Achieving Accurate BOD Results: What Your Questions Revealed

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Purpose

- Built from real lab questions
  - Focused on common challenges identified in the previous BOD webinar
- Addresses shared pain points
  - Dilutions, GGA performance, seeding, DO, and data interpretation
- Bridges method and practice
  - Emphasis on execution, troubleshooting, and defensible decision-making
- Focus on confidence and consistency
  - Practical guidance to improve real-world BOD results and lab performance
- https://info.eraqc.com/webinar-mastering-bod-analyses

Method overview – SM 5210 B, 24th Ed.

- What it is
  - Indirect indicator of biodegradable organic matter
  - Captures both carbonaceous and nitrogenous oxygen demand
- How it's run
  - Light-sensitive analysis → incubate in the dark
  - 5-day incubation at 20 ± 1^{0}C
- Interpreting results
  - BOD_5 includes nitrogenous demand
  - CBOD isolates carbon demand (nitrification inhibited)
- Context
  - Results often correlate with COD and TOC for PT samples and GGA standard

BOD & CBOD – what you need to know

- BOD is a biological test
  - Indirectly measures oxygen demand from biodegradable carbon and nitrogen – sensitive to setup and conditions
- CBOD isolates carbon demand
  - Uses a nitrification inhibitor to remove nitrogen effects – critical for regulatory reporting
- Expect variability, not precision
  - Results depend on biology; differences often reflect technique, not calculation errors
- Control reduces variability
  - Strict management of incubation time, temperature, light, and handling is essential

Dilution water – a common failure point

- Dilution water is a critical test environment
  - Supports microbial activity and is a common source of variability – not just “clean water”
- Water quality and container cleanliness matter
  - Distilled, DI, or clean tap water are acceptable; plastic or glass containers are fine if properly cleaned
- Proper equilibration and handling are essential
  - Target at least 7.5 mg/L DO; avoid over-aeration – natural equilibration improves stability and prevents drifting DO
- Focus on stability and trends, not single results
  - Drifting blanks often indicate instability; trending data helps identify and correct issues

Dilutions, depletion & bottle selection

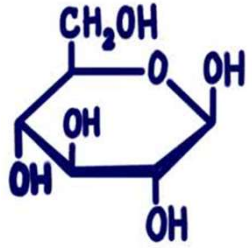
- Only use bottles that meet method criteria
  - Valid bottles must meet both depletion and residual DO requirements
- Dropping bottles is correct – not a failure
  - Fewer qualified bottles are better than more questionable ones
- Failed bottles = setup issue, not data issue
  - Adjust dilutions, seed, or conditions and rerun if needed
- Don’t force borderline results
  - Never round or stretch data – plan better dilutions to avoid edge cases

DO measurement & calibration

- Calibration limitations
  - Confirms accuracy at one point in time but does not guarantee stability during analysis (drift can still occur)
- Use DO reference materials to verify performance
  - DO CRMs confirm accuracy throughout the run and help detect drift early
- Avoid over-aeration and handle samples gently
  - Excessive mixing creates artificially high and unstable DO readings
- Improve data confidence through verification and control
  - Understanding calibration limits and monitoring stability reduces uncertainty in BOD results

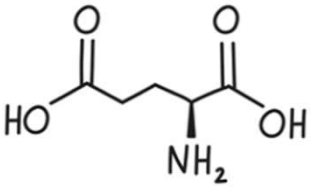
What is GGA?

- GGA is a standardized mixture with a known theoretical BOD_5 value
  - Biodegradability is predictable
- GGA recovery
  - $\textbf{GGA } \boldsymbol{Recovery}\ (\%) = \frac{Measured\ BOD\ of\ GGA}{198\ mg/L} \times 100$
  - Standard Methods acceptable results are 198 ± 30.5 mg/L; equivalent to ~84-116% recovery
- Common causes of poor GGA recovery
  - Low recovery (<84%)
    - Weak or unhealthy seed; toxic contaminants in dilution water; chlorine; insufficient nutrients; temperature and time
  - High recovery (>116%)
    - Contamination; overly strong seed; oxygen depletion errors; improper blank correction
- Why GGA recoveries are important
  - If GGA doesn't work, none of the sample BOD results can be trusted



Glucose

$C_6H_{12}O_6$



Glutamic acid

$C_5H_9NO_4$

GGA recovery is a quality control check on your BOD system, not on your samples

Seeding and GGA – why labs struggle

- Seeding drives GGA performance
  - GGA failures often point to seed issues – not calculations – especially when PT samples pass
- Seeding ensures biological activity
  - Added microorganisms enable oxygen consumption in low-activity, diluted, or toxic samples
- Consistency and correction are critical
  - Maintain consistent seed volume and apply seed correction to isolate true sample demand
- Focus on performance, not “perfect” seed
  - Evaluate seed sources, optimize conditions, and use GGA as a diagnostic tool

$$\text{BOD} = [(DO_i - DO_f)_{sample} - f(DO_i - DO_f)_{seed\ control}]/P$$

Troubleshoot low GGA recovery in BOD testing

- Start with the seed (most common cause)
  - Low GGA typically reflects weak/inactive seed – verify freshness, activity, and volume
- Check for toxicity and contamination
  - Dilution water, chlorine, or residues can suppress microbial activity
- Confirm dilution water quality and incubation conditions
  - Ensure proper nutrients, pH, temperature (20 ± 1^0C), and consistent handling
- Verify DO measurement and overall system performance
  - Check probe accuracy and technique – invalid GGA means troubleshoot and rerun

Low GGA recovery (<84%) requires investigation and rerun

Troubleshooting quick hits

- Chlorine removal with sodium sulfite
  - Using sodium sulfite effectively removes chlorine from samples to avoid interference in BOD testing
- pH adjustment concerns
  - Adjusting pH can dilute proficiency testing samples, impacting test accuracy if not done carefully
- Incubator temperature excursions
  - Incubator temperature fluctuations can affect BOD results, stressing the need for stable incubation conditions
- Bottle cleaning practices
  - Proper bottle cleaning is essential to prevent contamination and ensure accurate BOD measurements

Using BOD, CBOD, COD & TOC correlations

- Correlations can be useful – but only in controlled samples
  - Consistent relationships are typically limited to GGA and PT materials
- Real-world samples introduce variability
  - Complex matrices, non-biodegradable organics, and changing conditions break correlations
- Key drivers of COD-BOD relationships
  - Biodegradability, sample composition, nitrification, and toxicity
- Use correlations as a sanity check – not a calculation tool
  - Always rely on direct measurement for defensible BOD/CBOD results

Use correlations to support your understanding, but not as a substitute for proper testing

Understanding Waters ERA PT BOD acceptance

- BOD shows wider PT ranges by design
  - Biological variability from microorganisms leads to less precision than COD or TOC
- Acceptance limits reflect real-world performance
  - Based on regression across many labs – not relaxed standards
- Focus on consistency, not precision
  - Reliable seeding, dilution water, and technique drive acceptable results

Applying this in your lab

- BOD is a biological test
  - Variability is expected and must be managed, not eliminated
- Preparation and handling drive results
  - Dilution water, seed, incubation, and cleanliness matter most
- Routine quality checks
  - Use GGA, blanks, and seed controls and incorporate DO reference materials into routine checks for accurate measurements
- Methodical application
  - Strong BOD performance relies on consistent, thoughtful application of method principles without shortcuts
- Incremental improvements
  - Focus on small, manageable changes to enhance BOD performance and achieve consistent results

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THANK YOU!