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AN UPDATE OF PROCEDURES FOR THE MEASUREMENT OF COLOR IN PULP MILL WASTEWATERS

TECHNICAL BULLETIN NO. 803

MAY 2000

by

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Acknowledgments

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PRESIDENT'S NOTE

One of NCASI's roles in support of the pulp and paper industry's environmental programs is the evaluation and development of analytical methods useful in characterizing the constituents and properties of process effluents and wastewaters. In 1971, NCASI developed and published a method for the measurement of color in effluents from pulping operations and their receiving waters. Since then, the NCASI color method has been widely used in mill effluent monitoring and compliance assessment programs; it has also been used to evaluate receiving water properties. But in the nearly three decades since the color method was published, a number of innovations have occurred in pulp and papermaking techniques. The impact of these innovations on effluent quality has been significant enough to suggest that the NCASI color procedure be re-evaluated using modern pulping effluents.

This bulletin describes a recent evaluation of the method and provides an updated procedure for color measurement. The procedural updates were designed to enhance the repeatability and reproducibility of the method while maintaining the existing fundamental basis of historical pulp mill effluent color measurement.

Aspects of the color measurement procedure that were evaluated included wavelength for lower color effluents, turbidity removal, pH during and following filtration, and sample cell path length. The updated procedure was subjected to single- and multi-laboratory evaluations, and the results were used to determine quality assurance and quality control criteria for assessing method performance. The updated method was also compared to the 1971 version and found to yield similar values in most cases. The complete, updated method, NCASI Method Color 71.01, is included as an appendix to this report. The results of this investigation will be useful to mills that measure color in process effluents, treated final effluents, and receiving waters.

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May 2000

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ABSTRACT

NCASI Method 253 was originally developed in 1971 to provide a technique for color measurement in pulping wastewaters and their receiving waters. Because the processes of pulp and paper production have changed considerably since that time, a study was undertaken with the objective of updating the method. The color of pulp mill effluents is highly pH-dependent, and the pH adjustment procedure included in Method 253 sometimes resulted in less than optimum stability of pH during the analytical procedure. The use of a buffer to stabilize pH and reduce the erroneous increase in absorbance was investigated and successfully implemented. Experiments were conducted to investigate approaches for removing turbidity from pulp mill wastewater samples, since turbidity can interfere with the assessment of color by scattering light and introducing a high bias. Centrifugation was investigated as an alternative to filtration for the removal of turbidity. The percent decrease in turbidity was consistently higher for aliquots treated using filtration vs. centrifugation. In addition, the resultant color values were consistently higher for samples that were centrifuged. The current technique, use of a 0.8 µm membrane filter, proved to be an effective method of turbidity removal in pulp mill wastewaters. Pre-filtration of the samples using a 1 µm glass fiber filter was confirmed as an acceptable method for reducing filter plugging of the 0.8 µm membrane filters. Comparisons of three different types of membrane filters demonstrated that similar results could be obtained with any of the three filter types. The selection of a wavelength of 465 ηm was confirmed to be valid in low color effluents for the spectrophotometric measurement of absorbance. Finally, the method was assessed at both the single- and the inter-laboratory level for ruggedness, precision, and accuracy. The average relative standard deviations for the single laboratory precision were 2.8% in biologically treated effluents and 7.1% in treatment plant influents. The average relative standard deviations for the inter-laboratory precision were 15% in biologically treated effluents and 24.1% in treatment plant influents. A well-defined and documented method for the measurement of color was developed, including quality control and quality assurance criteria. The updated method is included in this report as Appendix B.

KEYWORDS

color units, true color, turbidity, filtration, pH stability, spectrophotometer, platinum cobalt

RELATED NCASI PUBLICATIONS

Technical Bulletin No. 253 (December 1971). *An investigation of improved procedures for measurement of mill effluent and receiving water color.*

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AN UPDATE OF PROCEDURES FOR THE MEASUREMENT OF COLOR IN PULP MILL WASTEWATERS

1.0 INTRODUCTION

Many mills routinely monitor color as a tool in assessing wasteload for Best Management Practices (BMP), to assist in evaluating process changes, as a requirement of a facility's National Pollution Discharge Elimination System (NPDES) permit, and to monitor effluent and receiving water quality. In 1971, in an effort to standardize the measurement of color in pulp mill wastewaters, NCASI published Technical Bulletin No. 253, *An investigation of improved procedures for measurement of mill effluent and receiving water color* (NCASI 1971). Since then, the nature of pulp mill wastewater samples has changed due to the application of process technologies such as oxygen delignification and chlorine dioxide substitution, as well as improvements in spent liquor management. Subsequently, the matrices currently being assessed differ from the matrices initially used to develop the NCASI method. In some instances, problems have been encountered with pH stability and turbidity interfering with the accurate assessment of true color, using Method 253. In addition, an effort was made to make the method more consistent with current NCASI method protocols and to define quality assurance and quality control (QA/QC) parameters. In response to these issues, NCASI initiated a task to optimize the NCASI color method for application to pulp mill wastewaters.

2.0 BACKGROUND

Color in pulp mill effluents may result from the presence of several substances in the wastewater that contribute different chromophoric characteristics to the matrix. The largest portion of this chromophoric component is thought to be the high molecular mass materials that result from degradation of lignin during pulping and bleaching processes. This high molecular mass material (molecular weights greater than1000) carries several chromophoric structures that impart light-absorbing qualities to the resulting effluents (Kringstad and Lindstrom 1984). The measurement of color in industrial wastewater samples is typically limited to true color, which is the color of samples from which turbidity has been removed. The measurement of color present in pulp mill wastewaters can be determined using several different methods, including the visual comparison method, spectrophotometric method, colorimetric method, and tristimulus filter method (*Standard Methods* 1998; USEPA 1971, 1978). Within the pulp and paper industry, the spectrophotometric methods, which include NCASI Method 253, are frequently used to assess true color in wastewaters. NCASI Method 253 measures the absorbance of a pulping mill wastewater or receiving water sample at a wavelength of 465 ηm, once the sample has been adjusted to pH 7.6 and filtered through a 0.8 µm membrane filter to remove turbidity.

The determination of a true color value is extremely pH dependent, with the color value increasing as the pH of the sample increases. Therefore, controlling the pH of the sample during filtration and measurement of absorbance is of great importance.

The turbidity of the sample can also influence the color value determined. Turbidity is an expression of optical properties that cause light to be scattered or absorbed rather than transmitted in a straight line through the sample. Suspended materials such as clay, fiber, titanium dioxide, precipitated calcium carbonate, humic materials, and lignin may cause turbidity in pulp mill wastewaters. Because much of the color in effluent samples is due to high molecular mass (HMM) materials which are byproducts of the degradation of lignin, it is difficult to devise an optimal method for removing turbidity without removing some of the true color. The true color in a sample, when determined using a spectrophotometric method, is the dissolved color which absorbs light as it is transmitted through the sample. It is desirable to remove the greatest amount of turbidity from the samples while limiting

the amount of true color removed. This optimum may vary from sample to sample, depending on the particles contributing to the overall turbidity and true color, and the ease with which they are removed. The majority of color measurement methods, including NCASI Method 253, use filtration or centrifugation for the removal of turbidity from a sample. The filtration technique can produce results that are consistent from day to day in the same lab and among different labs, but may also remove some of the true color. Centrifugation avoids the interaction of color with the filtering material, but it can be difficult to obtain consistent results due to variations in the nature of the sample, the size and speed of the centrifuge in use, and reintrainment of turbidity during sample transfer.

When a spectrophotometer is utilized for the determination of color, several instrumental parameters can also influence the overall results. These parameters include the wavelength selected for measurement of absorbance, differences in light path lengths, the condition of the cell, the presence of stray radiation, reflection losses, scattering losses, photocell fatigue, source fluctuations, and loss of wavelength calibration (Ingle and Crouch 1988). It is also critical that the cells used to measure absorbance are clean, free of scratches, and reproducibly placed in the spectrophotometer. For these reasons, use of properly calibrated equipment in good working order is important.

3.0 RESEARCH OBJECTIVES

The four primary objectives of this research project were to:

1. evaluate the performance of NCASI Method 253 for the measurement of color when used on influent and effluent samples from present-day pulp mills exhibiting low effluent color;
2. develop and evaluate various approaches to improve the color measurement procedure for the assessment of true color in influent and effluent samples from present-day pulp mills exhibiting low effluent color. This objective involves the development of techniques to stabilize sample pH during filtration and absorbance (color) measurement, and the investigation of potential interference in the color method (including different sources of turbidity);
3. provide a well-defined and documented method for the measurement of color that incorporates quality assurance and quality control (QA/QC) criteria and is consistent with EPA's method development guidelines, NCASI method protocols, and current NPDES requirements for the industry; and
4. assess precision and accuracy by conducting intra- and inter-laboratory investigations using the updated procedure.

4.0 EXPERIMENTS AND RESULTS

4.1 Sampling Site Selection and Sample Collection

Table 4.1 provides summary descriptions of the mills that provided samples for this research. Information in the table shows wood type, bleaching sequence, average daily production, average daily water usage, and wastewater treatment plant (WTP) type. Grab and composite samples were collected from six bleached kraft mills that utilize oxygen delignification and/or high chlorine dioxide substitution. During the course of this research, additional samples from a thermomechanical pulping mill and an unbleached kraft mill were also tested. Samples of biologically treated effluent, influent to the treatment system, primary effluent (effluent from primary treatment before secondary treatment), and receiving water were analyzed. The unpreserved samples were collected by mill personnel and shipped overnight on ice to the NCASI West Coast Regional Center (WCRC). The samples were stored at 4°C until analyzed. Prior to manipulations, the samples were removed from

the refrigerator and allowed to warm to room temperature. Settled solids were re-suspended by vigorously shaking the sample bottles prior to use.

Table 4.1. Description of Mills

Mill Code[a]	Wood Type[b]	Bleaching Sequence[c]	Average Daily Production[d]	Average Daily Water Usage[e]	WTP Type[f]
A	SW	D(EO)WDED	950	16	ASB
B	SW/HW	(CD)EHD	1450	44	AS
C	SW/HW	O(CD)ED	1850	28	ASB
D	SW/HW	(CD70)(EP)D	680	13.5	AS
E	SW/HW	CEDD	1700	32	AS
F	SW	unbleached	1090	24	ASB
G	SW	sodium hydrosulphite	750	5	ASB
H	SW	OM(EOP)MP	500	11	ASB

[a] The mills sampled in this study used the kraft process (with the exception of Mill G, which is a TMP mill).
[b] SW = softwood; HW = hardwood.
[c] D = chlorine dioxide; E = alkaline extraction; C = chlorine; H = hypochlorite; O = oxygen; M = chlorine monoxide; P = hydrogen peroxide; W = washing stage.
[d] Average daily production in air dried tons/day.
[e] Average daily water usage in million gallons/day.
[f] ASB = aeration stabilization basin; AS = activated sludge.

4.2 Analytical Methods and Instrumentation

4.2.1 *Brief Description of NCASI Method 253*

NCASI Method 253 (NCASI 1971) involves selecting a 200 mL sample of wastewater or water and adjusting the pH to 7.6 with hydrochloric acid (HCl) or sodium hydroxide (NaOH), while assuring less than a 1% change in the sample volume. A 50 mL aliquot of this pH-adjusted sample is filtered through a 0.8 μm porosity membrane filter pre-rinsed with distilled water. A portion of the filtered sample is transferred to an absorption cell, and the sample's absorbance is measured at 465 ηm using a spectrophotometer. The color is determined by spectrophotometric comparison of the sample with known concentrations of platinum cobalt solutions.

4.2.2 *Instrumentation*

Measurements were conducted at the NCASI WCRC using a Spectronic 21D spectrophotometer equipped with a digital readout. This spectrophotometer utilizes a tungsten light source and has a wavelength range of 340 to 1000 ηm, with a spectral slit width of 10 ηm. The color units for a sample are determined by comparing the absorbance reading with a standard curve prepared using solutions of platinum cobalt. Turbidity measurements were done on a Hach Model 18900 Ratio Turbidimeter with ranges of 1 to 2, 1 to 20, and 1 to 200 nephelometric turbidity units (ntu). The unit is equipped with a tungsten lamp, and was operated using the procedure in the instrument manual (Hach 1991).

4.3 Wavelength Selection Verification

Selection of the wavelength used to measure the absorbance of the samples influences the accuracy and precision of color measurements. Ideally, the absorbance observed for samples of similar color values should overlap the absorption of the standard selected to assess the color value (Ingle and Crouch 1988). NCASI conducted experiments during the development of Method 253 to determine

the wavelength at which the platinum cobalt standard overlapped the absorption curve of pulp mill wastewater samples. The experiments involved plotting the absorption curves for bleached kraft mill effluent samples in each of 18 sets of data from a survey representing 18 different instruments. The results of the experiments indicated that the areas of the visible spectrum yielding an exact match of sample with the platinum cobalt color standard occurred in the range of 450 to 480 ηm (NCASI 1971). The results also indicated that accuracy and precision could be achieved by measuring the absorbance at a wavelength of 465 ηm.

To verify this wavelength selection in current sample matrices, an experiment was performed which involved measuring the absorption curve for a 200 platinum cobalt unit (PCU) standard, a biologically treated effluent (Mill A), and a treatment system influent (Mill B). The absorption curves were prepared within a wavelength range of 400 to 700 ηm in intervals of 10 ηm using samples with color values of approximately 200 PCUs. The absorption curves for this experiment are plotted in Figure 4.1. Ideally, the plots for the absorbance vs. wavelength for the samples tested should show lines overlapping the platinum cobalt standard curve at the wavelength used for measuring sample absorbance in the method. The mill wastewater samples tested did not show distinct peaks, indicating that a mixture of chromophores are responsible for the resulting color observed. As wavelength increased, the absorbance of light gradually decreased. Optimum overlap occurred in the range of 460 to 470 ηm. The selection of a wavelength of 465 ηm for the measurement of effluent and influent color was valid.

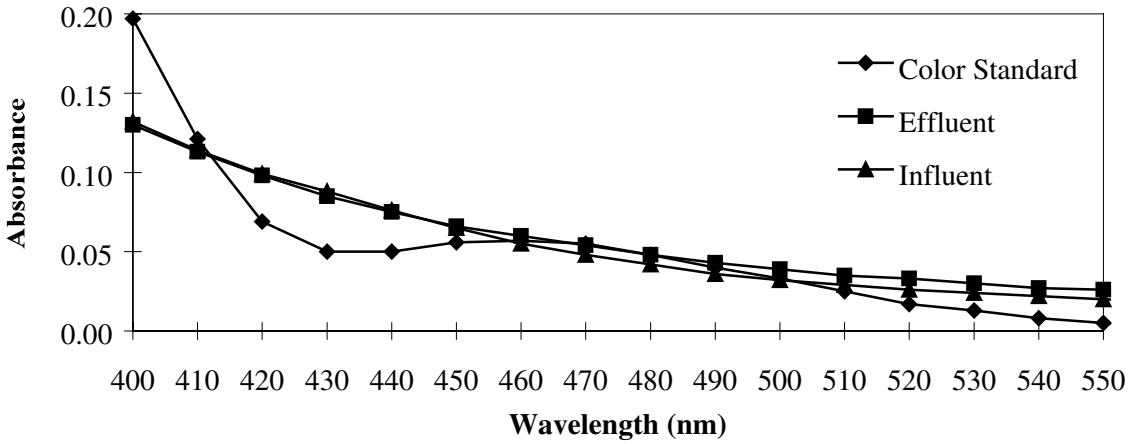

Figure 4.1. Absorption Curve Overlap.

4.4 pH Adjustment Validation Experiments

4.4.1 *The Effects of pH on Color Determinations*

In NCASI Method 253, the pH of the sample is to be adjusted to 7.6 using a solution of NaOH or HCl. As previously noted, users of this method sometimes find the pH difficult to stabilize. In most cases, the pH of the sample increases following filtration through a 0.8 µm membrane filter and over time. Comparative work conducted as part of an inter-laboratory evaluation of the updated NCASI Method Color 71.01 confirmed these observations (Section 5.5, Figure 5.1).

To examine the effects of pH on the color value determined for a biologically treated pulp mill effluent sample, the absorbance of a sample at various pH values was measured. A 200 mL aliquot of sample (Mill A) was adjusted to pH 7.6 and filtered through a 0.8 µm membrane filter. A 25 mL aliquot was taken from the filtrate and transferred to a beaker. The initial pH and absorbance were

measured. The sample pH was then adjusted from 7.0 to 8.4 in increments of 0.2. Absorbance was measured at each pH level and color values were calculated based on these measurements. This experiment was repeated with a biologically treated effluent from Mill C. The results of the experiments are illustrated in Figure 4.2. The color values for Mill A showed a consistent increase across the range of pH values tested. The color values for Mill C appeared to be somewhat stable between pH 7.6 and 8.0. Color values measured at pH 8.2 and 8.4 indicate a sharp increase, then decrease, which could not be effectively explained. The percent increases in the color values determined from pH 7.6 to 8.4 were 7.2% and 2.7% for Mill A and Mill C, respectively, while the percent decreases in the color values determined from pH 7.6 to 7.0 were 6.5% and 4.0% for Mill A and Mill C, respectively.

This experiment demonstrates the importance of stabilizing the pH of pulp mill effluent samples at a given value and recording the value at which color assessments are determined.

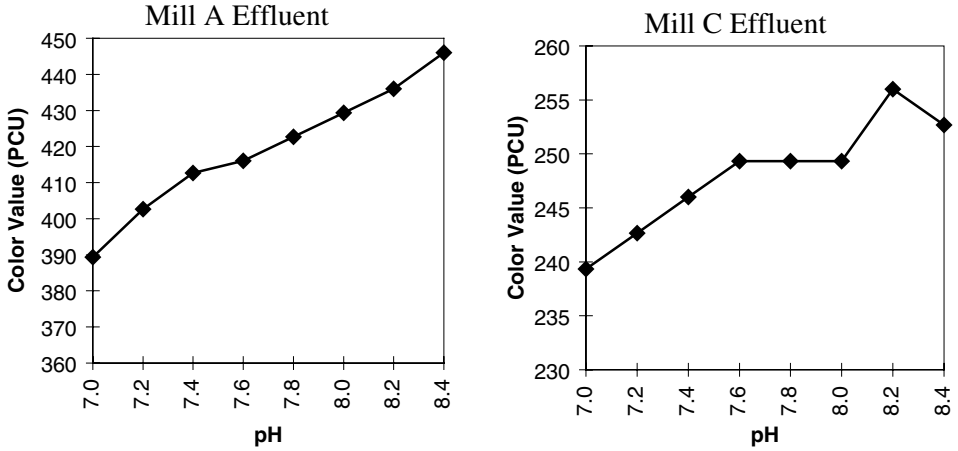

Figure 4.2. Effects of Sample pH on True Color Value Determined.

4.4.2 *pH Stabilization Experiments*

Given the importance of pH stabilization, NCASI investigated different buffering systems in an effort to stabilize the pH of samples, and thereby reduce errors in color measurement associated with fluctuations in pH. Several buffering systems were examined, including a phosphate buffer solution (dibasic sodium phosphate and monobasic sodium phosphate, pH 7.6) which was found to stabilize the pH of pulp mill effluents during research conducted by NCASI in 1981 (NCASI 1981). Additional buffers investigated include a dry phosphate buffer in the form of a capsulated powder (dibasic sodium phosphate and monobasic potassium phosphate, pH 7.0), and a biological buffer solution of beta, beta-dihydroxy-1, 4-piperazinebis (propanesulfonic acid) dihydrate (POPSO, pH 7.2 to 8.5) (Ferguson et al. 1980) prepared at a concentration of 3 g/L in organic free reagent grade deionized water (Barnstead™ water).

Each buffer was studied for its effectiveness in stabilizing the pH of the samples during the filtration process and over time. Four 50 mL aliquots of an effluent sample from Mill H with a color value in the range of 99 to 176 PCU were prepared for use in this experiment. The first aliquot was not treated with a buffer, the second was treated with 1 mL of the pH 7.6 phosphate buffer solution, the third was treated with 0.60 grams of the pH 7 phosphate buffer capsule, and the fourth was treated with 1 mL of the POPSO buffer solution. Each sample was then adjusted to pH 7.6 ±0.05 using a 20% sodium hydroxide solution or 10% hydrochloric acid solution, and filtered through a 0.8 µm membrane filter.

To determine the stability of sample pH, the pH was recorded initially, during adjustment, after filtration, at 15-minute intervals for one hour, and then hourly for three hours. After filtration the absorbance of each aliquot was measured using a spectrophotometer set at a wavelength of 465 ηm. The color value was determined from the measured absorbance and the equation derived from the calibration curve.

As indicated in Figure 4.3, the pH of the sample containing no buffer and the sample containing the POPSO buffer increased following filtration and continued to gradually increase over the three-hour period. In comparison, the pH of the samples buffered with phosphate remained relatively constant. The fluctuation in pH observed between the buffered and non-buffered solutions represents a 77% increase in the color values determined immediately following filtration of the sample.

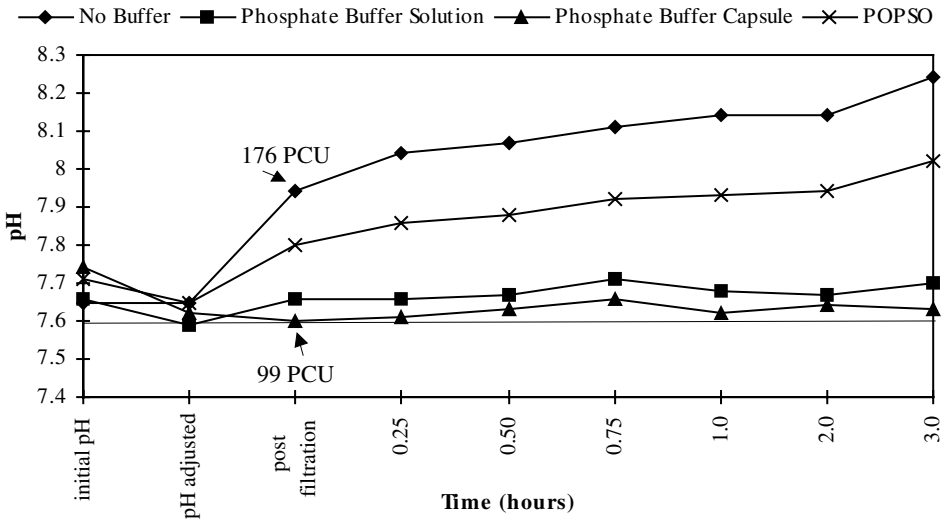

Figure 4.3. pH Stabilization Using Various Buffers in an Effluent Sample with a Color Value of Approximately 100 PCU.

This experiment was repeated using a biologically treated effluent from Mill A with a color value in the range of 362 to 420 PCU. The experiment followed the procedure outlined above, with two exceptions. The pH was measured every hour for four hours, then once again after a period of 24 hours. The results of this experiment are presented in Figure 4.4, and follow the same general trends in this effluent using the various buffers. The fluctuation in pH between the phosphate buffered and non-buffered samples represents a 16% increase in the color values determined immediately following filtration.

Based on the results of these experiments, the color procedure was modified to include the addition of a phosphate buffer prior to filtration. The phosphate buffer capsule proved to be a convenient and effective method for stabilizing the pH of effluent and influent samples. Additional experiments were conducted to determine if pH 7 or pH 8 phosphate buffer capsules stabilized the sample pH more effectively. The use of pH 7 or pH 8 buffer capsules followed by fine adjustment of the final pH to 7.6 ±0.05 with NaOH or HCl resulted in the stabilization of the sample pH at 7.6 ±0.05 following filtration. It was observed that the pH 7 buffer capsule dissolved more readily into the effluent samples; therefore it was utilized throughout the remainder of this study.

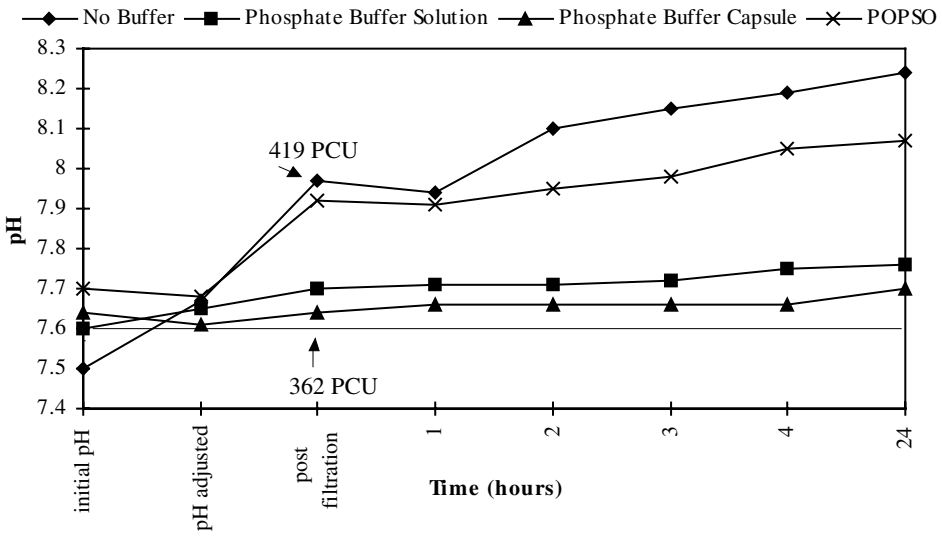

Figure 4.4. pH Stabilization Using Various Buffers in an Effluent Sample with a Color Value of Approximately 360 PCU.

4.5 Turbidity Removal Experiments

The presence of turbidity in a sample can cause light scattering and/or increase the absorption, thereby increasing the resultant color value. True color is the color due to dissolved substances that absorb light. The objective in this research was to determine a reliable method for removing a majority of sample turbidity with the smallest corresponding change in the true color value. Due to the nature of the multi-component matrix and the different methods used for assessing true color and turbidity, this can be a very difficult task. Several techniques for the removal of turbidity were investigated as part of this work. Experiments were conducted to examine the effects of a pre-filtration step, centrifugation vs. filtration, and filter porosity on the true color value of pulp mill wastewater samples.

4.5.1 *Pre-filtration Experiments*

Clogging of filters has been observed during filtration of pulp mill wastewater samples using the recommended 0.8 µm membrane filters (NCASI 1971). This can result in artificially low color values because clogging effectively reduces the pore size of the filter, resulting in the removal of true color bodies from the sample. Slow sample filtration can also increase the time required to process samples, and require the use of multiple 0.8 µm membrane filters. It is sometimes useful to remove large particulate matter by pre-filtering the sample through a 1.0 µm glass fiber filter. This allows the sample to pass more readily through the smaller porosity membrane filters, and reduces the likelihood that the filter will plug and remove true color along with turbidity.

An experiment to investigate pre-filtration was simultaneously conducted with the buffer comparison experiment. Samples were treated with the buffers as described in Section 4.4.2, but one set of samples was filtered with a 1.0 µm glass fiber filter before treatment, and one set of samples was not pre-filtered before treatment with the various buffers. As indicated in Table 4.2, less than 4.6% variation was observed in all cases between the results with and without pre-filtration with a 1.0 µm glass fiber filter.

The color values were slightly higher in the samples that were pre-filtered, indicating that the pre-filtration of samples may have decreased clogging of the 0.8 µm membrane filters, resulting in less true color removal.

Table 4.2. Effects of Pre-filtration vs. No Pre-filtration on True Color

Filtration Technique	No Buffer Added	Phosphate Buffer Solution Added	Phosphate Buffer Capsule Added	POPSO Buffer Added
Pre-filtered 1.0 µm GFF[a] then 0.8 µm MF[b]	419	396	362	396
0.8 µm MF[b]	402	379	346	396
Relative percent difference (RPD)	4.0%	4.4%	4.6%	0%

[a] GFF is a glass fiber filter.
[b] MF is a membrane filter.

4.5.2 *Centrifugation Experiments*

Centrifugation was also investigated as an alternative to filtration for the removal of turbidity from pulp mill wastewater samples. Samples of biologically treated effluent from Mills A, F, G, and H were utilized to determine the effectiveness of centrifugation vs. filtration through a 0.8 µm membrane filter for the removal of sample turbidity. Color values were also determined. The initial turbidities of the effluent samples were determined by measuring duplicate samples and calculating the average turbidity of the two samples. Then, 200 mL aliquots were buffered (pH 7 phosphate buffer capsule) and the pH was adjusted to 7.6 ±0.05 using a 10% sodium hydroxide solution. The aliquots were split into four aliquots of 50 mL each. Two aliquots were filtered using a 0.8 µm membrane filter, and the absorbances were measured at 465 ηm. The true color values were determined for the samples using the absorbance measurements. The turbidity of each aliquot was also measured. The remaining two aliquots were centrifuged for 30 minutes at 2100 revolutions per minute (rpm), and the absorbances and turbidities of the supernatant layer were measured. The results are presented in Figure 4.5, which shows the average color value determined for the duplicate samples from each mill on the Y axis. The color values are represented by bars, and the percent decreases in sample turbidity after each treatment are represented by lines, with corresponding values on the right axis.

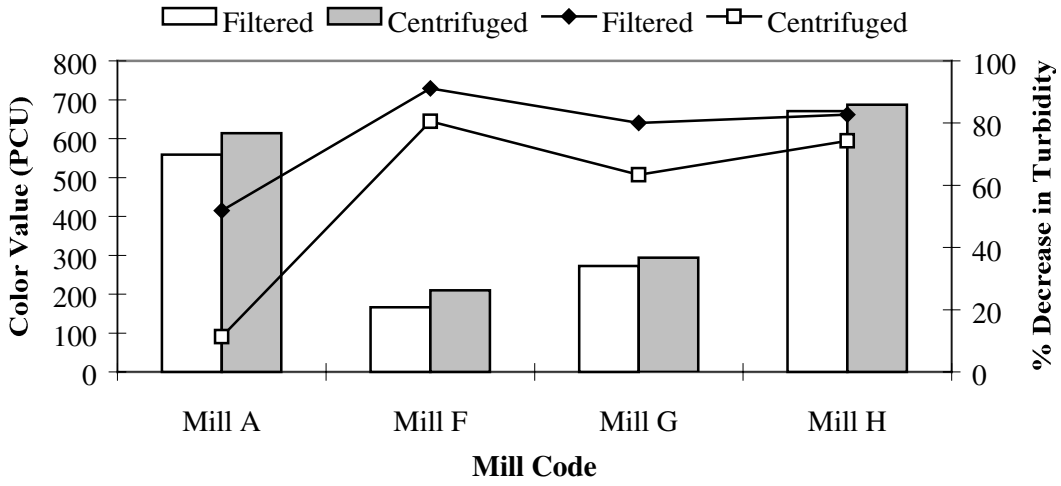

Figure 4.5. Effects on Sample Turbidity and Color: Filtration vs. Centrifugation.

The percent decrease in turbidity was consistently higher for aliquots treated using filtration through a 0.8 µm membrane filter than for samples treated using centrifugation for 30 minutes at 2,100 rpm. The resultant color values were consistently higher for the samples that were centrifuged to remove turbidity. The average increase in the color values determined for the centrifuged samples relative to the filtered samples was 21%, with a range of 2 to 32%.

4.5.3 *Filter Porosity Verification Experiments*

NCASI Method 253 recommends the use of a 0.8 µm membrane filter for removal of turbidity. To verify that this filter porosity is still appropriate for low color effluents, an experiment was conducted to evaluate the amount of color and turbidity being removed using filters of various porosities. This involved filtering the same 50 mL aliquot of sample through successively smaller filters to observe the change in color and turbidity determined with each filtration. The filtration series included 2.7, 1.5, and 1.0 µm glass fiber filters, followed by 0.8, 0.65, 0.45, and 0.22 µm membrane filters. Absorbance and turbidity were measured after each filtration. The macromolecules that constitute the true color in a pulp mill wastewater sample are in the range of 0.1 to 0.7 microns (NCASI 1971), therefore the color observed above 1 µm was assumed to represent color due to suspended and dissolved components (apparent color) as well as true color (dissolved components only). Figures 4.6 (effluent, Mill B) and 4.7 (influent, Mill C) illustrate the changes in sample color and turbidity following filtration through the 1 to 0.22 µm membrane filters (x-axis); the values for the 2.7 and 1.5 µm glass fiber filters are excluded. The graph on the left illustrates the changes in color observed following each filtration, while the graph on the right shows the effects on sample turbidity. The percentage of the initial turbidity removed following each filtration is indicated above the bars of the graph on the right. In all cases, as the filter porosity decreased, the resulting color value decreased. Due to the interactive nature of light scattering (due to turbidity) and absorbance (due to true color), this would be the anticipated trend.

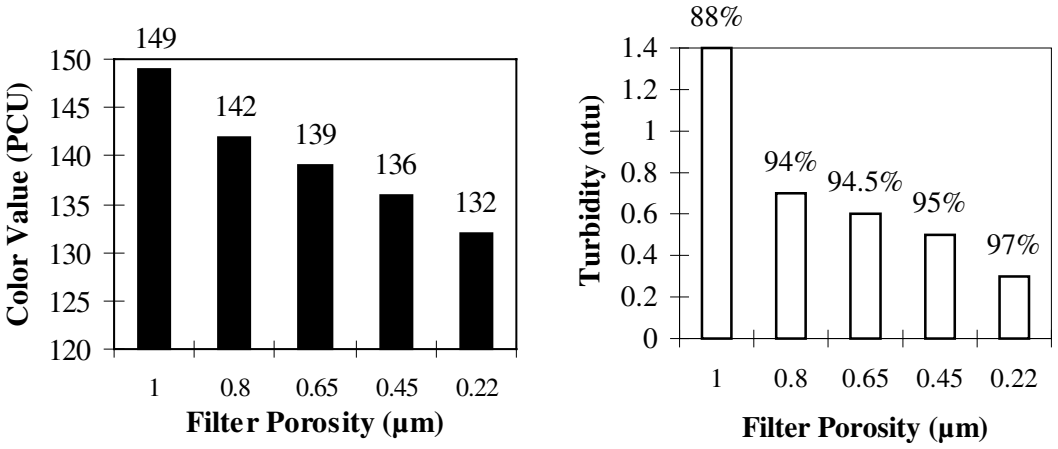

Figure 4.6. Filter Porosity Effects on Mill B Effluent Turbidity and Color.

It is difficult to determine whether the continual decrease in the color values was due to an increase in the turbidity removed or to the removal of color bodies from the samples by the filters. For the Mill B effluent sample, filtration below 0.8 µm resulted in a difference of 7% in the resultant color value, down to a filter porosity of 0.22 µm. Between the 1 µm and 0.8 µm filtrations, 95% of the color remained while 50% of the remaining turbidity was removed; and between the 0.8 µm and 0.65 µm filtrations, 97% of the color remained while 14% of the remaining turbidity was removed. Following filtration through the 0.8 µm filter, 94% of the initial turbidity was removed, as indicated by the numbers above the bars. The initial measurement of turbidity was 4.1 ntu.

Successive filtration continued to remove portions of the turbidity, but in smaller increments. This may be due to the removal of color bodies by the smaller porosity filters. A regression analysis of the change in turbidity vs. color did not show a significant relationship between the two (Appendix A, Section A2). In other words, an incremental change in turbidity did not correlate to an incremental change in color. Therefore, it is more likely that the changes noted in the color values were related to the removal of color bodies from the samples than to the removal of additional turbidity.

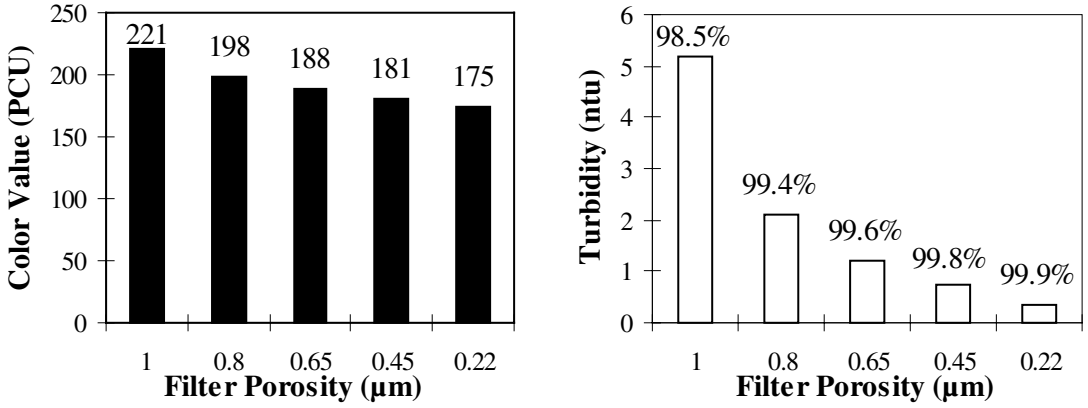

Figure 4.7. Filter Porosity Effects on Mill C Influent Turbidity and Color.

For the Mill C influent sample, filtration below 0.8 µm resulted in a difference of 12% in the resultant color value, down to a filter porosity of 0.22 µm. Between the 1 µm and 0.8 µm filtrations, 90% of the color remained while 60% of the remaining turbidity was removed; and between the 0.8 µm and 0.6 µm filtrations, 95% of the color remained while 43% of the remaining turbidity was removed. The overall decrease in the color value determined between the 0.8 µm and 0.22 µm filtrations was 13%, but the majority of the turbidity in the initial sample (99.4%) was removed following filtration through the 0.8 µm membrane filter. A regression analysis of the change in turbidity vs. color for the Mill C influent did not show a significant relationship between these two variables (Appendix A, Section A2). These results indicate that the 0.8 µm membrane filter removed the bulk of sample turbidity while having a minimal effect on the dissolved color bodies within the sample.

4.5.4 *Comparison of Filter Types*

There are several brands and types of membrane filters on the market, therefore this work included an evaluation of various types of filters. Comparative experiments were conducted using three brands of filters: Gelman Metricel™, Gelman Supor 800™, and Nucleopore Membra-Fil™ filters. Both Gelman Metricel and Nucleopore Membra-Fil filters are made of mixed cellulose esters. The Gelman Supor 800 filter is a hydrophilic polysulfone membrane filter.

A 400 mL aliquot of a biologically treated effluent sample from Mill D was adjusted to pH 7.6, then 50 mL portions were filtered through each membrane filter. Experiments involved three replicates using Supor filters and three replicates using Metricel filters. The samples were not buffered prior to pH adjustment.

The Nucleopore Membra-Fil was utilized because of the wide range of porosities commercially available for this filter type. An experiment was conducted to compare the Nucleopore Membra-Fil filter to the Gelman Metricel filter. A 400 mL aliquot of a biologically treated effluent sample from Mill F was adjusted to pH 7.6, then 50 mL portions of this sample were filtered through each membrane filter. Experiments involved three replicates using Nucleopore Membra-Fil filters and

three replicates using Metricel filters. The absorbances were measured and statistically compared for differences.

The results for these two filter comparison experiments were evaluated using analysis of variance (alpha = 0.05) to determine if filter type affected color value. The results, shown in Table 4.3, do not reveal a statistically significant impact of filter type on measured color.

Table 4.3. Comparison of Various Membrane Filters

Comparison	p-value
Metricel and Supor	0.13
Metricel and Nucleopore Membra-Fil	0.42

The Metricel filters were used in the majority of the color experiments. The Nucleopore Membra-Fil filters were used during the successive filtration experiments because they were available in a wider range of porosities.

4.5.5 *Investigations of Possible Turbidity Agents*

NCASI examined several different turbidity agents potentially present in pulp mill wastewaters which may interfere with the determination of true color. The purpose of these experiments was to determine if a 0.8 µm membrane filter was effective in removing the turbidity agent while having a minimal effect on true color, and to determine how different turbidity agents might interfere with the determination of true color in biologically treated effluents. The turbidity agents explored included precipitated calcium carbonate, titanium dioxide, lime mud, fiber, and green liquor dregs.

Each of these agents was added to separate aliquots of water in an amount adequate to achieve approximately 25 to 50 ntu of turbidity when 500 µL of the solution was spiked into a 50 mL volume. These spiking stocks were spiked into 50 mL of deionized water, which was then buffered, pH adjusted to 7.6 ±0.05, and filtered through a 0.8 µm membrane filter (Sample A). The same spike amount of the turbidity agent solution was then added to 50 mL of an effluent sample (Mill A), buffered, pH adjusted, and filtered (Sample B). Finally, 50 mL of an effluent sample without the added turbidity agent was buffered, pH adjusted, and filtered (Sample C). The absorbance and turbidity values were measured and recorded for each sample (A, B, and C) initially, after the addition of buffer and pH adjustment, and after filtration. The experiment was conducted with three replicates of each sample type (A, B, and C).

The average turbidity of each set of three replicates was calculated. The average value calculated for Sample C (no turbidity added) and Sample A (turbidity spike in water) were added together. This value was then compared to the turbidity value of Sample B, the turbidity-spiked effluent. This comparison was conducted to determine if the turbidity value was affected by the sample matrix. The turbidity values determined for Sample C plus Sample A were equivalent to the turbidity values measured for Sample B, indicating that the turbidity agent spike was not affected by the sample matrix.

An additional component of this experiment was to determine whether some turbidity agents are more readily removed by filtration than others. The presence of turbidity in the samples following filtration can cause erroneously high color values due to light scattering. Figure 4.8 illustrates the average true color value determined for the three replicates of each sample type following filtration through the 0.8 µm membrane filter. The color values are indicated on the y-axis, while the x-axis lists the turbidity agent used. The black bars represent the average true color value determined for Sample C (effluent only) and the white bars represent the average color value determined for Sample B (effluent

plus turbidity agent). The percentage of the turbidity removed by filtration is indicated above each set.

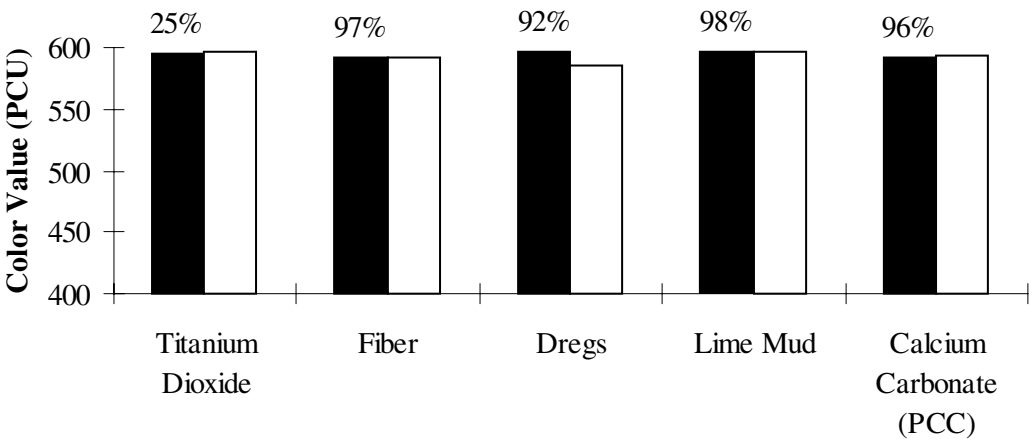

Figure 4.8. Turbidity Agent Removal Results: Effluent Sample.

The results indicate that the 0.8 µm membrane filter effectively removes the turbidity introduced by the indicated agents, with the exception of titanium dioxide. The results also indicate that the true color values determined for the samples with and without added turbidity varied by less than 3% following filtration through a 0.8 µm membrane filter.

The experiment was repeated using a solution of lignin which served as a surrogate for pulp mill wastewater true color to examine the trends in a solution that contained no other source of turbidity except the spiked turbidity agent. As indicated in Figure 4.9, turbidity was effectively removed for all of the agents tested except the titanium dioxide. In addition, the true color values determined for the lignin sample with and without added turbidity from fiber, dregs, lime mud, and precipitated calcium carbonate varied by less than 7%, although in general the color values determined in the turbidity agent spiked lignin samples resulted in slightly increased color values. The true color values determined for the lignin sample with and without added titanium dioxide varied by 25%.

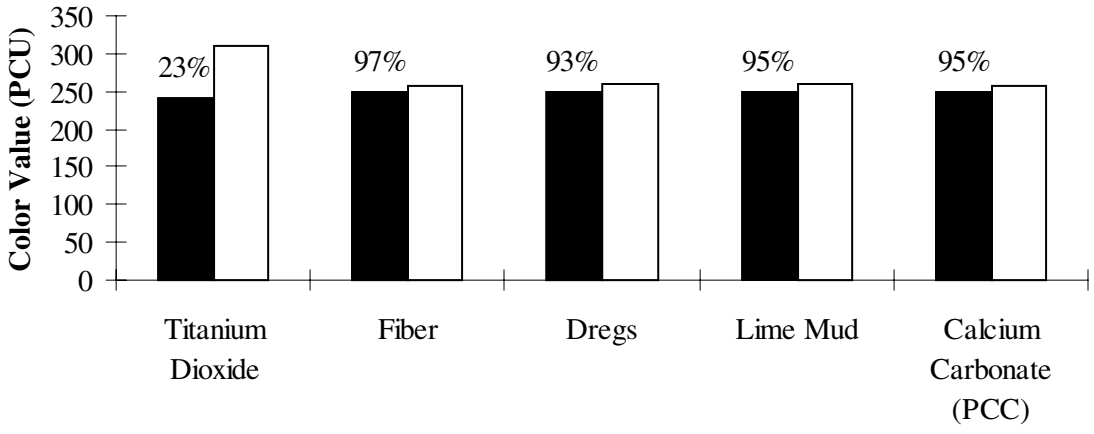

Figure 4.9. Turbidity Agent Removal Experiments: Lignin Solution Sample.

4.6 Sample Volume Selection

It was previously observed that the volume chosen for filtration by each analyst can significantly alter the final color reported for the sample and may account for increased variability in observed results (NCASI 1971). This occurrence is usually related to filter plugging that can occur when filtering a larger sample volume. The filter plugging reduces the effective filter pore size, which can remove some of the components contributing to the true color of a sample. To investigate this observation, duplicates of four aliquots of sample with volumes of 25, 50, 100, and 200 mL were treated identically (i.e., buffered, pH adjusted, and filtered through 0.8 µm membrane filter). This experiment was repeated using biologically treated effluents from Mill A and Mill B and an influent to the treatment system from Mill B. If the filtering rate slowed or foaming was observed, the filtration was stopped and another filter was used to complete the filtration of the total aliquot of sample. The results of this experiment are shown in Table 4.4. The percent relative standard deviation of the color values determined using the 25, 50, 100, and 200 mL volumes ranged from 0.7 to 5.6%, indicating that when using this technique, sample volume did not effect the overall color value determined. Therefore, a 50 mL sample volume was chosen for ease in manipulation and filtering, and the appropriate filtration technique is specified in the updated method (Appendix B). The 50 mL sample volume was selected over a 25 mL sample to allow for easier pH adjustment of the initial sample without changing the sample volume by more than 1%.

Table 4.4. Effects of Sample Volume on the True Color Value Determined

Sample	25 mL (PCU)	50 ml (PCU)	100 mL (PCU)	200 mL (PCU)	Average (PCU)	RSD[a] (%)
Mill A Effluent	617	580	597	583		
	603	603	580	[b]	595	2.2
Mill B Effluent	173	176	176	176		
	176	173	176	176	175	0.7
Mill B Influent	189	186	186	206		
	193	176	203	209	194	5.6

[a] Relative standard deviation expressed as a percent.
[b] This determination was not available due to a limited sample availability.

4.7 Effects of Various Light Path Lengths on Resultant Color Values

An experiment was conducted to investigate the effect of light path length on the determined color value. Samples from five different mills (Mills A, E, F, G, and H) were measured to determine the color value of each matrix using a 10, 20, 25, 50, and 100 mm light path length cell. The samples were analyzed in replicates of three, and the color values were calculated using the equations in Table 4.5 for each specific light path length cell. These calibration curve equations were obtained by analyzing a calibration curve ranging from 10 to 500 PCU using a curvette with the light path length indicated in Table 4.5.

Table 4.5. Calibration Curves for Various Light Path Length Cells

Linear Equation y = mx +b	R-squared	Light Path Length (mm)
y = 0.0003x – 0.0003	1.000	10
y = 0.0005x + 0.0028	0.9988	20
y = 0.0006x – 0.0004	0.9999	25
y = 0.0014x – 0.0003	1.000	50
y = 0.0027x + 0.0018	1.000	100

The average color values determined for the various samples, the range of color values reported, and the relative standard deviations of these averages expressed as a percent are presented in Table 4.6.

Table 4.6. Summary of Light Path Length Experiments

Mill Code	Average Color Value (PCU)	Range of Color Values (PCU)	Relative Standard Deviation (%)
A	514	486 - 547	5
E	184	178 - 190	3
F	78	75 - 81	3
G	274	265 - 288	4
H	1186	1112 - 1271	6

Figure 4.10 illustrates the results of this experiment. The color values of the samples used in this experiment ranged from 75 to 1,271 PCU. The values plotted in Figure 4.10 are the average PCUs determined for the three replicates using the indicated light path length cells. Due to limited sample volumes, Mill H was not measured in the 100 mm light path length cell.

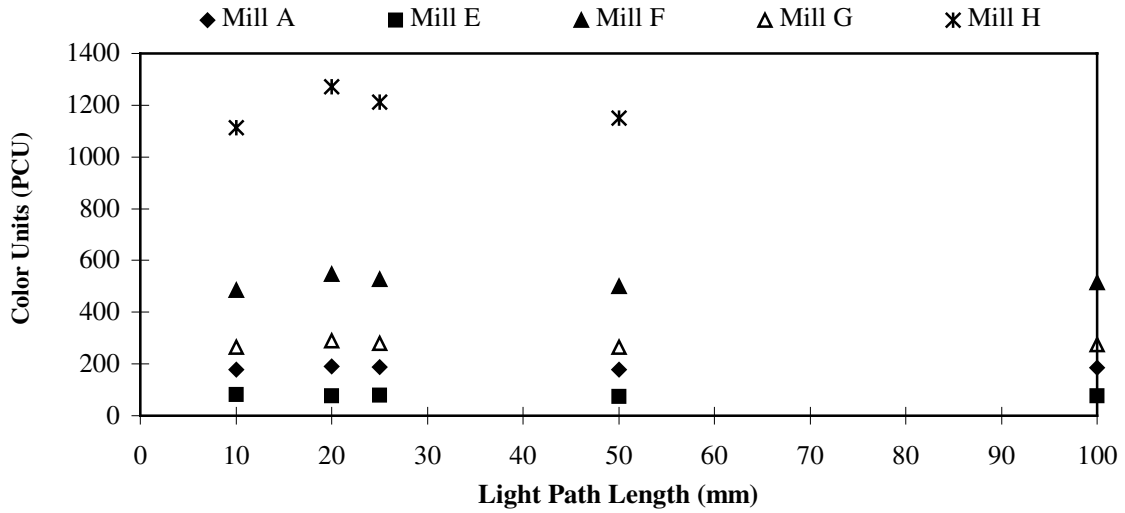

Figure 4.10. Color Units (PCU) of Five Effluent Samples Using Various Light Path Length Cells.

The relative standard deviations (RSD), expressed as percents, are similar to the intra- and inter-laboratory relative standard deviations determined for replicate analyses (Section 5.6). The RSDs are also below EPA's color method precision criteria of 10% listed in the *Federal Register* (*Federal*

Register 1997), indicating that for color value determinations of samples within the range of 70 to 1,300 PCU, cell light path length is not likely to be important.

5.0 UPDATED METHOD COLOR 71.01 VALIDATION AND QA/QC

The previous sections of this bulletin presented the results of experiments conducted to optimize the NCASI color measurement method in pulp mill wastewaters. This section presents information relating to the validation of the updated method (NCASI Method Color 71.01) and development of quality control and quality assurance criteria.

5.1 Brief Description of NCASI Method Color 71.01: The Updated Color Method

The updated procedure involves adding half of a pH 7 buffer capsule to a 50 mL sample of wastewater or water to stabilize the pH, adjusting the pH to 7.6 ±0.05 with HCl or NaOH (such that the volume change of the sample is less than 1%), and filtering the aliquot through a 0.8 µm porosity membrane filter pre-rinsed with distilled water. A portion of the filtered sample is then transferred to an absorption cell and the absorbance is measured at 465 ηm using a spectrophotometer with a tungsten lamp source and 10 ηm spectral slit width. The spectrophotometer capabilities should include an operating range of 400 to 700 ηm and an ability to emit light at a selectable wavelength of 465 ηm. The color units for the sample are determined by comparing the absorbance reading with a standard curve prepared using dilutions of a platinum cobalt stock solution. A full description of the updated method can be found in Appendix B.

5.2 Preparation of the Calibration Curve

The linearity of the spectrophotometer used for this work was assessed by analyzing a five-point calibration curve that ranged in concentration from 25 to 500 PCU. The calibration curve was prepared using a stock platinum cobalt solution with an initial color value of 500 in volumes of 2.5, 10, 20, and 25 mL. These volumes were diluted with Barnstead™ deionized water to 50 mL in a volumetric flask. The corresponding color unit values of these solutions were 25, 100, 200, and 250 PCU. In addition, an aliquot of the undiluted stock platinum cobalt solution with a color value of 500 PCU was measured and included in the curve. The absorbances of these solutions were measured at a wavelength of 465 ηm on a Spectronic 21D spectrophotometer with a tungsten lamp light source. The various curves analyzed in this research utilized cuvettes with 10, 20, 25, 50, or 100 mm light paths. Calibration curves were prepared using the results of these measurements by plotting the absorbance values against the color units of each of the standards measured. A linear regression equation was generated for each curve and used to calculate the color units of the samples from their measured absorbances. Calibration criteria (Appendix B, Sections 10 and 17) were determined by measuring several curves and developing acceptable criteria based on the coefficient of determination (r-squared) from the linear equations generated for each curve. Throughout the course of this study, a total of thirteen calibration curves were generated, with the results displayed in Appendix B, Table 6. All of the curves analyzed had an r-squared value greater than0.9968.

5.3 Daily Calibration Verification

Virtually all instrumental analysis methods require that some type of calibration verification be conducted at the beginning of each day or prior to analysis of samples. One objective of this project was to bolster the QA/QC requirements of the color method (NCASI 1971). Therefore, a mechanism for accomplishing daily calibration checks was employed during this investigation. The results from these experiments form the basis for daily calibration criteria incorporated in the updated color method (Appendix B, Section 9.2).

For all of the color analyses conducted as part of this project, the validity of the instrument calibration curve was verified prior to sample preparation. Different calibration points were selected on each day in order to verify the calibration over the full range of the curve. The results of the calibration verification were calculated by dividing the measured concentration by the known concentration of the standard and were reported as percent recovery.

During the course of this work, calibration verifications were completed on 47 occasions using various light path length cells and color ranges from 25 to 500 PCU. The results of these calibration verifications yielded an average percent recovery of 95%, a range of 88 to 118%, and a standard deviation of 6.7. The distribution of percent recovery results can be used to suggest limits around which the analyst should consider further evaluation of instrument performance or re-calibration of the instrument. For this method, warning and action limits were calculated as the mean plus and minus two standard deviations and three standard deviations, respectively. These limits are shown in Table 5.1. When warning limits are exceeded, the analyst should investigate possible instrument problems, perform a calibration verification of the instrument as recommended by the instrument manual, check for contamination and evaporation of standard solutions, examine glassware for scratches, re-zero the spectrophotometer, and reanalyze the standard. When the action limits are exceeded, the analyst should likewise investigate potential problems, prepare a new calibration curve, and reanalyze the curve as recommended in Section 5.2.

Table 5.1. Summary of Daily Instrument Calibration Checks

Average Recovery[a] (%)	Standard Deviation[b]	Warning Limits[c] (%)	Action Limits[d] (%)	Minimum Recovery (%)	Maximum Recovery (%)	Color Unit Range (PCU)
94.9	6.7	81 - 108	75 - 115	88	118	25 - 500

[a] Average recovery for 47 daily calibration checks.
[b] Standard deviation of the recoveries for 47 daily calibration checks.
[c] Average recovery plus and minus two times the standard deviation.
[d] Average recovery plus and minus three times the standard deviation.

5.4 Single Laboratory Precision

5.4.1 *Instrument Precision*

The instrument precision of the Spectronic 21D, which was utilized throughout the single laboratory validation of the updated method at NCASI's West Coast Regional Center, was verified by the analysis of the midpoint of the calibration curve a total of seven times over an eight-hour period. The results of the instrument precision experiment yielded a relative standard deviation of 1.4% for the seven replicates of the 100 PCU standard.

5.4.2 *Method Precision*

The precision of the method was characterized and continuously monitored using duplicate analyses. Throughout the single laboratory assessment of precision, a total of twelve different duplicate sample pairs of both biologically treated effluent and treatment system influent from the mills listed in Table 4.1, were analyzed. The relative percent differences for the duplicates ranged from 0.4 to 14.1% for the biologically treated effluents, with an average relative percent difference of 2.8%. The color values of the samples utilized in this assessment ranged from 81 to 1,112 PCU, indicating that the precision of the method is consistent over a wide range of color values. The relative percent differences observed for the treatment system influent duplicate pairs ranged from 0.1 to 13.2%, with an average relative percent difference of 7.1%. The average relative percent differences for the

effluent and influent sample duplicates were below EPA's standardized quality assurance and quality control acceptance criteria of 10% for precision in color methods listed in the *Federal Register* (*Federal Register* 1997).

5.5 Comparison of Updated NCASI Method Color 71.01 to NCASI Method 253

A comparison of the updated method and NCASI Method 253 was made using data from two different NCASI laboratories. This comparison was done using biologically treated effluents from Mills A and B, influents to the treatment system from Mills A and B, a prepared lignin solution, and a receiving water sample collected below the discharge of Mill D. Trends that suggest there may be some method differences were observed. The color values determined in five of the six matrices tested were lower using the updated procedure (Figure 5.1). This lower color value often corresponded to an observed difference in the post-filtration pH between the two methods (Figure 5.2). In the matrices tested, a pH of 7.6 ±0.05 was achieved using the updated method, but NCASI Method 253 samples were not buffered, and the post-filtration pH increased. The influent sample from Mill A was an exception to this trend. This sample yielded a higher color value using Color 71.01, although the pH of the sample remained at 7.6 ±0.05 using this method.

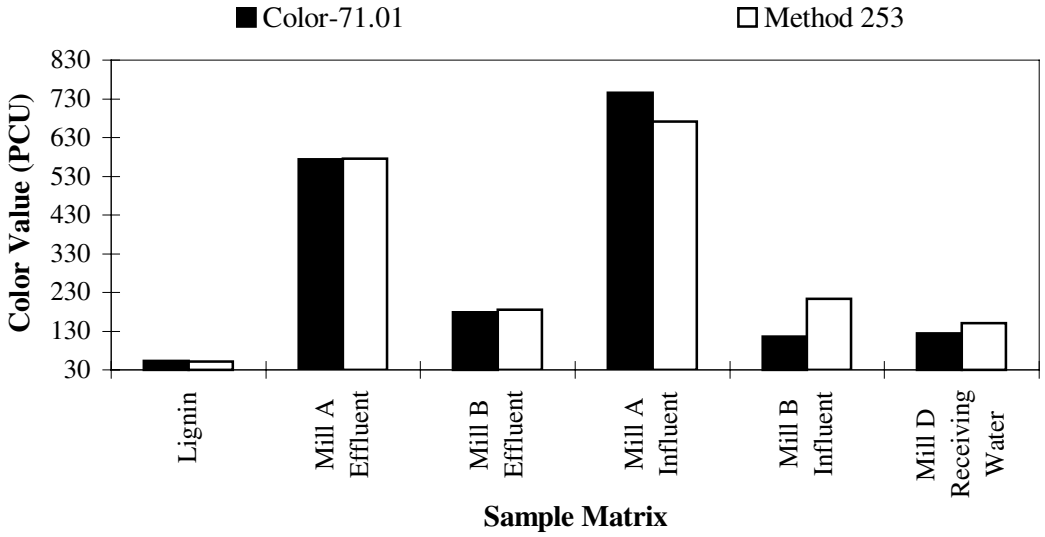

Figure 5.1. Comparison of Resultant Color Values in Different Sample Matrices Using NCASI Methods 253 and Color 71.01.

The statistical difference between the two methods was evaluated through the use of F-tests and p-values. The statistical calculations used for this evaluation utilized a one-tailed F test to determine if the updated Method Color 71.01 was more precise than Method 253. The F-test considers the ratio of the squares of the two sample standard deviations such that calculated F is always greater than one. If the F-ratio is less than the critical value of F, the variances are assumed to be equal (precision of the two methods is similar). The p-values were utilized to determine if the two methods generated comparable color values. The F-ratios, p-values ($\alpha = 0.05$), and critical values for F are presented in Table 5.2. The p-values were calculated assuming equal variances as justified by the F-test results. If the calculated p-values are less than the selected alpha of 0.05 for a 95% confidence level, the mean color values are not considered to be significantly different. A full report of the statistical analysis can be found in Appendix A, Section A3, along with definitions of the statistical terms.

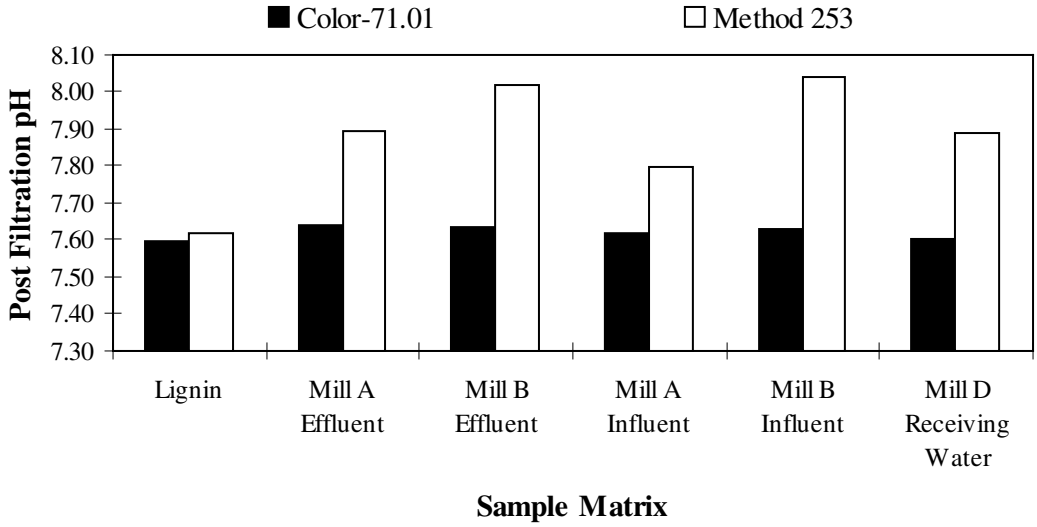

Figure 5.2. Comparison of Post-Filtration pH in Different Sample Matrices Using NCASI Methods 253 and Color 71.01.

As the results indicate, a significant difference in the resultant color determinations was observed for the influent from Mill B and the receiving water from Mill D, with higher color values reported when using Method 253.

Table 5.2. Summary of the Statistics Comparing NCASI Methods 253 and Color 71.01 in Different Matrices

Sample Matrix	F-Ratio	Critical Value of F	p-Value
Lignin Solution	0.098	9.27	0.572
Effluent Mill A	0.229	9.27	0.961
Effluent Mill B	0.304	9.27	0.655
Influent Mill A	0.159	9.27	0.053
Influent Mill B	1.52	9.27	0.009
Receiving Water Mill D	1.46	9.27	0.019

5.6 Inter-Laboratory Investigation of NCASI Method Color 71.01

In an effort to assess the ruggedness and precision of the updated method, an inter-laboratory study was conducted which included industry and NCASI laboratories. A total of eight laboratories participated in a study to determine the intra- and inter-laboratory precision of the updated color procedure using several matrices. The samples included biologically treated effluents from Mills A and B, influents to the treatment system from Mills A and B, portions of a prepared lignin solution, a receiving water sample collected from the discharge stream of Mill D, and a platinum cobalt color standard. Mill personnel collected the influent (24-hour composites), effluent, and receiving water (grab) samples into one-gallon amber glass bottles with Teflon™-lined screw caps. NCASI received the iced samples from the mills via overnight delivery.

To minimize the potential for bias, the samples were thoroughly mixed and split into separate 100 mL portions. This step ensured that any differences in study results could be attributed to analytical method variables and not to sampling or compositing procedures. The samples were then shipped on

ice via overnight carrier to the eight participating laboratories along with a study plan, analytical procedures, and a data sheet (Appendix C). The participating labs received the samples blind, along with a calibration check standard prepared at a color value of 125 PCU. The check standard was prepared by diluting a 500 PCU color standard using Barnstead™ deionized water. The color units and percent recoveries of the QA/QC check standard analyses are presented in Table 5.3. Each laboratory also received replicates of the various matrix types so that the intra-laboratory precision could be assessed. The laboratories were asked to analyze the samples on the date of receipt according to the procedure and study plan presented in Appendix C, and report the data on the data sheet also included in the appendix.

The inter-laboratory data were evaluated statistically using Youden matched pairs of similar matrices (Youden and Steiner 1975). The sample results are summarized in Table 5.4. The codes L1 through L8 identify the participating laboratories, and each matrix type is designated by a letter code. The table also includes information on the light path length of the cells used at each laboratory, although the use of a specific light path length was not part of the study plan.

Table 5.3. Summary of the True Color Values and Percent Recoveries for the QA/QC 125 PCU Standard

Laboratory Code	Color Units (PCU)	Recovery (%)
L1	106	85
L2	145[a]	116
L3	106	85
L4	113	90
L5	129	103
L6	123	98
L7	118	94
L8	103[b]	82
Average	118	94
Standard Deviation	13.2	10.6
Relative Standard Deviation (%)	11.2	11.3

[a] Although this value is high in relationship to the other laboratory results, it was not an outlier based on Grubb or Dixon tests.

[b] Although this value is low in relationship to the other laboratory results, it was not an outlier based on Grubb or Dixon tests.

The Youden matched pair analyses were initiated by preparing an xy plot of the data points for each sample type using one pair of data for the x-axis and one pair of data for the y-axis. Appendix D, Figures D1 through D6, present the sample plots for each matrix. This analysis is conducted in order to assess bias in the data and identify outlying data points. The average value obtained for the y-axis data is represented by the horizontal line and the average of the data plotted on the x-axis is represent by the vertical line. Random variability is demonstrated if the data are evenly distributed within the four quadrants around the intersection of the x and y averages. The closer the data points are to the intersection, the better the analytical precision. High and low laboratory bias is demonstrated when the sample results from a given laboratory are at an extreme along the 45-degree line of the graph. Points distant from the 45-degree line indicate a lack of agreement between results from the same laboratory. Careful review of these plots indicate that although the majority of data points lie near the intersection of the average values, some laboratory bias exists. Examination of the plots indicate that Labs 2 and 3 were biased high, while Lab 4 was biased low. Cochran's, Grubb's, and Dixon's outlier tests were utilized to determined the significance of these laboratory biases (AOAC 1989).

Table 5.4. Inter-laboratory Study: Summary of Results (Color Values Reported in PCU)

Laboratory Code:	L1	L2	L3	L4	L5	L6	L7	L8
Light Path Length[a]:	10 mm	10 mm (sq.)	10 mm	10 mm	28 mm	100 mm	16 mm	10 mm
Sample Type								
Lignin 1	49	90	140	46	59	57	75	43
Lignin 2	49	70	105	51	54	58	68	43
Effluent Mill A-1	509	695	745	454	646	605	515	509
Effluent Mill A-2	516	745	830	440	621	607	515	496
Effluent Mill B-1	162	290	305	147	199	194	195	153
Effluent Mill B-2	159	295	260	141	196	173	168	143
Influent Mill A-1	712	1040	970	556	811	746	640	623
Influent Mill A-2	686	990	1000	533	771	678	638	589
Influent Mill B-1	89	205	210	83	154	141	110	96
Influent Mill B-2	89	205	300	87	132	144	168	89
RW[b] Mill D-1	136	220	180	112	169	149	180	116
RW[b] Mill D-2	132	215	240	113	167	165	140	116

[a] All cells utilized in this study were cylindrical unless otherwise noted.
[b] RW is receiving water.

Dixon's test evaluates the occasional aberrant average value. Cochran's test is applied to test for the removal of laboratories showing significantly greater intra-laboratory variability than the other laboratories in the study for a given matrix type. Grubb's test is applied to assess whether a laboratory has an extreme average value. If no outliers are determined using a single Grubb's test, which is a determination of a single laboratory with high or low bias, then a double Grubb's test is performed to determine if the outliers are masked by the presence of both high and low extremes. Of the five pairs of data plotted, Lab 3 consistently fell outside the range of averages using the Dixon's test. These results may be due to the lack of experience of the analyst performing the measurements at this laboratory, since it was the analyst's first experience conducting measurements of color in pulp mill matrices. In addition, Lab 2 data fell outside the range of averages using the Dixon's tests for the Mill B effluent, Mill A influent, and Mill B receiving water samples.

Once the outliers were removed, statistical calculations were performed to assess the intra-laboratory (repeatability, r) and inter-laboratory (reproducibility, R) precision of the method. The intra-laboratory repeatability is a measure of the closeness of agreement between successive results obtained with the same method on identical matrices under the same conditions and is calculated based on the sum of squares of the laboratory ranges between the two duplicate samples (refer to Appendix A for definitions). The inter-laboratory reproducibility is the variation arising from different operators in different laboratories using different apparatus and is composed of the total within-sample variation. It is calculated by combining the between-laboratory variation, the laboratory-sample interaction, and the between-replicate variance (refer to Appendix A for definitions). Table 5.5 lists the results for the Youden pair analyses, which includes the mean of the laboratory averages, the repeatability and reproducibility standard deviations, and the repeatability and reproducibility relative standard deviations. The results indicated that the intra-laboratory precision (agreement of replicate values within a particular laboratory) of the method ranged from 2.3 to 13.8%. The inter-laboratory precision (agreement in values among laboratories) of the method ranged from 13.1 to 35%. The influent Mill B samples (35%) and the lignin stock solutions (24%) resulted in the largest variations.

Table 5.5. Results of the Inter-laboratory Study Youden Pair Analyses

Parameter	Lignin Solution	Effluent Mill A	Effluent Mill B	Influent Mill A	Influent Mill B	Receiving Water Mill D
Number of labs	7[a]	7[a]	6[b]	6[b]	7[a]	6[b]
Mean of lab averages	58.0	562	169	665	128	141
s (r) repeatability standard deviation	5.8	17.2	7.1	15.4	17.6	13.2
s (R) reproducibility standard deviation	13.9	95.2	22.1	87.7	44.8	25.6
RSD (r) repeatability relative standard deviation	10.0	3.1	4.2	2.3	13.8	9.3
RSD (R) reproducibility relative standard deviation	24.0	16.9	13.1	13.2	35.0	18.1

[a] Lab 3 was an outlier and was removed prior to completing the calculations in this table.
[b] Labs 2 and 3 were outliers and were removed prior to completing the calculations in this table.

6.0 SUMMARY AND CONCLUSIONS

The purpose of this study was to evaluate and optimize NCASI Method 253 when used on biologically treated effluents and treatment plant influents from low color pulp mill effluents. This work has resulted in a well-defined and documented method for the measurement of true color which includes quality control and quality assurance criteria. Appendix B contains the updated color method, NCASI Method Color 71.01, that was prepared as a result of this work.

The selection of a wavelength to measure sample absorbance influences the accuracy and precision of color measurements; therefore, the wavelength of 465 ηm used in NCASI Method 253 was verified in current sample matrices. Optimum overlap of absorbance curves for a 200 platinum cobalt standard, wastewater treatment system influent, and biologically treated effluent occurred in the range of 460 to 470 ηm, indicating that the use of a wavelength of 465 ηm was valid in modern mill effluents.

The color of pulp mill effluents is highly pH dependent, and the pH adjustment procedure included in Method 253 sometimes resulted in less than optimum stability of pH during the analytical procedure. As sample pH increases, the measured absorbance value increases, resulting in higher color values. One objective of updating Method 253 was to examine techniques to stabilize sample pH. Several buffering systems were examined, including a phosphate buffer solution, a dry phosphate buffer capsule, and a biological buffer solution. The buffer capsule (dibasic sodium phosphate and monobasic potassium phosphate, pH 7.0) stabilized the sample pH most effectively. The buffer capsule stabilized the sample pH at 7.6 ±0.05 for the duration of a 24-hour experiment. In addition, it is a convenient technique for pH stabilization that does not contribute to sample dilution.

Experiments to investigate methods for the removal of sample turbidity while minimizing effects on true color were also conducted. Pre-filtration of samples with a 1 µm glass fiber filter was determined to help reduce problems associated with clogging of the 0.8 µm membrane filter. Experiments to assess turbidity removal using centrifugation vs. filtration indicate that filtration using a 0.8 µm membrane filter removed a larger percentage of the turbidity in wastewater samples. These experiments also indicate that higher color values were determined in centrifuged samples.

Successive filtration experiments using a range of filter porosities between 1.0 and 0.22 µm indicate that the majority of sample turbidity, 94 to 99.4%, was removed using the 0.8 µm membrane filter. In addition, experiments to investigate the removal of turbidity agents including precipitated calcium

carbonate, titanium dioxide, lime mud, fiber, and green liquor dregs indicate that 92 to 98% of the turbidity was removed using the 0.8 µm membrane filter for each agent except titanium dioxide (23 to 25% removal). Therefore, the 0.8 µm membrane filter was verified as an effective method for the removal of turbidity in modern pulp mill wastewater samples. Several types of 0.8 µm membrane filters are available commercially. A comparison of color values determined using three different types of 0.8 µm membrane filters did not reveal a statistically significant impact of filter type on measured color value.

The volume of sample selected for filtration was investigated. It was determined that when using the techniques recommended in the updated method, sample volume did not effect the overall color value determined. Therefore, a 50 mL sample volume was chosen for ease of manipulation and filtering.

Samples from five different mills were used to investigate the effect of the light path length on the resultant color value. It was demonstrated that color values varied by a relative standard deviation of less than 6% when measured using light path lengths of 10, 20, 25, 50, and 100 mm.

Method ruggedness, precision, and accuracy of the updated method (Color 71.01) were determined at the single laboratory level. Instrument precision, expressed as the percent relative standard deviation of seven replicates of a 100 PCU color standard, was determined to be 1.4%. Precision was calculated in both biologically treated effluents and treatment plant influents. At the single laboratory level, the average relative percent difference in duplicate analyses for biologically treated effluents was 2.8% (n = 12 duplicate/sample sets), and for treatment plant influents it was 7.1% (n = 12 duplicate/sample sets). Calibration curves prepared using light path lengths of 10 to 100 mm yielded r-squared values of greater than 0.9968 for twelve different calibration curves. The average recovery for 43 daily calibration checks was 94.9%, with a standard deviation of 6.7.

Eight laboratories participated in a multi-laboratory study to assess the ruggedness, accuracy and precision of the updated color measurement procedure. The samples included biologically treated effluents from Mills A and B, influents to the treatment systems from Mills A and B, portions of a prepared lignin solution, a receiving water sample collected from the discharge stream of Mill D, and a platinum cobalt color standard. The platinum cobalt color standard data were utilized to assess method accuracy, and yielded an average recovery of 94% and a relative standard deviation of 11.3%. The precision for the inter-laboratory assessment resulted in a relative standard deviation of 15% for biologically treated effluents, 24.1% for treatment plant influents, and 18.1% for the receiving water sample. The intra-laboratory precision (agreement of replicate values within a particular laboratory) was also assessed during this multi-laboratory study and resulted in a relative standard deviation of 3.6% for biologically treated effluents, 8.1% for treatment plant influents, and 9.3% for the receiving water sample.

The precision and accuracy of NCASI Method 253 was determined to be similar for effluents from Mills A and B, a lignin solution, an influent from Mill A, and a receiving water from Mill D when compared to the NCASI Method Color 71.01 during studies conducted at two different laboratories. The only exception was found in an influent sample from Mill B which gave higher color values when NCASI Method 253 was utilized.

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APPENDIX A

STATISTICAL DATA

A1. DEFINITIONS AND DESCRIPTIONS OF STATISTICAL TERMS

The statistical procedures and tests utilized throughout this technical bulletin are defined as follows.

Relative Standard Deviation (RSD). A measure of the spread or dispersion of data expressed as a percentage.

Relative Percent Difference (RPD). A measure of the spread or dispersion of a sample and duplicate pair expressed as a percentage.

F-ratio. A ratio of the two sample set variances (i.e., the ratios of the squares of the standard deviations).

p-value. The probability that the difference in means is due to random variability of samples pulled from a normal distribution.

t-test. A test that determines whether two means from two independent, normally distributed samples differ.

Cochran's test. A statistical test for homogeneity of variance. The hypothesis is that the variances across different levels are equal; if the significance levels are greater than 0.05, do not reject the hypothesis that the variances are not different.

Grubb's test. A test that is applied to assess whether a laboratory has an extreme average value.

Dixon's test. A statistical test for assessing suspect measurements. Examines the ratio of the suspect value minus the value nearest it and the difference between the highest and lowest measurements.

Repeatability variance (s(r)^2). Sum of the squares of the laboratory ranges divided by two times the number of participating laboratories minus one.

Reproducibility variance (s(R)^2). The true variance between replicate determinations (σo^2) plus the true variance between laboratories (σL^2).

σo^2. Sum of squares of individual replicates minus one divided by the number of replicates times the sum of the squares of the replicate totals, all divided by the number of participating laboratories times the number of replicates minus one.

σL^2. Determined by finding the mean square of the between-laboratory variance which is $MS_L = r\sigma L^2 + \sigma o^2$ and using the result from above (σo^2), rearranging the equation and solving for σL^2.

MS_L. One divided by the number of replicates times the sum of the squares of the replicate totals minus one divided by the number of participating laboratories times the number of matrices times the number of replicates all times the square of the grand total of the results for all replicates divided by the number of participating laboratories minus one.

A2. REGRESSION ANALYSIS OF THE CHANGE IN TURBIDITY VS. COLOR VALUE

Effluent Mill B

Dependent variable - Color value (PCU)
Independent variable - Change in turbidity

Parameter	Estimate	Standard Error	t-statistic	p-value
Intercept	136.2	5.8	23.7	0.0018
Slope	0.03	0.17	0.20	0.8599

Analysis of Variance

Source	Sum of squares	Df	Mean square	F-ratio	p-value
Model	1.08	1	1.08	0.04	0.8599
Residual	53.7	2	26.8		
Total (corr.)	54.75	3			

Influent Mill C

Dependent variable - Color value (PCU)
Independent variable - Change in turbidity

Parameter	Estimate	Standard Error	t-statistic	p-value
Intercept	164.4	30.6	5.4	0.0329
Slope	0.44	0.62	0.69	0.5566

Analysis of Variance

Source	Sum of squares	Df	Mean square	F-ratio	p-value
Model	57.6	1	57.6	0.49	0.5566
Residual	235.4	2	117.7		
Total (corr.)	293.0	3			

A3. SUMMARY OF F-TEST AND T-TEST STATISTICS

Method 253 Compared to Method Color 71.01

Lignin Solution	Method 253	Color 71.01
Mean	51.3	52.7
Variance	2.3	22.9
Standard Deviation	1.5	4.8
Observations	4	4
df	3	3
F	0.098	
p-value of F	0.883	
t	-0.598	
p-value of t	0.571	

Effluent Mill A	Method 253	Color 71.01
Mean	575	573
Variance	1144	4992
Standard Deviation	33.8	70.6
Observations	4	4
df	3	3
F	0.229	
p-value of F	0.257	
t	0.051	
p-value of t	0.961	

Effluent Mill B	Method 253	Color 71.01
Mean	185	179
Variance	139	459
Standard Deviation	11.8	21.4
Observations	4	4
df	3	3
F	0.304	
p-value of F	0.354	
t	0.469	
p-value of t	0.655	

Influent Mill A	Method 253	Color 71.01
Mean	672	745
Variance	509	3201
Standard Deviation	22.6	56.6
Observations	4	4
df	3	3
F	0.159	
p-value of F	0.165	
t	2.40	
p-value of t	0.052	

Influent Mill B	Method 253	Color 71.01
Mean	213	116
Variance	1601	1053
Standard Deviation	40.0	32.4
Observations	4	4
df	3	3
F	1.52	
p-value of F	0.738	
t	3.77	
p-value of t	0.009	

Receiving Water Mill D	Method 253	Color 71.01
Mean	151	125
Variance	162	111
Standard Deviation	12.7	10.5
Observations	4	4
df	3	3
F	1.46	
p-value of F	0.761	
t	3.15	
p-value of t	0.019	

APPENDIX B

NCASI METHOD COLOR 71.01

COLOR MEASUREMENT IN PULP MILL WASTEWATERS BY SPECTROPHOTOMETRY

Acknowledgments

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NCASI's Mission

To serve the forest products industry as a center of excellence for providing technical information and scientific research needed to achieve the industry's environmental goals.

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This method is included in the NCASI Methods Manual as an update to NCASI Method 253. The purpose of including this updated method in the NCASI Methods Manual is to make it known that a method revision is under development and to solicit comments regarding the technical merit and applicability of the revised method. Those electing to apply the method are strongly encouraged to conduct rigorous QA/QC or validation so that the quality of the data generated can be evaluated.

NCASI METHOD COLOR 71.01

COLOR MEASUREMENT IN PULP MILL WASTEWATERS BY SPECTROPHOTOMETRY

1.0 Scope and Application

1.1 This procedure utilizes a spectrophotometer to measure the absorbance of light as it passes through a sample. The color is determined by comparison of the absorbance of the sample to the absorbance of colored solutions of known concentrations. The colored solutions used in this procedure are platinum cobalt stocks. The unit of color is the color produced by 1 mg platinum/liter in the form of the chloroplatinate ion, or PCU.

The term "color" represents the true color of an aqueous sample from which turbidity has been removed (1). Turbidity can cause the color value determined for a sample to be elevated due to an increase in light scattering. In this procedure, filtration is used to remove turbidity from the sample. The optimal method for removing turbidity without affecting the color value determined has not been found, but filtration using a 0.8-micron (µm) membrane filter has been demonstrated to be effective for most pulp mill effluent samples.

The color value determined for a specific sample is pH dependent and increases as the pH of an aqueous sample increases. Therefore, a buffer is used to stabilize pH during the filtration and measurement process.

1.2 This method has been validated at the single and inter-laboratory level in wastewater treatment plant influents, biologically treated effluents from kraft pulp and paper mills, and receiving waters. Demonstration of method performance for specific matrix types is recommended.

1.3 The estimated method detection limit (MDL) achievable is instrument and light path length dependent and was found to be 4 PCU for a river water sample using a 10-mm light path length (2). The MDL determined in a kraft mill treated effluent was found to be 7 PCU using a 10-mm light path length. These values are provided as guidance. Due to improvements in instrumentation and changes in matrix effects, each laboratory should establish its own MDL. The lower instrument calibration limit (LCL) for this method is approximately 10 PCU. Lower calibration levels can be obtained by using an increased light path length. The concentration range used during the single and inter-laboratory method validation was from 10 to 500 PCU. Sample values above 500 PCU may be determined by quantitative dilution.

2.0 Summary of Method

2.1 Biologically treated effluents and wastewater influents

Place a 50-mL aliquot of unpreserved influent (effluent) into a 100-mL beaker. Measure the initial pH of the sample and add approximately one-half of the solid from a pH 7 buffer capsule. Dissolve the buffer capsule completely using a mechanical stir plate and stir bar. Add an appropriate amount of sodium hydroxide solution (NaOH) or hydrochloric acid (HCl) to adjust the pH to 7.6 ±0.05. Filter the sample through a 0.8-µm membrane filter. If the analyst cannot filter the sample through the membrane because of significant clogging, a pre-filtration step may be added prior to pH measurement and adjustment. To verify pH stability, periodically measure the post-filtration pH.

2.2 Quantitative analysis

Assess the color load by measuring the absorbance of the solution in a spectrophotometer set at a wavelength of 465 nanometers (ηm). Calculate the color units using the linear regression equation developed in Section 12.1.

2.3 Quality assurance

Quality is assured through reproducible calibration and testing of the sample preparation and spectrophotometer system. A method blank is analyzed with each sample set (samples started through the process on a given day, to a maximum of 20, along with sample duplicates to ensure quality data). A complete description of quality control procedures, calculations, and method performance criteria are listed in Sections 9.0 and 10.

3.0 Definitions

3.1 These definitions are specific to this method, but conform to common usage as much as possible.

3.1.1 PCU—platinum cobalt color unit

3.1.2 May—this action, activity, or procedural step is neither required nor prohibited

3.1.3 May not—this action, activity, or procedural step is prohibited

3.1.4 Must—this action, activity, or procedural step is required

3.1.5 Should—this action, activity, or procedural step is suggested, but not required

4.0 Interferences

4.1 Reagents, glassware, and other sample processing hardware may contribute analytical interferences resulting in inaccurate absorbance readings. Run method blanks initially and with each subsequent sample set to demonstrate that the

reagents, glassware, and other sample processing hardware are free from interferences under the conditions of the method.

4.2 Turbidity causes the measured color value to be greater than the true color value. Therefore, it is necessary to remove turbidity in the sample prior to measuring the sample's absorbance in a spectrophotometer. Interferences will vary considerably from source to source, depending on the diversity of the site being sampled. This procedure recommends the use of a 0.8-µm membrane filter to remove turbidity prior to measuring the absorbance in the spectrophotometer. As needed, the analyst can pre-filter the sample with a 1.0 µm glass fiber filter or use more than one 0.8-µm membrane filter if clogging prevents the sample from readily passing through the membrane filter.

4.3 The color value determined for a given sample is highly pH dependent. As pH increases the color value determined for the sample also increases. Therefore, stabilization of the pH at a given value for all measurements is required. This procedure is conducted with the sample at a pH of 7.6 ±0.05 to maintain consistency with standard methods currently in use.

4.4 All glassware must be clean and free of scratches. In addition, the cells used for spectrophotometric measurement of the samples must be free of all oil or residue that may cause interference in the absorbance measurement. It is recommended that the same cell be utilized for the measurement of the calibration curve, daily calibration checks, blanks, and samples.

5.0 Safety

5.1 Each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to a level protective of human health. This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of data handling sheets should also be made available to all personnel involved in these analyses.

5.2 The platinum cobalt solution is corrosive and a suspected carcinogen. Hydrochloric acid (HCl) and sodium hydroxide (NaOH) are also corrosive and should be handled with caution. Take appropriate measures to avoid contact with the chemicals by wearing chemical-resistant gloves, eye protection, and other protective clothing.

5.3 As with all samples, precautions should be taken to avoid exposure to potentially toxic, caustic, or nuisance odor compounds. Samples should be handled with gloves and opened in a fume hood.

6.0 Equipment and Supplies

Note: *Brand names, suppliers, and part numbers are cited for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.*

6.1 A spectrophotometer with a tungsten lamp source and a 10-ηm spectral slit width is required. The instrument must be capable of emitting light at a selectable wavelength of 465 ηm with an operating range of 400 to 700 ηm. Verify that the spectrophotometer is calibrated correctly by following the directions from the manufacturer for your specific instrument.

6.2 Do not use glassware with any star fractures, cracks, or severe scratches. All glassware should be washed with detergent, rinsed with tap water, then rinsed with reagent grade water prior to use.

6.3 Equipment for sample preparation

6.3.1 Filtration apparatus consisting of a flask, a vacuum source, and a filtration holder that will accommodate a 47-mm filter

6.3.2 Pasteur pipettes

6.3.3 50-mL graduated cylinder

6.3.4 Teflon™-coated stir bar

6.3.5 100-mL beaker

6.3.6 47-mm, 1.0-µm glass fiber filters for pre-filtration

6.3.7 47-mm, 0.8-µm membrane filters

6.4 Other apparatus

6.4.1 pH meter calibrated using a two-point calibration procedure at pH 7 and pH 8 using the appropriate buffer solutions

6.4.2 Magnetic stirrer

6.4.3 Sample cuvettes (absorption cell) for the measurement of absorbance, cell path length is determined by the required MDL, refer to Table 1 for the path length specific MDLs (3)

7.0 Reagents and Standards

7.1 Reagents

7.1.1 Metrepak pHydrion buffer capsules, or an equivalent supplier (dibasic sodium phosphate and monobasic potassium phosphate, pH 7.0), to stabilize sample pH

7.1.2 Organic-free reagent water in which color is not detected by this method

7.2 Standards

7.2.1 Fisher or an equivalent supplier, platinum cobalt color standard, 500 PCU stock

7.2.2 If a reliable source of platinum cobalt color standard is not available, prepare a solution of potassium chloroplatinate. Dissolve 1.246 g of potassium hexachloroplatinate, K_2PtCl_6 (Aldrich or an equivalent supplier), and 1.00 g of crystallized cobalt (II) chloride hexahydrate, $CoCl_2 \bullet 6\ H_2O$ (Aldrich or an equivalent supplier), in a portion of organic free reagent water containing 100 mL of concentrated HCl. Dilute this solution with distilled water to the desired color value. For example, dilution to 1000 mL will provide a standard with a color value of 500 PCU. Dilution to 500 mL will provide a standard with a color value of 1000 PCU.

7.2.3 Prepare a five-point calibration curve encompassing the sample concentration range. Prepare a calibration curve by diluting 5, 10, 20, and 50 mL of 500 color unit stock solution of platinum cobalt in 100 mL of deionized water. Include the absorbance measurement of the 500 color unit stock solution of platinum cobalt in the curve. The prepared calibration standards will have color units of 25, 50, 100, 250, and 500. It is necessary to prepare a calibration curve that brackets the expected values of color in the samples.

7.2.4 Stock solutions of the color standards do not need to be refrigerated, but care should be taken to protect the standards from evaporation, light, and contamination when not in use. Remember that the color standard solutions have pHs less than 2.0 and should be handled with care. Stock solutions of all standards should be checked for signs of concentration or formation of precipitates prior to the preparation of calibration or performance test standards. Replace the stock solutions if a change in concentration is indicated by the inability to meet the criteria specified in Sections 9.2 and 10.3.

7.3 Reagents for sample preservation and pH adjustment

7.3.1 Sodium hydroxide, ACS reagent grade, is used to adjust sample pH during processing. Prepare a 20% solution by adding 20 grams of sodium hydroxide pellets very slowly to 100 mL of reagent grade water using a

stir bar and stir plate. Because this reaction is exothermic, take care that the heat generated by the addition of the NaOH to the water does not break the glassware.

7.3.2 Hydrochloric Acid, ACS reagent grade, is used to adjust sample pH during processing. Prepare a 10% solution by adding 10 mL of concentrated HCl to approximately 85 mL of reagent grade water using a stir bar and stir plate. Bring the volume to 100 mL once the heat has dissipated.

8.0 Sample Collection, Preservation, and Storage

8.1 Sample collection

Collect grab or composite samples using clean sampling containers that are free from contaminants which may interfere with the analyses. Composite samples should be refrigerated during the sampling period. The color determination should be made as soon as possible following sample collection. An assessment of sample stability should be done on a matrix specific basis since biological changes which can occur during storage may affect the color and alter the pH of the sample.

8.2 Sample preservation

Samples are not preserved prior to analysis, as a change in pH can greatly affect the resulting color determination. Samples should be refrigerated prior to analyses (4°C). Sample refrigeration should occur as soon as possible after sample collection.

9.0 Quality Control

9.1 Each laboratory that uses this method should operate a formal Quality Assurance Program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and ongoing analyses of standards and blanks as a test of continued performance. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

9.2 Spectrophotometer performance and calibration verification

9.2.1 Fill the sample cuvette with reagent grade water and place it in the spectrophotometer. Adjust the absorbance reading to zero. Verify zero after every four to six samples using the same process.

9.2.2 Determine that the spectrophotometer system is operating within acceptable parameters by conducting a calibration check before each set of analyses (samples started through the measurement process on a given day). The calibration check involves reanalyzing one of the standard calibration solutions used to prepare the calibration curve (Sections 7.2.3 and 10.3). The percent recovery determined for the calibration check should be within the calculated warning limits (Section 17, Table 2). The

color determination may be sensitive to spectrophotometer and cell conditions such as dirty glassware. If the calibration check fails to meet the acceptance criterion, locate a new sample cuvette or perform appropriate maintenance and reanalyze the calibration check sample. If this fails to correct the calibration verification difficulties, the calibration curve should be re-prepared and analyzed.

9.2.3 Verify that the spectrophotometer is calibrated properly by periodically measuring an independent color standard check sample. If all calibration checks and adjustments fail to correct the problem, calibrate the instrument electronically. Electronically calibrate the instrument by first selecting the transmittance option and setting the wavelength to 450 ηm. With the 100% T/Zero control, set the display to read 100.0, insert an occluder in the sample well, and close the cover. Adjust the %T adjustment knob to read exactly 0.0 and remove the occluder.

9.3 Frequency

One sample per analytical batch of no more than twenty samples of similar matrix type should be allocated for quality control (i.e., duplicate analyses). A representative sample from each new or untested source or sample matrix should be treated as a quality control sample.

9.4 Blanks

9.4.1 Demonstrate that the analytical system is free of color by preparing and analyzing a blank with each sample set (20 samples or less). Prepare a method blank using the same procedure outlined in Section 11.0 utilizing reagent grade water for the sample.

9.4.2 If color is found in the blank at a value greater than 10% of the method detection limit or the lowest calibration limit, analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination at this level.

9.5 Sample and duplicate precision

Analyze a sample and duplicate for each matrix type with each set of samples to assess the precision of the analyses. Calculate the relative percent difference (RPD) in color for each sample and duplicate pair using Equation 1. The calculated RPD should be less than 14%.

Equation 1

$$Relative\ Percent\ Difference = \frac{(Highest\ Color\ Value - Lowest\ Color\ Value)\ x100}{Average\ Color\ Value\ of\ the\ sample\ and\ duplicate}$$

A summary of the precision determined in the single laboratory validation is provided in Section 17, Table 3 for treatment system influent and biologically

treated effluent samples. The average relative standard deviation for the single laboratory precision was 2.8% in biologically treated effluents and 7.1% in treatment plant influents. A summary of the accuracy and precision determined during an inter-laboratory validation is provided in Section 17, Tables 4 and 5 for treatment system influent, biologically treated effluent, and a receiving water sample.

9.6 Field replicates and field spikes

Depending on specific program requirements, field replicates may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

10.0 Calibration and Standardization

10.1 Zero the spectrophotometer (Section 9.2.1) and establish the operating conditions outlined below. Use the same operating conditions to analyze all samples, blanks, calibration curves, and calibration verification samples.

10.2 Quantitation

10.2.1 Analyze the calibration standards (Section 7.2.3) at a wavelength of 465 ηm using the procedure described in Section 11.2.3. Construct a calibration curve by plotting the absorbances and the color units of the calibration curve points. An example of a calibration curve plot is located in Section 17, Figure 1.

10.2.2 If the r-squared value determined for the curve is 0.991 or greater, the calibration curve is assumed to be linear and acceptable. The linear equation determined from the curve can then be used to calculate sample color (Section 12.1). If the curve is not linear, evaluate the problem, undertake the appropriate remedial action, and reanalyze the calibration curve solutions. If remedial actions and reanalysis fail to produce an r-squared value of at least 0.991, prepare new calibration curve solutions and analyze them. The statistics for calibration curve linearity determined during a single laboratory validation of this method are included in Section 17, Table 6.

10.3 Verify calibration prior to the analysis of each set of samples (Sections 9.2). Analyze one of the calibration standards (Section 7.2.3) prior to the analysis of each set of samples. It is recommended that the selected calibration standard vary over time in order to verify the calibration of the instrument over the full calibration range of the method. Recalibrate if the percent recovery for the color standard of the analyzed calibration solution is outside of the warning criteria (Table 2).

10.4 Process a blank with the curve to confirm that the glassware, sample cuvette, reagents, and other components are free from contamination. Prepare the blank with deionized water using the procedure for the preparation of the samples (Section 11).

10.5 Demonstrate that color is detectable at the minimum level using the lowest level calibration curve solution and the same path length of the sample cuvette that will be used to analyze all curve points, calibration verifications, and samples.

11.0 Procedure

This section includes the procedures used to adjust pH, and filter the treatment plant influent and biologically treated effluent samples. The pH adjustment and filtering procedures are used for all types of samples and method blanks.

11.1 pH adjustment of the sample

11.1.1 Remove the sample from the refrigerator and allow the sample to come to room temperature. Calibrate the pH meter using a two-point calibration with pH 7 and pH 8 buffer solutions. Shake the sample to ensure homogeneity and immediately measure 50 mL of the sample using a 50-mL graduated cylinder. For method blanks, measure 50 mL of reagent grade water.

11.1.2 Measure and record the sample pH (initial pH). Gently open a pH 7 Metripak pHydrion (or equivalent) buffer capsule and add approximately one-half of the contents (powder only) to the sample. Stir until all of the buffer has dissolved. Reserve the remaining half of the buffer for the next sample. Adjust the sample pH to 7.6 ±0.05 by adding a small volume of sodium hydroxide solution (preferably 20%) dropwise. If the pH is adjusted slightly too high, hydrochloric acid (preferably 10%) may be added dropwise to readjust the sample pH. The sample aliquot must be discarded and re-prepared if the sample volume changes by more than 1% before the pH is within the desired range. Differing strength acid and/or base solutions may be used to meet this criteria. Record the adjusted pH.

11.1.3 Assemble an aspiration-type filtering apparatus and pre-wet a 0.8-µm membrane filter with approximately 1 mL of deionized and/or distilled water on each side of the membrane. Gently shake off the excess water, place the filter onto the filter support, secure the filter holder/funnel in place, turn the aspirator on, and slowly add the sample. A rapid decline in the rate of flow through the membrane or foam coming off the membrane filter, can indicate filter plugging. If the filter plugs, immediately replace the filter with a new filter pre-wet with deionized and/or distilled water, and continue filtering the remaining sample.

11.1.4 If filtration through the 0.8-µm membrane filter is excessively difficult, the 50 mL of sample can be pre-filtered through a 1.0-µm glass fiber filter prior to sample manipulation. Transfer the sample filtrate to a 100-mL beaker equipped with a Teflon stir bar. Place the beaker on a mechanical stir plate and gently stir the sample. Buffer, adjust pH, and filter through a 0.8-µm membrane filter as described in Sections 11.1.2 and 11.1.3.

11.2 Spectrophotometer analysis

11.2.1 The spectrophotometer conditions should be set according to the criteria described in Section 9.2.3.

11.2.2 Perform the calibration verification as outlined in Section 9.2.2.

11.2.3 Verify that the spectrophotometer is zeroed (Section 9.2.1). Rinse the cell with a small amount of the filtered sample. Discard the rinse. Transfer enough of the filtered samples to the sample cuvette, filling to the reference line. Measure and record the absorbance at 465 ηm and discard the sample. Rinse the cell thoroughly with deionized and/or distilled water. Periodically verify that the spectrophotometer is zeroed.

12.0 Data Analysis and Calculations

12.1 Quantitation

12.1.1 The linear regression equation from the calibration curve (Section 10.2) is used to calculate the corresponding color value of the samples. Calculate the color value in the sample using Equation 2.

Equation 2

$y=mx+b$

where:
y is the absorbance
m is the slope
b is the y-intercept
x is the calculated color value

Calculate the color units for each sample by utilizing the measured absorbance value and the linear equation derived from the calibration curve (Section 10.2).

12.1.2 The calibration curve slope and y-intercept will vary depending on the light path length of the sample cuvette used in each different spectrophotometer. Therefore it is important to use the same light path length for all measurements. The following is an example calculation from a calibration curve prepared in one laboratory.
Example Calculation:

Absorbance measured for the sample (y) 0.039

Equation $y = 0.0003x + 0.0016$

$$x = \frac{y - 0.0016}{0.0003}$$

Substitution	$x = \frac{0.039 - 0.0016}{0.0003}$
Calculated Color Units (x)	x = 125 PCU

12.2 Data review requirements

12.2.1 Review the data to assess the accuracy and precision of the determined color value, spectrophotometer problems, interferences, and bias using the guidance provided in Section 9.0 and 10.0. Correct any problems prior to reporting the analytical results.

12.2.2 Assess the need for sample dilutions. The procedure for conducting sample dilutions and reanalysis is described in Section 12.3.

12.2.3 Resolve inconsistencies between duplicates as necessary.

12.2.4 If review of the data shows any problems which could affect subsequent analyses, discontinue the analyses until the problems are resolved.

12.3 Results outside the calibration range

If the calculated color value exceeds the highest color calibration point, dilute an aliquot of the sample with reagent grade water prior to sample processing to bring the concentration within the calibration range of the method and continue the sample preparation process from Section 11.

13.0 Method Performance

Single laboratory performance for this method is detailed in Section 17, Tables 2, 3, and 6. Acceptance criteria were established from an inter-laboratory study using the draft method. The data from this study are given in Tables 4 and 5.

14.0 Pollution Prevention

Pollution prevention approaches have not been evaluated for this method.

15.0 Waste Management

15.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

15.2 Instructions for sample and waste handling and disposal

15.2.1 Dispose of all samples as required by federal, state and local regulations.

15.2.2 Neutralize the sodium hydroxide solution and pour it down the drain with copious amounts of water.

15.2.3 Neutralize the calibration standard solutions to pH 7 and pour the aqueous portion of the extracted sample aliquot down the drain with copious amounts of water.

15.3 For further information on waste management, the Environmental Protection Agency suggests you consult *The Waste Management Manual for Laboratory Personnel*, and *Less is Better: Laboratory Chemical Management for Waste Reduction*. Both are available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC, 20036.

16.0 References

1. *Standard Methods for the Examination of Water and Wastewater*, 20th Edition, American Public Health Association, Washington, DC, 1998, 2-1 to 2-8.

2. *Federal Register*, "Appendix B to Part 136-Definition and procedure for the determination of the method detection limit-revision 1.11." Vol. 49, No. 209. October 26, 1984.

3. National Council for Air and Stream Improvement, Inc. (NCASI). 1971. *An investigation of improved procedures for measurement of mill effluent and receiving water color.* Technical Bulletin No. 253. Research Triangle Park, NC: National Council for Air and Stream Improvement, Inc.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

Table 1. Minimum Detectable Color for Various Light Path Length Sample Cuvettes

Sample Cuvette Light Path Length	Minimum Detectable Color Units
30	1
20	5
10	7[a]
5	10
1	25

[a] Determined using a treated effluent from a kraft mill.

Table 2. Daily Calibration Verification Criteria

Average Recovery[a] (%)	Standard Deviation[b]	Warning Limits[c] (%)	Action Limits[d] (%)	Color Unit Range (PCU)
94.9	6.7	81 - 108	75 - 115	10 - 500

[a] Average recovery for 47 daily calibration checks.
[b] Standard deviation of the recoveries for 47 daily calibration checks.
[c] Average recovery plus or minus two times the standard deviation.
[d] Average recovery plus or minus three times the standard deviation.

Table 3. Single Laboratory Precision: NCASI Color-71.01

Sample Type[a]	Range RPD[b]	Average RPD[c]
Effluent[d]	0.4 - 14.1%	2.8%
Influent[e]	0.1 - 13.2%	7.1%

[a] Precision of the target analytes native to treatment system influents and biologically treated effluents.
[b] Range of relative percent differences observed between a sample and a duplicate.
[c] Pooled average relative percent difference for all sample and duplicate pairs analyzed.
[d] Range and average RDP for 12 duplicate pairs.
[e] Range and average RDP for 12 duplicate pairs.

Table 4. Inter-Laboratory Accuracy[a]

Average Percent Recovery	Standard Deviation	Relative Standard Deviation (%)
94	10.6	11.3

[a] Summary of the percent recoveries for a 125 PCU color standard analyzed by eight different laboratories using eight different spectrophotometers.

Table 5. Results of an Inter-Laboratory Study Youden Pair Analyses to Assess Intra- and Inter-Laboratory Precision

Parameter	Lignin Solution	Effluent Mill A	Effluent Mill B	Influent Mill A	Influent Mill B	Receiving Water Mill D
Number of Labs	7	7	6	6	7	6
Mean of Lab Averages	58.0	562	169	665	128	141
s(r) repeatability standard deviation	5.8	17.2	7.1	15.4	17.6	13.2
s(R) reproducibility standard deviation	13.9	95.2	22.1	87.7	44.8	25.6
RSD (r) repeatability relative standard deviation[a]	10.0	3.1	4.2	2.3	13.8	9.3
RSD (R) reproducibility relative standard deviation[b]	24.0	16.9	13.1	13.2	35.0	18.1

[a] Intra-laboratory (repeatability) precision of the method.

[b] Inter-laboratory (reproducibility) precision of the method.

Table 6. Calibration Curve Linearity

Linear Equation y = mx +b	R-squared	Light Path Length (mm)
y = 0.0153x – 0.0108	0.9981	10
y = 0.0003x + 0.0052	0.9968	10
y = 0.0003x + 0.0016	0.9999	10
y = 0.0003x + 0.0010	0.9997	10
y = 0.0005x + 0.0014	0.9999	20
y = 0.0003x + 0.0004	1.0000	10
y = 0.0005x + 0.0004	0.9999	20
y = 0.0003x + 0.0003	0.9999	10
y = 0.0003x – 0.0030	1.0000	10
y = 0.0005x + 0.0028	0.9988	20
y = 0.0006x – 0.0004	0.9999	25
y = 0.0014x – 0.0003	1.0000	50
y = 0.0027x + 0.0018	1.0000	100

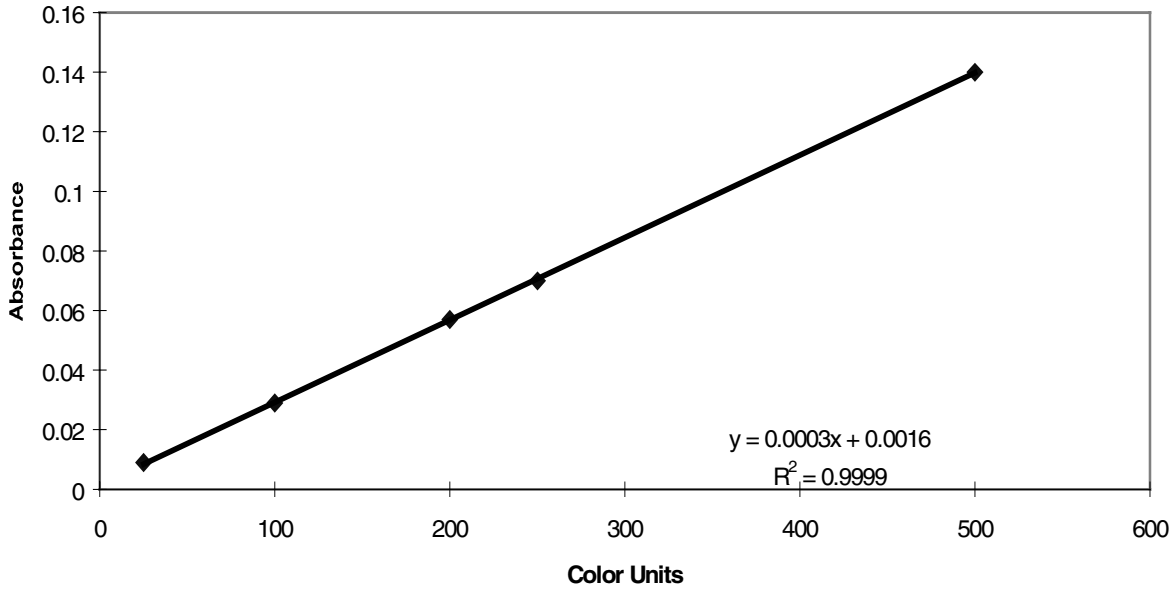

Figure 1. Typical Calibration Curve.

APPENDIX C

INTER-LABORATORY STUDY PLAN, PROCEDURE, AND DATA SHEET

1.0 SCOPE

This study is intended to evaluate the precision and accuracy of an updated color method when applied to pulp mill effluents. The following sections describe the sample collection, analysis requirements, and data reporting requirements for the study.

2.0 SAMPLE COLLECTION, STORAGE, AND SHIPMENT

Grab samples of pulp mill treatment system influents and final effluents will be collected from two kraft mills equipped with secondary biological treatment. Sample matrices will represent mills pulping hardwood and softwood, utilizing oxygen delignification and high chlorine dioxide substitution. In addition to the above samples, a receiving water sample will also be assessed. The samples will be collected and shipped via overnight FedEx on ice to the NCASI West Coast Regional Center where they will be homogenized and split into sample containers for shipment to the participating laboratories. In addition to the effluent and influent samples, a color reference material solution and platinum cobalt standard solution (color standard solution) will also be included in the shipment. All samples will be unpreserved and should be stored at 4°C until analyzed. Sampling will be scheduled to ensure sample shipment to each of the participating labs on the same day (Target Date: February 17). Upon receipt, the laboratory must maintain the samples at 0 to 4°C and analyze for color within 24 hours using the NCASI Updated Color Measurement Procedure (see attached). A total of twelve samples and one color solution check standard will be sent to each laboratory. For those laboratories that do not routinely measure color, a set of calibration curve solutions will also be included in order for the lab to measure a standard curve prior to sample analyses.

3.0 SAMPLE ANALYSIS REQUIREMENTS

Each sample matrix will be analyzed for color using the attached NCASI Updated Color Measurement Procedure. Since the objective of this study is to compare the results obtained using the procedure, it is important that the laboratory strictly adhere to the method requirements. These requirements are clearly defined in the attached procedure.

3.1 Method Requirements

All instruments should be zeroed using reagent grade water prior to standard and sample readings. The calibration check standard (Bottle 1) should be read prior to the samples. Each sample should be measured using the attached procedure. Please provide the information specified on the attached data sheet for each sample measured.

3.2 Data Deliverables

Complete the attached NCASI Color Measurement Study Data Sheet with the information requested and return it to our laboratory in the enclosed envelope. We realize that some laboratories utilize spectrophotometers with software programs that do not provide the option of viewing the calibration curve equation. If possible, we request that you provide NCASI with the absorbance readings for the calibration points measured. If this is not an option, leave this portion of the sheet blank. We also recognize that you may only be able to provide color units for samples A through J, but request that you also include the measured absorbance when possible. It would be helpful to include any

observations that you feel may have had an effect on your results (condition of cells, instrument function, clogging of filters, etc.) in the comment section.

3.3 Data Evaluation

The data will be evaluated for the following information:

- Completeness of the data sheet, including all data deliverables requested
- Correct reporting units and significant figures
- Results for the calibration check standard
- Results for the average concentration, standard deviation, and relative standard deviation for each sample matrix analyzed by the procedure
- Inter- and intra-laboratory precision using Youden pair analyses
- Method precision using ANOVA statistics

3.4 Report of Findings

The analytical results and statistical evaluation will be summarized in a report. Codes will be used to identify results associated with each lab. The names of the participating labs will not be identified. This study is not intended to endorse or verify a laboratory, but rather to evaluate the inter- and intra-laboratory precision using the updated color procedure. Participating laboratories will be provided with a final copy of the report when it is completed.

NCASI UPDATED COLOR MEASUREMENT PROCEDURE

1.0 GENERAL DISCUSSION

This procedure utilizes a spectrophotometer to measure the absorbance of light as it passes through a sample. The color is determined by comparison of the sample to colored solutions of known concentrations. The color solutions used in this procedure are platinum cobalt stocks. The unit of color is the color produced by 1 mg platinum/liter in the form of the chloroplatinate ion, PCU.
The term "color" represents the true color of an aqueous sample from which turbidity has been removed. Turbidity can cause the color value determined for a sample to be elevated due to an increase in light scattering. In this procedure, filtration is used to remove the turbidity in the sample which may interfere with the determination of color. The optimal method for removing turbidity without affecting the color value determined has not been found, but filtration using a 0.8 µm membrane filter has been demonstrated to be effective for most pulp mill effluent samples.
The color value determined for a specific sample is pH dependent and increases as the pH of the aqueous sample increases. Therefore, a buffer is used to stabilize the pH during the measurement process.

2.0 EQUIPMENT AND SUPPLIES

2.1 Equipment

- Spectrophotometer with a tungsten lamp source and 10 ηm spectral slit width; the operating range should include 400 to 700 ηm and the instrument should be capable of emitting light at a selectable wavelength of 465 ηm
- pH meter
- Mechanical stir plate
- Teflon™ stir bar
- Filtration system that will accommodate a 47 mm filter
- Pasteur pipettes
- 50-mL graduated cylinder
- 100-mL beaker

2.2 Supplies provided by NCASI in the sample kit

- 47 mm, 1.0 µm glass fiber filters from Gelman for prefiltration
- 47 mm, 0.8 µm membrane filters from Nucleopore, Membra-Fil
- pH 7 buffer capsules to stabilize sample pH
- Color standard calibration check solution
- Set of calibration standard stock solutions (if your lab requested them)

2.3 Reagents laboratories will need to supply

- Sodium hydroxide solution (preferably 20%, but 10% may be sufficient)
- Hydrochloric acid (preferably 4%, but a more dilute solution may be sufficient)

3.0 CALIBRATION STANDARDS

The calibration curve standards were prepared by making dilutions of a 500 color unit stock solution of platinum cobalt. The prepared calibration standards have color units of 10, 25, 50, 100, 250, and

500 PCU. Refrigeration of the calibration standard solutions is not necessary, but care should be taken to protect the standards from evaporation and contamination when not in use.

4.0 CALIBRATION CURVE

4.1 All calibration standard and sample measurements must be conducted with the spectrophotometer set at a wavelength of 465 ηm.

4.2 Make sure that the cell to be used is clean and free of scratches, oils, and/or dirt. It is recommended that the cell be placed in the spectrophotometer in the same position for each reading. This can be accomplished by orienting a mark on the tube in the same position for each reading. Allow the spectrophotometer to warm up as recommended in the instrument manual. Zero the spectrophotometer using deionized and/or distilled water.

4.3 Rinse the cell with a small amount of the color calibration standard to be measured. Discard this rinse and transfer a portion of the color calibration standard to the cell. Measure the absorbance of the sample in the spectrophotometer and record the reading. Repeat this process for each of the six calibration standards.

4.4 Plot the absorbance vs. the color value of the six calibration standards and fit the curve using a linear regression model. Record the R-squared value (correlation coefficient) and the equation on the data sheet. The curve is considered linear if the correlation coefficient is greater than or equal to 0.991. If the curve does not meet this criteria, the procedure should be repeated until this criteria is met.

4.5 The pH of the calibration stock solutions is below 2; therefore these solutions should be neutralized prior to disposal.

5.0 MEASUREMENT OF COLOR STANDARD CALIBRATION CHECK

5.1 Make sure that the cell to be used is clean and free of scratches, oils, and/or dirt. Allow the spectrophotometer to warm up as recommended in the instrument manual. Zero the spectrophotometer using deionized and/or distilled water.

5.2 Rinse the cell with a small amount of the color standard calibration check (Bottle #1) to be measured. Discard this rinse and transfer a portion of the color standard calibration check to the cell. Measure the absorbance of the sample in the spectrophotometer and record the reading. Calculate the color value of the color standard calibration check using the process described in Section 7.0. Record the value on the data sheet.

6.0 MEASUREMENT OF SAMPLES

6.1 Remove the sample bottles (Samples A through J) from the refrigerator and allow the samples to come to room temperature. Calibrate the pH meter using a two-point calibration with pH 7 and pH 8 buffer solutions.

6.2 Invert the sample bottle several times to re-suspend the solids that may have settled during storage. Measure 50 mL of the sample using a 50-mL graduated cylinder.

6.3 Pre-filter the 50 mL of sample through a 1.0-µm glass fiber filter prior to sample manipulation. Transfer the sample filtrate to a 100-mL beaker equipped with a Teflon stir bar. Place the beaker on a mechanical stir plate and gently stir the sample.

6.4 Measure and record the sample pH (initial pH). Gently open a buffer capsule and add approximately one-half of a pH 7 Metripak, pHydrion buffer capsule (powder only); stir until

all of the buffer has dissolved. Reserve the remaining half of the buffer for the next sample. Adjust the sample pH to 7.6 ±0.05 by adding a small volume of sodium hydroxide solution (preferably 20%) dropwise or hydrochloric acid (preferably 4%), depending on the initial pH of the sample. The sample aliquot must be discarded and re-prepared if the sample volume changes by more than 2% before the pH is within the desired range. Differing strength acid and/or base solutions may be used to meet this criteria. Record this adjusted pH on the data sheet.

6.5 Assemble an aspiration-type filtering apparatus and pre-wet a 0.8 µm membrane filter with approximately 1 mL of deionized and/or distilled water on each side of the membrane. Gently shake off the excess water, place the filter onto the filter support, secure the filter holder/funnel in place, turn the aspirator on, and slowly add the sample. Watch for a rapid decline in the rate of flow through the membrane or foam coming off the membrane filter, which can indicate filter plugging. If the filter plugs, immediately replace the filter with a new filter pre-wet with deionized and/or distilled water and continue filtering the remaining sample. Record the number of filters used to prepare each of the samples on the data sheet.

6.6 Verify that the spectrophotometer is still zeroed. Rinse the cell with a small amount of the filtered sample. Discard the rinse. Transfer a portion of the filtered sample to the cell and measure the absorbance at 465 ηm. Transfer all of the filtered sample back into the original beaker. Measure and record the sample's pH and record this value on the data sheet as the post-filtration pH. Rinse the cell thoroughly with deionized and/or distilled water. Verify once again that the spectrophotometer is zeroed.

7.0 CALCULATIONS AND REPORTING

7.1 Calculate the color units for each sample by utilizing the measured absorbance value and the linear equation derived from the calibration curve (Section 4.0) in *your* lab. The following is an example calculation from a calibration curve prepared in our laboratory.

7.2 The calibration curve slope and y-intercept will vary depending on the light path length of the cell used in each different spectrophotometer. Therefore it is important to use the same cell for all measurements.
Example Calculation:

Absorbance 0.039

Equation $y = 0.0003x + 0.0016$

$$x = \frac{y - 0.0016}{0.0003}$$

Substitution $$x = \frac{0.039 - 0.0016}{0.0003}$$

NCASI COLOR MEASUREMENT STUDY DATA SHEET

Participating Company _____

Address _____

Phone Number _____ Contact Person _____

Apparatus:

Instrument Manufacturer _____ Model _____

Wavelength Used _____ Cell Path Length _____

Calibration Curve:

Standard Level (CU)	Absorbance
10	
25	
50	
100	
250	
500	

**If your lab uses a spectrophotometer that stores calibration curve data, please record the stored absorbances or color units if possible.

Calibration Curve Equation	R^2 Value

Calibration Check:

Absorbance and/or color units of color standard calibration check _____

Date Analyzed _____

Samples:

Sample Code	Initial pH	Adjusted pH	Post-Filtration pH	Number of membranes used	Absorbance	Color Units (PCU)	Date Analyzed
A							
B							
C							
D							
E							
F							
G							
H							
I							
J							
K							
L							

Comments:

APPENDIX D

YOUDEN PAIR STATISTICAL ANALYSIS PLOTS

Figure D1 illustrates the raw data received for the two lignin samples analyzed as part of the inter-laboratory study. The plots are made by using the results for one sample as abscissa, and the results of the other sample as ordinate. The average of all the data points for Lignin 1 and Lignin 2 are represented by the vertical and horizontal lines in the middle of the graph. The ideal situation would result in data points found along the 45-degree line of the graph with the data clustered near the intersection of the two average lines. The closeness of the individual points to the 45-degree line reflects the within-laboratory precision, and the overall spread in either the abscissa or ordinate direction reflects the overall precision or reproducibility. The data from laboratory L3 is biased high, as indicated by its location removed from the other laboratory results to the high extreme on the x and y axes. Dixon's test for outliers indicated that the results from L3 could be removed from the data set.

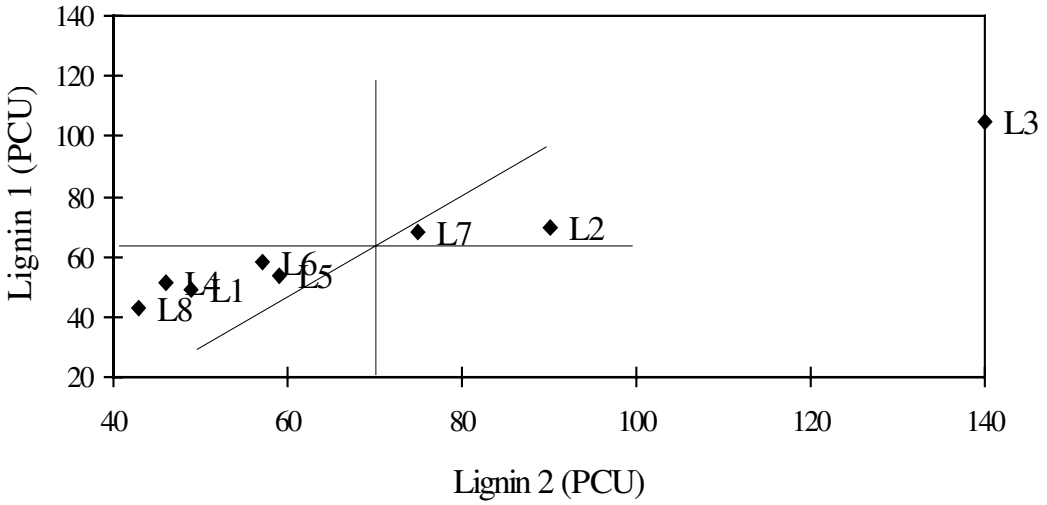

Figure D1. Youden Pair Plot for Lignin 1 and Lignin 2.

Figure D2 illustrates the raw data received for the two effluent samples (Mill A) analyzed as part of the inter-laboratory study. The data from laboratory L3 is biased high, as indicated by its location removed from the other laboratory results to the high extreme on the x and y axes. Dixon's test for outliers indicated that the results from L3 could be removed from the data set.

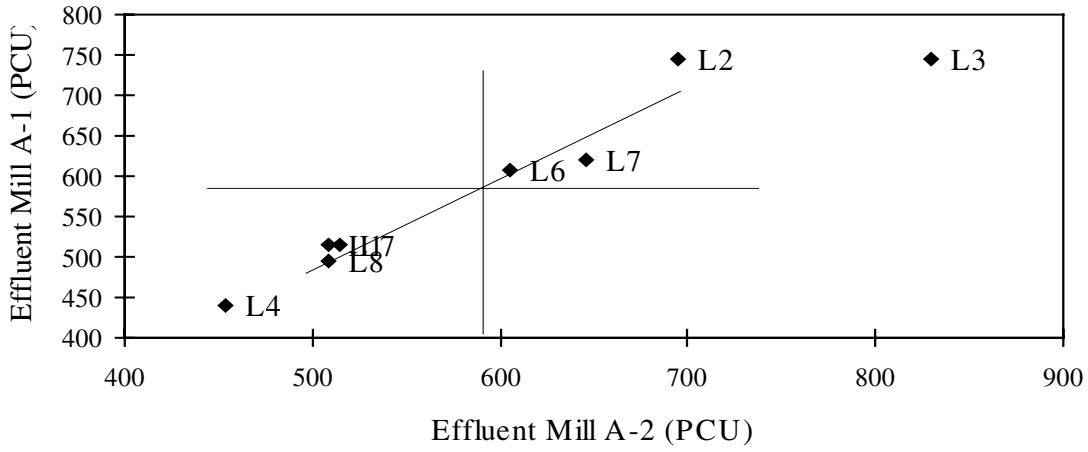

Figure D2. Youden Pair Plot for Effluent Mill A-1 and Effluent Mill A-2.

Figure D3 illustrates the raw data received for the two influent samples (Mill A) analyzed as part of the inter-laboratory study. The data from laboratories L3 and L2 are biased high, as indicated by their locations removed from the other laboratory results to the high extreme on the x and y axes. Dixon's test for outliers indicated that the results from L3 and L2 could be removed from the data set.

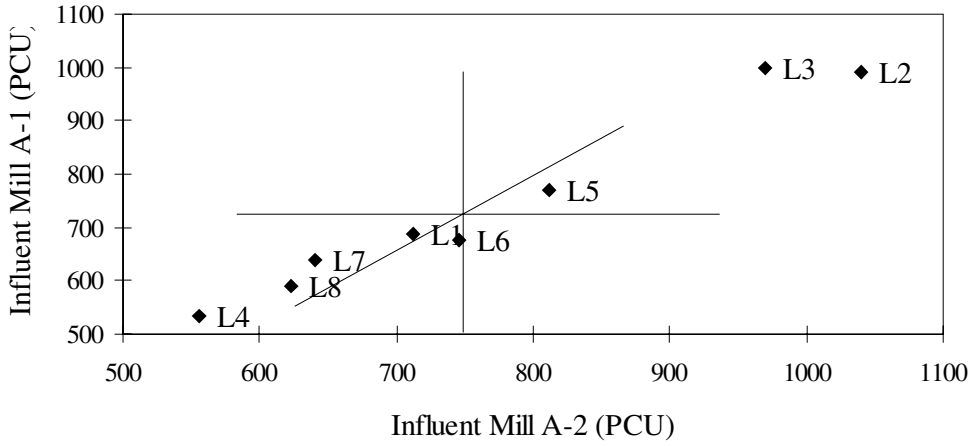

Figure D3. Youden Pair Plot for Influent Mill A-1 and Influent Mill A-2.

Figure D4 illustrates the raw data received for the two effluent samples (Mill B) analyzed as part of the inter-laboratory study. The data points plotted from laboratory L3 and L2 are biased high, as indicated by their locations removed from the other laboratory results to the high extreme on the x and y axes. Dixon's test for outliers indicated that this bias was significant and these laboratories were removed from the data set.

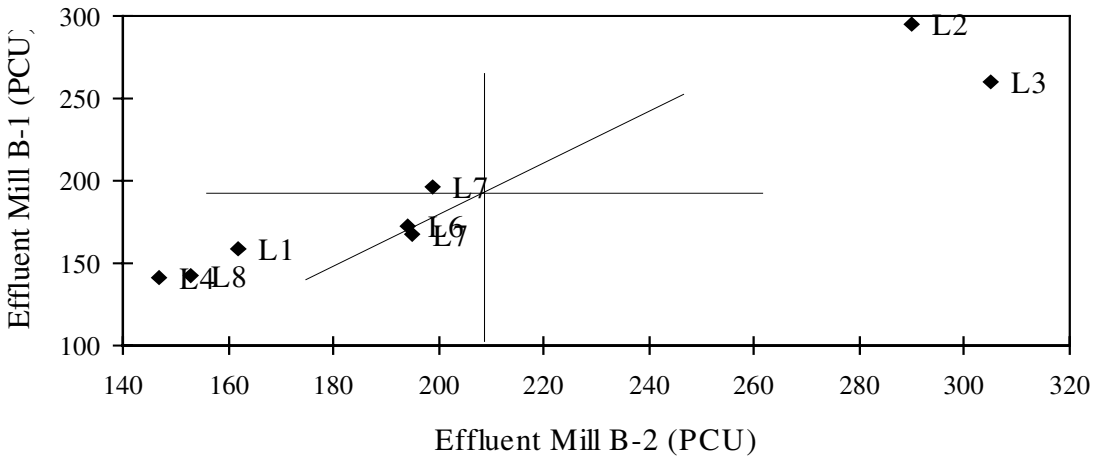

Figure D4. Youden Pair Plot for Effluent Mill B-1 and Effluent Mill B-2.

Figure D5 illustrates the raw data received for the two influent samples (Mill B) analyzed as part of the inter-laboratory study. The data from laboratory L3 is biased high, as indicated by its location removed from the other laboratory results to the high extreme on the x and y axes. Dixon's test for outliers indicated that this bias was significant and this laboratory was removed from the data set.

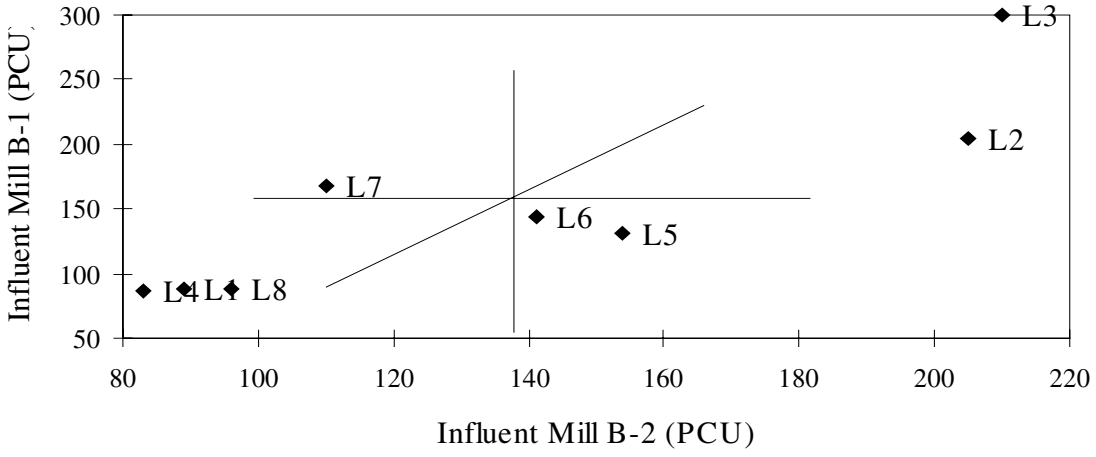

Figure D5. Youden Pair Plot for Influent Mill B-1 and Influent Mill B-2.

Figure D6 illustrates the raw data received for the two receiving water samples (Mill D) analyzed as part of the inter-laboratory study. The data points plotted from laboratory L3 and L2 are biased high, as indicated by their location removed from the other laboratory results to the high extreme on the x and y axes. Dixon's test for outliers indicated that this bias was significant and these laboratories were removed from the data set.

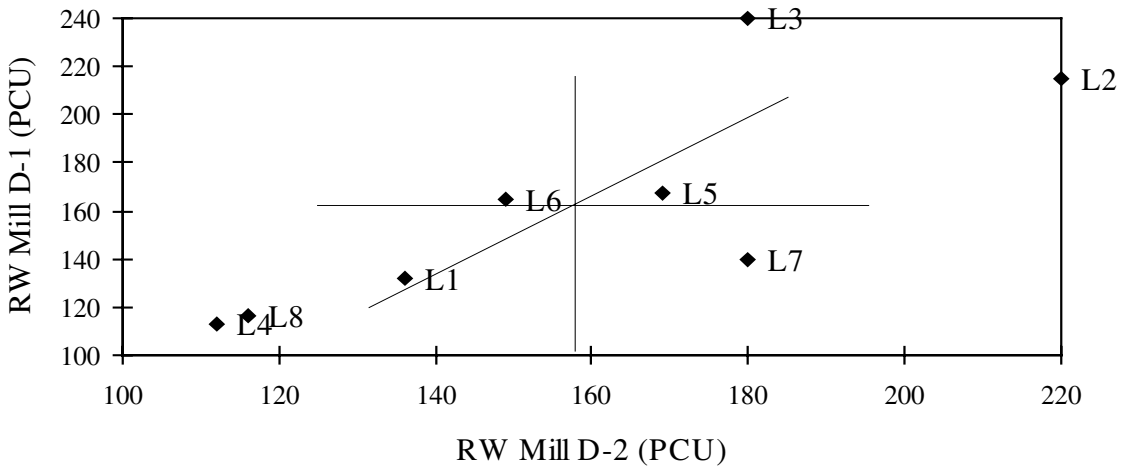

Figure D6. Youden Pair Plot for Receiving Water Mill D-1 and Receiving Water Mill D-2.