MICROBIAL ISSUES ENCOUNTERED IN WASTEWATER TREATMENT AT MOORHEAD FACTORY AND REMEDIAL MEASURES

Indrani S. Samaraweera*[1], Terry D. McGillivray[1], Diane L. Rheault[1] and Dennis Burthwick[2]
American Crystal Sugar Company, Technical Services Center
[1]1700 N. 11th Street and [2]2500 N. 11th Street, Moorhead, MN 56560

Introduction:

Wastewater treatment is an integral part of processing of sugar beets in the sugar industry. American Crystal Sugar Company (ACS) has five factories. Three of these factories, Moorhead (MHD), Hillsboro (HLB), and East Grand Forks (EGF) each have a 6.7 million gallon anaerobic contactor, aerobic basin, and ponds for processing of wastewater while the other two factories have lagoons and wetlands for the treatment of their wastewater.

During the 2007/2008 campaign our efforts were focused on microbial issues in wastewater treatment at the MHD factory. Therefore, this paper deals with problems encountered with filamentous bacteria, poor settling in treatment of the high strength wastewater and studies to circumvent these problems. In addition some differences observed in the MHD and HLB anaerobic systems will also be discussed.

Materials and Methods:

A) Microbiology

1) Sample collection

Weekly samples of wastewater were obtained aseptically in sterile screw cap containers from each of three locations: a) anaerobic influent from the new covered wastewater pond, b) anaerobic tank or anaerobic contactor, and c) aerobic basin or activated sludge system. These samples were observed microscopically at the ACS Technical Services Center Microbiology Lab. Samples from similar locations in the wastewater treatment systems at the Hillsboro and East Grand Forks factories were intermittently obtained for comparative purposes.

2) Microscopy and Photography

i) Wet Mounts, Staining, Floc formation and Higher Life Forms – Separate wastewater (WW) wet mounts on slides were observed microscopically with or without a drop of lactophenol cotton blue and/or India ink stain. This was for determination of higher life forms (amoeba, flagellates, ciliates, rotifers) in the aerobic basin WW sample and presence of filaments and motility, tetrads, bacterial polysaccharides, and floc characteristics in other samples. The wet mounts allow living microorganisms to be observed as they appear in the environment.

ii) Staining of Smears – Different WW samples were smeared on slides and air dried. Staining protocols for the Neisser and Gram stains were then carried out. This was for identification of filamentous bacterial types using a key provided by Environmental Leverage Inc. (www.EnvironmentalLeverage.com) (3). The Neisser stain provided a key for identification of bacterial filaments on the basis of branching, Neisser positive or negative, and other characteristics within this key. The gram stain provided a key for identification of bacterial filaments on the basis of whether it was

Gram negative, Gram positive, or Gram variable and showed the presence or absence of sheath (with and without attached growth), sulfur granules, and other characteristics within that key.

All above microscopy was carried out using an Olympus BHTU-001A microscope with the use of bright field, phase contrast, and dark field accessories. A Nikon Coolpix 4500 digital camera mounted on the microscope eyepiece tube was used for photographing of microscopic specimens.

iii) Fecal coliform analysis – The membrane filter method 9222D from standard methods for examination of water and wastewater (1992) 18th edition (5) was used.

B) Chemistry

1) Sample collection – Samples of wastewater (anaerobic tank influent, anaerobic tank, and aerobic basin) were obtained one, two, or three times per week during the campaign and analyzed at the factory lab or the Technical Services Center chemistry lab and analyzed for the tests listed below:
2) Temperature measurements were taken off the on-line Rosemont system.
3) pH value – Measurements were made using a Thermo Orion 520A+ pH meter.
4) Chemical Oxygen Demand or COD (Reactor digestion method) – Measurements were made using the Hach 8000 colorimetric method with the Hach DR/4000V Spectrophotometer.
5) Total Suspended Solids or TSS – Measurements were made using method 2540D from standard methods for examination of water and wastewater (1992), 18th edition (5).
6) Total Volatile Suspended Solids or TVSS – After the TSS test is carried out, the sample is weighed and burned in a muffle oven (Thermolyne Sybron Type 30400 furnace) at 550ºC for 1 hr. The sample is cooled in a desiccator and reweighed. The difference in sample weights gave the TVSS value.
7) % TVSS – The measurement was calculated using the TVSS value obtained.
8) Alkalinity – 15 ml of sample was titrated with 0.0356 N H_2SO_4acid to a pH of 4.8 and reported as mg per L of $CaCO_3$.
9) NH_4 - N – Measurement for ammonia nitrogen was made using Method 4500-NH_3F from Standard Methods for Examination of Water and Wastewater (5). An ammonia selective electrode Orion 720A+ was used.
10) P – The reactive phosphorus measurement was made using Method 4500-PE in Standard Methods for Examination of Water and Wastewater (5) which is equivalent to the Hach 8048 method. A Hach DR4000V Spectrophotometer was used.
11) Total VFA's or Volatile Fatty Acids – Measurements were made using an acid base titration with phenolphthalein indicator after 100 ml of sample (filtered and centrifuged) had been distilled in a 500 ml distillation flask. See Standard Methods for Examination of Water and Wastewater (5).
12) Organic acid measurements – Analyses were carried out for acetic propionic formic, butyric, valeric, and total lactic acid using a Waters HPLC with a refractive index detector. The column used was an Aminex HPX-87H from BioRad.
13) BOD_5 or Biological Oxygen Demand – Measurement was made using test method 5210B from Standard Methods for Examination of Water and Wastewater (5). A YSI 58 dissolved oxygen meter was used.

Results and Discussion:

The main purpose of wastewater treatment is to remove pollutants before discharging to the river by reducing the COD (Chemical Oxygen Demand) and odor. This allows the factory to meet strict regulatory requirements for discharge to the river.

The wastewater treatment (WW) process at the MHD factory consists of a primary flume clarifier from which the overflow goes to the new wastewater covered pond (holding pond). The underflow from the primary flume clarifier is dewatered in the three belt presses, and the mud is trucked out. Water from the WW holding pond goes to the anaerobic tank, followed by the anaerobic clarifier and the activated sludge system (aerobic tank and sedimentation basin). The water can be discharged from the sedimentation basin (final clarifier) or be sent to the stabilization polishing ponds before being discharged. The treated water is discharged to the Red River once all regulatory criteria are met. This is dependent on the time of the year as once the river freezes, water cannot be discharged under the ice.

In anaerobic digestion consortia of microorganisms mainly bacteria are involved in the transformation of complex high molecular weight organic compounds to methane by a series of microbiological processes (2).

Organic matter $\rightarrow CH_4 + CO_2 + H_2 + NH_3 + H_2S$

There are four categories of bacteria that are involved in the above process. See Table 1 (2). These are namely the 1) Hydrolytic bacteria; 2) Fermentative Acidogenic bacteria (i.e. acid forming), e.g. Clostridia; 3) Acetogenic bacteria (acetate and H_2 producing bacteria), e.g. Syntrobacter wolinii and Syntrophomonas wolfei (9); 4) Methanogens, Methanosarcina, and Methanothrix (11). There is a symbiotic relationship between the acetogenic and methanogenic bacteria. The methanogens help achieve a low hydrogen tension required by acidogenic bacteria (2). The acetogenic bacteria also grow faster than the methanogenic bacteria.

During the last couple of years the MHD factory has been having difficulties with processing of WW in the anaerobic digester. The main reason for this was due to low settling in the anaerobic tank resulting in the loss of the anaerobic biomass of microbes with the anaerobic effluent. This phenomenon became more apparent as the COD in the anaerobic influent increased through the campaign. See Fig. 1. The MHD factory would start the campaign with a low anaerobic influent COD of 1400 ppm in September (at the start of the beet slicing campaign) and increase to about 50,000 COD towards the end of the campaign in May of the following year. Hence the MHD factory anaerobic tank was relatively easy to operate from September to December. However, these operations became more difficult in January with decrease in ambient temperatures to single digit or subzero levels and coincided with the increase in COD levels to the anaerobic tank.

Therefore we began looking at the WW microbial profile closely at the MHD factory receiving weekly samples from the covered new wastewater pond (anaerobic influent), the anaerobic tank, and the aerobic basin of the activated sludge system. This was begun at the end of the 2006/2007 beet slice campaign and continued through the entire 2007/2008 campaign and presently at the Technical Services Microbiology Lab. We also obtained the same samples intermittently from the East Grand Forks (EGF) and Hillsboro (HLB) factories which had similar WW treatment systems for comparative purposes. The main characters we were looking for in these samples were: the floc characteristics, filamentous and other bacterial types, and higher life forms as these are diagnostic tools and pointers to problems in the anaerobic tank and the

activated sludge system (3). See Table 2. It should be noted that filamentous bacteria cannot be typically grown on agar media and therefore require the type of microscopic identification we have been carrying out.

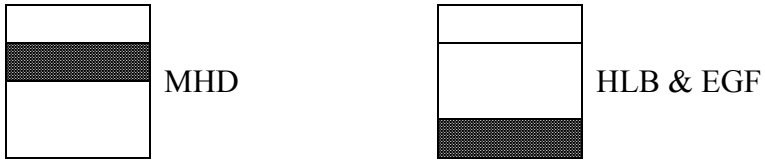
The successful removal of wastes from the water depends on how efficiently the bacteria consume the organic material and on the ability of the bacteria to stick together, form floc, and settle out the bulk fluid (4). The flocculation or clumping characteristics of the microbes enable them to form solid masses large enough to settle to the bottom of the anaerobic or aerobic tanks. The better the flocculation characteristics of the sludge, the better the settling and results in better wastewater treatment (4). See floc observed under dark field microscopy Fig. 2.

The higher life forms monitored consisted both of protozoa and metazoa. The protozoans looked at were classified on the basis of movement, e.g. amoebae, flagellates, ciliates, free swimming ciliates, crawling (grazing) ciliates, and stalked ciliates. The metazoans observed were the rotifers, nematodes, water bears, and bristle worms.

The types of protozoa present give some indication of the treatment system performance. In a steady state system there is a natural progression in the dominance of the different protozoan species (4). The activated sludge system and the anaerobic system are not usually in a steady state, as the type and concentration of organic and other nutrients, amount of available oxygen and temperature change constantly. However, the relative dominance of the protozoan and/or metazoan species gives an understanding of treatment system conditions. For instance the presence of worms in the aerobic basin shows the sludge is old and needs to be wasted or testate amoeba are found when the environment is too harsh for the naked amoebae to survive. The crawling ciliates begin to gain dominance after most soluble nutrients have been removed. During this time most of the dispersed bacteria have begun to clump together to form floc (4) and indicates the sludge age of the treatment system is adequate. An abundance of stalked ciliates indicates that most of the organic nutrients have been removed (4). As the sludge ages, the stalked ciliates change from single stalked to colonial species. See pictures of stalked ciliates, rotifers, and bristle worms from MHD aerobic basin in Fig. 3, 4, and 5 respectively.

At the start of the 2007/2008 campaign, water samples showed the presence of large amounts of filamentous bacteria in the MHD anaerobic tank. See Fig. 8. This was detrimental to the methanogenic bacteria which are slow growing and therefore unable to compete with the filamentous microbes (which have a large surface area) for the food.

In addition, a distinct difference in wastewater samples from the anaerobic tanks at MHD, HLB, and EGF in undisturbed containers on the bench were observed. The MHD samples showed the black biomass floating on the surface of the water with a lower clear layer of water indicating poor settling. However, the HLB and EGF samples showed the black biomass at the bottom of the container with a clear upper layer showing good settling. See Fig. given below.



The problem with the filamentous microbes when present at excessive levels is that they extend from the flocs into the bulk solution and interfere with compaction and settling of the sludge. However, in normal flocs a balance between the floc forming and filamentous bacteria

results in strong floc that keep their integrity and settle well. The excessive filamentous bacteria then followed through into the activated sludge system as well. See pictures of floc obtained from the MHD and EGF aeration basin which shows this difference clearly. See Fig. 6A and 6B.

Another problem was observed in wastewater wet mount slides from the MHD anaerobic tank. It showed the presence of viscous, slimy, or jelly-like material produced by bacteria, e.g. Zooglea when a drop of India ink was added to the wet mount and observed microscopically. See Fig. 7. This phenomenon is termed "Zoogleal bulking" or nonfilamentous bulking and is caused by the excessive production of a slimy exopolysaccharide by these bacteria. This also results in reduced settling and compaction of floc. See picture showing polysaccharide and filaments in Fig.8.

Therefore, in an effort to decrease both the filamentous and non filamentous bulking in the anaerobic tank three different remedial options such as wasting, increasing loading or Chemical Oxygen demand (COD) to the anaerobic tank and chlorination were evaluated at the beginning of the campaign.

The purpose of wasting is to get rid of the excess microbial population so have just enough for the food coming in. Although the wasting of sludge from the anaerobic tank underflow towards the beginning of campaign showed a decrease in the filaments (to some extent) on microscopic observation and comparison of pictures, it was not entirely successful. Also, this could be done only on very infrequent occasion and therefore was not a long term solution.

The second option was to increase the COD loading or F/M (food to microorganism) ratio to the anaerobic tank. This was achieved by having a mix of flume water (high COD) and water from the new waste water pond go into the anaerobic tank. As the COD loading increased, the filaments in the anaerobic tank showed a decrease and an increase in biogas was also observed. This was due to the methanogens now having a better chance of competing with the filamentous bacteria for food as there was more of it to go around. The F/M at this stage was 0.1 (10). This also was considered a relatively short term solution as it was not possible to mix the flume water with the water from the pond (influent) for an extended period of time.

Therefore we were left with the third option or chlorination. As such a series of chlorination trials to reduce the filamentous, bacteria, and zoogleal polysaccharide which was causing bulking and settling issues was begun. This was critical as the anaerobic process is the workhorse of the entire wastewater treatment system and removes 95% of the organic material, Rein et al (10). Both bench and factory chlorination trials were carried out.

Chlorine Studies: As the bench chlorination studies were successful, these studies were continued at the MHD factory with the use of sodium hypochlorite. This method selectively destroyed the filaments rather than the acetogens and methanogens due to their higher surface area when carried out at the correct dosage. The commonly used dose was 2 lbs of chlorine per 1,000 lbs of biomass, and it is recommended that the return activated sludge (RAS) be exposed three times per day (10). The initial feeding of chlorine was carried out just ahead of the anaerobic tank once/day at 0.625 lb/min. After feeding the tank with chlorine for 3-4 days, we allowed the tank to recover a couple of days monitoring the filaments present on a daily basis and then re-chlorinated. The target was to expose the entire inventory (VSS) to the dose at least once/day. The filament level was compared at time zero before start of chlorination microscopically. On the first experiment it looked as though we had reduced the filaments but were not entirely successful. Therefore, the chlorine addition point was changed, and

chlorination was carried out at the anaerobic clarifier return point. The microscopic pictures of filaments taken showed a greater impact on the decrease in filaments by the latter method of chlorination. See Fig. 9 and 10. All chemical parameters were also monitored carefully to make sure that nothing else was adversely affected. A total of four chlorination studies and additions were carried out during the campaign.

Jenkins, Richards, and Daigger (7) and also other workers (3) have associated filamentous and other bacteria that cause bulking and plant operating problems with conditions and WW characteristics at the plant. See Table 3.

Nutrient Addition Studies and pH Control:

A third problem we encountered in January was that the alkalinity in the anaerobic tank kept dropping. Therefore we had to add one truck load of caustic (NaOH) per week to the wastewater pond or EQ pond. However, the alkalinity continued to fall in January along with the pH. We then had to increase addition of caustic to two truck loads per week to the wastewater pond to maintain alkalinity in the anaerobic tank.

During the 2007/2008 beet slicing campaign for the first time, the macro nutrient content of the influent to the anaerobic tank, anaerobic tank, and the aerobic basin and other areas were monitored at MHD. For comparison purposes the nutrient content in the EGF and HLB wastewater samples also were looked at. Distinct different nutrient profiles in wastewater in the three factories were observed. At MHD the C:N:P ratio changed with increase in COD, while at HLB and EGF hardly any changes occurred throughout the campaign.

According to Henze and Harremoes (6) the theoretical minimum COD/N/P ratio is 350/7/1 for a highly loaded system, whereas for a lightly loaded system it is 1000/7/1. In addition Takashima and Speece (12) noted that the NH_4-N concentration in the reactor must be maintained in excess of 40-70 mg/L to prevent a reduction in activity of the biomass. Evaluation of the water from the MHD anaerobic tank also showed that the NH_4-N and phosphorous content was very low or hardly present while the HLB and EGF anaerobic tanks were not deficient in N and P. Also we were seeing cyanobacterial tetrads in the wastewater influent (EQ pond) to the anaerobic tank and the anaerobic tank at MHD as well. See Fig. 11 showing tetrads in the WW influent at MHD. This was not observed in the HLB and EGF wastewater. The presence of tetrads indicate nutrient deficiency, particularly nitrogen. Therefore the addition of nitrogen as urea (ammonium nitrate form) was begun on February 4, 2008, to the anaerobic tank. Micro nutrients from Environmental Leverage were also added. This is because trace metals have been shown to be stimulating to methanogens (12).

Nutrient deficiency conditions can lead to decreased removal of organic loading and/or cause sludge bulking. Therefore the wastewater treatment becomes limited due to loss of biological solids from the anaerobic tank with the subsequent overloading of the aerobic treatment system causing it to fail. The aerobic system could then pass the high solids to the stabilization ponds. This could result in odor issues in the spring as the wastewater warmed up and the microbial activity in the ponds increased.

When clarifier failure occurred, samples from the anaerobic tank could be degasified and the solids would settle in a settling test (10). However, the MHD factory did not have this option of settling using a de-gassifier on a long-term basis.

The urea (ammonium nitrate) addition indicated that we were not getting the ammonia anticipated and needed in the anaerobic tank. Therefore, we switched to the addition of NH_4OH on February 17, 2008. This showed more favorable results, and the dosing was increased to 450

gpd. At this time the anaerobic clarifier effluent was showing <0.16 mg/L of NH_3 and 0.52 mg/L of phosphorus. The ammonium hydroxide was being metered into the tank from totes and the anaerobic clarifier underflow solids from middle of January onwards was found to be consistently higher than that observed during previous campaign 06/07. Fig. 12 indicated better settling. However, again floaters began to appear. Therefore, it was critical to increase the phosphorus levels in the Anaerobic tank which were low and limiting treatment. We looked at potassium dihydrogen phosphate, urea phosphate, and then phosphoric acid as options.

A type of chemical precipitation that can be troublesome in anaerobic systems is struvite or $MgNH_4PO_4$ formation. Loewenthal et al. (8) examined struvite precipitation and found that it is commonly present in wastewater containing high concentrations of dissolved orthophosphates, NH_4-N and MG^{++} ions. We have observed that when ammonium hydroxide is added to the pond, the phosphorus or P content in the anaerobic tank also increases. This could be due to P in the form of struvite going back into solution.

During mid February and early March 2008 a significant increase in the filamentous microbe Type 021N was observed in the aerobic tank. The wastewater here was also rather thick and slimy. This could have been due to a number of reasons: low DO (dissolved oxygen), limited nutrients (N and/or P), low F/M ratio, or sulfide compounds. Therefore we considered starting chlorination of the aerobic tank but decided to wait and see if we could get the anaerobic tank under control.

The problem with filamentous microbes is that they out compete the nonfilamentous floc forming microbes for food or nutrients (C, N and P), both macro and micro nutrients. Therefore more food or nutrients should be available for the floc forming bacteria to grow and compete with the filaments. Once this occurred, the sludge automatically settled much better.

It should be noted that the cell wall of a gram positive bacterium is made up of three primary components: a polysaccharide layer, the cell wall, and the cell membrane. But gram negative bacteria have five primary components: a lipopolysaccharide layer, a phospholipid layer, a lipoprotein layer, the cell wall, and the cell membrane. Three main elements – N, P, and sulfur – are required to develop the gram negative cell wall. If any of these elements are lacking, the cell wall does not develop properly. For instance P is required to develop the phospholipid layer and N is required to develop the lipoprotein layer (4). If there is a deficiency of P, the phospholipid layer will not develop properly. Instead of a phospholipid layer, there will be a layer that is predominantly made up of lipid. The cell wall now has its lipopolysaccharide layer (which is already fat and slimy), along with an additional layer of lipid. If there is also a deficiency of N, the cell will have a significant addition to the lipid layer forming a triple lipid layer (4). This extra thick layer of lipid and slime interferes with the development of floc. The bacterial cells now do not compact closely together by a thin layer of slime but are now spaced farther apart by a much thick layer of lipid and slime. This extracellular lipopolysaccharide now resists water and causes the sludge to remain suspended and will not settle (4). The extra slime layer may also interfere with the cell's ability to remove nutrients from the water because it becomes difficult for nutrients to penetrate through the slime layer to enter the cell wall (4). See Fig. 13. This is because bacteria feed by adsorption by allowing food particles to stick to the cell wall, followed by absorption.

Up to this point the addition of different macronutrients (N and P) was carried out directly to the anaerobic tank. However, the greatest impact of nutrients was observed when a truck load of ammonium hydroxide was added to the wastewater new covered pond (EQ) on 4/4/08 and phosphoric acid to the anaerobic tank. About this time we also started heating the

new covered WW pond or EQ pond at MHD which resulted in pre-acidification of WW as well. When the addition of phosphorus (P) and nitrogen (N) reached established levels in the anaerobic tank marked improvements in treatment (never seen before) were observed (10). For instance:

- Filaments and lipopolysaccharides disappeared in the anaerobic tank.
- Concentration of the anaerobic clarifier overflow solids decreased.
- Concentration of the anaerobic clarifier underflow solids increased.
- The alkalinity in the anaerobic tank increased and could be sustained.
- The total volatile fatty acids (VFA's) in the anaerobic tank decreased.
- The percent methane in the biogas increased.
- The COD loading to the anaerobic tank was increased to a new sustained high level of 245,000 lbs/day.

The targeted N and P concentration in the anaerobic tank was reached at the same time the nitrogen addition of NH_4OH was changed to the new covered pond. The impact of addition of ammonia to the new covered pond is seen very quickly in the influent ammonia to the anaerobic tank. This indicates that there is probably a hydraulic short circuiting in the new covered pond or EQ pond at MHD. Due to the above changes, the COD loading to the anaerobic tank could be increased and a new sustained high loading of 245,000 lbs/day was achieved. See Table 4. This shows that once the NH_4-N content increased to 56.8 mg/L on 4/21/08 (previously 0.276 mg/L on 4/4/08) then the TSS in the An. Clarifier effluent decreased from 3,460 mg/L to 300 mg/L even with increased COD loading. The VFA's also decreased from 582 to 66 and the TSS in the Anaerobic Clarifier underflow increased (4/25/08).

Looking at all the data from the previous campaigns the following graph and table were put together. See Table 5. This shows that during the previous campaign as a result of the numerous changes made in the wastewater treatment at the MHD factory we were able to process 1,484,955 lbs of COD more during the 2008 campaign in 22 days less than in campaign 2007. This was a very significant difference from previous years. Fig. 14 shows that we were able to carry out sustained and better treatment in the 2008 campaign ('07/'08) as compared to the 2007 campaign ('06/'07).

Conclusions:

The microbiological and chemical studies carried out at the Moorhead factory wastewater treatment facility have shown that the following strategies were successful:

1) Chlorination – at the anaerobic clarifier underflow to reduce filamentous bacteria
2) Macro nutrient addition – Nitrogen as ammonium hydroxide to the wastewater new covered pond and phosphorous as phosphoric acid to the anaerobic tank were the most successful in maintaining continued settling in the anaerobic tank from mid-January to May 2008 without the loss of the anaerobic microbial biomass in the anaerobic clarifier effluent.
3) Temperature was increased in the EQ pond at the time resulting in some pre-acidification.

When the targeted N and P concentrations in the anaerobic tank were reached, marked improvements in treatment never seen before were observed. The changes seen were as follows:

a) Filaments and lipopolysaccharides disappeared in the anaerobic tank.
b) The concentration of the anaerobic clarifier overflow solids decreased.
c) The concentration of the anaerobic clarifier underflow solids increased.

d) The alkalinity in the anaerobic tank increased and could be sustained.
e) The total volatile fatty acids in the anaerobic tank decreased.
f) The percent methane in the biogas increased.
g) The COD loading to the anaerobic tank was increased to a new sustained high level of 245,000 lbs/day.

As a result of the above changes made in the wastewater treatment at the MHD facility, we were able to process: 1) 1,484,955 lbs of COD more during the 2008 campaign, 2) This was achieved in 22 days less than in campaign 2007, 3) A new sustained high loading of 245,000 lbs/day was processed. This was a very significant difference from previous years.

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TABLE 1

Metabolic Bacterial Groups Involved in Anaerobic Digestion of Wastes

Complex organic molecules
(e.g. polysaccharides fats)

↓ 1) Hydrolytic bacteria

Monomers
(e.g. glucose, amino acids, fatty acids)

↓ 2) Fermentative acidogenic bacteria

Organic acids, alcohols, ketones

↓ 3) Acetogenic bacteria

Acetate, CO_2, H_2

↓ 4) Methanogenic bacteria

Methane (CH_4)

FIGURE 1

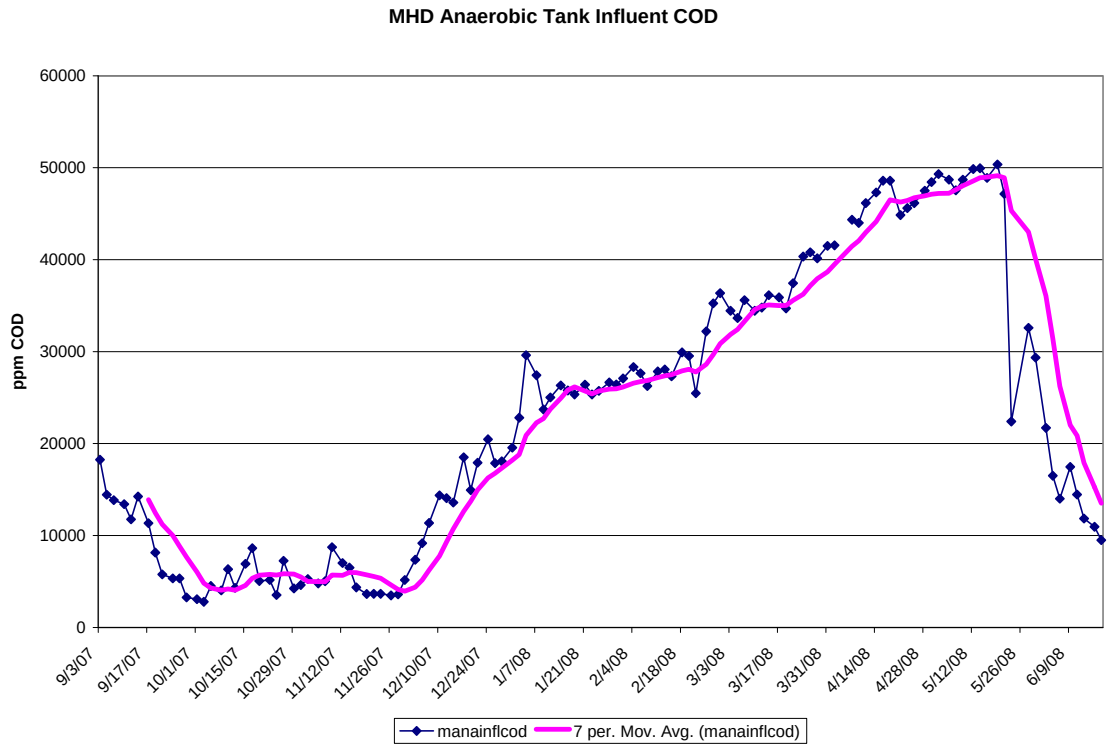


TABLE 2

Characteristics in Wastewater Monitored in Microbiology Lab

1. Floc Characteristics – compact, diffuse, bridging or internal bulking, clear, golden brown, or black.

2. Filamentous and Other Bacterial Identifications
   a) Wet Mount – Lactophenol cotton blue stain & India Ink stain (Zoogleal polysaccharide)

   b) Staining (of air dried smears) 1. Neisser Stain, 2. Gram Stain } Thiothrix, Zooglea, Tetrads, N. limicola, Mycothrix parvicella, S. natans, Type 021N etc.

3. Higher Life Forms – amoeba, flagellates, free swimming ciliates, stalked ciliates, rotifers, and worms.

[Techniques used at the Environmental Leverage Inc. workshops (3) were used.]

FIGURE 2

Floc (Dark field) 100x

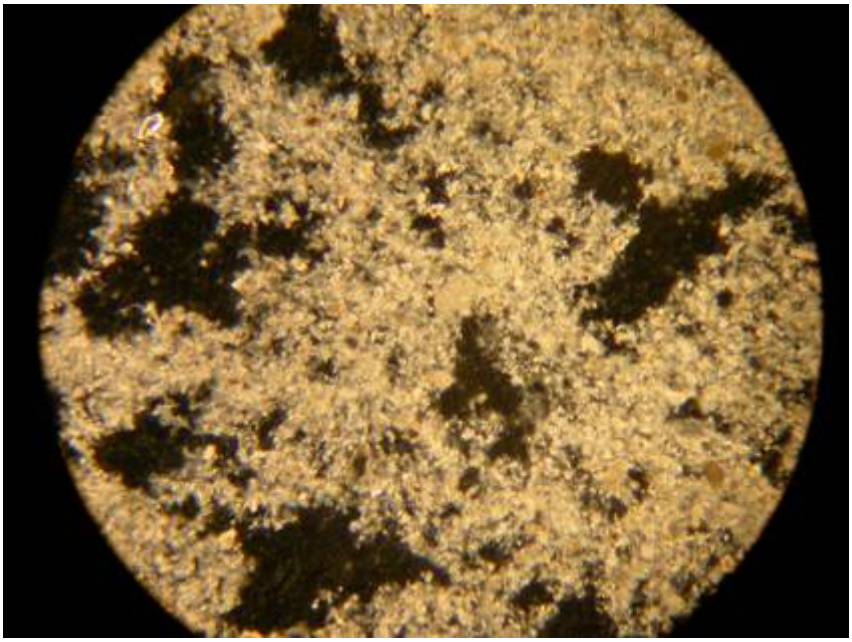


FIGURE 3

Moorhead Aerobic Basin (1-26-09) 400x Stalked ciliate cluster

FIGURE 4

Moorhead Aerobic Basin - Two rotifers 400x

FIGURE 5

Bristle Worms, Filaments & Floc (Dark field)

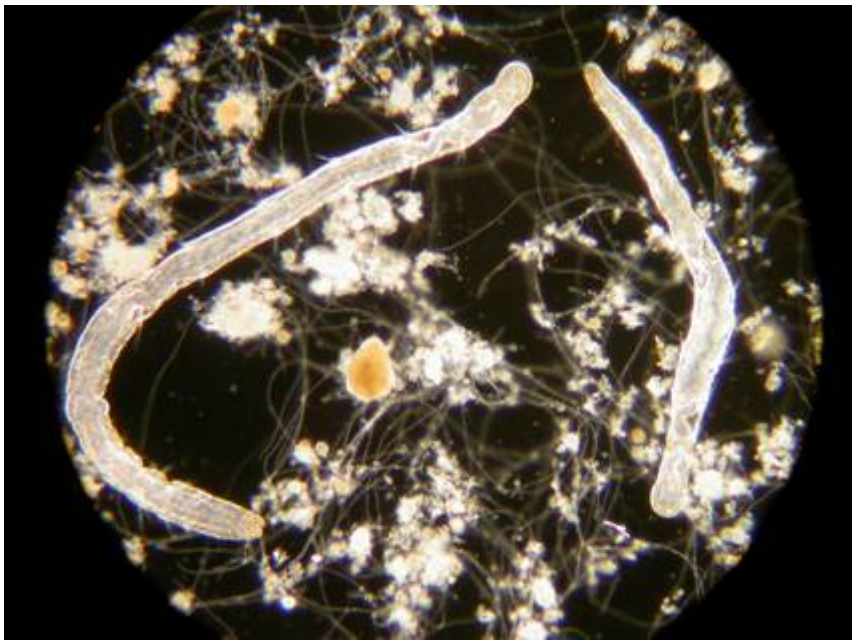


FIGURE 6A

MHD Aerobic Basin 1-14-08 (a lot of filaments)

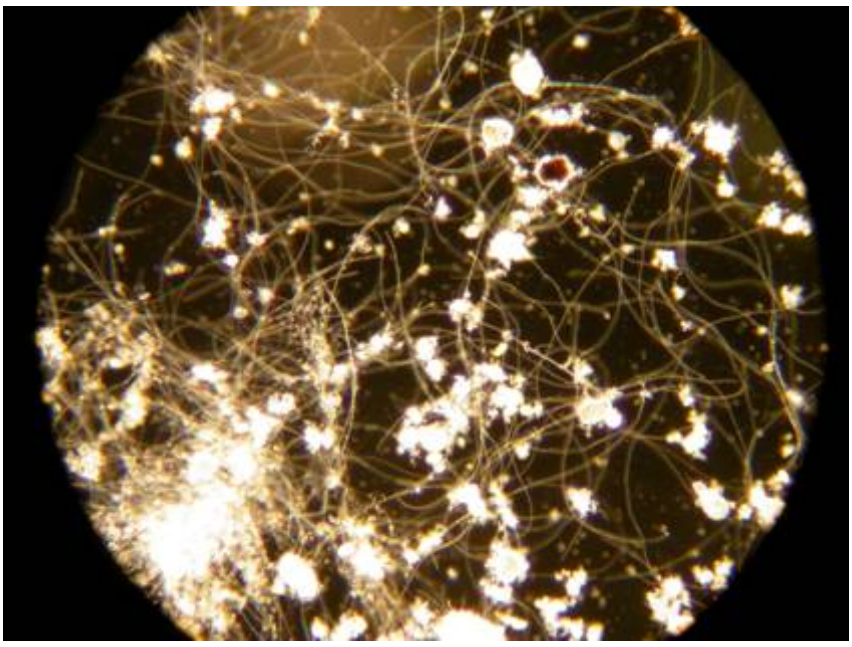


FIGURE 6B

EGF Aerobic Basin 1-14-08 (no filaments visible)

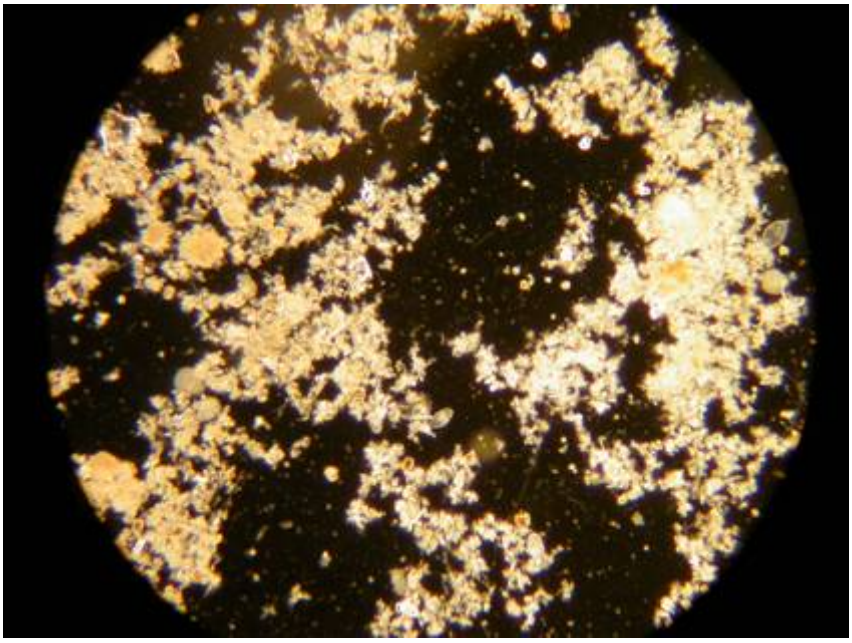


FIGURE 7

Moorhead Wastewater - India Ink stain 10-15-07 (polysaccharide) (100x)

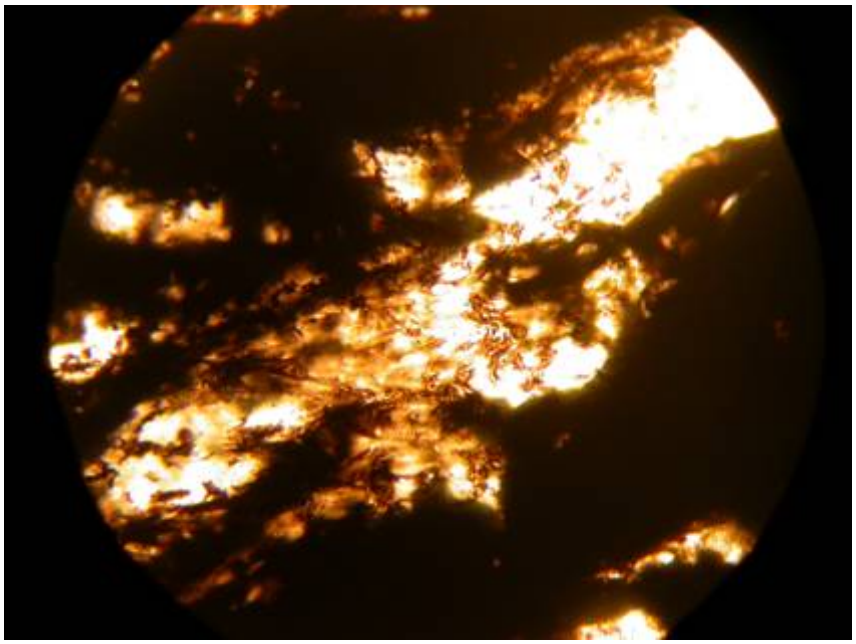


FIGURE 8

(9-17-07) 1000x Wet Mount (Lactophenol cotton blue)
Zero time - (1000x)

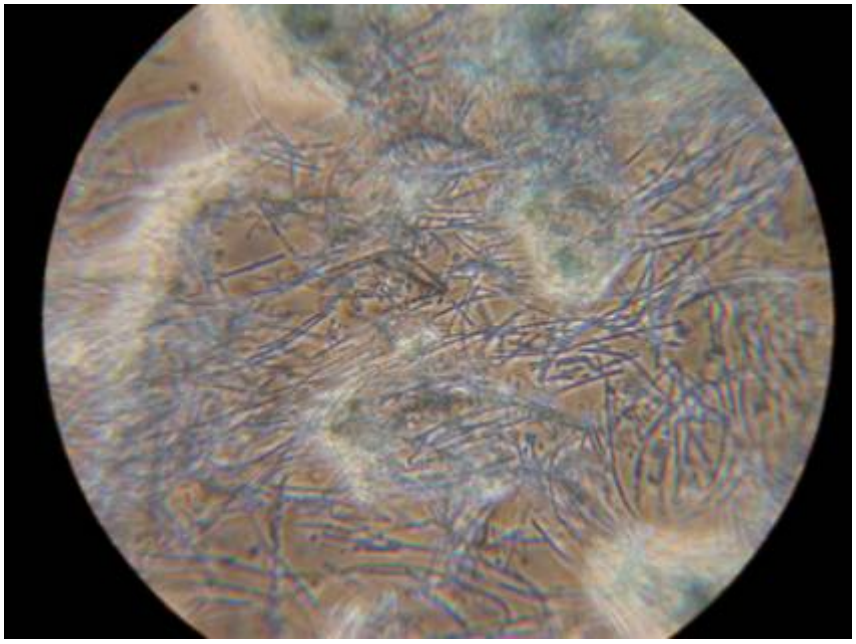


FIGURE 9

Start of Na hypochlorite Experiment - IV
Zero hr (Before chemical addition began)

MHD Anaerobic Tank (1-28-08) 1000x Wet Mount (Lactophenol cotton blue)

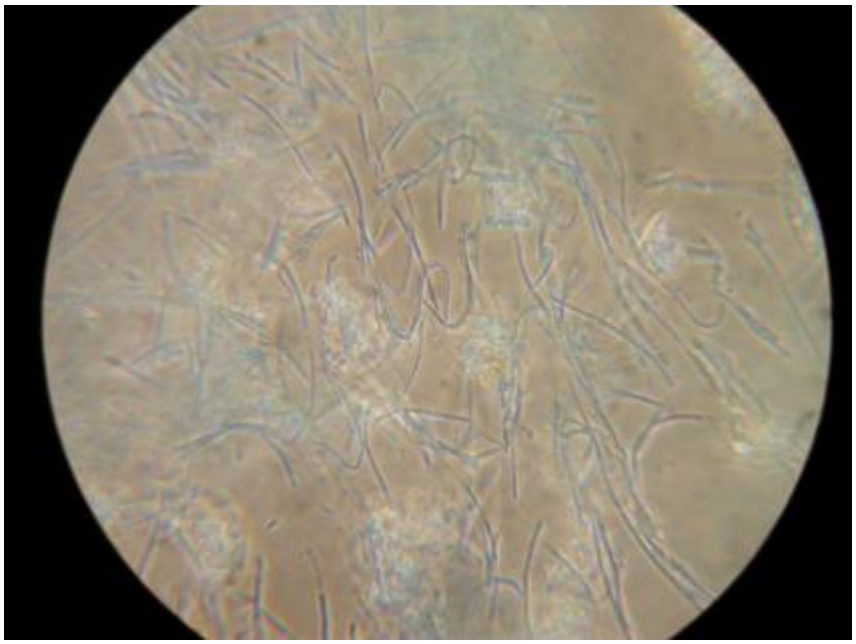


FIGURE 10

Na hypochlorite Experiment – IV End
MHD Anaerobic Tank (2-1-08) 1000x Wet Mount (Lactophenol cotton blue)

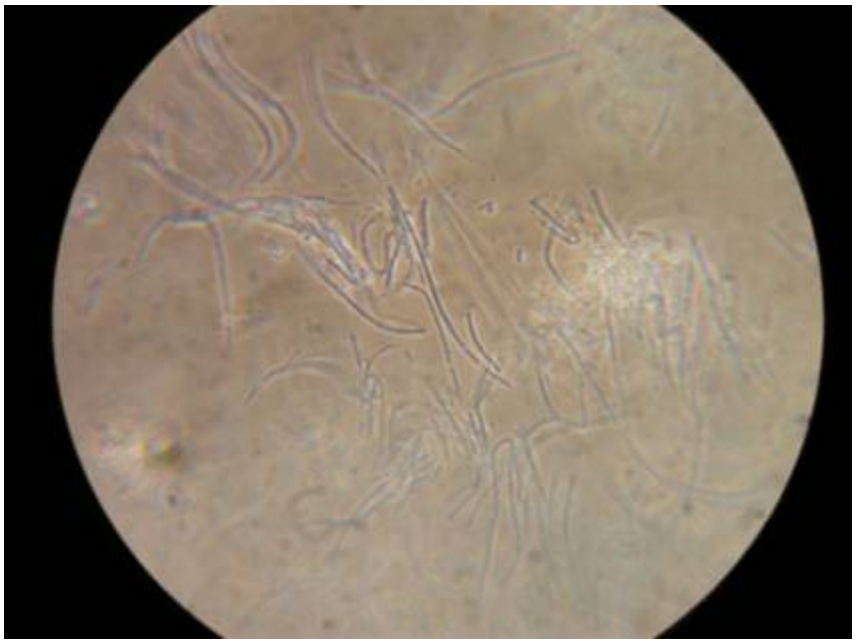


TABLE 3

Filamentous Bacteria, Other Bacteria, and Causes for Growth

1. Low DO Filaments – Type 1701, S. natans, Type 021N, Thiothrix, H. hydrossis, N. limicola

2. Limited Nutrients (N or P) – Thiothrix, S. natans, H. hydrossis, Types O21N, 0041 and 0675, Cyanobacterial tetrads

3. Low F/M Ratio – Type 0041, Nocardia, Types 0581, 021N, 1851, 0961, 0092, 0803, 0675, Microthrix parvicella, H. hydrossis

4. Sulfide Compounds or Septic Wastes – Thiothrix, N. limicola II, Beggiatoa, Types 021N, 0914, 0411, 0092, 0581, 0961, Spirillum

5. Low pH (<6.5) – Fungal filaments, masses of Zoogleal polysaccharide

6. High Grease and Oils – Nocardia, Microthrix parvicella, Type 1863

FIGURE 11

Moorhead WW Influent – Tetrads 1000x (Lactophenol cotton blue)

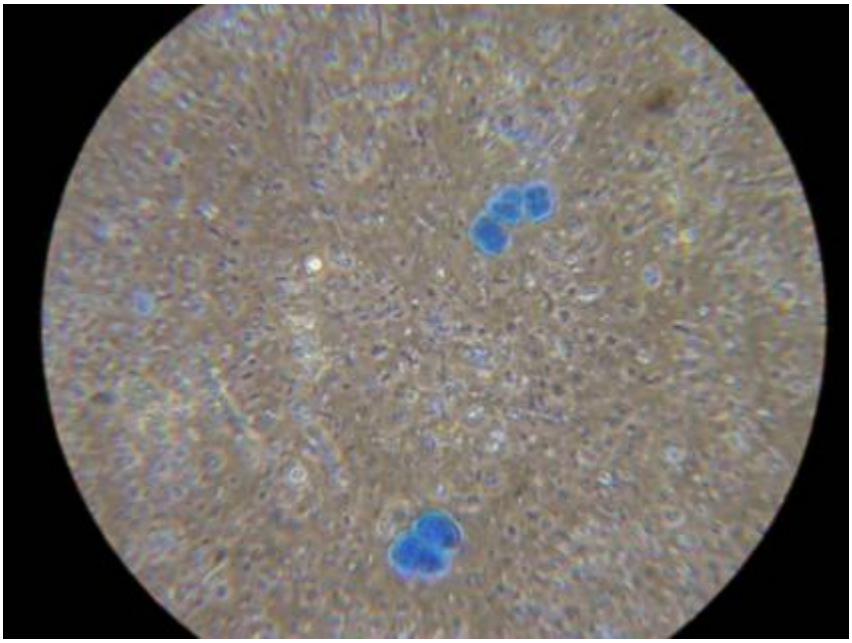


FIGURE 12

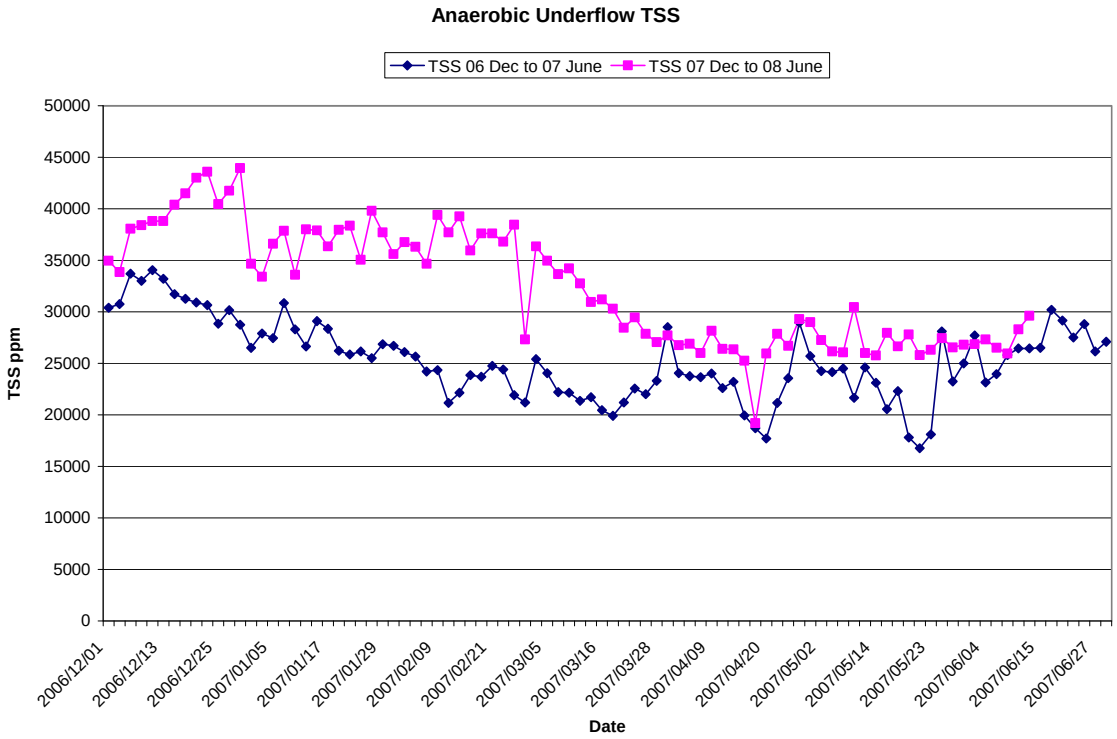
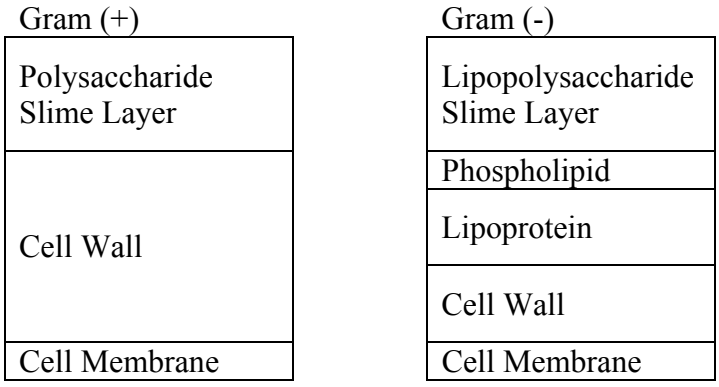
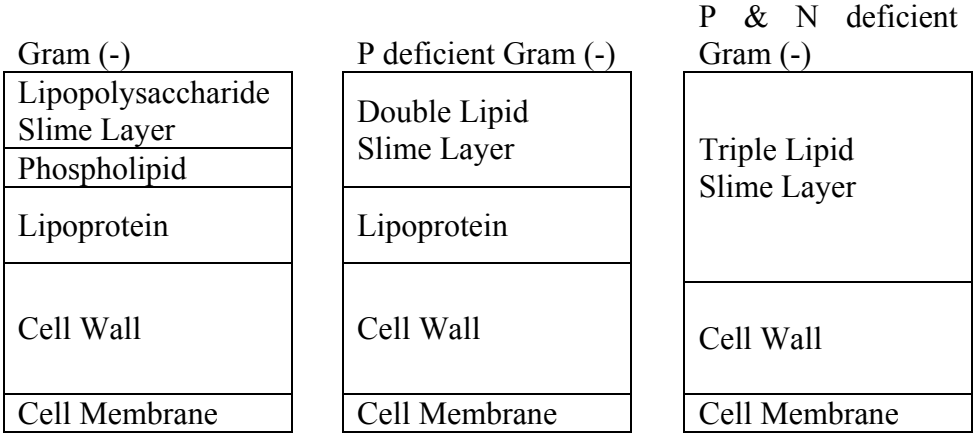


FIGURE 13 (4)

Gram Positive and Gram Negative Bacterial Cell Wall Differences



(Usually slime bulking is caused by the gram-negative bacteria.)



(When N & P are deficient, additional lipid is added to the slime layer, as the phospholipid and lipoprotein layers are not developed properly.)

TABLE 4

Moorhead Wastewater Data (Campaign 07/08)

Date	Location	pH	COD mg/L	TSS mg/L	TVSS % TVSS	Alkalinity	VFA	NH_4-N mg/L	P mg/L
4-4-08	An. Influent	4.4	40,350	660	540/81.8	-	1,925	40.8	-
	An. Tank	6.9	1,105	16,550	11,600/70.1	1,738	582	0.276	3.35
	Aerobic Tank	8.9	147	9,300	5,660/60.9	-	-	-	-
	An. Clarifier Effluent	7.3	3,604	3,460	2,560/74.0	-	-	-	-
	An. Clarifier Underflow	8.9	-	26,900	17,700/65.8	-	-	326.8	-
4-21-08	An. Influent	4.6	44,850	1,020	840/82.4	-	4,319	-	-
	An. Tank	7.2	284	16,250	11,750/72.3	2,869	66	56.8	5.9
	Aerobic Tank	8.0	46	9,040	5,780/63.9	-	-	6.08	2.55
	An. Clarifier Effluent	7.5	508	300	250/83.3	-	-	-	-
	An. Clarifier Underflow	7.8	-	25,950	17,750/68.4	-	-	464	5.7
4-25-08	An. Influent	4.3	46,150	900	800/88.9	-	3,737	-	-
	An. Tank	7.2	395	15,450	11,000/71.2	3,691	66	113.6	3.05
	Aerobic Tank	7.9	53	5,800	3,320/57.2	-	-	1.338	1.53
	An. Clarifier Effluent	7.5	593	347	287/82.7	-	-	-	-
	An. Clarifier Underflow	7.3		26,700	18,600/69.7	-	-	79.6	-

COD = Chemical Oxygen Demand
TSS = Total Suspended Solids
TVSS – Total Volatile Suspended Solids
NH_4-N = Ammonium Nitrogen
VFA = Volatile Fatty Acids
P = Phosphorus
An. = Anaerobic

TABLE 5

Differences in Wastewater COD Processed at Moorhead Factory during Campaign 2007 and 2008

Beet Campaign Year	Dates and Days for Processing of Wastewater	Total COD Processed through Campaign lbs COD
FY 2006/2007	9/1/06 – 7/21/07 = 324 days	29,626,528 (91,400 lbs/day – Average)
FY 2007/2008	8/23/07 – 6/19/08 = 302 days	31,111,483 (103,018 lbs/day – Average)
	Difference in days of processing = 22 days less	Difference in lbs COD processed F'2008 = 1,484,955 more

FIGURE 14