Biofilms and dispersants: A less-toxic approach to deposit control

Linda R. Robertson and Nicole R. Taylor

ABSTRACT: Slime deposits are complex, containing highly diverse microflora along with wood fibers, fines, calcium carbonate, pitch, clay, and additives. Mills routinely use biocides to control deposition. It may be possible to reduce the use of these toxic chemicals by adding appropriate dispersants to the papermaking system. However, dispersant activity in papermaking systems is poorly understood, partly because of a lack of screening methods for evaluating nontoxic chemicals. This report presents the results of laboratory efforts to study the effect of dispersants on in vitro *cultures of pure biofilms and biofilms containing calcium carbonate. Results from a mill trial of a nonionic polymer dispersant showed excellent inhibition of deposition.*

KEYWORDS: Bacteria, biochemical properties, biocides, calcium carbonate, calcium compounds, carbonates, deposits, dispersants, lignosulfonates, microorganisms, microscopy, toxicity.

Paper-machine waters provide an ideal environment for the growth of deposit-forming bacteria and fungi *(1–3)*. Deposits are typically complex, containing highly diverse microflora along with wood fibers, fines, calcium carbonate, pitch, latexes, clay, or other papermaking additives. When deposits break loose and fall into the paper furnish, they can cause end-product defects such as holes, spots, or even web breaks.

Mills commonly add biocides to their papermaking systems to control microbial growth within acceptable limits. Efforts to reduce the toxicity of deposit control have generated interest in the use of dispersants as an adjunct to conventional deposit-control programs.

The activity of nonionic dispersants against purely biological films has been demonstrated in cooling-water systems *(4, 5)*. However, the chemistry of paper processing systems is more complex, and the activity of nonionic dispersants in such systems is poorly understood.

Conventional wisdom has held that dispersants act as "biopenetrants," opening the biofilms and allowing biocides to penetrate the exopolysaccharides. There are additional claims that dispersants facilitate penetration of biocides into the cell *(6)* or that they trigger sloughing of biofilms. Several European workers contend that lignosulfonate dispersants prevent bacterial attachment, allowing mills to forego the use of toxic chemicals altogether *(7–9)*. According to these workers, the deposits that do form are loose, soft, fluffy, and easy to disperse. Despite these claims, the mode of action of these dispersants is poorly understood.

Among the difficulties in evaluating the effects of nontoxic agents is the lack of suitable measurement systems. Biocides are evaluated by measuring kill or inhibition. With nontoxic materials, alternative methods of assessing chemical efficacy must be developed.

This article describes experimental methods that have been developed to evaluate the effects of dispersants on pure bacterial biofilms and on biofilms containing particulate calcium carbonates. The methods included environmental scanning-electron microscopy

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(ESEM), confocal-laser scanning microscopy (CLSM), light microscopy, and gross measurement of biofilm diameter. The laboratory study examined the activity of six lignosulfonate dispersants and a single nonionic polymer dispersant. This article also presents the results of a subsequent mill trial using the nonionic polymer dispersant.

Methods

Laboratory study

Biofilms. A sheathed, filamentous bacterium commonly found in paper mill deposits was selected for the study. Bacterial cells were grown in a nutrient medium designed to promote biofilm formation. The medium was put into rinsed, 8-oz flush jars. After inoculation, jars were placed into an orbital shaker at 35°C at 210 rpm. At predetermined intervals, biofilms were rated for diameter and vigor. Ground calcium carbonate or precipitated calcium carbonate at 0.2% were added to some cultures.

Dispersants. The tested dispersants included six lignosulfonate dispersants (Lignosulfonates A–F). A nonionic polymer was also screened. Dispersants were added to the cultures at the rate of 20 ppm, 100 ppm, or 200 ppm.

Microscopy. Biofilms were carefully removed from glass surfaces using a Teflon™ cell scraper and floated onto glass slides (for light microscopy and CLSM) or on SEM stubs (for ESEM examination). Light-microscopy specimens were examined directly using a phase-contrast microscope. CLSM samples were stained before examination with acridine orange.

Mill trial

A biofouling monitor was installed on a white-water side stream of a clay-coated boxboard mill. Coupons were removed from the monitor at 14-day intervals, and 10-cm^2 areas of the coupons were analyzed on each coupon for colony-forming units of aerobic and sulfate-reducing anaerobic bacteria. Tryptone glucose extract agar was used for aerobic bacteria, and a ferric citrate agar was used for sulfate-reducing anaerobic bacteria. Dry weights were measured at 105°C.

Results and discussion

Laboratory work

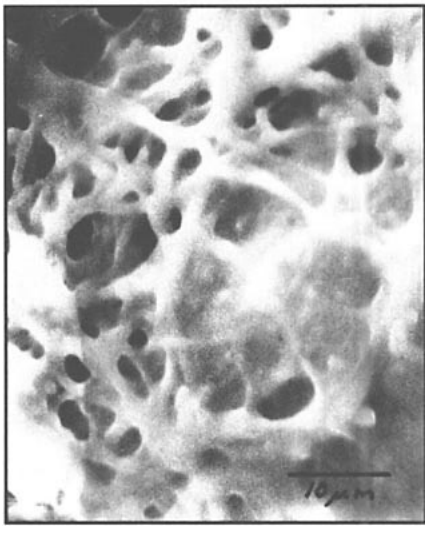
The primary purpose of the laboratory work was to develop a screening system that could be used to evaluate the effects of nontoxic chemicals in controlling paper-machine deposits. The bacterium used in the study easily forms biofilms on paper-machine surfaces. However, it did not consistently do so in the *in vitro* shake flask experiments.

Establishing the optimal growth conditions that enhanced cell attachment to surfaces was critical to the success of these experiments. At the end of the incubation period, the diameter, relative thickness, and vigor of the biofilm could be measured at the jar base, and these results could be correlated to dispersant efficacy. Vigor was measured on a scale of zero (no film) to 4 (heavy, vigorous growth).

Researchers at Montana State University *(10)* observed that when inert latex particles were added to an established biofilm, they were transported to the base layer of the film. This suggested that the biofilm might be more open than previously assumed. It also suggested that one theory for the mode of action of dispersants—that they open the film for penetration by biocides—might be inaccurate. To examine this hypothesis, biofilms were examined through use of an Electroscan environmental scanning-electron microscope (ESEM). The ESEM allowed observations to be made using wetted, viable samples. Standard SEM observations are done with killed, dried samples, resulting in distorted features and numerous artifacts.

The ESEM photomicrograph in

1. ESEM photomicrograph of live filamentous biofilm shows an open, weblike structure.



2. Effect of nonionic polymer dispersant on formation of *in vitro* cultures of pure filamentous biofilm and mixed biofilm containing calcium carbonate

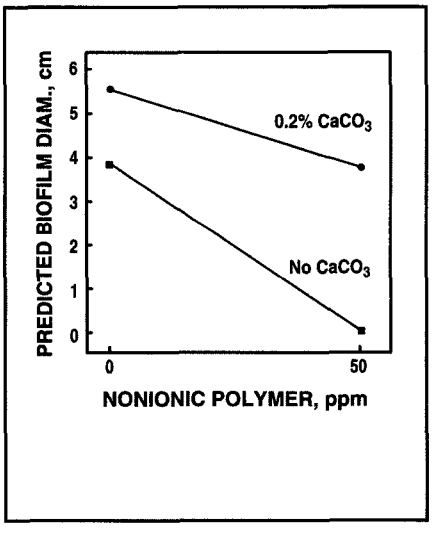
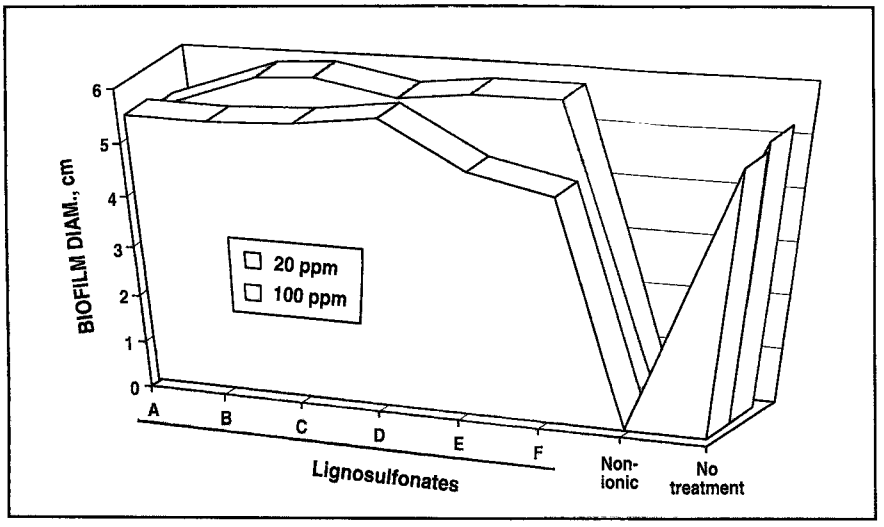


Fig. 1 shows the open and web-like film matrix of a viable, untreated biofilm when part of the fluid has been removed in the instrument. Given the openness of the structure, it is possible to imagine that materials might migrate to the biofilm base.

Another tool used to examine biofilm was a Leica confocal-laser scanning microscope (CLSM). With this microscope, a laser passes through the layers of the biofilm, exciting materials that have been

3. Effect of dispersant addition on formation of *in vitro* biofilm cultures



stained with fluorescent dyes. Sophisticated software techniques reassemble the biofilm, enhance the color, or produce contour plots. Using CLSM, it was possible to observe both the individual cells in the biofilm and the rough tower-like structures typical of filamentous bacterial biofilms. When dried, these "towers" ranged in depth from 1 μm to 2.5 μm. (Note that biofilms contain high levels of water and that drying the biofilms before measurement greatly decreased their thickness.) When the nonionic polymer dispersant was added to jars at the same time as the bacterial inoculum, the resulting biofilm was smaller in diameter, thinner, and less vigorous. It also appeared flatter in the contour plots, and the depth was an even 1 μm throughout the biofilm.

While the nonionic polymer dispersant showed good activity against the pure biofilms, increased concentrations were needed with mill-like biofilms containing calcium carbonate. **Figure 2** shows that the mixed system containing calcium carbonate required higher levels of nonionic dispersant to inhibit film formation.

In separate experiments, nonionic polymer dispersant was added at 10 ppm, 20 ppm, and 100 ppm to existing biofilms. The dispersant did not trigger sloughing or film removal, indicating that it could not be used to clean a dirty system at the levels examined in this study. However, this suggests that addition of this dispersant to a papermaking system will not cause existing deposits to break loose and induce sheet defects.

The use of lignosulfonates to control deposits has met with mixed success. While they have been used to reduce pitch deposition, there was no evidence of lignosulfonate activity against the purely bacterial biofilms in these studies. In fact, some lignosulfonates created larger, thicker biofilms than did the untreated controls, as seen in **Fig. 3**. Many lignosulfonates contain high levels of sugars, which would serve as nutrients for the bacterium.

Although lignosulfonates appeared to have no effect against biofilms, they may affect mixed calcium carbonate:bacterial deposits. In studies with jars containing lignosulfonates and ground calcium carbonate or precipitated calcium carbonate, no reduction in the attachment of biofilms was observed, as seen in **Figs. 4 and 5**. If anything, the relative vigor of the biofilm increased with the increased lignosulfonate concentration. Lignosulfonate A was used in this experiment.

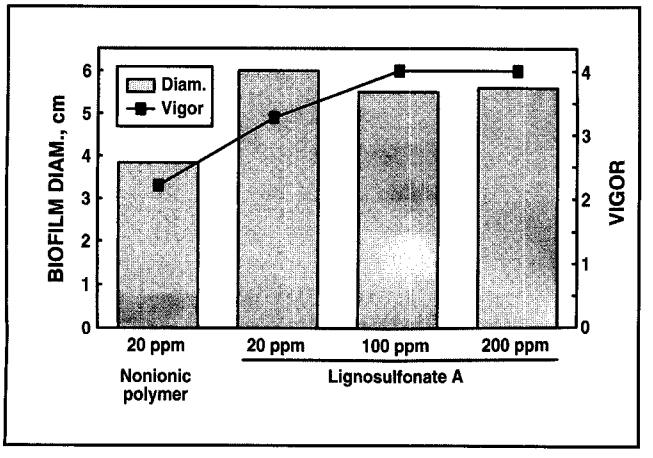
Mill trial

One concern of any laboratory study is the application of the data generated *in vitro* to the real world. To that end, a trial was conducted with the nonionic dispersant in a midwestern mill as part of a low-toxicity deposit-control program. **Figure 6** shows dry weights observed on 10-cm^2 areas of test coupons withdrawn at two-week intervals from a biofouling meter installed on a white-water side stream. Coupons collected before addition of the nonionic dispersant showed a heavy buildup of bacterial and chemical material and had a dry weight of 83 mg/10 cm^2. Coupons collected during the trial were very clean and showed only a slight film buildup, with a dry weight of 4 mg/10 cm^2.

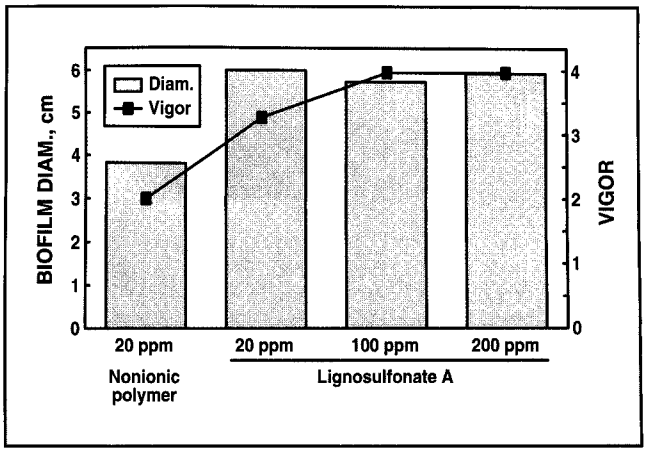
The ultimate test to determine whether a chemical is having an effect on a machine is to remove the chemical and look for negative effects. To that end, the nonionic dispersant was taken off the machine. Because of the closure of the system and the long half-life of the chemical, we did not expect to see immediate disappearance of the chemical from the system. However, heavy deposition was again observed on the coupons. The dry weight of a 10-cm^2 area was 46 mg at the end of the standard two-week interval, a tenfold increase over the trial period.

Figure 7 shows the plate counts for aerobic bacteria and sulfate-reducing anaerobic bacteria before, during, and after the trial. There was a 99% reduction in the counts of aerobic bacteria on coupons in the treated system as compared with the untreated system. In addition, the count of sulfate-reducing anaerobes was reduced by 99.99%. With heavy and thick deposition, the anaerobic counts may be a better indicator of the overall sessile population. This is because the number of aerobic bacteria may be limited in a heavy deposit, since their growth is either enhanced by

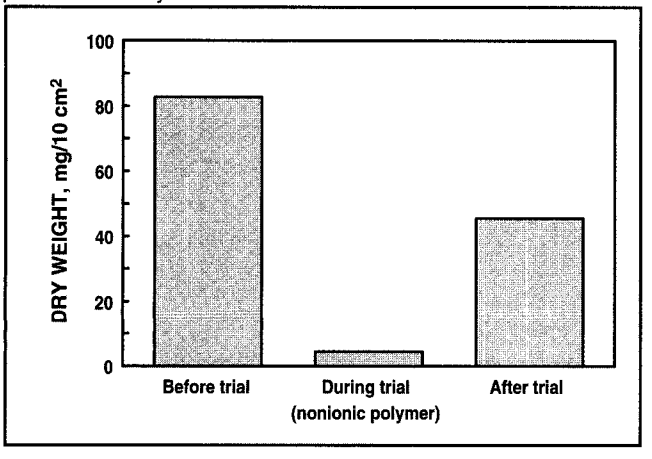
4. Effect of dispersant addition (nonionic polymer and Lignosulfonate A) on formation of *in vitro* biofilms containing ground calcium carbonate



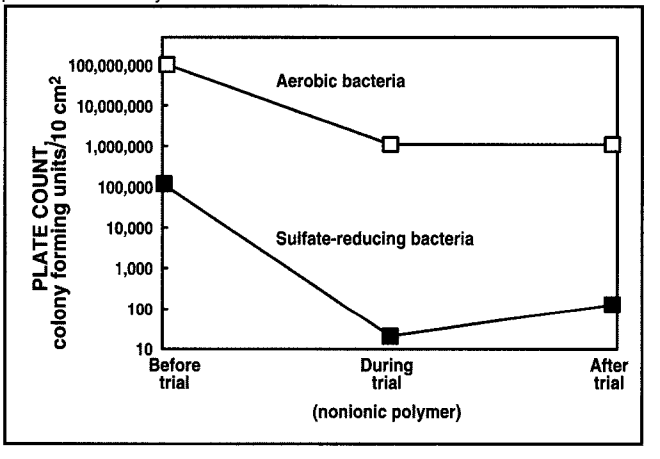
5. Effect of dispersant addition (nonionic polymer and Lignosulfonate A) on formation of *in vitro* biofilms containing precipitated calcium carbonate



6. Dry weight of deposits on test coupons after two-week exposures before, during, and after addition of nonionic polymer dispersant to mill system



7. Plate counts of deposits on test coupons after two-week exposures before, during, and after addition of nonionic polymer dispersant to mill system



or dependent on oxygen. Growth of anaerobes in a heavy deposit would not be affected by a lack of oxygen. Research indicates that the total viable count may reflect the living organisms, but protein levels may more accurately reflect total bioaccumulation *(11)*. Future studies should explore this issue.

In contrast to the dry-weight measurements, the number of bacteria on the coupons harvested during the post-trial period did not increase to the level observed on coupon surfaces during the pretrial period. This could be because the nonionic dispersant does not degrade quickly and should persist for some time in a closed system.

While the removal of toxicants from a paper-mill deposit-control program is appealing, this should not be done without carefully considering all safety factors. Uncontrolled growth of anaerobic bacteria in chests has been implicated in injuries and deaths *(12)*. These organisms produce hydrogen, methane, and hydrogen sulfide. Spoilage of chests, fiber strength degradation, and starch degradation also can occur when the growth of microorganisms is not controlled. The judicious use of biocides to control the adverse effects of microbial activity makes sense for environmental, safety, and economic reasons.

Conclusion

ESEM and CLSM are valuable techniques for examining and evaluating biofilms. However, our experiments indicate the need to develop additional methods of evaluating the effectiveness of nontoxic chemicals in controlling mill deposits. Such methods must be able to gather information on pure biofilms and on mixed deposits containing both inorganic and biological materials.

Lignosulfonate dispersants did not appear to inhibit the formation of bacterial biofilms in the laboratory. Indeed, lignosulfonate fractions containing sugars appeared to increase biofilm formation.

Addition of a nonionic polymer dispersant to the culture medium at the time of inoculation did inhibit biofilm formation. The films that formed tended to be thinner and less dense than the untreated biofilms. Addition of nonionic dispersant to existing biofilms did not trigger sloughing or film removal. Greater amounts of nonionic dispersant were needed to prevent or inhibit biofilm formation when particulate calcium carbonate was present in laboratory studies.

A field trial of the nonionic dispersant showed excellent inhibition of deposit formation.

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