Microtox®

Using Microtox® to ensure the Quality of Treated Effluents

MODERN WATER
A Deepverge Company

Abstract

Microtox is a valuable tool in managing plant operations to achieve and maintain effluent quality and to remain in compliance with discharge permit limits.

This guide describes how Microtox is effectively used to screen and monitor the quality of treated effluents and establish a Compliance Probability Range.

On the Microtox LX software there are two methods recommended for use with treated effluents: the Microtox Comparison Test and the Microtox Screening Test, either of which may be used to establish the appropriate operating goal: a Compliance Probability Range. The characteristics and data interpretation of these methods are covered, along with examples of data sheets and charts used to generate baseline monitoring data and establish the upper and lower limits of the Compliance Probability Range.

One waste treatment facility in the Midwest established their site-specific Compliance Probability Range. As a result of managing plant operations to achieve effluent quality that falls within this range, they have greatly increased their level of assurance in maintaining compliance with their discharge permit's toxicity limits. They now routinely pass their compliance bioassays where once they rarely did.

This increased level of assurance in acceptable effluent quality is derived from the high degree of correlation between the response of the Microtox test and the results of conventional bioassays.

Preface

Modern Water Inc. developed the Microtox Comparison Test to provide easy to perform tests with rapid and economical results. The development of these methods was spurred primarily by the need for a highly sensitive screen to monitor and evaluate the quality of effluents currently produced by municipal and industrial wastewater treatment plants.

These tests have been designed to provide very sensitive endpoints. This increased sensitivity allows for the measurement of small bioreactive responses which can alert the operating staff to any adverse changes in the treatment process, or indicate how well the plant is treating the effluent.

The Compliance Probability Range

One can establish site specific action limits (upper and lower) which, when exceeded, clearly indicate that the plant is not operating within compliance or when an adverse change has occurred. The action limits should be established by calibrating Microtox results with the results of compliance species testing. These upper and lower action limits define the boundaries of the Compliance Probability Range for the plant and should be carefully monitored for excursions which could result in compliance bioassay failures.

Test Guidelines

(The following assumes a level of familiarity with the Microtox LX software and capability with using the technology according to the procedures described in the protocol).

1. Collect 24 hour composite treated effluent samples.
2. Test treated effluent samples within 24 – 48 hours after collection. if not tested immediately after collection, keep samples stored at 4°c.
3. It is recommended that treated effluent samples be collected in new borosilicate glass containers (polycarbonate and polypropylene containers are also acceptable).
4. If chlorination is part of the treatment process, always collect the treated effluent prior to chlorination. (if this is not possible, dechlorinate the sample using sodium thiosulfate).

Characteristics of the Comparison Test

- The procedure compares 5 replicates of Test Sample (i.e. effluent) against 5 replicates of Reference sample (i.e. reconstitution solution).
- Testing is conducted at a single concentration which will normally be 82% since most effluent samples will require osmotic adjustment.
- Zero-time light level readings are taken for normalizing all timed readings.
- Statistical analysis is performed on the raw data and provided on the data report.
- Only one sample can be tested at a time.
- The result of the test is reported as "% Difference" (% Difference is equivalent to % Effect).

Characteristics of the Screening Test

- The procedure compares one concentration of Test Sample to a control (diluent or clean reference sample).
- Testing is conducted at a single concentration, defaults in the software are 2%, 45%, or 81.9%, depending on toxicity expectancy. 81.9% is typically the choice for most effluent samples.
- Screening test is easier and faster to perform than the Comparison Test.
- Up to 16 samples can be tested at a time.
- The result of the test is reported as "% Effect".

Which Test To Use?

Use the screening test when one, a combination of, or all of the following are desired:

- Ease of use and speed.
- Ability to test more than one sample at a time.
- Ability to test at the highest concentration (81.9%) possible.
- Emphasis on a biologically significant response over a statistically significant response.

Use the comparison test when one, a combination of, or all of the following are desired:

- High precision.
- Statistical analysis of the raw data.
- Emphasis on a statistically significant response over a biologically significant response.

Once a particular method is chosen, it should be used for both establishing the baseline and routine monitoring of the treated effluent. (do not use one test for establishing the baseline and then the other test for routine monitoring.)

Establishing the Effluent Baseline and the Compliance Probability Range

1. On nearly a daily basis for over a period of at least 30 to 60 days during normal plant operation, perform either a comparison or screening test (choose one and use it throughout the baseline development) on a 24-hour composite treated effluent sample to become familiar with the normal range of fluctuations that can occur in effluent quality.
2. Record the 15 minute result (% Effect) on a data sheet as shown in Table 1, and plot these onto the Microtox daily toxicity chart as shown in table 1. (if available, a spreadsheet program with graphing capabilities may be used for tabulating and graphing the data.)
3. Calculate the average % Effect for the 30 to 60 day period. This represents the baseline % Effect around which normal fluctuations in effluent quality (as observed during the 30-60 day baseline period) can be reasonably expected. Plot the baseline on the daily toxicity chart as shown in Table 1. (if available, a spreadsheet program with graphing capability may be used for calculating the average and graphing the baseline.)
4. Split samples for testing between the compliance species and the Microtox test to develop and become familiar with the Compliance Probability Range for Microtox. The Compliance Probability Range serves as a “safe operating zone” for final effluent quality and will mandate the limits of acceptable effluent quality fluctuations.

Table 1: Treated Effluent Baseline Data Sheet

Type of treated effluent:

_____ Primary __x__ Secondary _____ Tertiary

Date started: 07/01/06

Date finished: 09/30/06

Test type used:

_____ Comparison Test __x__ Screening Test

Month 1

	% Effect (15 min)
1	-8.53
2	-6.51
3	-8.49
4	-11.41
5	-19.05
6	-13.32
7	-12.5
8	-13.53
9	9.2
10	7.92
11	1
12	-10.3
13	5.87
14	28.9
15	-11.57
16	-2.11
17	-11.65
18	5.2
19	19.41
20	-6.05
21	11.53
22	-4.01
23	1.91
24	10.5
25	-21.3
26	1
27	3.18
28	17.34
29	23.18
30	17.34
31	20.8
Total	**0.87**
Month Average	**0**

Month 2

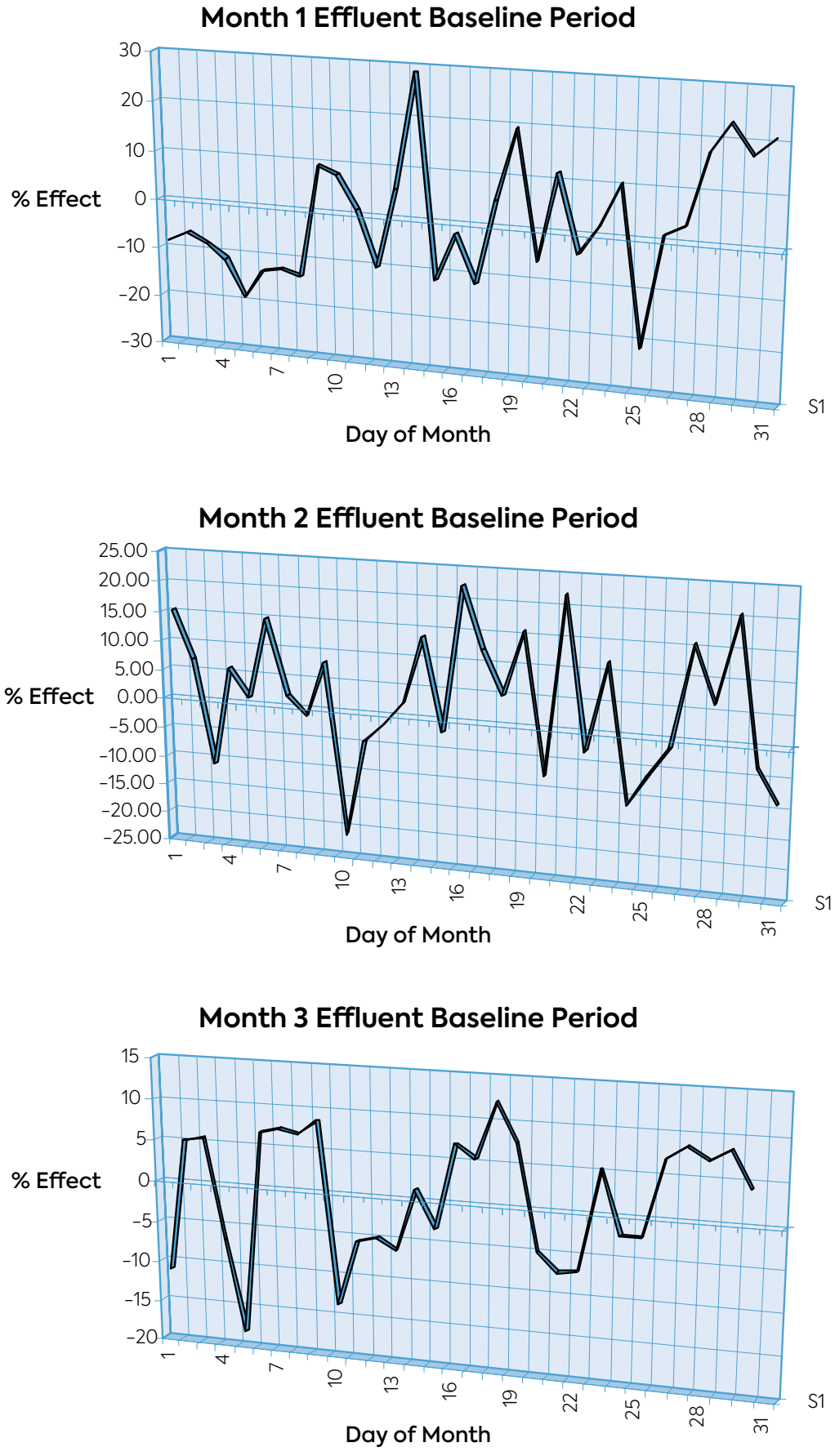
	% Effect (15 min)
1	15.20
2	6.87
3	-11.05
4	5.9
5	1
6	14.77
7	1.97
8	-1.24
9	7.94
10	-21
11	-4.75
12	-1.57
13	2.4
14	13.45
15	-1.97
16	22.34
17	12.12
18	5.07
19	15.55
20	-7.86
21	21.9
22	-3.06
23	11.46
24	-11.35
25	-6.07
26	-1.5
27	15.57
28	6.07
29	20.45
30	-3.51
31	-8.91
Total	**116.19**
Month Average	**3.7**

Month 3

	% Effect (15 min)
1	-11.1
2	5.1
3	5.6
4	-6.7
5	-18.1
6	6.7
7	7.3
8	6.8
9	8.6
10	-13.6
11	-5.5
12	-5.0
13	-6.2
14	1.2
15	-3.2
16	7
17	5.4
18	12.1
19	7.6
20	-5.0
21	-7.2
22	-6.9
23	5.3
24	-2.3
25	-2.2
26	7.0
27	8.6
28	7.1
29	8.4
30	4.3
31	
Total	**21.1**
Month Average	**0.7**

Baseline (Average) % Effect: 1.5

Figure 1


Month 1 Effluent Baseline Period
% Effect
30
20
10
0
-10
-20
-30
1
4
7
10
13
16
19
22
25
28
31
S1
Day of Month
Month 2 Effluent Baseline Period
% Effect
25.00
20.00
15.00
10.00
5.00
0.00
-5.00
-10.00
-15.00
-20.00
-25.00
1
4
7
10
13
16
19
22
25
28
31
S1
Day of Month
Month 3 Effluent Baseline Period
% Effect
15
10
5
0
-5
-10
-15
-20
1
4
7
10
13
16
19
22
25
28
31
S1
Day of Month

Routine Monitoring

Once the baseline and range of normal toxicity fluctuations in the effluent have been established, then 24-hour composite treated effluent samples can be analyzed on a routine basis with either the Comparison or Screening Test (use the test which was involved in establishing the baseline).

Plot the 15 minute Screening Test results on the Microtox Daily Toxicity Chart or use a spreadsheet with graphing capabilities and identify the area of the graph which represents the baseline. Any persistent trend away from the baseline may indicate that an adverse change is occurring that may soon impact the compliance status of the plant.

Data Quality

- For the Comparison Test, the 95% Confidence value (the value to the right of the "±" symbol) should be 15 or lower. If it is not, the test should be repeated.
- For the Screening Test, manually calculate 2 times the standard deviation for the combined control and sample readings. If the 2SD is 15 or higher, the test should be repeated.

Interpretation of the Comparison and Screening Test Results

- Both the Comparison Test and Screening Test provide results in terms of % Effect which is the percent difference (as measured by the amount of light lost or gained) that exists between the calculated means of the test and reference sample.
- A positive % Effect indicates an inhibitory response (based upon lower light output produced by the test organism in the test sample relative to the reference sample).
- A negative % Effect indicates a stimulatory response (based upon higher light output produced by the test organisms in the test sample relative to the reference sample).
- Light stimulation is normally a result of hormesis and is often associated with chronic toxicity. Hormesis is a term that describes the increased activity observed in organisms when they are exposed to low (chronic) levels of toxicants. Hormesis can also be described as an event in which the test organism is initially stressed exhibiting an increased metabolic rate and only later exhibits an inhibitory toxic response due to the cumulative effect upon other metabolic reactions and pathways.

% Effects greater than (+)5 are indicative of acute toxicity in the sample. (eg. +20%).

% Effects more negative than (-)5 may be indicative of chronic toxicity. (eg. -20%).

% Effects between zero to (+)5 or zero to (-)5 are usually indicative of a non-toxic response.

Commercial testing lab results comparing the Microtox Screening Test with the 7 day chronic ceriodaphnia test on 22 different treated effluents indicate approximately 95% agreement between the results of these two bioassays. (see table 2).

Table 2

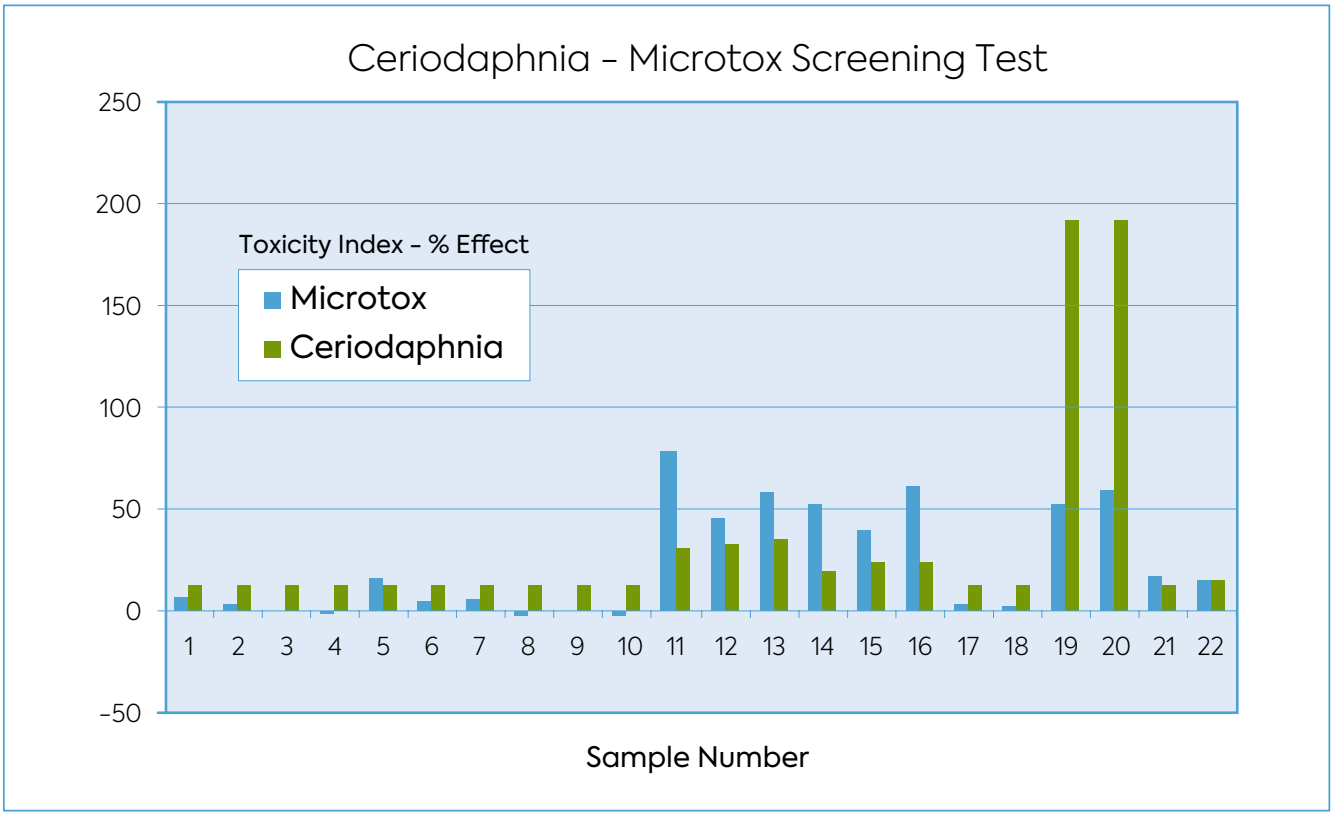
Sample Number	Ceriodaphnia (LC_{50})	Toxicity Index (1200/LC_{50})	Inhibition Test (% Effect)
1	>100	12	6.6
2	>100	12	3.1
3	>100	12	0.1
4	>100	12	-0.6
5	>100	12	16
6	>100	12	4.1
7	>100	12	5.6
8	>100	12	-2
9	>100	12	0.3
10	>100	12	-2.7
11	38.9	30.85	78.9
12	36.3	33.06	46
13	33.9	35.4	57.5
14	61.6	19.48	51.8
15	50	24	39.5
16	50	24	61.9
17	107	12	3.6
18	107	12	2.1
19	<6.25	192	52.2
20	<6.25	192	59.5
21	>100	12	16.6
22	81.2	14.78	14.6

Toxicity Index Values

To better visualize the relationship between % Effect and LC50, the LC50 can be converted into a Toxicity Index value which is more easily comparable to a % Effect value.

By dividing the LC50 into 1200, a Toxicity Index value greater than 12 indicates a Ceriodaphnia failure (if compliance is determined by an LC50≥100%). Likewise, a % Effect reading greater than 12 in the Screening Test corresponds to a probable compliance failure so a direct comparison of the two data sets is now possible in graphic form as shown in table 3 with values greater than 12 indicating a failure in both tests.

Table 3


Ceriodaphnia – Microtox Screening Test
250
200
150
100
50
0
-50
Toxicity Index - % Effect
Microtox
Ceriodaphnia
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
Sample Number

Summary:

The Microtox Screening Test and Comparison test are two toxicity test procedures which can be easily performed to assure waste treatment plant operators and management of final treated effluent quality. Establishment of an effluent base line and determination of the Compliance Probability Range, followed by routine monitoring, can assist plant personnel in achieving and maintaining compliance with discharge limits.

On-site programs of routine toxicity monitoring (of both influent and effluent) have been shown to greatly improve effluent quality. The information generated by the Microtox screening and comparison tests provide plant operators with the means by which limits can be set for current plant operations and goals set for future plant performance.

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