Factors Affecting Slime Accumulation in Fiberboard Mill Process Water

by

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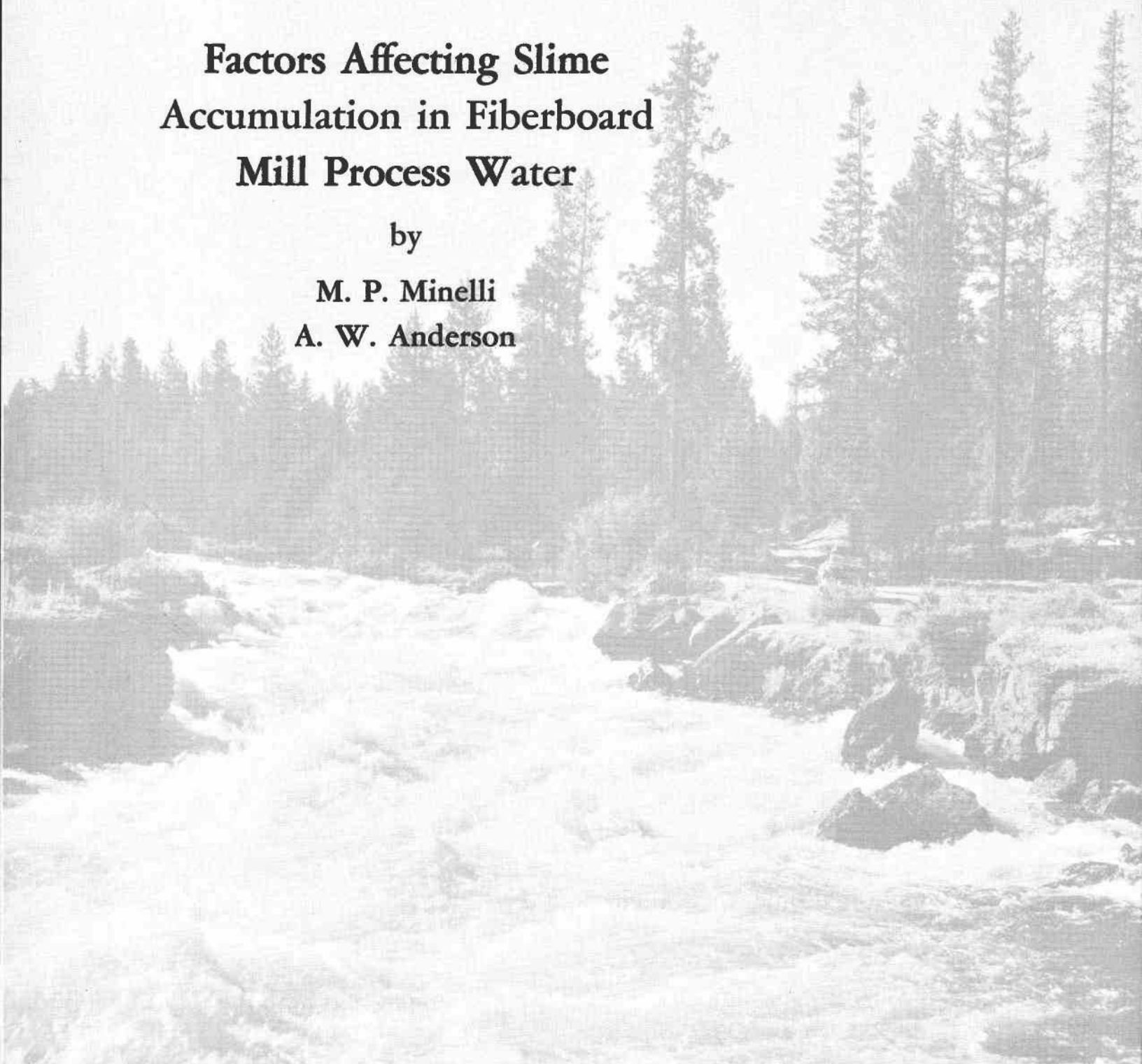

Water Resources Research Institute
Oregon State University
Corvallis, Oregon

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Completion Report

"Factors Affecting Slime Accumulation in Fiberboard Mill Process Water"

Funded by
Office of Water Research and Technology
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Department of Microbiology and
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Oregon State University
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A. RESEARCH PROJECT ACCOMPLISHMENT

The problem of filamentous bulking in the plant studied appears to be amenable to engineering design and a better understanding of the biological sequences occurring in a high sugar effluent. The use of coagulants, salts, chlorination, hydrogen peroxide and nutrient balance adjustments are aimed at treating the problem symtomatically. Their application involves a great deal of time and expense and yields only temporary relief.

The information we have collected was an attempt to characterize the nature of the wastewater and identify the source of the problem by simulating the plant treatment conditions and altering the physical and biological parameters to affect a positive change.

The character of the water was summarized as follows:

1. an adequate BOD removal for re-use process water was observed
2. sufficient nitrogen and other nutrients for microbial growth was observed
3. high carbohydrate concentrations was present
4. the pH for an activated sludge system was too low.

The cause of the bulking was G. candidum which was indentified microscopically as well as by the common identification techniques. The increased stability of the carbohydrates as analyzed by gas liquid

chromatography, was due to polymerization of free wood sugars beginning in the primary settling pond and completed in the aeration basin. The combination of a predominate filamentous microbial population and increased wastewater viscosity resulted in reduced clarifier efficiency or bulking.

Plant treatment was simulated in seven bench scale reactors varying in size from 500 ml to 10,000 ml. Each unit simulated: flow rate, retention time, nutrient additions, temperature, dissolved oxygen and pH. The following list summarizes the attempts to reduce the filamentous population and/or to promote coagulation for increased residue removal.

Parameter Tested	Result
1. Less than 1.0 mg/l D.O.	Increased slime production and turbidity. No settling.
2. Increase pH from 4-7 (4-6 mg/l D.O.)	Improved residue removal and lower total carbons. Poor settling.
3. Addition H_2O_2 (50-200 mg/l)	Filamentous population tripled.
4. $NaNO_3$ as nitrogen source	Slight decreases in filamentous population and total carbon. Increased total residue. Highly turbid.
5. Direct aeration of plant process water	Nonfilamentous, well balanced microbial population. Good settling and residue removal.

We conclude that the stability of the carbohydrates was increased as a result of free wood sugar polymerization. These biologically inert polymers increased the water viscosity and thereby contributed to the suspension of the predominately filamentous microbial population. Increasing the pH to about 7.0 and eliminating the primary settling pond by rapid screening, followed by direct aeration, should limit the filamentous flora and allow the free wood sugars to be transformed to carbon dioxide and water under well oxygenated conditions.

B. Publications

Title: Factors Affecting Slime Accumulation in Fiberboard Mill Process Water

Author: Michael Philip Minelli

Type of Manuscript: Thesis, M.S. Degree
Copy enclosed

C. Project status

Completed as of June 30, 1975. Report indicated under A and B above.

D. Application of Results:

This project was done with the cooperation of Mr. Keith Kruse of Forest Fiber Products Co., P. O. Box 68, Forest Grove, Oregon 97116. It is our understanding that he will seriously consider applying our research finding to the Forest Fiber plant in an attempt to solve their slime problem. We will cooperate with them during the tests.

E. Work Remaining:

The research has been completed. Putting our finding into practice is in progress as indicated above.

AN ABSTRACT OF THE THESIS OF

MICHAEL PHILIP MINELLI for the degree MASTER OF SCIENCE
(Name) (Degree)

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The objectives for this study were to determine the causes of slime formation in activated sludge treatment and to recommend possible control measures. The primary problem was a seasonal filamentous bulking which was caused by the mold, Geotrichum candidum. During the winter, mycelial fibers appeared and formed a free floating mesh which harbored a community of bacteria, yeasts and rotifers. The highly encapsulated mycelial filaments were assumed to utilize the free wood sugars to form its capsular polymer. The biological transformation of free wood sugars into polymerized slime could be followed through the treatment steps by gas-liquid chromatography.

The research data indicated that by by-passing the primary settling basin, thus avoiding the anaerobic degradation of the free wood sugars, the formation of the troublesome polysaccharides in

the aeration pond could be avoided. After a rapid screening to remove the particulate matter, direct aeration at a pH of approximately 7.0 allowed a well balanced, nonfilamentous, microbial population to predominate.

Factors Affecting Slime Accumulation in Fiberboard Mill Process Water

by

Michael Philip Minelli

A THESIS

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Date thesis is presented June 18, 1975

Typed by Clover Redfern for Michael Philip Minelli

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The process used at this mill is similar to that of most fiberboard production operations. High pressure steam removes the water soluable material from the wood leaving a fibrous skeleton which is placed under high pressures with a minimum of bonding material to form the fiberboard.

The predominate constituent of the wastewater was the soluable carbohydrates. Since the usual constituent of microbial slime is a polysaccharide complex, it seemed reasonable to assume the monomeric wood sugars were being polymerized. This would account for the slime accumulation and, in part, the large COD to BOD ratio due to the biologically resistant polysaccharides.

Assay of the free and polymerized sugars in each step of the treatment process was by gas-liquid chromatography. Results identified the primary settling basin as the site of free sugar disappearance to the anaerobic and facultative microorganism. The predominately filamentous microbial flora, which were heavily encapsulated, increased the stability of the water thus holding the microbial population in suspension with the dissolved residue.

In the laboratory we eliminated the anaerobic primary settling pond by rapidly screening the plant effluent followed directly by aeration basin treatment. In this manner, the wood sugars were not polymerized and the free sugars were easily removed by an excellent settling aerobic microbial population. Adjustment of the pH to

neutrality was also necessary to encourage the more expedient bacterial population to limit the filamentous organisms. Microscopic examination and plate count techniques verified the well balanced character of the sludge.

LITERATURE REVIEW

Introduction

The accumulation of slime in waste water is a common problem in the activated sludge and trickling filter treatment. The scope of the problem appears to be as diverse as the kinds of micro-organisms involved as well as the specific conditions under which they exist.

The Slime Matrix Community

"Sewage fungus," abwasser-pilz (32), heterotrophic biocoenosis (36), and "slime infestation" (15) are all names used to describe the slime matrix. This matrix, developed by the micro-organisms, forms a gelatin-like substance often referred to as zoogloea. To the microbiologist zoogloea refers to a specific genus of bacteria as well as the growth products produced. Anderson and McCoy (2) isolated a Zoogloea organism and found that matrix to be a polysaccharide which contained the monosaccharide components of a pentose and a hexosamine. Tezuka (31) has isolated a Zoogloea bacterium and has identified its gelatinous mucopolysaccharide matrix as primarily two amino sugars N-acetyl glucosamine and N-acetyl fucosamine. This polymer was quite resistant to water and soil microbial degradation. In contrast to the beliefs of many, Obayashi and Gaudy (23) demonstrated the oxidation of extracellular microbial polysaccharides

through extended treatment. However the ease of digestion will vary according to the varying chemical composition of the matrix, the microbial population present and the conditions under which they exist.

According to Cooke and Hirsch (5) and others, the matrix of filamentous organisms forms a community containing other nonfilamentous bacteria, protozoa, yeasts, algae and diatoms. Although nematodes and rotifers are also found on the periphery of the matrix they are possibly feeding off the slime. Ingrams et al. (17) describes several species of snails that occur in various wastewater treatment facilities which utilize slime as a food. They represent "crawling BOD removed."

Historical Filamentous Studies

The following references cited were chosen to describe phenomena which was relevant to slime production and filamentous bulking by fungi.

Historically text books dealing with water pollution microbiology have given little recognition to the role of fungi in their natural habitat or their importance in waste water treatment processes. Therefore, the following periodicals give a partial list of sources which were used as a background.

As early as 1906 in Waterbury, Connecticut, Rettger (25) studied growths on filters that changed from red to black. After washing most

of the bacteria away, mold colonies developed when innoculated on gelatin. The organisms referred to were later identified as Geotrichum candidum and the red color was attributed to Fusarium aquaeductuum. In 1914, Johnson (18) in England observed and reported on bacteria and seven fungi on trickling filters and chose five fungi for special studies. They found the fungal dominance changes depending upon the season from Penicillium sp. in the late summer to Geotrichum sp. in the winter and an unidentified fungus is mid spring. Other fungi mentioned were Pythium and Dictyuchus. Rudolphs and Trajkovich (27) studied the distribution of fungi in filter beds. They found among the fungi studied that the population increased as the temperature of the effluent decreased. Ingols and Heukelekian (16) could induce bulking by adding carbohydrates and subsequently could retard its action by the addition of urea. A ratio of 8:1 was determined to be the optimum carbon to nitrogen requirements for settling. They concluded that a highly nitrified sludge would bulk less in carbohydrate rich waste water. Lackey and Wattie (21) summarized previous work on the control of Sphaerotilus natans relevant to bulking of activated sludge. They found that bulking was greatest in the presence of excess monosaccharides and disaccharides. In addition, they found the dye malachite green to be specifically inhibitory for Sphaerotilus; however, due to its cost, they recommended chlorination of returned sludge for most treatment plants. Reynoldson

(26) correlated the growth of Oospora (Geotrichum) and the fly Psychoda with a seasonal pattern. The fungi blanket was thickest in the second of a double filtration filter during winter and spring months and decreased when the feeding fly population was greatest in the summer. In 1946, Pomeroy and Bowlus (24) showed that some attempts to control slimes resulted in excess sulfide production.

Recent Bulking Studies

Cooke (6) lists the most prevalent species of sewage fungi reported by various workers in the period of 1901-1953. They are: Leptomitus lacteus, Geotrichum candidum, Sepedonium sp., Fusarium aqua eductuum, Penicillium sp., Sphaerotilus natans, Beggiatoa alba, and Zoogloea ramigera. In 1957, Cooke identified and described the nine most common sewage fungi and characterized each according to morphological and physiological characteristics. He also presented information pertaining to the preference of these fungi to carbohydrate carbon sources over protein, keratin, some fats and organic acids. According to his studies the fungi are unable to fix atmospheric nitrogen and some are unable to utilize potassium and sodium nitrate. Curtis (10) has reviewed the literature dealing with sewage fungus. He used the term sewage fungus to include bacteria, fungi and protozoa which bind the slime community together. A survey that he conducted in the United Kingdom for the Water

Pollution Research Laboratory, showed that the following organisms were most common: bacteria; S. natans, Zoogloea sp., Flavobacterium sp., Beggiatoa alba; fungi; Geotrichum candidum, Leptomitus lacteus; protozoa; Carchesium polypinum; algae; Stigeoclonium tenue.

The condition of bulking has been a problem in water treatment since the beginning of its existence. Although several remedies have been prescribed, none of them function without severe limitations. Smith and Purdy (29) suggested in 1939, that several different filamentous organisms may cause bulking. Since this fact is now well established, Farquhar and Boyle (12) felt the key is to correctly identify the causative micro-organisms and apply measures specifically suitable for their control. They presented an identification key for 19 common bulking organisms. Specific methods are described for identifying these organisms in mixed cultures. Sladka et al. (28) have made similar contributions toward correctly classifying filamentous bacteria, algae, and fungi. This approach should enable different physiological efficiencies to be exposed.

Frequently, bulking and Sphaerotilus natans are used synonomously. Since S. natans refers only to a sheathed bacterium, its control may require specific inhibitors directed toward its way of life. Confusion between S. natans and other filamentous organisms is commonly found in the literature. The mold Geotrichum candidum has received 52 different names and has been referred to as

Sphaerotilus. There is also confusion about the genus Zoogloea and the species ramigera. Since it was proposed in 1867 all the original type strains have been lost. Crabtree and McCoy (9) proposed a new strain as a neotype. Since that time several investigations have produced a number of Zoogloea strains biochemically similar but morphologically unlike the proposed neotype (13, 34).

Until the filamentous organism's physiological and metabolic biochemistry is more completely elucidated, the "shotgun" approach to improve the settling quality of bulked sludge will persist. To date, the addition of Fe^{+3} and Al^{+3} salts as well as nonionic, ionic, and cationic polymers, chlorination, hydrogen peroxide and nutrient balance adjustments appear to be the only corrective measures commonly employed.

METHODS AND MATERIALS

Collection and Treatment of Samples

Each week 20 liter samples were collected directly from the effluent flow at the primary settling pond and once very 50 days from all stages of treatment. Time of collection, plant treatment, pH, and ambient pond temperatures were recorded. These samples were subjected to the following analysis:

1. Total carbon
2. Total reducing sugars
3. Nitrogen as ammonia and organic
4. Free and polymerized monomeric sugar analysis
5. Total and nonfilterable residues
6. Settling
7. Microscopic analysis and plating

In addition to properly characterizing the wastewater, the weekly 20 liter samples were used in the bench scale treatment units. Subsequently, untreated plant effluent was used in lieu of primary settling basin effluent. This change came about after recognizing the increased stability of the primary settling through anaerobic digestion.

Bench Scale Treatment Units

Bench scale treatment progressed from four 50 ml Belco spin flasks (Bellco Glass Co., Vineland, New Jersey) to four 2,000 ml suction flasks to one 10,000 ml laboratory fermentor, model MF-114 (New Brunswick Scientific Company Inc., New Brunswick, New Jersey) (Figures 1 and 2). Each unit simulated flow rate and retention time equivalent to that used at the fiberboard mill. Nutrients required were added as ammonium chloride and sodium phosphate yielding concentrations equivalent to that of the plant.

Effluents were pumped from refrigerated storage by four individually controlled electrolysis pumps in the 500 ml and 2000 ml units. Temperature approximating those common during the winter months was held constant at 14°C ± 1.0°C. The pH was then characteristic of the effluent as adjusted at the mill except when designated as an experimental parameter. Dissolved oxygen was maintained at a constant level of 2 to 4 mg/liter and monitored using a lead silver oxygen probe (Rustrak Miniature Chart Recorder, Rustrak Instrument Company, Inc., Manchester, New Hampshire). The oxygen probes were made according to the directions of Johnson et al. (19).

Microbiological Analysis

Wet mount preparations were made from bottom sludge and centrifuged wastewater. The results indicated an overwhelming dominance of filamentous fungi. The following staining procedures were employed to reveal more details of the fungal structure: Gram stain, sudan black B (fat vacuoles), Loefflers methylene blue with malachite green (endospores), Manevals capsule stain.

The following broths and medias were used to explore the pleomorphic character of Geotrichum candidum: acidified Sabouraud dextrose agar, dextrose peptone broth, nutrient broth, lactose broth, 1% tryptic soy agar, plate count agar, malt extract agar, acidified potatoe dextrose agar.

Colony counts for the mold, G. candidum, were made on media prepared from the effluent. Its preparation involved:

1. Filtered effluent, 1,000 ml.
2. Dehydration on steam to a syrup consistency.
3. Rehydrate with 500 ml distilled water and filter.
4. Add 70 mg of ammonium chloride.
5. Add 10 grams bacto agar.
6. Adjust pH to 3.0 with H_2SO_4.
7. Autoclave 15 minutes at 121°C at 15 psi pressure.

This medium inhibited all bacteria and most molds and yeasts in the wastewater studied. The colony morphology of G. candidum was clearly distinguishable from other contaminants. Correlation of colony counts with Sabouraud and plate count agar were satisfactory.

Settling

The methods used to accurately determine the settleability of the sludge were settled volume, sludge volume index, and sludge density index. Sixty minutes of settling time was allowed in the settled volume while 30 minutes settling for the sludge volume index (30).

Nitrogen

Nitrogen as ammonia was determined by preliminary distillation followed by standard acid titration. Organic nitrogen was determined by the classical Kjeldahl method. The procedure as outlined by Standard Methods (30) was followed.

Residue

Total residues on evaporation were determined for 30 ml samples in an ignited dish and were evaporated over steam. The aliquots were then dried at 103° C for 1 hour. The results were applied to the sludge volume index determinations (30).

Total Reducing Sugars

The colorimetric method for the determination of sugars by Dubois et al. (11) was followed. Reagents and apparatus used are as follows:

1. H_2SO_4 reagent grade 95.5%, Specific Gravity 1.84.
2. Phenol 80% in distilled water.
3. Standards from sucrose in distilled water (10 μg/ml to 60 μg/ml).
4. Bausch and Lomb Spectronic 20 spectophotometer.

Procedure:

1. 2 ml of sample.
2. .05 ml of 80% phenol.
3. 5 ml of Conc. H_2SO_4.
4. Mix and let stand at room temperature for 10 minutes.
5. Read at 490 mu.
6. Determine concentration from standard curve.

Sugar Chromatography

Silylation Method

Approximately 50 mg of lypholized wasted material and 1.0 ml dimenthylformamide, hexamethyldisilazane, trimethylchlorosilane mixture obtained from Applied Science Laboratories, Inc., State

College, Pennyslvania, were introduced into a plastic stoppered vial and shaken vigorously for about 30 seconds. The mixture was allowed to stand for at least 5 minutes; one or two microliter quantities were then injected into the gas chromatograph. The column consisted of 1% XE 60 on Ankrom ABS stainless steel 1/8 inch x 6 ft.

Alditol Acetate Derivatives (Modified Method of Albersheim et al. (31))

1. Precipitate the $SO_4^=$ from 70 ml of wastewater by adding approximately 1.5 g of $BaCl_2$. Mix for 5 minutes then centrifuge at 5,000 rpm for 5 minutes, and collect the clear supernatant. Sprinkle a small amount of $BaCl_2$ to check for complete $SO_4^=$ removal.
2. Decant 50 ml of clear supernatant and add 1 ml of inositol internal standard (10 mg/ml std.). Adjust the pH to 6 with a saturated solution of $Ba(OH)_2$.
3. Add about 0.2 grams of $NaBH_4$, mix, and allow to stand for 30 minutes at room temperature (~ 25°C).
4. Add glacial acetic acid dropwise until gas evolution ceases.
5. Remove boric acid by evaporation in a rotary evaporator to dryness or near dryness. Do not exceed 70°C. Wash 3 times with 10 ml of absolute ethanol and evaporate to dryness each time. Scrape off the dried residues and sugars from the

sides of the round bottom flask.

6. Add 10 ml of acetic anhydride and 0.5 g of sodium acetate and reflux for 20 minutes. (Do not exceed 130° C.) Rotate round bottom flask to bring refluxing material into contact with the residues on the side walls.
7. Add 10 ml of dichloromethane, centrifuge for 5 minutes at 5,000 rpm and evaporate to desired concentration.
8. Inject into chromatograph.

Polymerized Sugar Chromatography Procedure

1. Centrifuge 75 ml of wastewater at about 5,000 rpm for 5 minutes.
2. Measure exactly 50 ml of supernatant and precipitate the slime with 100 ml of ethanol (95%) or N-propyl alcohol.
3. After a precipitate has formed, centrifuge at 5,000 rpm for 10 minutes.
4. Pour off the supernatant and add about 2 ml of distilled water to the slime and resuspend. Add about 100 ml of alcohol and precipitate once again. Centrifuge and collect the pellet as before. Repeat twice more to assure adequate cleaning.
5. Suspend the slime in 50 ml of distilled water and add 2.5 ml of 72% H_2SO_4.

6. Autoclave briefly to hydrolyze the slime polymer (10 minutes at 120°C at 15 psi).
7. Complete sample preparation beginning at step 1 under free sugar chromatography--alditol acetate preparation.

Column preparation: One hundred mg of ethylene glycol succinate (EGS) were dissolved in 25 ml of chloroform and 100 mg ethylene glycol adipate (EGA) and 200 mg XF 1150 were dissolved in 25 ml of acetone. The above mixtures were poured together quickly, mixed with 10 gm of gas chrom P, and stirred gently occasionally. After 0.5 hour, it was poured into a fritted funnel and excess liquid allowed to drain off. The mixture was poured into a large petri dish and dried. A 4' x 1/8" stainless steel column was filled by vibration and equilibrated in the GC for about 2-6 hr at 188°C before used. It was advisable to flow a low level of helium gas through the column during equilibration (6 to 10 lbs). All analysis were made using a F & M High Efficiency Gas Chromatograph, Model 402, with a Honeywell Strip Chart Recorder, Electronik 16, and a Hewlett Packard 3370A Integrator. The column was run isothermally at 188°C. Injection port and detector temperatures were 215°C and 210°C respectively. The approximate gas flow rates were as follows: helium 70 ml/min, and air 230 ml/min. Two µl was the standard volume injected.

Total Carbon

Total carbons were determined on each stage of plant treatment. This method was employed to monitor the pollutional strength of the water. A Lindberg combustion tube furnace, model CF-2R (Lindberg Engineering Co., Chicago, Ill.) was modified to accept 20 µl liquid samples. The sample was ignited at 1150° C which formed CO_2 from all carbonaceous material. A Lira Infrared Analyzer, Model 300 (Mine Safety Appliances Company, Pittsburgh, Pa.) monitored CO_2 concentrations, and the results were recorded on a Heath Servo-Recorder, Model EU-20B (Heath Company, Benton Harbor, Mich.). Standard samples were prepared from primary standard benzoic acid within a range of 250 mg/l to 1,000 mg/l carbon.

RESULTS AND DISCUSSION

The plant selected for this study was located at Forest Grove, Oregon. Their highly stabilized, slime plagued wastewater was representative of activated sludge treatment in many fiberboard mills. The wastewater from the process is pumped to a primary settling basin at an average daily flow of 220,000 gpd. The temperature was 43° C and contained approximately 13,000 aerobic or facultative microorganisms per ml. These organism were largely non-filamentous, borderline mesophiles of Gram positive coci, Gram negative rod, and budding yeast character. The primary settling pond holds 0.5 million gallons of process water and contains approximately 9000 facultative organisms/ml. During the winter months centrifuged wastewater examined by wet mounts revealed a large population of filamentous organisms. Aeration is supplied by 5 surface aerators to a basin capacity of 2.1 million gallons and maintains 4 to 6 mg/l of dissolved oxygen. The average colony count was 60,000 organisms/ml and the population resembles that of the primary settling pond. The clarifier has a 9600 gallon capacity with a retention time of about 1 hour. Its effluent contained approximately 315,000 organisms/ml which consisted largely of floating filamentous communities.

Twenty percent of the input flow became excess bottom sludge and was pumped to an adjacent field. The clarified water passes

through metal weirs and into a storage pond for eventual reuse as processing water. The average detention time was 7 days.

Samples were collected at points A, B, C and D every 50 days and at points A and B at weekly intervals and assayed for settleable solids, total sugars, pH, organic nitrogen, ammonia nitrogen, total carbon and residues (Figure 3). In addition the samples collected at points A and B for use in the bench reactors were treated under conditions duplicating those of the aeration basin. This included nutrient additions, pH, dissolved oxygen and feed rate. The nutrients added at the mill were 600 lbs of NH_4Cl and 150 lbs of treble phosphate fertilizer per day. Slime formation, using a laboratory reactor, could be easily demonstrated (Figure 1).

Characterization of Process Water

Table I exhibits the results obtained during August, 1974, on the waste water. According to information obtained from the plant, adequate BOD removal with minimal slime accumulation occurred during this period. Table II shows the change from August to October at which time the water temperature had dropped from 19 to 15°C. It was during this period that a significant increase in slime build-up began.

High pressure steam applied to the wood splits off available acetyl groups and this lowers the pH values of the process water. The

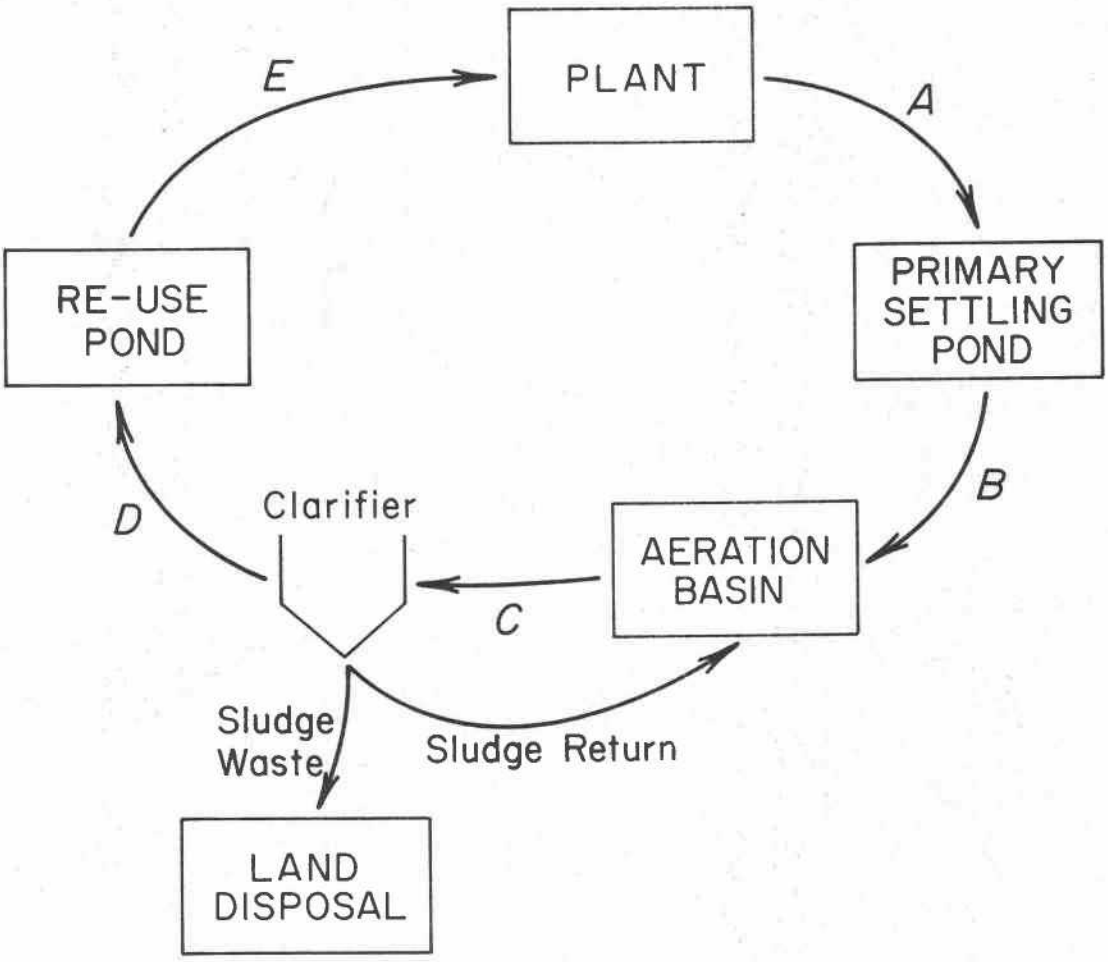

Figure 3. Activated sludge water treatment process with water reuse capability and land disposal of sludge waste.

Table I. Fiberboard process water (sample 8/5/74).

Sampling Point	pH	Total Sugars (g/l)	NH_3N (mg/l)	N-organic (mg/l)	N-total (mg/l)	Total Carbon (mg/l)	Settleable Solids (Imhoff) (ml)
A	4.6	4.05	33.3	17.5	50.8	5400	65
B	4.0	2.90	03.3	15.8	19 1	4100	0
C	4.4	1.25	66.7	241.7	308.4	2760	375
D	-	--	--	--	--	--	-
E	5.7	0.34	36.7	21.6	58.3	580	0

Table II. Fiberboard process water (sample 10/9/74).

Sampling Point	pH	Total Sugars (g/l)	NH_3N (mg/l)	N-organic (mg/l)	N-total (mg/l)	Total Solids (g/l)	Total Carbon (mg/l)
A	4.5	11.0	14.5	24.0	38.5	5.05	8000
B	4.1	3.4	1.3	21.5	22.8	4.26	3000
C	3.2	1.4	42.3	174.0	216.3		1840
D	3.5	1.3	38.4	144.0	182.4	2.40	1420
E	5.6	0 2	27.2	30.4	57.6		500

pH is not adjusted at this fiberboard mill.

High concentrations of sugars result from the steaming process since 60 to 80 percent of wood consists of cellulose and hemicellulose polysaccharides. Hemicellulose, which contains the shorter chained polysaccharides are more susceptible to hydrolysis than the cellulose. The major polymers of wood hemicellulose are: xylans, glucomannans, methylglucoronoarabinoxylan, galactoglucomannan and arabinogalactan (22). To compare the two tables as a function of temperature would be erronenous because of plant production changes and rainfall dilution. Therefore, these data serve as a general characterization of the wastewater involved.

In summary, we can say there was:

1. adequate BOD removal for re-use water
2. sufficient nitrogen for microbial growth
3. high carbohydrate concentrations
4. low pH for viable activated sludge.

Identification of Free Wood Sugars

The identification of the major wood sugars were by gas-liquid chromatographic analysis following the alditol acetate procedure described on page 17. Figure 4 shows a standard mixture of known sugars with those common to the effluent in the bench scale reactors. They are: rhamnose, arabinose, xylose, mannose, galactose and

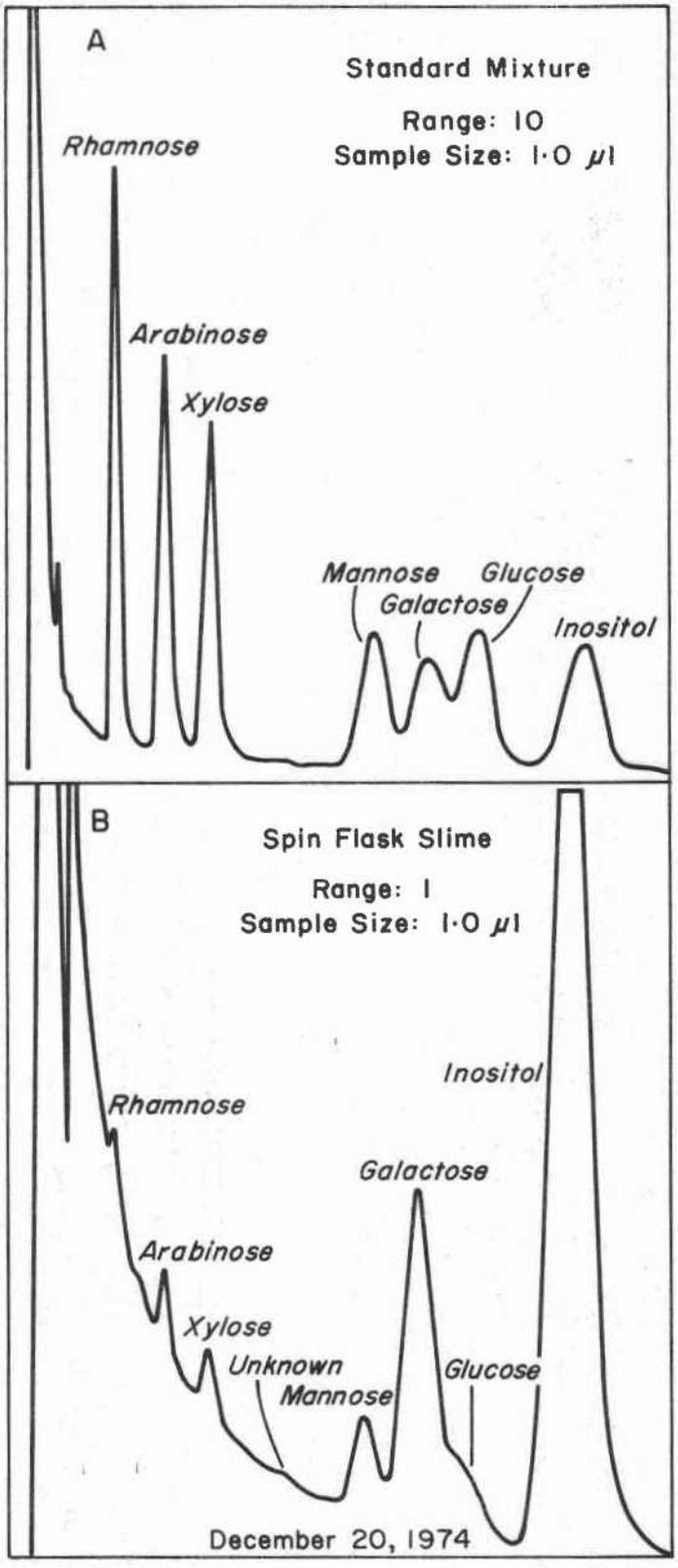

Figure 4. A chromatographic analysis of the constituent sugars produced in the bench scale reactors with Geotrichum candidum as the predominate microorganism.

glucose plus the internal standard inositol. Figure 4 shows the relation of standard sugars to free sugars from plant process water before treatment and following primary and secondary treatment. From the analysis, carbohydrates in the form of free sugar, are removed as such. However, they appear as polymerized sugar which can be demonstrated by acid hydrolysis to free the sugar and then followed by chromatography. Hence its presence can be demonstrated by gas chromatography (Figure 6). According to the data shown in Table III the initial production of slime began in the anaerobic primary settling pond with large additional gains in the aeration basin. The large concentration of galactose appearing in the plant polymerized sample is possibly a wood hemicellulose consisting primarily of galactose and/or slime residue from the transport pipes. The conditions in the anaerobic primary settling pond produce a predominance of the filamentous mold, Geotrichum candidum (Figure 8) during the winter months. The capsular slime produced by this organism is shown in Figure 9. Although the function of capsule material remains obscure it is thought to be a protection against such things as oxygen, viruses, feeding protozoa, and possibly stalked forms of bacteria.

The graph (Figure 7) shows the relationship between free and polymerized sugars within the treatment facility. The concentrations are as precise as is possible with the method employed. Hydrolysis, heat and pressure in the analysis reduced the amount of recoverable

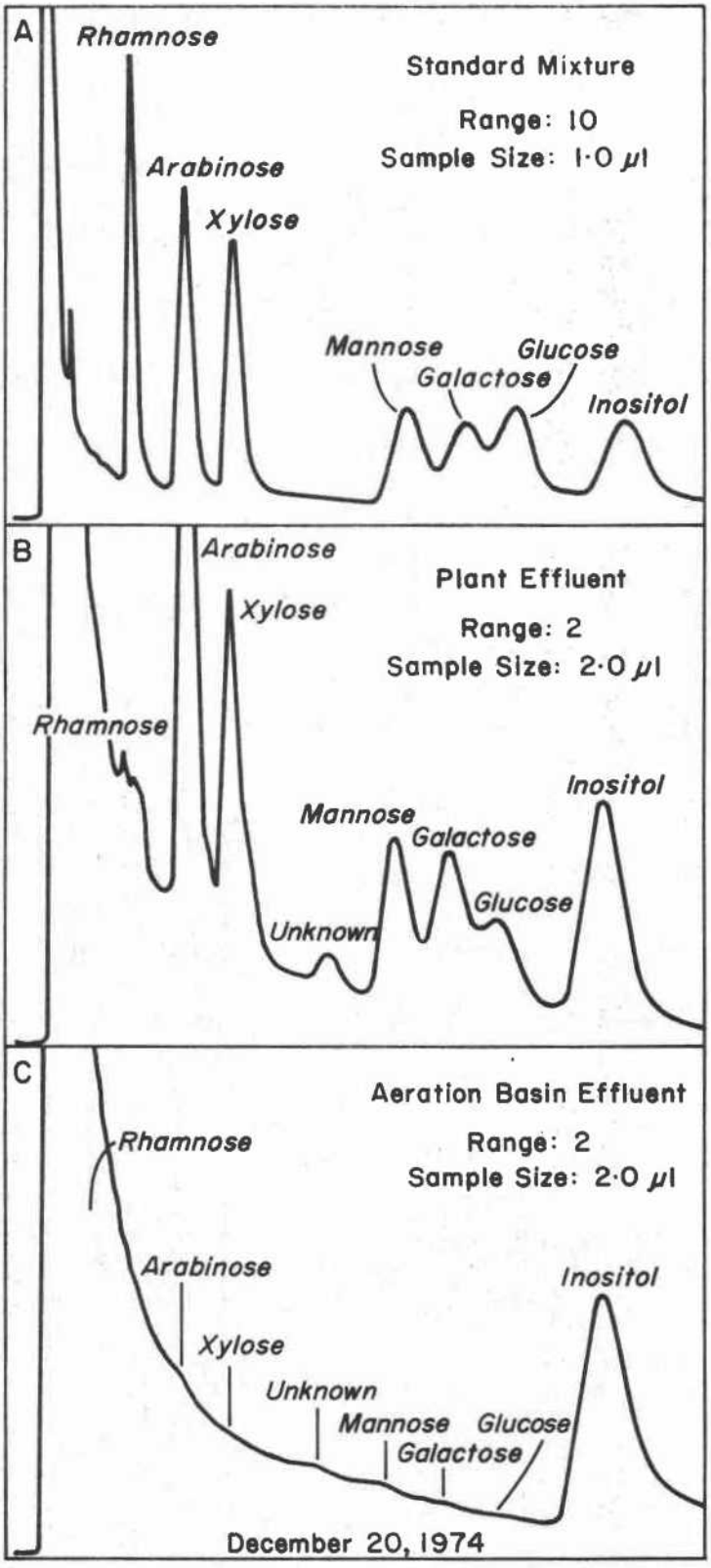

Figure 5. Free waste carbohydrates were identified by gas chromatographic analysis of plant process water and aeration basin effluent.

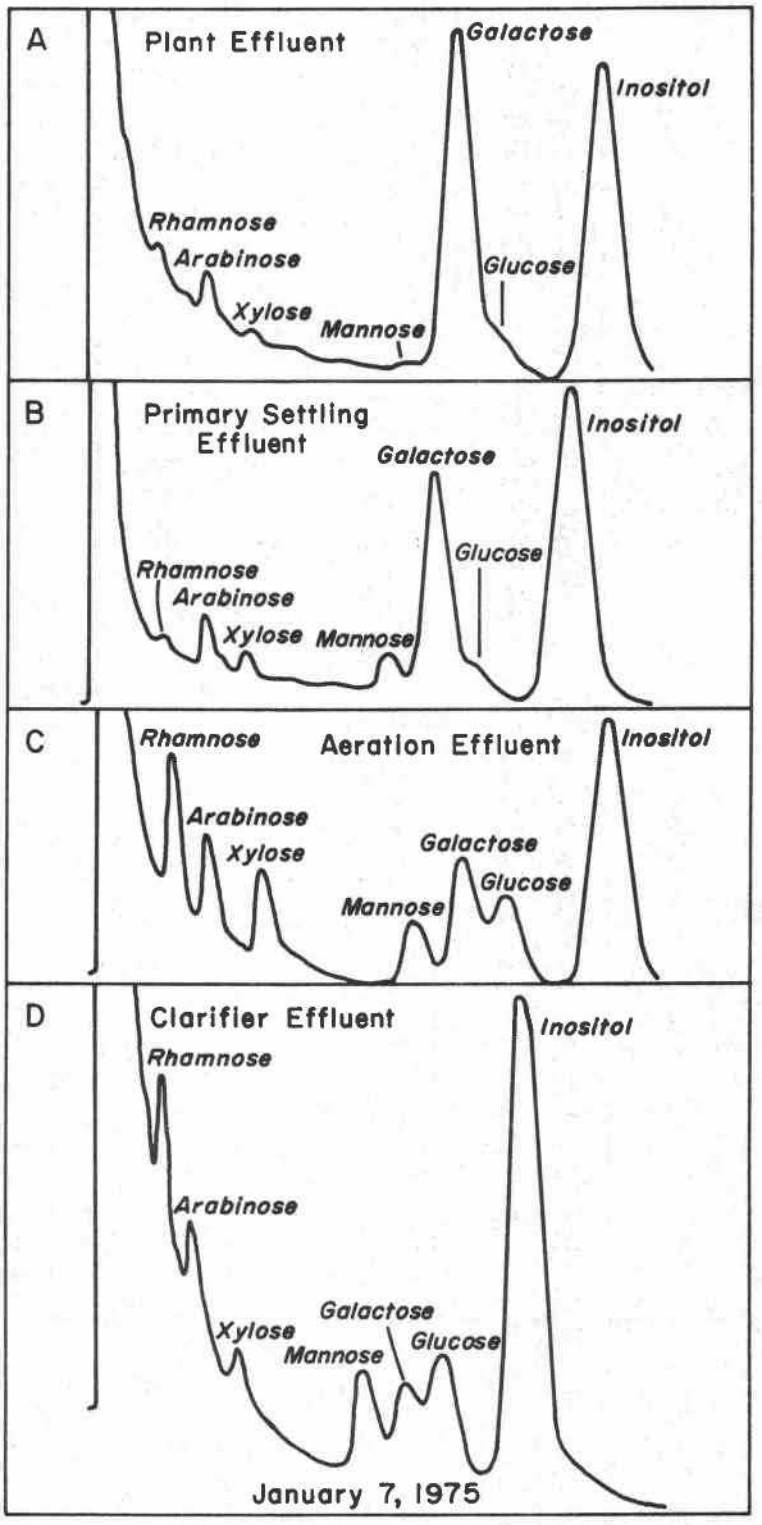

Figure 6. Polymerized carbohydrates were identified by gas chromatographic analysis on all treatment steps.

Table III. Relative carbohydrates in the free and polymerized forms from effluents of each treatment step.

	Rhamnose	Arabinose	Xylose	Unknown	Mannose	Galactose	Glucose	Total
Free carbohydrates (mg/l)								
Plant	1.68	712.00	181.32	21.64	194.00	104.94	10.18	1224.76
1° settling	0.13	11.53	2.33	12.74	8.82	13.40	---	48.95
Aeration	trace	trace	trace	trace	trace	trace	trace	
Clarifier	trace	trace	trace	trace	trace	trace	trace	
Re-use	trace	trace	trace	trace	trace	trace	trace	
Polymerized carbohydrates (mg/l)								
Plant	.87	5.91	trace		trace	176.72	---	183.50
1° settling	1.35	8.71	4.17		9.92	91.11	---	115.26
Aeration	20.31	18.05	25.17		20.25	56.86	6.53	147.17
Clarifier	8.12	5.75	4.26		26.69	24.41	19.22	88.45
Re-use	.02	2.80	2.40		5.02	1.57	6.06	17.87

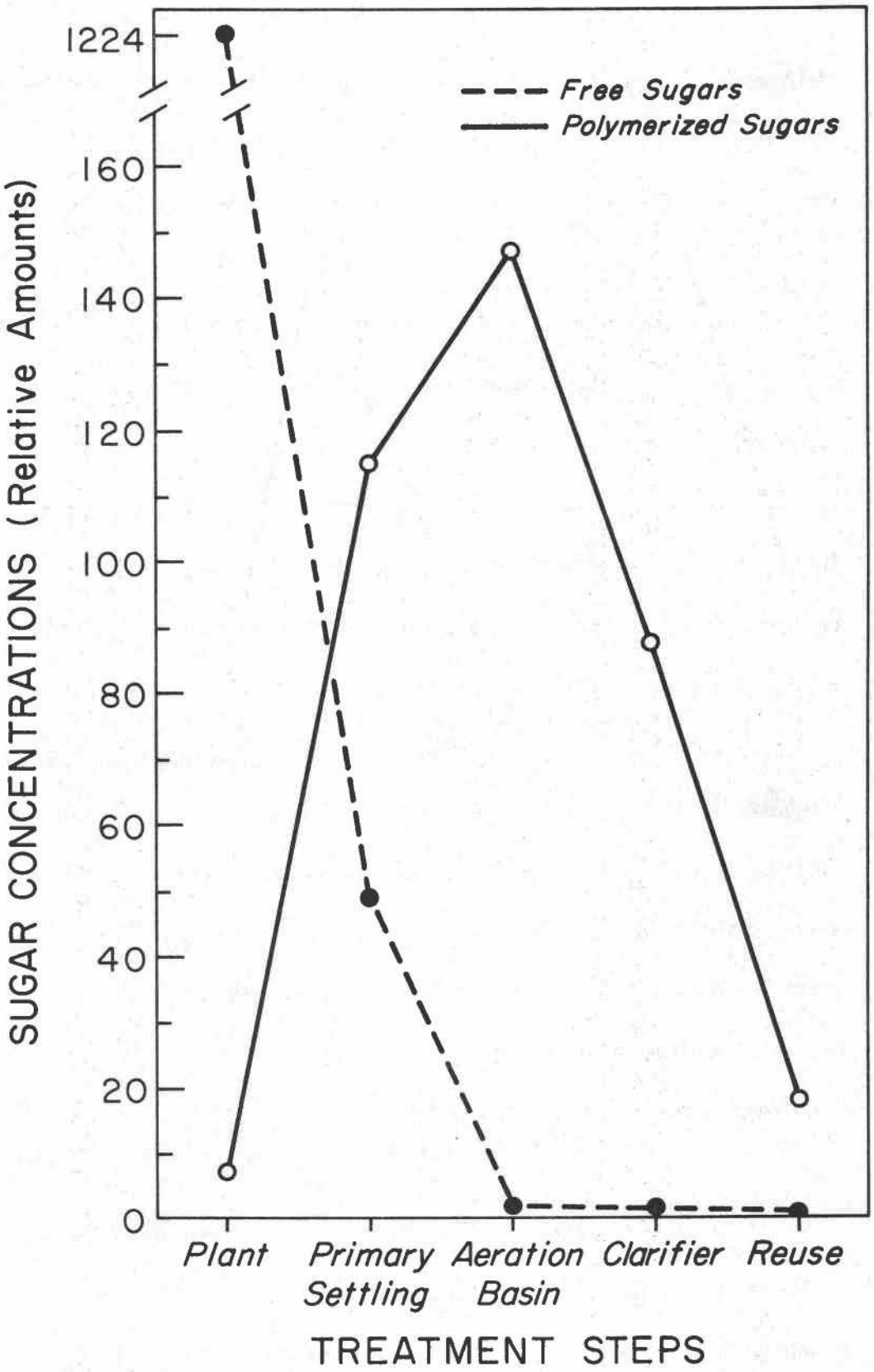

Figure 7. The rate of free and polymerized sugars within the treatment process.

polysaccharide. Insufficient hydrolysis prevents adequate release of polymerized sugars thereby yielding reduced concentrations. Although our procedure was not optimized for accuracy the conditions of hydrolysis were rigidly controlled for precision.

Table III identifies the sugars and their relative quantities in relation to the treatment steps. The free sugars are those known to exist in coniferous trees. The relative proportions would vary according to specific species. The polymerized sugars represents transitional stages from crude plant polysaccharides to a variety of bacterial transformation products. Toshio and Yasuta (33) have approximated ratios of crude fungal polysaccharide by DEAE cellulose column fractionation and zone electrophoresis for Cladosporium herbarum. They identified galactomannan as D-galactose, D-mannose and a trace of glucose in a molar ration of 1.0:1.5 respectively. However, the state of the art does not allow a complete biochemical description of a complex biological habitat system, but the fate of the sugars can be followed within the system.

Microscopic Analysis and Quantitation

Centrifuged samples of wastewater were observed microscopically as wet mounts and as stained preparations. The bulking was evidenced by a predominance of filamentous organisms. The mesh of mycelial filaments contained a host of other non-filamentous bacteria,

yeasts, and rotifers. The organism predominating during the winter months was the mold, G. candidum. This mold is now recognized as a common bulking organism in activated sludge and trickling filter systems. During the months of September through February, it represented approximately 65% of the total microbial population observed when aerobically grown on plate count agar at room temperature. Although plate counts do not give an accurate picture of the true microbial population, the relative changes in total numbers is indicative of the biological transformation potential. The average numbers for each treatment step were as follows:

Table IV. Quantitative bacterial counts for each treatment step. (Aerobic, 20°C, 24 hrs, PCA media).

1. Plant process water	13,000 Org./ml
2. Settling pond	9,000 Org./ml
3. Aeration basin	60,000 Org./ml
4. Clarifier	315,000 Org./ml
5. Re-use	290,000 Org./ml

The samples are grab samples of effluents of each treatment process. The large number of organisms spilling over the clarifier is characteristic of problems associated with bulking. Media made from the wastewater as outlined on page 14 was later used to give direct counts of Geotrichum candidum. Although not completely selective, the numbers of other bacteria were virtually eliminated without reduction of mold numbers. The characteristic colony

morphology of the bulking organism further facilitated enumeration.

Geotrichum candidum Character

Geotrichum candidum is a pleomorphic intermediate between filamentous molds and yeast. The following characteristics are useful for its identification.

1. most commonly found in soil
2. forms large "barrel shaped" arthrospores on solid media (plate count agar, malt extract agar and acidified potatoe dextrose agar)
3. reproduces vegetatively in wastewater and broths (nutrient broth, dextrose peptone broth)
4. colony morphology can range from white and mucoid to powdery and wrinkled, large or small
5. rapid growth on plate count agar at 20° C
6. sunken pellicles in dextrose peptone broth after 5 days
7. produces capsule and fat vacuoles in rich carbohydrate medias
8. cannot fix nitrogen
9. very limited growth on nitrate nitrogen while excellent growth on ammonium based nitrogen sources
10. no growth on sucrose while excellent growth on xylose, mannose, glucose and fructose

11. filaments may be erect or under the media surface. The dimensions very from 2-8 μ diameter and 3-50 μ in length
12. cannot use cellulose in the filter paper form
13. growth is excellent on carbohydrates however, proteins keratin and organic acids will also support more limited growth.

Seasonal Microbial Shift

Although the principal organism responsible for the bulking conditions during the winter was G. candidum, our preliminary investigations during August, 1974, yielded the bacterium Klebsiella pneumoniae. Like G. candidum, it can produce large quantities of extracellular polysaccharide. However, it is not filamentous and its ability to bulk is questionable. In the past, the settleability of the wastewater was much improved during the summer months, probably as a result of the low number of G. candidum observed during this period when the temperature was more favorable to the growth of the K. pneumoniae.

Problem Resolution Coagulation Studies

Wastewater effluents from the primary settling pond were initially used in our bench reactors. Coagulation studies employing cationic and nonionic polyelectrolytes proved economically

impractical since minimum dosages of 168 mg/l at pH 8.5 were necessary for adequate coagulation with the cationic coagulant. The quantity of nonionic coagulant required was 1 g/l at a pH of 8.0. The most economical coagulant to use was alum at 400 mg/l and pH 7.5 costing approximately $35.00 per day.

Bench Reactor Studies

Three 500 ml spin flask reactors were set up to study the effects of dissolved oxygen and pH on solid removal.

Table 5 shows the results for three weeks which were collected after all the flasks had stabilized. (All flasks received 17.4 mg/l/day NH_3Cl.)

Table V. pH and dissolved oxygen affects on residue removal.

	D.O.	Total Solids	pH	Total Carbon
Spin flask 1	<1.0	3.6 g/l	4.7	5000 mg/l
Spin flask 2	4-6 mg/l	3.4 g/l	5.0	3500 mg/l
Spin flask 3	4-6 mg/l	3.0 g/l	7.0	2100 mg/l

The results of Table V indicate that:

1. Oxygen below 1.0 mg/l resulted in a heavy slime accumulation on the bottom and sides of the container. The total solids remained unchanged while a significant reduction in total carbon occurred.

2. Increased pH from 4.5 to 7.0 resulted in slightly better removal of solids and total carbon. Flask #3 represents the combined effects of increased dissolved oxygen and pH over flask #1. Settling was not significant and the clarified water remained turbid.

Increased pH and dissolved oxygen appeared to have a significant affect on solids removal. However, the tendency to bulk and the characteristic filamentous population continued to predominate.

Additional studies using three, 2,000 ml suction flasks and controlling the mixing, dissolved oxygen, temperature, pH, flow rate, retention time and nutrient additions to simulate plant conditions were begun. The recommendation suggested by Cole et al. in "Hydrogen Peroxide Cures Filamentous Bulking in Activated Sludge" was followed (4). Doses varying from 500 mg/l to 200 mg/l were used following establishment of baseline uniformity in all flasks. The most favorable results occurred after two days at concentrations of 150 mg/l. Although only trace amounts of settling occurred, total residues decreased from 4.4 g/l to 4.0 g/l while total carbon decreased from 2205 mg/l to 1410 mg/l. The total sugars were reduced from 2.9 g/l to 1.1 g/l. However, plate counts showed a significant increase in G. candidum as a result of hydrogen peroxide treatment ranging initially from 846,000 molds/ml to greater than 2,500,000 molds/ml after treatment. The increased filamentous

population could account for the slightly increased settling as well as reductions in total residue, total carbon and total sugars. It became obvious that hydrogen peroxide does not cure filamentous bulking when the organism responsible is G. candidum.

According to Cooke (7) G. candidum cannot grow in the presence of KNO_3 or $NaNO_3$ and poorly in NH_3NO_2. When $NaNO_3$ was used as the nitrogen source small decreases in total carbon (2295 mg/l to 1725 mg/l) and mold counts (670,000 molds/ml to 520,000 molds/ml) occurred with a simultaneous increase in total residue (4.4 g/l to 6.4 g/l). Settleability was not enhanced and the effluent remained extremely turbid.

Additions of commercial filter grade alum (not less than 17% Al_2O_3) following hydrogen peroxide and sodium nitrate treatment required concentrations approximately equal to the amount necessary to coagulate primary settled effluents (400 mg/l).

The attempts to limit the growth of G. candidum in primary settled effluents with dissolved oxygen and pH adjustments, hydrogen peroxide and sodium nitrate additions, resulted in fungal count changes without adequate removal or settling. Detention in the primary settling pond alters the carbohydrates biologically by forming polymers and other short chained carbon compounds. Efforts to overcome this defect by nutrient additions was studied by CH_2M Hill engineers. They achieved satisfactory operation at bench level by maintaining the

BOD/N/P ratios at approximately 100/14/2 at 25°C with pH levels between 6.8 and 8.5. However, the solution proved economically impractical for the plant involved.

The following attempt to increase settling was based upon previous data which indicated low concentrations of polymerized carbohydrates in the process water directly from the plant. It was hoped that the free wood sugars would be aerobically neutralized by direct aeration of plant process water before the free sugars were removed anaerobically. This effluent was rapidly filtered through a screen in the laboratory to remove large particulate matter and the pH adjusted to about 7.0 with $CaCO_3$. The filtering prevents transport tubing from clogging between the refrigerated wastewater reservoir and the reactor. At full scale plant operation the particulate removal would also be necessary to prevent aeration fouling.

After filling the 2,000 ml reactor with process water the microbial population was allowed to establish itself until flocculation occurred. This was done while:

1. maintaining dissolved oxygen between 4-6 mg/l
2. maintaining the temperature at 13°C ± 1°C
3. adding nutrients as before
4. continually mixing the water in the reactor
5. controlling the pH to approximate neutrality.

Additional process water was not added to the reactor during the

adjusting process. After flocculation became visible (approximately five days) the flow rates equivalent to that characteristic of the plant were initiated. The reactor was permitted to select naturally the organisms most suited to the conditions.

Microscopic examination of the settled flocs revealed a minimum of filamentous growth with a balance of cocci, rod and budding yeast microorganisms. Without allowing complete reactor stabilization, 80% of the flocculated material (137 ml) settled within six minutes in a 1 liter Imhoff cone. The clarified water was somewhat transparent and contained 1575 mg/l carbon, SVI of 190 and an SDI of .53.

The favorable results prompted a scale-up to a 10 liter fermentor controlling the same parameters as above (Figure 2), using the established microbial population in the 2,000 ml flask for seed. Visible flocculation and settling began to occur within two weeks accompanied with a well balanced microbial population.

The following data summarizes the sludge character and water quality: SVI 27.4, SDI 3.6, total carbon 975 mg/l

Figure 10 shows the sludge settling profile of 10 liters of aeration water over a 19 minute period. A 50% sludge return was used for a period of 9 days prior to testing. All settling was completed within approximately 6.5 minutes followed by approximately 10 minutes of compaction to a stable level.

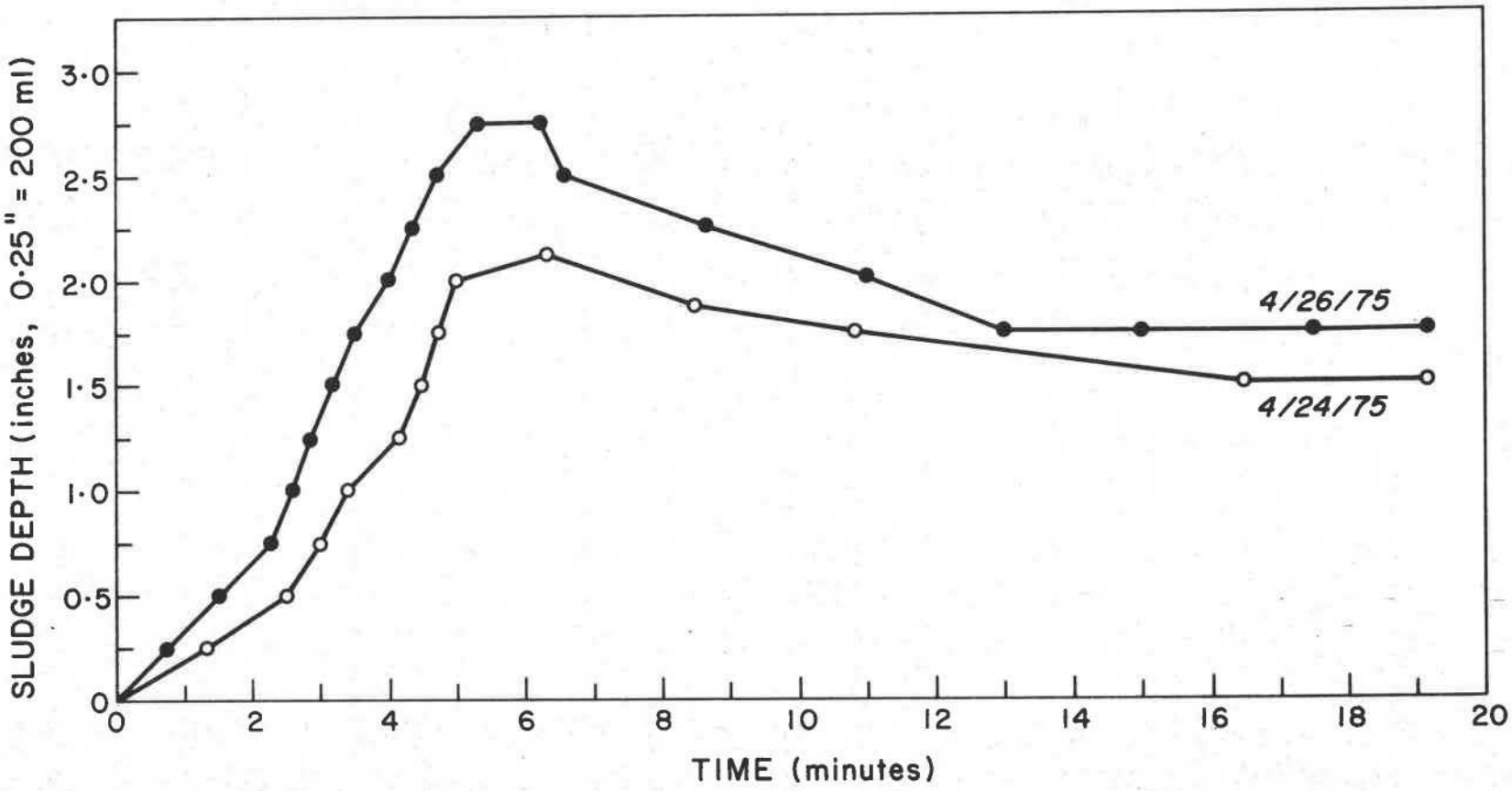

Figure 10. Sludge settling profile of 10 liters of aeration treated process water at aeration basin temperature.

SUMMARY AND CONCLUSION

The problem of filamentous bulking in the plant studied appears to be amenable to engineering design and a better understanding of the biological sequences occurring in a high sugar effluent. The use of coagulants, salts, chlorination, hydrogen peroxide and nutrient balance adjustments are aimed at treating the problem symtomatically. Their application involves a great deal of time and expense and yields only temporary relief.

The information we have collected was an attempt to characterize the nature of the wastewater and identify the source of the problem by simulating the plant treatment conditions and altering the physical and biological parameters to affect a positive change.

The character of the water was summarized as follows:

1. an adequate BOD removal for re-use process water was observed
2. sufficient nitrogen and other nutrients for microbial growth was observed
3. high carbohydrate concentrations was present
4. the pH for an activated sludge system was too low.

The cause of the bulking was G. candidum which was indentified microscopically as well as by the common identification techniques. The increased stability of the carbohydrates as analyzed by gas liquid

chromatography, was due to polymerization of free wood sugars beginning in the primary settling pond and completed in the aeration basin. The combination of a predominate filamentous microbial population and increased wastewater viscosity resulted in reduced clarifier efficiency or bulking.

Plant treatment was simulated in seven bench scale reactors varying in size from 500 ml to 10,000 ml. Each unit simulated: flow rate, retention time, nutrient additions, temperature, dissolved oxygen and pH. The following list summarizes the attempts to reduce the filamentous population and/or to promote coagulation for increased residue removal.

Parameter Tested	Result
1. Less than 1.0 mg/l D.O.	Increased slime production and turbidity. No settling
2. Increase pH from 4-7 (4-6 mg/l D.O.)	Improved residue removal and lower total carbons. Poor settling.
3. Addition H_2O_2 (50-200 mg/l)	Filamentous population tripled.
4. $NaNO_3$ as nitrogen source	Slight decreases in filamentous population and total carbon. Increased total residue. Highly turbid.
5. Direct aeration of plant process water	Nonfilamentous, well balanced microbial population. Good settling and residue removal.

We conclude that the stability of the carbohydrates was increased as a result of free wood sugar polymerization. These biologically inert polymers increased the water viscosity and thereby contributed to the suspension of the predominately filamentous microbial population. Increasing the pH to about 7.0 and eliminating the primary settling pond by rapid screening, followed by direct aeration, should limit the filamentous flora and allow the free wood sugars to be transformed to carbon dioxide and water under well oxygenated conditions.

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