

NC DEQ/DWR WASTEWATER/GROUNDWATER LABORATORY CERTIFICATION BRANCH

LABORATORY NAME:		CERT #:	
PRIMARY ANALYST:		DATE:	
NAME OF PERSON COMPLETING CHECKLIST (PRINT):			
SIGNATURE OF PERSON COMPLETING CHECKLIST:			

Parameter: **Vector Attraction Reduction**  
 Method: **Option 4: Specific Oxygen Uptake Rate (SOUR) [40 CFR 503.33(b)(4)]**  
**Pathogens and Vector Attraction in Sewage Sludge, EPA/600/R-22/194, January 2023**

Equipment:

300 ml BOD Bottle (if using DO meter)	Oil and particle-free air supply (Not Required)	Evaporating dishes of 100-mL capacity
Dissolved Oxygen Meter Make/Model:	Magnetic stir bar or DO meter with built-in stirrer	Drying oven, for operation at 103 to 105°C
Manometric or respirometric device with capacity of at least 300 mL	Analytical balance, capable of weighing to 10 mg	Desiccator, provided with a desiccant containing a color indicator of moisture concentration or an instrumental indicator
Temperature-measuring device accurate to ± 0.5 °C	Air Stone (Not Required)	

**PLEASE COMPLETE CHECKLIST IN INDELIBLE INK**  
 Please mark Y, N or NA in the column labeled LAB to indicate the common lab practice and in the column labeled SOP to indicate whether it is addressed in the SOP.

	GENERAL	L A B	S O P	EXPLANATION
1.	Is the SOP reviewed at least every 2 years? What is the most recent review/revision date of the SOP? [15A NCAC 02H .0805 (a) (7)]  <b>Date:</b>			Quality assurance, quality control, and Standard Operating Procedure documentation shall indicate the effective date of the document and be reviewed every two years and updated if changes in procedures are made. Verify proper method reference. During review notate deviations from the approved method and SOP.
2.	Are all review/revision dates and procedural edits tracked and documented? [15A NCAC 02H .0805 (a) (7)]			Each laboratory shall have a formal process to track and document review dates and any revisions made in all quality assurance, quality control and SOP documents.
3.	Has the laboratory developed and implemented a documented training program? [15A NCAC 02H .0805 (a) (7) (P)]			Each laboratory shall develop and implement a documented training program that includes documentation that: (i) that staff have the education, training, experience, or demonstrated skills needed to generate quality control results within method-specified limits and that meet the requirements of these Rules; (ii) that staff have read the laboratory quality assurance manual or applicable Standard Operating Procedures; (iii) that staff have obtained acceptable results on Proficiency Testing samples pursuant to Rule .0803(1) of this Section or other demonstrations of proficiency (e.g., side-by-side comparison with a trained analyst, acceptable results on a single-blind performance evaluation sample, an initial demonstration of capability study prescribed by the reference method).
4.	Is there North Carolina data available for review?			If not, review PT data
	<b>PROCEDURE – Sample Preparation</b>	<b>L A B</b>	<b>S O P</b>	<b>EXPLANATION</b>
5.	Are clean evaporating dishes heated at 103 to 105°C for 1 hour in an oven? [SM 2540 G-2020 (3) (a) (1)]			If only measuring total solids, heat dish for 1 h or longer in a 103 to 105 °C oven.
6.	Are dishes cooled to ambient temperature in a desiccator and weighed? [SM 2540 G-2020 (3) (a) (1)]			Cool in desiccator to ambient temperature and weigh.
7.	How are weighed dishes stored until needed? [SM 2540 G-2015 (3) (a) (1)]  <b>Answer:</b>			Store in desiccator or 103 to 105 °C oven until needed.

8.	Do samples qualify to be analyzed according to Option 4? [Pathogens and Vector Attraction in Sewage Sludge, EPA/600/R-22/194 (January 2023), Section 9.6] [ <a href="#">Specific Oxygen Uptake Rate Compliance letter, 20090923, NCDENR</a> ]			<p>For an aerobically digested sewage sludge with a total solids content equal to or less than 2% which has been processed at a temperature between 10°- 30°C, reduction in vector attraction can also be demonstrated using the SOUR test. The SOUR test should not be used on sewage sludge products such as heat or air-dried sludge or compost. Because of the reduction of microbial populations that occur in these processes, the SOUR results are not accurate and should not be used.</p> <p>SOUR Compliance Letter 4. <u>Continuous Aeration</u>. The SOUR test may only be used on sludges which have been stabilized in aerobic environments. Sludge holding tanks which are used primarily for sludge gravity thickening and only periodically aerated to minimize odor generation may not use the SOUR test to indicate compliance with the VAR requirements.</p>
9.	Are samples collected as a composite of grab samples taken within a period of a few minutes duration? [Pathogens and Vector Attraction in Sewage Sludge, EPA/600/R-22/194 (January 2023), Appendix C (2)]			The collection of the sample and the time between sample collection and measurement of the SOUR are important. The sample should be a composite of grab samples taken within a period of a few minutes duration.
10.	Are sample containers filled no more than half full? [Pathogens and Vector Attraction in Sewage Sludge, EPA/600/R-22/194 (January 2023), Section 10.9]			If the sewage sludge is to be used for the oxygen uptake test, the sample bottle should not be more than half full to provide some oxygen for respiration of the microorganisms in the sewage sludge.
11.	Is a maximum holding time of 2 hours observed? [Pathogens and Vector Attraction in Sewage Sludge, EPA/600/R-22/194 (January 2023), Table 10.2 in Section 10.12 and footnote 3] [ <a href="#">Specific Oxygen Uptake Rate Compliance letter, 20090923, NCDENR</a> ]			The maximum holding time between sampling and actual analysis, including shipping time, is two hours.
12.	Is the sample kept at the temperature of the digester from which it was drawn and aerated thoroughly before it is poured into the BOD bottle for the test? [Pathogens and Vector Attraction in Sewage Sludge, EPA/600/R-22/194 (January 2023), Appendix C (2)]			The sludge should be kept at the temperature of the digester from which it was drawn and aerated thoroughly before it is poured into the BOD bottle for the test. If the temperature differs from 20°C (68°F) by more than ±10°C (±18°F), the temperature correction may be inappropriate and the result should not be used to prove that the sewage sludge meets the SOUR requirement.
13.	If the test cannot be started within 15 minutes, is the sample kept at the digester temperature and aerated until the test can be completed? [Pathogens and Vector Attraction in Sewage Sludge, EPA/600/R-22/194 (January 2023), Appendix C (2)] [ <a href="#">Specific Oxygen Uptake Rate Compliance letter, 20090923, NCDENR</a> ]			The sample should be transported to the laboratory expeditiously and kept under aeration if the SOUR test cannot be run immediately.
14.	Are the percent solids of the samples verified to be between 0.5 and 2%? [Pathogens and Vector Attraction in Sewage Sludge, EPA/600/R-22/194 (January 2023) Section 9.6]			If the percent solids are not between 0.5 and 2%, this VAR method is not allowed to be used.
15.	Is this information documented? [15A NCAC 02H .0805 (a) (7) (E)]			All analytical records, including original observations and information necessary to facilitate historical reconstruction of the calculated results, shall be maintained for five years.
16.	If the total solids content of the sewage sludge is less than 0.5%, and the sample is thickened to less than 2% solids, is the thickening period limited to two hours? [Pathogens and Vector Attraction in Sewage Sludge, EPA/600/R-22/194 (January 2023) Section 9.6]			The SOUR testing on sewage sludges with a total solids content below 0.5% may give inaccurately high results. It is therefore suggested that a dilute sewage sludge could be thickened to a solids content less than 2% solid and then tested, provided that the thickening period is not in excess of two hours.
17.	Is this information documented? [15A NCAC 02H .0805 (a) (7) (E)]			All analytical records, including original observations and information necessary to facilitate historical reconstruction of the calculated results, shall be maintained for five years.
	<b>PROCEDURE – Meter Calibration</b>	<b>L A B</b>	<b>S O P</b>	<b>EXPLANATION</b>
18.	How is the DO meter calibrated? [SM 2710B-2021 (3) (a) (1)]  <b>Answer:</b>			1) Calibrate the oxygen probe and meter according to the method given in Section 4500-O G, or 2) Calibrate the manometric or respirometric device according to manufacturer's instructions.

19.	Is the meter calibration documented each day analyses are performed? [15A NCAC 02H .0805 (a) (7) (E)]			All analytical data pertinent to each certified analysis must be filed in an orderly manner so as to be readily available for inspection upon request.
	<b>PROCEDURE – SOUR Test</b>	<b>L A B</b>	<b>S O P</b>	<b>EXPLANATION</b>
20.	Is the BOD bottle filled to overflowing with a representative sample of the biological suspension to be tested? [SM 2710 B-2021 (3) (d) (1)]			Fill sample container to overflowing with an appropriate volume of a representative sample of the biological suspension to be tested.
21.	When the DO probe is inserted, is enough sample displaced to fill the flared top of bottle and its contents isolated from the atmosphere? [SM 2710 B-2021 (3) (d) (2)]			If an oxygen-sensing probe is used, immediately insert it into a BOD bottle containing a magnetic stirring bar and the biological suspension. Displace enough suspension with probe to fill flared top of bottle and isolate its contents from the atmosphere.
22.	Is the stirring speed high enough to promote adequate mixing? [SM 2710 B-2021 (3) (d) (2)]			Activate probe stirring mechanism or magnetic stirrer. [NOTE: Adequate mixing is essential. For suspensions with high concentrations of suspended solids (i.e., >5000 mg/L) more vigorous mixing than that provided by the probe stirring mechanism and magnetic stirrer may be required.]
23.	After the meter reading has stabilized, is the initial DO concentration documented? [SM 2710 B-2021 (3) (d) (3)]			After meter reading has stabilized, record initial DO reading, and start timing device.
24.	If the initial DO concentration is < 2 mg/L, is the test started over with a sample having higher initial DO levels? [SM 2710 B-2021 (3) (d) (3)]			Low DO (< 2 mg/L at the start of the test) may limit oxygen uptake by the biological suspension and will be indicated by a decreasing rate of oxygen consumption as the test progresses. Reject such data as being unrepresentative of suspension oxygen consumption rate and repeat test beginning with higher initial DO levels.
25.	Is the DO concentration documented at time intervals of less than 1 min, depending on rate of consumption, over a 15-min period or until DO becomes limiting, whichever occurs first? [SM 2710 B-2021 (3) (d) (3)]			Record appropriate DO data at time intervals of less than 1 min, depending on rate of consumption. Record data over a 15-min period or until DO becomes limiting, whichever occurs first. The oxygen probe may not be accurate below 1 mg DO/L.
26.	Is the sample temperature held constant during the test? [SM 2710 B-2021 (3) (d) (3)]			The results of this determination are quite sensitive to temperature variations and poor precision is obtained unless replicate determinations are made at the same temperature. When oxygen consumption is used as a plant control test, run periodic (at least monthly) replicate determinations to establish the precision of the technique. This determination also is sensitive to the time lag between sample collection and test initiation.
27.	Are the observed readings (DO, milligrams per liter) plotted versus time (minutes) on arithmetic graph paper to determine the slope of the line of best fit? [SM 2710 B-2021 (4)]			Plot observed readings (DO, milligrams per liter) versus time (minutes) on arithmetic graph paper and determine the slope of the line of best fit. The slope is the oxygen consumption rate in milligrams per liter per minute.
28.	How is the specific oxygen consumption rate calculated? [SM 2710 B-2021 (4)]			Calculate specific oxygen consumption rate in milligrams per gram per hour as follows:  Specific oxygen consumption rate, (mg/g)/h =  $\frac{O_2 \text{ consumption rate, (mg/L)/min} \times 60 \text{ min}}{\text{total solids (g/L)} \quad \quad \quad \text{h}}$  A spreadsheet calculator can be found on the Non-discharge Permitting website here, under 'Residuals Forms': <a href="https://deq.nc.gov/about/divisions/water-resources/water-resources-permits/wastewater-branch/non-discharge-permitting-unit/reporting-forms">https://deq.nc.gov/about/divisions/water-resources/water-resources-permits/wastewater-branch/non-discharge-permitting-unit/reporting-forms</a>
29.	Is this information documented? [15A NCAC 02H .0805 (a) (7) (E)]			All analytical records, including original observations and information necessary to facilitate historical reconstruction of the calculated results, shall be maintained for five years.
30.	Is the result temperature-corrected to an equivalent 20 °C value? [Specific Oxygen Uptake Rate Compliance letter, 20090923, NCDENR]			The SOUR test must be conducted at the same temperature that the digester is operating. The SOUR test must be temperature corrected to an equivalent 20°C value. The temperature correction equation is as follows:

				$SOUR_{20} = SOUR_T \times \Theta^{(20-T)}$ <p>Where:                      T = sludge temperature in degrees Celsius.  <math>\Theta = 1.05</math> for temperatures above 20°C  <math>\Theta = 1.07</math> for temperatures below 20°C</p>
	<b>PROCEDURE – Total Solids Determination</b>	<b>L A B</b>	<b>S O P</b>	<b>EXPLANATION</b>
31.	Is 25 to 50 grams of homogenized sample placed in a prepared evaporating dish and weighed? [SM 2540 G-2020 (3) (a) (2) (a)]			Stir to homogenize, place 25 to 50 g in a prepared evaporating dish, and weigh.
32.	Are samples evaporated to dryness on a water bath, hot plate or block, or in an oven prior to drying? [SM 2540 G-2020 (3) (a) (2) (a)]			It is important to evaporate samples at a lower temperature prior to drying at 104 °C to avoid splattering and loss of solids.
33.	Are evaporated samples dried in an oven at 103 to 105 °C for 1 h or longer? [SM 2540 G-2015 2020 (3) (2) (a)]			Evaporate to dryness on a water bath, on a hot plate or block, or in a drying oven, then dry the evaporated sample at 103 to 105 °C for 1 h or longer, cool to ambient temperature in a desiccator, and weigh.
34.	Are dried samples cooled to ambient temperature in a desiccator containing fresh desiccant, and weighed? [SM 2540 G-2020 (3) (a) (2) (a)]			See explanation above.
35.	Is the heating, cooling, desiccating, and weighing procedure repeated until the weight change is less than 50 mg? [SM 2540 G-2020 (3) (a) (2) (a)]			Repeat successive cycles of drying, cooling, desiccating, and weighing until the weight change is <50 mg.
36.	Are percent total solids calculated according to the formula in SM 2540 G-2020 (4)? [Pathogens and Vector Attraction in Sewage Sludge, EPA/600/R-22/194 (January 2023), Appendix C (2)]			Total solids concentration is determined by Standard Method 2540 G.  $\% \text{ total solids} = \frac{(A - B) \times 100}{C - B}$ <p>where:                      A = final weight of dried residue + dish (mg),                      B = weight of dish (mg),                      C = weight of wet sample + dish (mg), and</p>
	<b>QUALITY ASSURANCE</b>	<b>L A B</b>	<b>S O P</b>	<b>EXPLANATION</b>
37.	Does the laboratory analyze total solids duplicate samples at a rate of 5% per batch? [SM-2540 A-2020 (5)]			Analyze ≥5% of all samples in duplicate or at least one duplicate sample with each batch of ≤20 samples.
38.	What is the acceptance criterion for total solids duplicates? [15A NCAC 02H .0805 (a) (7)] [SM-2540 A-2020 (5)] <b>Answer:</b>			SM: The laboratory may plot duplicate determinations on a control chart for evaluation. Typically, the relative percent difference (RPD) of duplicates should not exceed 10%, but RPDs may vary considerably due to sample matrix and concentration.
39.	What corrective action does the laboratory take if the duplicate samples results are outside of established control limits or method accuracy limits? [15A NCAC 02H .0805 (a) (7) (B)] <b>Answer:</b>			If quality control results fall outside established limits or show an analytical problem, the laboratory shall identify the Root Cause of the failure. The problem shall be resolved through corrective action, the corrective action process documented, and any samples involved shall be reanalyzed, if possible. If the sample cannot be reanalyzed, or if the quality control results continue to fall outside established limits or show an analytical problem, the results shall be qualified as such.
40.	Is the analytical balance being serviced at least every 12 months by a qualified vendor/technician? [15A NCAC 02H .0805 (a) (7) (J)]			Laboratory analytical balances shall be serviced by a metrology vendor or technician every 12 months to verify that the balance is functioning within manufacturer's specifications.
41.	Does the laboratory have documentation to verify that the balance has been serviced? [15A NCAC 02H .0805 (a) (7)]			Supporting Records shall be maintained as evidence that these practices are implemented.
42.	Is the laboratory using Class S or ASTM Class 1 and/or Class 2 weights? [15A NCAC 02H .0805 (a) (7) (J)]			The analytical balance shall be checked with one ASTM Type 1, Class 1 or 2, or equivalent standard weight each day used.

43.	Are the weights being verified at least every 5 years? [15A NCAC 02H .0805 (a) (7) (J)]		These weights shall be verified every five years.
44.	Does the laboratory have documentation indicating that the weights were verified? [15A NCAC 02H .0805 (a) (7)]		<b>Date Verified:</b> Documentation of weight verifications or recertification must be maintained for 5 years. If the condition of a weight(s) is in question at any time due to damage (e.g., corrosion, nicks, scratching, etc.), the laboratory must have that weight(s) re-verified as described above.
45.	Is the balance checked with a single weight each day of use? [15A NCAC 02H .0805 (a) (7) (J)]		<b>Weight:</b>
46.	Is this documented? [15A NCAC 02H .0805 (a) (7) (J)]		
47.	Is the balance checked with at least three weights monthly? [15A NCAC 02H .0805 (a) (7) (J)]		<b>Weights:</b>
48.	Is this documented? [15A NCAC 02H .0805 (a) (7) (J)]		The values obtained shall be recorded, dated, and initialed.

Additional Comments:

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Inspector: \_\_\_\_\_ Date: \_\_\_\_\_

## Luminescence DO (LDO) Daily Check

Although many manufacturers of LDO probes indicate that the probes require calibration only every 6 months or so, NC WW/GW LC requires that an LDO probe must be calibrated or have the calibration verified each day of use. Below is a procedure for verifying the calibration of an LDO probe.

- 1) Place probe in a plastic bag with a wet sponge or a BOD bottle partially filled with water
- 2) Make sure the bag is effectively sealed (zip, rubber band, or twist tie)
- 3) Allow appropriate instrument warm up time
- 4) Read D.O. and temperature
- 5) Check the reading vs. the Solubility Table below and apply appropriate atmospheric (barometric) pressure or altitude correction factor
- 6) Calculated D.O. value must verify meter reading within  $\pm 0.5$  mg/L (do NOT calculate and apply a correction factor to calculated D.O.).

Temp. °C	D.O. mg/L	Temp. °C	D.O. mg/L
4	13.11	19.5	9.18
4.5	12.94	20	9.09
5	12.77	20.5	9.00
5.5	12.61	21	8.92
6	12.45	21.5	8.83
6.5	12.30	22	8.74
7	12.14	22.5	8.66
7.5	11.99	23	8.58
8	11.84	23.5	8.50
8.5	11.70	24	8.42
9	11.56	24.5	8.34
9.5	11.42	25	8.26
10	11.29	25.5	8.18
10.5	11.16	26	8.11
11	11.03	26.5	8.04
11.5	10.90	27	7.97
12	10.78	27.5	7.90
12.5	10.66	28	7.83
13	10.54	28.5	7.76
13.5	10.42	29	7.69
14	10.31	29.5	7.62
14.5	10.20	30	7.56
15	10.08	30.5	7.50
15.5	9.98	31	7.43
16	9.87	31.5	7.37
16.5	9.77	32	7.31
17	9.67	32.5	7.24
17.5	9.57	33	7.18
18	9.47	33.5	7.12
18.5	9.38	34	7.07
19	9.28	34.5	7.01

Atmospheric Pressure mm Hg	Equivalent Altitude Ft.	Correction Factor
760	0	1.00
752	278	.99
745	558	.98
737	841	.97
730	1126	.96
722	1413	.95
714	1703	.94
707	1995	.93
699	2290	.92
692	2587	.91
684	2887	.90
676	3190	.89
669	3496	.88
661	3804	.87
654	4115	.86
646	4430	.85
638	4747	.84
631	5067	.83
623	5391	.82
616	5717	.81
608	6047	.80
600	6381	.79
593	6717	.78

Ref: YSI Model 5000/5100 DO Meter Manual. Slight variations in DO, pressure, and/or altitude may be found in other manuals.

Example: If ambient temperature is 21°C and elevation is approximately 1126 ft, the theoretical DO would be:

$$8.92 \times 0.96 = 8.56 \text{ mg/L}$$

or, If ambient temperature is 21°C and the atmospheric (barometric) pressure is 745 mm Hg, the theoretical DO would be:

$$8.92 \times 0.98 = 8.74 \text{ mg/L}$$