The Effect of Anaerobiosis on Measurement of Sulfite Pulp Mill Effluent Concentration in Estuarine Water by U.V. Spectrophotometry

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In studies designed to characterize and model the mixing behaviour of pulp mill effluents (PME) in natural receiving waters and in laboratory bioassays to test their environmental side effects, a conservative measure of PME concentration is necessary. Dissolved substances in effluents from the sulfite pulping process consist of approximately one third soluble carbohydrates, principally hexoses and pentoses and two thirds lignin and lignosulfonates (LEITHE 1973). Soluble carbohydrates are readily available for microbial utilization, whereas lignins are resistant to microbial attack (BROCK 1966). Sulfonation of the side chains in the lignin molecule and formation of new carbon to carbon linkages which occur during sulfite pulping increases resistance to microbial attack (WATKINS 1970).

Because of their apparently conservative nature, lignins and lignosulfonates have frequently been used to measure PME concentration with the following non-specific methods: chemical (see LEITHE 1973), fluorometric (BAUMGARTNER et al 1971; WILANDER et al 1974), or U.V. spectrophotometric (WEXLER 1964; MRKVA 1969). Wet oxidation techniques for determination of C.O.D. are not a practical alternative because of interference by halides in seawater. DOBBS et al (1972) suggested absorbance measurements at 254 nm to determine total organic carbon.

The work reported here investigates two possible sources of error which may occur in monitoring the mixing characteristics of PME in seawater with the U.V. spectrophotometric method.

MATERIALS AND METHODS

All field samples were obtained from the L'Etang Inlet, a 17 km long marine dominated estuary in the Bay of Fundy. This inlet receives effluent from a hardwood pulpmill utilizing the sodium sulfite chemical digestion process (see WILDISH et al 1972).

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U.V. spectra of water samples from various regions of L'Etang Inlet showed qualitative and quantitative differences. To test whether biological or chemical factors were responsible, the following experiment was carried out. Culture solutions were prepared containing 2.7 g ammonium sulfate and 0.3 g Difco yeast extract made up in 1 litre of filtered sea water (S = 30 ± 1‰). Anaerobic cultures contained 1.0 mℓ of a sodium thioglycollate solution (10% w/v), an ingredient omitted in the aerobic cultures. Sodium lignosulfonate (Aldrich Co.) at a final concentration of 126 mg/ℓ or PME at 10 mℓ/ℓ was added to each of the culture types. The pH was adjusted with NaOH or HCl to 7.2. The "seed" consisted of the micro-organisms contained in 10 mℓ/ℓ of L'Etang Inlet mud taken from an anaerobic part of the system. Aliquots (100 mℓ) of these solutions were then dispersed in 500 mℓ conical flasks of seawater (S = 30±1‰). Anaerobic cultures were flushed for 10 minutes with oxygen free-nitrogen. Controls were sterilized by .45μ membrane filtration and the seed mud by autoclaving. The flasks were incubated without shaking at 4°C, 15°C and room temperature (25-28°C) for 63 days. U.V. absorbance was measured at wavelengths of 280 and 250 nm.

To test whether sulfides, nitrates, or sugars interfered in determinations at 280, 254, or 250 nm, 0.1 or 0.01% solutions of technical products were made up in PME at various dilutions and absorbance measurements made. Purging experiments were with compressed air or oxygen-free nitrogen. Sulfide ion concentration was determined with a specific ion electrode (Orion #94-16) using the known subtraction method (Orion Instruction Manual 1974).

A Beckman DBGT spectrophotometer equipped with a recorder was used for absorbance measurements and spectral recording. One cm silica cells with distilled water as the reference were used throughout.

RESULTS AND DISCUSSION

Since absorbance measurements in the culture experiment had stabilized by 29 days, results are given as percentage absorbance remaining then (see Table 1). Reductions in absorbance occurred in all the culture flasks, but most markedly for PME and in anaerobic conditions. The U.V. absorbance of PME controls did not change throughout incubation, although the sodium lignosulfonate control at 4°C showed an increased absorbance (aerobic 58%, anaerobic 16% after 29 days). In interpreting this data, the experiments of WATKINS (1970) with model lignin compounds are pertinent. Some fractions from the bacterial utilization of vanillyl sulfonate and aromatic compounds showed increases in

TABLE 1.

Percentage absorbance at 280 and 250 nm remaining after 29 days in cultures grown under different incubation conditions.

Temp. °C	Incubation condition	Sodium lignosulfonate 280 nm	Sodium lignosulfonate 250 nm	PME 280 nm	PME 250 nm
4	aerobic	90	95	56	47
	anaerobic	84	92	56	62
15	aerobic	90	91	100*	100*
	anaerobic	75	79	67	77
25-28	aerobic	91	94	61	60
	anaerobic	75	83	48	53

*Lag effect: 47 and 52% respectively after 63 days.

absorbance at 280 and 260 nm on culturing with bacteria from river water, making calculation of utilization rates difficult. Soil and river bacteria capable of utilizing monomeric sulfonated aromatics were considered by WATKINS (1970) to be rare. GANCZARCZYK and OBIAGA (1974) describe poorly understood physical, chemical and biological factors influencing the removal of lignosulfonates in activated sludge treatment of pulp mill effluents. In these experiments a maximum of 15.2% lignin removal by biological treatment in fresh water was achieved.

Some water samples from the L'Etang Inlet smelled strongly of hydrogen sulfide. When they were flushed with compressed air (see Table 2) a colour change from dull to yellow brown and a reduction in U.V. absorbance occurred. Purging a previously aerated sample with oxygen-free nitrogen had no effect on absorbance at these wavelengths.

The effect of added sulfide on U.V. absorbance is shown in Table 3. In solution the hydrogen ion complexes sulfide to form bisulfide ion and hydrogen sulfide. In acidic conditions hydrogen sulfide is present whilst at intermediate pH the bisulfide ion is the major species present. GOLDHABER and KAPLAN (1975) mention a strong

TABLE 2.

Effect of purging with air on U.V. absorbance.

Sample	Sulfide mg/ℓ	A280 nm	A254 nm	A250 nm
L'Etang Inlet sample aged 27 days	25.60	1.92	3.24	4.29
Same, flushed 10 minutes	1.12	2.43	2.82	3.06
Same, flushed 60 minutes	0	2.34	2.70	2.88

absorbance by the bisulfide ion at 25°C and pH 6-9 with a broad peak at 230 nm. It is probably this which differentially interferes, the effect being stronger at 250 than at 280 nm.

TABLE 3.

Effect of sodium sulfide on U.V. absorbance.

Sample	Sulfide mg/ℓ	A280 nm	A254 nm	A250 nm
10^{-3}% Na_2S in PME	122.00	0.80	5.16	8.22
10^{-4}% Na_2S in PME	12.80	0.65	1.20	1.62
PME	0	0.60	0.69	0.74

Nitrates, as sodium nitrate, and various sugars (glucose, ribose) absorb in the U.V. region. Such interference was not serious at the highest concentrations of these substances found in the L'Etang Inlet. Other interfering species not evaluated here include suspended matter and humic acids (see WILANDER et al. 1974).

U.V. spectra of seawater and sodium lignosulfonate are shown in Fig. 1 Two field samples are also shown. The effluent sample IV, shows two interfering peaks, one of which may be the bisulfide ion (230 nm). Sample V was taken after retention in the L'Etang Inlet and shows disappearance of the 200-204 peak.

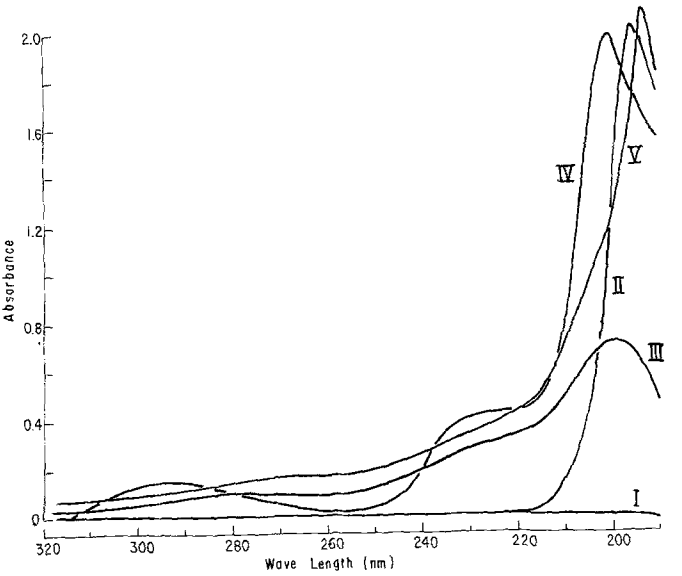

Fig. 1. U.V. spectra of: I distilled water blank; II seawater blank, diluted 5x; III sodium lignosulfonate ≈0.1 mg/ℓ in sea water; IV PME at inlet (fresh water) dilution 40x; V PME in sea water (20.6‰) .45μ membrane filtered, dilution 15x.

CONCLUSIONS

Our results demonstrate two possible sources of error in the field determination of lignins and lignosulfonates. They are caused by:

1. Possible microbial degradation of PME, particularly under anaerobic conditions.

2. Interference by the bisulfide ion under anaerobic conditions.

One practical application of these observations is that lignins and lignosulfonates as measured by U.V. spectrophotometry cannot be used as a conservative indication of PME in receiving waters, particularly where part of the system is anaerobic. This conclusion probably applies also to other non-specific techniques. Lignins and lignosulfonates have been used as natural tracers in preference to fluorescent dyes (see, for example, WILANDER et al. 1974) because dyes are expensive, they do not change physical conditions of the system and, because they have been previously regarded as con-

servative, allow ample time for observations of dispersion. AUNINS and TUPREINE (1974) also report coagulation of lignosulfonic complexes on addition to seawater, the degree of this effect depending on salinity, temperature, and degree of turbulent mixing.

Interference from the bisulfide ion can be removed by purging with air or nitrogen. If it is necessary to store water samples before analysis, they must be sterilized effectively, such as by membrane filtration. Field samples filtered through .22μ or .45μ membrane filters and held in covered test tubes maintained their U.V. absorbance values unchanged for at least 3 months.

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