Using catalase activity to measure microbiological activity in pulp and paper systems

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Microbiological problems in paper mills

Microbiological contaminants enter pulp and paper mill systems either through fresh water or incremental re-use of white water. The fibrous and nonfibrous furnish and the presence of other chemicals in the mill system serve as an abundant source of nutrients for the rapid propagation of microorganisms. Bacteria and fungi seek out these ideal environmental conditions for their growth. In addition, mill temperatures and pH ranges also encourage the rapid incubation of bacteria and fungi.

Accumulations of microorganisms are referred to as "slime deposits." Such deposits cause serious trouble in paper manufacturing, clogging the filters, screens, showers, the wire, and pipe lines. In addition, the quality of the finished product suffers from slime spots, discoloration, pinholes, and poor sheet formation.

Control approaches

Chemical treatment is the general practice for effective control of biological fouling in pulp and paper mill systems. In developing a biomonitoring program, it is essential to determine the capability of the microbiocide against the resistance offered by the microbe. Hence, a periodical examination of microbial concentration from the different sources of the system is important in determining the performance of the microbiocide or the ability of organism to develop immunity.

The most commonly used methods of bacterial evaluation are the standard plate count and the use of dipsticks. The inherent advantages and disadvantages of these methods for determining microbial concentrations have been documented (*1*). The greatest drawback of these methods has been the 48-h incubation time. In places where the microbiological examination facilities are not available on site, samples are shipped to an outside laboratory. The changes that occur in microbial density during transit have to be taken into consideration.

To overcome these difficulties, attempts have been made recently to obtain test results in a shorter time frame. One such attempt is the measurement of microbial concentrations through the quantity of adenosine triphosphate (ATP) in a liquid. ATP luminescence is a rapid and precise method, but it is fairly complicated and expensive to perform routinely.

Another simple and rapid method for routine control is the Bio-Lert method (*2*). This method uses an indicator system with a sterile, stabilized nutrient broth in a convenient vial. The time required for the color to change from blue to pink is correlated with a standard plate count. However, this method is best suited for the samples that contain excessive biological activity; it is not well suited for samples with minimal biological activity.

A new method

A new rapid method, sensitive enough for evaluating total bacterial content, has now been developed as a routine test method. The test is based on the assay of the enzyme "catalase," which is generally present in all aerobic living systems. Although this method is quite effective in estimating the microbial activity in sewerage effluent (*3*) and fresh water (*4*), it has not been used in the pulp and paper industry. How effective, then, is the use of catalase assay in determining bacterial content in the pulp and paper mill?

Experimental procedure

In determining catalase activity, the method of Euler and Josephson (*5*) was used. To a 25-mL sample, 250 micromoles of hydrogen peroxide was added and incubated for 1 h at room temperature (25–28°C). At the end of this period, the reaction was stopped by adding 5 mL of 2*N* sulfuric acid, and the residual hydrogen peroxide was titrated against 0.1*N* potassium permanganate. The activity of the enzyme is expressed as micromoles of hydrogen peroxide decomposed per hour per 100 mL of sample.

Catalase assay and slime deposit analysis

The common points where biological fouling poses a major problem are:

1. Make-up water
2. White water
3. Machine chest
4. Broke chest
5. Talc storage.

These sites were chosen for study.

The slime samples were collected

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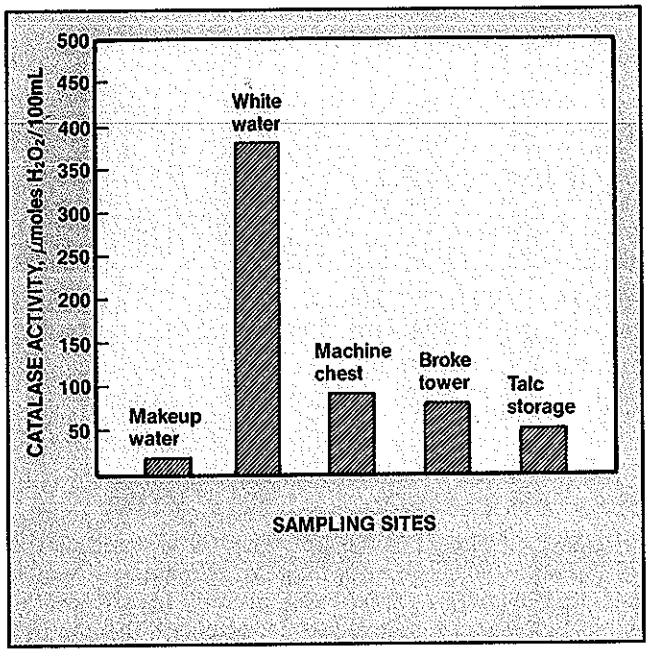
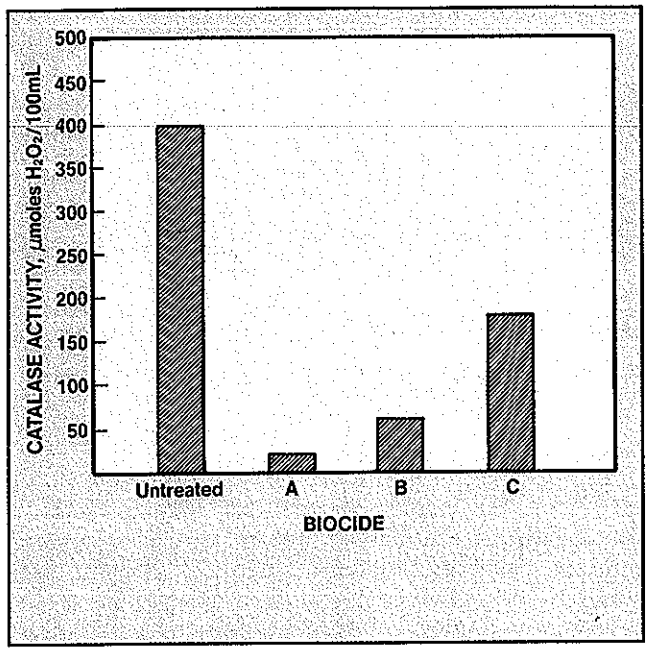
1. Catalase activity at different sites



2. Catalase activity in white water before and after treatment with biocides



I. Comparison of test methods on biological activity

Method	*Incubation time, h*	*Accuracy*	*Inexpensive equipment and reagents*	*Quantitative results*	*Remarks*
Catalase assay	30 min	Very good	Yes	Yes	Rapid, simple procedure useful to measure different levels of biological activity
Bio-Lert	1–4	Very good	Yes	Yes	Rapid, simple procedure mainly intended to measure high levels of biological activity
Standard plates	48–72	Excellent	Moderate	Yes	Time consuming, results not rapid
Dipstick	24	Fair	Yes	Yes	Simple, test results not rapid
ATP luminescense	<30 min	Good	No	Maybe	Rapid test, expensive equipment and reagents

and washed three times with sterile water. Aliquots of slime deposits were added to 100 mL of sterile water. The mixture was kept on a rotary shaker (200 rpm) for 1 h and was then filtered through cotton wool. The filtrates were used for catalase activity measurements.

Results and discussion

The method proposed is rapid and sensitive. **Figure 1** represents the microbial activity as assessed by catalase assay. The maximal activity was in the recirculated white water, while the least activity was observed in makeup water. The rest of the samples had low biomass concentrations. This finding suggests that white water is the primary source of the microbiological deposit on a paper machine. Hence, all further studies were carried out only on this sample.

Three biocides were used for control of biofouling. **Figure 2** represents their impact. The catalase activity before and after treatment indicated that Biocide A was the most effective.

To confirm these observations, representative samples from different sites in Fig. 1 were simultaneously examined by the standard plate count method. The correlation between catalase activity and microbial population is presented in **Fig. 3**, which shows a good correlation between catalase activity and bacterial count.

3. Correlation between catalase activity and total plate count

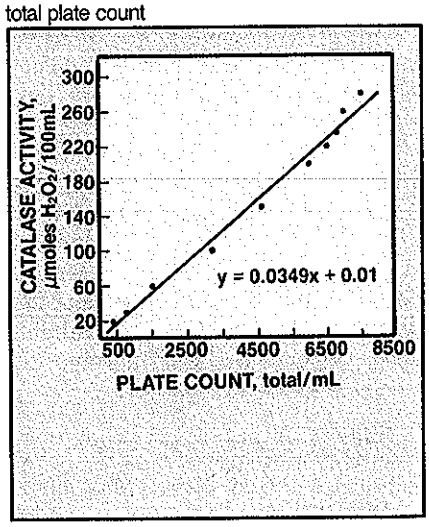


Table I compares the proposed technique with other methods, highlighting the advantages of the catalase assay method.

Conclusions

This method for assessing the effect of various biocides on microbial activity is simple and rapid. Unlike other methods, this assay method can be easily adapted for routine analysis and can serve as a simple technique in monitoring the biocide program.

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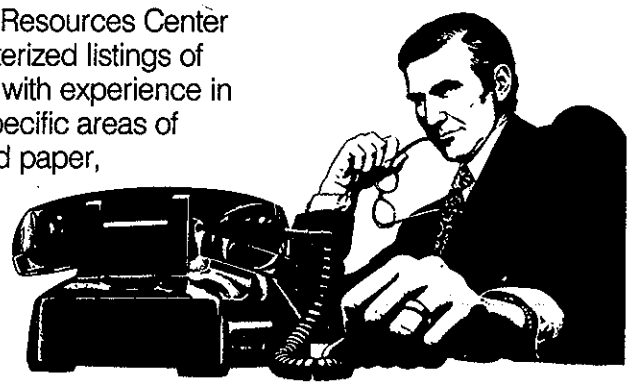

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