InterLab®

APPLICATION PROCEDURE

PolyTox® Rapid Toxicity Test

In 1997, 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.

An attractive solution to analyzing the quality of wastewater is PolyTox. PolyTox provides a simple, rapid test for measuring the toxicity of wastewater to biological wastewater treatment systems. PolyTox contains specialized microbial cultures and can determine the toxicity of wastewaters and chemicals in biological treatment systems in 30 minutes, with no expensive instrumentation required.

The process described in this "Application Procedure" evaluates the inhibitory effect of the wastewater or chemical(s) to the specialized bacterial cultures by measuring the respiration rate under defined conditions in the presence of different concentrations of that wastewater or chemical. The respiration rate is the oxygen consumed by the aerobic bacterial cultures and is expressed in mg O_2 per liter per minute.

PolyTox is designed to provide a rapid screening method whereby wastewaters and chemicals, which may adversely affect the biomass of a wastewater treatment facility, can be determined and non-inhibitory concentrations for wastewaters and chemicals prescribed. This test kit is most applicable to wastewaters and chemicals that are likely to remain in solution. The Lethal Concentration, LC_{30}, in this procedure is the concentration of the wastewater or chemical at which the respiration rate is 30% of that exhibited by the baseline or control. The inhibitory or toxic effect of the wastewater or chemical at a specific concentration is expressed as a percent of the baseline respiration rate. A testing procedure utilizing at least five different concentrations is recommended.

The LC30 value should be regarded merely as a guideline of toxicity for that particular wastewater or chemical to its own wastewater microorganisms, since the naturally occurring environment cannot be duplicated exactly under laboratory conditions.

EQUIPMENT REQUIRED

- Standard (300 ml) BOD bottle(s).
- Dissolved oxygen probe and meter. The probe must fit snugly into the neck of the BOD bottle, elimination all headspace.
- One-inch magnetic stirring bar and magnetic stirrer or self-stirring dissolved oxygen probe capable of suspending the PolyTox populations in the BOD bottle.
- Aeration device (e.g., aquarium pump, tubing and air stone).
- One and two liter containers to be used for aeration of the distilled or deionized water (control) and wastewater or chemical (test) samples.
- pH adjusting solution(s) (e.g., dilute sodium hydroxide or sulfuric acid).
- Thermometer.
- Funnel.
- Stopwatch.
- 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.

TEST CONDITIONS

- Duration/contact time: 19 and 21 minutes
- Containers: 1 liter size for the aeration of the controls(s), 2 liter size for the aeration of the test(s)
- Air Supply: clean, oil-free air
- Water: Deionized and/or distilled water
- Reactor Vessel: BOD bottle(s)
- Test Solution: The freshly prepared wastewater or chemical solution (e.g., aerated solution with pH and temperature adjusted)
- Control: Baseline respiration rate for the PolyTox populations only
- Temperature: 20 $\pm$ 2°C.

PROCEDURE FOR BASELINE ACTIVITY

1. Calibrate the dissolved oxygen probe and meter according to the manufacturer's specifications.
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±2°C).
3. Pour 50 mls of the aerated, pH adjusted water into a small beaker and set side.
4. Remove the cap from one of the PolyTox vials. Place a funnel into the neck of a clean, dry BOD bottle and pour the contents of the vial into the BOD bottle.
5. Add the magnetic stirring bar to the BOD bottle if a self-stirring probe is not available.
6. With stopwatch in hand, pour the pre-measured 50 mls of water into the BOD bottle containing the PolyTox vial contents. **IMMEDIATELY START THE STOPWATCH.**
7. Pick up the BOD bottle and swirl the contents for 25 to 30 seconds, making sure that the PolyTox populations are thoroughly wet and thus activated.
8. 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.
9. Place the bottle on a flat surface and tap gently to remove bubbles.
10. 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.
11. Initiate the stirring in the BOD bottle.
12. The dissolved oxygen level should be at least 6.5 mg/l at this time. Record the dissolved oxygen reading continuously with the optional recorder or every two minutes by hand. 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 60-90 seconds after adding the first 50ml of pre-aerated water.
13. Use the following equation to calculate the dissolved oxygen uptake rate or the baseline activity of the PolyTox populations:

    Equation 1:

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

    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.

PROCEDURE FOR BACKGROUND ACTIVITY OF SAMPLE

To account for any background oxygen depletion caused by either microbes present in the 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.
2. Air-saturate one liter of wastewater or test solution (full strength) by aerating the sample for at least 30 minutes at a relatively constant temperature (20±2°C).
3. If necessary, adjust the pH of the wastewater or solution to 7.0 with dilute sodium hydroxide or sulfuric acid.
4. Add the magnetic stirring bar to the BOD bottle if a self-stirring probe is not available.
5. Hold the BOD bottle at a 45° angle and pour pre-aerated solution 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.
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.
8. Initiate the stirring in the BOD bottle.
9. The dissolved oxygen level should be at least 8.0 mg/L at this time. Record the dissolved oxygen reading continuously with the optional recorder or every two minutes by hand. 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 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.
10. Use the following equation to calculate the dissolved oxygen uptake rate for the background activity of the sample:

Equation 2:

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

Where:
$DOUR_B$ = Baseline Dissolved Oxygen Uptake Rate
$DO19_B$ = Dissolved Oxygen (mg/L) at 19 minutes
$DO21_B$ = Dissolved Oxygen (mg/L) at 21 minutes

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

PROCEDURE FOR TOXICITY TEST

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:

Step

1. Pour 50mls of the sample into a small beaker and set aside.
2. Remove the cap from one of the PolyTox vials. Place a funnel into the neck of a clean, dry BOD bottle. Pour the dry bacterial contents into the BOD bottle.
3. Add the magnetic stirring bar to the BOD bottle if a self-stirring probe is not available.
4. With stopwatch in hand, pour the pre-measured 50 mls of water into the BOD bottle containing the PolyTox vial contents. **IMMEDIATELY START THE STOPWATCH.**
5. Pick up the BOD bottle and swirl the contents for 25 to 30 seconds, making sure that the PolyTox populations are thoroughly wet and thus activated.
6. Hold the BOD bottle at a 45° angle and pour additional per-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.

7. Place the bottle on a flat surface and tap gently to remove bubbles.
8. 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.
9. Initiate the stirring in the BOD bottle.
10. The dissolved oxygen level should be at least 6.5 mg/l at this time. Record the dissolved oxygen reading continuously with the optional recorder or every two minutes by hand. After you are familiar with the procedure, the dissolved oxygen level can be recorded at the pertinent times of 19 and 21 minutes only.
11. Use the following equation to calculate the dissolved oxygen uptake rate for the test sample:

Equation 3:

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

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

12. Use the following equation to calculate the corrected dissolved oxygen uptake rate for the sample to account for any background activity ($DOUR_B$)

Equation 4:

$$DOUR_C = DOUR_T - DOUR_B$$

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

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

13. Use the following equation to calculate the percent inhibition of the test sample to the PolyTox populations:

Equation 5:

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

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

If the inhibition is significant, it may be necessary to dilute the test wastewater or chemical solution and repeat the PolyTox test procedure. Testing at various dilutions can be used to determine the concentration at which 30% inhibition of the microorganisms occurs, LC_{30}. (For the purposes of the PolyTox toxicity testing procedure, inhibition of microorganisms is equated to reduction in dissolved oxygen utilization by the microorganisms).

For more information, call:

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