O PAPEL vol. 84, N.o 5, pp. 84 - 89 - MAY 2023

# NOVEL BIOFILM CONTROL TECHNOLOGY FOR PAPER MACHINES

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## ABSTRACT

Paper manufacturing in the neutral-alkaline pH range requires a rigorous microbiological control program in order to avoid runability issues caused by slime growth on machine surfaces. Oxidative biocides, especially stabilized-chlorine compounds, are most commonly used in wet-end for controlling pink slime growth. However, chlorine-based programs do contain the risk of causing machine corrosion damages as an unintended and costly side effect. Running with significantly lower chlorine levels would naturally alleviate the corrosion issue, however, it can result in massive uncontrolled slime growth. Therefore, it would be beneficial to discover a new antibiofilm chemistry, specifically targeting surface-attached bacteria. Using this kind of biofilm-targeted chemistry would enable reducing the chlorine dosing, without facing pink-slime issues. In a university collaboration project, a large library of molecules was screened with the purpose of identifying a promising new antibiofilm chemistry. The project led to the identification of a new molecule, B11. This paper shows the results obtained from the first B11 tests in authentic paper making conditions. It demonstrated biofilm inhibition at a uniquely low concentration of 0.05 mg/l. At this dosage level, its magnitudes are more effective than any organic biocide molecules currently on the market. This new technology has great potential to become the antibiofilm product of choice, when reduction of chlorine-chemistries is perceived as beneficial. This could become necessary due to on-going corrosion issues with machinery or in connection with water system closure project. A biocide program including a very low daily consumption of B11 and reduced dose of chlorine-compounds enables running a paper machine with maximized corrosion safety and effective biofilm control.

***Keywords:*** *paper machine, microbiological control, biocide, biofilm, slime, chlorine, corrosion*

## INTRODUCTION

Paper machine wet-end is a good growth environment for moderately thermophilic bacteria. Bacteria can be present as free-swimming (planktonic) cells in the process flows, or as attached cells on machine surfaces. Attached cells can multiply on surfaces, creating a structure where cells are embedded in an extracellular polymeric matrix, a biofilm. In time, the biofilm will grow to a size that it is clearly visible to the naked eye as a slime layer. Excess slime formation can cause costly problems such as sheet breaks and production losses. Defects such as dirt spots and holes in paper will lower the quality of finished paper.

Biocides are commonly applied to prevent, or at least, reduce slime formation (Kolari, 2007). This paper focuses on production processes that use bleached fibers and the wet-end has a neutral-to-slightly alkaline pH. It appears that unless adequately controlled, these processes are especially prone to slime formation. For papermakers of these grades, the expression "pink slime" is, unfortunately, quite familiar. The color of slime is due to the presence of bacteria that contain colored pigments (Ekman et al. 2007).

In the past, paper makers applied organic biocides and/or free chlorine to the wet-end. Programs based on organic biocides were costly. On the other hand, side reactions with additives limited the use quantities of free chlorine. Therefore, many mills had a situation that, due to budget constraints, only modest control of pink slime was achieved. A significant change occurred approximately 20 years ago with the emergence of stabilized-chlorine technologies which do not interfere with other chemical additives in papermaking. The first stabilizer on the market was ammonium bromide (Barak, 1999), soon followed by other chlorine-stabilizer chemistries. Common to all these technologies is that there are two precursor products, the active biocidal sodium hypochlorite and a stabilizing compound. These precursors are mixed on-site to produce a mild oxidizer which is dosed to the wet-end. These oxidative biocides presented a better cost-benefit ratio when compared

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to traditional organic biocides. Machines were now able to achieve proper slime control and subsequently evidence of how cleanliness improved runability started to build up.

Great, isn't it? In the beginning, yes. However, after extensive use, corrosion issues were observed in paper machines. When ammonium-based stabilizers are mixed with hypochlorite, they generate haloamines, such as monochloramine (MCA). Due to its polarity and low molecular weight, it has high volatility and poses potential vapor phase corrosion issues in the press and drying section of paper machines (Keegan et al. 2010). Solutions for the corrosion issues have been proposed. One can try to reduce the usage of chlorine compounds in general and apply more organic biocides. One proposed approach is to use in the same process two different stabilizer-chlorine compounds, monochloramine (MCA) and monochloro-5,5-dimethylhydantoin (MCDMH), in a manner that they are applied in different areas of the process. This holistic application strategy limits the use of volatile MCA in the areas most sensitive to corrosion, while still benefiting from the MCA kill power in the long loop, and the short loop biofilm control is done by using non-volatile MCDMH (Nelson et all. 2015).

Reducing the use of chlorine creates a risk for a situation where planktonic growth still remains under control, but biofilm can be excessive. Very quickly, the machine surfaces can have intense pink-slime formation. This is due the fact that in general, compared to the planktonic cells, biofilms are more difficult to control (Kolari, 2007). This is due to two factors. Firstly, the surface-attached cells produce an extracellular polymer matrix around the cells, limiting diffusion and protecting cells from biocides that have been dosed to process flow. Secondly, the scientific community has demonstrated that the same bacterial cell, whether in planktonic state, or growing surface-attached, is metabolically different. Therefore, it would be beneficial to discover an antibiofilm chemistry that is more targeted against the surface-attached form of bacteria. The use of this kind of biofilm-targeted chemistry would hopefully enable reducing chlorine dosing, without facing pink-slime issues.

With this target in mind, Kemira initiated a university collaboration project where a large library of molecules was screened in attempt to identify promising new antibiofilm chemistries. The project utilized *Meiothermus silvanus* as one model organism. This colored moderately-thermophilic bacteria has demonstrated to be a very common primary slime-former in paper machines, discovered from pink slimes all over the world (Ekman et al. 2007). The project led to the identification of one new molecule, B11. In laboratory experiments it inhibited biofilm formation at very low concentrations and its use as an antibiofilm agent in paper industry was patented (Simell et al. 2020). This paper shows results for the first B11 tests under authentic paper-making conditions.

## METHODS

### Chemistry

A new product synthesis route was developed (Hiltunen et al. 2020) and material was produced in quantities sufficient for the first field test run in authentic paper making process.

### Field test in fine paper machine

A field test was done on a fine paper machine producing uncoated free sheet at an average of 1,400 tons per day. The base-biocide program of the paper machine had stable performance. During the field test, the paper machine base-biocide program was kept unchanged in all areas except in the super clear filtrate (SCF) tank, where controlled changes in chemistry dosing were done. The SCF tank volume was 80 $m^3$ and average flow 60 L/sec. Field testing comprised four subsequent run cycles. Chemistry was added to the SCF tank with a regular chemical dosing pump. The dosing was continuous, except during the run cycle of the reference month when there was no biocide dosing.

After every run cycle the machine had a maintenance shutdown. At the beginning of the shutdown the SCF tank was drained, and the cleanliness of tank surfaces was inspected immediately, before surfaces started to dry. Visual inspection was complemented by photographs.

In addition, a small side-flow device was installed. The SCF water was continuously flowing through the device with a 10 L/min flow. This created a fast flow passing through a small device chamber holding eight AIS316 stainless steel coupons with a size of 25 mm x 75 mm. Before use, the coupons were prepared by washing them for 1 hour with warm soap water and brush, polishing with GRID 1200, washing with acetone and ethanol. After cleaning, the coupons were dried and weighted to get the initial value. During the run cycle, two coupons were removed each week, placed in a sterile plastic centrifuge tube and transported to laboratory for analyses. Nikon 50i epifluorescence microscope with a digital camera was used for inspection of the coupons. Living attached cells on steel coupons were stained with a SYTO 9 green-fluorescent nucleic acid stain (Invitrogen) for 15 min in the dark. Each coupon was thoroughly scanned with 100x and 1000x magnification. For dry-weight measurement, the coupon was kept in a 105°C oven for overnight drying. The coupon was cooled down, the dry weight recorded, and the difference was calculated to the dry weight of the same coupon measured before installation in the side-flow device.

## RESULTS AND CONCLUSIONS

The field test was done on a fine paper machine during four consecutive run cycles. In this machine the biocide program included two stabilized oxidizers, monochloramine (MCA) and monochloro-dimethylhydantoin (MCDMH), used as described in an earlier ABTCP paper (Santos et al. 2020). This dual oxidizing biocides system had worked well for several years.

However, the process had a specific deposition issue in one filtrate tank, the super clear filtrate tank (SCF). The deposits were composed of pink slime and sizing hydrolysates. These deposits had been very persistent against oxidizing biocides and, therefore, organic biocides had to be specifically dosed in the SCF tank. In time, via trialing of different products, the organic biocide product well-established in use was a mixture of DBNPA and DDAC, a quaternary ammonium compound.

With this situation, the fine paper machine with well-performing and stable base-biocide program, but with one challenging tank, provided an excellent location for this research. In the process industry it is difficult to arrange such a well-controlled testing environment for a new antibiofilm technology. This was an exceptional opportunity, because it was known that running this SCF tank without any biocide would create conditions where some biofilm would show up. This acted as an untreated reference. By running the incumbent product, we were able to quantify the performance of the existing technology. Subsequently we were able to quantify the performance of this new antibiofilm technology by running it at different concentrations.

Table 1 shows the testing scheme. The overall paper machine production performance was at a very similar level during the entire field test, i.e., the four consecutive months, making the run cycles well comparable. Overall performance and biocide consumption of the base program was also stable during the four-month testing period. The only exception was that, during the last test month, the paper machine received unexceptionally high quantities of coated broke from the neighboring machine. This showed up as slightly increased deposit formation in the wet-end. In other words, the microbial load in the system was slightly higher during the last test cycle with a lower B11 dose.

Figure 1. shows photographs taken from the SCF tank during monthly maintenance shutdowns. The SCF tank was always inspected immediately after draining, before surfaces had dried. The incumbent biocide product demonstrated a biofilm control effect, however, tank surfaces were not completely clean (Fig 1A). Some areas of the tank surfaces were covered by a thin layer of deposits, whereas other parts of the tank surfaces were clean. The run cycle without any additional biocide dosed in the SCF tank resulted in extensive deposit formation all over the tank (Fig 1B). Deposits were visible to the naked eye and in some areas had a slimy and yellowish appearance. The run cycle with a higher dose of B11 resulted in a completely clean SCF tank (Fig 1C). To the naked eye, it was not possible to detect any fresh deposits on

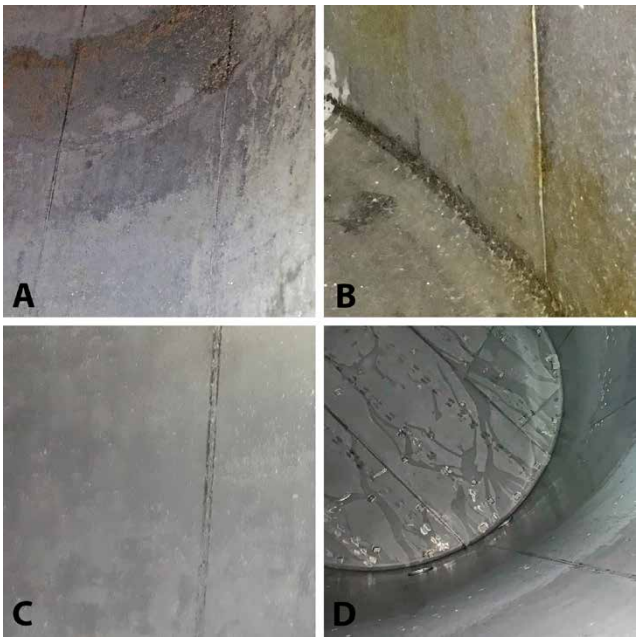


**Figure 1.** Photographs of surface cleanliness in the SCF tank after approximately one month of running with incumbent biocide product (A), without any biocide addition (B), with B11 at a higher dose (C) and with B11 at a lower dose (D).

**Table 1.** Information about the biocide program during the four consecutive test run cycles. Table shows sum dosages of the entire paper machine. Different treatments of SCF tank are shown in Table 2.

| Run cycle | Length of run cycle (days) | Average dose (kg/ton of paper produced) | | |
|---|---|---|---|---|
| | | Hypochlorite product | Stabilizer products | Organic biocidal products |
| Incumbent biocide dosed direct in SCF tank | 24 | 2.66 | 1.11 | 0.08 |
| Reference – No biocide dosed direct in SCF tank | 32 | 2.43 | 1.05 | 0.06 |
| High B11 dose direct in SCF tank | 33 | 2.23 | 1.03 | 0.05 |
| Low B11 dose direct in SCF tank | 30 | 2.36 | 1.03 | 0.06 |

the tank surfaces. On some areas of the tank surface a thin layer of firmly adhered white material was observed. This was assumed to be carbonate scaling that had deposited on the tank surfaces during years. This material was scraped off from the surface into a sterile plastic tube and analyzed in laboratory with the FTIR method. The results showed that it was mainly composed of calcium carbonate, with some minor amounts of fibers and ASA hydrolysate present. The run cycle with a lower dose of B11 also resulted in a clean SCF tank (Fig 1D).

In addition to shutdown inspections of the SCF tank, biofilm formation over time was monitored via a side-flow device containing several stainless-steel test coupons immersed in continuous flow of SCF water. At the start of a cycle, the device contained eight sterile stainless-steel coupons and each week two coupons were removed for laboratory analysis with an epifluorescence microscope.

Figures 2 and 3 show a collection of epifluorescence microscopic pictures taken from the steel coupons after three weeks of immersion to side flow of SCF tank. These coupons were stained with a DNA stain. Living micro-organisms on the steel surface emit green fluorescence. It is noteworthy that all green-colored objects in the pictures are not microorganisms, because a part of fluorescence originates from non-biological deposits, either non-specifically stained or due to autofluorescence. Therefore, the shape of the fluorescent objects played a big role in results interpretation, which is a well-recognized phenomena with fluorescent staining techniques.

During the run cycles with higher B11 dosing (Fig. 2C) and lower B11 dosing (Fig. 2D), a large part of the examined surfaces were completely black under fluorescent microscopic imaging. This indicates areas of clean steel surface with a complete absence of any microorganisms or organic deposits. During the incumbent biocide product dosing, the surfaces contained significantly more deposited material (Fig 2A). When the SCF tank went untreated with any biocide, the surfaces were almost completely covered with deposits (Fig 2B).

When the coupons that were exposed to the lower B11 dosage were inspected at maximum 1000x magnification, it was possible to detect a few isolated single bacterial cells only, sporadically on the steel surface (Fig 3D). All the observed living bacterial cells were attached direct to steel surface, no cell clusters were present. On the other hand, the coupons from the incumbent biocide product run (Fig 3A) and reference run (Fig 3B) contained a lot of deposited material, creating multilayer deposits. The deposits were much thicker than optical focus layer of the fluorescence microscope and it was not possible to focus sharply on individual bacterial cells. Piles of cells and other deposited material are seen as intensively fluorescent clusters on the steel surface.

Fluorescence microscopic analysis of the steel coupons was completed by assessing coverage percentage and relative thickness of deposits on the whole surface area of the stainless-steel coupons. Table 2 shows that, after the Reference run cycle, the coverage percentage was 85%. This indicated that at only 15% of the entire coupon surface area was clean, not covered by biofilm. After the Low B11 dosage run cycle, up to 70% of the steel surface was completely clean. This was remarkable cleanliness compared to earlier best product, providing only 25% of surface with complete cleanliness.

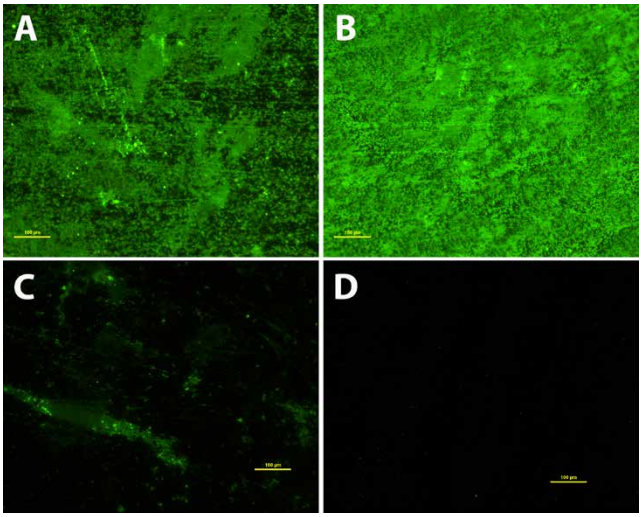


**Figure 2.** Epifluorescence microscopy pictures from the stainless-steel coupon surfaces after running three weeks with incumbent biocide product (A), without any biocide addition (B), with B11 at higher dose (C) and with B11 at lower dose (D). DNA staining. Magnification 100x.

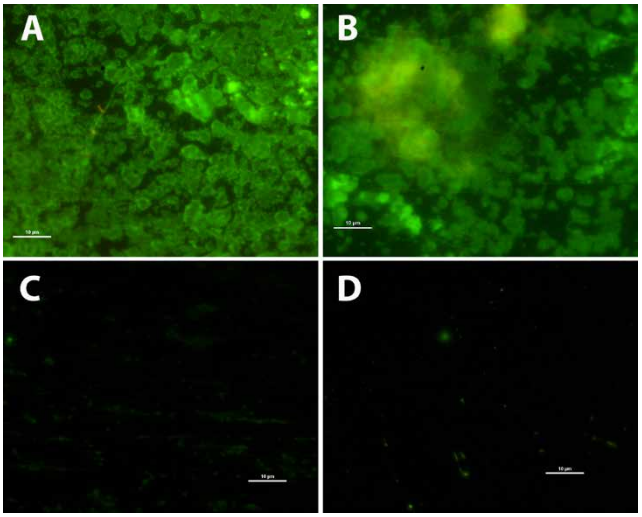


**Figure 3.** Epifluorescence microscopy pictures from the stainless-steel coupon surfaces after running three weeks with incumbent biocide product (A), without any biocide addition (B), with B11 at higher dose (C) and with B11 at lower dose (D). DNA staining. Magnification 1000x.

**Table 2**. Comparison of SCF tank cleanliness during different run cycles

| Run cycle | Chemistry dose as active (mg/L) | Observations from tank surfaces during shutdown inspection | Stainless-steel test coupons in side flow | | |
|---|---|---|---|---|---|
| | | | Coverage (%)[1] | Thickness rating [0 to 3] | Deposit weight |
| Incumbent biocide dosed directly in the SCF tank | 1.14 | Control effect, but surfaces were not completely clean, some deposits were evident | 75 | 2 | 1.39 |
| Reference – No biocide dosed direct in SCF tank | 0 | Tank dirty, slimy | 85 | 2 – 3 | 1.53 |
| High B11 dosed direct in SCF tank | 0.19 | Tank clean | 45 | 0 – 1 | 0.29 |
| Low B11 dosed direct in SCF tank | 0.05 | Tank clean | 30 | 0 – 1 | 0.53 |

[1] Deposition (chemical and biological) to process surfaces is never constant film-like, instead it is unevenly distributed. This is described by coverage percentage and average thickness. These values were assessed by fluorescence microscopic analysis of surface area of the whole steel coupon at the end of run cycle, from duplicate coupons.

Table 2 also shows relative thickness of the deposits on the steel coupons. Thickness was measured by first focusing the microscope objective to the level of the steel surface, writing down the number in focus wheel, and then re-focusing the microscope to the upper-most point of a deposit, again writing down the number in focus wheel and calculating the difference. In this manner, it was possible to evaluate height of the deposits in all areas of the steel coupon and assess a relative thickness rating number for the inspected coupon. After the Reference run cycle, the thickness rating range was between 2 and 3. This indicates that, depending on the location on the steel surface, the thickness of deposits varied between 2 and 3 in this relative scaling. After the Low B11 dosing run cycle, the thickness rating varied between 0 and 1, and 70% of the area being completely clean with no attached material on it.

After the microscopic examinations, the analyses were completed by measuring the dry weight of material that had deposited on the coupon surface during their immersion time in SCF water side flow. It is worthy to note that this is a sum analysis, not differentiating possible carbonate scaling, organic deposition and living biomass from each other. Because test periods had slightly different lengths, the results are reported as dry weight increase trend, milligrams per month. Aligned with the microscopic observations, there was significantly less deposition on steel coupons when B11 was dosed (Table 2.).

## DISCUSSION

The field testing of B11 provided encouraging results, indicating high performance at inhibiting biofilm formation on surfaces. This testing confirmed earlier laboratory observations that B11 is effective in very low concentrations. During the four consecutive run cycles under authentic paper machine conditions, the best cleanliness of the SCF tank was achieved in run cycles with B11. This happened even though the overall oxidizer dosing in the base-biocide program was slightly lower during the B11 run cycles (Tables 1 and 2).

This encouraged scaling up the synthesis of B11 to larger volumes and now it is possible to continue with full-scale trials to collect further evidence of the high performance. At this stage, we can report first indicative results suggesting that it is possible to maintain good surface cleanliness while reducing stabilized-chlorine dosages by 40%. This results in 69–90% lowered corrosion rates of carbon steel corrosion coupons placed in the first groups of drying section. Longer trials will demonstrate by how much the chlorine dosage can be reduced.

It is a rare event that a new antimicrobial compound is introduced in the pulp and paper industry. The same reality also exists in the medical antimicrobial compounds field, even though the need is enormous due to an increasing number of resistant bacterial strains being diagnosed. Awareness of this situation with medical antimicrobials raised our interest to confirm that we are not dealing with a chemistry that bacteria would easily develop resistance against when taken in wider use. In co-operation with the University of Copenhagen's Costerton Biofilm Center, a global leading institution in biofilm research, a study was conducted to study this phenomena using the industry-relevant bacterium species *Meiothermus silvanus* as model organism. After extensive studies with different approaches, it can be concluded that the B11 molecule has

multiple targets in this biofilm-forming species (manuscript in preparation). Resistance to medical antimicrobials evolve due to the fact that they typically have just one target in the bacteria. In contrast, a molecule such as B11, with multiple targets in a bacterial cell, is unlikely to develop resistant mutants even after long exposure.

This paper demonstrated that B11 can control biofilms at a uniquely low concentration of 0.05 mg/l. At this dosage level, its magnitudes are more effective than any organic biocide molecules currently on the market. Therefore, this new technology is very promising to become the antibiofilm product of choice, when reduction of chlorine-chemistries is seen as a necessity. This can be due to on-going corrosion issues of machinery materials. System closure projects is another case where this kind of novel technology would be beneficial. It is known that significant reduction of freshwater consumption will accelerate microbial activity and managing it by intensifying chlorine-based programs in closed loop may accelerate corrosion. A biocide program including a very low daily consumption of B11 and a significantly reduced dose of chlorine-compounds enables running the paper machine with effective biofilm control.

Sustainability analysis: Sustainability increases if productivity of existing assets improves without the need to build new equipment. Improved cleanliness can have a direct positive impact on productivity. For example, a 30-minute shorter break time per week can improve productivity +0.3%. In some machines, patching of holes and slime defects at rewinder occasionally force the paper machine to slow down. In those machines, debottlenecking the rewinder via increased machine cleanliness may erase, for example, six unplanned 4-hour stops per year, improving productivity an additional +0.3%. Lowered corrosion rate provides two kinds of benefits: reduced maintenance costs of machine structures and reduced losses of production time due to corrosion repairs. These machines that have already experienced severe corrosion issues caused by intense use of chlorine-based biocides, have seen the cyclic nature of corrosion damages. First, only mild issues arise due to corrosion, such as sporadic holes or breaks due to corrosion flakes and some minor repair costs. Then issues intensify, more production time is lost due to holes and breaks, some dryer cylinders and ventilation systems require renewal, lifetime of fabrics is shortened, and time needed for repairs starts to prolong shutdowns. At the worst, tens of cylinders and/or headbox can need a renewal and several production days are lost. In case of not removing the cause of corrosion, this cycle can repeat. In a machine with ca. 1,000 TPD production example, when summing up costs along a four-year cycle, from the appearance of corrosion issues to the larger repair costs, the total cost of renewals and lost production time sums up to almost 2 M€. As an annualized cost, it is almost 0.5 M€. To conclude, the cost of corrosion may be difficult to understand due to its slow evolution, however, lowering the corrosion rate is a clear economic benefit. In addition, less need for new machine components improves sustainability.

## ACKNOWLEDGEMENTS

*Kemira Oyj supported the development of B11 described in this paper.* ■

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