Designation: D 6592 – 01

An American National Standard

Standard Test Method for Portable Chemiluminescent Water Quality Determination[1]

This standard is issued under the fixed designation D 6592; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers the detection of water and wastewater contaminants which inhibit chemiluminescence.

1.2 This test method may be applied to ambient waters including but not exclusively river water and water from sewage treatment plants. It is the responsibility of the user of this standard to ensure the validity of the test method for waters of untested matrices.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

D 1129 Terminology Relating to Water[2]

D 1192 Specification for Equipment for Sampling Water and Steam in Closed Conduits[2]

D 1193 Specification for Reagent Water[2]

D 2777 Determination of Precision and Bias of Applicable Methods of Committee D19 on Water[2]

D 3370 Standard Practices for Committee D19 on Sampling Water[2]

D 3856 Standard Guide for Good Laboratory Practices in Laboratories Engaged in Sampling and Analysis of Water[2]

D 4210 Practice for Interlaboratory Quality Control Procedures and Discussion on Reporting Low Level Data[2]

D 5847 Standard Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis[2]

3. Terminology

3.1 *Definitions*—For definitions of other terms used in this test method, refer to D 1129 Terminology Relating To Water.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *chemiluminescence*—the generation of light by a chemical reaction.

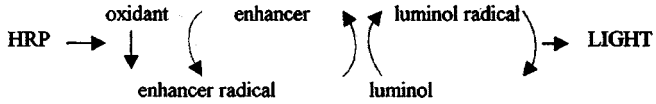

FIG. 1 Output of Luminol Radicals

3.2.2 *luminometer*—an instrument capable of measuring light emitted during a luminescent reaction, over a wide range of wavelengths.

3.2.3 *photodiode*—a semiconductor light sensor that generates a current or voltage when illuminated by light.

4. Summary of Test Method

4.1 This analytical test method is an enhanced chemiluminescent method based on the procedure of Whitehead et al.[3] Luminol is oxidized by a complex formed between an oxidant and peroxidase (HRP) to form a luminol radical. This decomposes via an endoperoxide intermediate to form the excited state 3-aminophthalate dianion which decays to its ground state with the emission of light. The presence of enhancers results in a steady output of luminol radicals during the reaction (see Fig. 1). Contaminating substances (such as sewage, silage effluent, heavy metals, detergents, cyanides and pesticides containing organic residues) react with the assay reagents by scavenging radicals from the luminol or enhancer radicals, or by inhibiting the catalytic action of HRP. This results in a reduction of light output to a degree which is proportional to the amount of contaminant present. Light output is measured using a portable luminometer, which detects light by means of a silicon photodiode.

5. Significance and Use

5.1 This test method was developed for the purpose of screening for contamination in water. It is used for the analysis of waste water, process water and environmental water. Results of testing performed in the pulp and paper industry, sewage treatment plants and river water systems are presented in Appendix X1.

5.2 This method is not suitable for determining the precise concentration of specific analytes.

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[1] This test method is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

Current edition approved March 10, 2001. Published May 2001.

[2] *Annual Book of ASTM Standards*, Vol 11.01.

[3] Whitehead T.P. Thorpe G., Lane M., Watson A., Billings C. in Bioluminescence and Chemiluminescence: status report, Szalay A.Z, Kricka L.N. and Stanley PE. Wiley 1993, pp. 425-429.

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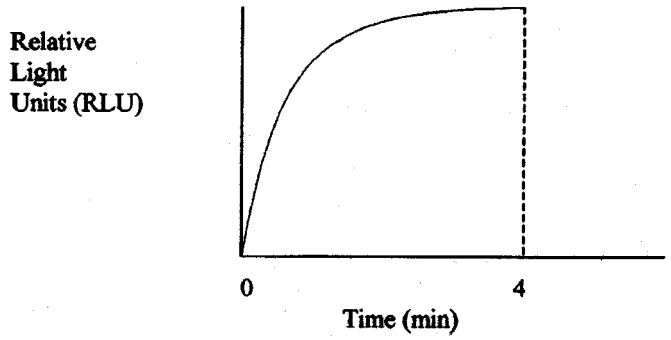

FIG. 2 Example Graph of Light Output vs Time for the Chemiluminescent Reaction

5.3 This test method may be used to develop trend data, which can be useful in assessing the performance characteristics of treatment process or changes in the raw water.

6. Interferences

6.1 *Particulate Matter*—Samples containing a high degree of particulate matter[4] can interfere with the test. Particulate or suspended matter may be removed by centrifugation or membrane filtration if components of interest are not altered.

7. Apparatus

7.1 A hand-held portable luminometer with key pad and LCD display capable of measuring light output over a 4 minute period, and integrating the result. See Fig. 2.

8. Reagents

8.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean Reagent Grade Water conforming to Specification D 1193 Type II. Other reagent water types may be used, providing it is first ascertained that the water is of sufficiently high purity without adversely affecting the precision and bias of the determination.

8.3 *Buffer*—Borate buffer, pH 8.5.[5]

8.4 *Enzyme Reagent*—Reagent containing peroxidase.[5] Enzyme reagent is prepared for use by diluting 100µl of enzyme reagent with 1 ml of borate buffer.

8.5 *Signal Reagent*—Consisting of luminol, enhancer and oxidant.[5] The signal reagent is prepared for use by reconstituting with an appropriate volume of borate buffer, section 8.3, as detailed in section 10.3.

8.6 *Reference Sample*—The Reference Sample consists of Reagent Grade Water conforming to Specification D 1193 Type II.

8.7 *Quality Control Sample*—The Quality Control Sample (QCS) is a solution of known composition with a known inhibition level. A suggested QCS is phenol, 5mg/L in reagent water (55 % inhibition).

9. Sampling

9.1 Collect all samples in accordance with Specification D 1192 and Practice D 3370, as applicable.

9.2 Samples must be collected in glass or plastic containers that are clean and free from artifacts and or interferences. The suitability of the containers should be demonstrated for each new lot by performing a container blank.

9.3 Preserve samples by cooling to 4°C for up to 24 h after sampling. If it is necessary to hold samples for longer than 24 hours, it is the responsibility of the user of the test to determine the maximum holding time for individual samples.

10. Calibration

10.1 The instrument is calibrated using Reagent Grade Water as the Reference Sample in the procedure detailed in section 10.4.

10.2 Switch on the luminometer and allow a warm-up time in accordance with manufacturer's instructions.

10.3 Reconstitute a vial of signal reagent with 5 ml of borate buffer, pH 8.5, using a pipette. Carefully remove the stopper from the reagent vial and add the buffer solution. Replace the stopper and mix the vial contents by inverting the vial several times. Prepare a 1 + 10 dilution of enzyme reagent by diluting 100 µl of enzyme reagent with 1 ml of borate buffer.

10.4 Set reference light level.

10.4.1 Adjust the luminometer to measure the reference light output level in accordance with the manufacturer's instructions.

10.4.2 If the luminometer has an appropriate menu, select "Reference", ensuring that the chamber lid is closed, and enter appropriate information as per the LCD screen prompts.

10.4.3 Add a 100 µl aliquot of reconstituted signal reagent and 1000 µl of reagent water to a high optical precision cuvette, suitable for use in a luminometer. Mix the contents of the cuvette by gentle agitation.

10.4.4 Add 20 µl of diluted enzyme reagent to the cuvette and mix the contents by gentle agitation.

10.4.5 Insert the cuvette into the open luminometer chamber and close the chamber lid. Initiate measurement of light output according to luminometer manufacturer's instructions.[6] Light output should be measured over a period of 4 minutes, and the area under the curve should be integrated to determine the result.[7]

[4] Greater than 8000 NTU.

[5] Exact composition of reagents is proprietary information and is covered by a patent. Reagents suitable for use and prepared under patent license are available from Randox Laboratories Ltd., 55 Diamond Road, Crumlin, Co. Antrim, N. Ireland. Interested parties are invited to submit information regarding the identification of an alternative(s) to this patented item to the ASTM Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee, which you may attend.

[6] Procedures 10.4.4 and 10.4.5 must be performed over a period not exceeding 5 seconds.

[7] A luminometer capable of measuring light output over a period of 4 minutes and calculating the integral of the result is available from Randox Laboratories Ltd., Diamond Road, Crumlin, Co. Antrim, N. Ireland.

TABLE 1 Overall Interlaboratory Precision (St), Recovery and Bias Data for the Chemiluminescent Detection Method

No. of Reported Values	No. of Retained Values	True Value (%)	Mean Recovery X (%)	Percent Recovery (%)	Bias (%)	Overall Standard Deviation (St)	Sample Composition
9	8	23	14.50	63.04	–36.96	7.31	Potassium Hydrogen Phthalate 10210 mg/L
9	8	47	41.75	88.83	–11.17	4.65	Cadmium 102 mg/L
9	8	47	41.38	88.03	–11.97	4.66	Phenol 3 mg/L
9	7	47	50.43	107.29	7.29	2.99	Potassium Hydrogen Phthalate 12250 mg/L
9	8	48	43.38	90.36	–9.64	4.75	Cadmium 102 mg/L
9	7	50	50.71	101.43	1.43	3.25	Phenol 3.5 mg/L
9	8	50	54.63	109.25	9.25	4.10	Phenol 3.5 mg/L
9	7	52	45.57	87.64	–12.36	4.08	Calcium 1000 mg/L
9	8	55	41.50	75.45	–24.55	10.38	Phenol 5 mg/L
9	7	55	61.57	111.95	11.95	4.54	Iron 20.2 mg/L
9	8	55	52.25	95.00	–5.00	5.92	Phenol 5 mg/L
9	8	55	46.50	84.55	–15.45	5.48	Magnesium 1.94 mg/L
9	8	55	56.00	101.82	1.82	7.27	Iron 20.2 mg/L
9	8	57	42.88	75.22	–24.78	7.32	Magnesium 243 mg/L
9	8	61	58.75	96.31	–3.69	3.01	Phenol 7.5 mg/L
9	8	62	54.63	88.10	–11.90	5.18	Phenol 2.5 mg/L + Potassium Hydrogen Phthalate 511 mg/L
9	8	69	60.63	87.86	–12.14	3.58	Chromium 20.1 mg/L
9	8	69	61.50	89.13	–10.87	3.42	Chromium 20.1 mg/L
9	8	73	70.13	96.06	–3.94	3.98	Phenol 2.5 mg/L + Chromium 10.05 mg/L
9	7	75	73.29	97.71	–2.29	2.87	Phenol 10 mg/L
			Mean	91.75	–8.25	5.28	(pooled St)
							QC sample—phenol 5 ppm

10.4.6 If the luminometer is capable of saving the result, follow the manufacturer's instructions in order to do so. Remove cuvette.

11. Procedure

11.1 Adjust the luminometer to measure the test light output level in accordance with the manufacturer's instructions. If the luminometer has an appropriate menu, select "Test", ensuring that the chamber lid is closed, and enter appropriate information as per the LCD screen prompts.

11.2 Add a 200 µl aliquot of test sample to a cuvette containing 100 µl of reconstituted signal reagent and 800 µl of reagent water.

11.3 Gently mix the contents of the cuvette, and initiate the reaction by adding 20 µl of diluted enzyme reagent.

11.4 Insert the cuvette into the open luminometer chamber and close the chamber lid. Initiate measurement of light output according to luminometer manufacturer's instructions. Light output should be measured over a period of 4 minutes, and the area under the curve should be integrated to determine the result.

11.5 If the luminometer is capable of saving the result, follow the manufacturer's instructions in order to do so. Remove cuvette.

12. Calculation

12.1 The reaction produces maximum light output where no contamination is present, as represented by the Reference Sample. Light output is calculated as the area under the curve and expressed as the percentage inhibition compared to the light output from a Reference Sample.

Reference:

$$REF = \sum_{nR=1}^{240} nRi \quad (1)$$

where:

nR = a reading from the ADC (Analogue-Digital Converter).

Test (4 minute reading):

$$T4min = (1–((\sum_{nT=1}^{240} nTi) / REF)) \times 100\ \% \quad (2)$$

where:

nT = a reading from the ADC. Result is expressed as % inhibition of the integrated results.

A rapid 2 minute value may be obtained from the following formula:

$$T2min = (1–(nR_{120}/nR_{120})) \times 100\ \% \quad (3)$$

where:

nR_{120} = the reading from the ADC for the Reference Sample at two minutes, and

nT_{120} = the reading from the ADC for the Test Sample at two minutes. The result is the % inhibition at two minutes from the initiation of the reaction.

12.2 The instrument displays the percentage of inhibition result. If this figure is greater than 75 %, dilute the test sample with reagent water, and re-test.

TABLE 2 Single Operator Precision Data

NOTE 1—Precision was evaluated using various contaminants in a reagent water mix.

Amount Added (%)	Number of Retained Pairs	So (%)	Analyst Relative Deviation (%)
50	7	1.96	3.73
50			
55	8	6.92	16.20
47			
55	7	6.67	11.37
55			
61	7	1.99	3.02
75			

12.3 If a dilution has been performed, calculate the total inhibition by multiplying the result by the relevant dilution factor.

13. Precision and Bias [8]

13.1 Nine laboratories participated in a collaborative study to determine the precision and bias of this procedure. The precision and bias values determined in this study include variability due to shipping and (where relevant) dilution of the samples used. The collaborative study and data analysis was performed using Practice D 2777. Each sample was analysed in singlicate. Since this procedure pertains to a screening test, test samples consisted of a variety of analytes in a single matrix (reagent water).

13.2 The test data were obtained on reagent grade water and these precision and bias values may not be applicable to more complex matrices. It is the user's responsibility to ensure the validity of this test method to waters of untested matrices.

13.3 The overall precision of this test method within its designated range for reagent water varies as shown in Table 1.

13.4 The single-operator precision of this test method within its designated range for reagent water varies with the material tested as shown in Table 2.

13.5 *Bias*—Recoveries of known percentage inhibitions from 20 samples containing a variety of contaminants types are shown in Table 1.

13.6 These data may not apply to waters of other matrices, therefore it is the responsibility of the analyst to ensure the validity of the test method in a particular matrix.

[8] Supporting data are available from ASTM headquarters, Request RR: D19–1169.

14. Quality Control

14.1 Before this test method is applied to the analysis of samples, the analyst must establish quality control by the procedures recommended in Practice D 4210 and Guide D 3856.

14.2 Quality control (QC) requirements are the initial demonstration of laboratory capability followed by regular analyses of quality control standard material. Other criteria may be more appropriate in a given situation depending on the data quality objectives.

14.3 A Reference Sample should be run when the luminometer is first switched on, each day that an analysis is to be performed, or if a fresh batch of reagents is prepared. A Reference Sample should also be run with each sample batch (maximum of 20 samples) to check for contamination introduced by the laboratory or use of the Test Method.

14.4 The laboratory using this test should perform an initial demonstration of laboratory capability. Seven replicates of Reagent Grade Water should be analysed. Inhibition should not exceed ± 17 %, compared to the value of the Reference Sample (TV = 0 for reagent water). If data quality objectives cannot be achieved, the problem must be located and corrected before further samples are analysed.

14.5 To monitor accuracy, one Quality Control Sample (QCS), consisting of a sample of known inhibition level, should be run with each sample batch (maximum of 20 samples). The QCS is a solution of known composition with a known inhibition level. A suggested QCS is phenol, 5mg/L in reagent water (55 % inhibition).

14.5.1 Calculate the measured inhibition of each replicate, the mean inhibition, mean accuracy (as mean percentage of true value) and the precision (as relative standard deviation, RSD) of the QCS. The accuracy of the control sample, expressed as a percentage of the true value should be 75 to 125 %, and the RSD should be < 20 %. The results must meet the before the data for that batch of samples are deemed acceptable. If accuracy and precision limits consistent with data quality objectives cannot be achieved, the problem must be located and corrected before further samples are analysed.

14.6 The laboratory may perform additional quality control as desired or appropriate.

15. Keywords

15.1 beta-emitter; borate buffer; chemiluminescence; contaminant; luminometer; oxidant; pollutant; reagent; signal reagent

APPENDIX

(Nonmandatory Information)

X1. FIELD STUDIES USING THE PORTABLE CHEMILUMINESCENT METHOD OF WATER QUALITY DETERMINATION

INTRODUCTION

The chemiluminescent method has been used for the detection of contamination in a range of field studies. Chemiluminescent screening has produced valuable trend data on the quality of the sampled water. In addition, results of chemiluminescent screening have been correlated with those of BOD and COD testing, allowing a rapid calculated estimation of BOD and COD levels.

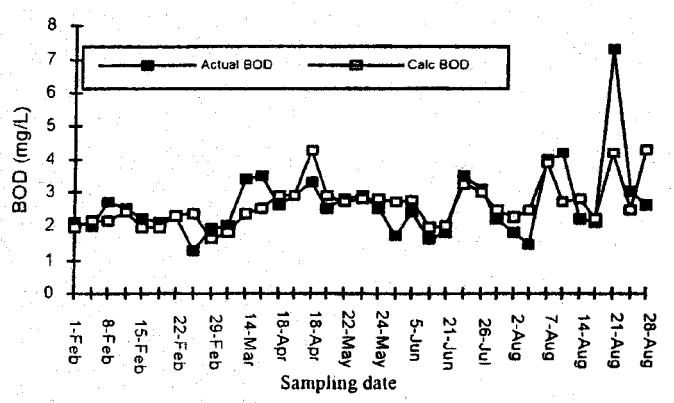

FIG. X1.1 Actual and Calculated BOD Values for Water Samples from the River Maine

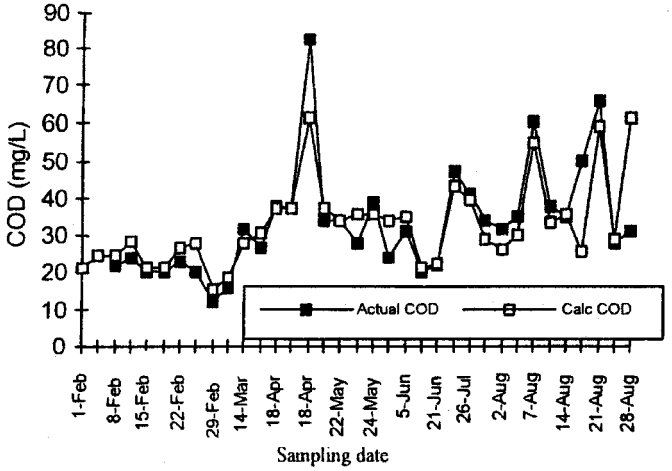

FIG. X1.2 Actual and Calculated COD Values for Water Samples from the River Maine

X1.1 *Monitoring of River Systems:*

X1.1.1 Water samples were taken from a named river over a three month period. Samples were taken at approximately 0.5 m below the surface of the water and around 1 m from the river bank, and chemiluminescent screening, BOD and COD testing was performed.

X1.1.2 BOD measurement by conventional methods has reported poor sensitivity at low levels, although there was good agreement between the actual and the calculated BOD values as shown in Fig. X1.1. The results in Fig. X1.2 also show good agreement between the actual and calculated COD values. The results indicate that the chemiluminescent method of water quality determination is as effective at detecting pollutants in river water as BOD and COD laboratory based methods.

X1.1.3 *Pollution "Hotspot" Incident*—An extensive "fish kill" was reported in a local river and was thought to be linked to the release of effluent from a nearby distillery. River water samples were promptly analysed using the chemiluminescent water quality determination system.

Sample	% Inhibition
Reference sample	0
Upstream	21
Distillery tributary	92
River mouth	46

The results show that the distillery tributary was the source of the pollution.The results show that the distillery tributary was the source of the pollution.

X1.2 *Monitoring of Pollution in Sea Water Samples:*

X1.2.1 A series of experiments were performed to determine the effectiveness of the chemiluminescent screening method in detecting pollutants in sea water samples, compared to conventional methods.

X1.2.2 A large volume of sea water (3 % salinity) was collected from the seashore and stored between +2°C to +8°C to await analysis. Varying concentrations of raw sewage effluent (from 0–20 % sewage, v/v) were added to a selected volume of sea water. The samples were then analysed at room temperature using the chemiluminescent screening method. BOD and COD levels of the samples were measured using standard methods.

X1.2.3 The graphs show variations in BOD, COD and the chemiluminescent screening method results with added sewage concentration.

X1.2.4 Results of the chemiluminescent screening method agree closely with BOD and COD levels of the samples in the range 0–130 mg/L.

X1.2.5 Although these sea water samples are not from a real pollution incident, the results give a clear indication that the chemiluminescent screening method is effective for detecting pollutants in sea water. Salt concentrations up to 7 % do not affect the measurements.

X1.3 *Monitoring of Effluent in Animal Waste Rendering Plants:*

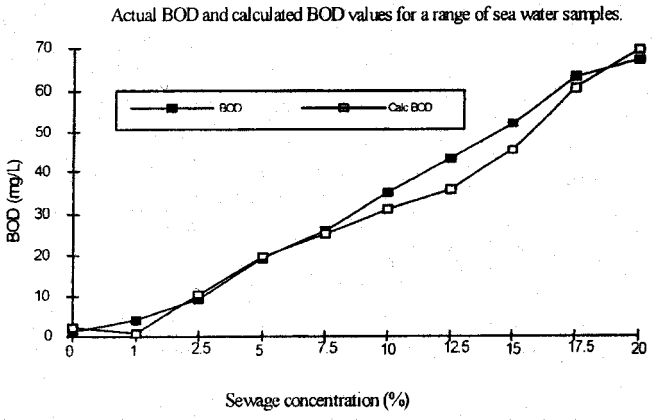

FIG. X1.3 Actual BOD and Calculated BOD Values for a Range of Sea Water Samples

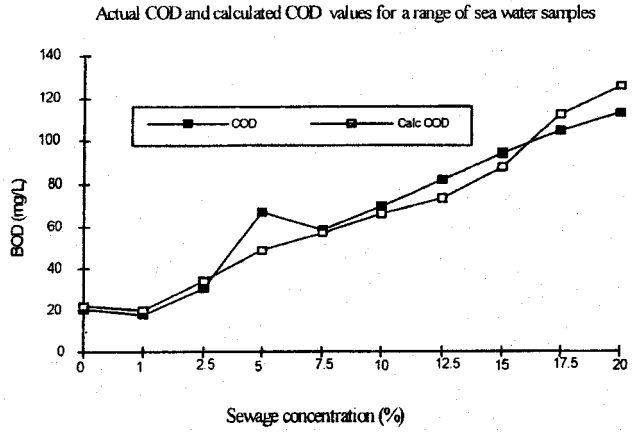

FIG. X1.4 Actual COD and Calculated COD Values for a Range of Sea Water Samples

X1.3.1 Comparison of the chemiluminescent screening method results with BOD and COD measurements in the large balance tanks used in animal waste rendering plants.

X1.3.2 Samples were taken at regular intervals from a balance tank at an animal rendering plant. The samples were analysed using the chemiluminescent screening method and BOD and COD levels were determined by conventional methods. Results of the chemiluminescent screening method were statistically analysed and calculated BOD and COD levels determined.

X1.3.3 The graphs show good agreement between calculated values from the chemiluminescent screening method results and both BOD and COD, as determined by laboratory methods. The chemiluminescent screening method was effective at detecting fluctuations in the levels of pollutants in water samples.

X1.3.4 Processing of animal waste in the balance tank at this rendering plant is a continuous process and a constant water level is maintained. The BOD, COD and values from the chemiluminescent screening method levels progressively increased between March and September which coincided with an increase in the volume of waste processed by the plant.

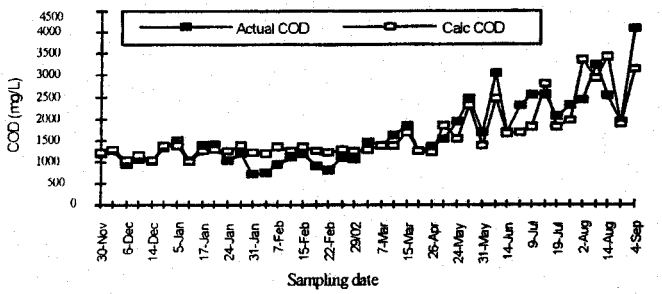

FIG. X1.5 Actual and Calculated BOD Values for Water Samples from a Balance Tank at an Animal Waste Rendering Plant

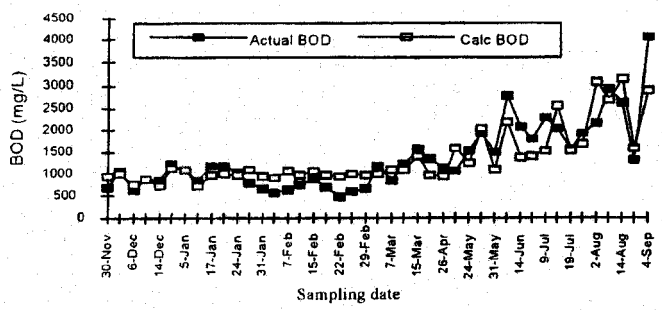

FIG. X1.6 Actual and Calculated COD Values for Water Samples from a Balance Tank at an Animal Waste Rendering Plant

X1.4 *Monitoring of Effluent in the Pulp and Paper Industry:*

X1.4.1 Most effluent from the pulp and paper industry undergoes treatment prior to release into receiving waters, however untreated effluent discharge from pulp and paper mills may constitute a serious source of water pollution in countries around the world. Untreated effluent from this source is lethal to fish and may contain three major types of constituents, which include dissolved organic compounds, suspended solids and an inorganic component.

X1.4.2 Water samples were collected from different lagoons at a pulp and paper mill over a 6 month period. Six 1 litre samples were collected at each sampling, pooled, and 2 × 100 ml aliquots were removed for testing. A cup sampler device was used to draw the samples. Water samples were stored in compliance with STM SW846 Standard Method for Handling and were analysed within 24 hours of having been drawn. Samples were analysed in duplicate using the chemiluminescent screening system.

X1.4.3 Results of the chemiluminescent screening method showed good agreement with both BOD and COD, as determined by laboratory methods. The chemiluminescent screening method was effective at detecting fluctuations in pollutant levels.

X1.4.4 "BOD shock loadings" at a pulp and paper mill were rapidly detected with the chemiluminescent screening method, after a suspected black liquor loss into the wastewater treatment system, with a predicted peak BOD value within 5 % of the actual BOD value.

X1.5 *Monitoring in the Sewage Treatment Industry:*

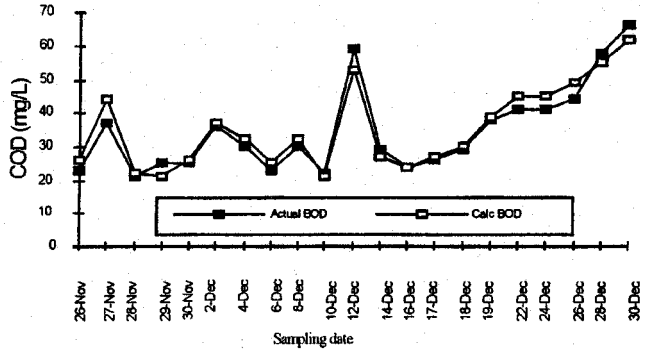

FIG. X1.7 Actual and Calculated BOD Values for Water Samples from a Pulp and Paper Plant

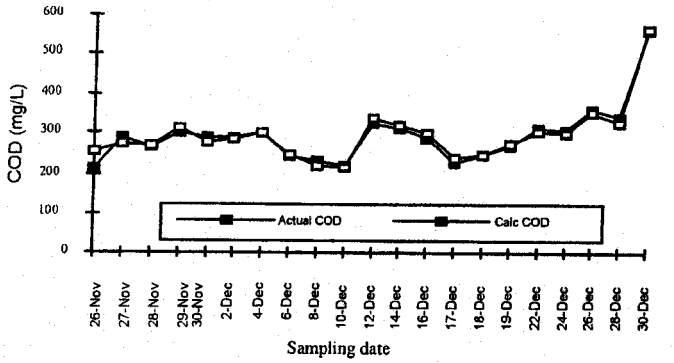

FIG. X1.8 Actual and Calculated COD Values for Water Samples from a Pulp and Paper Plant

X1.5.1 Studies were set up to determine whether the chemiluminescent screening method was as effective as BOD for detecting pollutants in water samples.

X1.5.2 Raw sewage samples were taken at regular intervals. These samples were untreated and had high levels of suspended solids. Water samples were taken at around 0.5 M below the surface of the water using a sampling cup. The samples were analysed using the chemiluminescent screening method, and BOD and COD levels measured. Results from the chemiluminescent screening method were then statistically analysed and calculated BOD and COD levels were determined.

X1.5.3 The BOD and COD values calculated from the chemiluminescent screening method results closely followed the actual values, as determined by laboratory methods. The results indicate that the chemiluminescent screening method is as effective as conventional methods for the determination of pollutant levels in sewage samples.

X1.6 *Use in Industrial Processes:*

X1.6.1 Water samples were taken daily from a waste water treatment plant at a chemical works, using a cup sampler device. Samples were stored in compliance with STM SW846 Standard Method for Handling and were analysed within 24 hours of having been drawn. Samples were analysed in duplicate using the chemiluminescent screening system and COD levels were then determined using a conventional laboratory method.

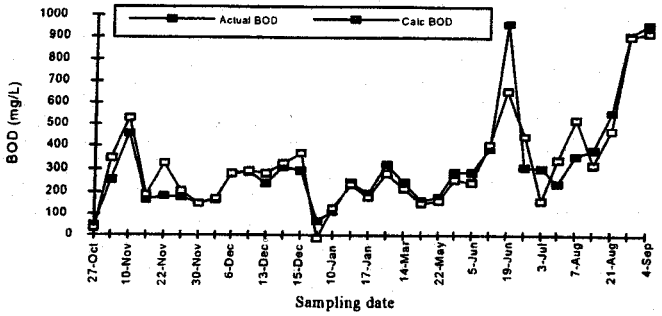

FIG. X1.9 Actual and Calculated BOD Values for Water Samples from a Sewage Treatment Plant

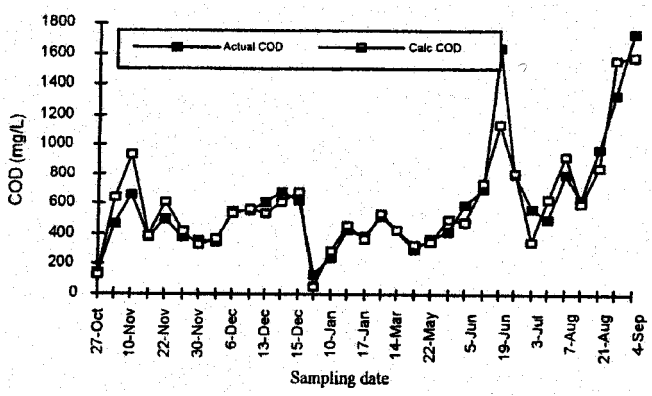

FIG. X1.10 Actual and Calculated COD Values for Influent Samples from a Sewage Treatment Plant

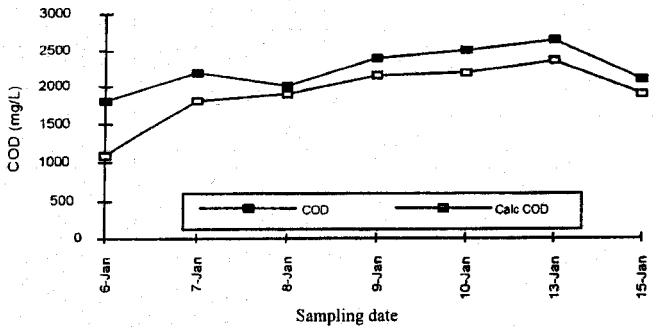

FIG. X1.11 Actual and Calculated COD Values for Samples of Influent from a Chemical Plant

X1.6.2 Variations in actual COD levels in the samples were detected by the chemiluminescent screening method, as there was good agreement between actual and calculated values (Figs. X1.11 and X1.12).

X1.6.3 Detection of cleaning agents in water samples from an industrial process.

X1.6.4 Small volumes of water samples were taken from a wastewater tank at a large industrial plant. The samples contained a cocktail of chemicals including D-limonine, dipropylene glycol, nonylphenol ethoxylate and isopropyl alcohol.

X1.6.5 The chemiluminescent screening method was used to measure the levels of pollutants in the samples and the results were compared with COD values, determined using laboratory methods.

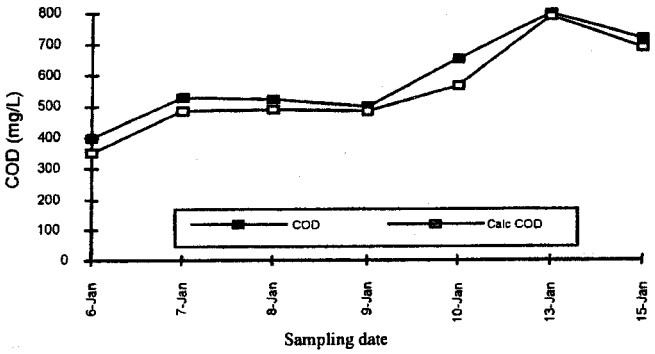

FIG. X1.12 Actual and Calculated COD Values for Samples of Effluent from a Chemical Plant

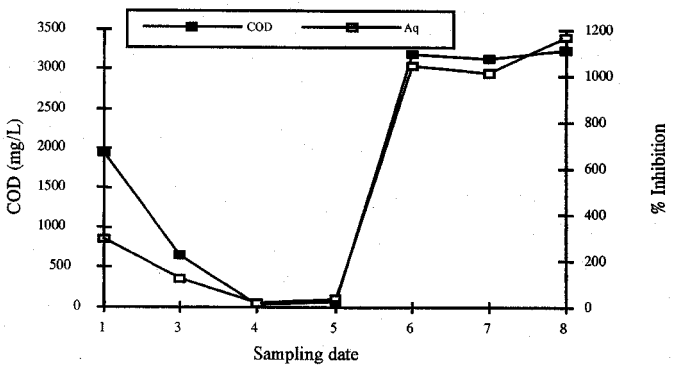

FIG. X1.13 Actual and Calculated COD Values for Water Samples from an Industrial Process

X1.6.6 Calculated COD values could not be determined as the data was not available, therefore results were represented as percentage inhibition. The wastewater samples analysed in this experiment contained high levels of cleaning agents and surfactants. These chemicals were readily detected using the chemiluminescent screening system and the results agreed with COD values, determined by laboratory methods (Fig. X1.13).

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