POLYTOX®

RAPID TOXICITY TEST

Features and Benefits of using POLYTOX®

Features:

- **Easy to Use**
- **Fast – About 30 minutes per test**
- **Readily available through major distribution companies**
- **Field Adaptable for quick testing**
- **Easy to automate in Laboratory**

Benefits:

- **Economical**
- **Accurate**
- **Consistent**
- **Reliable**

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InterLab®

POLYTOX® Rapid Toxicity Test
Testing Procedures and Instructions

In 1972, the United States Clean Water Act declared a need to regulate the discharge of toxic pollutants into the nation's water supply. This Act allowed the Environmental Protection Agency (EPA), through the use of permits, to establish rules and regulations governing the "pretreatment" of industrial wastewaters prior to their discharge.

In the early 1980's researchers developed **POLYTOX®** specifically to determine if a water or wastewater stream had toxic effects on known biological activity. **POLYTOX®** provides a simple, rapid test for measuring the toxicity of a water or wastewater stream using the respiration of known microorganisms as the indicator. **POLYTOX®** contains specialized microbial cultures that can determine the relative toxicity of water and wastewaters streams in about 30 minutes, with no expensive instrumentation required.

The **PolyTox®** Rapid Toxicity Test described in this document evaluates the inhibitory effect of toxicants in potable tap water (i.e. TW) to specialized bacterial cultures by measuring the respiration rate under defined conditions in the presence of different concentrations of toxicants in the tap water. The respiration rate is the oxygen consumed by aerobic and facultative bacterial cultures and is expressed as mg of O_2 consumed per liter per minute (called the Dissolved Oxygen Update Rate [DOUR]).

The POLYTOX® test kit remains most applicable to toxicants and chemicals that are likely to remain in solution. The "Inhibition Concentration", IC_{30}, in this procedure is the concentration of toxicants in the water phase that causes a 30% reduction in respiration rate related to that exhibited by the baseline or control. The inhibitory or toxic effect of a specific concentration of toxicant is expressed as a percent of the baseline respiration rate. In general, for unknown toxicants a testing procedure utilizing at least five different concentrations is recommended.

Since the naturally occurring environment cannot be duplicated exactly under laboratory conditions, the IC_{30} value in this test should be regarded merely as a guideline of toxicity for that particular tap water sample as compared to natural microorganisms. If, for example, a specific tap water exhibits an IC_{30} on a reoccurring basis this "baseline" is only affected when and if the toxic nature of the sample stream is altered.

EQUIPMENT REQUIRED

1. **POLYTOX**® Test Kit
2. Standard (300 ml) BOD bottle(s).
3. Dissolved oxygen probe and meter. The probe must fit snugly into the neck of the BOD bottle, eliminating all headspace.
4. One-inch magnetic stirring bar and magnetic stirrer or self-stirring dissolved oxygen probe capable of suspending the **POLYTOX**® populations in the BOD bottle.
5. Aeration device (e.g., aquarium pump, tubing and air stone). Aeration of background water can be accomplished by hand agitation, but is not recommended.
6. One and two liter containers to be used for aeration of the deionized water (control) and sample water, wastewater or chemical (test) samples.
7. pH adjusting solution(s) (e.g., dilute sodium hydroxide or sulfuric acid), if required.
8. Thermometer.
9. Funnel (optional)
10. Stopwatch.
11. Optional – A single channel recorder connected to the dissolved oxygen meter to provide a continuous strip chart recording of the dissolved oxygen level in the BOD bottle versus time.

TESTING CONDITIONS

- Duration/contact time: 30 minutes for ultimate DOUR; 19 and 21 minutes for base test.
- Containers: 1 liter size for the aeration of the controls, 2 liter size for the aeration of the test(s)
- Air Supply: clean, oil-free air. An aquarium pump is recommended.
- Water: Deionized or distilled water. DI water is recommended.
- Reactor Vessel: BOD bottle(s)
- Test Solution: A freshly collected sample of tap water. Alter the TW with the specific toxicant necessary to determine its' toxic effect. Aerate the test solution with pH and temperature adjusted.
- Control: Baseline respiration rate for the **POLYTOX**® populations only.
- Temperature: 20°C $\pm$ 2°C.

BRIEF OVERVIEW OF TESTING PROCEDURES

The **POLYTOX**® Rapid Toxicity Test is a four-step process as outlined below. Detailed, procedural steps and calculations are explained beginning in Section I.

It is important to note that tap water samples can be tested either with chlorine residual intact or the residual chlorine can be eliminated with sodium thiosulfate. Please be sure not to overfeed the sodium thiosulfate or the DO of the sample could be effected.

I. **DETERMINE BASELINE ACTIVITY**: First, test for the ***BASELINE ACTIVITY*** for the **POLYTOX**® microbial populations. This accounts for any oxygen depletion caused by the **POLYTOX**® microbial population using aerated deionized or distilled water with **POLYTOX**® as the standard. Note: for an example of what your baseline should look like see graph 1

II. **DETERMINE BACKGROUND ACTIVITY**: To account for any ***BACKGROUND ACTIVITY*** in the sample to be tested, test for oxygen depletion caused by either microbes present in the sample itself or by the stripping away of COD (Chemical Oxygen Demand) during aeration. This sample(s) is tested in the **absence** of the **POLYTOX**® population.

III. **CONDUCT FINAL TESTING** of the actual sample of tap water on which toxicity will be determined. This TW sample can be neat or "spiked" with the toxicant in question. This is the ***SAMPLE TOXICITY TEST*** outlined in Section III.

IV. **DETERMINE CORRECTED RESPIRATION RATE**: Finally, all ***CALCULATIONS*** are made to determine the corrected DOUR of the sample in question and the percent inhibition, or IC_{30}, of the sample in question.

I. PROCEDURE FOR BASELINE ACTIVITY

To account for any oxygen depletion caused by the **POLYTOX®** microbial population, aerated deionized or distilled water is used with **POLYTOX®** to determine a ***"baseline activity"***.

1. Calibrate the dissolved oxygen probe and meter according to the manufacturer's specifications.

Figure 1

2. Air-saturate 500 mls of pH adjusted (7.0) deionized or distilled water by aerating the water for at least 30 minutes at a relatively constant temperature (20°C ±2°C).

Figure 2

3. Pour 50 mls of the aerated, pH adjusted (pH adjustment is optional) water into a BOD bottle and set side.

Figure 3

4. Add the magnetic stirring bar to the BOD bottle if a self-stirring probe is not available.

Figure 4

5. With stopwatch in hand, remove the cap from one of the **POLYTOX®** vials. Pour the contents of the **POLYTOX®** vial into the BOD bottle containing the aerated, pH adjusted (pH adjustment is optional) water. A funnel may be used if desired. **IMMEDIATELY START THE STOPWATCH.**

Figure 5

6. Pick up the BOD bottle and swirl the contents for 10 to 15 seconds, making sure that the **POLYTOX®** populations are thoroughly wet and thus activated.

Figure 6

7. Hold the BOD bottle at a 45° angle and pour additional pre-aerated water into the BOD bottle. Pour the water down the side of the bottle to avoid the formation of excess air bubbles. Fill the bottle to a level just above the bottom of the ground glass joint.

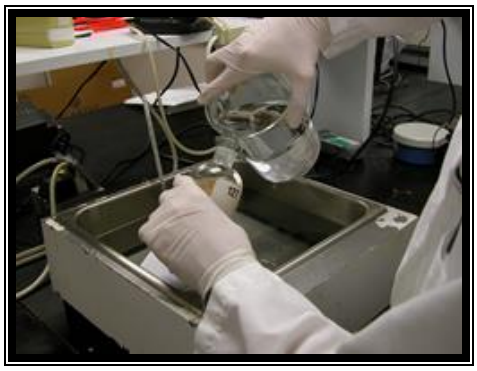


Figure 7

8. Place the bottle on a flat surface and tap gently to remove bubbles.

Figure 8

9. Insert the dissolved oxygen probe into the BOD bottle, carefully displacing all bubbles from the bottle. It helps to tilt the bottle to the side so that bubbles will slide off the face of the dissolved oxygen probe membrane. Initiate the stirring in the BOD bottle.

Figure 9

10. The initial dissolved oxygen level should be at least 6.5 mg/l at this time. Starting at the 2 minute interval, record the dissolved oxygen reading continuously (with an optional recorder) or every two minutes by hand. Be sure you record the pertinent times of 19 and 21 minutes. After you are familiar with the procedure, the dissolved oxygen level can be recorded at the pertinent times of 19 and 21 minutes only.

NOTE: With practice, the dissolved oxygen probe can be placed in the bottle within 45 to 60 seconds after adding the first 50mls of pre-aerated water.

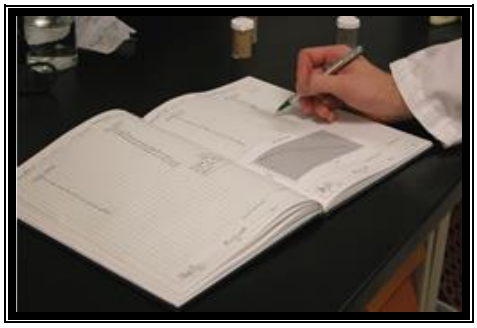


Figure 10

11. Use Equation 1 in the "Calculations" section (section IV) towards the end of this document to calculate the dissolved oxygen uptake rate (DOUR) or the ***BASELINE ACTIVITY*** of the **POLYTOX®** populations.
Note: see graph 1

II. PROCEDURE FOR BACKGROUND ACTIVITY OF SAMPLE (BLANK)

To account for any ***background oxygen depletion*** caused by either microbes present in the tap water sample itself or by the stripping away of COD (Chemical Oxygen Demand) during aeration, the sample(s) must also be tested in the ***ABSENCE*** of the **POLYTOX®** population.

1. Calibrate the dissolved oxygen probe and meter according to the manufacturer's specification.

Figure 11

2. Air-saturate one liter of tap water sample (full strength) by aerating the sample for at least 30 minutes at a relatively constant temperature (20±2°C). Any chlorine residual can be removed with sodium thiosulfate prior to air-saturation, if necessary.

Figure 12

3. If necessary, adjust the pH of the tap water to 7.0 with dilute sodium hydroxide or sulfuric acid.

Figure 13

4. Add the magnetic stirring bar to the BOD bottle if a self-stirring probe is not available.

Figure 14

5. Hold the BOD bottle at a 45° angle and pour pre-aerated tap water sample into the BOD bottle. Pour the sample down the side of the bottle to avoid the formation of excess air bubbles. Fill the bottle to a level just above the bottom of the ground glass joint.

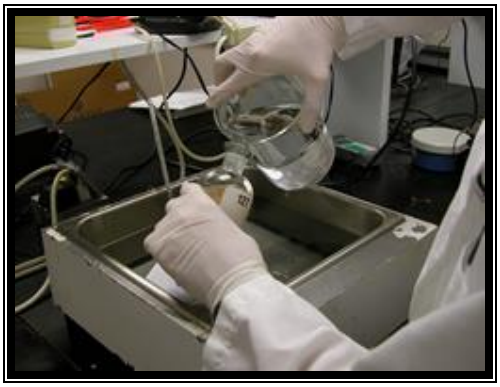


Figure 15

6. Place the bottle on a flat surface and tap gently to remove bubbles.

7. Insert the dissolved oxygen probe into the BOD bottle, carefully displacing all bubbles from the bottle. It helps to tilt the bottle to the side so that bubbles will slide off the face of the dissolved oxygen probe membrane. Initiate the stirring in the BOD bottle.

Figure 16

8. The initial dissolved oxygen level should be at least 8.0 mg/l at this time. Starting at the 2 minute interval, record the dissolved oxygen reading continuously (with an optional recorder) or every two minutes by hand. Be sure you record the pertinent times of 19 and 21 minutes. After you are familiar with the procedure, the dissolved oxygen level can be recorded at the pertinent times of 19 and 21 minutes only.

NOTE: If the initial dissolved oxygen is less than 8.0 mg/L, the 30-minute pre-aeration procedure must be repeated and the dissolved oxygen level rechecked. If it is still less than 8.0 mg/L, it is likely that a significant chemical oxygen demand exists in the test solution. This will interfere with the POLYTOX® test and must be eliminated. Over night aeration of the sample may be sufficient to remove the immediate oxygen demand. It should also be noted that removal of the chemical oxygen demand by air stripping methods could change the levels of inhibition exhibited by the sample.

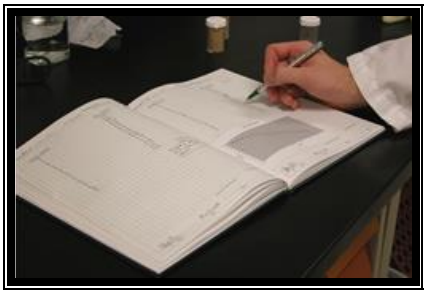


Figure 17

9. Use the Equation 2 to calculate the dissolved oxygen uptake rate for the ***background activity*** of the sample.

III. PROCEDURE FOR TAP WATER SAMPLE TOXICITY TESTING

The final testing is of the ***actual sample of tap water*** on which toxicity is to be determined. Calculate using Equation 3.

With the dissolved oxygen probe and meter calibrated according to the manufacturer's specifications and the test sample pre-aerated, pH and temperature adjusted, proceed onto the following steps. Any chlorine residual can be removed with sodium thiosulfate prior to air-saturation, if necessary.

1. Pour 50mls of the tap water at 20°C±2°C sample into a BOD bottle and set aside.

Figure 18

2. Add the magnetic stirring bar to the BOD bottle if a self-stirring probe is not available.

Figure 19

3. With stopwatch in hand, remove the cap from one of the **POLYTOX**® vials. Pour the contents of the **POLYTOX**® vial into the BOD bottle with aerated, pH adjusted (optional) tap water sample. A funnel may be used if desired. IMMEDIATELY START THE STOPWATCH.

Figure 20

Figure 20a

4. Pick up the BOD bottle and swirl the contents for 10 to 15 seconds, making sure that the **POLYTOX**® populations are thoroughly wet and thus activated.

Figure 21

5. Hold the BOD bottle at a 45° angle and pour additional pre-aerated tap water sample into the BOD bottle. Pour the water down the side of the bottle to avoid the formation of excess air bubbles. Fill the bottle to a level just above the bottom of the ground glass joint.

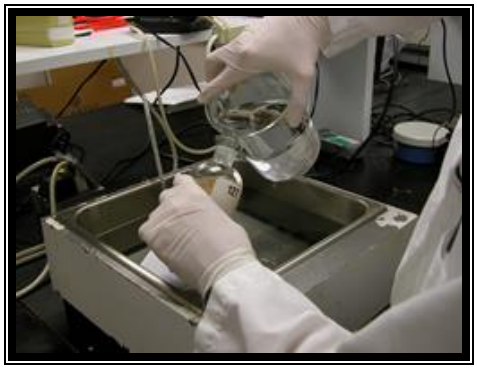


Figure 22

6. Place the bottle on a flat surface and tap gently to remove bubbles.

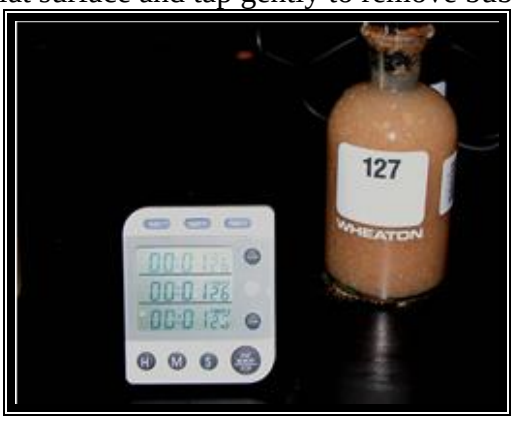


Figure 23

7. Insert the dissolved oxygen probe into the BOD bottle, carefully displacing all bubbles form the bottle. It helps to tilt the bottle to the side so that bubbles will slide off the face of the dissolved oxygen probe membrane. Initiate the stirring in the BOD bottle.

Figure 24

8. The initial dissolved oxygen level should be at least 6.5 mg/l at this time. Starting at the 2 minute interval, record the dissolved oxygen reading continuously (with an optional recorder) or every two minutes by hand. Be sure you record the pertinent times of 19 and 21 minutes. After you are familiar with the procedure, the dissolved oxygen level can be recorded at the pertinent times of 19 and 21 minutes only.

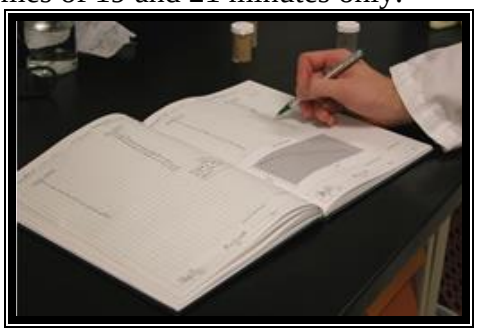


Figure 25

9. Use ***Equation 3*** to calculate the dissolved oxygen uptake rate (DOUR) for the toxicity of the tap water sample.

IV. Calculations and Interpretation of Results

The following equations are used to calculate the *"Percent Inhibition"* of the test sample to the **POLYTOX®** populations (Equation 5). The *"Percent Inhibition"* is a relative number that exemplifies the toxicity of a tap water sample.

In general, if the *"Percent Inhibition"* is 30% of less, the relative toxicity is the sample is negligible. If the *"Percent Inhibition"* falls between 30% and 50% the sample enters a "slightly toxic" zone. If the *"Percent Inhibition"* runs over 50% we classify the sample as "toxic to very toxic" to the **POLYTOX®** microorganisms.

Since each tap water source is unique, the TW sample curves and relative *"Percent Inhibition"* of that TW source is unique. We recommend that the responsible laboratory or operator run enough TW sample tests to develop a *"Percent Inhibition"* history that is unique to that tap water. With that information any noticeable variance from that data base will indicate some kind of toxic effect inherent to that particular sample.

➡ Equation 1

Equation 1 calculates the ***BASELINE ACTIVITY*** for the **POLYTOX®** microbial populations. This accounts for any oxygen depletion caused by the **POLYTOX®** microbial population using aerated deionized or distilled water with **POLYTOX®** as the standard. Note: see graph 1

$$DOUR_S = \frac{DO19_S - DO21_S}{2 \text{ minutes}} = mg/L/min$$

Where:
$DOUR_S$ = Baseline Dissolved Oxygen Uptake Rate
$DO19_S$ = Dissolved Oxygen (mg/L) at 19 minutes
$DO21_S$ = Dissolved Oxygen (mg/L) at 21 minutes.

For any POLYTOX® **kit, the baseline rate of respiration in deionized or distilled water should range between 0.20 to 0.50 mg/L/min. The baseline respiration rate for your** POLYTOX® **should remain within this range for three months if the kit is stored (20±5°C). (DO NOT FREEZE OR REFRIGERATE.) A baseline should be run for each series of tests.**

➡ Equation 2

Equation 2 accounts for any ***BACKGROUND ACTIVITY*** oxygen depletion caused by either microbes present in the tap water sample itself or by the stripping away of COD (Chemical Oxygen Demand) during aeration, the sample(s) must also be tested in the

absence of the **POLYTOX®** population. Under ideal conditions this is close to 0.0 mg/L/minute.

$$DOUR_B = \frac{DO19_B - DO21_B}{2 \text{ minutes}} = \text{mg/L/min}$$

Where:
$DOUR_B$ = Background Dissolved Oxygen Uptake Rate
$DO19_B$ = Dissolved Oxygen (mg/L) at 19 minutes
$DO21_B$ = Dissolved Oxygen (mg/L) at 21 minutes
Note: see graph 2

For any given sample, the background rate of respiration should be less than 0.05mg/l/min.

➡ Equation 3

Equation 3 calculates the dissolved oxygen uptake rate (DOUR) for the ***ACTUAL TEST sample.***

$$DOUR_T = \frac{DO19_T - DO21_T}{2 \text{ minutes}} = \text{mg/L/min}$$

Where:
$DOUR_T$= Dissolved Oxygen Uptake Rate for the Test Solution
$DO19_T$ = Dissolved Oxygen (mg/L) at 19 minutes
$DO21_T$ = Dissolved Oxygen (mg/L) at 21 minutes

➡ Equation 4

Equation 4, or $DOUR_C$, calculates the ***CORRECTED dissolved oxygen uptake rate (DOUR) for the tap water sample*** to account for any background activity ($DOUR_B$).

$$DOUR_C = DOUR_T - DOUR_B$$

Where:
$DOUR_C$ = Corrected Dissolved Oxygen Uptake Rate for the Test Solution
$DOUR_T$ = Dissolved Oxygen Uptake Rate for the Test Solution
$DOUR_B$ = Background Dissolved Oxygen Uptake Rate

Note: If the respiration of the test solution is lower than the baseline rate, then the test solution is considered inhibitory to the microorganisms.

➡ Equation 5

Equation 6 calculates the ***"Percent Inhibition"*** of the test sample to the **POLYTOX®** populations.

$$\% \text{ INHIBITION} = 1 - \left\{ \frac{DOUR_C}{DOUR_S} \right\} \times 100$$

Where:
$DOUR_C$ = Corrected Dissolved Oxygen Uptake Rate
$DOUR_S$ = Baseline Dissolved Oxygen Uptake Rate.

Testing at various dilutions can be used to determine the concentration at which 30% inhibition of the microorganisms occurs, IC_{30}. (For the purposes of the **POLYTOX®** toxicity testing procedure, inhibition of microorganisms is equated to reduction in dissolved oxygen utilization by the microorganisms).

V. Frequently Asked Questions

The following are questions that have been asked over the years that may give some insight to actual testing and interpretation of results.

Q. How important is the cleanliness of the BOD bottles?

A. Although the cleanliness of the BOD bottles is not as important as in BOD testing, it is still important. We recommend the following:

1. Allow to soak (around an hour) in mild soapy water (we suggest Alconox), scrub with bottlebrush (designated for BOD_5 bottles only).
2. Allow bottles to soak in 9% Nitric acid solution or 1:1 HCl solution for about 1 hour.
3. Rinse bottles by allowing tap water to flow into bottles while shaking. When you notice no more bubbles appearing while you shake the bottle, rinse 2 more times.
4. Finally, 3 rinses with DI or sample water.

Q. When I run baseline samples with POLYTOX® I get different DOUR numbers. Is there something wrong with the product?

A. Probably not. As long as you are using POLYTOX® from the same batch number there is a very high degree of consistency from vial to vial. We find that this issue occurs mostly with new POLYTOX® users. Technique is everything. Once you get your technique perfected you will find that both baseline and live sample results will be repetitive and accurate.

Q. Must I run the baseline and background samples every time?

A. No. We recommend running baseline samples on every new batch of POLYTOX® that you use. Since this is simply determining the effect of the base POLYTOX® product on the DOUR we find that it remains very consistent for every batch.
Once you achieve a data history for the background TW sample this test can also be run on a "spot-check" basis in order to determine the microbial effect of the TW sample. In tap water this background should be almost zero.

Q. Do you have a spreadsheet that will automatically calculate DOUR's?

A. Yes. We can send you an Excel spreadsheet that can easily calculate DOUR's and a TW's % inhibition. Below is a picture of the spreadsheet. We can send you the file or it can be downloaded from the section of our website at www.polyseed.com.

BASELINE	**INPUT D.O. Reading from Meter at:**			
		19 minutes:	**6.00**	**mg/L**
		21 minutes:	**5.40**	**mg/L**
	Eq.1:	***DOUR(S)=***	**0.30**	**mg/L**
BACKGROUND	**INPUT D.O. Reading from Meter at:**			
		19 minutes:	**8.00**	**mg/L**
		21 minutes:	**7.90**	**mg/L**
	Eq. 2:	***DOUR(B)=***	**0.05**	**mg/L**
SAMPLE TEST	**INPUT D.O. Reading from Meter at:**			
		19 minutes:	**7.20**	**mg/L**
		21 minutes:	**6.65**	**mg/L**
	Eq. 3:	***DOUR(T)=***	**0.28**	**mg/L**
CORRECTED				
	Eq. 4:	***DOUR(C)=***	**0.23**	**mg/L**
PERCENT INHIBITION				
	Eq. 5:	***Inhibition=***	**25.00**	**%**

Q. I ran a Baseline POLYTOX® test that depleted in 21 minutes. What does this mean?

A. The POLYTOX® test is designed for the baseline test (with DI water) to deplete in approximately 30 minutes. The time normally falls between 28 and 32 minutes. If your Baseline test is depleting in 21 minutes do the following:

1. Re-clean the BOD bottles, DO probe and associated glassware
2. Check the DO meter for malfunction
3. Check the DI water to make sure the pH is about 7.0 and the conductivity is low
4. Make sure the water temperature is +- 20^{o}C
5. Re-run the test

Q. What does it mean when the initial dissolved oxygen level is at least 8.0 mg/I, but the background rate of respiration ($DOUR_B$) is greater than 0.05 mg/I/min.?

A. This is very unlikely when the sample is tap water. But, is it should occur, a $DOUR_B$ that is greater than 0.05 mg/I/min. is likely to be caused by a combination of biological and chemical oxygen demand (i.e., sample composition and microscopic examination could verify biological activity). This will be normalized in the final equations.

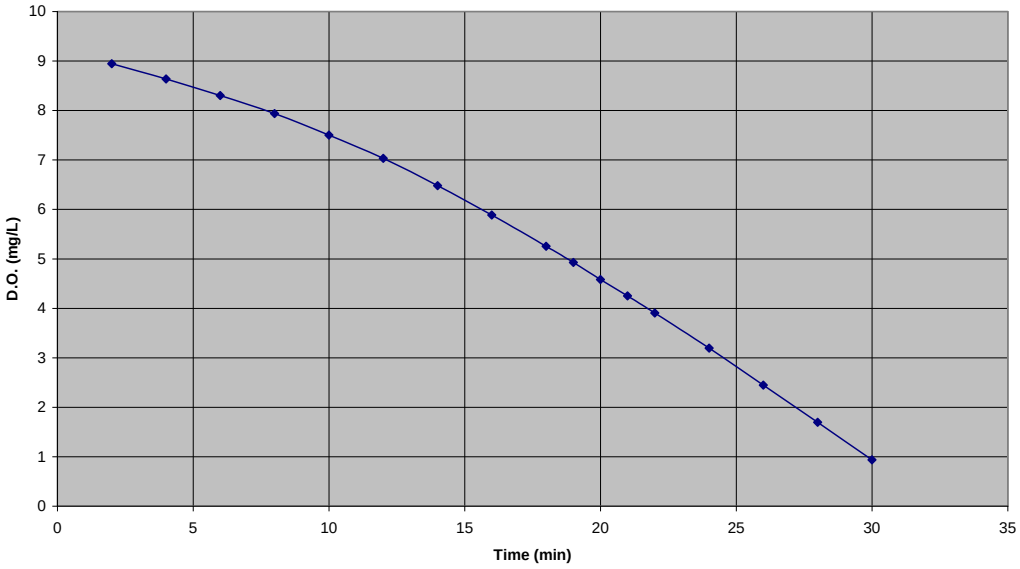
Q. What do I do when the dissolved oxygen has been completely depleted prior to the 19 minute reading?

A. This is very unlikely when the sample is tap water. But, if it should occur, record the pertinent times in which dissolved oxygen of at least 1.0 mg/L remains for the baseline (DOURs) and tests ($DOUR_T$). Using those times (i.e., 15 and 17 minute readings) calculate the various activity rates.

Q. What does it mean when the dissolved oxygen rate of the test ($DOUR_T$) is greater than the baseline rate of respiration (DOURs)?

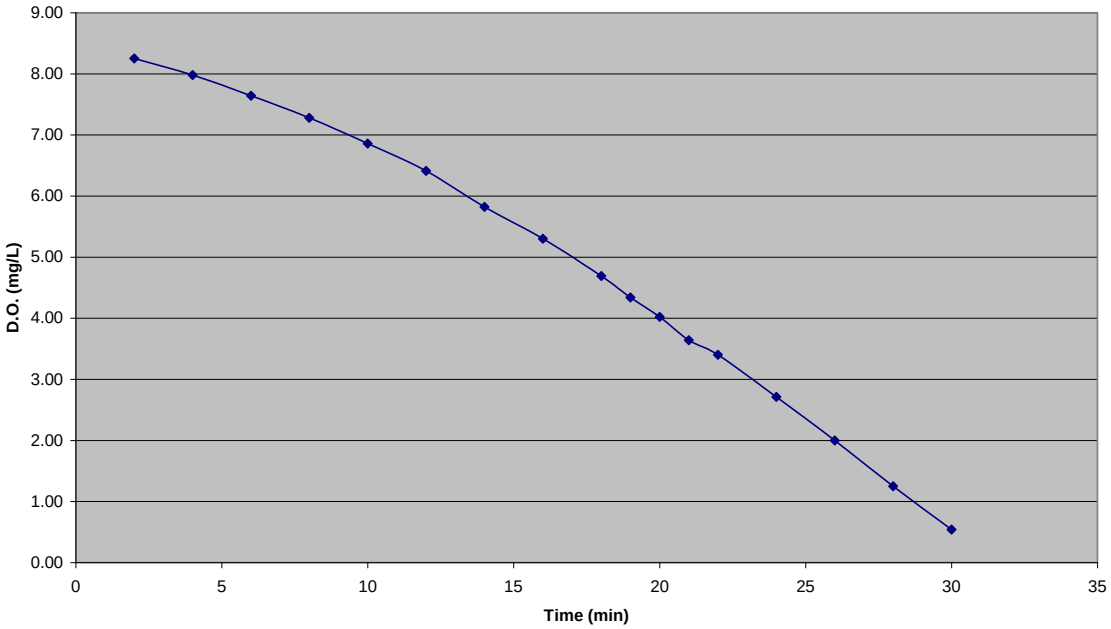
A. This is very unlikely when the sample is tap water. Depending upon the homogeneity of the contaminants within the sample, areas of low organic levels may even experience some enhancement of biological oxygen uptake activity. At concentration of samples where there is no toxic effect upon the microbial oxygen uptake rate, uptake rates greater than the baseline rate (DOURs) are sometimes experienced. These enhanced rates are represented as negative inhibition values and usually indicate the presence of certain readily degradable compounds which the "POLYTOX®" bacteria can immediately utilize as a good source, thereby increasing cellular metabolic activity and the uptake of dissolved oxygen.

Baseline Test Average with DI Water, LOT #PT 110306-1B
DOUR at 19 and 21 Minutes = 0.3375
Ultimate 30 Minute DOUR = 0.2861



Graph 1

Baseline Test Average with Texas Tap Water, LOT #PT 110306-1B
DOUR at 19 and 21 Minutes = 0.35
Ultimate 30 Minute DOUR = 0.28



Graph 2

For more information, call:

Technical Service Department

InterLab®

26018 Budde Road

Spring, TX 77380

281.298.9410

888.876.2844

Fax 281.298.9411

www.interlabsupply.com

Email: technical@polyseed.com