be more like globules, sometimes occupying a large portion of the filament.

5. Crystal Violet Sheath Stain

- Use dry mount stain to observe filament abundance.
- Wet mount for sheath observation.

B. IDENTIFICATION PROCEDURE

1. Stress transport and storage - pg. 24

2. Use Paskur pipette, don't blot dry slide for 100x, since it will dry out fast otherwise.

3. Difficult to assess the number of different types of filaments present at 100x; 400x much better.

4. See modified I.D. procedures

   - Seems to be a more logical progression of observations and procedures.

   - Step 2 - some observation repeated at different powers since it is not always easy to ascertain some characteristics at one particular magnification and circumstance.

5. Key and flow chart are generally "average" or "typical characteristics".

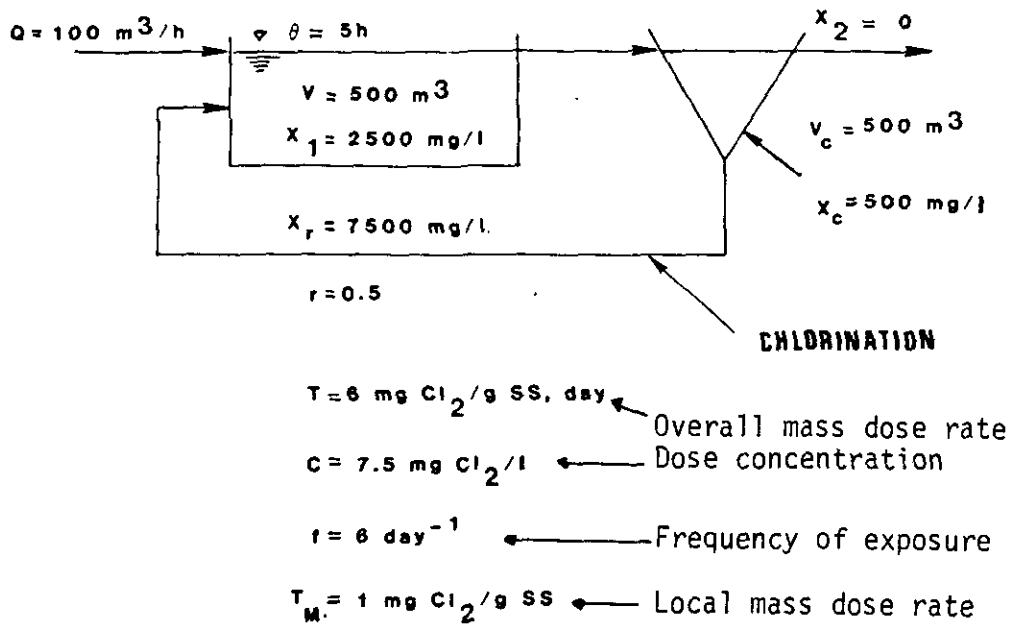
   - Should check text descriptions and descriptions by others for variations of characteristics observed by others.

6. Crystal violet or methylene blue good to observe filament abundance, effects

7. Should sketch observations for filaments.

Appendix D. Chlorination Parameters

a. "DOMESTIC WASTE" CASE



b. "INDUSTRIAL WASTE" (LONG AERATION TIME) CASE

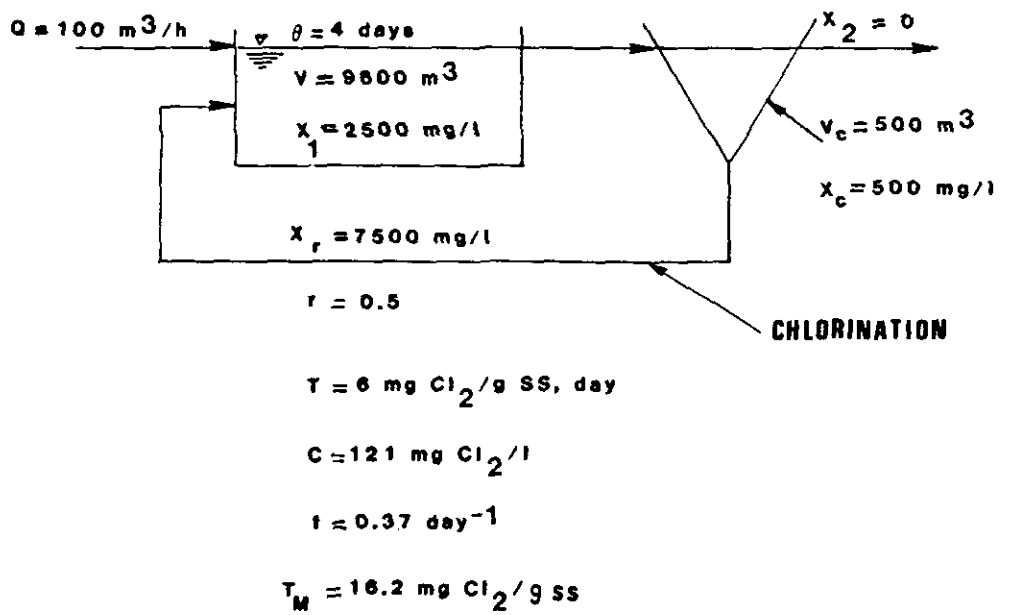


Figure 15. Chlorination parameters for (a) a typical domestic waste treatment plant and (b) a long aeration time treatment plant (Reference 4)