YCT (Yeast, Cereal Leaves, Flake-Fish Food, or Trout chow) Preparation

1. SCOPE AND APPLICATION

    1.1. This method is based on EPA-821-R-02-012 *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 5th Edition. It is used to prepare food for the maintenance of the bioassay test organisms, *Ceriodaphnia dubia, Daphnia pulex* and *Daphnia magna*.

2. SUMMARY OF METHOD

    2.1. Flake food is fermented. Cereal leaves and yeast are added to fermented flakes. The yeast may be added immediately or later. The mixture is filtered through a ~110 µm Nitrex screen. Aliquots are poured into bottles and frozen until used.

3. EQUIPMENT AND SUPPLIES

    3.1. Mettler Toledo 403TS/00 balance, calibrated per SOP TA-06.34

    3.2. Class 1 weights

    3.3. Weigh boat, 1 per ingredient (3)

    3.4. 1 L Graduated cylinder, 1 per ingredient (2-3)

    3.5. 250 mL Graduated cylinder, 1

    3.6. 1 L Erlenmeyer flask, 1

    3.7. 2 L Separatory Funnel, 1

    3.8. Aeration device with sterile glass pipette

    3.9. Blender

    3.10. Stir plate

    3.11. Parafilm®

    3.12. 250 mL Nalgene plastic bottles, 12

        3.10.1. Label bottles with “YCT” “batch#” and the preparer’s initials. “Batch #” is the date the ingredients are mixed and poured into the bottles. Format is mm/dd/yy. Add an expiration date to the label of 1 year after preparation date.

        3.10.2. Container size and the number needed can be changed to meet the needs of the laboratory.

    3.13. ~110 µm Nitex screen

    3.14. 2 or 4 L Flask, 1

    3.15. Computer with access to the Laboratory Information Management System (LIMS)

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4. REAGENTS AND STANDARDS

4.1. Deionized Water

4.2. Distilled Water

4.3. Tetramin® or Aquatox® flake fish food

4.4. Dried cereal leaves (wheat grass)

4.5. Fleishmann's Yeast

5. PREPARATION

5.1. Day 0 (Initiation Day)

5.1.1. Using a weigh boat and the Mettler balance, weigh out 5.0 g of flake fish food.

5.1.2. Measure 1 L of distilled water using a 1 L graduated cylinder.

5.1.3. Combine fish food and distilled water in a 2 L separatory funnel.

5.1.3.1. Alternative Method (preferred): Measure 1 L of distilled water using a 1 L graduated cylinder. Add the fish food and 350-400 mL of distilled water into the blender (more than this volume will blow liquid around the blender lid). Blend on high speed for 3-5 minutes. Stop the blender.

5.1.3.2. Pour fish food/water mixture into a 2 L separatory funnel (marked at the 1 L point). Use the remainder of the original liter of water to rinse any remaining fish food from the blender (put into cylinder containing fish food).

5.1.4. Place separatory funnel under the hood. Cover with parafilm. Aerate moderately for 5 or 7 days.

5.1.4.1. Aeration aids decomposition, while the hood removes offensive odors.

5.1.4.2. Set-up date is day 0. Current testing indicates 5-day aeration is preferred.

5.1.5. Note: Depending on which day decomposition occurs, make sure to adjust the references to 'days' below (i.e. Day 4, Day, 5, Day 6, etc.) when following the remaining steps.

5.1.6. Monitor the separatory funnel occasionally to ensure that it doesn't dry out. Add distilled water as needed (not to exceed total volume in the funnel of one liter).

5.2. Day 4 or 6

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5.2.1. Using a weigh boat and the Mettler balance, weigh out 5.0 g of dried cereal leaves (wheat grass).

5.2.1.1. Day 4 if 5-day aeration (preferred), or day 6 if 7-day aeration.

5.2.2. Measure 1 L of distilled water using a 1 L graduated cylinder. Add the cereal leaves and 1 L of distilled water into a 1 L flask. Use a magnetic stir bar and stir wheat grass solution overnight on a stir plate.

5.2.2.1. Alternative method (preferred): Measure 1 L of distilled water using a 1 L graduated cylinder. Add the cereal leaves and 350-400 mL of distilled water into the blender (more than this volume will blow liquid around the blender lid). Blend on high speed for 3-5 minutes. Stop the blender.

5.2.2.2. Pour cereal leaves/water mixture into a 1 L flask. Use the remainder of the original liter of water to rinse any remaining cereal leaves from the blender (put into cylinder containing cereal leaves) and to bring the volume of the cereal leaves cylinder to 1 L.

5.2.2.3. Cover the cylinder with Parafilm® and let settle in the refrigerator overnight.

5.3. Day 5 or 7

5.3.1. Stop aeration of fish food mixture and bring volume of liquid in funnel back to 1 L with distilled water (to replace water lost due to evaporation).

5.3.2. Cover the funnel containing fish food with Parafilm® and place the funnel in the walk-in refrigerator for at least 1 hour. This allows the solids to settle to the bottom of the funnel.

5.3.3. Stop spinning the cereal leaves and remove the stir bar. Place the flask in the walk-in refrigerator for 1 hour to allow the solids to settle. (If spinning overnight; alternative method has the cereal leaves already settling in the refrigerator.)

Note: The following steps 5.3.4 – 5.3.9 apply if yeast is being added at a later date (preferred). The alternative method is adding yeast immediately. Skip to step 5.3.10 if yeast is being added immediately.

5.3.4. Remove the funnel containing fish food and flask containing the cereal leaves from the refrigerator and place both on the counter in the culture room. Filter equal volumes of the fish food supernatant and the cereal leaves supernatant through a 110 µm Nitex screen into a 2 L flask. Filter each separately, discarding solids as needed to keep the screen open.

5.3.5. Mix thoroughly and pour into 250 mL plastic bottles (or best size for lab use). Fill bottles two thirds full to allow room for freezing and to add in the remaining one third of the food—the yeast solution. Place bottles in walk-in freezer for later use; or, the walk-in fridge if being used immediately.

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5.3.5.1. Do not fill the bottles to the top because a third of the remaining space is needed for the yeast mixture, if added later.

5.3.6. As needed, allow the frozen food to thaw in the walk-in refrigerator. It is recommended that the food be given 48 hours to completely thaw.

5.3.6.1. Attempt to keep the YCT bottles on a 2-week rotation, taking a new bottle out on Friday every other week, and making up the new bottle on Monday.

5.3.7. Refrigerate at 0-6 ºC in the walk-in refrigerator. Add a two-week discard date to the label. Use thawed YCT within two weeks.

5.3.7.1. Discard all thawed YCT chow after two weeks.

5.3.7.2. Discard thawed YCT chow if it has a putrid (rotten, sour) odor. Good YCT chow should have a “sickly-sweet” odor.

5.3.7.3. YCT should be discarded if “chunks” (usually an off-white color) appear in thawed YCT prior to or during use. This could be fungal or bacterial growth.

5.3.7.4. If there are still chunks that are not fungal or bacterial growth in the thawed YCT, the YCT can be filtered a second time using a 110 µm Nitex screen.

5.3.8. Record YCT preparation in LIMS Standard Prep Tracker. Under Components, add the ingredients as 5 g of yeast, 5 g of cereal leaves, and 5 g of fish flakes.

5.3.9. Opening a new bottle without yeast

5.3.9.1. Shake the thawed-out bottle well. Pour the contents into a 250 mL graduated cylinder. Divide the volume by half. The number you get will be the amount of yeast infused distilled water you will be adding. If the initial volume is either an uneven amount for division, or the volume plus the additional mixture to be added exceeds 250 mL, dump out excess liquid before moving onto the yeast mixture.

5.3.9.1.1. Ex. Filtered liquid volume comes out to 152 mL. Dividing by half, we can conclude roughly 76 mL of the bottle is the flake-fish infused water, while the other 76 mL is the cereal leaves infused water. Thus, we will need to add 76 mL of yeast mixture to keep the ratio even.

5.3.9.2. Calculate the amount of yeast you will need to weigh out. A 1-L mixture of yeast requires 5 g of yeast. To calculate the amount of yeast (x) needed for the new volume, use the following formula:

$$\frac{x}{Volume\ needed} = \frac{5\ g}{1000\ mL}$$

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5.3.9.3. Pour the mixture back into the YCT bottle. Measure the volume of distilled water needed with the same 250 mL graduated cylinder. Use less water than needed to allow for rinsing after blending.

5.3.9.4. Add the yeast and distilled water to the blender. Mix on low speed for about 1 minute until the yeast is well dispersed and the mixture has turned milky. Pour yeast mixture into the 250 mL cylinder. Rinse blender by adding distilled rinse water and use rinsewater to bring the cylinder to volume.

IMPORTANT: Do not allow the yeast mixture to settle.

5.3.9.5. Pour the yeast mixture from the cylinder directly into the YCT bottle. Cap and shake well to create a uniform mixture.

5.3.9.6. Add two additional labels to the bottle. Once the YCT has been filtered, add a label that says “Filtered”. When the bottle is thawed and opened, add a label with “Open Date”, “Volume Ratio”, and “g of Yeast Added”.

5.3.9.6.1. Ex. mm/dd/yy; 76:76:76 mL; 0.325 g of yeast

5.3.9.6.2. Remember to discard an opened bottle after 2 weeks.

5.3.10. Combining all three YCT ingredients at once

5.3.10.1. Using a weigh boat and the Mettler balance, weigh out 5.0 g of yeast.

5.3.10.2. Measure 1 L of distilled water using a 1 L graduated cylinder.

5.3.10.3. Add the yeast and distilled water to the blender. Mix on low speed until the yeast is well dispersed and the mixture has turned milky. Pour yeast mixture into a one-liter flask. Rinse blender by adding distilled rinse water and remaining water from the cylinder to the flask. Shake/swirl occasionally to prevent settling.

IMPORTANT: Do not allow the yeast mixture to settle.

5.3.10.4. Remove the flask containing fish food and flask containing the cereal leaves from the refrigerator and place both on the counter in the culture room. Filter equal volumes of the fish food supernatant and the cereal leaves supernatant through a 110 µm Nitex screen, and add an equal volume of the yeast mixture into a 4 L flask. Filter and measure each ingredient separately, discarding solids as needed to keep the screen open.

5.3.10.4.1. The yeast is generally not filtered but can be if determined to be necessary by the Tox Supervisor.

5.3.10.5. Mix thoroughly and pour into 250 mL plastic bottles. Leave 1 inch of air space as a minimum. Cap the bottles. Freeze until needed.

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5.3.10.6. Do not fill the bottles to the top or they will break when the contents freeze.

5.3.10.7. Same as step 5.3.6.

5.3.10.8. Same as step 5.3.7.

5.3.10.9. Record YCT Preparation in LIMS Standard Prep Tracker.

6. DATA ARCHIVAL

6.1. Use a black, waterproof pen for all bench sheets and logbooks.

6.2. Record preparation in LIMS Standard Prep Tracker. In accordance with SOP TA-06.42 (LIMS entry for Laboratory Parameters).

6.3. To correct raw data entries, place a single line through the incorrect or illegible entry, write the corrected entry near the error, initial and date the correction. Do not overwrite entries.

6.3.1. The corrected entry is written as near as possible to the error or else in the comment box on the appropriate bench sheet.

6.3.2. If the correction requires an explanation, write a comment and reference the error with a letter. Initial and date the explanation. Do not attempt to put all information (correction and comments) into a small space.

6.3.3. Use sequential alphabetical annotation to identify errors and corresponding comments (use A for first error and use A for corresponding explanatory comment; use B for second error, etc.).

7. QUALITY CONTROL

7.1. New batches of YCT **should** be tested per SOP TA-01.07; however, ongoing culture performance and/or control performance in scheduled chronic bioassays may be used to verify suitability of the new batch of YCT.

8. SAFETY/HAZARDOUS WASTE MANAGEMENT

8.1. Proper laboratory protection—including lab hood or ventilation system, lab coat, gloves, and safety glasses—is required when working with chemicals and unprocessed samples.

8.2. Refer to FDEP Laboratory Quality Manual for sample disposal.

8.3. Refer to the FDEP Laboratory Health and Safety Manual.

8.4. Read MSDS information for reagent handling and disposal.

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9. REFERENCES

9.1. FDEP Laboratory Quality Manual

9.2. FDEP Laboratory Health and Safety Plan

9.3. EPA 821-R-02-012, *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 5th Edition.

9.4. DEP SOP TA-01.07, *YCT (Yeast, Cereal Leaves, Flake-Fish Food, or Trout Chow) and Artemia Suitability Tests*

9.5. DEP SOP TA-06.34, *Calibration and Use of the Mettler Toledo 403TS/00 Balance*

9.6. DEP SOP TA-06.42, *LIMS Entry for Laboratory Parameters*

9.7. MSDS information sheets

APPENDIX OF CHANGES

12/12/2007 Added Scope and Application; added Summary of Method. 10/30/2008 Added References. Changed 07 to 08 in comment for Materials—Day 7—Step 6.

05/24/2013 Reformatted SOP.

02/09/2015 Formatted for 508 compliance.

04/06/2015 Changed YCT container sizes. Clarified procedure when using blender. Deleted trout chow from list of ingredients in our procedure. Changed equipment list from each day to a single list.

03/28/2016 Updated links and references.

03/30/2017 Updated links.

03/19/2018 Updated links and added hyperlinks; referenced LIMS Standard Prep Tracker; changed font to Calibri

03/12/2019 Updated references

03/17/2020 Updated preparation to include using a separatory funnel, not allowing the yeast mixture to settle, and using a stir plate to spin wheat grass instead of a blender. Updated links.

02/25/2021 Updated for universal language and checked for grammar and spelling accuracy.

07/26/2021 Updated with new instructions for both 5-day aeration method and adding yeast to bottles at a later date. Updated links.

08/01/2022 Clarified aeration days. Checked links.

07/25/2023 Checked for readability, added Class 1 weights to materials, added an SOP for LIMS uploads.

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