# 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 organism *Ceriodaphnia dubia*.

2. SUMMARY OF METHOD

    2.1. Flake fish food is fermented. Equal parts cereal leaves and yeast are added to fermented flakes. 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. Plastic Distilled Water Rinse Bottle

    3.5. 1 L Graduated cylinder (1)

    3.6. 500 mL Graduated cylinder, (2)

    3.7. 1 L Erlenmeyer flask, (1)

    3.8. 500 mL Erlenmeyer Flask, (1)

    3.9. 2 L Separatory Funnel, (1)

    3.10. 1 L Glass Jar, (1)

    3.11. Aeration device with sterile glass pipette

    3.12. Blender

    3.13. Stir plate

    3.14. Parafilm®

    3.15. Nalgene plastic bottles for finished YCT, as needed

    3.16. ~110 µm Nitex screen

    3.17. Large Plastic Funnel

    3.18. 2 L Flask, (1)

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



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## 4. REAGENTS AND STANDARDS

4.1. Deionized Water (DI)

4.2. Tetramin® or Aquatox® flake fish food

4.3. Dried cereal leaves (wheat grass)

4.4. Fleishmann's Yeast

## 5. PREPARATION

5.1. Day 0 (Initiation Day)

5.1.1. Weigh 5.0 g of flake fish food.

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

5.1.3. Pour the DI water into the clean separatory funnel and mark the 1 L line with a piece of tape. Return the water to the graduated cylinder.

5.1.4. Add the fish food and 350 – 400 mL of distilled water to the blender. Blend on low speed for 5 minutes.

5.1.5. Pour the fish food / water mixture into the separatory funnel. Use the remaining water to rinse the blender, letting the blades run for at least 15 seconds. Add the rinse water and any remaining water to the funnel.

5.1.5.1. The total volume in the funnel should reach the pre-marked 1 L line.

5.1.6. Place the separatory funnel in a 1 L glass jar inside the fume hood. Insert an airline with a sterile glass pipette tip, cover the top with Parafilm®, and aerate moderately for 7 days.

5.1.7. Monitor the separatory funnel at least once daily to ensure that it doesn't dry out. Add DI water as needed (not to exceed a final volume of 1 liter), making sure to add water on Saturday to last until Monday.

5.2. Day 6

5.2.1. Weigh 2.5 g of dried cereal leaves (wheat grass).

5.2.2. Measure 500 mL of DI water using a 500 mL graduated cylinder.

5.2.3. Add the cereal leaves and 250 mL of DI water into the blender. Blend on low speed for 5 minutes.

5.2.4. Pour cereal leaves / water mixture into a 1 L flask. Use the remaining water to rinse the blender, letting the blades run for at least 15 seconds. Add the rinse water and any remaining water to the funnel.

5.2.4.1. Use a 1 L flask because the blended cereal leaves produce a large amount of foam. This should dissipate during the overnight settling.



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5.2.5. Cover the cylinder with Parafilm® and let settle in the walk-in refrigerator overnight.

5.3. Day 7

5.3.1. Stop aeration of the fish food mixture and bring volume of liquid in funnel back to 1 L with DI water.

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 to settle.

5.3.3. Once settled, remove the mixture from the walk-in refrigerator and place on the lab bench being careful not to agitate the liquid.

5.3.4. Weigh out 2.5 g of yeast. Use freshly opened packet or sealable glass jar.

5.3.4.1. Yeast should be kept in the freezer when not in use to preserve freshness.

5.3.5. Measure 500 mL of DI water using a 500 mL graduated cylinder.

5.3.6. Add the yeast and 250 mL of DI water to the blender. Blend on low speed for 5 minutes.

5.3.7. Pour yeast / water mixture into a 500 mL flask. Use the remaining water to rinse the blender, letting the blades run for at least 15 seconds. Add the rinse water and any remaining water to the funnel.

5.3.7.1. Do not filter or allow yeast mixture to settle before combining ingredients.

5.3.8. Pour the suspended yeast mixture into a graduated cylinder and note the final volume. Return liquid to the flask.

5.3.9. Filter and measure the fish food mixture and the cereal leaves mixture separately as follows:

5.3.9.1. Using a ~110 µm Nitex screen and a plastic funnel, pour the settled mixture into a graduated cylinder. Allow the screen to catch particulates floating in the mixture but try not to dump the settled solids in the bottom of the flask / funnel. The screen can be cleaned of particulates as needed to maintain easy filtration.

5.3.9.2. Make note of the final volume of filtered liquid for both the fish food and the cereal leaves. They can then be poured into clean flasks to await combining.

5.3.10. Determine which of the three liquid ingredients has the lowest final volume. Equal parts of all three ingredients need to be combined to make the final YCT product.

5.3.11. Measure out each of the three ingredients to the selected volume and combine in one flask, mixing until fully incorporated.



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5.3.12. Pour the YCT mixture into individual bottles leaving sufficient headspace to allow for liquid expansion during freezing.

5.3.12.1. The caps of the bottles can be left loose until frozen to prevent the bottles from cracking during freezing.

5.3.12.2. Preferable volumes are 50 – 100 mL aliquots in 60 – 125 mL bottles.

5.3.13. Record YCT Preparation in LIMS Standard Prep Tracker. Under Components, add the ingredients as 2.5 g of yeast, 2.5 g of cereal leaves, and 2.5 g of fish flakes. Use the generated LIMS# on the bottle labels and place the generated label on the freezer bin that contains all the prepared bottles.

5.3.14. Label the bottles with tape that includes: “YCT”, “LIMS#”, “Batch#”, “Expiration Date”. “Open Date expiration” (leave blank to be filled in when the bottle is first opened after thawing), and “Analyst initials”.

5.3.14.1. The Batch# is the date the YCT is bottled (Day 7), this is also the day the LIMS label should be created.

5.3.14.2. Set expiration date in LIMS as 100 days from creation. If food is performing well, can extend expiration date.

5.3.14.3. The open-date expiration is two weeks from when the bottle is thawed and opened. Place a new bottle in the walk-in refrigerator with enough time to thaw (24- 48 hours) before needed.

5.3.15. Place the bottles of YCT in a plastic storage bin and set in the walk-in freezer, thawing bottles as needed.

5.3.15.1. Discard all thawed YCT after two weeks.

5.3.15.2. Make new YCT and perform the suitability test before the frozen YCT expires (~ 3 months). Thaw and dispose of all expired YCT once the new YCT has been proven suitable for use.

## 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 per SOP TA-06.42.

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.



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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 Assurance Manual for sample disposal.

8.3. Refer to the FDEP Health and Safety Manual.

8.4. Read MSDS information for reagent handling and disposal.

## 9. REFERENCES

9.1. FDEP Laboratory Quality Assurance Manual

9.2. FDEP Health and Safety Manual

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.

01/18/2025 Rewrote section 5 to reflect the current method for making YCT and removing all alternate options.



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