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pH Maintenance of Municipal Wastewater Effluent by CO_2 Recycling During Trout Lethality Testing

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In Canada, trout lethality testing must be performed with strict adherence to the Environment Canada, Trout Reference Method (Environment Canada 2000). A requirement of the method is continuous aeration at a specified rate for the duration of the 96 h trout lethality test. Aeration of effluents can cause an upward drift in the pH of the effluent sample from stripping of carbon dioxide (CO_2). This phenomenon can be significant when testing effluents from municipal wastewater treatment facilities because these effluents may contain high levels of ammonia, which will become un-ionized as pH rises (Servizi and Gordon 1986). An increasing concentration of un-ionized ammonia could make the effluent more toxic to the test fish (Schnell et al. 1990) and would not be indicative of a discharge to the receiving environment. The pH maintained test would solely 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.

Materials and Methods

Test fish were rainbow trout (*Oncorhynchus mykiss*) fingerlings received from Sun Valley Trout Farms (Mission, BC). All fish were maintained for a minimum 2-week period at prescribed conditions and met all health criteria for husbandry of fish identified in the method (Environment Canada 2000). Trout were held under a photoperiod of 16 h light and 8 h dark with a lighting intensity of (mean ± SD) 238 ± 30 lux. Fish were fed at least once a day with a ration of approximately 5% of their body weight. Fish were not fed for at least 16 h prior to testing nor during the test. Mean (±SD) mortality (%) in the trout stock acclimation tank was 0.4 ± 0.3 during the 7 days prior to testing.

Water used 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 daily measurements for seven days prior to each test: temperature 15.0 ± 0.2°C; dissolved oxygen 8.8 ± 0.1 mg/L; conductivity 363 ± 6 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 3.1 L/g of trout per day and there were at least 3.5 L of water in the tank per 10 g of trout fingerlings.

Edmonton municipal wastewater final effluent grab samples were collected with Goldbar treatment facility staff in 20 L containers, which were completely filled to exclude air. Approximately 220 L total volume was collected, and returned to the Prairie and Northern Laboratory for Environmental Testing, Ecotoxicology Laboratory, in Edmonton. The duration of sample storage prior to testing for the Nov 2, Nov 17 and Dec 5, 2005 samples were 70, 21 and 96 h, respectively. Samples were stored at 4 ± 2°C until the test day. Prior to testing, all sample containers were mixed to form one composite sample. The composite sample was then spiked with an ammonium chloride solution. The Edmonton municipal effluent was sampled on three different occasions with initial chemical and physical measurements being made on the composite sample, just prior to trout lethality testing.

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Trout lethality tests including controls, were run in accordance with the Environment Canada Reference Method, Single Concentration Procedure (Environment Canada 2000). In summary, tests were conducted at 15 ± 1°C with a photoperiod of 16 h light and 8 h dark. All tests were static, had three replicates and were performed using approximately 52 L of 100% sample concentration in polyethylene lined aquariums, to which ten fish that had not been fed for at least 16 h were added. Aeration was controlled with a rotometer and was continuous throughout the test at a rate of 6.5 ± 1 mL/min/L 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-h intervals for the duration of the 96 h trout lethality test. Trout lengths and weights were estimated prior to testing to ensure a loading density of less than 0.5 g/L and recorded at the end of testing in compliance with the reference method (Environment Canada 2000). Fish loading densities were maintained at less than 0.5 g/L of test solution.

A recycle procedure test using 100% concentration of Edmonton municipal effluent and a control was run concurrently with each reference method test. The recycle procedure employed an Elite 800 115-volt, aquarium aeration pump that provided continuous aeration throughout the test at a rate of 6.5 ± 1 mL/min/L, controlled with a rotometer. Ten fish were added into each test solution. Each test aquarium, including the aeration pump, was sealed with a 6 mm thick, clear acrylic lid (Fig. 1). The sealed lid created a closed test 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. The headspace was 7,800 cm^3 in the test aquaria. All other conditions remained the same as in the reference method.

Reference toxicant tests following the reference method were conducted using reagent grade phenol. The median lethal concentration to 50% of fish during the 96 h exposure period (LC_{50}) and the corresponding 95% confidence limits, were calculated by probit analysis (Stephan 1977).

Reference toxicant test results were compared with historical data to measure precision of the method (Environment Canada 1990), and judged acceptable if the LC_{50} is within the established warning limits (95% confidence interval).

Results and Discussion

Two reference toxicant tests were analyzed within the same time period as the municipal effluent toxicity tests. Reagent

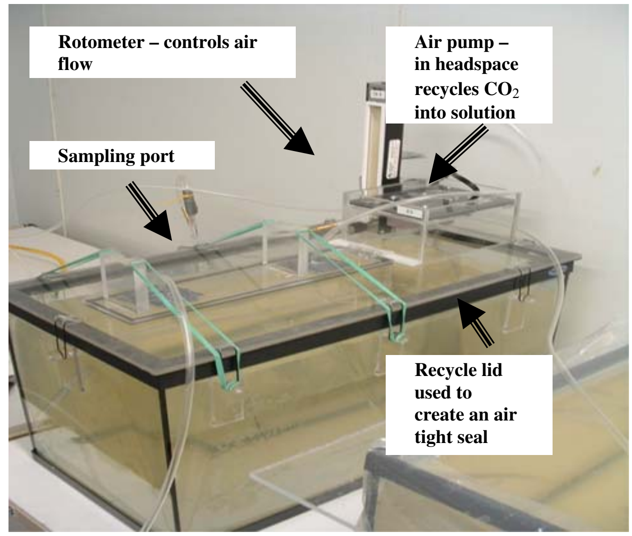

Fig. 1 Recycle procedure design

grade phenol tested in November 2005, produced 96 h median lethal concentrations (LC_{50}) of 10.6 and 12.3 mg/L, respectively. All reference toxicant LC_{50} values fell within the historical warning limits (95% confidence interval), of 8.23–13.1 mg/L. This data indicated that the test fish were suitable for valid acute toxicity testing (Environment Canada 1990).

For each of the municipal effluent sampling dates, the contents of all sample containers were mixed to form one composite sample and the chemical and physical characteristics determined (Table 1). The different samples showed slight variations of all parameters measured, with pH ranging from 7.3 to 7.4. Composite samples of 100% concentration were then spiked with an ammonium chloride solution directly into the composite container and mixed thoroughly before splitting into approximately 52 L used for the reference method test and 52 L used for the recycle procedure test. Loading densities of the ten trout added to each test solution had a mean value of 0.46 g/L.

Physicochemical results (mean ± SD) from testing control water, following the reference method and using the recycle procedure, are presented in Table 2.

Table 1 Edmonton municipal effluent physicochemical measurements

Test date	Composite effluent sample 100% concentration			
	Temperature (°C)	DO[a] (mg/L)	Conductivity (μmhos/cm)	pH
02 Nov 05	14.4	8.4	1,030	7.3
17 Nov 05	15.2	7.9	1,070	7.3
05 Dec 05	14.8	8.5	1,020	7.4

[a] Dissolved oxygen

The values of pH during trout lethality testing using the single concentration procedure in accordance with the reference method are shown in Fig. 2. Edmonton municipal effluent pH increased from an initial range of 7.5–7.6, in three replicate runs, to a final pH range of 8.4–8.5 during the 96 h test, as illustrated in Fig. 2. At the 5% level of significance ($\alpha = 0.05$) the mean pH of the wastewater effluent at 96 h was significantly greater (critical value – 2.015) than the pH at the start of reference method trout testing. It is important to note that the pH drift is most significant during the first 24 h of testing as the pH increased 0.7, 0.8 and 0.8 units in each of the three replicates, respectively.

Trout lethality testing using the recycle procedure determined that pH measurements at the start of the trout test for each of three replicate runs, ranged from 7.4 to 7.6; approximately the same values as found in the reference method (Fig. 2). The mean effluent pH using the recycle procedure was, however, maintained for the duration of the 96-h tests in the range of 7.5–7.6 units (Fig. 2). At the 5% level of significance ($\alpha = 0.05$) the pH at 96 h is equal to the pH at the start of trout testing, using the 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 1.0 and 0.1 units for the reference method and recycle procedure, respectively. At the 5% level of significance ($\alpha = 0.05$), the data indicated that the mean increase in pH during the reference method tests and the recycle procedure

Table 2 Control water physicochemical measurements (mean ± SD)

Physicochemical measurements		Time (h)				
		Start-0 h	24 h	48 h	72 h	96 h
Temperature (°C)	a	15.2 ± 0.1	15.1 ± 0.5	15.1 ± 0.6	15.2 ± 0.2	15.2 ± 0.2
	b	15.5 ± 0.5	15.6 ± 0.3	15.6 ± 0.3	15.9 ± 0.0	15.9 ± 0.3
Dissolved oxygen (mg/L)	a	9.3 ± 0.0	9.0 ± 0.3	9.0 ± 0.3	8.9 ± 0.3	8.9 ± 0.3
	b	9.2 ± 0.1	8.9 ± 0.3	8.9 ± 0.3	8.8 ± 0.2	8.8 ± 0.4
Conductivity (μmhos/cm)	a	365 ± 3	364 ± 3	366 ± 3	365 ± 3	366 ± 2
	b	359 ± 19	367 ± 4	368 ± 4	367 ± 5	370 ± 9
pH	a	8.2 ± 0.1	8.3 ± 0.2	8.3 ± 0.1	8.4 ± 0.1	8.4 ± 0.1
	b	8.1 ± 0.0	8.0 ± 0.1	8.0 ± 0.1	8.0 ± 0.2	8.0 ± 0.2

a Reference method and b recycle procedure

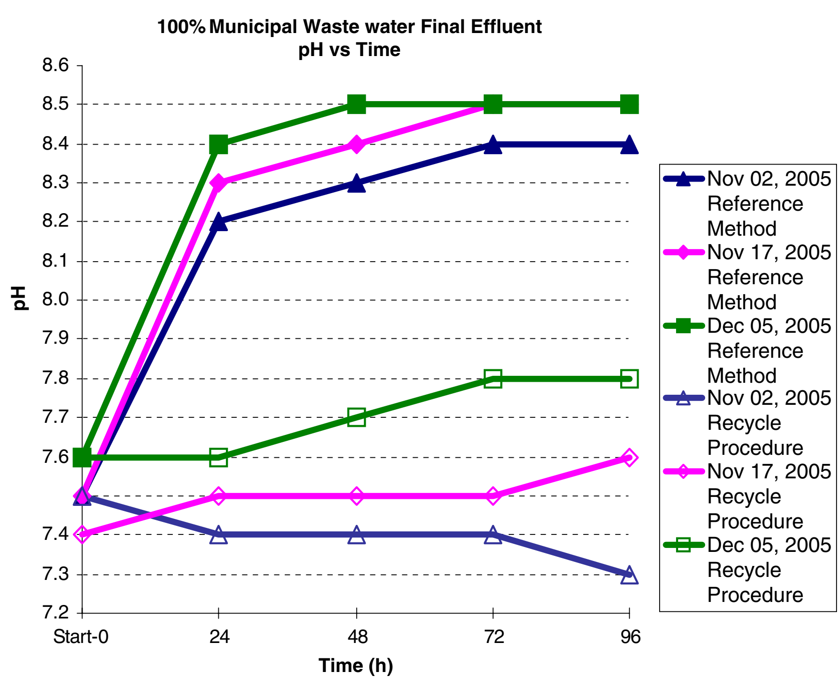

Fig. 2 pH drift during reference method and recycle procedure

Table 3 Edmonton municipal effluent dissolved oxygen (DO mg/L)

Test date		Time (h)				
		Start-0 h	24 h	48 h	72 h	96 h
02 Nov 2005	a	9.3	8.7	8.7	8.6	8.6
	b	9.1	8.6	8.6	8.6	8.4
17 Nov 2005	a	9.3	9.2	9.0	9.1	9.2
	b	9.3	9.0	9.0	8.9	9.1
05 Dec 2005	a	9.3	9.2	9.2	8.9	9.0
	b	9.1	9.1	9.0	8.8	8.8

a Reference method and b recycle procedure

Table 4 Edmonton municipal effluent ammonia concentration (mg/L)

Test date	Composite effluent sample ammonia concentration	
	Non-spiked	Spiked
02 Nov 2005	0.18	16.2
17 Nov 2005	0.45	16.4
05 Dec 2005	3.10	18.1

tests are not equal. This significant difference is illustrated in Fig. 2.

Dissolved oxygen levels were maintained during all 96 h testing with the lowest value at 90.3% air saturation during the recycle procedure on 2 November 2005. The initial values of dissolve oxygen ranged from 9.1 to 9.3 mg/L to a final 96 h dissolved oxygen range of 8.4–9.1 mg/L for the recycle procedure (Table 3).

The Environment Canada Reference Method (Environment Canada 2000), has explicit instructions and conditions for completing toxicity testing, and requires that the test must be conducted without adjustment of sample or solution pH. A pH-adjusted 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. 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). Their (Mount and Mount 1992) study flooded the headspace of a non-aerated test using an external source of CO_2. This study however, used CO_2 stripped from the effluent and reintroduced it back into the effluent with an aeration pump. Toxic municipal effluent with a high total ammonia level showed no acute toxicity when tested under a CO_2-controlled atmosphere.

Previous work using Chemi-Thermo-Mechanical Pulp (CTMP) mill effluent suggest that the CO_2 recycle procedure offers a practical means to evaluate the effects that pH extremes have on toxicity of effluents to trout (Elliott et al. 2003).

In this study, a typical upward pH drift during aeration of the Edmonton municipal effluent during reference method trout lethality testing was observed in all three replicate runs. Effluent pH increased an average of 1.0 units from an initial range of 7.5–7.6 to a final pH range of 8.4–8.5, during the 96 h trout lethality test. This increase in pH could significantly change the mean ratio of un-ionized ammonia from 0.86% to 8.01%, as calculated using the formula by Alabaster and Loyd (1980) and a pK_a of 9.56 for un-ionized ammonia calculations at 15.0°C and zero salinity (Emerson et al. 1975). Ammonia concentrations for spiked and non-spiked effluent samples were measured prior to the start of each test (Table 4). Testing of 100% effluent spiked with approximately 16 mg/L ammonia (ammonium chloride) showed that ammonia toxicity was significantly reduced by pH maintenance.

The municipal effluent spiked with a high ammonia level was determined to be toxic (67% mean mortality) using the reference method, however, no trout mortality was observed when tested using the recycle procedure. The shift in ammonia species to the toxic form may cause some samples that were non-lethal at the time of receipt to become lethal from an upward pH drift during the 96 h trout lethality test.

The recycle procedure tested herein maintained pH of municipal effluent during the 96 h trout lethality test to an observed average increase of 0.1 pH units from the three trials. Analysis of variance (ANOVA) determined that at the 5% level of significance (α = 0.05), the pH increase during the 96 h reference method and recycle procedure tests were significantly different. During recycling of CO_2, dissolved oxygen in the effluent was maintained at an average level of 90.9% air saturation. Dissolved oxygen was therefore held well within the prescribed reference method limits of 70%–100% of air saturation using a rate of 6.5 ± 1 mL/min/L during trout lethality testing. Alabaster et al. (1957) showed that increased concentrations of carbon dioxide requires 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 from 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 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 pH of the effluent was maintained for the duration of the 96-h trout lethality test.

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 the toxicity of municipal effluents, when run in parallel with the reference method for regulatory testing.

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