Microtox™ and *Ceriodaphnia dubia* toxicity of BKME with powdered activated carbon treatment™

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Abstract

This paper compares treatment of bleached kraft pulp mill effluent (BKME) using activated sludge vs. PACT™, with respect to removal of toxicity as measured using two different assays: Microtox™ and *Ceriodaphnia dubia*. Both activated sludge and PACT™ treatment processes were operated over a range of solids and hydraulic retention times using BKME. Various doses of powdered activated carbon were applied in the PACT™ process.

Activated sludge treatment is sufficient to remove nearly all detectable Microtox™ toxicity for bleached kraft pulp mill effluent with initial low to moderate toxicity. Based on the Microtox™ toxicity assay, PACT™ was found to slightly improve the toxicity level of highly toxic bleached kraft pulp mill effluent.

The chronic *Ceriodaphnia dubia* toxicity assay was more sensitive than the Microtox™ assay in determining toxicity. Significant residual chronic toxicity towards *Ceriodaphnia dubia* remained in all bleached kraft pulp mill effluents, irrespective of the treatment process. Both activated sludge and PACT™ remove toxicity, but PACT™ effluents are more toxic. Powdered activated carbon alone shows chronic toxicity towards *Ceriodaphnia dubia*, probably due to physical ingestion of powdered activated carbon particles.

Introduction

Effluents from the pulp and paper industry are complex wastes that are difficult to treat. Toxicity and organic loading of this waste pose a hazard to aquatic organisms. Currently secondary treatment using the aerobic activated sludge process is required for pulp mill wastewater. The powdered activated carbon treatment™ (PACT™) process, is a modification of the activated sludge process in which powdered activated carbon (PAC) is added to the mixed liquor. Biomass grows directly on the carbon particles, thus the mixed liquor suspended solids (MLSS) is a combined mass of carbon and biomass.

PACT™ has been shown to improve the treatment of a variety of toxic wastewaters and a number of advantages of PACT™ over standard AS treatment has been cited (Deitrich et al., 1988; Hutton, 1990; Lankford and Eckenfelder, 1990; Meidl, 1990). These include:

- Improved removal of chemical and biochemical oxygen demand (COD and BOD respectively).
- Stability of operation with variability in influent concentration and composition.
- Enhanced removal of toxic substances and priority pollutants.
- Effective colour removal.
- Improved solids settling.
- Suppressed stripping of volatile organics.

Only one example of applying PACT™ to the treatment of pulp and paper industry wastewater has been reported (Verrault and Depuyt, 1992). However, the particular application was paper making rather than wood pulping. The study reported on the use of a 56 m^3 PACT™ to treat spent cooking liquors from cotton fibre and cellulose fibre pulping at a fine paper mill (18 000 to 25 000 mgCOD/ℓ, and 9 000 to 10 000 mgBOD/ℓ) with a PAC dosage of 1.21 g/ℓ. The authors reported good treatment, 90 to 92% removal of COD and >98% removal of BOD. However, no comparison of PACT™ and activated sludge (AS) was made and the study did not concern itself with toxicity removal. The objective of this paper is to compare the performance of the activated sludge process and the PACT™ process in terms of toxicity removal as measured by Microtox™ and *Ceriodaphnia* chronic toxicity test assays.

Microtox™ and *Ceriodaphnia dubia* toxicity assays

Microtox™ is a patented toxicity assay (Microbics Corporation Carlsbad, CA) that uses a light-emitting marine bacterium, *Photobacterium phosphoreum* and measures the decrease in light output in response to a 5 or 15 min exposure to toxicant. Toxicity of a sample is generally reported as an *effective concentration 50%* (EC_{50}), meaning the concentration of test sample at which there is a 50% reduction in bacterial light production. Values may also be reported as EC_{10} or EC_{20}, the concentrations at which respectively 10% and 20% reductions in light output are observed. Reported EC values are inversely proportional to toxicity, i.e. the lower the EC value, the more toxic the sample. The main advantage of the Microtox™ assay is its speed and convenience as it can be conducted in less than 1 h. Its disadvantage is its relative insensitivity compared to other bioassays.

A number of studies have investigated the use of Microtox™ for evaluating pulp and paper wastewater toxicity (Table 1). Many of these tried to correlate Microtox™ with standard bioassays (i.e., fish and algae) in the hope that the latter more expensive and cumbersome tests could be replaced. Blaise et al. (1987) compared Microtox™ to algal and fish toxicity tests and reported that biological treatment of chemithermomechanical pulp (CTMP) effluent reduced Microtox™ toxicity from about 5% to >100%. Very low toxicity remained with respect to trout, and moderate toxicity with respect to algae. Fish were generally the least sensi-

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Received 20 July 1999; accepted in revised form 9 November 1999.

TABLE 1
TOXICITY REPORTED FOR PRIMARY AND SECONDARY MILL EFFLUENTS

Type	Treatment	Microtox™: 15 min. EC_{50} (%)		*Ceriodaphnia* chronic: NOEC[c] (%) except where noted		Reference
		1° [a]	2° [b]	1°	2°	
BKME	AS				10,30,32,75	Kraus,1990
BKME	none	11.5 to 37				Lavallee et al., 1992
BKME	AS				56	Kraus, 1990
BKME	secondary	~ 17	100	2	75	Firth and Backman,1990
Various	secondary		8,45,60,75,100		2,12,25,43,75, 100	Firth and Backman,1990
BKME	AS			IC25[d]: 1.2 to 10.2 (ave 4.3, 4 mills)	IC25: 1.4 to 83 (ave 26.1, 7 mills)	O'Connor et al., 1992
BKME	AS	7.9	93	0.1	75	Fein et al., 1992

[a] 1° = primary effluent
[b] 2° = secondary effluent
[c] NOEC = No observed effect concentration
[d] IC_{25} = Inhibiting concentration for 25% inhibition of reproduction

tive, Microtox™ was moderate, and algae were most sensitive to toxic agents. They remarked that reliance on a single toxicity test is insufficient and that to accurately assess the total effects of an effluent on the receiving environment, a gamut of tests should be considered.

McLeay (1987) stated that with regard to secondary treated pulp mill effluent, "Microtox™ is relatively insensitive and cannot detect any residual, sublethal activity" and that "... the relative insensitivity of acute lethal (or Microtox™) bioassays renders them of little value in assessing residual toxicity for samples of receiving waters or biotreated ("detoxified") effluents".

Given the insensitivity of the Microtox™ assay to low levels of toxicity, another test is needed to differentiate PACT™ and AS pulp mill effluent treatment. The *Ceriodaphnia* chronic toxicity test assesses the effect of longer-term exposure of toxins on animal reproduction. The advantage is that this test can assess sublethal, chronic toxicity for a complete life cycle in less than 7 d by growing *Ceriodaphnia* in various concentrations of effluent sample while a control group is grown in pure water. The number of offspring produced by animals exposed to each sample concentration is compared to the control group. A reduction in the number of offspring provides a measure of toxicity. Results are typically reported as IC_{50} (inhibiting concentration 50%), that is, the concentration of sample that will cause a 50% reduction in the number of offspring.

Always of concern is the handling and storage of pulp effluent samples used for toxicity testing (Fein et al., 1993). Microtox™ toxicity was unstable if samples were allowed to remain at room temperature for 24 h causing toxicity to decrease from ~15 to 100%, whereas maintaining samples at 4°C resulted in a decrease from only 15 to 35% toxicity after 9 d. Immediate analysis or proper storage is imperative in order to produce valid results.

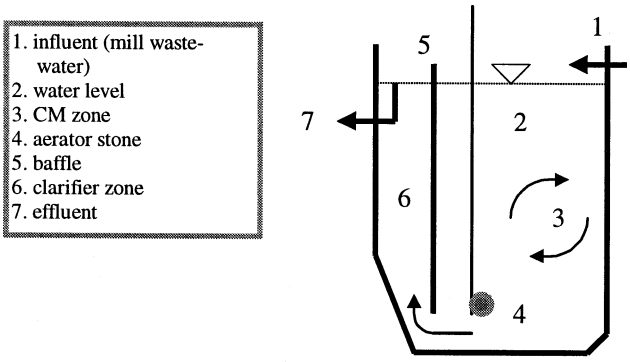


Figure 1
AS and PACT™ reactor schematic, side view. Flow proceeds from right to left.

PACT™ Process

Experimental approach

General

Whole mill BKME was collected in several batches from the James MacLaren Inc. mill in Thurso, Que., which uses wet drum debarking and 11% of softwood furnish. Typical pulp production at the mill was 735 air-dried t/d with water usage of 71 000 m^3/d. The mill used chlorine dioxide bleaching followed by caustic extraction in the following bleach sequence DEDED (D = chlorine dioxide, E = caustic extraction). Wastewater was collected at the pipeline discharge into the mill's treatment lagoons and stored in 200 ℓ plastic barrels, frozen at 20°C. Individual barrels were thawed as needed and wastewater stored at 2ºC prior to use. To mimic primary treatment (minimise suspended solids), thawed samples were settled for 24 h then decanted prior to use. Phosphorus and nitrogen were added to maintain a degradable-COD:N:P ratio of 100:5:1. Phosphorus was added as K_3PO_4 or K_2HPO_4, nitrogen as NH_4Cl or

TABLE 2
REACTOR OPERATING CONDITIONS[a]

HRT (h)	4	8	24	72				
SRT (d)	5	5	10	15	5	10	15	5
PAC (g/ℓ)								
0	CR17,D	CR13,C & CR4,A	CR5,A	CR6,A		CR2,A	CR3,A	CR1,A
0.1		PR7,A	PR9,A					
0.2		PR8,A						
0.5	PR18,D	PR14,C	R16,C					
1.0		PR15,C			PR10,B	PR11,B	PR12,B	

[a] PR# AND CR# refer to PACT™ and control AS reactors respectively
Shading indicates condition tested. Numbers in each box (CR1,CR2,PR12 etc) are unique identifying numbers for each reactor operated at a given condition, following letters refer to feed Batch A, B, C or D.

$(NH_4)_2SO_4$. One sample batch of wastewater was acidic, pH less than 4, and was neutralised to approximately pH 6.7 with KOH. Wastewater samples received no other pretreatment.

Six 5 ℓ completely mixed (CM) activated sludge reactors were operated in continuous mode (at least one was a non-PAC control). Initial inoculum was obtained from a municipal wastewater facility (Outaouais Urban Community Wastewater Treatment Plant, Gatineau, Que.). Thereafter when converting to new operating conditions of hydraulic retention time (HRT), solids retention time (SRT) and PAC dose, reactors were seeded with mixed liquor from previous batches. Wastewater feed was continuously delivered to the reactors with peristaltic pumps. Reactors were a combined CM chamber/clarifier design. The nominal 5 ℓ operating volume of the reactors is defined as the volume in the CM zone when the baffle is completely closed (lowered). Clarifier volume is approximately 2.2 ℓ. Mixing and aeration in the CM zone were provided by a double airstone connected to a filtered air supply. Dissolved oxygen levels in the reactors were maintained above 2 mg/ℓ, with one exception discussed later. Reactors were covered with Plexiglas™ to minimise losses from splatter and evaporation. Reactor design is shown in Fig. 1 and the various operating conditions are shown in Table 2. In total 17 conditions were examined over 18 separate runs.

Because wastewater was collected at four different times, influent feed quality varied over the runs. Each of the four different batches of feed is identified by A, B, C and D. Each reactor run is given a unique number from CR1 to PR18. All reactors in a given feed group received the same feed during that portion of the run which was analysed for determining average COD and solids values. Each data point represents the value of a two-day or three-day composite sample. Sample analyses were based on stability of MLTSS and whether the SRT calculated from the observed solids levels was close to the target nominal value, that is, the portion of the run which was as close to steady state as possible. The PAC used was WPX-Z grade (Calgon Carbon Corporation[1]), produced from reactivated (recycled) granular coal-based carbon. PAC was dried at 105ºC before weighing. Make-up PAC was added to the PACT™ reactors once per day, after the daily solids wasting, as a PAC-effluent slurry.

Analyses

Feed, mixed liquor and effluent were sampled daily for solids and COD. Unless otherwise noted, procedures used were those in *Standard Methods* (1989). Typically two-day and three-day composite samples were measured for solids and COD. Samples were ashed at 550ºC for 2 h to ensure complete volatilisation of PAC. COD measurements employed the ferrous ammonium sulfate titrimetric method. Soluble COD was measured on supernatants of samples centrifuged for 20 min at 12 100 G. Elsewhere the term soluble refers to filtrate from samples filtered through Whatman™ GF/C filters.

Samples for other analyses were composites taken during the final 2 or 3 d of a particular reactor run. Final samples for adsorbable organic halide (AOX), carbonaceous BOD (CBOD), metals and toxicity were blended, filtered and stored at 20ºC. AOX analyses were performed on soluble samples only, using a Dohrman AOX analyser (Seprotech Laboratories, Ottawa, ON). CBOD was the standard 5 d, 20ºC BOD test, using 2-chloro-6-trichloromethyl pyridine as nitrification inhibitor.

PAC dosage as reported is based on the known make-up rate of PAC (i.e. the nominal dosage) and the average HRT during a particular reactor run.

An M500 Microtox™ system as well as reagents and protocols followed were obtained from Microbics Corporation. Data reduction and statistical analysis were performed by Microbics statistical software.

Ceriodaphnia dubia *toxicity assay*

Linda Olde of the Pulp and Paper Research Institute of Canada (PAPRICAN) donated *C. dubia* starter cultures. The protocol used was essentially that of Environment Canada (1992a). Any deviations are noted below.

C. dubia *food preparation*

C. dubia were fed a modified version of the standard, two-part diet of yeast-Cerophyll™-trout chow (YCT) mixture and algae. Following Environment Canada (1992a) recommendations YCT was replaced by a bakers yeast-alfalfa-Tetramin™ (YAT) mixture. YAT was stored frozen in 200 mℓ batches until needed. Thawed

portions of YAT for daily feeding were stored at 4ºC.

For the algal portion of the diet, two types of algal culture *Ankistrodesmus* sp. and *Selenastrum capricornutum* were cultivated and used (Environment Canada, 1992a). Algal cultures were concentrated to an optimum concentration of 3.0 to 3.5 x 10^7 cells/ml prior to feeding to daphnids (Cerda and Olive, 1993). Algal concentrate was stored at 4ºC.

Test conditions

Culture and dilution water was municipal tap water, dechlorinated by autoclaving in 20 ℓ glass carboys for 90 min at 121°C, then cooled and aerated for at least 24 h using a diaphragm aquarium pump.

Samples of untreated pulp mill wastewater and treated reactor effluents were prepared for testing by adjusting for concentration, dissolved oxygen (minimum of 90% saturation) and pH (adjusted to range 7.5±0.3 with HCl or KOH as required). Dilution water, prepared as described above, was used for dilution of test samples. Each test concentration employed 10 neonate *Ceriodaphnia*, one neonate per test cup. Test duration for the required three broods in 60% of control animals was typically 7 d. During the test period, test solutions were renewed daily in each test cup.

Statistical procedures

The accepted statistical method for calculating IC_{50} values is that of probit analysis (Environment Canada, 1990, 1992a, 1992b, Finney, 1964). A probit is equivalent to the normal standard variate $Z+5$ and results are given in terms of Z. The relation between the observed data and Z is as follows:

If N is the number of offspring produced per *Ceriodaphnia* in a test group and N_0 that of the control group, then the standardised variate, Z, is related to reproduction, N/N_0, as follows:

$$\frac{N}{N_0} = \Phi(z) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{z} e^{-u^2/2} du$$

The underlying assumption in this analysis is that the dose-response relationship between the toxicant and test animal follows a log-normal distribution. Extreme values of Z, outside the range -2.5 to 2.5, carry little weight and can be disregarded (Finney, 1964). Points within this range are used to calculate a weighted regression line from which the IC_{50} and corresponding confidence limits are interpolated. IC_{50} values were interpolated from plots of Z vs. the logarithmic sample concentration. The IC_{50} corresponds to the concentration at which Z is zero.

Results and discussion

Microtox™ acute toxicity

Operating conditions and performance of the AS and PACT™ reactors are summarised in Tables 2 to 4. The toxicity of feeds and reactor effluents as measured with the Microtox™ assay is summarised in Table 1. Only EC_{50} values have been reported. For some samples, the EC_{20} and EC_{10} were calculated by the Microtox™ software but were deemed unreliable because of large confidence

TABLE 3
SUMMARY OF AVERAGE OPERATING CONDITIONS, SOLIDS AND COD VALUES FOR REACTOR RUNS[a]

Run	Nominal[b] Conditions	HRT (h)	SRT) (d	Std. dev. (%)	PAC (mg/ℓ)	SCOD in (mg/ℓ)	Std. dev. (%)	SCOD out (mg/ℓ)	Std. dev. (%)	SCOD[f] removal (mg/ℓ)	SCOD % removal	Std. dev. (%)
CR1	A/72/5/0	69.2	5.0	17	0	541	8	303	14	237	44.0	5
CR2	A/24/10/0	24.8	7.5	18	0	583	29	181	21	402	69.0	7
CR3	A/24/15/0	24.1	12.0	7	0	601	34	124	37	477	79.4	8
CR4	A/8/5/0	7.7	5.0	6	0	344	26	114	13	230	66.9	8
CR5	A/8/10/0	7.8	8.9	16	0	344	26	106	19	238	69.3	3
CR6	A/8/15/0	7.9	15.0	8	0	343	25	117	23	226	66.0	9
PR7	A/8/5/0.1	7.7	5.0	3	96	344	26	104	21	240	69.8	9
PR8	A/8/5/0.2	7.8	5.1	5	194	342	24	100	29	242	70.8	10
PR9	A/8/10/0.1	7.9	9.5	6	99	344	26	119	16	225	65.5	10
PR10	B/24/5/1	24.8	4.8	6	1032	566	4	113	17	452	79.9	5
PR11	B/24/10/1	24.9	10.0	10	1038	569	20	141	27	428	75.2	3
PR12	B/24/15/1	23.4	16.3	7	975	554	4	82	14	471	85.1	6
CR13	C/8/5/0	7.8	5.5	10	0	569	35	213	26	356	62.5	5
PR14	C/8/5/0.5	8.3	5.1	3	517	569	35	132	22	437	76.8	17
PR15	C/8/5/1	8.3	5.1	3	1038	606	33	72	30	534	88.1	8
PR16	C/8/10/0.5	8.2	10.8	8	513	569	35	114	22	455	80.0	6
CR17	D/4/5/0	4.1	5.0	9	0	791	24	350	26	442	55.8	13
PR18	D/4/5/0.5	3.8	4.9	4	480	766	25	165	28	601	78.5	5

[a] Averages are taken over the period "analysed days."
[b] Nominal conditions refer to feed batch, and target values for HRT (hr), SRT (d) and PAC dose (g/ℓ) respectively.
[C] FTSS = feed total suspended solids
[d] MLVSS = mixed liquor volatile suspended solids
[e] NM = not measured
[f] $SCOD_{removal} = SCOD_{in} \quad SCOD_{out}$

TABLE 4
SUMMARY OF BOD AND AOX[a]

R	Nominal[b] conditions	Soluble CBOD in (mg/ℓ)	Soluble CBOD out (mg/ℓ)	Soluble CBOD % removal	AOX in (mg/ℓ)	AOX out (mg/ℓ)	AOX% removal
CR1	A/72/5/0	NM[d]	NM		10.3[e]	NM	
CR2	A/24/10/0	355	8.4	97.6	NM	5.3	
CR3	A/24/15/0	355	6.7	98.1	NM	5.0	
CR4	A/8/5/0	NM	NM		4.1	3.3	20
CR5	A/8/10/0	NM	NM		4.1	3.5	15
CR6	A/8/15/0	NM	NM		4.1	3.3	20
PR7	A/8/5/0.1	NM	NM		4.1	3.2	22
PR8	A/8/5/0.2	NM	NM		4.1	2.4	41
PR9	A/8/10/0.1	NM	NM		4.1	2.7	34
PR10	B/24/5/1	289	5.6	98.1	9.8	NM	
PR11	B/24/10/1	289	11.6	96.0	9.8	1.4	86
PR12	B/24/15/1	289	3.7	98.7	10.3[e]	2.1[e]	80
CR13	C/8/5/0	333	4.5	98.6	12.0	5.9	51
PR14	C/8/5/0.5	333	3.1	99.1	12.0	3.2	73
PR15	C/8/5/1	333	2.1	99.4	12.0	1.9	84
PR16[f]	C/8/10/0.5	333	79.3	76.2	12.0	5.5	54
CR17	D/4/5/0	506	11.9	97.6	10.5	8.0	24
PR18	D/4/5/0.5	506	16.7	96.7	10.5	6.4	39

[a] Samples for these analyses were typically composites from the final 3 d of a reactor run.
[B] Nominal conditions refer to feed batch, and target values for HRT (hr), SRT (d) and PAC dose (g/ℓ) respectively.
[D] NM = not measured
[e] AOX analysis on sample taken from middle of run.
[F] PR16 suffered from biomass washout during the final 3 d of the run. Thus performance appears uncharacteristically poor.

intervals. This is to be expected in samples with low toxicity for two reasons. First, the Microtox™ protocol is able to measure toxic response only in samples of 100% concentration. Since EC_{20} or EC_{10} values in samples of low toxicity lie near the upper end of the test range, they are subject to wider confidence intervals. Second, it is difficult to assess whether an observed 10 or 20% reduction in light output in a given sample (a small relative effect), is due to toxicity or normal variation. The measurement of an effect, whose magnitude is the same as the "noise" expected in the analysis, will exhibit poor reproducibility.

An important feature of the data is the range of toxicity in the feed. In the 15 min test data, Feed B was least toxic with an EC_{50} of 25%, Feeds A and C were equal at 14% and D was most toxic at 3.1%. Although it is well known that toxicity roughly correlates with wastewater strength (COD or BOD), organic strength is not strictly predictive of the absolute toxicity of a given sample. The progression of toxicity in Batches A, B and D is parallel to increasing feed strength, 340, 570 and 770 mg/ℓ SCOD respectively. However Feeds B and C, which were of equal strength at 570 mg/ℓ SCOD, had different EC_{50} values, 25 and 14% respectively. Furthermore, although the trend of higher strength/higher toxicity may hold true, it is not a direct relationship. For example, the strength of Batches A and D differs by a factor of 2.3 but their EC_{50} values differ by a factor of 4.5. Nor does the ratio of CBOD to SCOD strongly correlate with toxicity. For Batches A, B, C and D, this ratio was 0.60, 0.52, 0.56 and 0.65 respectively. Once again considering Batches A and D, these showed similar CBOD:SCOD ratios despite having a wide disparity in toxicities. This suggests that variability in feed toxicity reflects not just changes in wastewater strength but in composition as well. It should be noted that often the most important contributors to mill toxicity are one-time events (e.g. spills) rather than on-line processes operating at steady-state. The high toxicity in Feed D may be such a case.

In contrast to organic strength, AOX content of the feed did not correlate with toxicity. This too has been observed in other studies (Graham, 1996). In the present case, Feeds A and C had identical toxicity despite AOX differing by a factor of 3.

The 15 min Microtox™ test is slightly more sensitive than the 5 min test (cf. EC_{50} values for feeds) and is more suited to analysis of low-toxicity samples. For Batches A through C (CR1 through PR16), Microtox™ could not differentiate between the effluents. Out of a total of 30 measurements, 24 produced the same result, an EC_{50} greater than 100%. The 6 samples which did show EC_{50} values of less than 100% had very wide confidence intervals, hence they may well have EC_{50} values greater than 100%. In the majority of cases activated sludge alone was sufficient to remove observable Microtox™ toxicity. For feed Batches A and B, effluent EC_{50} values from all runs were greater than 100% (with only one exception). It is important to understand that an EC_{50} of greater than 100% does not imply that a sample is non-toxic, but rather that the stated response to toxicity could not be detected. Thus these results show only that both AS and PAC treatment removed Microtox™

TABLE 5
MICROTOX™ TOXICITY OF FEED AND REACTOR EFFLUENTS

Reactor		EC_{50} (15 min test)		EC_{50} (5 min test)	
R	**Parameters[a]**	**Feed**	**Effluent**	**Feed**	**Effluent**
CR1	A/72/5/0	14% (12-16)[b]	NM[c]	16% (16-20)	NM
CR2	A/24/10/0		>100%		>100%
CR3	A/24/15/0		>100%		>100%
CR4	A/8/5/0		>100%		>100%
CR5	A/8/10/0	**A**	>100%	**A**	>100%
CR6	A/8/15/0		>100%		>100%
PR7	A/8/5/0.1		>100%		92% (59-141)
PR8	A/8/5/0.2		>100%		>100%
PR9	A/8/10/0.1		>100%		>100%
PR10	B/24/5/1	25% (16-62)	>100%	25% (19-34)	>100%
PR11	B/24/10/1	**B**	>100%	**B**	>100%
PR12	B/24/15/1		>100%		>100%
CR13	C/8/5/0	14% (7-25)	75% (26-217)	17% (11-27)	[d]76% (22-271)
PR14	C/8/5/0.5		91% (60-137)		67% (49-93)
PR15	C/8/5/1	**C**	>100%	**C**	[d]99% (30-327)
PR16	C/8/10/0.5		>100%		>100%
CR17	D/4/5/0	3.1% (3-3.3)	44% (37-53)	5% (4.6-5.5)	46% (39-53)
PR18	D/4/5/0.5	**D**	66% (51-85)	**D**	50% (44-57)

[a] Parameters refer to feed batch, HRT (h), SRT (d) and PAC dose (g/ℓ) respectively.
[b] Values in parentheses are 95% confidence limits calculated by Microtox™ software.
[c] NM = not measured
[d] Estimates. Microtox™ software calculated EC_{50} by extrapolation.

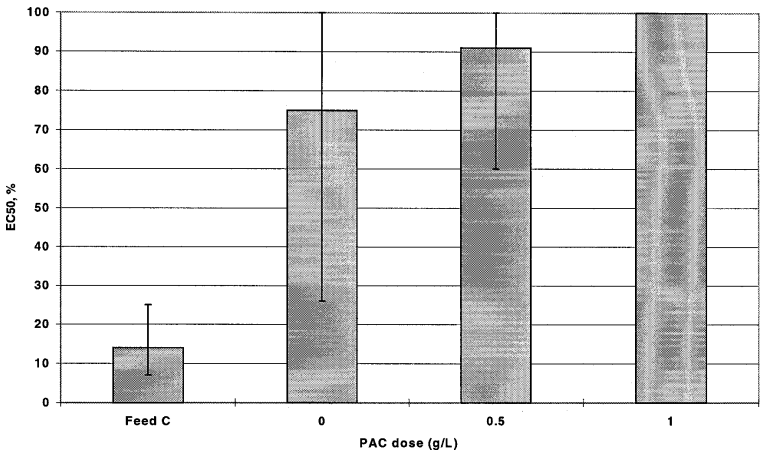


Figure 2
Microtox™ toxicity for feed and effluents of Batch C reactors, 15 min test

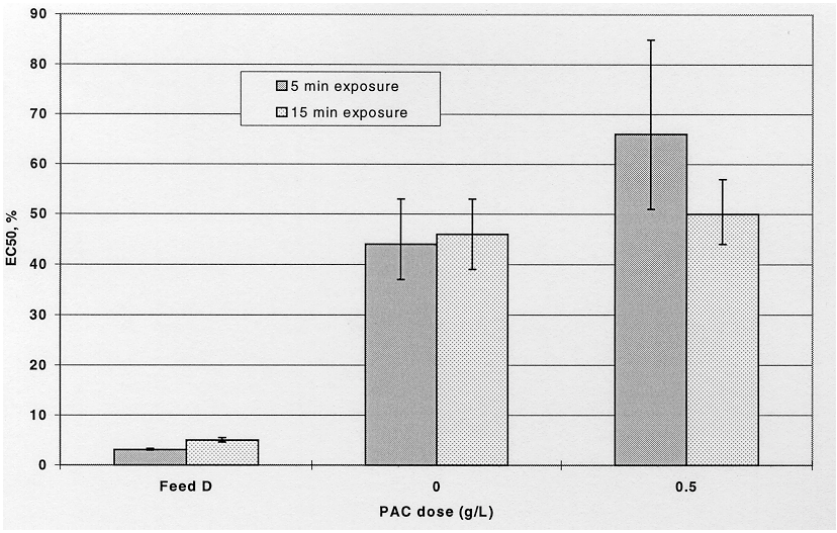
toxicity to non-detectable levels. Any additional improvement by PAC cannot be detected. There may still exist differences in effluent quality between control and PAC reactors, but in these cases Microtox™ sensitivity was insufficient to detect them.

However, feed Batches C and D suggest that PACT™ may differ from AS with respect to toxicity removal. For runs with each of these batches, toxicity was lower in PACT™ effluents compared to AS. Figure 2 describing runs with feed Batch C shows the improvement in effluent toxicity with increasing PAC dose. However, Batch C alone does not produce conclusive evidence because the 95% confidence intervals overlap (Table 5). The most toxic, Batch, D, provides more convincing evidence. Figure 3 shows the same trend of greater toxicity removal with increasing PAC dose, but confidence intervals are tighter. This suggests that PACT™ does indeed improve toxicity removal compared to AS.

Although the Microtox™ assay demonstrates that secondary treatment removes toxicity from pulp mill wastewater, the low sensitivity of the test limits its utility as a means of comparing effluent quality (toxicity removal) among different treatment systems.

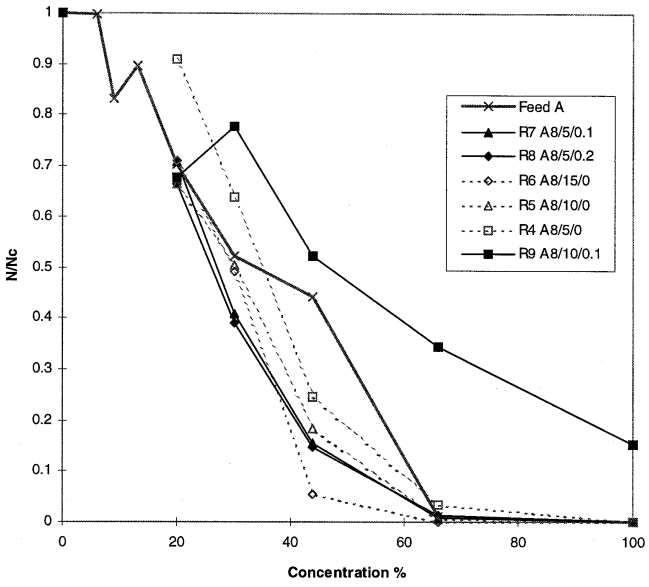
Ceriodaphnia dubia chronic toxicity

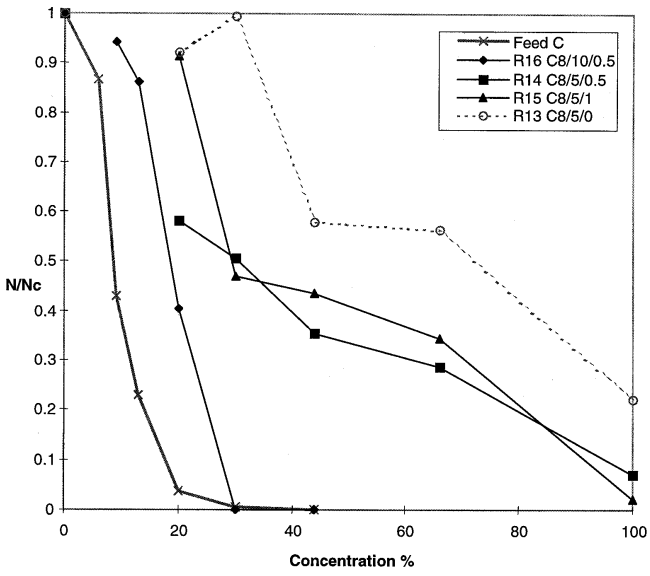
In contrast to Microtox™, the *C. dubia* chronic toxicity assay detected significant residual toxicity in effluents and was able to clearly differentiate between control and PACT™ treated effluents, but results were surprising. Three groups were tested: Batch A is shown in Fig. 4, Batch C in Fig. 5 and Batch D in Fig. 6. These plots show daphnid reproduction as a function of sample concentration. Each line represents the reproduction vs. concentration for a particular feed or effluent. Lines indicate high



toxicity where reproduction decreases sharply (from the control value of 1.0) in response to slight increases in concentration.

IC_{50} values were determined to compare samples. Figure 7 shows probit plots with good linearity, while Fig. 8 shows plots with poorer fit. Recall that the probit method assumes that the toxicity response follows a log-normal distribution, that is, untransformed data should present an approximately sigmoidal response curve (sigmoidal if log concentration is plotted on the abscissa). Examination of the untransformed data in the plots of raw data reveals that the relationship does not fit well at low concentrations. The curves were not always symmetrically sigmoidal, but rather drop sharply at concentrations where toxic response begins. Also, some of the reactor effluent toxicities trail off almost linearly at high concentration. It is difficult to say if such a departure from the classic sigmoid response is anomalous behaviour or whether this is typical for *Ceriodaphnia* assays. Other studies do not report raw data, only final *no observed effect concentration* (NOEC) or IC_{50} values. Many of the data which support the sigmoidal response model are based on single toxin experiments under strictly controlled conditions, e.g. testing of pesticides on insects, or rats. However, in the present case, the substances investigated are complex mixtures of compounds, therefore the toxic response may not be as clear-cut. Nevertheless, because the probit method remains the standard for interpolation of IC_{xx}, it is used here.





Despite the deviation at the extrema, the transformed plots show good linearity in the middle concentration range, which is most important for determination of IC_{50} values. In IC_{50} plots where endpoints departed from linearity, only those values close to 50% were used for interpolation. Extrema have less statistical weight and can be ignored in the analysis (Environment Canada, 1990; Finney, 1964) and the temptation to fit all data to the equation must be avoided.

In order to compare whether differences in *Ceriodaphnia* toxicity were statistically significant, T-tests were performed on reproduction data at each test concentration for each feed batch (Table 6). In the analysis of Batch A, which was the least toxic feed

Figure 3 (top)
Microtox™ toxicity for feed and effluents for Batch D of 4 h HRT reactors (CR17 and PR18)

Figure 4 (middle)
Plot of reproduction vs. test sample concentration for Ceriodaphnia dubia *chronic toxicity assay, Batch A, feed and reactor effluents. (N/Nc is the ratio of offspring produced at a given test concentration vs. the number of offspring produced in the control. Reactor parameters (i.e. A8/5/0.1) refer to feed batch, HRT (h), SRT (d) and PAC dose (g/ℓ) respectively)*

Figure 5 (bottom)
Plot of reproduction vs. test sample concentration for Ceriodaphnia dubia *chronic toxicity assay, Batch C, feed and reactor effluents. (Thick line is feed, thin lines are PACT effluents, dashed lines are control AS effluents. Reactor parameters (i.e. C8/10/0.5) refer to feed batch, HRT (h), SRT (d) and PAC dose (g/ℓ) respectively)*

TABLE 6
T-TEST OF *CERIODAPHNIA DUBIA* CHRONIC TOXICITY ASSAY RESULTS[a]

Reactor		Sample conc. (%)	N/Nc		N	t-stat	t-crit at 5%	Signifi-cant at 5%
R	Parameters		mean	std. dev.				
PR13	C8/5/0	100	0.351	0.152	10			
PR14	C8/5/0.5	100	0.070	0.044	10	5.62	2.10	Yes
PR15	C8/5/1	100	0.020	0.050	9	2.35	2.11	Yes
CR13	C8/5/0	66	0.895	0.280	10			
PR14	C8/5/0.5	66	0.288	0.168	9	5.65	2.11	Yes
PR15	C8/5/1	66	0.345	0.109	10	0.89	2.11	No
PR13	C8/5/0	66	0.895	0.280	10	5.79	2.10	Yes
PR13	C8/5/0	44	0.772	0.317	10			
PR14	C8/5/0.5	44	0.355	0.171	9	3.51	2.11	Yes
PR15	C8/5/1	44	0.455	0.077	10	1.68	2.11	No
CR13	C8/5/0	44	0.772	0.317	10	3.08	2.10	Yes
CR13	C8/5/0	30	1.044	0.322	10			
PR14	C8/5/0.5	30	0.505	0.181	10	4.61	2.10	Yes
PR15	C8/5/1	30	0.471	0.154	10	0.46	2.10	No
CR13	C8/5/0	30	1.044	0.322	10	5.08	2.10	Yes
CR17	D4/5/0	30	0.368	0.159	10			
PR18	D4/5/0.5	30	0.181	0.249	10	2.00	2.10	No
CR17	D4/5/0	20	0.098	0.190	10			
PR18	D4/5/0.5	20	0.526	0.297	10	3.84	2.10	Yes
CR4	A8/5/0	20	0.909	0.222	10			
CR5	A8/10/0	20	0.665	0.175	9	2.65	2.11	Yes
CR6	A8/15/0	20	0.712	0.314	10	0.40	2.11	No
PR7	A8/5/0.1	20	0.709	0.169	10	0.03	2.10	No
PR8	A8/5/0.2	20	0.665	0.168	10	0.57	2.10	No
PR9	A8/10/0.1	20	0.678	0.294	9	0.11	2.11	No
	Feed A	20	0.691	0.381	9	0.08	2.12	No
CR4	A8/5/0	20	0.909	0.222	10	1.54	2.11	No
CR4	A8/5/0	30	0.639	0.195	10			
CR5	A8/10/0	30	0.503	0.178	10	1.63	2.10	No
CR6	A8/15/0	30	0.490	0.260	10	0.13	2.10	No
PR7	A8/5/0.1	30	0.409	0.209	10	0.77	2.10	No
PR8	A8/5/0.2	30	0.389	0.173	10	0.23	2.10	No
PR9	A8/10/0.1	30	0.777	0.191	9	4.64	2.11	Yes
	Feed A	30	0.556	0.351	10	1.67	2.11	No
CR4	A8/5/0	30	0.639	0.195	10	0.65	2.10	No
PR8	A8/5/0.2	30	0.389	0.173	10	3.03	2.10	Yes
	Feed A	30	0.556	0.351	10	1.35	2.10	No
PR9	A8/10/0.1	30	0.777	0.191	9	1.67	2.11	No
CR4	A8/5/0	30	0.639	0.195	10	1.55	2.11	No
CR4	A8/5/0	44	0.245	0.084	10			
CR5	A8/10/0	44	0.182	0.070	10	1.81	2.10	No
CR6	A8/15/0	44	0.054	0.059	10	4.38	2.10	Yes
PR7	A8/5/0.1	44	0.154	0.087	10	2.97	2.10	Yes
PR8	A8/5/0.2	44	0.148	0.084	10	0.15	2.10	No
PR9	A8/10/0.1	44	0.523	0.133	9	7.43	2.11	Yes
Feed A		44	0.442	0.277	10	0.80	2.11	No
CR4	A8/5/0	44	0.245	0.084	10	2.16	2.10	Yes
PR8	A8/5/0.2	44	0.148	0.084	10	2.59	2.10	Yes
Feed A		44	0.442	0.277	10	3.22	2.10	Yes
PR9	A8/10/0.1	44	0.523	0.133	9	0.80	2.11	No
CR4	A8/5/0	44	0.245	0.084	10	5.50	2.11	Yes
CR6	A8/15/0	44	0.054	0.059	10	5.86	2.10	Yes
Feed A		44	0.442	0.277	10	4.33	2.10	Yes
CR5	A8/10/0	44	0.182	0.070	10	2.88	2.10	Yes

[a] The t-statistic shown in each row compares the reactor in that row to the one immediately above. Reactor parameters refer to feed batch, HRT (h), SRT (d) and PAC dose (g/ℓ) respectively.

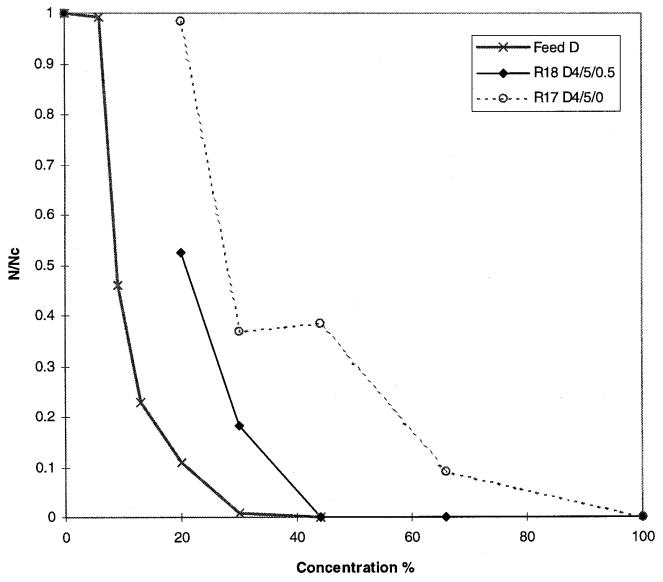
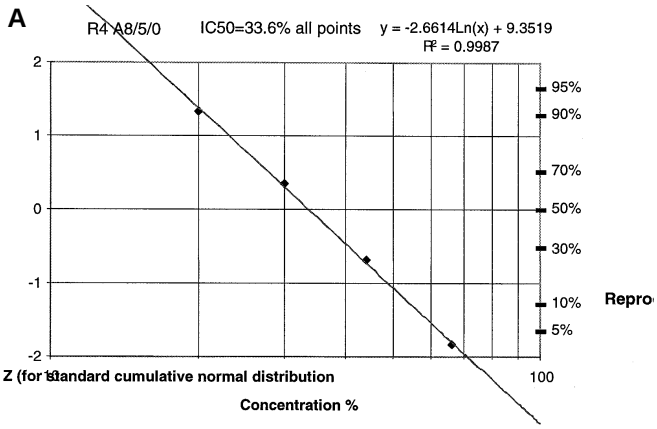


Figure 6

Plot of reproduction vs. test sample concentration for Ceriodaphnia dubia chronic toxicity assay, Batch D, feed and reactor effluents. (Thick line is feed, thin line is PACT effluent, dashed line is control AS effluent. Reactor parameters (i.e. D4/5/0.5) refer to feed batch, HRT (h), SRT (d) and PAC dose (g/ℓ) respectively)



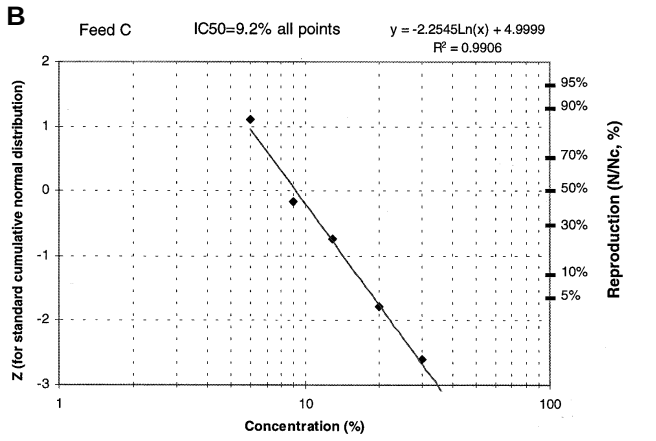
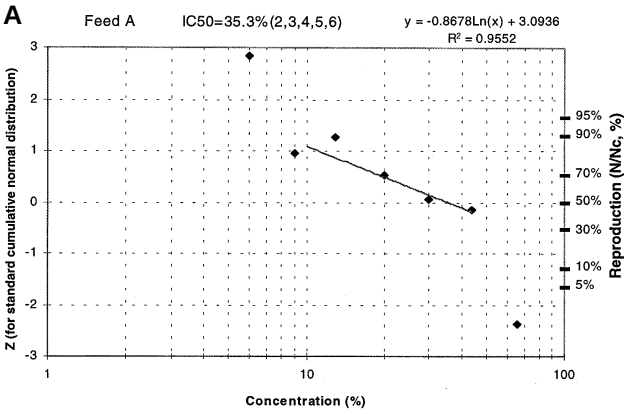


Figure 7

Probability-log (probit) plots for determining IC_{50} of Ceriodaphnia *for run CR4 A8/5/0 (Fig. 7A) and Feed C (Fig 7B),. IC_{50} was interpolated from the regression line shown.*



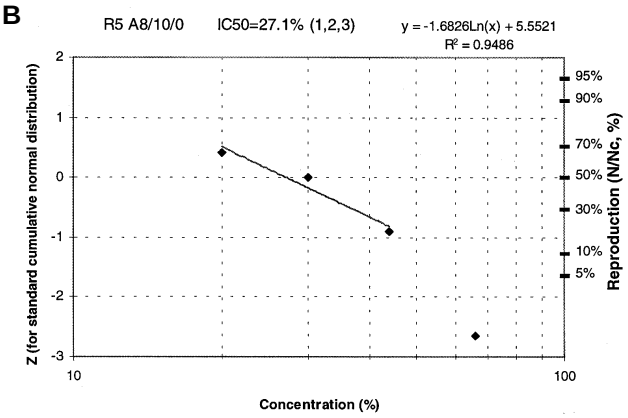


Figure 8

Probability-log (probit) plots for determining IC50 of Ceriodaphnia for Feed A (Fig. 8A) and run CR5 A8/10/0 (Fig. 8B). Numbers in parentheses indicate which data points were included in the regression analysis.

with IC_{50} of 35.3%, the majority of effluent toxicity levels were not significantly different. Five of the six effluent samples tested showed little difference in toxicity. With the exception of PR8 and CR4 at 30%, toxicity in effluents from CR4, CR5, CR6 and PR8 was not significantly different at any concentration tested. Feed toxicity was not significantly different from these effluents at any concentration except at 44%. One reactor effluent, PR9, was noticeably less toxic than the others, particularly at higher concentrations of 66 and 100%. However this same effluent was not significantly different from the feed at lower concentrations. IC_{50} values for Batches A, C and D are summarised in Table 7 and plotted in Fig. 9. No clear trend was observed in Batch A; values were within 10% of one another. These results show that for Batch A there was no appreciable difference in toxicity between control and test reactors, nor indeed between feed and reactor effluents. Batch A had the lowest organic concentration of the four feeds, averaging about 340 mg/ℓ SCOD. Although treatment removed 70% of SCOD, effluent toxicity was equal to that of the feed, suggesting that the wastewater components responsible for toxicity are poorly or slowly biodegradable.

In contrast to Batch A, Batches C and D showed differences in feed and effluent toxicity, but the comparison between control and PAC reactors was surprising. Feeds C and D were considerably more toxic than A, with IC_{50} values of 9.2 and 8.2% respectively. Both control and PAC reactors removed significant toxicity from the feed. In contrast to Feed A, this observation suggests that labile

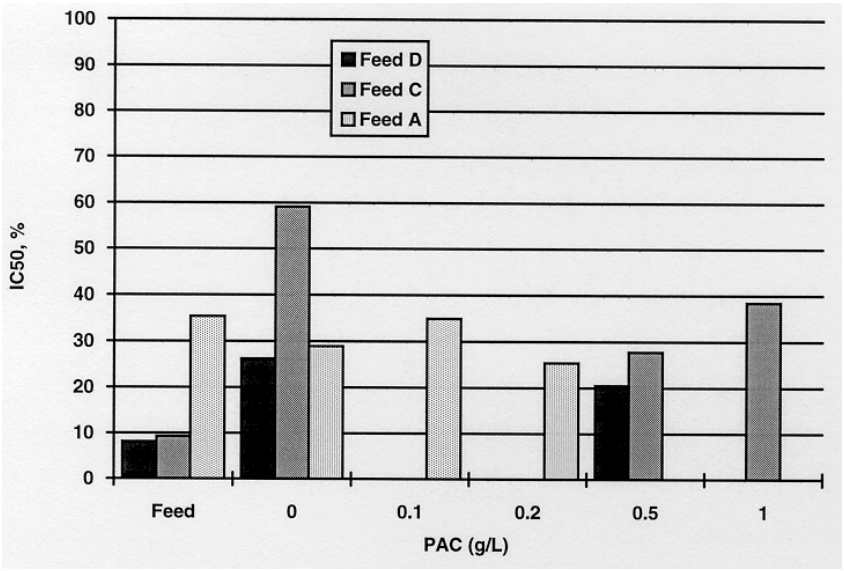


Figure 9
Summary of IC_{50} values for Ceriodaphnia *chronic toxicity assays.(Toxicity reported for Batch A control reactor represents the average of CR4, CR5 and CR6)*

TABLE 7
SUMMARY OF *CERIODAPHNIA DUBIA* CHRONIC TOXICITY ASSAY RESULTS[a]

Reactor		*C. dubia* chronic IC_{50}, %	
R	Parameters	Feed	Effluent
CR4	A/8/5/0	35.3	33.6
CR5	A/8/10/0		27.1
CR6	A/8/15/0		26.1
PR7	A/8/5/0.1		26.5
PR8	A/8/5/0.2		25.4
PR9	A/8/10/0.1		43.2
CR13	C/8/5/0	9.2	59.1
PR14	C/8/5/0.5		27.8
PR15	C/8/5/1		38.4
PR16	C/8/10/0.5		18.9[b]
CR17	D/4/5/0	8.1	26.2
PR18	D/4/5/0.5		20.5

[a] The IC_{50} values were determined by interpolation of probability-log plots.[b] PR16 suffered washout. Toxicity probably not representative for operating conditions.

toxins are also present in the wastewater and these can be removed by biodegradation.

Considering Batch C first, note that PR16 effluent toxicity was high relative to the other effluents. Recall that PR16 produced generally poorer quality effluent (Tables 2 and 3) due to washout, therefore toxicity observations are probably not representative for this reactor. Despite this, the IC_{50} of PR16 effluent was 18.9%, still less toxic than the feed. The surprising result for Batch C is that the control reactor, not PAC reactors as expected, produced the best quality effluent. PAC reactors PR14 and PR15, operating with 0.5 and 1.0 g/ℓ PAC doses, respectively, produced effluents that were not significantly different from one another over the concentration range of 30 to 60%. The IC_{50} values were at the low end of this range, 27.8 and 38.4% respectively, implying a greater difference than what the data suggest as a whole. The control reactor effluent was least toxic with an IC_{50} of 59.1%. Differences observed between the two test reactors with respect to the control were significant at all concentrations above 20%, and the control reactor effluent toxicity was lower at all concentrations.

Results from Batch D confirm the observations for Batch C. Both the test reactor, at 0.5 g/ℓ PAC dose, and the control reactor removed toxicity, but the control effluent was least toxic. Control effluent IC_{50} was 26.2%, less toxic than that for the PACT™ reactor effluent at 20.5%. As in Batch C, control effluent was less toxic at all concentrations below 100%, and differences between control and test effluent at three of the four sample concentrations were statistically significant (at 20, 40 and 66%). The data from Batches C and D demonstrate that PACT™ effluents are more toxic than control effluents, as measured with the *Ceriodaphnia dubia* chronic toxicity assay.

The observation that PACT™ effluents have greater toxicity seems paradoxical in light of other data. Recall that the Microtox™ results implied that treatment of highly toxic wastewater with high PAC doses of 0.5 to 1 g/ℓ improves toxicity reduction. The SCOD data also showed that high PAC doses increase COD removal, which is known to correlate with toxicity. Despite this, PAC reactor effluents clearly showed more toxicity towards *Ceriodaphnia*. This suggests that PAC itself may be responsible for toxicity towards *Ceriodaphnia*.

A test was conducted to assess what effects, if any, virgin PAC alone had on *Ceriodaphnia*. Three groups of daphnids were grown and compared for reproduction, as in the standard chronic toxicity test protocol. A control group, grown in plain dilution water, was compared with groups grown in (i) the same water with 0.1 g/ℓ virgin PAC added and (ii) GF/C filtrate of the 0.1 g/ℓ virgin PAC solution prepared in (i). GF/C filter paper has a nominal pore size of 1.2 μm and is the same filter paper used to prepare effluent samples for toxicity testing. Results are shown in Fig.10. Differences in test groups were statistically significant. Reproduction among daphnids grown in 0.1 g/ℓ PAC was markedly depressed, only 42% of that in the control. Additionally, microscopic examination revealed that 60% of these animals had accumulated deposits of a black material in their ventral cavities. Deposits were not observed in the controls, suggesting that the material ingested was PAC. Reproduction among animals grown in the GF/C filtrate was also significantly reduced, only 73% of the control value. Unlike

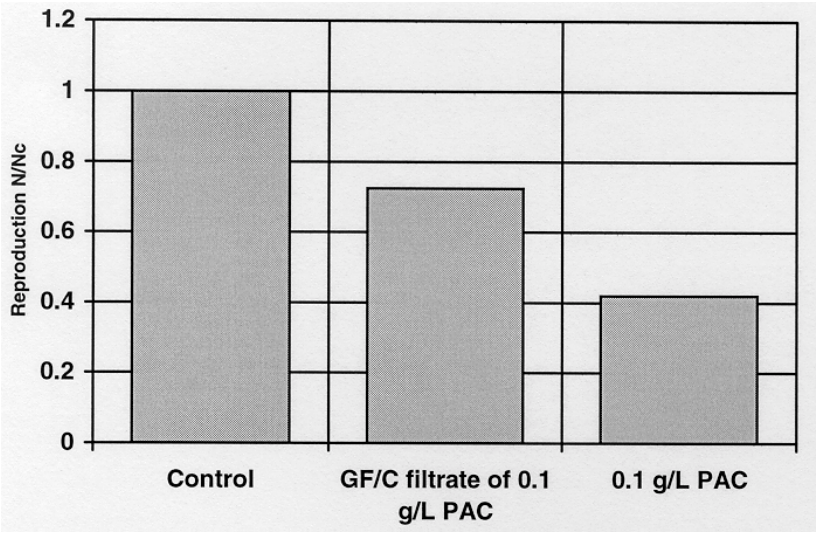


Figure 10
Comparison of the effects of PAC alone on Ceriodaphnia *chronic toxicity assay*

the other test group, no accumulation of particles was observed in these daphnids. Further filtration of GF/C filtrate across 0.45µm pore filters revealed that some PAC was present. It is clear from these observations that PAC has a toxic effect on *Ceriodaphnia*. The data suggest that the toxicity is primarily due to direct ingestion of PAC, rather than uptake of some soluble component of PAC released into the water, because the soluble fraction is the same in each test solution, they differ only in the amount of PAC removed on the filter. Indeed, activated carbon treatment is recommended as a method of removing chlorine from tap water used for culturing of daphnids, so it seems unlikely that soluble components of PAC are toxic. In light of the other results demonstrating PAC's improvement of effluent quality (i.e. COD, AOX, Microtox™), PACT™ may in fact reduce the soluble portion of toxicity but any beneficial effects are masked by the physical action of PAC on *Ceriodaphnia*. Further tests with smaller pore size filtrates could answer this question. But whether the mechanism, which underlies PAC toxicity, is physical or chemical is perhaps irrelevant. The practical significance is that GF/C filtrate represents a better than best case scenario in terms of solids removal from effluent, a level not likely to be achieved in a clarifier, yet toxicity remained in PACT™ effluent. Tertiary filtration may be required.

Conclusions

In most cases, Microtox™ is not sensitive enough to assess the efficiency of toxicity removal in secondary treated kraft mill effluents. AS treatment is sufficient to remove nearly all detectable Microtox™ toxicity for wastewaters of low to moderate toxicity. Compared to AS, PACT™ slightly improves treatment of highly toxic wastewaters.

Significant residual chronic toxicity towards *C. dubia* remains in all effluents, irrespective of treatment type. Both AS and PACT™ remove toxicity, but PACT™ effluents are more toxic, probably due to PAC ingestion.

PAC alone shows chronic toxicity towards *C. dubia*, probably due to physical ingestion of PAC.

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