OPTIMIZATION OF BIOCIDE STRATEGIES ON FINE PAPER MACHINES

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In this study a rapid at-line ATP (adenosine triphosphate) analysis is applied in papermaking. This ATP analysis takes less than a minute, and the information can be utilized instantly to adapt the biocide program. The study shows the effect of different biocide strategies at paper mills. Comparison is made between oxidative and reductive biocides on the one hand, and on the other hand between continuous vs. batch additions of biocide. Continuous biocide addition keeps the microbial activity at a constant level. However, a long production period without a boil-out might result in accumulation of resistant bacteria, which cannot be eliminated without changing the biocide strategy. Batch addition of biocide creates a high temporary concentration of biocide in the process. This causes lower temporary microbial activity in the process, but between the doses the microbial activity may rise to an intolerable level. Batch addition causes chemical variation to the wet end of a paper machine more easily than continuous addition. This can affect the performance of papermaking chemicals and cause problems with retention, fixing, etc. Both biocide addition strategies can be used if they are monitored and optimized properly. Rapid ATP analysis is a suitable tool for both purposes.

Keywords: Microbiology; Papermaking; Biocide; ATP; Wet end chemistry

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INTRODUCTION

Microbial activity can cause severe quality and runnability problems on paper machines (Edwards 1996). Since they have an aquatic environment, a temperature of 30-60°C, and a pH range of 4.5-9.0, paper machines offer an ideal environment for microbes to grow and reproduce (Kolari 2003). In addition, cellulose and various degradable additives present in paper machine waters offer a good source of nutrition for microbes. The reduction of fresh water consumption and closing up the water cycles causes the buildup of dissolved organic material used by microbes as nutrition. This, together with increased usage of recycled fibers and the move from acidic to neutral or alkaline paper manufacturing processes, are factors that have increased the amounts of microbes in paper machine systems, as well as the extent of problems related to these microbes (Blanco et al. 1997).

Biocides are products used to control the growth of microbes. They act either by killing microorganisms (the biocidal effect) or by inhibiting the growth of micro-organisms (biostatic effect). An ideal biocide should:

- Be applicable over a wide range of operating conditions, such as pH and temperature.
- Not interfere with other paper mill additives.
- Have a broad spectrum of activity towards microbes.
- Be efficient and fast-acting.
- Be environmentally friendly and non-toxic (no organic solvents, heavy metals, dioxins or furans) and also safe for the operator.
- Be low-cost and easy-to-handle.
- Be cost-competitive.

There are no biocides that can encompass all the requirements, and none of the biocides is suitable for all applications. The selection of biocides must always be made site-specifically (Edwards 1996). Proper estimation of reduction in the microbial count and in the number of fungi are essential parts of a successful biocide program.

Annually, over 200 million Euros are spent on slime prevention by the paper industry in Europe. The total sales of (non-oxidizing) slime preventing chemicals has started to decrease after the peak in 2003 (Finnish Environment institute 2007). Contrary to the traditional biocides, in the last few years the sales of oxidizing biocides have radically increased (Kolari 2007). The increased interest directed towards oxidizing biocides is due to their low cost. Oxidizing biocides also degrade faster, and the degradation products are not harmful or toxic but are environmentally sound. Typical biocide costs at paper machines vary between 1 and 4 €/paper ton. For average sized fine paper machine this means 0.5-1 million Euros annual costs for microbial control. Responsibility for maintenance of microbial control systems has drifted to chemical suppliers, and therefore all the interactions with process, other chemicals, etc. are seldom taken into account. If they were, further optimization and cost reductions would be possible (Sievänen 2008).

The development of a biocide strategy for a paper mill is always a compromise between the costs and performance. An insufficient use of biocides endangers the machine runnability and product quality. On the other hand, too extensive use of biocides is not only expensive, but may result in unwanted interactions with the process and other chemicals. Papermaking is a dynamic process in a continuous state of change. Thus, evaluation of biocide performance is difficult and the results should be available instantly. This is not possible with traditional plating methods for determination of microbial counts.

The quantification of adenosine triphosphate (ATP) has been used in industries as a bacterial cell viability assay method (Kramer et al. 2008; Najafpour 2007). In their studies related to the paper industry Mentu et al. (1997) concluded that "the measurement of biomass using an adenosine triphosphate (ATP) assay gave results immediately but was only suitable for high levels of microbial contamination." During recent years, the sensitivity of ATP assays has significantly improved. There are several portable devices that make ATP measurement easy to perform. Contrary to traditional plating, which takes three days, the results of the ATP assay are received in less than one minute. These devices have been developed mainly for the hygiene monitoring, and have not yet been

widely used in papermaking. This paper describes the usability of rapid ATP assay in papermaking as complementary test to the bacterial cultivation for evaluating the biocide strategies.

EXPERIMENTAL

Experiments for this study were performed both at a mill scale and at a laboratory scale. In the laboratory trials tests were performed to optimize the biocide dosage. Two mill-scale trials were performed. In the first trial the response of ATP measurement to changing conditions was addressed in detail. In the second trial the response of the papermaking environment to continuous and batch addition of biocides was studied.

ATP Measurement

Total and free ATP contents were measured by using an Aquasnap™ testing device (Hygiena, USA) according to manufacturer's instructions. The Aquasnap collects 100 µl of water sample in a honeycomb-shaped dipper. The sample is placed into a chamber in which reagents that react with ATP are released. The device measures the ATP contents in the solution and uses no internal or external standards. Both total and free ATP was measured. The Aquasnap for free ATP lacks the enzyme needed for cell lysis and therefore measures only free ATP. The Hygiena Aquasnap sampling devices were chosen for this study based on the information provided by the producer, that the devices are suitable for different industrial applications (Easter 2009).

The dipper collects only the water phase from the sample and therefore no filtration is needed. The light was measured and displayed in relative luminescence units (RLU) with a SystemSURE II portable luminometer (Hygiena, USA).

The range of this assay was from 10^{-11} M to 10^{-7} M ATP as determined based on calibration using known amount of ATP.

Laboratory Trials

Bromine-based biocide was used in the laboratory trials for dosage optimization. The biocide was generated from sodium bromide (NaBr) (Sigma-Aldrich, Finland) by electrolysis. The electrolysis was carried out using Electro MP Cell (ElectroCell, Denmark) according to manufacturer's instructions. NaBr concentration in electrolyzed solution was 0.3M. The solution was pumped through the cell with a flow rate 8 L/h. The applied current density was 1.5 kA/m^2. The main active compound in the product was sodium hypobromite (NaOBr). Active bromine content was measured according to manufacturer's instructions using a Dulcotest DT1 (Prominent, Finland) portable microprocessor-controlled photometer.

Laboratory experiments were conducted using a clear filtrate sample from a fine paper machine. Experiments were performed in 250 ml flasks with 50 ml samples. After addition of biocide the samples were incubated on rotary shaker at 37°C for 30 min. After 30-min contact time sub-samples were taken for ATP content determination and bacterial count. The results from the laboratory experiments are presented as an average of at least three measurements.

The effect of biocides on the ATP assay was tested in the clear filtrate. Bacteria were removed from the sample by filtering through a sterile 0.45 µm filter (Whatman GmbH, Germany) and ATP ($5 \cdot 10^{-9}$ M) was added. The ATP levels were measured after 4 and 15 minutes of exposure to different amounts of hypochlorite.

The amount of total heterotrophs and bacterial spores in paper machine samples was determined by a conventional plating technique. In short, samples were serially diluted (10-fold dilutions) with Ringer's solution (Merck KGaA, Germany) and plated on Nutrient Agar (Merck KGaA). Samples were incubated at 37°C for 48 h after then the colony forming units (CFU) were counted. The detection limit was 10 CFU/ml. To determine the amount of bacterial spores, the water samples were heated at 80°C for 20 min before plating.

Mill Trials

Trials were done at paper mills producing fine paper under neutral/alkaline conditions. During these trials 3 different biocide concepts were reviewed: an oxidative system based on hypochlorite and hydantoin, an oxidative system based on hypochlorite, and a reductive system using glutaraldehyde and DBNPA (2,2-Dibromo-3-nitrilopropionamide). Data were analyzed using the Wedge software (Kajanto 2002) and Microsoft Excel tools. The ATP measurement at each point of time was performed at least twice. The validity of the data was followed by comparing the results from the sequential measurements.

RESULTS AND DISCUSSION

Effect of Biocide on ATP assay

ATP assay is based on an enzymatic reaction and can be disturbed by aggressive chemicals. Moreover, when ATP is measured using a portable luminometer, no internal standard is used. Therefore any disturbance of the bioluminescent reaction can give false results.

Hypochlorite at a dosage of 10 ppm led to an increase in luminescence when the ATP level was measured four minutes after the biocide addition (Fig.1). Trials with hypobromite gave similar results; the luminescence increased at the biocide dosages of 8-9 ppm. Hypochlorite dosages higher than 10 ppm inhibited the enzymatic reaction, decreasing the emitted light.

Laboratory trials also indicated that a 20 ppm concentration of hypochlorite or hypobromite decomposes in less than 5 minutes to a level lower than the detection limit (results are not shown). Thus, in practical applications biocides (hypochlorite and hypobromite) at amounts usually used at paper mills do not disturb the ATP assay.

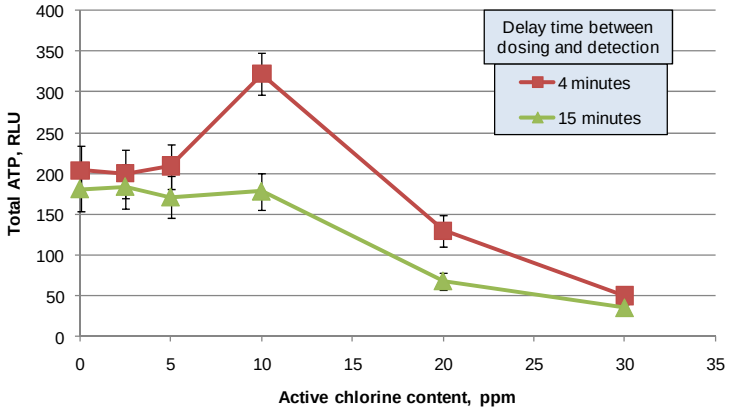

Fig. 1. Total ATP as a function of active chlorine content in a bacteria free sample. ATP values are averages of at least three measurements.

Water in a paper machine system is a complex medium, possibly containing residues from several chemicals. These residues may interfere with the ATP assay. Therefore, the reproducibility of the method is good only when the same process is followed. Any comparison between different processes should not be done.

Optimization of Biocide Dosage at Laboratory Scale

The rapid ATP measuring method is suitable for on-site optimization of the biocide dose. These experiments were done to demonstrate that the optimization of biocide dose can be done at a laboratory scale with actual mill samples. The water sample was taken from a sampler just before the biocide addition to the clear filtrate at the paper machine. Different dosages of bromine-based biocide were added to the sample. Total ATP contents were measured and compared to total viable and spore count (Fig. 2).

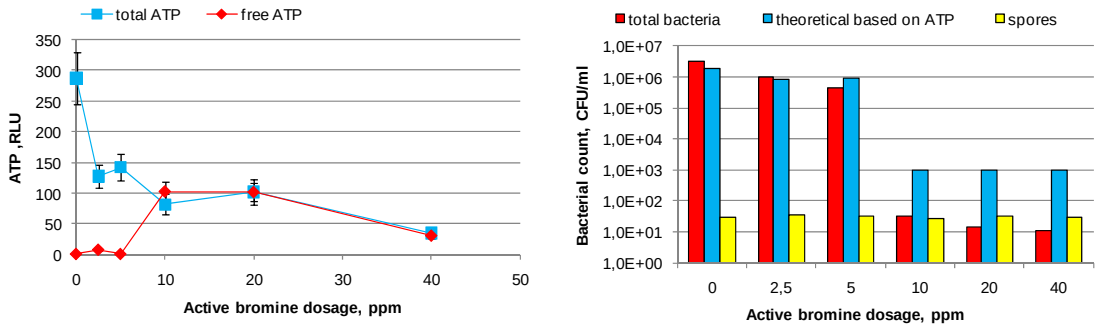

Fig. 2. Optimization of biocide dosage with a portable ATP luminometer. Biocide was dosed to fine paper machine's clear filtrate. Viable bacteria were calculated on the basis of intracellular ATP, which was estimated by subtraction of free ATP from total ATP content. ATP values are averages of at least three measurements.

Figure 2 shows that 10 ppm of a bromine-based biocide was able to reduce the amount of bacteria in a clear filtrate sample from 10^6 to 10^1 cfu/ml. It can be seen that the

optimal dosage range was very narrow. A dosage of 10 ppm of the biocide was able to reduce the amount of culturable bacteria to the limit of detection (10 cfu/ml), whereas a 5 ppm dosage only decreased the bacteria amount slightly. Thus, an optimization is important in order to avoid overdosing, while maintaining proper efficiency. In practice, dosages of more than 10 ppm are not realistic. Normally the dosages at paper machines are in a range of a few ppm's.

A decrease in the total ATP content was seen already after a 2.5 ppm biocide addition, while free ATP level remained low. When the biocide was added at an amount of 10 ppm the level of free ATP increased, indicating cell lysis. The concentration of intracellular ATP depends upon the cell size and the metabolic activity (Nilsson et al. 2002; Russel and Cook 1995). A small amount of biocide can disturb the activity of bacteria and reduce the cell volume, reducing the ATP level per cell.

Different bacteria have different amounts of ATP. The average ATP content for gram-negative and gram-positive bacteria is approximately $1.5 \cdot 10^{-18}$ and $5 \cdot 10^{-18}$ mol/cell, respectively, giving an ATP content ratio of 1:4-5 (Hattori et al. 2003; Mujunen et al. 1998; La Duc et al. 2007). This may result in deviations in the correlation between ATP content and bacterial cultivations. In this respect, ATP is not a good indicator to estimate the amount of spores, since bacterial spores contain only trace amounts of ATP (La Duc et al. 2007).

On the basis of the published data, the average ATP content is about $4 \cdot 10^{-18}$ mol/cfu. This average was calculated based on an assumption that the bacterial population in a paper machine system consists of gram-positive and gram-negative bacteria at equal amount, even though the ratio of gram-potitive to gram-negative bacteria may depends on the degree of closure (Öqvist et al. 2008; Lahtinen et al. 2006). Thus, an assay reading of 100 RLU equals to $6 \cdot 10^{5}$ cfu/ml.

The amount of culturable cells has been reported to range from less than 1 % to more than 50% of total viable bacterial cells in different environments (Colwell and Grimes 2000; Hammes et al. 2008; La Duc et al. 2007; Yoshida and Hiraishi 2004). The bacterial population may differ between paper machines and depend on many factors such as degree of closure, type of fibers, and production conditions (Öqvist et al. 2008; Desjardins and Beaulieu 2003; Lahtinen et al. 2006). Therefore, one may expect the number of viable bacteria (measured based on ATP level) to be much higher than that of culturable cells. Surprisingly, in our study we found that the ATP level in the clear filtrate correlated with bacterial count. This means that in this case most of bacterial population was represented by bacteria that were culturable on NA. The only disagreement between the two methods was at high dosages of the biocide. However, as mentioned above, the biocide at high dosages has a negative effect on the ATP assay.

When performing such optimization trials, one should always calculate a correlation factor for a given paper machine. The correlation factor describes the relation between ATP and bacterial count and is specific to each process. It is possible to replace the cultivations and utilize the full potential of rapid ATP assays by using this factor. Both methods, viable cell estimation based on ATP and bacterial counting, are biased.

Evaluation of Oxidative Biocide Program Using Analysis of Total and Free ATP

A test was carried out in which oxidative biocide (hypochlorite from a container) was added to a short loop of a fine paper machine producing specialty papers with batch dosing. Both free and total ATPs were measured from the wire water (filtrate as the paper is formed). The effect of the biocide feed on the ATP content of the wire water is presented in Fig. 3. The figure shows that 40 minutes of batch feed of the biocide was able to decrease the microbial activity to a slightly lower level. However, the level started to increase instantly after the dosing was stopped. Based on a theoretical calculation, the drop in cell counts was not very significant (Hattori et al. 2003; Mujunen et al. 1998). The level before the biocide feed was around 10^7 cfu/ml, and it dropped to slightly above 10^6 cfu/ml.

The results also indicate that the biocide program could be further optimized. Dosing batches should be applied more frequently, or pulp should be treated better before it enters the short loop. The wire water is probably not the best point to apply the biocide in this case.

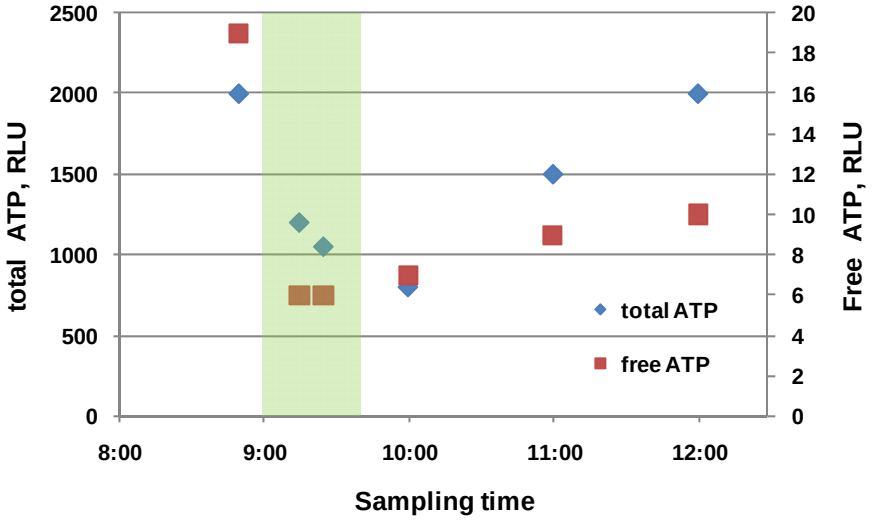

Fig. 3. ATP (free and total) measurements on wire water to monitor the effect of biocide performance. Colouring presents the dosing of biocide (hypochlorite was added for 40 minutes time).

Some authors have shown that exogenous ATP may persist for a long period of time even under harsh conditions (Schuerger et al. 2008), resulting in overestimation of microbial mass. As seen in Fig. 3, the level of free ATP in our trial was low. A very low amount of free ATP indicates that total ATP represented mostly the cellular ATP. The low level of free ATP in the process water could be due to rapid consumption of ATP by bacteria. As shown for Antarctic mineral soil, even at a temperature of 15°C ATP was utilized by microorganisms within 30 min (Cowan and Casanueva 2007).

Evaluation of Two Different Biocide Strategies at a Fine Paper Mill

The measurements performed in this study showed that by using rapid ATP analysis, it is possible to map the biocide system in one to two days, draw conclusions, do corrective actions, and monitor the effect of the actions. The performance of two different biocide strategies at the same mill is presented based on the ATP content results (see Fig. 4). Batch addition used a reductive biocide, whereas continuous addition used an oxidative biocide. Batch addition of the biocide corresponds to a dosing strategy in which the biocide is pumped to a process for a certain pre-determined time, followed by the time period without any biocide addition. In continuous addition the biocide is pumped to a process continuously. Batch addition showed a significant increase of microbial activity between the batches. During the dosing of biocide in batch addition, the ATP content dropped to a very low level. In continuous dosing mode the bacterial activity was rather constant, but higher than during biocide feeding in batch mode. The batch addition turned out to be inefficient. The switch to continuous mode was made based on a general overview of the machine and the results from the ATP analysis. A continuous mode was able, thus, to maintain a cleaner process. The biocide was added to broke filtrate. ATP was measured from the broke filtrate after the biocide addition point.

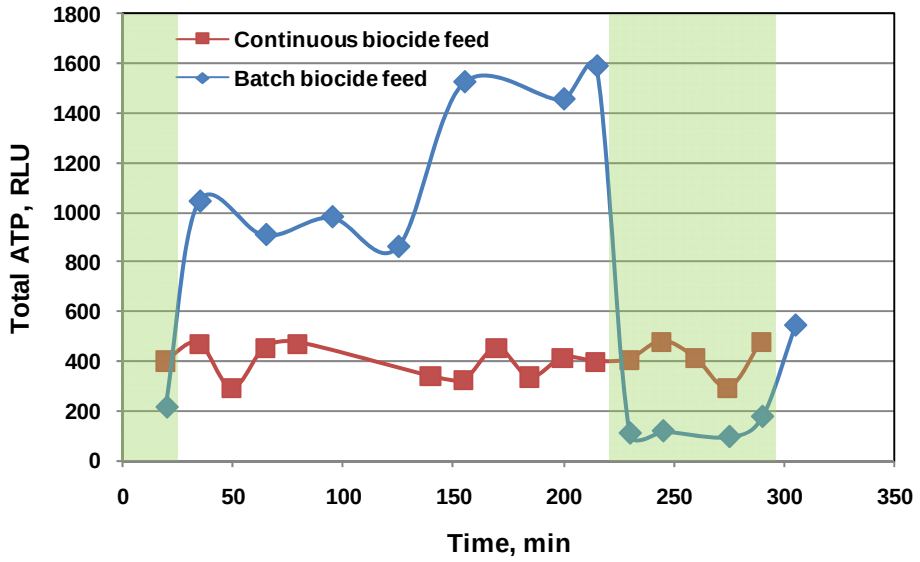

Fig. 4. Analysis of the ATP content for comparing different biocide strategies at paper mills. Colouring presents the dosing of biocide in batch mode.

Figure 5 shows how the biocide dosing in the batch mode caused chemical fluctuations in the wet end of a paper machine. The addition of slightly alkaline hypochlorite solution increased the process pH. At the same time, sodium and chloride ions in the hypochlorite solution increased process conductivity. When using the batch dosing mode, one should select the proper chemicals very carefully according to the process conditions. Unstable process due to such fluctuations might cause problems with chemicals such as fixatives and retention aids and can also cause other problems such as deposits.

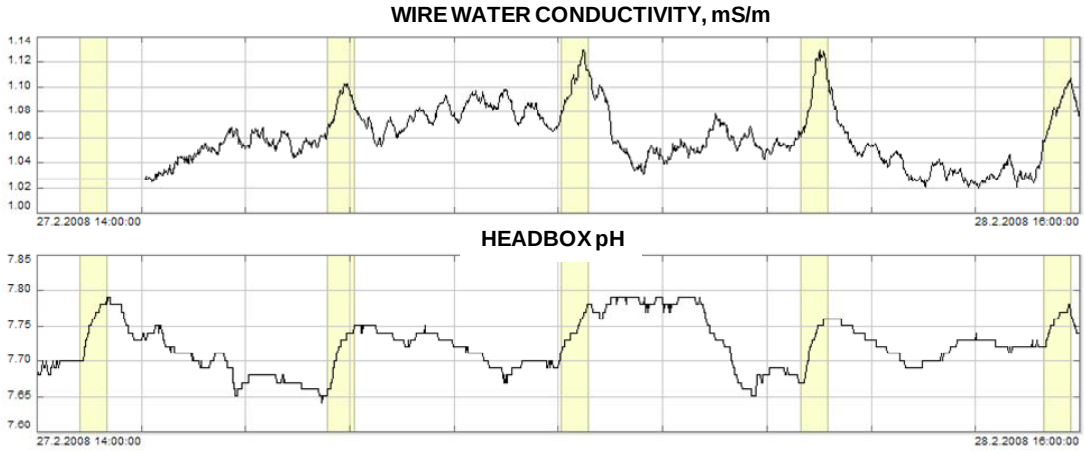

Fig. 5. Fluctuations in process conductivity and pH caused by the batch addition of a biocide. The biocide (hypochlorite) was added for 40 minutes time every 6th hour. The colourings present the dosing sequence.

CONCLUSIONS

1. ATP content measurement using a portable luminometer is a useful and easy-to-use method for evaluating microbial activity in paper mills. The rapid ATP assay offers the potential to augment or replace the traditional bacterial cultivation for evaluation of biocide program efficiency. Within one day, it is possible to map the biocide system. Based on the mapping, it is possible to draw conclusions, take corrective actions, and to monitor the effect of the actions.
2. Total ATP content is a good measure of bacterial activity. The combination of two assays, total ATP and free ATP, may provide additional information on biocide action. In our case study the low free ATP level in paper machine systems indicated that total ATP content was mostly represented by intracellular ATP.
3. Batch dosing of biocide to the process allows temporarily high local concentrations of biocide in the process, but the delay time between the batches allows microbes to grow. If the residues of biocide do not circulate back to the system, this might cause microbial problems. This is a question of optimization of the dosing strategy, i.e. the time between dosages, biocide amount, and optimal dosing points. Optimization can be done with ATP measurements and proper process monitoring tools.
4. A continuous flow of biocide creates stable conditions, but it may allow the formation of a resistant strain - continuous flow does not generate high local biocide concentrations. On the other hand, the amount of microbes in the system remains at a rather constant level. Whether this level is tolerable depends on the selection of biocide, the dosage amount, and the dosage point.
5. Biocides are chemicals, and they interact with other chemicals. Oxidative biocides lose their efficiency in reductive circumstances, such as in the presence of dithionite. Biocides also increase the load of ions in the process, which influences the functioning of fixatives and retention aids. This behavior is especially critical in the

case of batch dosing of biocides. A batch dose also generates chemical variations which might be detrimental to the process.

ACKNOWLEDGMENTS

The financial support and participation to the project by UPM-Kymmene, Stora-Enso, M-Real, and Metsä-Botnia are gratefully acknowledged.

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Article submitted: Nov. 11, 2009; Peer review completed: Dec. 18, 2009; Revised version received and accepted: Jan. 28, 2010.