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Toxicity Testing of Refinery Wastewater Using Microtox

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The disposal of municipal and industrial wastewaters without adequate treatment into receiving waters has resulted in a reduction in the assimilative capacity of these receiving waters. Through enforcement of laws and regulations, the regulatory agencies are changing this scenario slowly. Many regulations now require reduction in toxicity from an industrial effluent. Effluent aquatic toxicity is becoming a general pollutant like biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total suspended solids (TSS). The two approaches that are used for identifying a toxic stream are specific chemical analysis and the use of biological organisms (bioassays). The use of bioassays, such as Microtox is on the rise in recent years. Till date, conventional parameters such as BOD and TSS are used to determine effluent standards. Often they do not reflect the toxic nature of the wastewater. For example, Huber et al. (1979) could not get a significant correlation between effluent toxicity based on bioassay results and parameters such as ammonia, oil and BOD for a coastal refinery effluent. Maynard (1989) indicated the possibility of using “surrogate parameters” such as oil and grease, total suspended solids, nitrate, ammonia and total phenols to assess effluent quality, provided a large database with the surrogate parameter at varying degrees of effluent quality was developed. But Backman and Firth (1990) with a limited database, established a significant statistical relationship between a few parameters of pulp and paper mill effluent such as adsorbable organic halogens (AOX) and a bioassay using rainbow trout.

Multivaried products and demand result in significant differences in the quantity and quality of different wastewater streams within an industry such as a petroleum refinery. In such a case, a large database containing surrogate parameters may not help in establishing statistical correlations between surrogate parameters and toxicity analysis using a bioassay even in a particular industry. This was realized in studies conducted on refinery effluents. by the Petroleum Association for Conservation of the Canadian Enviromnent (PACE) (1985a). Therefore, it was decided to investigate this aspect further in relation to toxicity of a few wastewater streams in a local refinery.

MATERIALS AND METHODS

Wastewater used in this study was collected at three different locations in the wastewater treatment plant of Consumer’s Co-operative Refinery Ltd, Regina, Canada. These locations (Figure 1) are (a) influent to American Petroleum Institute (API) separator. (b) effluent from API separator and (c) stabilization pond eflluent or the final effluent discharged to Regina municipal sewer system. Samples were collected once a week, for a period of 11 weeks during January to April 1995.

Microtox is a bacterial bioassay which is based on monitoring changes in natural light emissions from a luminescent bacterium (*Photobacterium phosphoreum*). Toxicity and end point was measured as the effective concentration of a test sample that causes a 50% decrease in light output

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(IC_{50}) usually after 30 minutes of contact time. Except for the stabilization pond effluent samples, samples at the other two locations were prepared by primary dilution (dilution factor (1:10) and adjusted to an initial concentration of 4.5%. The stabilization pond effluent samples were tested at an initial concentration of 45%. All the three samples were concentrated using a centrifuge (300 rpm for 3 to 5 minuutes) to prevent interference of suspended particles during the bioassay. Experiments were run according to standard procedures (i.e.) duplicate basic test, as recommended in the Microbics operating manual (1992).

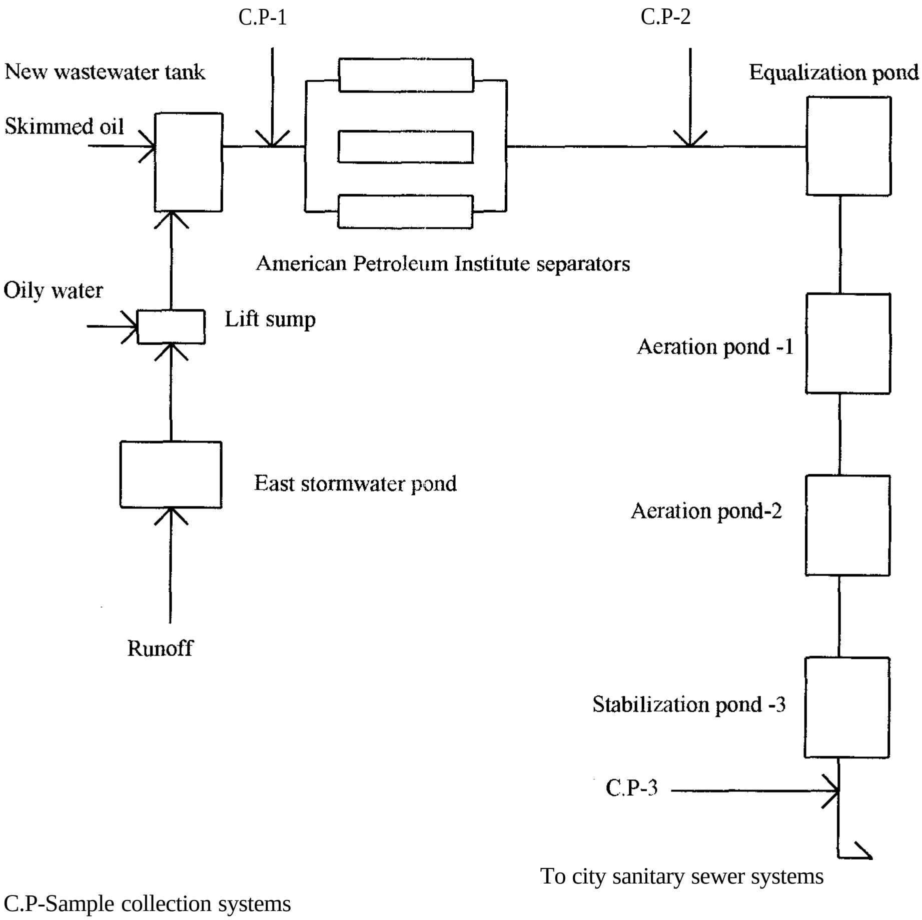

Figure 1. Wastewater collection and treatment system in Consumer's Co-operative Refinery Ltd, Regina, Canada.

The wastewater analysis involved the determination of pH, TSS, total dissolved soilds (TDS), COD, ammonia nitrogen and total petroleum hydrocarbons (TPH). The pH of the wastewater samples was determined using a Fisher Accumet pH meter (model 620) manufactured by Fisher Scientific Ltd. Total ammonia nitrogen was measured using a titrimetric method after preliminary distillation (APHA, 1992). Distillation was done in a 1002 distillation unit of kjeltec system manufactured by Tecator, Hoganaas, Sweden. Chemical oxygen demand, TDS and TSS of the samples were determined as per procedures 5220-C, 2540-C and 2540-D respectively described in "Standard Methods"(APHA, 1992). Soluble oil and grease identified as TPH in this study were measured using a Horiba-400 oil analyzer with an integral extractor. manufactured by Horiba

Instruments Inc., Irvine, California, USA. Carbon tetrachloride was used as a solvent in TPH analyses in view of its high extraction efficiency.

RESULTS AND DISCUSSION

The characteristics of refinery wastewater samples are provided in Table 1. Most of the wastewater samples, in particular effluents from API separator and stabilization pond had pH and COD which were found to be similar to those reported in other studies. Generally appreciable removal of SS is expected to be associated with API separators. Total suspended soilds content of samples observed in this study showed that the performance of API separators was not consistent. Similarly. the performance of API separators in the removal of TPH was also found to be not consistent during the sampling period. The expected reduction (> 60%) reported by Environment Canada (1990) was achieved only in two samples. In 6 of the 10 samples, an increase (10-20%) in NH_3^+N concentration was observed between the effluents of API separator and stabilization pond. This type of inconsistency may be related to the capacity of aerators set up in the aeration pond. The oxygen introduced could have been used mainly in the oxidation of carbon compounds.

Table 1. Characteristics of refinery wastewater samples

Wastewater sample	Characteristics	Range[a]	Mean[a]	Standard deviation[a]
Influent to API separator (CP-1)	pH	5.1-7.5	6.3	0.8
	COD	439-2390	1591	717
	TSS	40-633	375	231
	TDS	2390-11543	5148	3474
	NH_3-N	7-16	11	3
	TPH	24-558	350	223
Effluent from API separator (CP-2)	pH	5.2-7.5	6.5	0.7
	COD	239-2145	1290	605
	TSS	40-533	272	181
	TDS	1753-11480	4794	3420
	NH_3-N	7-16	11	4
	TPH	14-438	193	170
Effluent from Stabilization pond (CP-3)	pH	5.8-6.7	6.05	0.3
	COD	95-222	141	46
	TSS	10-130	45	35
	TDS	1430-13993	4960	3693
	NH_3-N	9-15	12	2
	TPH	5-13	9	3

[a]All values are in mg/l except for pH.

Different toxic patterns were observed in samples from the three locations (Table 2). On many occasions, the effluent from the API separator was found to be more toxic than the influent. More than half of the stabilization pond samples produced an IC_{50} of less than 50%. In a study which included chemical fractionation, Hansen (1989) reported that final effluent toxicity could be attributed to organic constituents. Statistical evaluation of data from Hansen's study indicated a mean toxicity (20 min IC_{50}) of 29 percent effluent with a standard deviation of 12 % whereas the mean toxicity (30 min IC_{50}) was 40% effluent with a standard deviation of 16% for the final effluent in the present study. Apart from the differences in types of effluents, the other reason for this difference could be the contact time (20 min), type of sampling (24h composite sampling) and number of samples (34) in Hansen's study compared with a contact time of 30 min, grab sampling and 11 samples in this study. The characteristics of the effluent from the present study were found to be almost similar to a secondary refinery effluent from another study where an inhibition value of 12.4±1.9 % was observed for an enzyme bioassay (G6PDH) (Armant et al.1980).

Table 2. IC_{50} (in %) of the various refinery wastewater samples

Week Numbers	Date of sample collection	Contact time between reagent and sample (minutes)	IC_{50} (in %) (CP-1)	CP-2	CP-3
1	Jan 17/95	1	4.53	1.48	32.34
		15	4.85	1.72	29.99
		30	4.89	2.02	30.18
2	Jan 25/95	1	4.76	7.75	57.96
		15	4.35	6.97	47.07
		30	4.89	7.01	36.61
3	Feb 01/95	1	2.48	3.03	30.83
		15	2.79	3.12	27.16
		30	3.05	3.42	27.73
4	Feb 09/95	1	2.46	2.45	34.72
		15	2.61	2.77	27.92
		30	2.65	3.39	28.99
5	Feb 17/95	1	2.27	2.30	12.79
		15	2.30	2.16	25.93
		30	2.60	2.50	26.63
6	Mar 23/95	1	1.90	3.02	61.86
		15	2.77	3.19	57.75
		30	2.58	3.48	56.36
7	Mar 30/95	1	2.42	3.21	52.61
		15	2.67	3.24	55.38
		30	2.60	3.60	52.48
8	Apr 06/95	1	2.76	1.74	68.34
		15	2.55	1.74	61.90
		30	2.48	1.97	56.63
9	Apr 13/95	1	1.28	1.78	39.08
		15	1.45	1.94	20.32
		30	1.40	2.22	12.83
10	Apr 17/95	1	0.86	0.98	72.74
		15	0.76	0.91	71.90
		30	0.74	0.86	63.26
11	Apr 27/95	1	1.50	1.57	55.42
		15	1.38	1.32	52.32
		30	1.49	1.40	50.31

CP-1 -influent to API separator; CP-2- effluent from API separator; CP-3- effluent from stabilization pond

The collected data were statistically analyzed to identify the existence of any relationship between wastewater characteristics and Microtox IC_{50} values. A software package "Statistica" produced by Statsoft™, Inc. for Windows was used to analyze data by a simple regression analysis. 't' statistic test was used for analysis and the statistical parameter of interest was prob >F (P value for F statistic). Prob > F is the 'P' value for the 'F' statistic which indicates that there would be a significant linear relationship at 0.05 level of significance (Dowdy and Wearden, 1985). Tables 3, 4 and 5 show the results of statistical analysis between refinery wastewater parameters and their corresponding Microtox IC_{50} values combining the data from all the eleven samples in each case. Of the six wastewater parameters, one or two exhibited a strong relationship with toxicity values. None of them was found to be associated with API separator effluent. Among the four parameters that exhibited a relationship with toxicity. COD and NH_3^+N were strongly identified with both API influent and waste stabilization pond effluent. This could be seen from their correlation values and significance at all three exposures. Though pH could not be considered a true reflector of wastewater toxicity in this case. association between pH of stabilization pond effluent and toxicity was observed. Though TSS was identified to be an indicator of the toxicity of API influent, this relationship could raise some doubts due to past records related to TSS in the refinery effluent. Vignon et al. (1989) observed at a wastewater treatment system for a military munitions facility particulate toxicity to be a major factor in toxicity reduction evaluation efforts. It was found that secondarily precipitated aluminum floc was responsible for the observed particulate toxicity. The idea of TSS as an indicator of toxicity has not been suggested anywhere, because inconsistency in processes and bad house keeping could result in a frequent fluctuation in the original concentration. In the refinery under discussion, the sump which receives process wastewaters is also an occasional recipient of storm water.

Table 3. Application of wastewater quality parameters for predicting toxicity response (Influent to API separator)

Linear regression statistics for bioassay test [a]	Wastewater quality parameters for influent to API Separator						
Microtox (acute) IC_{50}	pH	COD[b]	TSS[c]	TDS[d]	NH_3-N[e]	TPH[f]	NH_3[g]
<u>1min exposure</u>							
r	0.24	0.62	0.61	0.08	0.54	0.55	0.39
F	0.47	4.43	4.04	0.05	3.29	3.0	0.90
t-ratio	0.69	-2.11	-2.01	0.21	-1.81	-1.73	0.95
Significant($p \leq 0.05$)[h]	...	...	...	...	...	...	...
<u>15min exposure</u>							
r	0.19	0.69	0.73	0.02	0.62	0.68	0.23
F	0.3	6.18	8.08	0.002	5.08	5.91	0.28
t-ratio	0.55	-2.49	-2.84	0.05	-2.25	-2.43	0.53
Significant($p \leq 0.05$)[h]	...	√	√	...	√	√	...
<u>30 min exposure</u>							
r	0.2	0.7	0.75	0.08	0.64	0.66	0.34
F	0.35	6.7	8.8	0.06	5.44	5.35	0.63
t-ratio	0.59	-2.59	-2.97	0.24	-2.33	-2.31	0.80
Significant($p \leq 0.05$)[h]	...	√	√	...	√	√	...

[a] Microtox values expressed as % toxicity values for correlation with wastewater quality parameters

[b]Chemical oxygen demand; [c]Total suspended solids; [d]Total dissolved solids; [e]Ammonia Nitrogen NH_3^+N

[f]Total petroleum hydrocarbons; [g]Unionized ammonia; [h]Check mark indicates regression statistically significant at the 5 percent level.

Table 4. Application of wastewater quality parameters for predicting toxicity response (effluent from API separator)

Linear regression statistics for bioassay test [a]	Wastewater quality parameters for effluent from API separator						
Microtox (acute) IC_{50}	pH	COD [b]	TSS [c]	TDS [d]	NH_3-N [e]	TPH [f]	NH_3 [g]
1min exposure							
r	0.32	0.43	0.49	0.17	0.29	0.63	0.76
F	0.92	1.61	2.22	0.24	0.71	4.64	8.10
t-ratio	0.96	-1.27	-1.49	0.48	-0.85	-2.16	2.85
Significant($p \leq 0.05$) [h]	...	...	...	...	...	...	√
15min exposure							
r	0.25	0.48	0.55	0.21	0.36	0.63	0.71
F	0.54	2.12	3.0	0.38	1.18	4.67	6.0
t-ratio	0.73	-1.46	-1.73	0.62	-1.08	-2.16	2.45
Significant($p \leq 0.05$) [h]	...	...	...	...	...	...	√
30 min exposure							
r	0.18	0.55	0.60	0.25	0.40	0.63	0.65
F	0.26	3.0	3.87	0.52	1.53	4.56	4.49
t-ratio	0.51	-1.73	-1.97	0.62	-1.24	-2.14	2.12
Significant ($p \leq 0.05$) [h]	...	...	...	...	...	...	...

Table 5 Application of wastewater quality parameters for predicting toxicity response (efflluent from stabilization pond)

Linear regression statistics for bioassay test [a]	Wastewater quality parameters for effluent from stabilization pond						
Microtox (acute) IC_{50}	pH	COD [b]	TSS [c]	TDS [d]	NH_3-N [e]	TPH [f]	NH_3 [g]
1min exposure							
r	0.82	0.66	0.001	0.53	0.73	0.06	0.85
F	16.62	5.48	0	3.11	8.85	0.03	17.68
t-ratio	4.08	2.34	-0.004	-1.76	2.98	-0.16	4.21
Significant($p \leq 0.05$) [h]	√	√	...	...	√	...	√
15 min exposure							
r	0.67	0.76	0.25	0.73	0.73	0.08	0.73
F	6.43	9.41	0.47	9.12	9.37	0.05	7.78
t-ratio	2.54	3.07	-0.69	-3.02	3.06	-0.22	2.79
Significant($p \leq 0.05$) [h]	√	√	...	√	√	...	√
30 min exposure							
r	0.53	0.76	0.4	0.81	0.72	0.72	0.61
F	3.12	9.73	1.32	15.64	8.56	8.56	4.07
t-ratio	1.77	3.12	-1.15	-3.95	2.93	2.93	2.02
Significant($p \leq 0.05$) [h]	...	√	...	√	√	...	...

Note for Tables 4 and 5

[a] Microtox values expressed as % toxicity values for correlation with wastewater quality parameters;
[b] Chemical oxygen demand; [c] Total suspended solids; [d] total dissolved solids; [e] Ammonia Nitrogen NH_3^+N;
[f] Total petroleum hydrocarbons; [g] Unionized ammonia, [h] Check mark indicates regression statistically significant at 5 percent level.

Significance of ammonia as a toxicity contributor in wastewater depends mostly on the concentration of un-ionized form, which is dependent on pH and temperature. Realizing the toxic contribution of this parameter in aquatic streams, USEPA (1976) established a limit of 0.02 mg/L for unionized ammonia in aquatic environment for temperatures above 5°C and pH values below 8.5. Unionized ammonia concentration far exceeded this criterion in 6 out of 10 samples collected from the stabilization pond. Surprisingly more than 50% of stabilization pond effluent IC_{50} measured in this study did not seem to reflect the lethality of ammonia; based on a number of earlier studies, the Microtox test was Found to be quite sensitive to toxicants in fresh water medium although the test uses a marine bacterium. Cote reported (1976) an increase in toxicity of ammonia when pH rose from 7 to 8. Although there was marginal increase in pH between samples la and 2a and substantial rise in NH_3^+N concentration reflected in their respective unionized ammonia concentrations (0.003 and 0.14 mg/L NH_3), distinct difference in toxicity values between these two samples was not observed. However, the results from the study showed that ammonia may be recognized as an indicator of refinery wastewater (influent to API separator) toxicity.

Higher concentrations of TPH mean sufficient amount of napthenic acid or other unsaturated straight chain/cyclic acids in wastewater. which would certainly give an idea about its lethality irrespective of any bioassay. Reasonable statistical values (r=0.68 and 0.75, F=5.9 and 5.4 at 15 and 30 min IC_{50}) for API separator influent showed a linear relationship, thus raising the possibility of TPH as a surrogate for refinery effluent toxicity. Statistically TDS was found to be a surrogate for stabilization pond effluent toxicity only at 15 and 30 minutes exposure. All the three wastewaters from the refinery selected for this study were found to be toxic. It is natural for the wastewaters that are from the head of a treatment scheme to have high toxic strength. Statistical results have shown that conventional parameters of the refinery wastewater can be used to determine its toxic nature, Microtox seemed to be an adequate screening tool for a quick evaluation of the nature of the refinery wastewater.

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