In-Situ Inc.
Innovations in Water Monitoring
White Paper

In-Situ® RDO® Methods for Analysis of BOD, CBOD, and DO

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Background — Dissolved Oxygen Measurement

Since the publication of the Winkler method for measuring dissolved oxygen (DO) (Winkler 1888), the analysis of DO levels for water has been key to determining surface water purity and ecological wellness. The Winkler method is still one of only two analytical techniques used to calibrate oxygen electrode meters, the other procedure being based on oxygen solubility at saturation as per Henry's Law. Though many researchers have refined the Winkler analysis to DO levels in the low µg/L range (Potter 1957; Potter and White 1957; Potter and White 1957), the method does not lend itself to automation, continuous monitoring, or process control due to the nature of the method.

The development of an analytical instrument that uses the reduction-oxidation (redox) chemistry of oxygen was introduced during the 1950s (Kemula and Siekierski 1950). This redox electrode uses an oxygen-permeable membrane to allow the diffusion of the gas into the electrochemical cell and the redox potential is measured by the sensor. This analytical method is sensitive and accurate down to levels of ±0.1 mg/L DO (APHA 2005). However, the sensing technology is susceptible to drift and requires membrane replacement and regular maintenance and calibration.

During the last two decades, a new form of electrode has been developed. It is based on the luminescence emission of a photoactive chemical compound and the quenching of that emission by oxygen (Mingoarranz, Moreno-Bondi et al. 1995; Garcia-Fresnadillo, Marazuela et al. 1999). This quenching, photophysics mechanism is described by the Stern-Volmer equation for DO in a solution (Garcia-Fresnadillo, Marazuela et al. 1999):

$$\frac{I_0}{I} = 1 + K_{SV}[O_2]$$

I and I_0 are luminescence in the presence and absence of oxygen
K_{SV} is the Stern-Volmer constant for oxygen quenching
$[O_2]$ is the dissolved oxygen concentration

This equation has been expanded for the fixed photomer on an oxygen optical probe to take into account the two-dimensional photophysics (Borisov and Klimant 2007):

$$\frac{I_0}{I} = \frac{1 + K_{SV}[O_2]}{f_1} + \frac{1 + K_{SV}[O_2]}{f_2}$$

I and I_0 are luminescence in the presence and absence of oxygen

K_{SV} is the Stern-Volmer constant for oxygen quenching

$[O_2]$ is the dissolved oxygen concentration

f_x = fraction of each solid state photomer

The determination of oxygen concentration by luminescence quenching has a linear response over a broad range of oxygen concentrations and has excellent accuracy and reproducibility (Titze et al. 2008).

The membrane electrode and the optical probe measure the same molecular property of oxygen through different means. The membrane electrode measures oxygen via an electrochemical reaction. The optical probe uses a photochemical interaction to detect oxygen. The difference between a reaction and an interaction is that the former is consumptive and the latter is not. Calibration procedures (Winkler, etc.), Lower Reporting Limit chemistry (sodium sulfite/cobalt chloride) and application of Henry's Law are identical in both probes.

The optical probe provides an alternate technology that is at least as sensitive to low DO concentrations as a Winkler titration. The optical method has the same accuracy and reproducibility as a membrane electrode, with many of the same sampling requirements. Analysts are easily trained on the use of an optical DO sensor.

RDO Method Data Results

The EPA Office of Science and Technology has recommended the In-Situ® Inc. RDO® methods for inclusion at 40 CFR Part 136.3. The RDO methods use the In-Situ Inc. optical DO sensor in a stirred and non-stirred mode for DO, biochemical oxygen demand (BOD), and carbonaceous biochemical oxygen demand (CBOD). In the interim, NPDES permit holders can begin monitoring with the In-Situ Inc. RDO methods after seeking approval from their regional U.S. Environmental Protection Agency (EPA) authority.

The ATP validation process implemented by In-Situ Inc. for the RDO® sensor was extensive and provided over 1,300 individual data sets comparing the RDO sensor, both in stirred and non-stirred samples, with the Clark cell membrane electrode. Ten federal- or state-certified wastewater laboratories performed testing of nine categories of water and wastewater sample matrices.

Tables 1 and 2 provide a summary of the matrix types and a breakdown of the industrial matrices into specific industrial categories.

Table 1. Matrix Types Analyzed
1. DO meter calibration check sample (both high and low)
2. BOD dilution water blank
3. CBOD dilution water blank
4. BOD seed correction sample
5. CBOD seed correction sample
6. BOD glucose/glutamic acid (GGA) sample
7. CBOD glucose/glutamic acid sample
8. POTW raw plant influent
9. POTW finished plant effluent
10. Surface or ground source water
11. Finished tap water
13. Industrial effluent from categorical or signficant pretreatment industry

Matrix Types

The Tier 3 ATP Validation Study requires a minimum of nine laboratories with a different matrix type for a total of nine samples. Types of matrices analyzed were:

- **Influent:** This matrix consists of the untreated wastewater at the headworks of a wastewater treatment plant. Each sample will be unique as the composition and consistency of influent changes daily and varies with each wastewater treatment plant.
- **Effluent:** This matrix is the final product of the wastewater treatment process and is discharged to a receiving water body. Each sample will be unique as the composition and consistency of effluent changes daily and varies with each wastewater treatment plant.
- **Blank:** This matrix is the BOD or CBOD water used to produce the GGA check sample and for dilution of any BOD or CBOD sample aliquot. As per *Standard Methods for the Examination of Water and Wastewater®*, Method 5210 B (Eaton, et al. 2005, 5-2 – 5.7)., the overall depletion of this blank should be less than 0.2 mg/L over a five-day period. This matrix should be fairly uniform across the 10 participating laboratories.

Table 2. Industrial User Matrices for BOD and CBOD	
Significant Industry User Type	**Production Description**
Animal blood products	Dehydration, extraction and processing of animal blood
Animal slaughter facility	Slaughter and processing of beef, pork, and poultry
Brewery	Production of beer
Candy manufacturer	Production of candy
Dairy	Production of milk, cheese, and other dairy products
Ethanol plant	Production of ethanol from corn
Food processors	Production of bread, rolls, pasta, and other food items
Gelatin processor	Production of gelatin from beef hides
Meat byproduct processor	Rendering plant for slaughter houses
Meat processor	Production of meat products from beef, pork, and poultry
Soda pop manufacturer	Production of carbonated beverages
Wet corn mill	Production of starch, corn fructose, and dextrose from wet milled corn
Wood products processing	Production of secondary wood products from mill waste
Categorical Industry User Type	**40 CFR Chapter I, Subchapter N Category**
Iron and steel manufacturing	40 CFR 420
Metal finishing	40 CFR 433
Centralized waste treatment	40 CFR 437
Transportation equipment cleaning	40 CFR 442

- **Glucose/Glutamic Acid (GGA):** The GGA matrix consists of a known amount of a bacterial food source in dilution water. It is a measurement of the quality of seed used for seeded BOD and CBOD samples. The depletion of 198 ±30.5 mg/L is listed in Method 5210 B as the expected depletion for a five-day BOD or CBOD. This matrix should be fairly uniform across the 10 participating laboratories.
- **Seed:** This matrix consists of a known amount of active bacteria in dilution water. The values from the seed matrix are used to correct for seed oxygen uptake in the BOD and CBOD calculation. Each sample will be unique as the composition and consistency of the active bacteria changes.
- **Surface water:** This matrix consists of the surface water used for receiving effluent from a wastewater treatment plant and/or for drinking water source water. Each sample will be unique as the composition and consistency of surface water changes with site location.
- **Tap water:** This matrix consists of finished drinking water. Each sample will be unique as the composition and consistency of tap water changes with site location.
- **Industrials:** A minimum of two different industrial matrices are analyzed. Each matrix is either a Significant Industrial User (SIU) or a Categorical Industrial User (CIU). Each sample will be unique as the composition and consistency of an industry sample changes with industry type, date and location of the discharge. The definitions of these industrial matrices are:
- **Significant Industrial User (SIU)**
  - Any discharger subject to categorical pretreatment standards
  - Any other industrial user that discharges an average of 25,000 gallons per day or more of process wastewater (excluding sanitary, noncontact cooling, and boiler blowdown wastewaters) to the POTW.
    - That contributes a process wastestream which makes up five percent or more of the average dry weather hydraulic or organic capacity of the POTW treatment plant; or
    - That is designated as such by the control authority on the basis that the industrial user has a reasonable probability for adversely affecting the POTW's operation or violating any pretreatment standard or requirement.

- **Categorical Industrial User (CIU)**
  - SIU that includes: "All industrial users subject to Categorical Pretreatment Standards under 40 CFR Part 403.6 and 40 CFR Chapter I, Subchapter N" (40 CFR Part 403.3(t)(1)). For this purpose, an industrial user is deemed to be a categorical industrial user (CIU) when it meets the applicability requirements for a specific category and is subject to pretreatment standards for existing sources (PSES) or pretreatment standards for new sources (PSNS).

The laboratories that participated in In-Situ® Inc.'s RDO® sensor testing were specifically charged in the ATP protocol to select their most challenging samples in order to determine whether the RDO sensor was robust enough to stand up to daily use in the laboratory. Results showed extremely positive correlation between the membrane technology and the RDO sensor.

Some of the statistical measurements used to test the validity and ruggedness of the RDO sensor were the comparison of average GGA recovery for BOD/CBOD and the precision determined from Relative Percent Difference (RPD) between the DO values of the stirred and non-stirred RDO sensor and the Clark cell membrane electrode.

The use of GGA as a QA/QC standard in BOD/CBOD analysis is standard for wastewater laboratories. Over the years, wastewater professionals have debated about the specific interpretation of GGA results, although it is commonly agreed that the GGA results do indicate whether or not a wastewater sample has a viable bacteria seed. Comparison of each sample read by both the RDO sensor and Clark cell membrane electrode should agree within statistical limits set by Method 5210 B (198 mg/L ±30.5 mg/L). Figures 1 and 2 summarize the GGA standard deviation results for each participating laboratory.

The GGA calculated values for the stirred and non-stirred RDO sensor and the Clark cell membrane electrode showed good agreement with the recommended value and limits listed in Method 5210 B. Three laboratories did have BOD/CBOD values in excess of the 228.5 mg/L upper

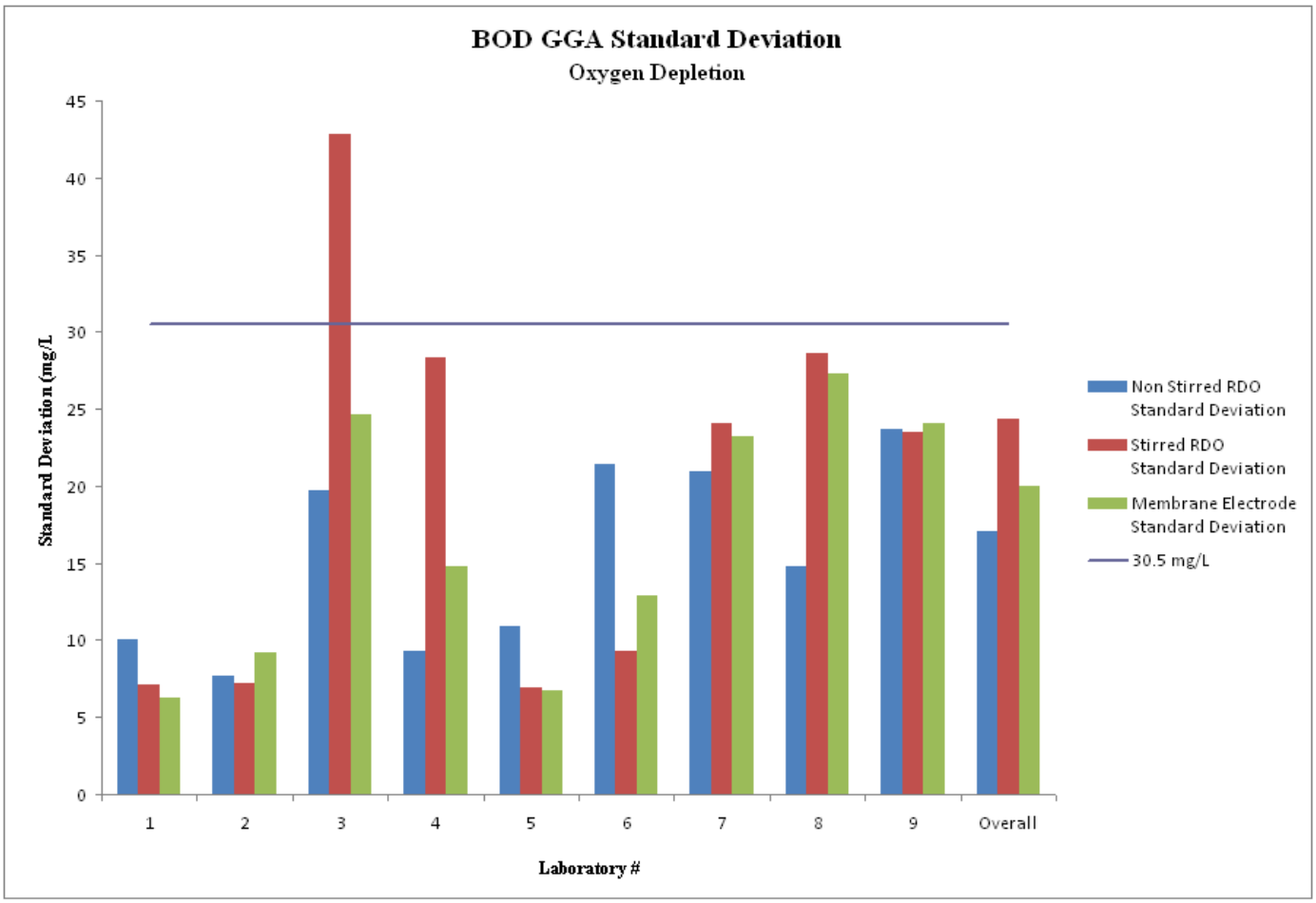

Figure 1. BOD GGA standard deviation, non-sitrred RDO method vs. stirred RDO method vs. membrane electrode

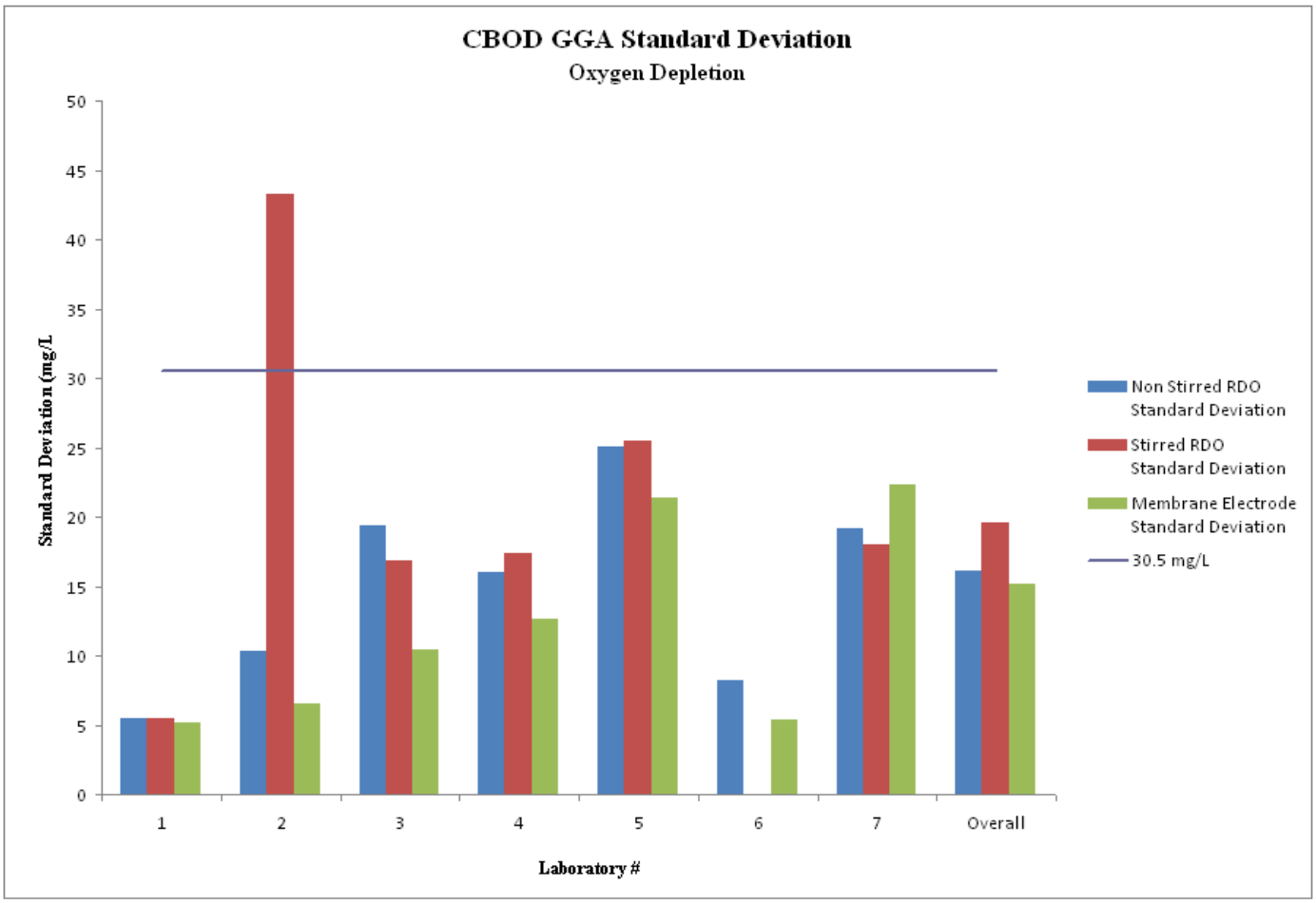

Figure 2. CBOD GGA standard deviation, non-stirred RDO method vs. stirred RDO method vs. membrane electrode

limit, indicating extremely active seed since both the RDO sensor and the Clark cell membrane had these high values. As no outliers were rejected for any GGA data, the overall standard deviation from all the laboratory GGA values for both BOD/CBOD were well within the required recovery values as stated in Method 5210 B.

Percent relative standard deviation (percent RSD) was calculated for the GGA data to compare the normalized standard deviation and to determine whether significant variation occurred within the GGA sample set. Typically, a percent RSD that is less than 20 percent is considered to show little variation within each sample set. Figures 3 and 4 illustrate the GGA sample sets for all BOD and CBOD, with outliers included.

There is only one CBOD data set from laboratory #2 where the percent RSD is greater than 20 percent. This CBOD data set from laboratory #2 consisted of only three data groups with a standard deviation significant enough that the percent RSD would indicate the variation seen. However, when this data set was included in the overall calculation, the percent RSD was reduced by approximately 50 percent. This indicates that the large variation seen with laboratory #2 did not impact the complete data set.

Though outlier calculation would have removed the data variation from laboratory #2, environmental analysis present in BOD or CBOD is known to have this type of data variation. In fact, inclusion of these outliers in the percent RSD overall calculation confirms that there is no significant variation between the RDO (whether stirred or non-stirred) and the membrane electrode.

DO was evaluated by comparing the stirred or non-stirred RDO® sensor reading to the same sample reading from a Clark cell membrane electrode and calculating the RPD. Current QA/QC standards recommend RPD values of 20 percent or less when comparing precision from duplicate samples. The RPD values obtained from each matrix type are summarized in Figure 5.

In most all matrices, the RPD was below the recommended RPD value. BOD sample RPD variation

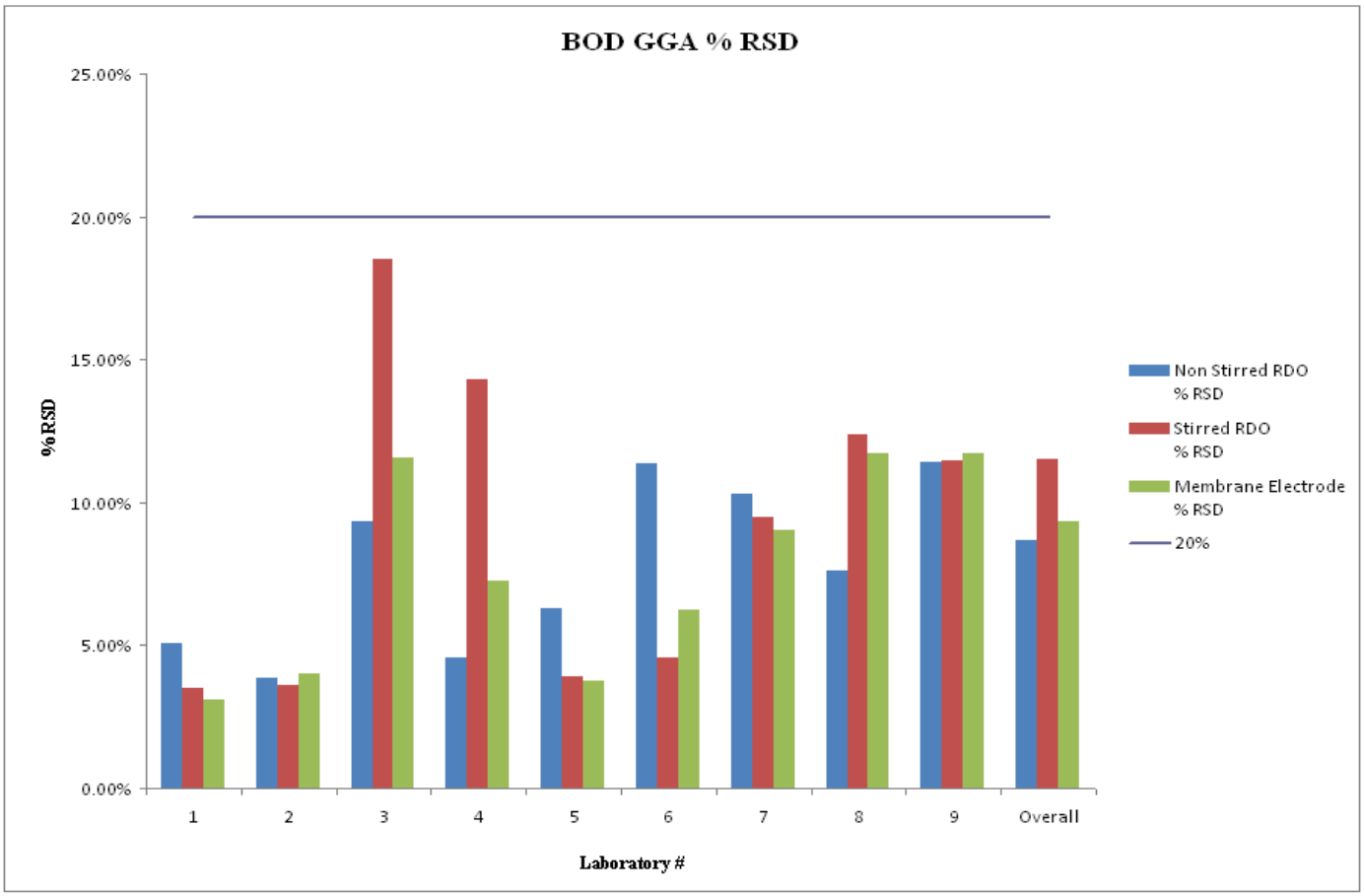

Figure 3. BOD GGA percent RSD, non-stirred RDO method vs. stirred RDO method vs. membrane electrode

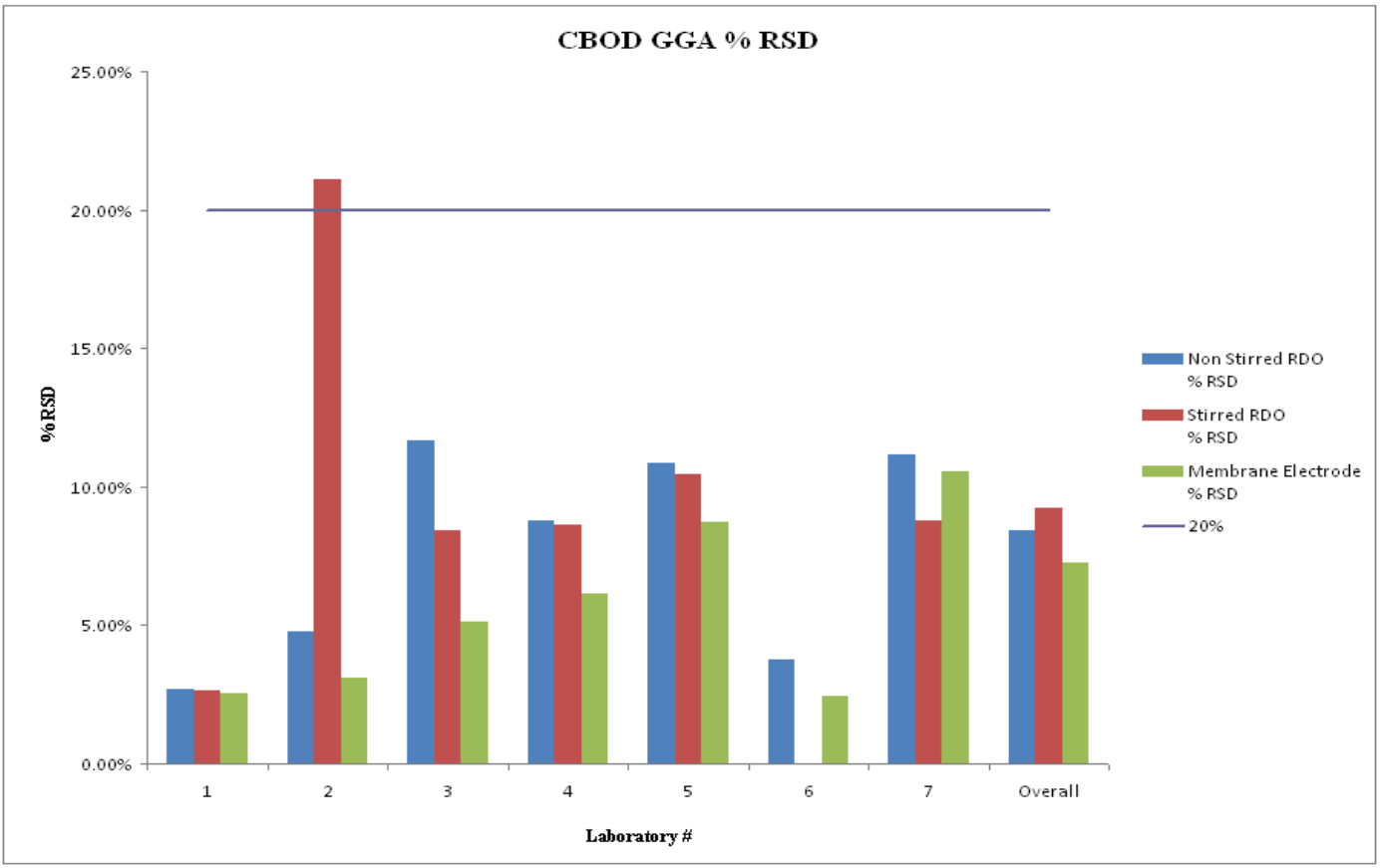

Figure 4. CBOD GGA percent RSD, non-stirred RDO method vs. stirred RDO method vs. membrane electrode

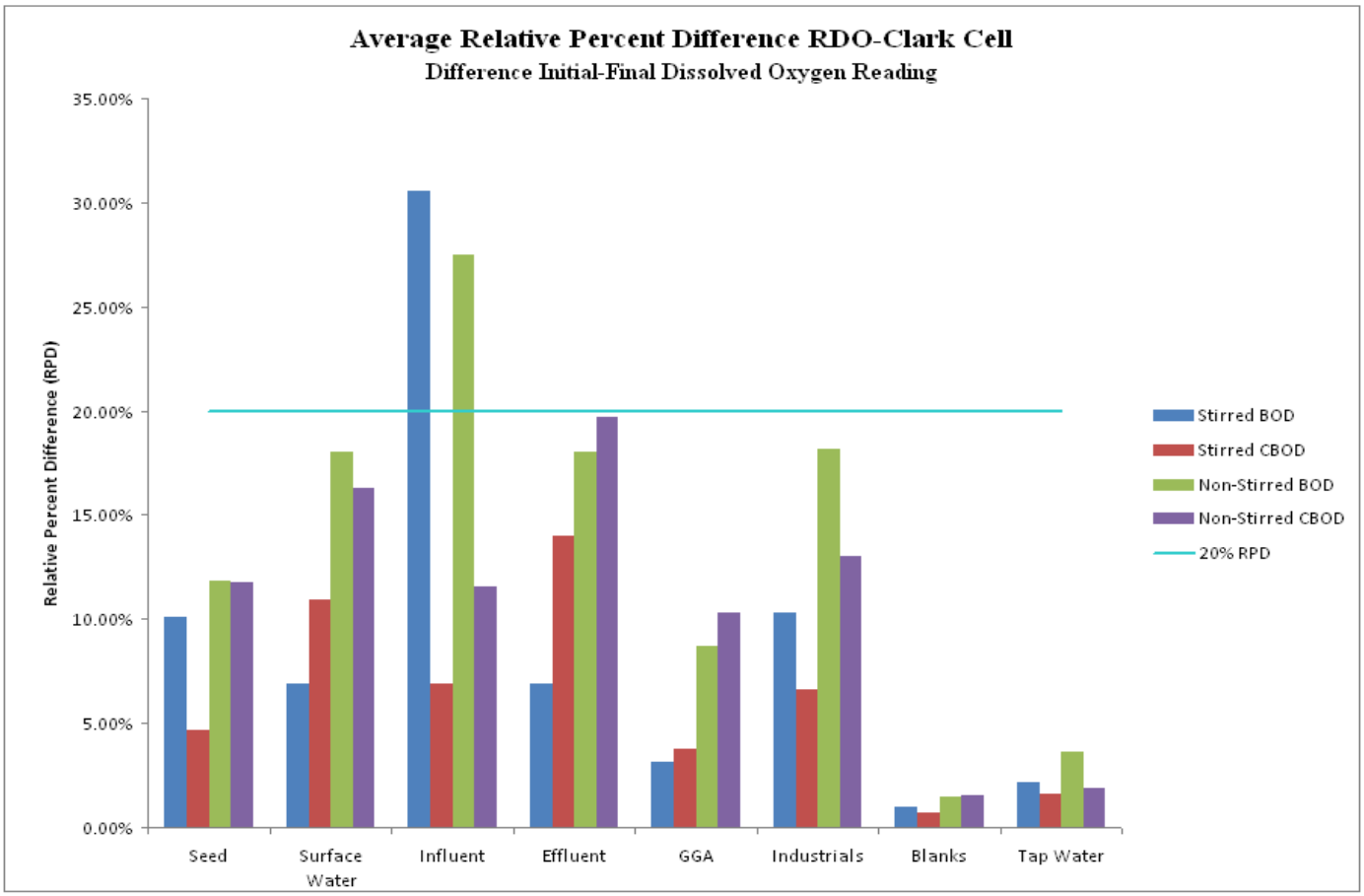

Figure 5. RPD average for DO difference, RDO method vs. membrane electrode

was likely caused by either inhomogeneity seen with influent matrices or the presence of nitrifying bacteria in the sample. As the CBOD RPD values did not show the same high RPD values, it can be assumed that nitrifying bacteria were present.

F-Test Results

Tables 3 and 4 summarize the F-test calculations for each individual laboratory and the overall GGA sample set. F-values were set at the 95 percent level (5 percent significance). The "Upper F-level 95%" column in tables 3 and 4 is the limit set for each non-stirred RDO method (NSRDO) and stirred RDO method (SRDO) values. The EPA uses the overall values to determine pass/fail. In Table 3, for example, the overall limit for BOD is 1.52. The overall NSRDO is 1.37, and the overall SRDO is 1.49. This demonstrates that both non-stirred and stirred RDO methods pass the EPA's acceptance criteria.

Table 3. Glucose/Glutamic Acid BOD F-Tests

Laboratory	Upper F-level 95%	F-test NSRDO	F-test SRDO	Upper F-level
1	2.48	2.61	1.30	2.48
2	19.00	1.45	0.61	19.00
3	6.39	1.57	3.02	6.39
4	3.79	2.57	3.66	3.79
5	9.28	0.38	1.08	9.28
6	2.82	2.74	1.92	2.82
7	5.05	1.24	1.08	5.05
8	3.18	3.42	1.10	3.18
9	3.79	1.04	0.95	3.79
Overall	**1.52**	**1.37**	**1.49**	**1.52**

Table 4. Glucose/Glutamic Acid CBOD F-Tests				
Laboratory	**Upper F-level 95%**	**F-test NSRDO**	**F-test SRDO**	**Upper F-level**
1	2.577	1.144	1.135	2.577
2	†	†	†	†
3	6.388	2.511	43.881	6.388
4	3.787	3.466	2.612	3.787
5	‡	‡	‡	‡
6	2.818	1.612	1.909	2.818
7	5.050	1.380	1.427	5.050
8	19.000	2.382	‡	‡
9	2.484	0.737	0.652	2.484
Overall	**1.550**	**1.120**	**1.299**	**1.560**

† Results are too few to calculate a standard deviation. ‡ Laboratory did not submit data.

The pooled standard deviations were calculated on each laboratory and for the complete data set. The F-value was calculated using the standard deviation from the RDO® sensor and membrane electrode. These calculations were adjusted so the lesser number was always in the denominator in order to produce a quotient greater than one. This is recognized as the most robust application of the F-test for method comparability. Values for F were obtained by calculation or table from http://stattrek.com/Tables/F.aspx or http://www.itl.nist.gov/div898/handbook/eda/section3/eda3673.htm#ONE-05-1-10.

With the exception of two laboratories whose individual F-test values for the non-stirred RDO samples were outside of the 95 percent level, all the other laboratory sample sets for the non-stirred RDO samples and the stirred RDO samples passed the F-test requirements for method comparability with the referenced method. Specifically, the overall F-test results show that both non-stirred and stirred RDO samples meet the requirements for method comparability with Method 5210 B.

Conclusion

These results, along with more detailed individual lab RPD data, provided the EPA with the scientific basis to issue the ATP letters, indicating that the RDO sensor, either in stirred or non-stirred mode, meets the requirements for the determination of DO, BOD, and CBOD. It also indicates that the RDO sensor is similar in performance to the methods listed in 40 CFR 136.3. Complete and accurate names of the In-Situ® methods are:

- In-Situ Inc. Method 1003-8-2009 Biochemical Oxygen Demand (BOD) Measurement by Optical Probe (ATP Case No. N09-0020)
- In-Situ Inc. Method 1004-8-2009 Carbonaceous Biochemical Oxygen Demand (CBOD) Measurement by Optical Probe (ATP Case No. N09-0021)
- In-Situ Inc. Method 1002-8-2009 DO Measurement by Optical Probe (ATP Case No. N05-0014)

These methods can be downloaded, along with the EPA ATP letters, at: http://www.in-situ.com/ApprovalLetters.

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