A Biological Analysis of a Sequence Batch Reactor, Investigating the Use of Fixed-Film Media to Increase Treatment Efficiency

Presented to the Faculty of Lycoming College in partial fulfillment of the requirements for Departmental Honors in Biology

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May 1, 2009

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Lycoming College Clean Water Institute in partnership with the Cromaglass®

International Wastewater Corporation

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ABSTRACT

The Cromaglass® Corporation, based in Williamsport, PA, manufactures Sequence Batch Reactor (SBR) systems for domestic wastewater treatment. A Cromaglass® CA-60, capable of treating 6,000 gallons of wastewater per day, was installed in 2005 at Hepburn-Lycoming Elementary School, part of the Williamsport School District. This SBR was the subject of a study by the Lycoming College Clean Water Institute, in conjunction with Cromaglass®. The purpose of this study was to examine the effects of the insertion of fixed-film media into the mixed liquor chamber of the SBR. Fixed-film media provides a large surface area for the growth of bacteria and protozoans that aid in the treatment process. It was hypothesized that this media would lead to increased treatment efficiency through the cultivation of these biological treatment organisms. This study focused primarily on the biological analysis of the mixed liquor, examining changes in protozoan populations after the insertion of fixed-film media and the corresponding biofilm growth. It was determined that the populations of stalked ciliates, rotifers, and suctorians increased significantly after the insertion of the fixed-film media, which indicates a healthier treatment system. Stalked ciliates increased from approximately 3000/mL to over 16,000/mL. Rotifers increased from 266/mL to over 2600/mL. Finally, suctorians increased from 0/mL to almost 900/mL.

INTRODUCTION

Sewage treatment plants are the biggest source of point-source pollution entering our nation's waterways (Chesapeake Bay Foundation, 2009). The nitrogen and phosphorus that remains in treated sewage effluent cause eutrophication, toxic algal blooms, dead zones, and other problems in the bodies of water into which they are released. Since the signing of the Chesapeake 2000 Agreement, emphasis has been placed on sewage treatment plants to upgrade their aging infrastructure and reduce the

amount of pollution they discharge into local streams (Chesapeake Bay Foundation, 2009). In more recent years, an increasing amount of pressure has been put on sewage treatment plants as they fail to meet the provisions of the agreement. Dozens of sewage treatment plants along the Susquehanna River are now being forced to upgrade equipment in an effort to reduce nitrogen and phosphorus levels going to the Chesapeake Bay.

With all of the pressure being placed on sewage treatment plants to improve their effluent quality, other methods of sewage treatment are being investigated. One such alternative method of sewage treatment is a Sequence Batch Reactor (SBR) unit, which has shown promise in its ability to remove nitrogen and phosphorus from wastewater, along with other pollutants (Mahvi, 2008). The Cromaglass® Corporation in Williamsport, Pennsylvania is one company that manufactures these SBR units. The Cromaglass® Corporation has been manufacturing fiberglass SBR units since 1965, and builds units of varying sizes, from those capable of treating 500 gallons per day to those capable up treating up to 200,000 gallons per day. These SBR units are designed for use in residential wastewater treatment as an alternative to traditional septic tank systems, and have been installed at businesses, homes, schools, and small communities (Cromaglass®, 2006).

Sequence Batch Reactor technology differs from that of a traditional sewage treatment plant because the SBR operates in time rather than in space. Sewage is treated on a batch basis, with a predetermined amount of sewage entering the unit at the beginning of every treatment cycle. The Cromaglass® SBRs have three chambers where the wastewater goes through five treatment steps: fill, aeration, denitrification (optional), transfer/settle, and discharge (See Figure 1). Each step in the cycle is timed so that a certain number of cycles can be completed per day. Wastewater enters the system and first flows through a non-corrosive screen which traps any inorganic solids. The filling chamber is also aerated and mixed in order to break up large organic solids so that they pass through the screen. Liquids and organic solids move into the aeration chamber or mixed liquor chamber, which is continuously

aerated and mixed. In the optional denitrification step, aeration to the chamber stops, but mixing continues and the chamber becomes anoxic. Under these conditions, anaerobic bacteria work to break down the organic nitrogen compounds in the sewage. The mixed liquor is then transferred to the clarification chamber, which is not aerated or mixed. Under these quiescent conditions, solids will settle to the bottom of the chamber, leaving clear, treated effluent on top. This final effluent can then be discharged to a leach field, sand mound, or permitted surface water. The sludge created in the clarification chamber can be returned into the aeration chamber for further treatment, or can be pumped to a sludge wasting tank (Cromaglass, 2006).

For this study, the Lycoming College Clean Water Institute (CWI) paired with Cromaglass® in order to test the use of fixed-film media to improve the treatment efficiency of their SBRs. Fixed-film media can be any sort of plastic, ceramic, or other substrate that is inserted into the SBR to increase surface area for bacterial growth. Since the SBR treatment process is dependent on the bacteria within the system, it was hypothesized that the addition of fixed-film media and the accompanying biofilm growth would increase the numbers of nitrifying and denitrifying bacteria, thereby increasing the amount of nitrogen removed by the system. Previous studies conducted by the CWI support that hypothesis, and also showed decreased BOD and solids (Yuda, 2008). Previous studies examined a CA-5 with a denitrification cycle, while this study investigated the effects of fixed-film media on a CA-60 with constant aeration and no denitrification cycle. This study hypothesized that inserting a fixed-film media would also improve treatment efficiency under these conditions. The SBR used in this study was installed in 2005 at Hepburn-Lycoming Elementary School and treats all of the wastewater generated at the school, including both restroom and kitchen waste.

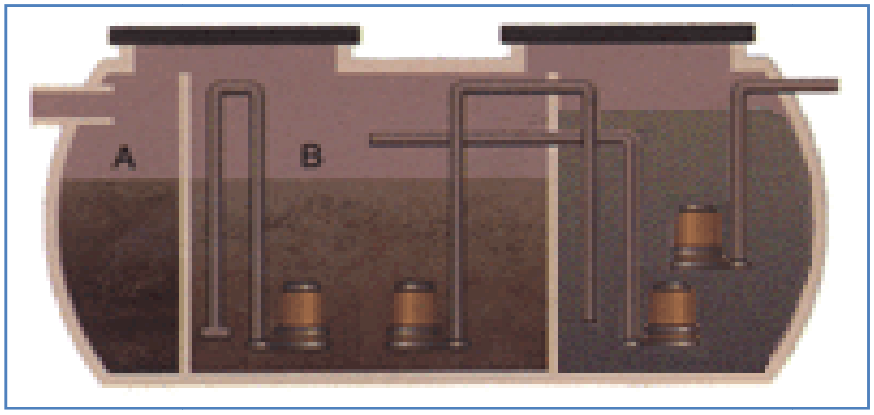
A second reason for conducting this study was the potential financial benefit for the Williamsport Area School District. The CA-60 at Hepburn-Lycoming Elementary School, because it treats both restroom and kitchen waste, has a chronic problem with foaming due to high amounts of lipids in

the wastewater. This foaming is primarily caused by the filamentous bacteria *Nocardia*, and can lead to treatment problems. The school district has been paying for bioaugmentation products, which are bacterial cultures that must be added to the system daily in order to digest lipids and reduce foaming. These cultures contain *Bacillus* and *Pseudomonas* bacteria that are designed to reduce fats, oil, and grease within the system. The cultures are manufactured by Maryland Biochemical Company, Inc. (http://www.marylandbiochemical.com/about.htm). During this study, those cultures were not added to the system so that the effects of the biofilm could be examined. If the foaming decreased as a result of the biofilm, then the biofilm could be left in the system indefinitely, providing the same benefits as the purchased cultures, but at virtually no cost.

Two studies were conducted simultaneously at the Hepburn-Lycoming site, one that involved chemical analysis of the water quality and one that involved biological analysis of the mixed liquor. This paper focuses on the biological analysis, using protozoans and filamentous organisms as an indicator of treatment efficiency. Previous studies have shown that the presence of certain types of protozoans within the mixed liquor can indicate healthy mixed liquor (Martin-Cereceda et al, 2006). Previous research has also found that the nitrifying and denitrifying bacteria in the mixed liquor are dependent on the protozoan community, so by studying the protozoans it is possible to gain information about the health of the bacterial community (Petropoulos et al, 2005). Since the bacteria perform the work of breaking down organic compounds and reducing nitrogen and phosphorus, a healthy bacterial community can indicate good quality effluent. Protozoans help to create this healthy environment in two major ways: they secrete polysaccharides and mucoproteins which help bacteria and other suspended matter to stick to the floc particle, and they crop the bacteria population. Since protozoans eat bacteria, they remove excess bacteria from the system, which stimulates the growth of more active floc particles that can assimilate more organic matter (Johannes, 1965 and Curds, 1965).

Finally, filamentous organisms, which can be bacteria or fungi, are also important in the treatment process. The long filaments connect floc particles in the mixed liquor, making larger particles that settle out more easily in the clarifier. The problem at Hepburn-Lycoming is that the primary filamentous organism is *Nocardia*, which has very short, branched filaments that do not create good floc particles. With the insertion of the biofilm, it is hypothesized that other types of filaments can gain abundance and compete with *Nocardia* to create a healthier treatment system. At the end of the study, the biological analysis will be compared with the chemical analysis to determine if changes in the biological community lead to an improvement in effluent quality, providing future Cromaglass® operators with a quick and simple way to evaluate how well their system is working.

Figure 1. Schematic of a Cromaglass® SBR. Chamber A is the fill chamber, B is the aeration/denitrification chamber, and the chamber farthest to the right is the clarification chamber.



MATERIALS AND METHODS

The sequence batch reactor unit used in this study was a Cromaglass® Corporation CA-60, which is capable of treating 6000 gallons of wastewater per day. This unit was installed in 2005 at Hepburn-Lycoming Elementary School in Williamsport, PA, and treats all wastewater generated at that property. Hepburn-Lycoming houses approximately 350 students and 35 faculty which generate an average wastewater flow of 2,600 gallons per day. The system operates on 144-minute cycles without an influent holding tank; instead all wastewater feeds directly into the treatment unit. The wastewater first enters the mixed liquor/aeration chamber, where it is constantly aerated and mixed. The mixed liquor is pumped into the clarification chamber or clarifier, where it is not aerated. The stillness of the water in this tank allows solids to settle to the bottom of the tank and create sludge, which is wasted into a separate sludge wasting tank. The wastewater remains in this chamber for a total of 70 minutes, and then is discharged into the chlorine tank, where it is chlorinated to kill any remaining bacteria. The final effluent is discharged into a pipe leading under the road, eventually emptying into Lycoming Creek (see Figures 2-3). The Williamsport Area School District has an NPDES permit to allow surface water discharge. Bacterial cultures were being added to the unit every day in order to control foaming and to break down excess lipids resulting from kitchen waste.

Testing of this unit began on January 27, 2009, at which point the bacterial cultures were no longer added to the system. The system was allowed to re-establish for just over two weeks in the absence of the cultures before biological analysis began on February 5, 2009. After a total of four weeks of sampling, fixed-film media was added to the system on February 26, 2009. The fixed-film media, designed in order to increase the bacterial and protozoan content of the SBR, consisted of "coffee can" Bio-Rings® created by Jaeger. Eight of the coffee cans (see Figure 4) were inserted into a container made of PVC pipe (see Figure 5). Two PVC pipe containers were suspended in the mixed liquor chamber and two were suspended in the clarifier. One container in each chamber had a screw-top lid

that could be removed for inspection of the fixed-film media. The fixed-film media were allowed to colonize for one week before sampling resumed. The first day of sampling with the fixed-film media installed was March 10, 2009. The study continued with the fixed-film media in place until April 14, 2009, at which point they were removed and sampled.

Samples were taken twice a week for the duration of the study. Samples were drawn from the mixed liquor chamber, the clarifier, and the chlorination tank on Tuesdays and Thursdays. Samples were taken as closely as possible to the end of the settling phase of the cycle, right before discharge. This time frame was chosen so that the sample would be as close to the actual effluent quality as possible. The samples were taken using a "grab sampler" (See Figure 6). The grab sampler consisted of a plastic sampling head connected to an eight-foot pole for ease of sampling the underground units. A sample jar could be attached to the head, which was inserted approximately one foot below water level in the chamber. A metal ring at the top of the pole was pulled, which opened holes in the sampling head and allowed the wastewater to fill the jar. The sampling jars held 500mL, and two jars were collected from each chamber. Also, an extra 250mL sample (approximately) was collected from the mixed liquor chamber to be used for the biological analysis. The temperature and dissolved oxygen level in the mixed liquor chamber was also recorded using a probe.

In the lab, the samples were evaluated for many water quality parameters, including total suspended solids, mixed liquor volatile suspended solids, nitrate, nitrite, ammonia, orthophosphate, and total phosphorus. The chemical analysis was conducted as a separate study and is examined in the corresponding paper *The Use of Fixed Film Media to Reduce Nutrient Levels in a Sequence Batch Reactor*, written by Amanda Lane (2009).

Along with the chemical analyses, the protozoan community within the mixed liquor was studied and is the main focus of this paper. The mixed liquor sample was lightly shaken to re-suspend any settled particles, and a small sample was drawn with a 1 mL transfer pipette. Two to three drops of

sample was placed on a microscope slide and a coverslip was placed on top, being careful to avoid air bubbles underneath. Extra sample was removed using a KimWipe until the coverslip no longer floated on the sample. Once that was accomplished, the sample remaining under the coverslip was approximately 0.05mL (Gerardi, 2008). The sample was then observed using a phase-contrast microscope. A phase-contrast microscope makes the protozoans within the sample easier to see. Motile protozoans were counted if they moved into or through the field of view during counting. Ten fields of view were chosen at random, and every protozoan within the field of view was counted and identified to a group based on method of locomotion. The protozoan groupings used were swimming ciliates, crawling ciliates, stalked ciliates, flagellates, suctorians, rotifers, and nematodes. Rotifers and nematodes are actually classified as metazoans because they are multicellular organisms, but are referred to as protozoans in this paper for the sake of simplicity. If possible, each protozoan was identified to genus level, though the identification proved difficult during the course of this study. This process was repeated two more times for a total of three slides.

After the examination of three slides for a total of 30 fields of view, the relative percentage of each group of protozoans was calculated. First, the average number of protozoans per field of view was calculated. This number was then multiplied by 600 (the number of fields of view under a 200x objective), and then by 20 (to expand the 0.05 mL to 1.0 mL) in order to find the number of organisms per milliliter of mixed liquor. The total number of organisms in one mL was found by adding the totals of all groups. After these calculations were completed, then the average number of organisms per mL was found by averaging the totals for each slide. The total number of organisms per group was also averaged across the three slides. Finally, the number of organisms per group was divided by the total number of organisms to find the relative percentage of each protozoan group within the sample. At the end of the study, a rank sum test was performed to determine if there was a significant difference between the relative percentages of each protozoan group before and after the insertion of the biofilm.

The rank sum test is described in Glase et al (1979). At the end of the study, the results of the biological analysis were compared with the chemical analysis from the accompanying study in order to determine if protozoan community structure could be used as an indicator of treatment efficiency and final effluent quality.

A second portion of the microscopic analysis examined the characteristics of the floc particles within the mixed liquor, along with the filamentous organisms found associated with those flocs. The floc particles were listed as either round or irregular, open or closed, firm or weak, and as small, medium, or large. These classifications were based on those explained in Eikelboom's book (2000). The abundance of filamentous organisms within the sample was ranked according to Eikelboom's Filament Index, with 0 being scarce and 5 being overly abundant. It was attempted to indentify the filamentous organisms to genus or type, however this also proved difficult due to the short length of the filaments in the samples, which were not easily seen past the edges of the floc particles.

2. View of Cromaglass® CA-60 SBR at Hepburn-Lycoming Elementary School. The two large chambers in the middle are the mixed liquor chamber and clarifier, and the small round opening in the foreground is the chlorine tank. Samples were taken from those three chambers.

Figure 3. View of Lycoming Creek across the street from Hepburn-Lycoming Elementary School. The effluent from the Cromaglass® SBR is discharged directly into Lycoming Creek.

Figure 4. "Coffee Can" Fixed-film media before and after insertion into an SBR. Note the large amount of surface area created in the center of the media, designed to promote as much biofilm growth as possible. This growth can be seen on the right. This photo was taken during the preliminary study at the Williamsport Sanitary Authority site in the fall of 2008.

Figure 5. Colonized PVC-pipe container designed to hold "coffee can" fixed-film media, shown after removal from SBR. This photo was taken during the preliminary study at the Williamsport Sanitary Authority site in the fall of 2008.

Figure 6. Grab Sampler with attached sampling jar being lowered into Cromaglass® SBR.

RESULTS

Over the course of the study, the SBR at Hepburn-Lycoming Elementary School was sampled nineteen times, nine times before the insertion of the fixed-film media and ten times after insertion. Biological analysis did not begin until the February 5th sampling, when it was estimated that all of the previously used biological cultures had been flushed out of the system. Also, the final sample on April 9th could not be used for biological analysis due to a broken pipe which stopped mixing in the aeration chamber. The mixed liquor had begun to settle in the absence of mixing, and therefore the sample contained very little floc particles or protozoans. In total, there were six samples analyzed before insertion of the biofilm and nine afterwards. The charts and tables given below summarize the results of this study.

Chart 1. This chart shows the relative percentages of the major groups of protozoans found within the mixed liquor samples of the SBR. The two most apparent changes over the course of the study are the increase in the number of stalked ciliates and the decrease in the number of flagellates. Also note the appearance of suctorians only after the fixed-film media was inserted on February 26th. The insertion of the media is marked by a black line. The large gap in the chart represents the week after the media was inserted, when no sampling was done in order for the biofilm to colonize.

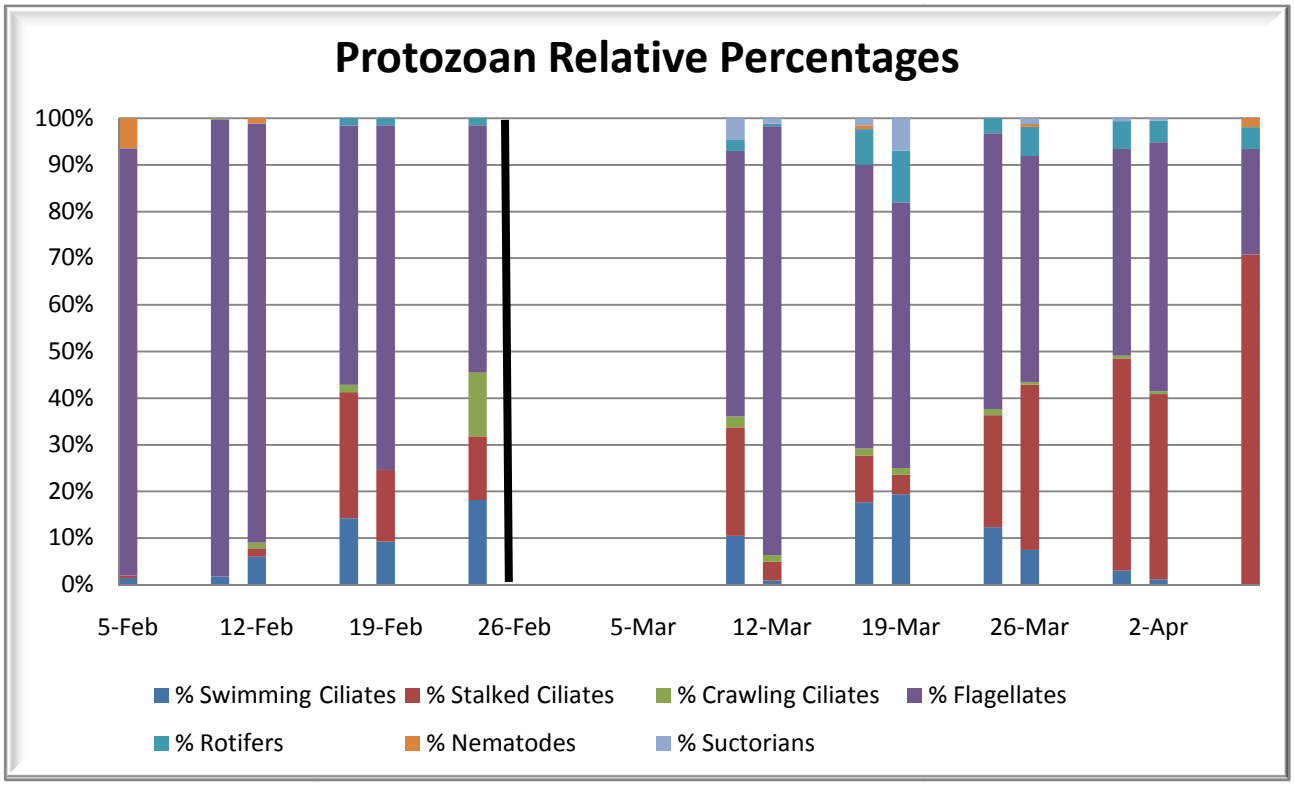


Chart 2. This chart displays the total number of protozoans per milliliter of sample. At the beginning of the study, the number of protozoans was close to 300,000 per mL, but dropped very quickly. Except for one point on March 12, the number of protozoans seemed to stabilize just above or below 50,000 per mL. The fixed-film media was inserted on February 26th and is marked with a black line.

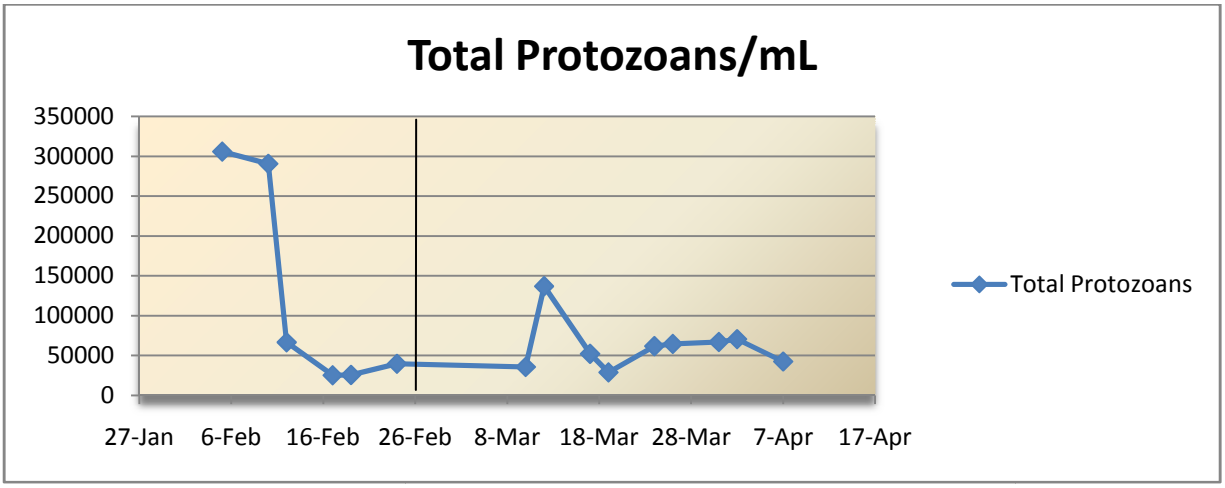


Chart 3. This chart graphs the total number of flagellates per milliliter of sample. Note how this graph is very similar to the graph of total protozoans, with the only deviation towards the end of the study, when the number of flagellates decreases slightly more than the total protozoans. The fixed-film media was inserted on February 26th and is marked with a black line.

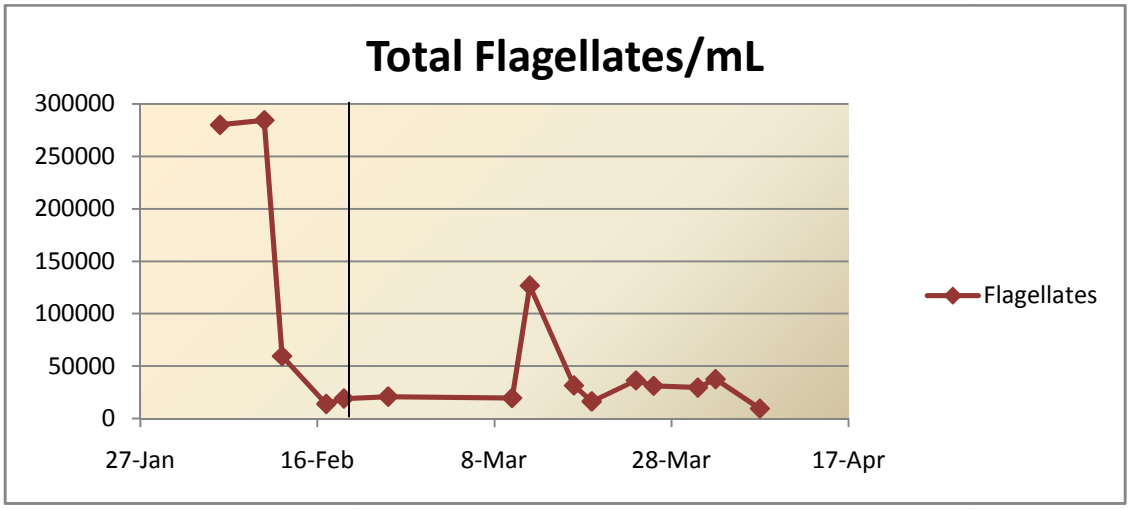


Chart 4. This chart shows the total number of stalked ciliates per milliliter of sample. The number of stalked ciliates increased slightly at the beginning of the study, leveled out, and then increased dramatically after March 20th. This trend is the exact opposite of the total protozoans and the flagellates. The fixed-film media was inserted on February 26th and is marked with a black line.

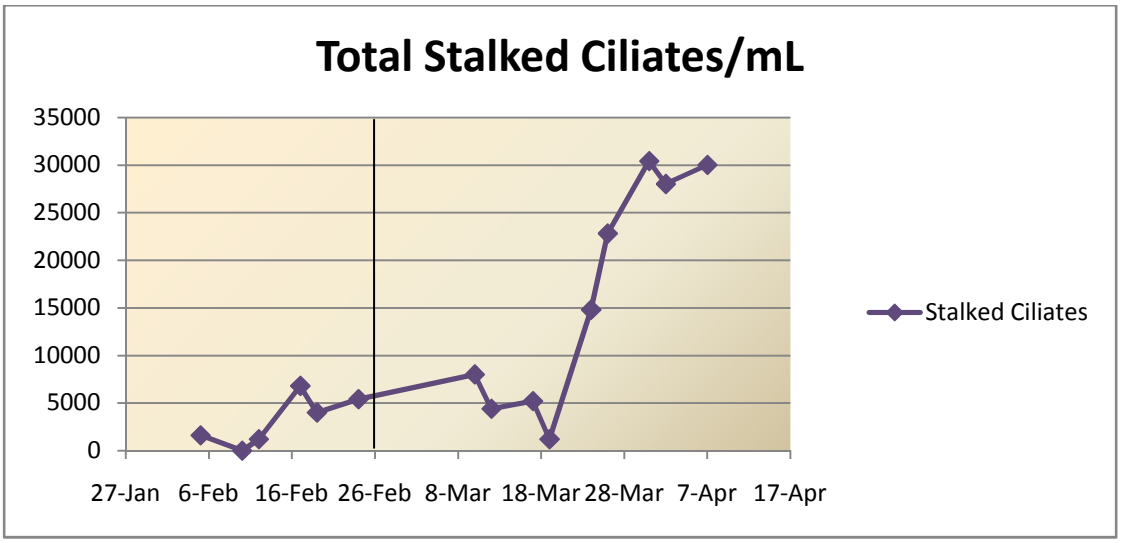


Chart 5. This chart displays the total number of swimming ciliates per mL of sample. This group showed the most variability throughout the study, and then declined sharply after March 20th. The fixed-film media was inserted on February 26th and is marked with a black line.

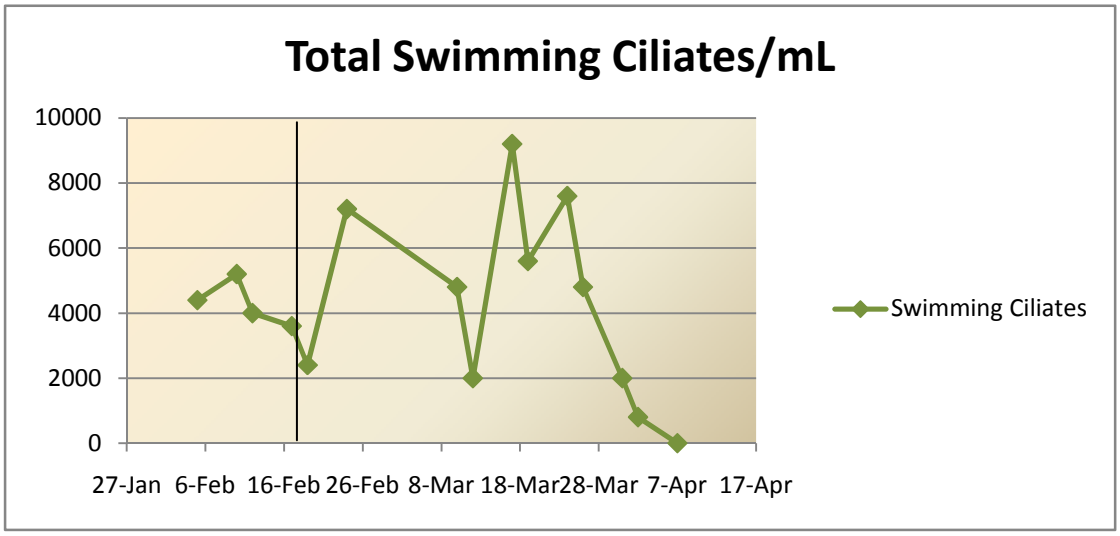


Chart 6. This chart shows the total number of rotifers per milliliter of sample. For the first few weeks, the numbers increased very slightly, then increased sharply after March 12th. After this date, the numbers showed considerable variability, but remained higher than previously. The fixed-film media was inserted on February 26th and is marked with a black line.

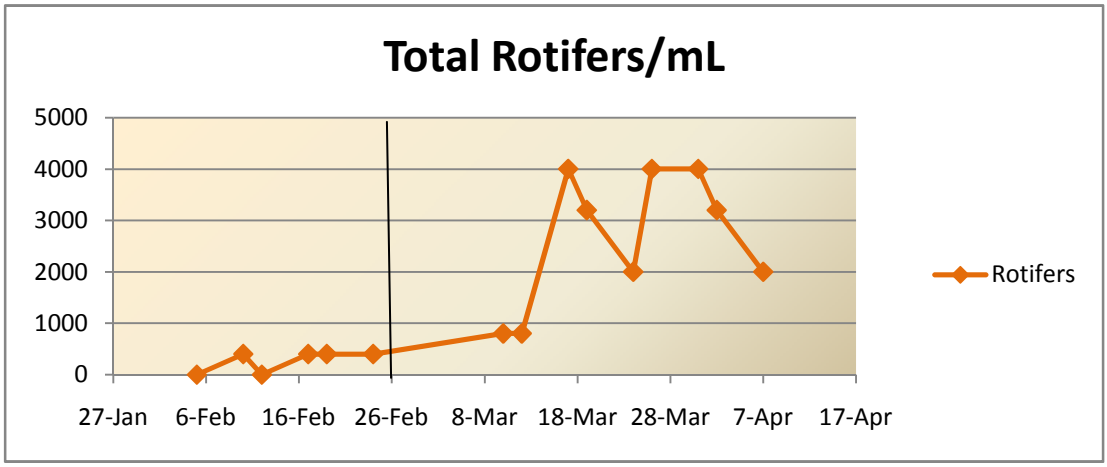


Chart 7. This chart shows the total number of crawling ciliates per milliliter of sample. This group mostly remained below 1,000 per mL, but displayed a large increase on February 24th. The fixed-film media was inserted on February 26th and is marked with a black line.

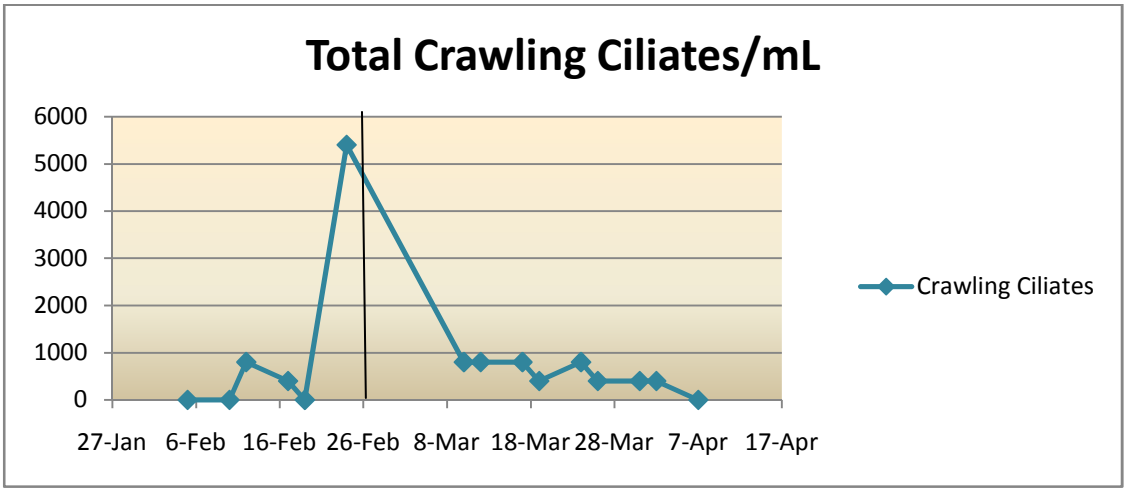


Chart 8. This chart shows the total number of nematodes per milliliter of sample. Except for a very high number at the beginning of the study, nematodes were not prevalent in the system. There were many days when zero nematodes were observed in the sample. The fixed-film media was inserted on February 26th and is marked with a black line.

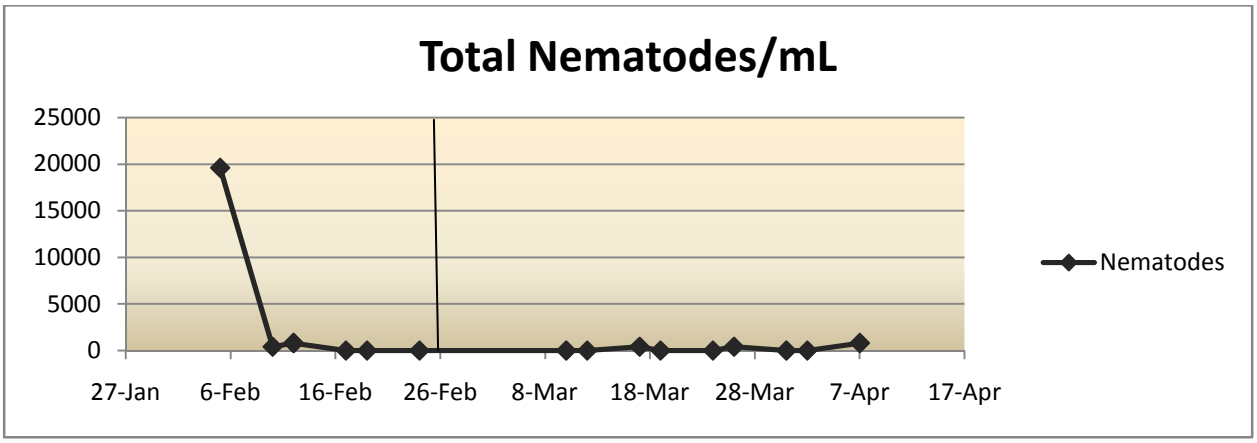
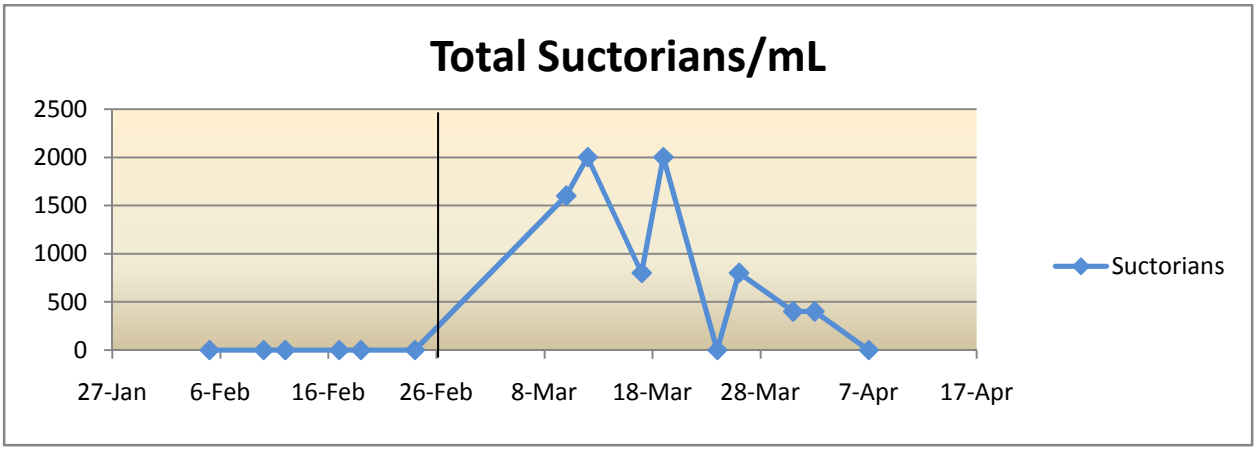


Chart 9. This chart displays the total number of suctorians per milliliter. Zero suctorians were found in samples taken before insertion of the fixed-film media. After insertion of the biofilm, however, the number of suctorians increased dramatically, then decreased towards the end of the study. The fixed-film media was inserted on February 26th and is marked with a black line.



In attempting to identify the protozoans present, many of the protozoans were identified to genus level. The predominant flagellate genus present at the beginning of the study was *Bodo*, and as the study progressed other, larger flagellates such as *Peranema* became more common, as well as others that could not be identified. The stalked ciliates consisted of mostly genus *Vorticella*, although a few other genera, such as *Opercularia*, were observed, mostly at the beginning of the study. The swimming ciliates found at the beginning of the study were mostly of the genera *Chilodonella* and *Trachelophyllum*. After introduction of the biofilm, however, *Chilodonella* populations decreased and eventually disappeared from the mixed liquor, leaving *Trachelophyllum* as the only swimming ciliate observed. Finally, the suctorians observed in the latter half of the study were primarily of the genus *Acineta*.

Table 1. This table summarizes the rank sum tests which were performed to determine the statistical significance of the results. The number of total protozoans, flagellates, stalked ciliates, swimming ciliates, and rotifers per mL of sample were compared before and after insertion of the biofilm. N_1 is the number of sampling dates before insertion of the fixed-film media, and N_2 is the number of sample dates after insertion. At an alpha-level of 0.05, the statistically significant results were for stalked ciliates, rotifers, and suctorians, which showed a significant increase during the course of the study. The null hypothesis was accepted for all other groups, indicating that the numbers of protozoans in these groups were similar before and after insertion of the biofilm.

	N_1	N_2	Mean Before +/- SD	Mean After +/- SD	α- level	Significance
Total Protozoans	6	9	12.54×10^4 +/- 13.46×10^4	62.09×10^3 +/- 31.58×10^3	0.05	No Significant Difference
Flagellates	6	9	11.30×10^4 +/- 13.20×10^4	37.64×10^3 +/- 34.74×10^3	0.05	Non Significant Difference
Stalked Ciliates	6	9	31.67×10^2 +/- 26.55×10^2	16.09×10^3 +/- 11.88×10^3	0.05	Significant
Swimming Ciliates	6	9	44.67×10^2 +/- 16.28×10^2	40.89×10^2 +/- 31.23×10^2	0.05	No Significant Difference
Rotifers	6	9	266.67 +/- 206.56	26.67×10^2 +/- 13.11×10^2	0.05	Significant
Crawling Ciliates	6	9	1100.00 +/- 2130.73	533.33 +/- 282.84	0.05	No Significant Difference
Nematodes	6	9	34.67×10^2 +/- 79.10×10^2	177.78 +/- 290.59	0.05	No Significant Difference
Suctorians	6	9	0.0 +/- 0.0	888.89 +/- 794.43	0.05	Significant

The other biological indicator that was evaluated was the filamentous organisms found in the mixed liquor. The most abundant filamentous organism by far was *Nocardia*, as previously mentioned. Two to three other filamentous organisms were observed within the floc particles; however the filaments did not protrude very far past the edges of the floc, making identification difficult. Due to the problems with filament identification, the only observation recorded was the filament index (FI). The filament index was developed by Eikelboom, as previously mentioned. For the duration of the study, the mixed liquor had a filament index ranging between 0 and 1. On March 10th and April 7th, a higher filament index was recorded (between 2 and 3). This change was primarily due to an increase in *Nocardia* on those dates.

Finally, observations were made about the general condition of the floc particles. Table 2 shows the recorded characteristics of the floc particles.

Table 2. This table displays observations made on the characteristics of the floc particles found in the mixed liquor samples. The flocs were always round and were typically firm, except for the week right before and right after the biofilm was inserted. The floc particles were also usually medium in size, though occasionally there were an abundance of small flocs. Large flocs were also seen occasionally, although they typically formed around some sort of inorganic material in the mixed liquor. Finally, the structure of the floc showed some variability, even within one sample. Some flocs were very compact, while others were more open.

	Shape	Structure	Strength	Size
5 Feb	Round	Open/compact	Firm	medium
10 Feb	Round	Open/compact	Firm	Medium/Large
12 Feb	Round	Open/compact	Firm	Small/Medium
17 Feb	Round	Open/compact	Firm	Small
19 Feb	Round	Open/compact	Slightly weak	Medium
23 Feb	No data			
10 March	Round	Open/compact	Weak	Medium
12 March	Round	Open/compact	Weak	Small
17 March	Round	Open/compact	Weak	Medium
19 March	Round	Open	Firm	Medium
23 March	Round	Open	Firm	Medium
31 March	Round	Open/compact	Firm	Medium
2 April	Round	Compact	Firm	Medium
7 April	Round	Compact	Firm	Medium

Figure 7. This photograph shows a view of the top of the fixed-film media container removed from the mixed liquor. The biofilm growth is visible on and around the coffee can.

Figure 8. This photograph shows the top coffee can after removal from the PVC container. The biofilm growth is clearly shown.

Figure 9. This figure shows the bottom seven coffee cans after they were dumped out of the PVC container. The top three coffee cans had a lot of biofilm growth, but as is apparent from this photograph, the bottom cans had very little, if any, biofilm growth. The fixed-film media containers placed in the clarifier displayed similar results.

DISCUSSION

In this study, it was found that the insertion of a fixed-film media did produce statistically significant changes in stalked ciliate, rotifer, and suctorian populations. These results partially support the hypothesis that biofilm growth creates a healthier community of bacteria and protozoans. Although there appeared to be large trends in the relative abundance of flagellates and total protozoans, the trends were not significant. A significant decrease in flagellates and a significant increase in crawling ciliates would have supported this hypothesis as well, and it is curious that those changes were not observed during this study.

Throughout the duration of this study, various system malfunctions occurred with the Cromaglass® unit at Hepburn-Lycoming. Some of these problems could have contributed to the variability seen in the graphs of protozoans. On Tuesday, February 24th, the clarification chamber was filling, and was therefore very turbid, during the normal sampling time, indicating a change in the timing cycle. The Williamsport School District (WSD) maintenance supervisor was contacted and confirmed a power outage during the previous weekend that had changed all of the timing cycles. An engineer from Cromaglass® reset the timer so that sampling could continue at the same time as previously. The fact that the clarification chamber was filling did not affect the biological analysis, which only relied on the mixed liquor chamber. A similar event occurred between March 10 and March 12, and the clarifier was again filling during the normal sampling time. This time cycle change was due to a flow-through cycle that occurred due to a clogged toilet in the school building. In a high-water event, such as a toilet that keeps flowing, the SBR is programmed to enter “flow-through” mode, where wastewater flows through the unit with no settling phase. This cycle occurs in order to prevent wastewater from flooding the unit, spilling out onto the ground. The cycle timing was not reset after this event, and the sampling time had to be adjusted. A second flow-through occurred the following weekend, but did not significantly alter the cycle timing. Power outages and flow-through cycles affect the timing of the treatment cycles

because, once the cycle begins again, it starts over at minute 1. For example, if a power outage occurred in minute 133 of the cycle, the cycle would begin again at minute 1 once power was returned.

Aeration pump failures also occurred in the mixed liquor chamber, which may have been responsible for some of the variability in the protozoan community. Pump 2 failed sometime in the week after the fixed-film media was inserted, and was replaced on March 9, 2009. The pump failure was noticed by a WSD maintenance employee who observed that the mixed liquor chamber was not aerating/mixing. It is unknown how long the mixed liquor chamber was quiescent before the second pump turned on. Pump 1 later failed sometime between April 7 and April 9, 2009. The mixed liquor chamber was not mixing or aerating during sampling time on April 9, and WSD employees were fixing the pump. Samples were taken for biological analysis, but the solids had already settled to the bottom of the chamber and there was very little to observe under the microscope. Due to this circumstance, protozoan counts were not performed on this day. By examining Figures 2-5, it should be noted that many of the significant changes in protozoan populations were observed in the two weeks after fixed-film media insertion. These changes may have been due to the beginning growth of the biofilm, or the changes could have resulted from the system malfunctions occurring during that time. In a real-world situation such as was used in this study, there is always the possibility of other factors influencing the results. Since the protozoan populations did not return to their previous levels after the system malfunctions, it can be stated that the population trends occurred due to the removal of the cultures and/or due to biofilm growth.

Although the trends in protozoan populations were not statistically significant, just their presence can indicate certain characteristics about the health of the treatment system. The flagellate *Bodo* indicates a high sludge load (>0.4 kg BOD/kg MLSS per day) and/or a lack of oxygen (Eikelboom, 2000). The stalked ciliate *Opercularia* also usually occurs at higher sludge loading levels, between 0.2 and 0.3 kg BOD/kg MLSS per day. The stalked ciliate *Vorticella* and the swimming ciliate *Trachelophyllum*

both occur commonly at sludge loading levels that are less than 0.4 kg BOD/kg MLSS per day (Eikelboom, 2000). Based on this data and the observations made during the study, it can be concluded that the sludge loading level decreased over the course of the study, leading to an increase in protozoans favoring lower sludge loading levels. The only observed protozoan that does not support this conclusion is *Chilodonella*, which was observed at the beginning of the study but disappeared in one to two weeks. *Chilodonella* typically occurs in activated sludge where the sludge load is less than 0.2 kg BOD/kg MLSS per day. The other protozoans observed at this time, however, point to a much higher sludge loading level, which may be the reason for the disappearance of this genus. Finally, *Peranema* was also observed during the latter half of the study; however, this genus is regularly observed in activated sludge and does not indicate any certain condition (Eikelboom, 2000). Also, although there was a significant increase in suctorian populations, Eikelboom states that these protozoans are regularly observed in activated sludge in small populations, and are not indicative of any certain condition.

Michael Gerardi, in his book Microscopic Examination of the Activated Sludge Process, presents a saprobic index which uses protozoans to determine the health of the mixed liquor biota and the final effluent quality. Protzoans such as *Bodo* and *Peranema*, among others, indicate a polysaprobic condition, which has high flow, severe organic overload, low dissolved oxygen, poor biota health, and poor effluent quality. *Chilodonella* and one species of *Vorticella*, among others, indicate an alpha-polysaprobic condition, which has high flow, organic overload, low dissolved oxygen, poor biota health, and poor effluent quality. The beta-mesosaprobic condition is indicated by *Opercularia*, *Trachelophyllum*, and a different species of *Vorticella*, among others. This condition is characterized by low flow, high organic loading, adequate dissolved oxygen, moderate biota health, and acceptable effluent quality. Finally, the oligosaprobic condition is indicated by rotifers, nematodes, and two different species of *Vorticella*. This condition is characterized by low flow, moderate organic loading, adequate dissolved oxygen, excellent biota health, “polished” effluent quality, and significant

concentrations of nitrate and sulfate. Due to the difficulty in identifying Vorticella to species level, it cannot be determined exactly which condition is indicated by the Vorticella found in this study. Based on the other protozoan populations observed and their changes throughout the study, it can be stated that the mixed liquor in the Hepburn-Lycoming SBR changed from a polysaprobic/alpha-polysaprobic condition to a beta-mesosaprobic and possibly oligosaprobic condition after insertion of the biofilm.

While researching and identifying protozoans, it was discovered that some books classify *Trachelophyllum* as a crawling ciliate, even though it also swims through the mixed liquor (Eikelboom, 2000). In the samples from Hepburn-Lycoming, *Trachelophyllum* was observed to be swimming much more often than crawling, so it was counted as a swimming ciliate. Other sources list *Trachelophyllum* as a free-swimming ciliate (Gerardi, 2008). *Chilodonella* is also listed as a free-swimming ciliate in some sources and as a crawling ciliate in others (Eikelboom, 2000 and Gerardi, 2008), but also were counted as swimming ciliates in this study because they were observed swimming in the mixed liquor. These conflicting sources are a potential source of error in this study, since it is not certain how to classify these two organisms. It is possible that the number of crawling ciliates should be much higher, while the number of swimming ciliates should be lower. One other possible source of error is that *Vorticella* has a free-swimming stage, which also was not learned until after the study (Barlow and Finley, 1976). After doing some additional research, it was discovered that the free-swimming stage looks very similar to a few organisms that were identified as swimming ciliates in the study, but could not be identified to genus level. It cannot be determined for sure, however, if those organisms were actually swimming ciliates or if they were the free-swimming stage of *Vorticella*.

The most surprising result of this study was that the biofilm in either chamber did not colonize as well as was hypothesized. Only the top 2-3 coffee cans (out of 8) had any sort of measurable growth on them when removed at the end of the study. The coffee cans which did have growth on them were closest to the surface, and biological analysis proved that the “growth” on the biofilm was more similar

to what is found in the foam that exists on the surface of the mixed liquor. The only organism indentified from the swabs of the coffee cans was *Nocardia*, which was found to be overly abundant. The possible reason for this lack of colonization could be that the SBR is programmed to aerate constantly. It is hypothesized that there was too much mixing occurring in the mixed liquor chamber, which could have prevented the bacteria from attaching to the coffee can, or it could have sheared all of the growth off of the coffee cans during a high flow event. The top few coffee cans that did experience biofilm growth were somewhat protected by the design of the container, because the very top of the container does not have any holes for mixed liquor to move through (See Figure 5).

The primary filamentous organism observed in the mixed liquor and the surface foam was *Nocardia*, and the levels of this organism varied throughout the study. *Nocardia* is an Actinomycete bacterium that grows best under the following conditions: a high fat content in the influent, surface-active materials in the influent, internal recycling of floating material, and a water temperature higher than 15°C (Eikelboom, 2000). At Hepburn-Lycoming, there is a high fat content in the influent because of the kitchen waste generated by the school, and the temperature of the unit increased from approximately 14°C in January to over 19°C in April. These two characteristics of the SBR provide perfect conditions for *Nocardia* growth. In the activated sludge process, *Nocardia* performs beneficial roles in feeding on wastes and assisting in floc formation (Jenkins et al, 2004). The branching filaments of *Nocardia* make great substrate for floc formation. As mentioned previously, however, *Nocardia* also has many detrimental roles in the activated sludge process, including creating large amounts of foam and contributing to a loss of secondary solids (Jenkins et al, 2004). Typically, once *Nocardia* has become established in an activated sludge system, bioaugmentation products or defoaming agents must be used.

In 1971, Curds created a computer model displaying how protozoan communities change with increasing sludge age (See Figure 10). Amoebae and flagellates appear when the sludge is new, since

there are few bacteria present and therefore little competition. Amoebae and flagellates are succeeded by free-swimming ciliates which eat the bacteria that are increasing in number as time progresses. These free-swimming ciliates secrete polysaccharides and mucoproteins, which aid in floc formation. Bacterial populations peak at approximately the same time as free-swimming ciliates. Crawling and stalked ciliates succeed the free-swimming ciliates because the now-mature floc particles hinder movement of the free-swimming ciliates. The larger, mature flocs also provide habitat for the crawling and stalked ciliates, which add weight to floc particles and improve their settleability. A healthy activated sludge system is considered to be one that is dominated by the "higher forms" of protozoans, the crawling and stalked ciliates. In this study, there were not a large number of crawling ciliates; however the stalked ciliates did increase significantly, indicating an overall improvement in sludge quality. The number of flagellates also decreased, though not significantly, which is another sign of increasing quality of the mixed liquor.

The large drop in flagellates that occurred at the beginning of this study, although it was not statistically significant, warrants further examination. Higher protozoan forms, such as ciliates, eat smaller protozoans such as flagellates, so the increasing number of larger protozoans may have resulted in the decrease in flagellate population. It also should be noted that the one large spike in flagellate population occurred on March 12, immediately after the flow-through event. It is possible that this flow-through washed out many of the established protozoans, and the system reverted back to one of primarily flagellates until the larger protozoans could re-establish themselves. The statistically significant increases in rotifers, stalked ciliates, and suctorians could be attributed to the insertion of the fixed-film media. It is hypothesized that the large amounts of surface area added by the coffee cans provided substrate for these organisms, which use stalks or "feet" to attach to a substrate. The enclosure of the coffee cans inside the PVC pipe also acted as a refuge for these organisms, where they could attach and

feed. The mixing action of the aeration chamber then occasionally sheared these organisms from the coffee cans and carried them into the mixed liquor, where samples were taken.

The final objective of this study was to compare the biological results with the chemical results, which are presented in the corresponding paper *The Use of Fixed Film Media to Reduce Nutrient Levels in a Sequence Batch Reactor*, written by Amanda Lane. In this study, a significant increase in nitrate and ammonia was observed. These results are the opposite of what was expected based on the changes in the protozoan community. An increase in stalked ciliates and rotifers should have correlated with an increase in effluent quality through a decrease in nitrogen compounds present in the effluent. One possible explanation for these results is the large increase in rotifer biomass. Rotifers are multicellular organisms, and all multicellular organisms excrete ammonia, which is converted by bacteria into nitrite and nitrate. It is hypothesized that the increase in ammonia is due to the increase in rotifers present in the mixed liquor.

Figure 10. This is the graph developed by Curds in 1971, showing the relative numbers of the different groups of protozoans as sludge age increases.

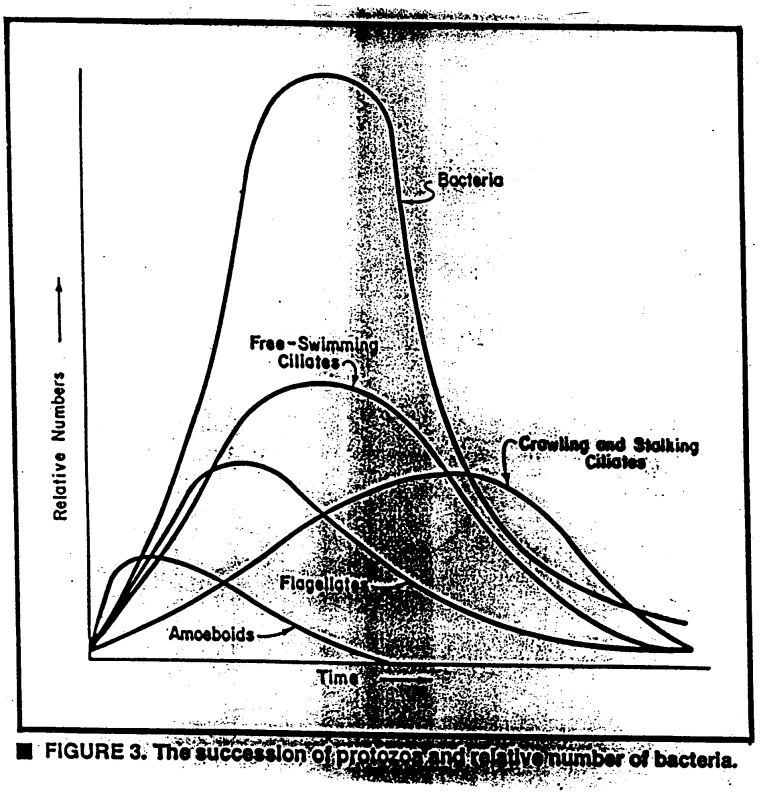


FIGURE 3. The succession of protozoa and relative number of bacteria.

CONCLUSIONS

In conclusion, significant changes in three protozoan groups within the SBR were observed after the insertion of the fixed-film media. Populations of stalked ciliates, rotifers, and suctorians increased significantly after the addition of the fixed-film media; however populations of the other major types of protozoans were not significantly altered. The increase in stalked ciliates and rotifers especially is indicates increasing health of the activated sludge system in the Hepburn-Lycoming SBR. The protozoans that were identified to genus were also indicative of certain treatment conditions. The protozoans observed at the beginning of the project indicated an unhealthy system, and over the course of the studies those populations shifted to genera indicative of more healthy conditions. The biofilm, however, did not colonize as was hypothesized, which may be due to the constant aeration shearing attached

growth from the biofilm. Rotifers, stalked ciliates, and suctorians may have used the fixed-film media as a substrate and refuge for attachment, causing the noted increase in their populations. While the concurrent study did not observe a decrease in nitrogen compounds, the recorded increase in ammonia may have been caused by the increase in rotifers, since rotifers are multicellular organisms that excrete ammonia. This project lends itself to many possible future studies on the effects of fixed-film media and biofilm colonization on Sequence Batch Reactor treatment systems.

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Acknowledgements

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