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The Evaluation of Excess Biomass Growth in Sewers

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Abstract−A few sections of the sewers in Watertown were experiencing clogging due to an excess biomass growth. It was suspected that the wastewaters discharged from Fisher-Barton's two facilities were causing excess biomass growth. Madison raw sewage had a slightly lower biomass growth than the mixture of industrial wastewater and domestic sewage samples. From microscopic examination of biomass, the main cause of excess biomass growth is thought to be toilet tissue. Biomass is attached to toilet tissue, decomposing it gradually and generating extracellular polymeric substances (EPS). Then, suspended particulates including graphite and precipitates are attached to the biomass and filamentous organisms, especially *Sphaerotilus natans*, leading to the increase in the biomass volume. We found that the filamentous bacteria were present in biofilm by FISH. A significant amount of graphite was embedded to biomass grown in sewer. Since the major cause of excess biomass appeared to be toilet tissue, it would not be economical to employ a filtration system for complete graphite separation. Instead, it is recommended that the manufacturing process where graphite is used be assessed and a best management practice is in place.

Key words: Excess Biomass Growth, FISH, Sewer, Evaluation, Wastewater

INTRODUCTION

Watertown has been experiencing excess biomass growth in a few sections of sewers, which seem to be influenced by wastewater discharged from Fisher-Barton's two facilities: Falcon Court and Fredrick. Because of excess biomass growth, the city crews have had to clean the sewers frequently. Therefore, the University of Wisconsin-Madison conducted a series of experiments to investigate the reason for the excess biomass growth in the sewers of Watertown, Wisconsin.

Attached microbial communities are found frequently on the surfaces of natural and engineered aquatic systems. Although they can be used for beneficial applications, such as wastewater treatment systems, there are many problems associated with biofilm buildup on their surface. These problems include reduced heat transfer efficiency within heat exchangers, increased frictional resistance in pipes, the deterioration of drinking water quality, and the plugging of pipes [McFeters et al., 1999]. Because of such unwanted biofilms, the operating cost of the system involved increases significantly [Bott, 1999]. Yoo et al. [2001] described and explained the wastewater treatment plant, then reviewed the applications of modeling, advanced process control, parameter estimation, expert system, monitoring and diagnosis in WWTP reported in the literature and used in practice. From an ecological point of view, biofilms are held together by EPS that allow the microorganisms to form stable aggregates with different cells, leading to synergistic microconsortia. This facilitates the sequential degradation of substances that are not easily biodegradable [Flemming et al., 1999] due to their cometabolic activity. In a study on microbial succession of biofilm by Manz et al. [1999], a monolayer was formed by single attached bacteria, cell chains, and microcolonies after 7 days. In terms of bacterial populations in aquatic environments, most bacteria are gram-negative and many of them belong to the class Proteobacteria [Stackebrandt et al., 1988; Woese, 1987]. Proteobacteria are subdivided into the α-, β-, γ- and δ-subclasses [Manz et al., 1992]. Most recently, the direct identification method, named fluorescent *in situ* hybridization (FISH), has been employed to characterize bacterial populations in the environment.

This method would greatly facilitate their identification, independent of time-consuming cultures, and physiological and biochemical tests [Manz et al., 1992]. In addition, this in situ technique overcame the bias that the bacterial populations isolated on culture medium could only be less than 1-10% of the whole population [Reasoner and Geldreich, 1985; Manz et al., 1994; Amann et al., 1995]. For the analysis of Proteobacteria, and *Cytophaga-Flavobacterium* subgroup of Bacteroides-Flavobacterium group and filamentous bacteria, such as, *Sphaerotilus natans*, that grows on the inner surface of a sewer, which dominated in mature biofilm, [Cao and Alaerts, 1995; Manz et al., 1999], FISH with rRNA targeted oligonucleotides were performed.

The main objective of this study was to evaluate the effect of wastewater discharged from Fisher-Barton's Fredrick, Falcon Court facilities and other sewages on biomass growth in a sewer. Laboratory-scale experiments were conducted to simulate the biomass growth in a sewer. Microscopic examination, microbial counting, and microbial group identification were conducted. The conventional parameters for monitoring water quality were examined such as total suspended solids (TSS), volatile suspended solids (VSS), total and soluble COD, total Kjeldahl nitrogen (TKN), ammonia, organic nitrogen, nitrate/nitrite, and total phosphorus.

EXPERIMENTAL

It is not easy to simulate sewer conditions in a laboratory, be-

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Table 1. Experimental conditions

Run no.	Sample	Temp., °C	Remarks
1	A	20±4	No treatment
2	B	20±4	No treatment
3	C	20±4	No treatment
4	D	20±4	No treatment
5	E	20±4	No treatment
6	A+B+E (1 : 0.5 : 0.5)	20±4	No treatment
7	A+B+E (1 : 0.5 : 0.5)	8±4	No treatment
8	A+B+E (1 : 0.5 : 0.5)	20±4	A's pH raised to 7.5
9	A+B+E (1 : 0.5 : 0.5)	20±4	A's pH lowered to 5.5
10	A+B+E (1 : 0.5 : 0.5)	8±4	A's pH lowered to 5.5
11	A+B+E (1 : 0.5 : 0.5)	20±4	A's pH raised to 7.5 and only supernatant added
12	B+E (1 : 1)	20±4	No treatment
13	A+D+E (1 : 0.5 : 0.5)	20±4	No treatment
14	Madison raw sewage	20±4	No treatment

Site A (26B-053B): Fisher-Barton Falcon Court facility wastewater. Site B (26B-054): Industrial wastewater without Fisher-Barton wastewater. Site C (26B-047): Fisher-Baton, industrial, and domestic wastewater. Site D (15-009): Fisher-Barton Fredrick facility wastewater. Site E (15-011 or 018): Domestic sewage.

cause the sewage flowrate and water quality vary significantly over time. Furthermore, different sewages have to be evaluated to determine the effect of each sewage on excess biomass growth in sewers. Therefore, it was decided to conduct a laboratory-scale experiment in a semi-batch mode.

1. Semi-Batch Sewer Simulation Experiment

The experimental conditions are summarized in Table 1. The experiments were performed in 21 cm×29 cm×9 cm plastic containers. Samples were received weekly and a total of 4 L was added to each container. The containers were left open to maintain an aerobic condition. Due to high evaporation, the containers had plastic covers, but air was allowed to get in through a small opening after from week 3. The samples were measured for conventional water quality parameters. The samples were analyzed for TSS, VSS, total COD, soluble COD, TKN, ammonia, organic nitrogen, nitrite/nitrate, and total phosphorus. In addition, the samples were microbiologically examined to qualitatively characterize microbial populations using *in situ* analysis with rRNA-targeted oligonucleotide probes. At the beginning of the experiment, 490 mL of the different wastewater mixtures (Table 1) and 10 mL of biomass at a Watertown sewer were added to a container. The biomass contained a significant amount of graphite in biomass. Due to the presence of a significant amount of graphite in the biomass, graphite had to be physically removed from biomass before addition. The biomass was mixed homogeneously.

2. Microbiological Characterization

The sewage and biofilm samples were examined microbiologically to qualitatively characterize microbial populations by using the FISH method both in the beginning prior to addition of sewer slime and at the end of experiment. The results were expressed in terms of the ratio (%) of the number of individual group-specific bacteria to the number of total bacteria. The total cell counts of bacteria on each run were enumerated biweekly.

2-1. Total Cell Counts

An aliquot of 10 μL of fixed and diluted sample was spotted on the well of a slide to enumerate the number of bacterial cells. After air-drying, it was stained with 8 μL of DAPI (4,6-diamidine-2-phenylindole, 033 μg/mL) for 5 min in the dark.

2-2. Cell Fixation

Each sample was fixed in a 1 to 3 ratio with 4% paraformaldehyde solution for at least 1 hour. After fixation, it was centrifuged at 14,000 rpm for 5 min. Using a 1× PBS (phosphate-buffered saline), the washed pelletted cells were resuspended and spotted on Teflon-coated slides (Cel-Line), dried at 46 °C for 30 min, and dehydrated in 50%, 80%, and 96% (v/v) ethanol for 3 min each.

2-3. Oligonucleotide Probes

Probe sequences [Manz et al., 1992; Wagner et al., 1994], target sites, and target organisms used in this study are summarized in Table 2. Oligonucleotides complementary to selected regions were synthesized by the University of Wisconsin Biotechnology Center. The fluorescent molecule, Cy3 (the indocarbocyanine dye), was attached to the 5' end of ALF1b, BET42a, GAM42a and 5(6)-carboxy-fluorescein-N-hydroxysuccinimide-ester to CF319a/b and SNA, respectively.

2-4. Whole Cell Hybridization

Air-dried, fixed samples on the Teflon-coated slide were hybridized with 8 μL hybridization solution (formamide - 15% for probe CF319a/b, 20% for probe ALF1b, 35% for probe BET42a and GAM42a, 50% for probe SNA, 0.9 M NaCl, 20 mM Tris/HCl pH 7.2, 0.01% SDS) and 50 ng probe [Manz et al., 1992] in an isotonically equilibrated humidity chamber at 46 °C for 1.5 hours. The addition of formamide is required for the optimal hybridization stringency. After incubation, the excess probe from the slide was removed with prewarmed washing solution (probe ALF1b: 20 mM Tris, 0.01% SDS, 180 mM NaCl, 5 mM EDTA; probes BET42a, GAM42a and CF319a/b: 20 mM Tris, 0.01% SDS, 40 mM NaCl, 5 mM EDTA; probe SNA: 20 mM Tris, 0.01% SDS, 45 mM NaCl, 5 mM EDTA) and immediately immersed in the same solution at 48 °C for 20 min.

Table 2. Oligonucleotide probe sequences, target sites, target organisms and formamide concentration in the hybridization buffer required for *in situ* hybridization

Probe	Sequence	Target site (rRNA positions)	% formamide	Target organisms
ALF1b	5'-CGTTCG(C/T)TCTGAGCCAG-3'	16S, 19-35	20	α-subclass of Proteobacteria
BET42a	5'-GCCTTCCCACTTCGTTT-3'	23S, 1027-1043	35	β-subclass of Proteobacteria
GAM42a	5'-GCCTTCCCACATCGTTT-3'	23S, 1027-1043	35	γ-subclass of Proteobacteria
CF319a/b	5'-TGGTCCGTGTCTCAGTAC-3'	16S, 319-336	35	*Cytophaga-Flavobacteria* cluster
SNA	5'-CATCCCCCTCTACCGTAC-3'	16S, 656-673	50	Sphaerotilus natans

Slides were rinsed gently with sterilized distilled water, air-dried and mounted in Anti-fade reagent (Fluoro-guard, Bio-Rad).

2-5. Observation

Cells stained with DAPI and labeled oligonucleotide probe were directly counted by using an epifluorescence microscopy (Axioplan 2, ×600-1,000, Zeiss).

RESULT AND DISCUSSION

1. Semi-Batch Sewer Simulation Experiment

In general, the biomass growth was not high, which was probably due to the depletion of biodegradable organic substrate several hours after addition of sewage. This result is unlike an actual sewer where fresh sewage flows almost continuously. Precipitates formed after the Fisher-Barton's Falcon Court facility wastewater was mixed with domestic sewage was not a major contributing factor to an excess biomass growth in the Watertown's sewer. Madison raw sewage and Site C sample had the greatest TSS and VSS values among the 14 runs. It was not possible to accurately simulate the excess biomass growth through this experiment that was observed in the Watertown sewers because in the experiment the sewage was added twice a week rather than continuously as in the Watertown sewers. Biomass growth was much greater when other Site B and Site E were mixed than when the Fisher-Barton's Falcon Court facility wastewater was mixed with domestic sewage. The changes in the bacterial community's structure were studied by comparing the sample in week 6 with the initial samples. By FISH technique, we found that the structures of biomasses cultured with Site C sample and the sample from the mixture of Sites A, B, and E were most similar to the structure of biofilm. As expected, the microbial community grown with the Madison raw wastewater sample was different from the biofilm obtained at a Watertown sewer. This indicates that a specific sewage induces a unique pattern of microbial community structure. Lower numbers of bacterial groups in total cell counts in the two Fisher-Barton Facilities wastewater and samples from the two Fisher-Barton Facilities mixed with domestic sewage implies that both Fisher-Barton's wastewaters are not optimal for bacterial growth. Actually, Site B sample appears to cause more biomass growth than Sites A and D wastewaters because the cell count were greatest when Site B sample was mixed with Site E.

From the TSS and VSS results, it was found that sites A and D had lower TSS and VSS values than other sewage samples. Site E had a significant variation in TSS and VSS. The TSS and VSS values were generally greater than other sewage samples.

The experiment performed during 6-weeks showed that sites A and D had lower total and soluble COD values than other sewage samples. Site E had the greatest total and soluble COD values among the five samples. Site B had the second highest COD values. Considering medium strength COD is 500 mg/L [Metcalf and Eddy, 1991], Site E sample is considered a rather strong strength COD. The COD : N : P ratio for the six-week average of all five samples was 107 : 88 : 7. The optimum ratio of COD : N : P for microbial growth is 100 : 5 : 1 [Metcalf and Eddy, 1991]. Therefore, it can be said that the biomass growth is limited by carbon source in Watertown sewer samples.

Fisher-Barton's samples had lower TKN and ammonia values than other sewage samples. Fisher-Barton's samples had lower organic nitrogen but much greater nitrite/nitrate values than other the sewage samples. Fisher-Barton's Site A sample had higher total phosphorus concentration than the other sewage samples for the first two weeks. Then, the phosphorus concentration dropped to 0 mg/L for weeks 3 and 4 and to 2 mg/L for weeks 5 and 6. In order to evaluate the effect of nitrite/nitrate on biomass growth, the nitrite/nitrate and ammonia concentrations were measured at week 4 after addition of sewages from Run 13. The ammonia and nitrite/nitrate concentration did not change over time, indicating that there is no noticeable nitrification or denitrification.

Furthermore, the evaporation rate was different for each run as has been seen from the results. Runs 7 and 10 were performed at 8 °C; thus, the evaporation rates were low. In general, Runs 12, 13, and 14 had greater evaporation rates than other runs. During the first week of the test, the Run 11 container was accidentally dropped losing all the content so that the run was started again. Because of this, the evaporation rate was lower than other runs. The containers were randomly arranged twice a week to eliminate any condition affecting evaporation.

Since the evaporation rate was different, the final biomass was measured by using the entire content in each container. The results are shown in Fig. 1. Run 14 had the highest TSS, followed by Run 3, Run 5 and Run 8 [Sites A (pH 7.5), B, and E]. In terms of VSS, Run 3 had the greatest, followed by Run 14, Run 8, and Run 10 [Sites A (pH 5.5), B, and E]. Site B sample had the lowest biomass growth over six weeks. There was no significant difference in TSS and VSS between Run 12 and Run 13. Run 10 appeared to have the densest biomass among the 14 runs. The biomass in Runs 13 and 14 formed less dense biofilm than others. Nine Springs' primary sedimentation tank influent (Madison raw sewage) lacks large pieces of toilet tissues. Because of this, it appears that Run 14 biomass had less EPS than other runs containing Watertown domestic sewage. Similarly, Run 13 biomass was less dense than Run 14 biomass. Over the course of the experiments, it appears that the best condition for biomass growth is the mixture of Sites B and E samples, which coincides with the high COD values of these samples.

During the experiments, it was observed that biomass became thick and sticky for the first few hours and then became less dense and looked loose, probably due to endogenous decay resulting from depletion of readily biodegradable COD. Because of this, it was

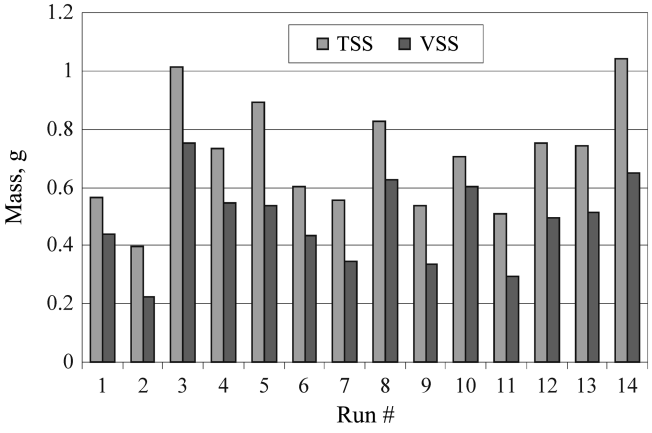


Fig. 1. Total TSS and VSS accumulated for six weeks.

not possible to reproduce the excess biomass growth observed in the Watertown sewer.

2. Microbiological Characterization

2-1. Total Cell Counts

The biomass composition in biofilms consists of not only bacterial cell biomass but also extracellular polymeric substances. Even though the cell biomass was only a minor fraction of the organic matter in the biofilms [Jahn and Nielsen, 1998], the bacterial number may indicate the biofilm forming potential in each run. Epifluorescence micrographs of the bacterial populations determined by DAPI-stained cells (total cell counts) in Runs 1 and 14 (magnification: 1,000×) are shown in Fig. 2. Variations of total cell counts versus time in for the 14 runs are shown in Fig. 3. The initial number of total bacteria stained with DAPI in each run varied from 1.2×10^6 (Run 1; Fisher-Barton facility wastewater) to 55.1×10^6 cells/mL (Run 14; Madison raw sewage). In general, the bacterial number was higher in solely municipal wastewater (Runs 5 and 14), especially those numbers in combined wastewater with municipal sewage than in Fisher-Barton facility wastewater (Runs 1 and 4). The bacterial number in other industrial wastewater (Run 2) was relatively high (6.2×10^6 cells/mL).

The increase in total cell counts from weeks 2 to 6 may be due to the fact that raw sewage was added twice a week and the biomass was concentrated because of evaporation during the test. The minimal value was obtained from Run 4 (Fisher-Barton Fredrick facility wastewater) (0.7×10^{10} cells/mL) and maximum value from Run 9, (Fisher-Barton facility wastewater adjusted pH to 5.5 at 20 °C, other industrial wastewater, and domestic sewage) (9.2×10^{10} cells/mL). The second highest run was, which Run 10 (7.6×10^{10} cells/mL) had the same conditions as Run 9, but the temperature was 8 °C instead of 20 °C. Therefore, it can be said that Site B sewage may also cause more biomass growth. The numbers of total bacteria were greater when five individual samples were mixed (Runs 6 through 13) than when individual samples were tested separately (Runs 1 through 5). It appears that there is a synergistic effect of individual samples on biomass growth. The effect of pH, temperature, and precipitates from site A on growth of biomass was not clearly demonstrated.

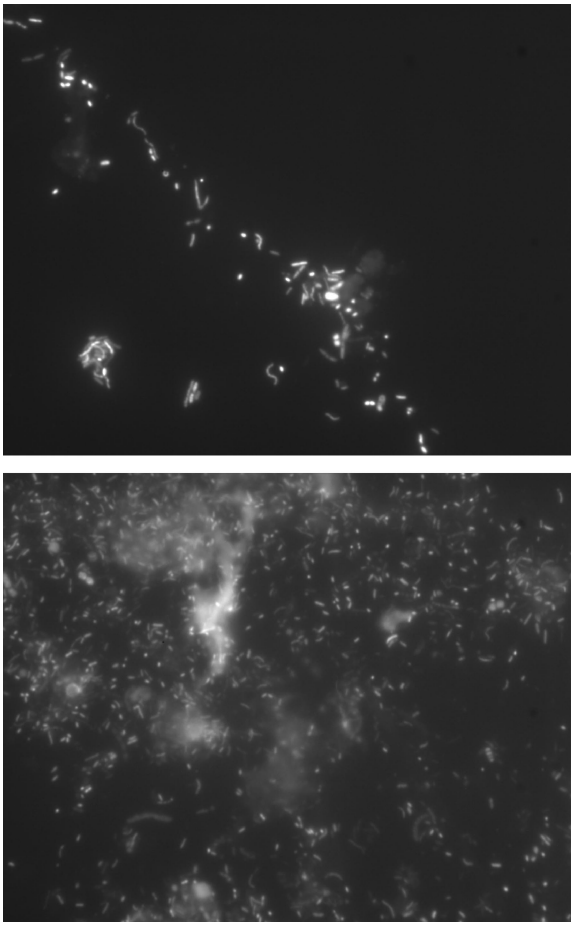


Fig. 2. Epifluorescence micrographs of the bacterial populations determined by DAPI-stained cells (total cell counts) in Runs 1 and 14 (magnification: 1,000×).

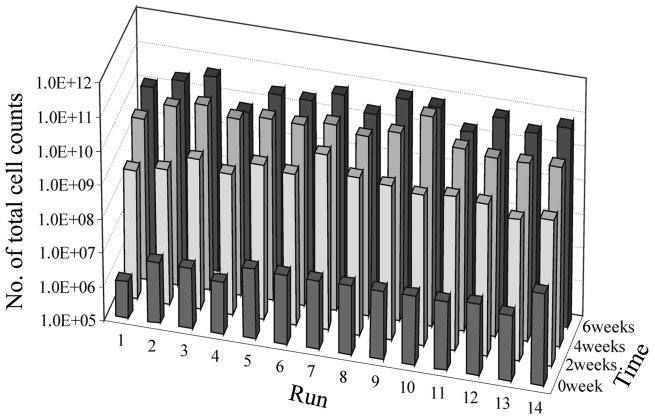


Fig. 3. Variations cell counts versus time, resulting from DAPI-stained cells using epifluorescence microscopy in 14 runs.

2-2. Comparison of *in situ* Microbial Community Structure in Sewage and Biofilm

In order to compare bacterial community structures of biomass cultured with specific combinations of five wastewater samples with

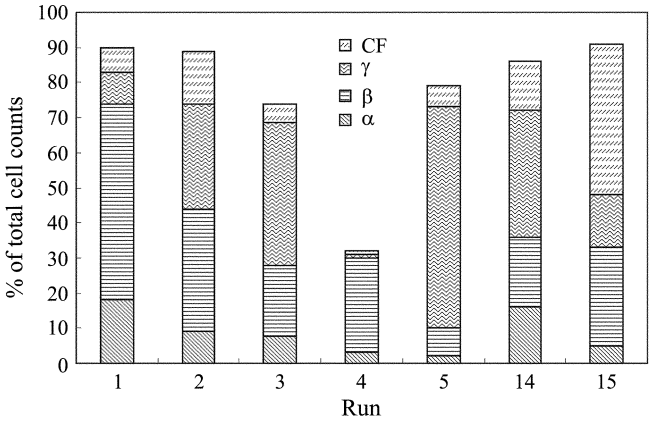


Fig. 4. Comparisons of *in situ* microbial community structure in sewage from different sites (A-E: 1-5; Madison raw sewage: 14) and biofilm (15), expressed in ratio (%) of the number of individual group-specific bacteria [α-, β-, γ-subclasses and *Cytophaga-Flavobacterium* (CF)] to the number of total bacteria in the initial samples.

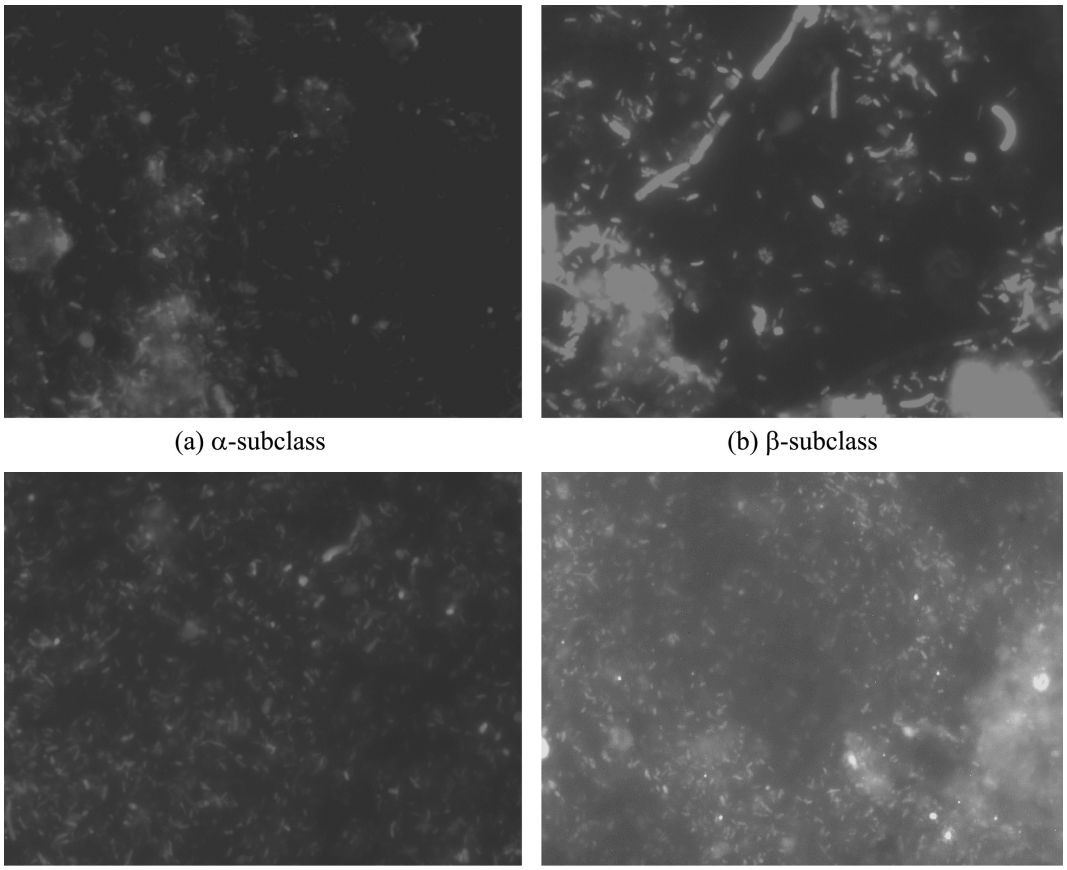


Fig. 5. Epifluorescence micrographs of the bacterial population in biofilm determined by group-specific bacteria using FISH (magnification: 1,000×).

those of biofilm taken from the sewer that has a plugging problem due to excess biomass growth, *in situ* identification technique using FISH was introduced. Fig. 4. shows the percentage of the α-, β- and γ-subclasses of Proteobacteria, and *Cytophaga-Flavobacterium* subgroup to total DAPI cell counts at the beginning of the experiment. While the β-subclass of Proteobacteria was the dominant group in Fisher-Barton facility wastewater (Run 1: 66%, Run 4: 27%) and in other industrial wastewater (Run 2: 45%), the γ-subclass was more abundant than β-subclass in mainly municipal sewage (Run 5: 83% and Run 14: 46%) and domestic sewage mixed with Fisher-Barton facility wastewater (Run 3: 41%).

Manz et al. [1994] performed a comparative analysis of *in situ* and nutrient-medium-cultured bacterial community in municipal and dairy wastewater using the FISH method. The nutrient-rich medium favored growth of γ-subclass that was in fact only a minor fraction in both municipal and dairy sludge bacterial communities. On the contrary, the prevalent bacterial groups were β-subclass in municipal sludge and *Cytophaga-Flavobacterium* (CF) subgroup in dairy sludge [Manz et al., 1994]. The prevalent occurrence of the bacteria belonging to the γ-subclass indicates their competence to grow in such a nutrient-rich environment. In contrast to the numerical domination of β- and γ-subclass in raw wastewater, CF subgroup accounted for 47% of bacterial populations in biofilm. The α-subclass accounted for 5%, β-subclass 31%, and γ-subclass16%, respectively. The photos of each subgroup are given in Fig. 5. As shown in the photo of β-subclass (Fig. 5b), the filamentous bacteria were present in biofilm. It was morphologically identified as *Sphaerotilus natans* according to the key of Eikelboom [1973] and Jenkins et al. [1994]. However, it did not hybridize with the gene probe SNA, even though *S. natans* belongs to β-subclass. The reason might be either the inability of the probe to penetrate the cell because of the slimes surrounding these filamentous bacteria or their low cellular ribosome content caused by their physiological inactivity [Nielsen et al., 1998].

As reported by other studies on marine biofilm [Burchard and Sorongon, 1998], reed biofilm in lake [Borsodi et al., 1998], and biofilm in river [Manz et al., 1999], CF-subgroup make up the substantial proportions in biofilms. Their presence is usually connected with their activities in the decomposition of different biopolymers found excessively in biofilm [Borsodi et al., 1998; Manz et al., 1999].

After 6 weeks of biofilm culturing with the addition of raw sewage twice a week, the changes of bacterial community structure were compared with the structure in the initial sample (Fig. 6). It can be seen that the dominant CF-subgroup in biofilm strongly influenced the inherent bacterial community structure. At week 0, β- were dominant in Sites A, B, and D and γ-subclasses were dominant in Site E and Nine Springs wastewater; however, at week 6, the fraction of β- and γ-subclasses decreased in all samples. In all cases, except Runs 3, 4, and 5, the percent of the four group specific bacteria to the total cell counts decreased after 6 weeks. It is probably related

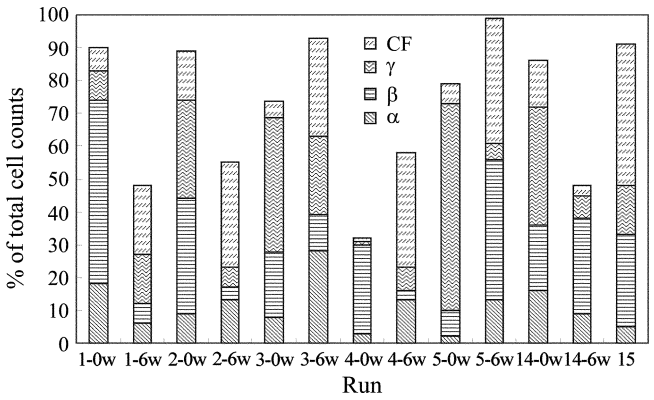


Fig. 6. Changes of *in situ* microbial community structure in sewage from the initial sample (0w) to 6-week's sample (6w) at different sites (A-E: 1-5; Madison raw sewage; 14) and biofilm (15) expressed in ratio (%) of the number of individual group-specific bacteria [α-, β-, γ-subclasses and *Cytophaga-Flavobacterium* (CF)] to the number of total bacteria.

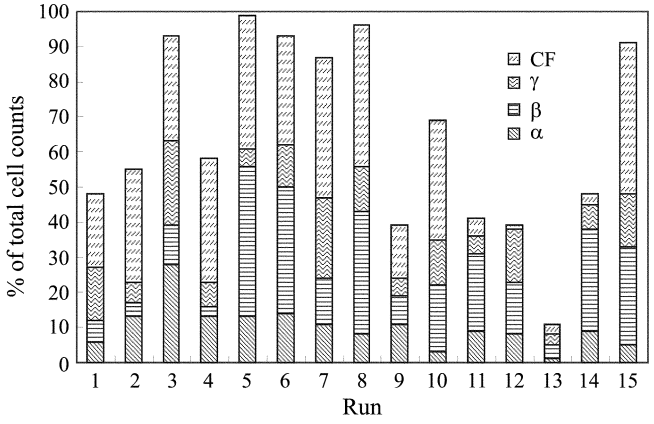
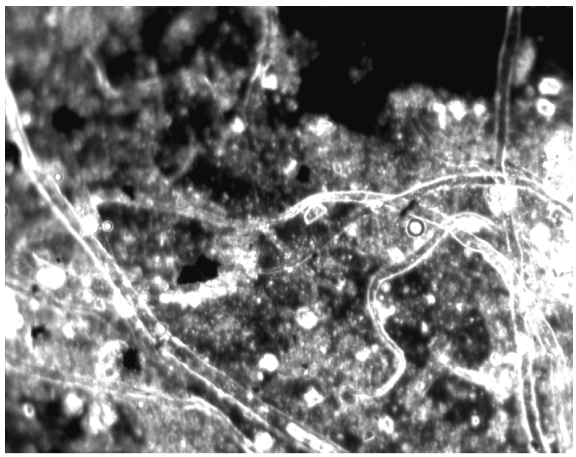


Fig. 7. Comparisons of *in situ* microbial community structure in each run including biofilm (15) expressed in ratio (%) of the number of individual group-specific bacteria [α-, β-, γ-subclasses and *Cytophaga-Flavobacterium* (CF)] to the number of total bacteria.

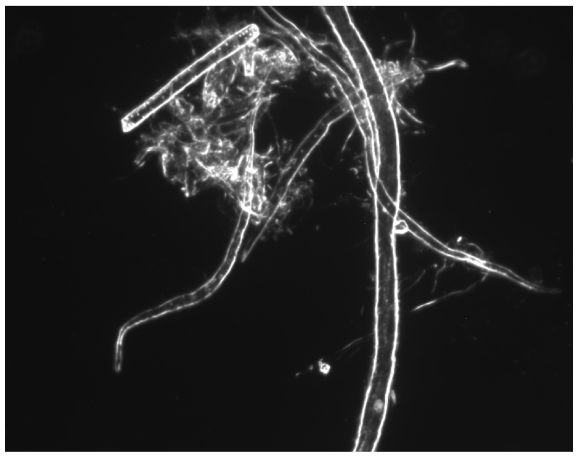
to the reduced detectability of bacteria by oligonucleotide probes that are dependent on the presence of sufficient ribosomes per cell [Nielsen et al., 1998]. Therefore, the reduced ribosome contents in cell could account for decreased metabolic activities of the bacteria.

The bacterial community structure was also compared among the 14 runs and biofilm sample. The results are summarized in Fig. 7. In terms of similarity of bacterial community structure in each run to those of biofilm, the structures in Runs 3, 6, and 8 (at pH 7.5) were most similar to the structure of biofilm. As expected, biomass grown with the Madison raw wastewater sample was different from the biofilm obtained at a Watertown sewer. This indicates that a specific sewage induces a unique pattern of microbial community structure. Lower numbers of bacterial groups to total cell counts in the two Fisher-Barton Facilities wastewater (Runs 1 and 4) and samples from the two Fisher-Barton Facilities mixed with domestic sewage (Run 13) indicate that both Fisher-Barton's wastewaters are not optimal for bacterial growth and subsequently yielded comparably lower detectability by using the FISH method. Furthermore, Run 13 had the lowest percent of total cell counts among the 14 runs. This indicates that the Site B sample (Run 12) may actually cause more biomass growth than Site A and D wastewaters since Run 12 had the greatest number of the total cell counts. When the pH was lowered to 5.5 (Runs 9 at 20 °C and Run 10 at 8 °C), the percent of the total cell counts was lower than those at neutral pH due to reduced metabolic activities of the cells. The percentages of the total cell counts were greater at 20 °C than at 8 °C when the pH was neutral while the percentages of the total cell counts were lower at 20 °C than at 8 °C when the pH was 5.5.

To elucidate the biofilm forming material, the biofilm was dissected and observed under phase-contrast microscope. The large amounts of filaments, similar to toilet tissue fiber that makes the backbone of the biofilm, were observed from microscopic examination. In order to confirm that filaments shown in Fig. 8 originated from toilet tissue fiber, toilet tissue was dissolved in water and examined through microscope (Fig. 8). MacDonald [2000] also observed wood fiber in a similar sample. The accumulation of toilet tissue in the sewer probably induced the excessive growth of cellulolytic, filamentous bacteria that produce both EPS and extracellular enzymes to degrade the EPS [Jahn and Nielsen, 1998]. Addi-



(a)



(b)

Fig. 8. Phase-contrast micrographs of toilet tissue fiber in biofilm (a) and dissolved toilet tissue sample (b) (magnification: 100×).

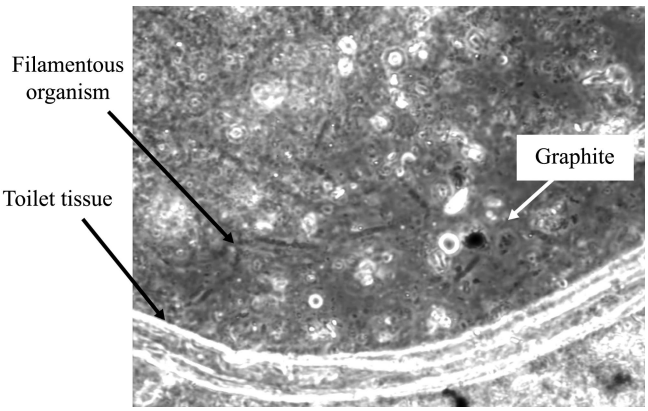


Fig. 9. Phase-contrast micrographs of biofilm (magnification: 670×).

tionally, the viscosity of EPS might have caused the accumulation of inorganic compounds and heavy metals in the biofilm, such as graphite particles produced by Fisher-Barton facility in Watertown's wastewater. Furthermore, as reported by Bott [1999], the flow velocity should be greater than 1 m/sec to reduce the growth of biofilm due to the shear force. The flow rate of sewage in Watertown could contribute to the accumulation of the above-mentioned compounds and excess growth of biofilm inside the sewer.

A photo taken with a phase-contrast microscope is shown in Fig. 9. The black dots are graphite. The bottom thick line is toilet tissue fiber and the thin black lines are filamentous organisms. The role of graphite is not clear; however, it appears that graphite attaches to biomass. From Fig. 8(a) and 9, it can be said that the main cause of excess biomass growth is toilet tissue. Biomass is attached to toilet tissue, decomposing it gradually and generating EPS. Then, graphite, suspended particulates, and precipitates are attached to the biomass. Filamentous organisms, especially *Sphaerotilus natans*, grow leading to the increase in the biomass volume.

CONCLUSIONS AND RECOMMENDATIONS

From the laboratory experiments and microbiological characterization, the following conclusions can be drawn:

The mixture of other industry wastewater (Site B) and Watertown domestic sewage (Site E) had the highest VSS value, followed by Madison raw sewage. Fisher-Baron's Fredrick facility (Site D) sample had slightly more biomass growth than the Falcon Court (Site A) sample. Precipitates formed after the Fisher-Barton's Falcon Court facility wastewater was mixed with domestic sewage were not a major contributing factor to excess biomass growth in the Watertown's sewer. The structures of the cultured biomass with the Site C sample and the sample from the mixture of Sites A, B, and E were most similar to the structure of the biofilm. As expected, the biomass grown with the Madison raw wastewater sample was different from the biofilm obtained at a Watertown sewer. This indicates that a specific sewage induces a unique pattern of microbial community structure. Lower numbers of bacterial groups to total cell counts in the two Fisher-Barton Facilities wastewater and samples from the two Fisher-Barton Facilities mixed with domestic sewage implies that both Fisher-Barton wastewaters are not optimal for bacterial growth. Actually, the Site B sample appears to cause more biomass growth than Sites A and D wastewaters because the cell counts were greatest when the Site B sample was mixed with domestic sewage (Site E). Fisher-Barton's wastewaters did not appear to provide favorable substrates for microbial growth.

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