Comparative Study of The Effect of Water Sample Preservation Variations and Spectrophotometer Types on *Chemical Oxygen Demand (COD)* Testing Results

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Abstract.

Water is a primary need for all living things, especially humans, to support survival and daily activities. However, not all water can be used directly by humans. Wastewater cannot be reused without treatment because it is produced from various industrial and domestic activities. Before treatment, water samples must be examined first in the laboratory to determine their quality. However, because the nature of wastewater is easily changed, special handling is needed to maintain its condition. The water samples used were industrial wastewater (AI) and domestic wastewater (AD) with variations of preservatives (P) and without preservatives (TP). Meanwhile, the spectrophotometers used were the DR 5000 and Pharo 300 types. The lowest COD levels were produced in wastewater samples without preservatives (TP), namely 234.4 mg O2/l AI and 8,441.5 mg O2/l AD. The lowest RPD percentage was 0.287% AD-P using a DR 5000 spectrophotometer.

Keywords : *Spectrophotometer; %RPD; COD and Preservatives.*

I. INTRODUCTION

Water pollution is a hot topic that is widely discussed today because of its very important role in human life. Water pollution can be sourced from wastewater that is directly discharged into water bodies without a treatment process first. Wastewater can come from industrial or domestic waste. Industrial wastewater is wastewater or waste water from industrial processes. And, domestic wastewater is wastewater that cannot be reused from residential activities, offices and markets.Environmental Statistics Data (SLH) of the Indonesian Central Statistics Agency (BPS) in 2020 shows that 65.56% of households in Indonesia dispose of used wastewater and do not use it. And, 57.42% of households in Indonesia dispose of wastewater directly into sewers/canals/rivers. Only 1.28% disposed of waste into WWTP and 10.26% through septic tanks. Meanwhile, the most discharged wastewater final disposal directly into sewers/channels/rivers is households in urban areas (67.48%). This shows that domestic waste is one of the sources that contribute to the pollution of river bodies.Water pollution can have a detrimental impact on both the environment and humans, such as decreasing the amount of oxygen in water, disrupting soil fertility, and impacting human health (can trigger diarrhea, dengue fever, hepatitis A and E, skin lesions, skin cancer, bladder and lung disease) (BPS, 2020). Therefore, before being discharged into a water body, it is necessary to treat it first. Wastewater treatment can be done physically, chemically and biologically. Before processing, a check in the laboratory needs to be carried out to determine the condition and quality of the wastewater.

Based on SNI 8995 of 2021 concerning Water Extraction Methods, it is stated that the maximum storage time for wastewater samples is ranging from 6-24 hours depending on the parameters. For testing the COD parameters should be analyzed as soon as possible or add preservatives such as H2SO4 to pH <2 then refrigerated ≤ 6 oC, the recommended storage time is a maximum of 7 days.COD (*Chemical Oxygen Demand*) is a parameter that measures the amount of oxygen needed to decompose chemicals in water (Yunisari, Y.D, et al. 2024). COD is one of the 10 Water Quality Indexes (IKA) that shows the level of water quality in an area. In addition to COD, other parameters are DO (*Dissolved Oxygen*), pH, *Fecal Coliform*, Total Phosphorus, TSS (*Total Suspended Solid*), NH3-N, NO3-N and TDS (*Total Dissolved Solid*) based on *the National Sanitation Foundation Water Quality Index* (NSF-WQI) (BPS, 2020) method.Various studies have been conducted to test wastewater through various parameters such as those conducted by Harahap, M.R., et al (2020) analyzing COD and TSS levels in liquid waste using a Uv-vis spectrophotometer. Mushofa, N and Febriyana, L (2024) analyzed COD levels in domestic wastewater using the reflux method using a Uv-vis spectrophotometer, according to the results of their research the COD levels produced met the requirements that had been set.

A similar study was also conducted by Aryana, Hera (2025) which stated that the results of testing domestic water samples showed that COD levels were in the vulnerable range of 82.2-87.7 mg/l (below the water quality standard). Oktaviani, T and Purnamasari, E.S (2024) also examined domestic water using a spectrophotometer, but did not analyze COD levels but analyzed phenol levels found in domestic wastewater at the Bojongsari WWTP outlet, resulting in phenol levels below quality standards. Not only COD tests on domestic wastewater, spectrophotometers can also be used to measure iron (Fe) levels in coal mine wastewater, as carried out by Suryani, M., D. et al. (2022) who tested wastewater samples from 3 mining industry locations with iron content test results in A waste of 0.721 mg/l; B waste 2,786 mg/l;, and C waste 4,961 mg/l. However, the studies above only analyzed using one type of spectrophotometer, no one has compared the 2 types of spectrophotometers. Therefore, it is necessary to conduct a comparative study of COD testing using 2 types of tools such as the DR5000 and Pharo 300 spectrophotometers.This study has the objectives of testing cod levels in industrial wastewater (AI) and domestic wastewater (AD) using spectrophotometers, comparing the results of cod testing on preserved and non-preservative wastewater, comparing the results of cod testing on the use of different types of spectrophotometers based on repeatability values .

II. METHODS

Types of Research

The research was carried out in the laboratory with a chemical testing method using tools so that this research is categorized as quantitative research.

Tools and Materials Used

The DR 5000 and Pharo 300 spectrophotometers were the main equipment used. The materials used were organic-free water, H_2SO_4, Digestion Solution, Ag_2SO_4, Potassium Hydrogen Phthalate (KHP) standard solution, PT.X's industrial wastewater (AI), and domestic wastewater (AD).

Research Procedure

COD testing is carried out based on SNI 6989.2.2019 starting from preparation, manufacture of standard solutions, manufacture of blank solutions, *Digestion Solution process*, measurement of test samples, calculations.

1. **Preparation**

Prepare the necessary tools and materials, perform tests as soon as possible. For the preserved water sample, add 1-2 drops of H_2SO_4 to the AI and AD samples.

2. **Manufacture of Standard Solutions**

Standard solutions with concentrations of 300 ppm and 500 ppm that have been made are fed into the test tube. Then add 1.5 ml of *Digestion Solution* and 3.5 sulfate reagents.

3. **Manufacture of Blank Solution**

A total of 2.5 ml volume of organic free water is placed into the tube then a *Digestion Solution* and a sulfate reagent solution are added.

4. **Process *Digestion Solution***

Prepare the water sample into a tube then add *the Digestion Solution* and the sulfuric acid reagent solution. Close the tube and shake gently until homogeneous. Place the tube on a preheated heater at 150 oC ± 2oC, then reflux for up to 2 hours. Cool the sample and working solution to room temperature. Allow the suspension to settle and make sure that the part to be measured is completely clear.

5. **Calibration Curve Creation**

Turn on the equipment and optimize the spectrophotometer according to the instructions for COD testing. Set the wavelength to 600 nm or 420 nm. Then, measure the absorbance of each working solution, record it, and plot it against the COD content. Create a calibration curve from the data and determine the equation of the straight line. If the linear regression correlation coefficient (r) is <0.995, check the equipment's condition and repeat the steps above until the r coefficient value is ≥0.995.

6. Test Sample Measurement

Optimize the tool according to the instructions for COD testing, set the wavelength at 600 nm. Measure the absorption of the working solution then record and plot it against COD levels. Create a calibration curve and determine its straight-line equation.

7. Calculations

The results of the sample absorption readings obtained from the calibration curve are entered into linear regression. Calculate using equations

$$\text{COD level } (mg\ O2/l) = C \times f \text{ (SNI 6989.2.2019)}$$

Where C is the COD value in the test sample (mg/l) and f is the dilution factor.

Research Process Diagram

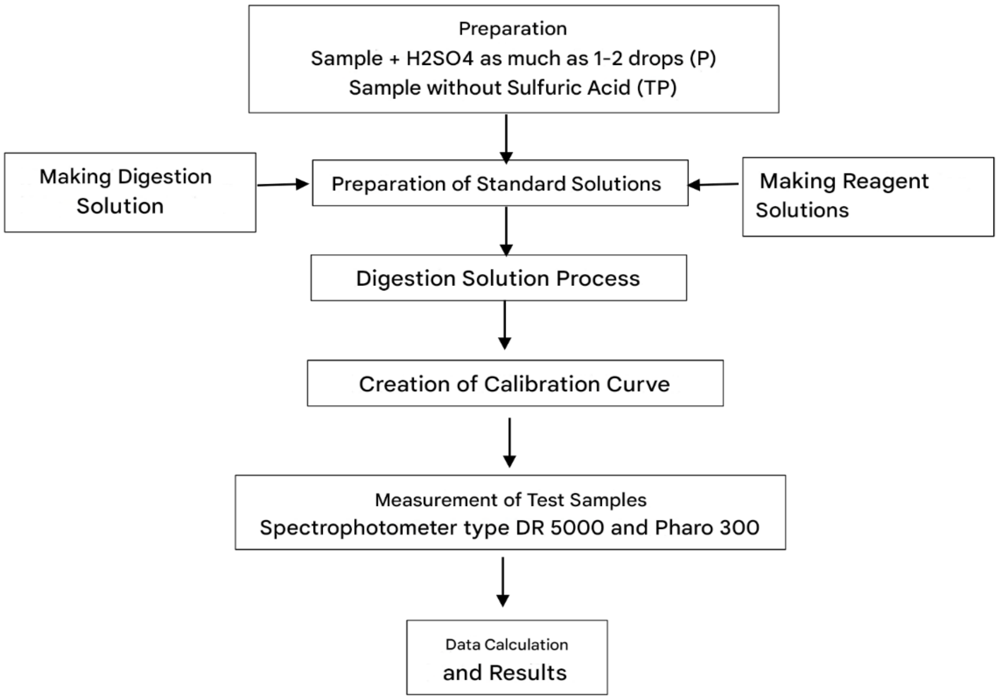

Fig 1. AI and AD Testing Process Diagram Using Spectrophotometer

III. RESULT AND DISCUSSION

COD testing using a Uv-vis spectrophotometer refers to SNI 6989.2.2019 on how to test the chemical requirement (COD) of Closed Reflux.

COD Test Result Data Using DR 5000 Spectrophotometer

Table 1. COD Testing Results Using DR 5000 Spectrophotometer

Sample	COD (mg O2/l)	%RPD	Std Rate	% Bias	%R
AI-P	**241,707**	**1,009**	-	-	-
AI-TP	**234,390**	**3,122**	-	-	-
Std 1	277,073	-	300	7,642	92,358
Std 2	491,707	-	300	1,658	98,341
Blank	1,463	-	-	-	-
AD-P	**849,024**	**0,287**	-	-	-
AD-TP	**844,146**	**0,289**	-	-	-
Std 1	306,341	-	300	2,114	102,114
Std 2	516,098	-	300	3,219	103,219
Blank	1,463	-	-	-	-

Table 1 above presents the data of COD testing results using the DR 5000 Spectrophotometer. The samples used were industrial wastewater (AI) and domestic wastewater (AD) with variations without preservatives (TP) and added preservatives (P). The DR 5000 spectrophotometer operates under Lambert-Beer's law and works on light absorption (Uv-vis) by using *deutrium* (Uv) and *wolfram* (vis) lamps to produce a broad spectrum, break light, and as a detector to convert the absorption into digital. The DR 5000 Uv-vis spectrophotometer is able to analyze light absorption with a sensitivity to the spectrum of 100-800 nm, while the UV spectrophotometer is able to analyze ultraviolet light absorption (with an ultraviolet spectrum of 190-360nm) (Suhartati, T., 2017 and Marbu, F.K., et al. 2023). And the vis spectrophotometer analyzes the colored solution formed by the addition of reagents (wavelength 320-1100 nm) (Suhartati, T., 2017 and Aryana, H. 2025). This mixture of reagents serves to decompose and break down the organic matter in the sample known as *the Digestion Solution.*

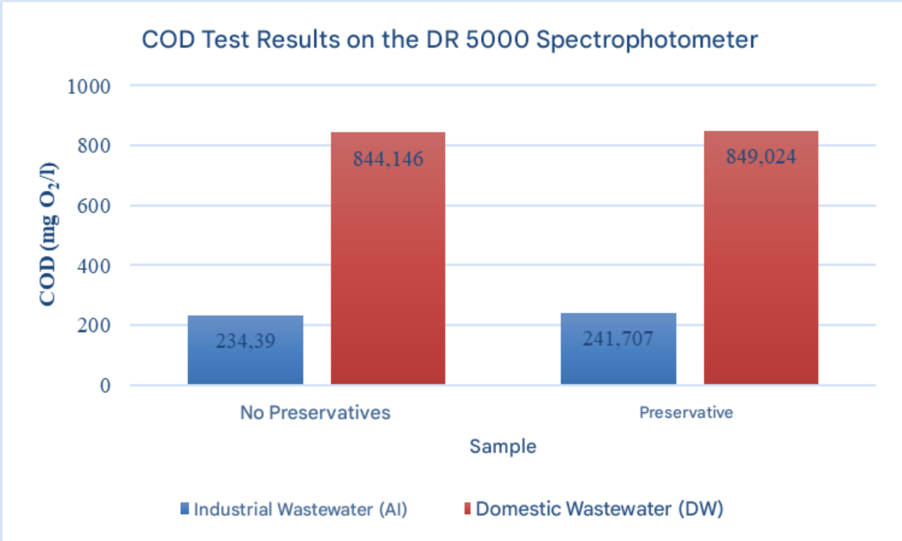

Fig 2. COD Test Results on DR 5000 Spectrophotometer

Figure 2 shows the results of COD AI and AD testing using the DR 5000 spectrophotometer. The results were obtained that the lowest COD levels were in samples without preservatives in both AI (234.39 mg O2/l) and AD (844.146 mg O2/l). Sample preservation was carried out with the addition of 1-2 drops of concentrated H2SO4. This is done to lower the pH to <2 so that the activity of microorganisms is stopped and changes in the concentration of organic substances during storage can be prevented (Kolb, et al.2017). This method is an alternative testing technique if the sample cannot be tested immediately. It is proven by the test results that do not provide a significant difference in COD values.

COD Test Result Data Using Pharo 300 Spectrophotometer

Table 2. COD Testing Results Using *Pharo* 300 Spectrophotometer

Sample	COD (mg O2/l)	%RPD	Std Rate	% Bias	%R
AI-P	**252,789**	**2,716**	-	-	-
AI-TP	**234,764**	**3,656**	-	-	-
Std 1	278,541	-	300	7,153	92,847
Std 2	493,133	-	500	1,373	98,627
Blank	5,579	-	-	-	-
AD-P	**977,253**	**0,351**	-	-	-
AD-TP	**958,369**	**0,358**	-	-	-
Std 1	311,159	-	300	3,719	103,719
Std 2	520,601	-	500	4,120	104,120
Blank	5,579	-	-	-	-

Table 2 above presents the data from COD testing using the *Pharo* 300 Spectrophotometer. This type of spectrophotometer tests the concentration of a component by determining the wavelength. The wavelength area is 190-1000 nm (Uv-vis). The lowest wavelength is 190-210 nm and the highest is 800-1000 nm. The *Pharo* 300 spectrophotometer belongs to the type *of single-beam instrument* with the principle of working the device using a single path of light and alternately passing through blanks and samples. This instrument measures absorbance at a single wavelength (Suhartati, T., 2017).

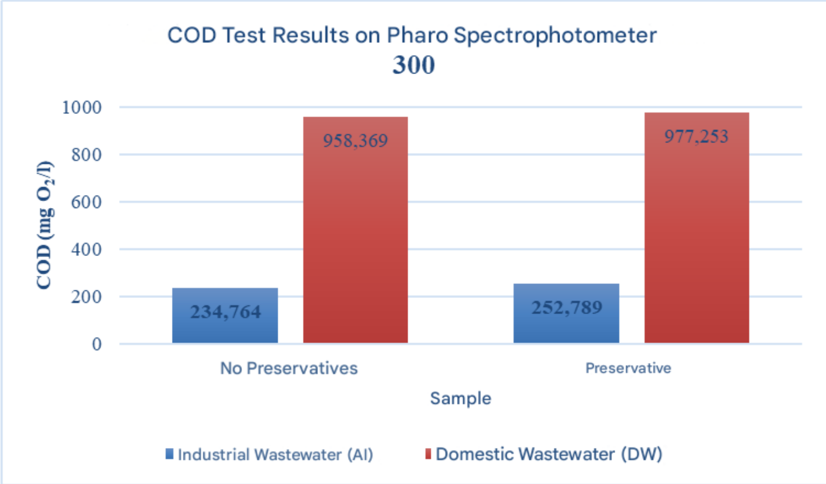

Fig 3. COD Test Results on Pharo 300 Spectrophotometer

Figure 3 shows the test results on COD AI and AD using *the Pharo* 300 spectrophotometer. The results were almost similar to tests using the DR 5000 spectrophotometer. The results of the COD test between samples that were given preservatives and those that were not given preservatives did not experience significant differences. So that this preservation method can be carried out on both types of spectrophotometers in accordance with SNI 6989.2.2019 concerning methods for testing chemical oxygen needs in water.

Comparison of COD and RPD Test Results (%) on DR 5000 and Pharo 300 Spectrophotometers

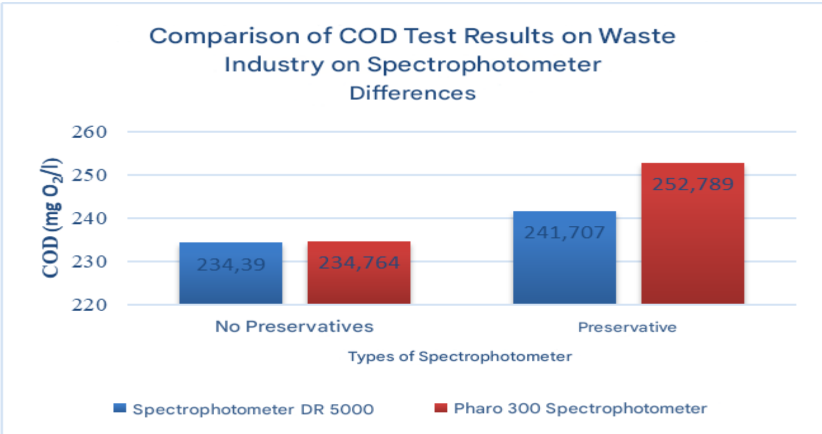

Fig 4. Comparison of COD Test Results in Industrial Wastewater to Spectrophotometer Differences

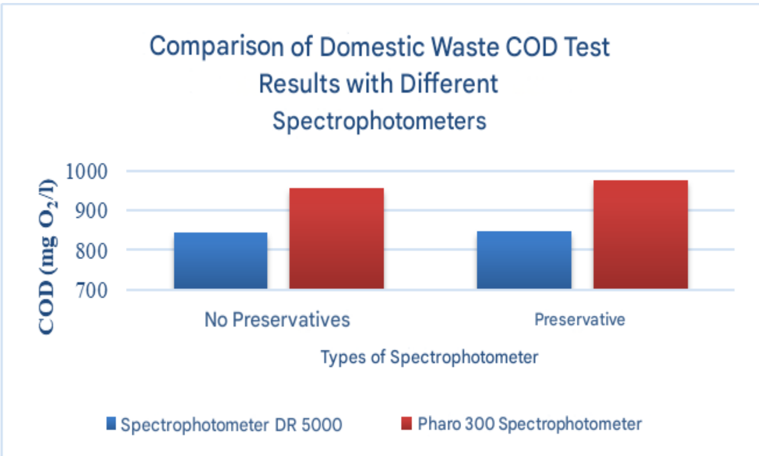

Fig 5. Comparison of COD Test Results in Domestic Wastewater to Spectrophotometer Differences

The comparison of COD test results with the difference in spectrophotometers is shown in figures 4 and 5. The two graphs state almost the same trend that COD test results are lowest in the use of DR 5000 type spectrophotometers in both cured and untreated wastewater testing. This type of spectrophotometer adopts *the working principle of a double beam instrument*, working in a wavelength range of 190-1100 nm covering the region of ulltraviolet (Uv) and visible light (vis). The DR 5000 spectrophotometer has two rays formed by a mirror cut. The blank solution is passed by the first ray while the second light simultaneously passes through the sample (Suhartati, T., 2017).The interaction of Uv-vis rays results in the transfer of electrons that are on the outside of the organic molecule from the base orbital to the excited level. This event requires a large amount of energy according to the type of electron. The difference in energy of each electron to excite is related to the wavelength (Suhartati, T., 2017).

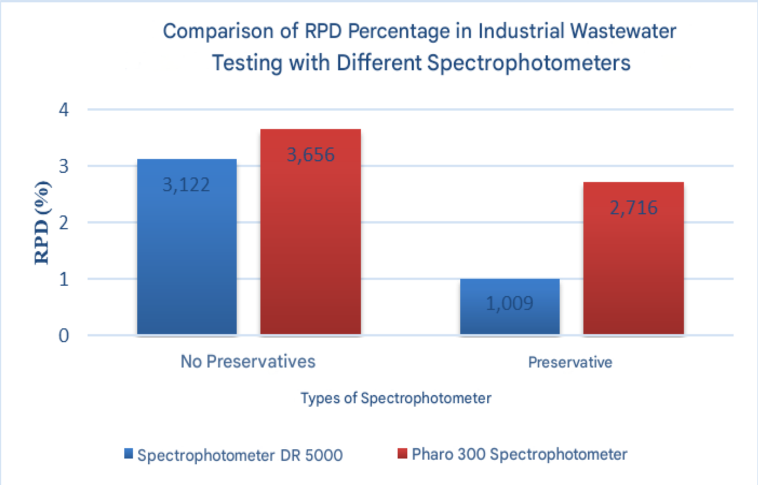

Fig 6. Comparison of RPD Percentage in Industrial Wastewater Testing to Spectrophotometer Differences

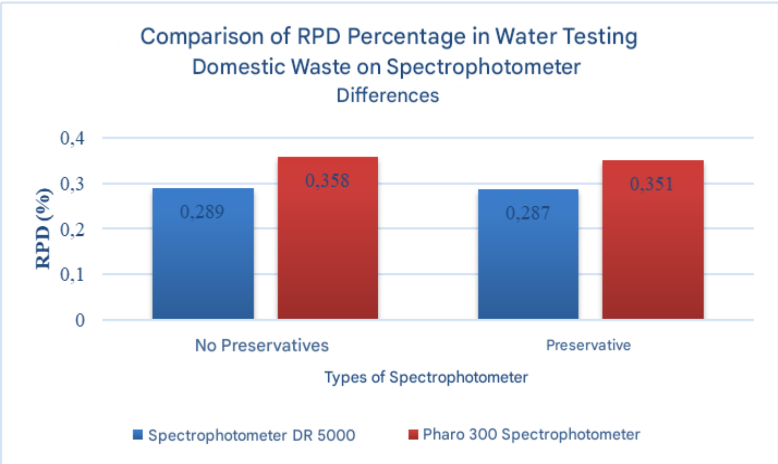

Fig 7. Comparison of RPD Percentage in Domestic Wastewater Testing to Spectrophotometer Differences

Figures 6 and 7 show a comparative trend of almost the same percentage of RPD in both industrial wastes with the lowest percentage of RPD, namely in the use of DR 5000 type spectrophotometers, both in industrial wastewater (AI) and domestic wastewater (AD) testing. A low %RPD value (≤ 10%) indicates that the data is valid or high-precision. However, if the %RPD value > 10%, the test needs to be repeated because it is considered *an outlier (Habibi, Y. 2019)*In the use of the DR 5000 spectrophotometer, the lowest percentage of RPD is in domestic wastewater testing. The difference in %RPD AD without preservatives was not significantly different (0.289%) from preserved AD (0.287%). This RPD percentage shows the percentage of the average difference between the two tests (Habibi, Y. 2019). The two graphs above show a lower %RPD even close to zero when using a DR 5000 spectrophotometer, so it can be concluded that this equipment is more precise. Due to its level of precision, the DR 5000 spectrophotometer is known as a highly accurate, stable and high-performance testing tool.

IV. CONCLUSION

The conclusions of this study are :

1. The results of COD testing on industrial wastewater (AI) samples using the DR5000 spectrophotometer were 241.707 mgO2/l (with curing) and 234.390 mgO2/l (without curing). Meanwhile, using a pharo 300 spectrophotometer, the COD test results were obtained which were 252,789 mgO2/l (with preservation) and 234,764 mgO2/l (without preservation).
2. The results of COD testing on domestic wastewater (AD) samples using the DR5000 spectrophotometer were 849.024 mgO2/l (with curing) and 844.146 mgO2/l (without curing). Meanwhile, using the pharo 300 spectrophotometer, the COD test results were obtained namely 977.253 mgO2/l (with preservation) and 958.369 mgO2/l (without preservation).
3. COD levels in wastewater samples without preservatives tended to be lower than in treated water samples.
4. The Repeatability value is lower (≤ 10%) with the use of the DR5000 spectrophotometer, so the COD test results using this tool are more accurate (0.287 AD-P and 0.289 AD-TP).

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