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Use of Immunoassays to Monitor In-mill and Waste Water Concentrations of Hydrophobic Organic Material

by

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ABSTRACT

The pulp and paper industry is moving towards mill closure for environmental and economic reasons. As mill waters are recycled, extractives concentrations are expected to increase significantly and methods to monitor their levels will be needed. One class of extractives of concern is the resin acids because of their toxic nature and detrimental effects to pulp and paper making processes. A simple, accurate, fast, reliable analytical method will be necessary to monitor their levels throughout pulp mill process waters. Previous work established dehydroabietic acid (DHA) as a good marker for the total resin acid content of treated pulp mill effluent samples. The ability of DHA to be a marker for total resin acid content of in-mill process lines of a TMP/CTMP pulp mill using a spruce/pine/fir furnish was tested. Concurrently the adequacy of using an enzyme-linked immunosorbent assay (ELISA) to monitor DHA from in-mill process lines was tested. In previous work an ELISA for DHA was developed using polyclonal antibodies raised in rabbits. These antibodies were determined to be very sensitive for DHA, with an IC_{50} of $7.2 \pm 2.0 \text{ ng}\cdot\text{ml}^{-1}$ (ppb). The assay was tested with resin acid free process water samples from a highly closed pulp mill. The samples were spiked with known amounts of DHA and directly purified by a solid phase extraction (SPE) method. At present several samples are being analyzed and results will be provided in a forthcoming paper. However, DHA may not be the best resin acid to use as a marker for total resin acid content of in-mill process water samples. A GC analysis of the resin acid content of 167 process water samples established abietic acid as the best single marker for total resin acid content. A correlation of 0.928 was found between the abietic acid concentration and the total resin acid content of these samples. An improved correlation of 0.957 was obtained if a combination of isopimaric and abietic acid contents was used as the marker for total resin acid content. A correlation of only 0.664 was obtained between DHA and total resin acid content of these samples.

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To reduce fresh water consumption and decrease energy costs as well as the amount of effluent released into the environment pulp and paper mills worldwide are moving towards water systems closure. However, mill closure will cause increased accumulation of dissolved and colloidal substances (DCS). These substances are known to be released from wood during mechanical pulping processes (Allen, 1975; Järvinen, et al., 1980), therefore, mills must improve management of their process water systems to maintain productivity and product quality (Cronin, 1996; Farlow, 1996). The dissolved and colloidal substances are mostly composed of lipophilic extractives, lignins, polysaccharides and inorganic materials (Sjöström, 1990; Ekman, et al., 1990). High levels of these materials are associated with problems such as pitch deposits, reduced paper strength and decreased brightness (Brandal and Lindheim, 1966; Lindström, et al., 1977). Accumulation of DCS in white water will be detrimental to paper machine runnability and paper quality.

The various components in DCS have been shown to cause different effects on the paper making process and paper quality (Wearing, et al., 1985; Francis and Ouchi, 1997). For example, resin acids are a class of lipid extractives associated with DCS that reduce wet web strength properties (Zhang, et al., 1999). Furthermore resin acids are known to be toxic to aquatic life. Their 96h-LC₅₀ to rainbow trout ranges from 0.2 to 1.7 mg·ml⁻¹ at neutral pH (McLeay and Associates Ltd. 1986). Therefore, monitoring resin acid levels in process lines of mills moving toward water systems closure may become increasingly important. Pulp and paper mills using softwoods as a furnish release resin acids from wood chips into process waters regardless of the pulping method (McLeay and Associates Ltd. 1986).

Gas chromatography (GC) is still the method of choice for monitoring the levels of individual resin acids and several methods with flame ionization have been developed to analyze them in pulp mill effluents (NCASI, 1986; Foster and Zinkel, 1982; Voss and Rapsomatiotis, 1985). Unfortunately these techniques are tedious and require extensive sample pretreatment. The samples must be extracted, and typically require clean-up procedures and/or derivatization steps before they are finally quantified. Large numbers of samples cannot be routinely and rapidly analyzed and the above methods are not amenable to on-line analysis. An alternative procedure would be to analyze for one or two components that are representative of the total amount of resin acids present. Such an approach could be simpler if a fast and accurate analytical method was available.

DHA has been demonstrated to be a useful indicator of total resin acids in biologically treated effluents. Chow et al. (1996) developed an HPLC method for the direct analysis of DHA from pulp mill effluent. This method requires no hazardous chemicals during sample pretreatment and DHA concentrations as low 100 ng·ml⁻¹ are detected. These researchers obtained a good correlation of their results with both the DHA and total resin acid content determined by a GC method. Immunoassay methods are another good alternative to GC because in general they are simple, sensitive and adaptable to laboratory or field situations. They usually

require little sample pretreatment before analysis and tend to be inexpensive. They are a semi-quantitative technique that have been successfully applied to a range of pesticides, (Rittenburg, et al., 1990) industrial chemicals (Eck, et al., 1990) and microbial toxins (Ramakrishna, et al., 1990). Li et al. (1997) developed a direct immunoassay based on ELISA that measures the amount of DHA-like resin acids in pulp mill effluent samples (see appendix 1). A DHA-conjugate was used as the immunogen and antibodies produced against this conjugate significantly cross-reacted with other resin acids that had a similar chemical structure to DHA. It was shown that knowledge of the levels of DHA-like resin acids could be used to determine total resin acid content in pulp mill effluents.

These antibodies were used to develop an indirect competitive ELISA to measure levels of DHA-like resin acids from process waters of pulp and paper mills. Before testing the assay on mill samples it was tested on buffered water samples spiked with known amounts of DHA. Recovery results for these samples ranged from 75-118%, which is an acceptable range for ELISA. The assay was then tested on resin acid free process water samples from a highly closed pulp mill. The recovery determined by ELISA for DHA in these samples was 130-250%. This indicated that components present in the sample were interfering with the assay. A solid phase extraction procedure was developed as pretreatment for the samples prior to analysis. The ability of the pretreatment to remove the interfering components was tested and the results will be presented in a forthcoming paper. For information on the ELISA and development of the SPE pretreatment see appendices 2 and 3.

At the same time we attempted to establish the ability of a single resin acid to be a marker for total resin acid content of in-mill process water samples. The resin acid content of 25 in-mill process water samples from various sites of the Quesnel River Pulp Mill was determined by GC. The ability of one or two resin acids to be a marker for the total resin acid content of the samples was tested. It was determined that abietic acid was the single best marker while DHA was the poorest marker. This unexpected result led to the examination of results from a resin acid audit carried out at the Quesnel River Pulp Mill by Bicho et al. (1996). The resin acid content of 167 in-mill process water samples was examined and once again abietic acid proved to be the best marker for total resin acid content while DHA was the poorest. Results of this work are presented in appendix 4.

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Quesnel River Pulp, Quesnel, BC.
Millar Western, Meadowlake, Sask.
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APPENDICES (Hard copy only)

- Appendix 1: **Li, K., A.N. Serreqi, C. Breuil, and J.N. Saddler.** 1997. Quantification of resin acids in CTMP effluents using an enzyme-linked immunosorbent assay. *Water Sci. Technol.* 35, 93-99.
- Appendix 2: **Gamboa, H.R., A.N. Serreqi, R.P. Beatson, J.N. Saddler and C. Breuil.** 1998. Development of an immunoassay procedure for monitoring dehydroabietic acid levels in pulp mill process waters. Sustainable Forest Management Network Western Student Workshop, October 7-8, Edmonton, AB
- Appendix 3: **Serreqi, A.N., H. Gamboa, R. Arao, R.P. Beatson, J.N. Saddler and C. Breuil.** 1999. ELISA of abietanes in process waters of a TMP pulp and paper mill. Proceedings of the Sustainable Forest Management Network Conference, Edmonton, AB, February 14-17.
- Appendix 4: **A.N. Serreqi, H. Gamboa, K. Stark, J.N. Saddler, and C. Breuil.** 1999. Resin acids markers for total resin acid content of in-mill process lines of a TMP/CTMP pulp mill. *Water Res.* Submitted.