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pH Maintenance of Chemi-Thermo-Mechanical Pulp Mill Effluent by CO_2 Recycling During Trout Lethality Testing

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In Canada, Environment Canada is responsible for enforcing the Fisheries Act, Pulp and Paper Effluent Regulations (Government of Canada 1992). The Regulations require monthly acute lethality testing of the discharge effluent, using rainbow trout (*Oncorhynchus mykiss*). An effluent is in compliance with the regulation when at 100% concentration, 50% or less test fish die. If 60% or more of the test fish die during the 96-hour exposure time, the effluent is defined as acutely lethal and an enforcement investigation would commence.

Trout lethality testing must be performed with strict adherence to the Environment Canada, Trout Reference Method (1990a). A requirement of the method is continuous aeration of the test sample at a specified rate for the duration of the 96-hour test period. Aeration of pulp mill effluent can cause an upward drift in the pH of the effluent sample due to the stripping of carbon dioxide (CO_2). This phenomenon can be significant when testing the effluents from Chemi-Thermo-Mechanical Pulp (CTMP) and Thermo-Mechanical Pulp (TMP) mills because these effluents are likely to contain high levels of ammonia, which will become un-ionized as pH rises (Servizi and Gordon 1986). An increasing concentration of un-ionized ammonia increases the toxicity and could make the effluent acutely toxic to the test fish (Schnell et al. 1990), resulting in a noncompliance to the regulation. Because the pH increase was an artifact of the test and not indicative of a discharge to the receiving environment, Environment Canada commenced work on a procedure to stabilize and maintain pH during the 96-hour trout lethality test. The pH-maintained test would solely be run in parallel to only evaluate what effect sample pH extremes may have on toxicity. Results for regulatory purposes are derived from testing the non-adjusted pH sample.

This portion of work evaluated the effect of CO_2 recycling within a closed system. Stripped CO_2 during test aeration was contained in a closed system and then recycled back into the test effluent via aeration. The impact of CO_2 recycling on pH and dissolved oxygen concentration was measured and compared to the measurements made during trout lethality testing using the Environment Canada, Reference Method.

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MATERIALS AND METHODS

Test fish were rainbow trout (*Oncorhynchus mykiss*) fingerlings received from Ackenberry Trout Farms (Sherwood Park, Alberta). Fish were maintained for a minimum 2-week period at prescribed conditions (Environment Canada 1990a) and exceeded all health criteria for husbandry of fish identified in the Reference Method. Trout were held under a photoperiod of 16 hours light and 8 hours dark with a lighting intensity of (mean ± SD) 216 ± 40 lux. Fish were fed 4 times a day with starter feed at a daily ration of approximately 5% of body weight, however, were not fed for at least 16 hours prior to testing nor during the test. No mortality in the trout stock acclimation tank was observed during the seven days prior to testing.

Water for acclimation of trout and controls during lethality testing was de-chlorinated City of Edmonton drinking water. Water had the following chemical and physical characteristics (mean ± SD), as determined from 14 daily measurements prior to each test: temperature 14.7 ± 0.4 ° Celsius; dissolved oxygen 8.7 ± 0.2 mg/L; conductivity 368 ± 16 µmhos/cm; and pH 8.2 ± 0.1. The flow of fresh water through the fish holding tank was no less than a rate of 4.7 L/g of trout per day and there were at least 3.9 litres of water in the tank per 10 g of trout fingerlings.

Chemi-Thermo-Mechanical Pulp (CTMP) final effluent grab samples were collected by mill staff in new 20 litre polypropylene drinking water containers, which were completely filled and sealed to exclude air. Approximately 100 litres total volume was collected, and received the same day by Environmental Protection Ecotoxicology Laboratory, in Edmonton. Testing was initiated the same day as received, or samples were stored overnight at 4 ± 2°C and tested the following day. Prior to testing all sample containers were mixed to form one composite 100% concentration sample.

The CTMP mill effluent was sampled on 3 different occasions with initial chemical and physical measurements being made on the composite sample, just prior to trout lethality testing.

Trout lethality tests including controls, were run in accordance with the Environment Canada Reference Method, Single Concentration Procedure (Environment Canada (1990a). In summary, all tests were conducted at 15 ± 1°C and were performed using approximately 50 litres of 100% sample concentration in new polyethylene lined aquariums, to which 10 fish were added. Test containers were covered and aeration using an Elite 799 aeration pump was continuous throughout the test at a rate of 6.5 ± 1mL/min./L, controlled with a rotometer. Physicochemical measurements including, temperature, dissolved oxygen, conductivity and pH, were made on all test solutions at the start of the test and on aliquots of test solution at 24 hour intervals for the duration of the 96 hour

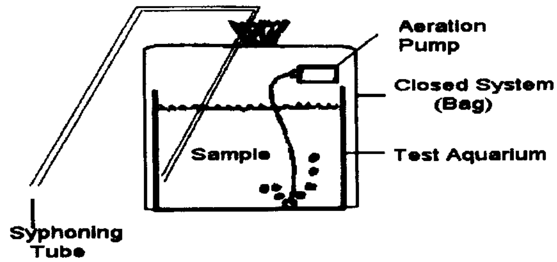

Figure 1. Recycle procedure setup.

test. Trout lengths and weights were recorded at the end of testing, and fish loading densities calculated and determined to be less than 0.5 g/L of test solution.

A Recycle Procedure test using 50 litres of 100% concentration of CTMP effluent and a control was run in polyethylene lined aquariums, concurrently with each Reference Method test. The Recycle Procedure employed an Elite799, 115-volt, aquarium aeration pump. The aeration rate of each Elite 799 pump was set at 6.5 ± 1mL/min./L and controlled with a rotometer. Ten fish were added into each test solution. Each test aquarium including the aeration pump was encompassed by a large (76cm. x 1.22m and 1.2mm thick) sealed clear polyethylene bag (Figure 1). The sealed bag created a closed system in which carbon dioxide stripped from aeration of the effluent was contained and reintroduced into the test solution by the aeration pump. A siphon line was introduced to the system to draw off aliquots of test solutions for chemical and physical measurements, while maintaining the integrity of the closed system. All other conditions remained the same as in the Reference Method.

RESULTS AND DISCUSSION

Three reference toxicant trout lethality tests were completed and run with the corresponding CTMP effluent toxicity tests. The median lethal concentration to 50% of fish during the 96-hour exposure period (LC_{50}) and the corresponding 95% confidence limits, were calculated by probit analysis (Stephan 1977). Reagent grade phenol was tested on May 10, May 28 and July 9, 2001, and produced 96-hour median lethal concentrations (LC_{50}) of 9.97 ppm, 9.97 ppm and 10.5 ppm, respectively. All reference toxicant LC_{50} values fell within the historical warning limits (95% Confidence Interval), of 8.69-11.1 ppm. Results therefore provide a measure of precision within the laboratory and the reference toxicant data falls within an acceptable range of variability (Environment Canada 1990b).

For each of the CTMP effluent sampling dates the composite sample chemical and physical characteristics were determined (Table 1). The different samples showed some variations of all parameters measured, with pH ranging from 7.9 to 8.2.

Loading densities of the 10 trout added to each test solution were less than 0.5 g/L and ranged from 0.34 g/L to 0.47 g/L.

Table 1. Composite CTMP effluent physicochemical measurements.

Test date	Composite effluent sample 100% concentration			
	Temperature °C	D.O.[a] mg/L	Conductivity µmhos/cm	pH
10-May-2001	15.6	6.2	2,930	8.2
28-May-2001	16.0	7.2	3,570	7.9
10-July-2001	15.6	7.1	3,350	8.0

[a]D.O. = Dissolved oxygen

Table 2. Control water physicochemical measurements (Mean ± SD).

Physicochemical measurement [a]		Time (hours)				
		Start-0h	24h	48h	72h	96h
Temperature (°C)	a.	14.7±0.6	15.3±0.1	15.2±0.3	15.4±0.2	15.4±0.1
	b.	14.6±0.2	15.4±0.1	15.6±0.1	15.6±0.1	15.6±0.1
Dissolved oxygen (mg/L)	a.	9.2±0.1	8.7±0.2	8.8±0.3	8.8±0.3	8.9±0.2
	b.	9.3±0.1	8.6±0.1	8.8±0.2	8.6±0.3	8.6±0.2
Conductivity (µmhos/cm)	a.	366±6	366±9	368±6	368±8	369±6
	b.	368±8	368±7	368±12	373±12	371±8
pH	a.	8.2±0.1	8.2±0.1	8.3±0.0	8.3±0.0	8.3±0.1
	b.	8.3±0.1	8.0±0.1	7.9±0.1	7.8±0.1	7.7±0.1

[a] a. Reference Method and b. Recycle Procedure.

The same source of treated water for trout acclimation was used for controls run with each test. Physicochemical results (Mean ± SD) from testing control water, following the Reference Method and using the Recycle Procedure, are presented in Table 2. The dissolved oxygen decrease during testing was comparable for both methods. CTMP mill effluent pH increased from an initial level of 8.1 - 8.3, in 3 replicate runs, to a final pH level of 8.9 - 9.0 during the 96 hour test (Figure 2). At the 5% level of significance (α =0.05) the mean pH at 96 hours was significantly greater (critical value -2.015) than the pH at the start of trout testing.

Trout lethality testing using the Recycle Procedure determined pH measurements at the start of trout testing for the 3 replicate runs, ranged from 8.1 to 8.3; the same values as found in the Reference Method (Fig. 2).

The effluent pH using the Recycle Procedure, however, was maintained for the duration of the 96-hour tests in the range of 8.2 to 8.4 units (Fig. 2). At the 5% level of significance (α =0.05) the pH at 96 hours (critical value of -2.015) was the same as the pH at the start of trout testing, using the Recycle Procedure.

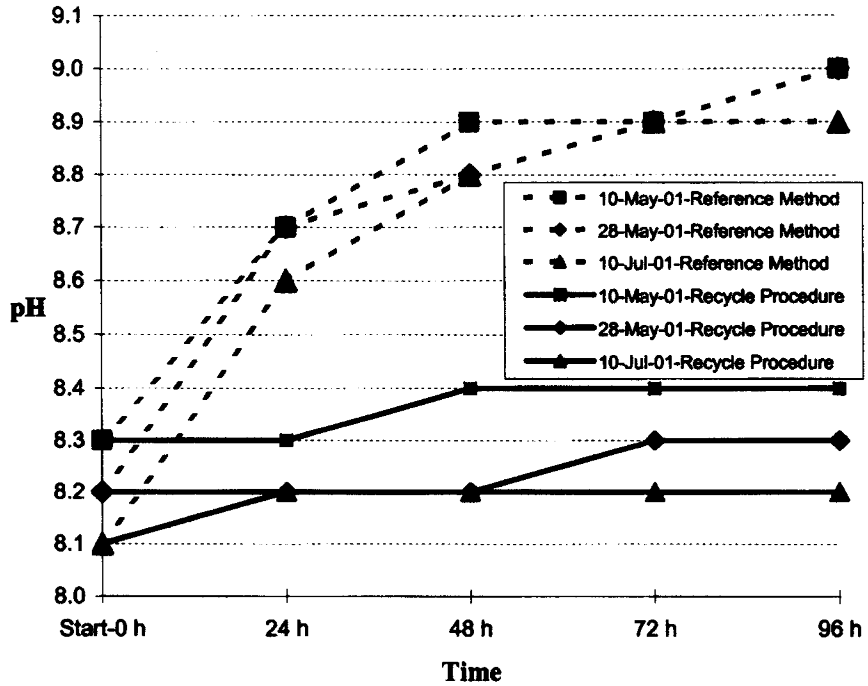

Figure 2. pH drift during reference method and recycle procedure.

Analysis of variance (ANOVA) was used to compare the mean change in pH during testing using the Reference Method and Recycle Procedure. The mean change in pH was 0.8 units and 0.1 units for the Reference Method and Recycle Procedure, respectively. At the 5% level of significance (α =0.05), the data suggests that the mean increase in pH during the Reference Method tests and the Recycle Procedure tests are not equal.

Dissolved oxygen levels increased in each effluent during both 96 hour test methods. The average increase in dissolved oxygen was 0.6 ppm during Reference Method tests while an average increase in the Recycle Procedure tests was 0.3 ppm (Table 5).

The Reference Method (Environment Canada 1990a), states that the test must be conducted without adjustment of sample or solution pH. A pH-adjusted or maintained test may be run in parallel to only evaluate what effect sample pH extremes may have on toxicity. The methods offer little direction on procedural details regarding pH adjusted testing. Some guidelines for adjusting pH using sodium hydroxide or hydrochloric acid are available for whole effluent tests (Abernethy and Westlake 1991). Samples chemically altered for pH are considered non-representative of the actual effluent discharged because addition of acid or base can result in the formation of new toxic agents and release sorbed and bound chemicals into solution. Addition of week acid or bases could also cause dilution of the 100% concentration of sample. A CO_2-controlled atmosphere has been used successfully in controlling test solution pH when testing a variety of static and static-renewal toxicity tests (Mount and Mount 1992). A toxic municipal effluent with a high total ammonia level showed no acute toxicity when tested under a CO_2-controlled atmosphere.

Table 5. CTMP effluent dissolved oxygen (D.O. mg/L).

	Time (hours)					
Test date[a]	Start-0h	24h	48h	72h	96h	
10-May-2001 a.	8.0	8.6	8.6	8.5	8.5	D.O. (mg/L)
b.	7.9	8.1	8.1	8.1	8.1	
28-May-2001 a.	8.3	8.6	8.8	8.8	8.8	
b.	8.3	8.5	8.5	8.4	8.5	
10-July-2001 a.	7.7	8.3	8.6	8.4	8.6	
b.	7.8	8.3	8.3	8.3	8.2	

[a] a. Reference Method and b. Recycle Procedure.

In this study, a typical pH drift upwards during aeration of CTMP mill effluent during Reference Method trout lethality testing was observed in all 3 replicate runs. Effluent pH increased an average of 0.8 units from an initial range of 8.1 - 8.3 to a final pH range of 8.9 - 9.0, during the 96 hour test. This increase in pH could significantly change the mean ratio of un-ionized ammonia from 4.2% to 20.4%, as calculated using the formula by Alabaster (1980) and a pK_a of 9.56 for un-ionized ammonia calculations at 15.0°C and zero salinity (Emerson et al.1975). The shift in ammonia species to the toxic form may convert some samples that were not acutely lethal at the time of discharge to acutely lethal samples during the 96-hour trout lethality test. No trout mortality was observed in any of the Reference Method or Recycle Procedure tests. Testing of 100% effluent spiked with ammonium hydroxide showed that ammonia toxicity was significantly reduced by pH maintenance. Effluent containing 4.5 ± 0.2 total ammonia measured at test completion and following the Reference Method, resulted in 100% trout mortality. At a final pH of 9.0 the calculated un-ionized ammonia concentration was 0.97 ppm. Using the Recycle Procedure a final pH of 8.3 and calculated un-ionized ammonia of 0.23 ppm were determined. No trout mortality was observed during the test.

The Recycle Procedure tested herein maintained pH of CTMP mill effluent during the 96 hour trout lethality test. A consistent increase of only 0.1 pH units was observed in each of the 3 trials. In none of the trials did the pH drift to below the original pH during testing. This criterion is important in determining toxicity of the effluent at the discharged level. The pH decrease of control water during the Recycle Procedure is likely due to increased levels of carbon dioxide from respiration of fish. Dissolved oxygen was maintained at an average level of 91.5% during testing using the Reference Method, and during the Recycle

Procedure, dissolved oxygen in the effluent was maintained at an average level of 88.3% air saturation. Dissolved oxygen was therefore held well within the prescribed Reference Method limits of 70 to 100% of air saturation using a rate of 6.5 ± 1mL/min./L. during trout lethality testing. Maintenance of dissolved oxygen is essential for respiration of the fish, to avoid hypoxia, and for oxygen demands due to Biochemical Oxygen Demand (BOD). Alabaster et al. (1957) showed that increased concentrations of carbon dioxide required increased concentrations of dissolved oxygen necessary for the survival of rainbow trout fingerlings. In addition, Merkens and Downing (1957) and Thurston et al. (1981), found that decreases in concentration of dissolved oxygen increased the toxicity of un-ionized ammonia.

Carbon dioxide stripped by aeration of the effluent in this study was contained in a closed system and the CO_2 reintroduced back into the test solution by an aeration pump using the Recycle Procedure. The pH of the effluent was maintained for the duration of the 96-hour trout lethality test. Results showed the pH increased consistently only 0.1 units for the 3 trials, despite slight variations of effluent quality. The concentration of CO_2 being emitted from the effluent, as a result of aeration during trout testing, would dictate the concentration of CO_2 reintroduced back into the effluent to maintain pH.

The results of these trials suggest that the CO_2 Recycle Procedure offers a practical means to evaluate the effects that pH extremes may have on toxicity, when run in parallel with the Reference Method for regulatory testing.

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