

Cell Surface and Exopolymer Characterization of Laboratory Stabilized Activated Sludge from a Beverage Bottling Plant

Authors: S. M. Boyette, J. M. Lovett, W. G. Gaboda and J. A. Soares

Abstract

Fermentor-stabilized activated sludge from an industrial beverage bottling plant was grown on three different food sources: normal plant wastewater, plant wastewater containing high sucrose concentrations, and a synthetic glucose-based feed stock. Surface charge, hydrophobicity, and exopolysaccharide composition were measured on the stabilized bacterial flocs. Cell surface charge was measured by electrophoretic mobility, dye exchange titration, and a standard colloid titration, while cell hydrophobicity was determined using the bacterial adhesion to hydrocarbons (BATH) test. Exopolysaccharide profiles were determined by measuring concentrations of glucose, galactose, mannose, glucuronic, and galacturonic acids in digested exopolymer extractions using HPLC.

Changes in the physical surface properties of the bacteria and the chemical composition of the extracted exopolymers were correlated with differences in the three food sources. Cell surface hydrophobicity was similar for cultures grown on different plant wastewaters, while the culture grown on synthetic food produced less floc hydrophobicity. Electrophoretic mobility measurements, charge titrations, and dye exchange titrations showed different total surface charge as well as varying charge availability. Additionally, total surface charge and total exopolysaccharide concentrations appeared less dependent on food source than the food-to-mass ratio. High concen-

trations of biodegradable food produced dispersed growth and high concentrations of exopolysaccharides that contributed to poor settling.

Introduction

Settling in an industrial activated sludge system requires mixed bacterial cultures with the appropriate chemical and physical properties to agglomerate and settle in the secondary clarifier. A small portion of this settled sludge is wasted to produce the desired solids residence time in the basin, and these wasted solids are often dewatered with the aid of a polymeric flocculant. The efficiency of this dewatering process depends on properly matching the polymeric flocculant to the surface characteristics of the bacterial flocs. Therefore, knowledge of floc surface properties as a function of wastewater composition helps in selecting the best flocculant.

Surface charge and hydrophobicity are important properties used to map flocculants to secondary sludge. These properties can be measured by several methods, but all methods require the biological floc to be perturbed from its normal environment before taking the measurement. Similar properties that are measured using different procedures often disagree, and these differences can be caused by perturbations in floc environments as well as nuances associated with the different molecular probes and conditions used to make the measurements. Fortunately, these dissimilar methods can yield complimentary information that creates a clearer picture of the bacterial surface when combined. Additional chemical meas-



Find a contact near you by
visiting gewater.com or
e-mailing custhelp@ge.com.

Global Headquarters
Trevose, PA
+1-215-355-3300

Americas
Watertown, MA
+1-617-926-2500

Europe/Middle East/Africa
Heverlee, Belgium
+32-16-40-20-00

Asia/Pacific
Shanghai, China
+86-21-5298-4573

ures can identify chemical components that contribute to these physical properties, and any correlation between physical properties and chemical composition can be used to help define how engineering operations affect bacterial surfaces.

Bacterial exopolysaccharides (EPS) are the major exopolymer on the bacterial surface and are present as heterogeneous mixtures of branched polysaccharides. Although exopolysaccharide concentrations are a major component in the exopolymer layer, they alone do not directly correlate with either floc density or settling properties (Andreadakis, 1992). These exopolymers are responsible for increased bridging flocculation that helps create good settling floc (Jorand et al., 1995), and they are known to help retain floc structures and minimize shear effects (Wahlberg et al., 1992). Additional effects from lipid components are believed to determine floc-to-floc adhesion through hydrophobic interactions (Goodwin and Forster, 1985). The combined activity of these exopolymers determines floc structure and stability by balancing hydrophobic and hydrophilic interactions (Eriksson and Axberg, 1981), with exopolysaccharides and lipids playing important roles. Floc surface charge and hydrophobicity are the sum effect of these exopolymer interactions and are the key measures used to estimate sludge settling efficiency (Urbain et al., 1993). These same properties help determine dewatering efficiency.

One way to unravel the intertwined effects of these different exopolymers is to identify the chemical components that contribute to charge and hydrophobic interactions. Most exopolysaccharides found in activated sludge systems are composed of five major sugars: glucose, galactose, mannose, glucuronic acid and galacturonic acid (Horan and Eccles, 1986; Horan et al., 1988). The monosaccharide composition and secondary structure of these branched biopolymers help determine the overall surface charge, with the uronic acids playing a major role. Measuring total uronic acids will not create a complete picture of bacterial surface charge, but measuring exopolysaccharide composition will begin to describe the complex exopolysaccharide structure that determines the effective charge on the surface. This effective charge character is the primary determinant for selecting a cationic flocculant, and when

supplemented with measures of cell surface hydrophobicity, begins to paint a better picture of floc surfaces.

Methods

Laboratory studies have shown that relative sludge dewatering performance can be evaluated on samples that have been stored at 39.2°F (4°C) for several days, but the flocculant concentration required for field application can not be determined with anaerobically stored sludge. Researchers have shown that anaerobic storage of sludge causes the release of dissolved organic carbon believed to be EPS and fermentation byproducts (Bruus et al., 1993). Loss of EPS in the exopolymer layer will decrease the charge required to flocculate the sludge and will affect flocculant selection. The objectives of this research project was to determine cell surface properties that might affect settling properties in full-scale activated sludge systems and to avoid sludge storage prior to surface characterization.

Sludge and wastewater samples were taken from a beverage bottling plant that uses a small activated sludge system to process wastewater containing high concentrations of readily degradable dissolved organic matter. Sludge was taken from the aeration chamber and wastewater was taken from the pH controlled equalization chamber. Samples were transported to the research laboratory and prepared by further adjusting the pH to 7.0 and then autoclaving the wastewater in storage carboys so that it could be used as a food source for the month-long experiment. These autoclaved wastewater samples, which allowed the sludge to be acclimated to a single wastewater sample, remained free from bacterial growth for more than a month. The wastewater was tested for chemical oxygen demand (COD), 5-day biological oxygen demand (BOD₅), 20-day biological oxygen demand (BOD₂₀), nitrate, nitrite, phosphorus (as PO₄), free ammonia (as N), and free and fixed ammonia (as N). Samples were also tested for total suspended solids (TSS), volatile suspended solids (VSS), and total solids. The mixed liquor samples were tested for TSS, VSS, and total solids. All tests followed standard methods (Clesceri et al., 1998).

The mixed liquor was stabilized in a BioFlow 3000 laboratory fermentor (New Brunswick Scientific)

fitted with an external cross flow CFP-2-E-6A microfilter (AG Technology) or a mini-clarifier used to maintain solids in the system. Solids were removed batch-wise daily to maintain a constant solids residence time (SRT). The mini-clarifier was used on the normal wastewater experiment because the mixed liquor in this experiment clogged the microfilter. Unfortunately, clarifier performance was sporadic and resulted in cell residence times fluctuating during the experiment. Wastewater flow into the fermentor was matched to the filter or clarifier effluent flow using a dual pump system. This flow rate was set to maintain a hydraulic residence time (HRT) that matched field conditions. Dissolved oxygen was controlled above 0.002 kg/m³ (2 ppm) in the fermentor, and the pH was controlled automatically with dilute sulfuric acid and sodium hydroxide solutions. Temperature was maintained at 86°F (30°C) throughout the experiments. Nephelometric turbidity and TSS were measured daily on fermentor samples and used to track culture stabilization in the laboratory system. Once a culture stabilized, usually occurring between 3 and 4 weeks, samples were extracted for cell surface characterization and exopolysaccharide analysis.

Fermentor sludge systems were run in three different batches, each with a different feed composition and with field samples taken at different times. The first fermentor experiment used field mixed liquor to fill the fermentor and fed a synthetic wastewater composed of glucose (0.2 kg/m³), peptone (0.4 kg/m³), methanol (0.2 kg/m³), and yeast (0.15 kg/m³) that had been buffered with phosphate and adjusted to pH 7. Ammonia, salts, vitamins, and essential metals were added to encourage culture growth. The second fermentor experiment used plant wastewater obtained during normal operation. The third experiment used plant wastewater obtained during an outage period where the waste treatment system was fed from a holding tank containing operation discards and vessel washouts. This wastewater contained detergents, surfactants, and high sucrose concentrations.

Surface analyses were performed on the three different mixed liquors after they stabilized in the fermentors. On one day, surface properties were analyzed on a sample removed from the fermentor, and on a second day exopolymer extractions

were performed on a new sample. Cell surface charge was determined by three different methods: electrophoretic mobility, colloid titration, and a dye exchange titration. Electrophoretic mobility was measured using either a Delsa 440SX (Coulter) or a Zeta Reader Mark 21 (Zeta Meter Instruments). All samples were diluted 1 to 20 in 0.001 N potassium nitrate to increase detection of negative electrophoretic mobilities, and instrument selection was based on floc size in the sample. Dispersed growth resulted in floc sizes less than 3 mm and required measurement on the Delsa 440SX instrument, while larger flocs greater than 5 mm could be measured using the Zeta Reader Mark 21 instrument. Charge titrations were performed using ruthenium red (Figueroa and Silverstein, 1987) where dye absorption (537 nm) on the supernate from centrifuged mixtures provided an indirect measure of the amount of dye adsorbed on the culture solids. The colloid titration was a classic PVSK titration (Kawamura, 1967), except cationic chitosan was replaced with a synthetic epichlorohydrin/dimethylamine copolymer. This PVSK titration was used to determine the total surface charge available to a high-molecular-weight, charged polymer.

Surface hydrophobicity was determined by bacterial adhesion to hydrocarbon (BATH) (Rosenberg, 1984; van der Mei et al., 1991). In the BATH test, the mixed liquors were diluted in 0.001 N potassium nitrate to give an optical density (600 nm) near unity. Once adjusted to the proper OD600 range, 5 × 10⁻⁶ m³ (5 mL) of diluted mixed liquor was mixed with 1 × 10⁻⁶ m³ (1 mL) of hydrocarbon and tested by the referenced procedure. Controls were performed by using 5 × 10⁻⁶ m³ (5 mL) of diluted liquor that experienced the same procedure, but without any hydrocarbon. Results were reported as the percent change in OD600 relative to the control experiment, and were adjusted to constant TSS.

Isopropyl alcohol extractions of EPS were performed by ultrasonically treating 1 × 10⁻⁴ - 1.5 × 10⁻⁴ m³ (100 - 150 ml) of mixed liquor for 5 min. at 30W. This dispersed sample was then centrifuged at 3000 g for 30 min. (Sanin and Vesilind, 1994), and the supernate was reacted with 2 volumes of isopropyl alcohol. This alcohol mixture was stored at 39.2°F (4°C) for 24 hours to allow the polysaccharides to precipitate, and then centrifuged at

Table 1: Fermentor operating conditions, wastewater, and mixed liquor seedstock analysis

		Wastewater		
		Synthetic	Normal	High Sugar
Operating System	HRT (days)	0.83	1.46	1.46
	SRT (days)	46	20	68
Recycle System		microfilter	Mini-clarifier	microfilter
Wastewater	BOD ₅ (kg/m ³)		1.24	6.14
	BOD ₂₀ (kg/m ³)		1.13	7.44
	COD (kg/m ³)	1.31	2.05	16.4
	NO ₃ (kg/m ³)		ND	ND
	NO ₂ (kg/m ³)		ND	ND
	Total P (@PO ₄) (kg/m ³)		0.0196	0.00676
	Free NH ₃ (@N) (kg/m ³)		0.0004	0.0026
	Free & Fixed NH ₃ (@N)		0.0117	0.0197
	TSS (kg/m ³)		0.170	0.423
	VSS (kg/m ³)		0.04	0.217
Total Solids (%)			0.24	0.74
Mixed Liquor Seed	TSS (kg/m ³)		14.970	10.835
	VSS (kg/m ³)		12.765	8.730
	Total Solids (%)		1.67	0.95

* ND = none detected.

3000 g and 39.2°F (4°C) for 15 minutes. The EPS solids were resuspended in water and analyzed for total sugar concentration using the phenol-sulfuric acid method (Dubois et al., 1956). The total sugar concentration was used to estimate extract monosaccharide concentrations sent for analysis. Monosaccharide analysis was performed by first digesting an extract sample in 2 M trifluoroacetic acid for 18–24 hours at 95°C (203°F). 100 mL of the digested EPS extract was analyzed in an isocratic elution chromatography (HP-1050) system with an Varex Mk III evaporative light scattering detector (Alltech). The system used a Supelcogel C-611 (300 mm x 7.8 mm) analytical column and a Supelcogel Ca/C611 Carbohydrate (50 mm x 4.6 mm) guard column. These columns were maintained at 212°F (100°C) during the analysis, while the drift tube in the detector system was maintained at 239°F (115°C). 0.05% (v/v) of 88% formic acid (ACS reagent grade) was used as the eluent at 0.75 mL/min. ACS grade mono-

saccharide standards of glucose, galactose, mannose, glucuronic acid, and galacturonic acid are used to determine relative concentrations of monosaccharides in the EPS extracts. Peak area chromatogram of a mixture with known monosaccharide concentrations.

Results

Table 1 lists the operating conditions, wastewater concentrations, and mixed liquor seed concentrations used in the three fermentor experiments. The synthetic wastewater (SW) experiment was performed in a smaller fermentor vessel and this resulted in a smaller HRT. The SRT matched plant operating conditions except for the normal wastewater (NW) experiment, where the use of the mini-clarifier limited the SRT to 20 days. All three experiments approached or fell within the extended aeration conditions used by the plant to minimize sludge production and reduce the vol-

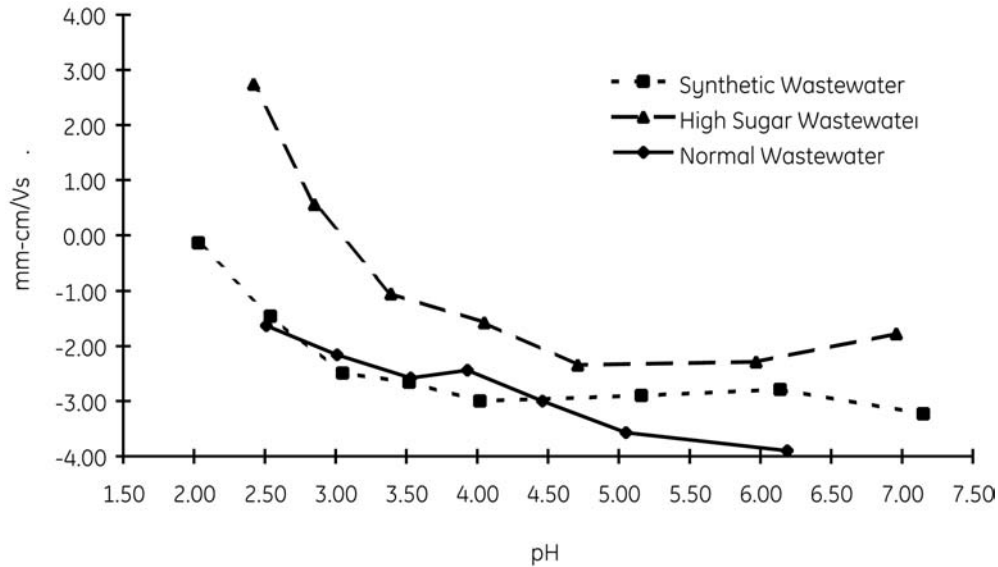


Figure 1. Electrophoretic Mobilities (mm-cm/Vs)

ume of sludge sent to a publicly-owned treatment works.

The wastewater profiles listed in Table 1 include the COD measure on the synthetic wastewater (SW) used in the first fermenter experiment. SW was composed of soluble material balanced to yield a carbon to nitrogen to phosphorous ratio of 100:1:1. The nitrogen-limited conditions in this design reflect the nitrogen-limited plant operating conditions expressed in the plant wastewater profiles. Fortunately, the plant has no history of filamentous bulking and showed normal distribution of filaments and microlife expected for operations balanced to a higher nitrogen ratio. The synthetic medium was mostly composed of readily degradable organic matter and the COD should be near the BOD₂₀ concentration listed for the NW sample. This synthetic medium was designed to approximate normal wastewater organic concentrations.

Table 1 also lists the plant wastewater profiles used in the two subsequent fermenter experiments. The high sugar wastewater (HSW) profile reflects wastewater taken during a plant outage where the activated sludge system was kept operational by feeding wastewater from a secondary holding tank that receives reactor washouts shortly after the plant shutdown. This wastewater contains very high concentrations of sucrose from previous bottling operations as well as cleaners used to washout the reactor vessels. This waste

water also contains a six-fold greater concentration of BOD₂₀ and an eight-fold greater concentration of COD. The higher COD is believed to reflect the additional cleaning chemicals. The nitrate, nitrite, free ammonia, and free and fixed ammonia concentrations show that the nitrogen concentration in the wastewater is lower than prescribed for normal activated sludge operation and in the form of complex nitrogen-containing molecules. No nitrate or nitrite concentrations were noted in the wastewater, but this may be partially a function of the 1 hr. delay between wastewater sampling and receipt in the laboratory. The wastewater solids in the two samples were proportional to the BOD and COD concentrations, and combined with the soluble concentration ratios, suggests that the two wastewaters were composed of similar materials but at different concentrations.

The solids of the two mixed liquor seed stocks used to fill the fermentors were similar, but the mixed liquor acclimated to NW possessed greater reactor solids. The ratios of TSS, VSS, and total solids were similar, but the solids were notably lower when the system was fed the HSW. This reflects the dispersed growth found under high sugar conditions. The NW-experiment fermentor produced larger flocs that clogged the microfilter and required an alternate mini-clarifier to be used. The reason this floc clogged the filter is unknown, but the larger flocs should have a lower clogging

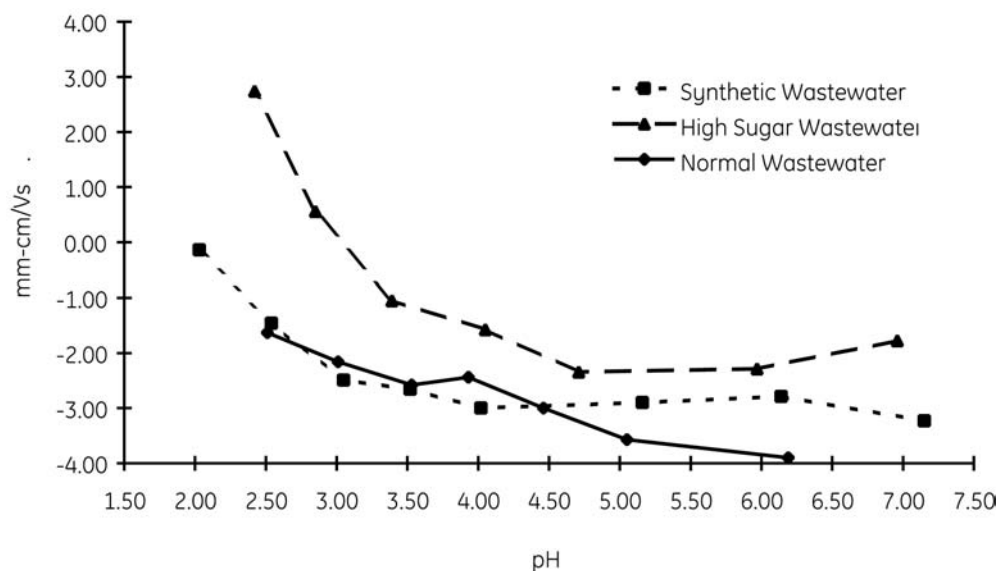


Figure 2. Dye Exchange Studies (Absorbance at 537 nm)

tendency than dispersed growth. This suggests that either smaller floc components are present in this liquor or that some extracellular material has unexpected filter-clogging tendencies.

Figure 1 shows electrophoretic mobility measures from the three fermentor experiments as a function of pH. All three samples were electronegative at neutral pH and slowly changed to mobilities that are more positive as the pH decreases. The SW and NW samples produced mobility curves that were very similar in shape and magnitude, but neither showed an isoelectric point near the 3.5 expected for uronic acids. In contrast, the HSW produced dispersed flocs, and showed less negative mobilities and an isoelectric point between pH 3.0 and 3.5. The magnitude of the electrophoretic mobilities may reflect a difference between the two test instruments, but the change in mobilities with pH was stronger with the HSW-grown culture. This suggests that charges are more easily neutralized in this sample.

Figure 2 shows results from the dye exchange studies. In this study, the cationic dye interacts with anionic charges on the sludge, and an increase in absorbance reflects fewer dye-sludge charge interactions. As the pH decreases, the anionic groups should become protonated and the dye should absorb less on the solids and result in higher absorbance readings. The HSW sample

produced greater dye absorbance at higher and medium pHs, suggesting that the charge interactions with the dye are weaker in this sample. In contrast, the NW and SW samples both showed strong dye absorption at neutral pHs and suggest that these samples possess charge sites readily available to the dye. Curiously, only the SW sample showed a curved pH profile characteristic of charge site protonation, and this suggests that charges were more easily neutralized in this sample than in the NW sample.

Figure 3 shows the colloid titration results for the same three samples. These colloid titrations reflect the surface charge that is available to interact with a charged cationic polymer. Clearly, the culture grown on HSW has the greatest interaction with the charged polymer, suggesting that the available surface charge on this sample's exopolymer layer is greater than on the other two samples. The cationic polymer will have difficulty diffusing into the exopolymer layer and will preferentially select anionic sites on the outer edges of the exopolymer layer. This sample was also dispersed and should have greater surface area, but the other two samples are diluted before titrating, and this should also produce greater surface area. The charge-pH curves for both the NW and SW experiments were both flat, suggesting that the charge sites reside inside the exopolymer

	Synthetic Wastewater*	Normal Wastewater*	High Sugar Wastewater*
Glucose	0.4	1.26	24.3
Galactose	1.12	0.84	13.2
Mannose	0.48	0.36	7.2
Glucuronic Acid	0.28	0.12	3.6
Galacturonic Acid	ND	0.15	ND

ND = none detected

Table 2: Extracted monosaccharide concentrations ([kg/m³]/ [kg/m³ TSS])

layer and are not available to interact with the cationic polymer.

Table 2 lists the monosaccharide composition of the extracted exopolymers. The results are listed as the ratio of the extract monosaccharide concentrations to the mixed liquor TSS concentrations. The table lists the relative concentrations of the five major monosaccharides: three nonionic monosaccharides and two uronic acids. The HSW sample shows much higher concentrations of all the monosaccharides except galacturonic acid. This corresponds with dispersed growth and a "slimed" condition. This sample shows that total exopolysaccharide production is more closely tied to food-to-mass (F:M) ratio than to food source concentrations. The other two samples show similar total monosaccharide concentrations, but with very different distributions. Both the NW and SW extracts contain similar total concentrations of uronic acids, but the NW sample is evenly distributed between glucuronic acid and galacturonic acid, while the SW sample contains only glucuronic acid. The similar total uronic acid concentration with the SW and NW extracts correlate with their overlapping electrophoretic mobility profiles.

Table 3 shows the relative hydrophobicity of the three mixed liquor samples grown on the different wastewaters. The NW and HSW samples matched within estimated errors and this suggests that the hydrophobic character of the two mixed liquors was the same. This was unanticipated since one of the liquor samples possessed large flocs while the other was the product of dispersed growth. The dilutions required to obtain similar OD 600 values may have disrupted the floc

structure, but this similarity was surprising. The lack of hydrophobicity in the SW sample was also unexpected, and this suggests that the exopolymer layer on this culture is notably different from the others.

Discussion

The purpose of this study was to compare similar activated sludge cultures grown on different feed stocks. The C:N:P ratio was kept constant throughout the experiments, and the readily degradable organic concentrations in the NW and SW were nearly equivalent, although the food sources were very different. Microscopic examination of the sludge grown on these two wastewaters showed adequate filament and microlife growth, and neither culture showed signs of bulked conditions. In contrast, the HSW-fed culture was dispersed and showed minimal filament and microlife growth, but this was expected for these extreme conditions.

Surface charge was measured by three independent methods, with each technique selected to provide different measures of the charge availability at different depths in the exopolymer layer. Electrophoretic mobility was chosen to measure the total charge experienced on the periphery of the exopolymer layer and should include contributions from charges buried inside the exopolymer layer. Some of these interior charges may be screened by surrounding molecules, but they should provide some contribution to the total measured charge. The colloid titration was chosen because it detects charges available on the outer exopolymer layer, and diffusion limitations should prevent the cationic polymer from diffusing into the inner exopolymer layers. Ruthenium red was used as an alternate charge detection method because previous work (Figueroa and

	Synthetic Wastewater	Normal Wastewater	High Sugar Wastewater
n-hexadecane	6	-18	-21
n-octane	-2	-9.9	-7.5

Table 3. Relative hydrophobicity (percent decrease in OD 600, normalized to constant TSS concentrations)

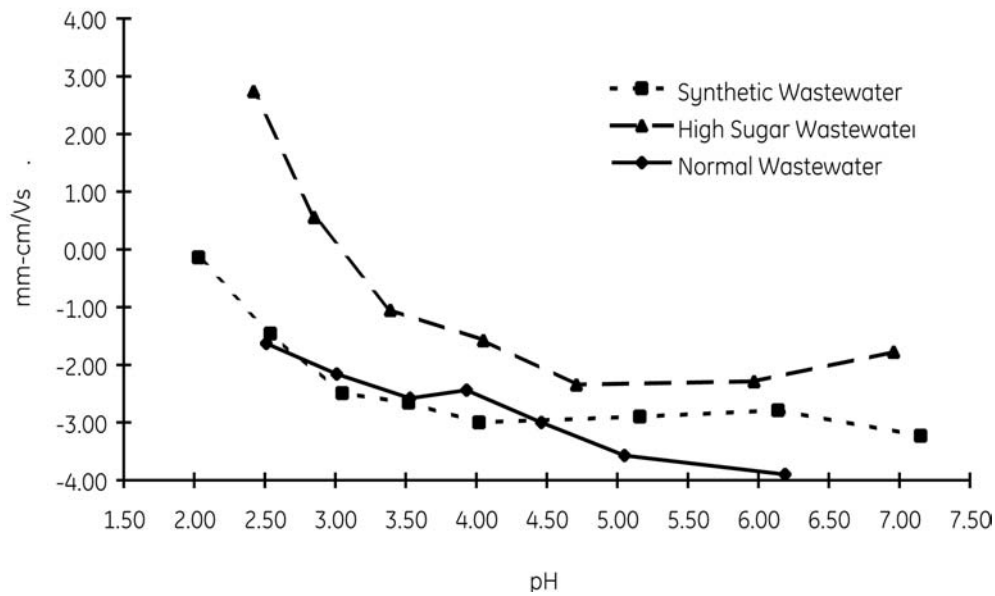


Figure 3. Colloid Titration (Anionic Charge in meq/g)

Silverstein, 1989) described this dye as having limited diffusion into the exopolymer layer. This implied that the dye would detect some charges buried in the exopolymer layer but would not be able to detect all the charge that electrophoretic mobility could detect.

These charge-measuring methods showed distinct differences between the three cultures examined in this study. The SW- and NW-fed samples showed almost identical electrophoretic mobility profiles. In contrast, the HSW-fed sample showed less total charge, but a charge that was more readily protonated when the pH was decreased. Additionally, the colloid titration showed a larger surface charge density with the culture grown on HSW. This suggests that there is a high outer charge concentration on this sample and that the charges are easily neutralized with the large cationic polymer. The dye exchange study shows the same profiles as the electrophoretic mobilities, and shows decreased dye release with the SW- and NW-fed samples. These results indicate that the net charge on these two samples was higher, but that the charge was contained deeper in the exopolymer layer and was better detected by electrophoretic mobility or dye exchange. The three charge measures on the HSW-fed sample suggest that the net charge was lower within the exopolymer layer but that there was greater surface charge on the outer layers.

This observation may be a result of the larger exopolymer concentration that would result in larger charged surfaces capable of interacting with the large cationic polymers used in the colloid titration. The other two samples would also have surface charge available for interaction with the cationic polymer, but their tendency to floc and their lower exopolymer concentration would result in a lower probability of a dramatic polymer to floc response. The limited response from the dye titration with the HSW-fed sample could also reflect limited diffusion into the large exopolymer layer, since this method produces relative charge measures and not absolute charge concentrations.

The charge titration measures showed similar charge profiles for the SW- and NW-fed samples. However, there was a slight difference in the dye exchange titrations. The SW-fed sample showed a pH response when the pH was decreased, while the NW-fed sample showed no change with pH. The monosaccharide analysis showed similar total uronic acid concentrations for these two samples, but the charged monosaccharide concentrations were different. The SW-fed sample was composed exclusively of glucuronic acid while the NW-fed sample was evenly split between glucuronic and galacturonic acids. This difference may partially explain the pH response seen at lower pH's in the dye exchange titration.

One surprising result from this study was that the SW produced a monosaccharide profile different from the two samples fed plant wastewater. Both the NW and HSW produced exopolysaccharides with glucose, galactose, and mannose in the same relative proportion and with glucose having the largest monosaccharide concentration in the extract. In contrast, the synthetic wastewater produced an exopolymer extract that had greater concentrations of galactose and mannose than glucose. Pure culture studies using glucose as the dominant carbohydrate source produced exopolysaccharides composed of glucose and galactose, with the later dominating (Grobben et al, 1996; Gammer-Nourani et al., 1998). This suggests that synthetic medium produces exopolymers with different monosaccharide profiles than cultures grown on plant wastewater. This exopolysaccharide difference did not appear to effect the charge profile of the flocs at normal operating conditions, but parallel hydrophobicity testing produced different results.

The relative hydrophobicity results from this study show cell surface properties with opposite food source correlations than the charge studies. It should be noted that the BATH test significantly alters the cell surface of tested bacteria (Pembrey et. al., 1999) and care must be taken when using this test for absolute measurements, but its purpose in this study was to give relative measures of surface hydrophobicity. The relative hydrophobicities of the cultures grown on plant wastewater had very similar hydrophobicities in spite of their very different exopolysaccharide concentrations. Additionally, one of these cultures grew in a dispersed form and the other produced large flocs with good settling properties. In contrast, the culture grown on synthetic medium had limited hydrophobic character. This result suggests that some additional component in the plant wastewater produces the hydrophobic character in the exopolymer layer. In support of these results, other researchers (Jorand et al., 1994) have shown that the food source can influence the hydrophobic character of a floc.

Conclusions

Cell surface properties measured in this study of laboratory-stabilized activated sludge have shown that activated sludge cultures grown on

synthetic media are significantly different from those grown on plant wastewater. These results suggest that the surface charge properties observed with synthetic media-grown mixed liquors are similar to those grown on normal plant wastewater, but that the monosaccharide composition and relative hydrophobicities are different. These latter two variables are known to have significant effect on sludge settling and dewatering properties and any study based on synthetic feed-based sludges should be interpreted with great caution. This also suggests that performing studies in an operating field system is preferred, but if this is not practical, laboratory-based activated sludge studies should use plant wastewater. Additionally, these results suggest that correlating settling and dewatering properties only to surface charge measurements could yield limited profiles of the bacterial cell surface properties in an activated sludge system. Additional studies correlating both surface charge and hydrophobicity with settling and dewatering properties should provide useful information on the complexity of these systems and should suggest ways to further optimize activated sludge operations.

References

- Andreadakis A. (1992). Physical and chemical properties of activated sludge floc. *Wat. Res.* 27(12), 1707-1714.
- Bruus J., Christensen J. and Rasmussen H. (1993). Anaerobic storage of activated sludge: effects on conditioning and dewatering performance. *Wat. Sci. Tech.*, 28(1), 109-116 .
- Clesceri L., Greenberg A. and Eaton A.(ed.). *Standard Methods for the Examination of Water and Wastewater* (1998). 20th edn, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.
- Dubois M., Gilles K., Hamilton J. and Rebers P. (1956). Colorimetric determination of sugars and related substances. *Anal. Chem.* 28, 350-352.
- Eriksson L. and Axberg C. (1981). Direct influence of wastewater pollutants on flocculation and sedimentation behavior in biological wastewater treatment. I Model system *Escherichia coli* B. *Wat. Res.* 15, 421-431.

- Figuerola L. and Silverstein J. (1989). Ruthenium red adsorption method for measurement of extracellular polysaccharides in sludge flocs. *Bio-technol. Bioeng.* 33, 941-947.
- Gammer-Nourani L., Blondeau K. and Simonet J.-M. (1998). Influence of culture conditions on exopolysaccharide production by *Lactobacillus rhamnosus* strain C83. *J. Appl. Microbiol.* 85, 664-672.
- Goodwin J. and Forster C. (1985). A further examination into the composition of activated sludge surfaces in relation to their settlement characteristics. *Wat. Res.* 19(4), 527-533.
- Grobben G., Smith M., Sikkema J. and deBont J. (1996). Influence of fructose and glucose on the production of exopolysaccharides and the activities of enzymes involved in the sugar metabolism and the synthesis of sugar nucleotides in *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772. *Appl. Microbiol. Biotechnol.* 46, 279-284.
- Horan N. and Eccles C. (1986). Purification and characterization of extracellular polysaccharides from activated sludges. *Wat. Res.* 20(11), 1427.
- Horan N., Bu'Ali A. and Eccles C. (1988). Isolation, identification and characterization of filamentous and floc-forming bacteria from activated sludge flocs. *Environ. Technol. Lett.* 9, 449.
- Jorrand F., Guicherd P., Urbain V., Manem J., and Block J. (1994). Hydrophobicity of activated sludge flocs and laboratory-grown bacteria. *Wat. Sci. Tech.* 30(11), 211-218.
- Jorrand F., Zartarian F., Thomas F., Block J., Bottero J., Villemin G., Urbain V. and Manem J. (1995). Chemical and structural (2D) linkage between bacteria within activated sludge flocs. *Wat. Res.* 29(7), 1639-1647.
- Kawamura S., Hanna Jr. G. and Shumate K. (1967). Application of colloid titration technique to flocculation control. *JAWWA.* Aug, 1003-1013.
- Pembrey R., Marshall K. and Schneider R. (1999). Cell surface analysis techniques: What do cell surface preparation protocols do to cell surface properties. *Appl. Environ. Microbiol.* 65(7), 2877-2894.
- Rosenberg M. (1984). Bacterial adhesion to hydrocarbons: a useful technique for studying cell surface hydrophobicity. *FEMS Microbiol. Lett.* 22, 289-295.
- Sanin F. and Vesilind P. (1994). Effect of centrifugation on the removal of extracellular polymers and physical properties of activated sludges. *Wat. Sci. Tech.* 30(8), 117-127.
- Urbain V., Block J. and Manem J. (1993). Bioflocculation in activated sludge: an analytic approach. *Wat. Res.* 27(5), 829-838.
- van der Mei H., Rosenberg M. and Busscher, H. (1991). Assessment of microbial cell surface hydrophobicity. In: *Microbial Cell Surface Analysis: Structural and Physicochemical Methods*, Mozes N., et. al. (ed.), VCH Publishers, New York, pp. 263-287.
- Wahlberg E., Keinath T. and Parker D. (1992). Relationship between activated sludge flocculation characteristics and cell-surface polysaccharide concentration. *Wat. Sci. Tech.* 26(9-11), 2527-2530.