

Independent Evaluation of a Mercury-free Commercial Chemical Oxygen Demand Test Kit for the Analysis of Ethanol Distillery Wastewater

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ABSTRACT

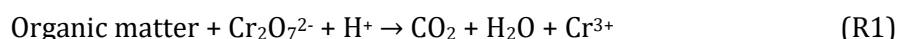
Chemical oxygen demand (COD) is a measure of the oxidizable organic compounds present in a sample and is commonly used as an indicator of water quality. Chloride ions, especially in high concentrations, present a significant interference in COD analysis, and could lead to positively or negatively deviated results. To counteract this interference, mercury-containing reagents, despite being known to be hazardous, are used. In this study, the performance of a commercial Hg-free COD test kit was compared to that of standard protocols from the American Public Health Association (APHA) (colorimetric and titrimetric methods) through the analysis of synthetic samples with known COD values and of actual wastewater samples from an ethanol distillery. Results indicate no significant difference ($P < 0.05$) between COD percent recoveries when synthetic samples without chloride (Cl^-) were analyzed: titrimetric method = $(95.02 \pm 2.84)\%$; colorimetric method = $(94.05 \pm 2.44)\%$; and commercial test kit = $(97.57 \pm 3.19)\%$. However, the COD is severely underestimated ($P < 0.05$) in the presence of a high concentration of Cl^- (1000 mg/L) titrimetric method $(91.21 \pm 4.80)\%$; colorimetric method $(89.82 \pm 4.41)\%$; and commercial test kit $(77.39 \pm 0.99)\%$. In the analysis of the two wastewater samples (Mill Wastewater and Raw Spent Wash), the COD values from the test kit are notably higher compared to the colorimetric and titrimetric methods. Specifically, for the Mill Wastewater sample (with 5535 mg/L Cl^-), the COD values obtained from the kit are $(18.66 \pm 7.23)\%$ and $(35.26 \pm 8.24)\%$ higher than the values obtained from the titrimetric and colorimetric methods, respectively. Following a similar trend for the Raw Spent Wash samples (with 22140 mg/L Cl^-), the COD values obtained from the kit are $(16.50 \pm 9.02)\%$ and $(19.22 \pm 15.59)\%$ higher than the values obtained from the titrimetric and colorimetric methods, respectively. Therefore, despite the Hg-free COD kit's advantage of being environmentally friendly, it is not recommended for the analysis of wastewater samples with very high chloride content.

Keywords: *chemical oxygen demand; commercial kit; titrimetric method; colorimetric method*

INTRODUCTION

Water is critical to the proliferation of life, and ensuring its quality is important for a lot of processes. Chemical oxygen demand (COD) is one of the numerous parameters for water quality assessment. It is a measure of the amount of a specific oxidant that reacts with a sample. Because oxidation of organic compounds is prevalent, COD is an important index for characterizing water. Specifically for wastewater, COD is a measure of the amount of oxygen needed to break down the organic pollutants present.

COD determination commonly involves the digestion of the sample with a strong oxidizing agent such as dichromate ($\text{Cr}_2\text{O}_7^{2-}$) (R1), with silver sulfate (Ag_2SO_4) as the catalyst (American Public Health Association, 2017).



In the titrimetric method of COD analysis, the unreduced $\text{Cr}_2\text{O}_7^{2-}$ after digestion will be titrated with ferrous ammonium sulfate or FAS ($\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$) (R2) (Li et al., 2017).



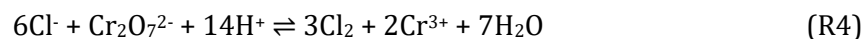
Ferroin can serve as the indicator, and the endpoint will be signaled by the sharp color change from blue-green to reddish-brown that persists for 1 min or longer (APHA, 2017). Potentiometric titration can also be used to determine the endpoint (Ma et al., 2016).

On the other hand, in the colorimetric method, COD can be estimated by absorbance measurements at two possible wavelengths: 420 nm and 600 nm. At 420 nm, the decrease in the $\text{Cr}_2\text{O}_7^{2-}$ concentration is being traced, while at 600 nm, the increase in Cr^{3+} is being measured. The commonly used calibration standard is potassium acid phthalate (KHP), with a theoretical COD of 1.176 mg O_2 /mg KHP.

The most common form of interference during COD determination is due to chlorides. Precipitation of AgCl can occur, which inactivates the catalyst (R3). This results in a negative deviation in the COD (APHA, 2017).



Cl^- can also react with the $\text{Cr}_2\text{O}_7^{2-}$ oxidant, which results in positive deviations in the COD (R4) (Li et al., 2017).

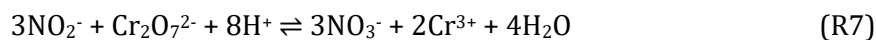


For the standard COD methods, the maximum allowable Cl^- concentration is 2000 mg/L. Chloride interference can be overcome largely, but not completely, by complexation with mercury (II) sulfate (HgSO_4) before reflux (R5) (APHA, 2017).



Other inorganic species can also interfere with COD analyses. Wastewaters are known to contain other halogens like F^- (Chaudhari et al., 2007) which could deactivate the Ag^+ similar to chlorides (R6), Fe^{2+} (Chaudhari et al., 2007) which could be oxidized by the dichromate (R2), and nitrite (NO_2^-) (Hadavifar et al., 2016) which could also be oxidized by the dichromate (R7).





There are reports of modifications of the standard COD methods. Some of these modifications include microwave and ultrasound-assisted digestion with Mn(III) as oxidizing agent (Domini et al., 2006; Domini et al., 2009) for the optimization of digestion conditions with more environmentally benign reagents, HgSO₄:Cl⁻ ratio adjustments (Kayaalp et al., 2010) for high salinity samples, and variable pathlength approach in measurements coupled with multivariate analysis (Chen et al., 2014) for predicting COD in wastewater. Several studies involve the elimination of Hg use (Vaidya et al. 1997; Geerdink et al. 2009; Kolb et al. 2017; Kishimoto and Okumura et al. 2018) owing to the known negative impacts of mercury on health and environment such as contamination, bioaccumulation, and biomagnification, developmental and neurological problems, and risks of cardiovascular diseases (Sundseth et al. 2017; Chen et al. 2018; Gworek et al. 2020). There are also newly-developed technologies that are totally mercury-free, such as the boron-doped diamond electrode method of Wang et al. (2012), and the visible light-assisted photocatalytic system of Li et al. (2013).

COD determination is a tedious analysis since the preparation of numerous reagent mixtures is needed. Thus, there is also a demand for commercially available and ready-to-use COD test kits. Of interest to this study are low-range COD kits, which are claimed to be mercury-free. Some of its manufacturer-reported properties are shown in Table 1.

Table 1. Properties of the mercury-free commercial COD test kit used in this study.

Parameter	Value
Working range	0 - 150 mg/L COD
Permissible chloride	<100 ppm
Vial composition	
Sulfuric Acid	65 - 87 wt. %
Water	10 - 34 wt. %
Silver Sulfate	≤1
Potassium dichromate	≤1
Handheld Photometer Wavelength	420 nm
Calibration equation	COD (ppm) = (-331)(absorbance) - 0.6

This study aims to independently verify the reliability of a commercially available mercury-free low-range COD test kit, specifically when extended to the analysis of ethanol distillery wastewater. It aims to compare the performance of the kit to the COD standard methods (closed reflux, colorimetric method, and open reflux, titrimetric method) from the American Public Health Association (APHA, 2017) in synthetic samples (in the presence and absence of Cl⁻), and in ethanol distillery wastewater samples.

METHODS

Materials and Equipment. The following analytical grade or primary standard grade reagents were used in this study: potassium dichromate (K₂Cr₂O₇), FAS hexahydrate ((Fe(NH₄)₂(SO₄)₂·6H₂O), Ag₂SO₄, and sodium chloride (NaCl) (Merck); HgSO₄ (Ajax Finechem); KHP (Carlo Erba); and 98% concentrated sulfuric acid (H₂SO₄) (RCI Labscan Limited). The following apparatuses were used in this study: (1) thermoreactors: Thermoreactor CR 2200 – WTW and Merck Thermoreaktor TR 300; and (2) UV-vis spectrophotometer: Shimadzu UVmini-1240 with quartz cuvette.

Preparation of Synthetic Samples. Synthetic samples of known COD were prepared from primary standard grade KHP based on the theoretical COD of 1.176 mg O₂/mg KHP (APHA, 2017).

Thus, around 0.0425 g KHP was dissolved in 500 mL distilled water, which is equivalent to a COD concentration of 100 mg/L. To simulate high chloride conditions, synthetic samples with 100 mg/L COD and 1000 mg/L Cl⁻ were also prepared. For this, around 0.0425 g KHP and 0.82 g NaCl were mixed and dissolved in 500 mL distilled water. The chloride concentration of 1000 mg/L was utilized following the work of Kolb et al. (2017) who considered this level as highly chlorinated and above the complexing capacity of HgSO₄.

Preparation of Wastewater Samples. The wastewater samples were obtained from a local ethanol distillery. Table 2 summarizes some information about the wastewater samples.

Table 2. Information on the wastewater samples.

Parameter	Mill Wastewater	Raw Spent Wash
Type of Sample	Waste from sugar milling and extraction of sugarcane juice (milling is done in a separate facility within the distillery).	Waste from the end of the EtOH production process; contains molasses, sugarcane juice, etc. Waste that enters the wastewater management.
Initial pH	4.144	3.996
Salinity, ‰	10	40
Chloride, mg/L*	5535.106	22140.426

*Estimated from salinity following Lewis (1980).

The wastewater samples (around 1 L each) were filtered to remove large solid particles and suspended matter (Whatman qualitative filter paper, Grade 1, 11 µm pore size). The samples were then preserved by acidification with concentrated H₂SO₄ to pH ≤ 2. Prior to the analysis, the samples were diluted accordingly with distilled water so that the COD and Cl⁻ levels were within the allowable range of each method. For the standard methods (titrimetric and colorimetric), 100x dilution was used (10.00 mL sample diluted to 100.00 mL with distilled water), while for the commercial kit method, 500x dilution was performed (0.20 mL sample diluted to 100.00 mL with distilled water).

Open reflux, titrimetric method. The open reflux, titrimetric method was performed according to the APHA Method 5220B (2017), which is suitable for the analysis of samples with COD values >50 mg O₂/L, with all the prescribed amounts of the samples and reagents reduced to 40%. Briefly, 20.00 mL sample (distilled water for blank) was placed in a reflux flask, followed by the addition of 0.4 g HgSO₄, 2 mL (initial) sulfuric acid reagent (10 g Ag₂SO₄/1 L H₂SO₄), 10.00 mL standard 0.25 N standard K₂Cr₂O₇, and another 28 mL (final) H₂SO₄ reagent. The mixture was cooled in an ice bath with thorough mixing. The flask and its contents were heated under reflux for 2 hours. Afterward, the setup was allowed to cool, and the inner walls of the reflux condenser were washed down with distilled water. The contents of the flask were diluted to around twice the volume with distilled water and then allowed to cool to room temperature. Finally, the mixture was titrated with standard 0.25 N FAS titrant to a ferroin indicator endpoint (color change is from blue-green to reddish-brown).

Closed reflux, colorimetric method. The closed reflux, colorimetric method was performed according to the APHA Method 5220D (2017), which is suitable for COD values >50 mg O₂/L. First, 0.51 g K₂Cr₂O₇, 1.67 g HgSO₄, and 8.35 mL H₂SO₄ were mixed, dissolved, and diluted with distilled water to give a 50 mL solution. This mixture served as the digestion solution. A 2.50 mL sample (distilled water for blank), 1.50 mL digestion solution, and 3.5 mL sulfuric acid reagent were added into a COD tube and thoroughly mixed. For the calibration standards, KHP solutions of varying COD were prepared. The COD tubes containing the mixtures were placed in a thermoreactor preheated to 150 °C and refluxed for 2 hours. After digestion, the samples were slowly cooled to room temperature and mixed to combine the contents. Suspended matter was

then allowed to settle. Finally, all samples were diluted to 25.00 mL, and the absorbances were measured at 420 nm using a UV-vis spectrophotometer.

Commercial Hg-Free COD Kit Method. Exactly 2.00 mL sample was added into the commercial COD kit vial and mixed thoroughly. Distilled water was used for the blank sample. The vials containing the mixtures were refluxed in a thermoreactor at 150 °C for 2 hours. The samples were then allowed to cool slowly, mixed, and allowed to settle. The COD values were finally measured at 420 nm using the handheld photometer that came with the kit.

Calculations and Data Treatment. The COD for the titrimetric and colorimetric methods, respectively, were calculated using equations E1 and E2 (APHA, 2017).

$$COD \text{ (titrimetric)} = \frac{(B-A)(M)(8000)}{\text{mL sample}} \times DF \quad (E1)$$

where B = mL FAS in blank titration; A = mL FAS in sample titration; M = molarity of FAS; 8000 = mEW of oxygen x 1000 mL/L; and DF = dilution factor.

$$COD \text{ (colorimetric)} = (x)(DF) \quad (E2)$$

where x = COD from linear regression.

The percent recovery was calculated using equation (E3).

$$\% \text{Recovery} = \frac{\text{Experimental value}}{\text{Reference value}} \times 100 \quad (E3)$$

For the comparison of the COD values of the wastewater samples obtained from the commercial kit and the standard methods, the percent difference was calculated using equation (E4.1). For the comparison of two standard methods (titrimetric vs colorimetric), the percent difference was calculated with respect to the titrimetric method (E4.2).

$$\% \text{Difference} = \frac{|COD_{\text{kit}} - COD_{\text{std method}}|}{COD_{\text{std method}}} \times 100 \quad (E4.1)$$

$$\% \text{Difference} = \frac{|COD_{\text{colorimetric}} - COD_{\text{titrimetric}}|}{COD_{\text{titrimetric}}} \times 100 \quad (E4.2)$$

Basic statistical parameters such as the mean, standard deviation, and relative standard deviation (RSD) were calculated. Statistical tests were also performed, particularly F-tests (to compare variances, i.e., precision) and t-tests (to compare means, i.e., accuracy). The t-tests performed assumed either equal or unequal variances, depending on the results of the F-tests.

RESULTS AND DISCUSSION

Analysis of Synthetic Samples. Synthetic samples of known COD were subjected to three different COD determination methods, and the accuracy of each method was assessed through the percent recovery. Some samples were also intentionally spiked with a high chloride concentration of 1000 mg/L (Kolb et al. 2017) to assess the effect of chloride interference on the analysis.

Table 3 summarizes the COD and percent recoveries obtained for the synthetic samples. As shown, there is good correspondence between the COD values obtained by the three different methods for the samples without Cl⁻. This is to be expected since chloride interference is not

present. However, a negative deviation is observed in the samples containing 1000 mg/L Cl⁻, which is possibly due to the deactivation of the Ag₂SO₄ catalyst (R3) (Taromsary et al., 2019).

Table 4 summarizes the statistical comparison of the percent recoveries in the presence and absence of Cl⁻ for each method performed. For the titrimetric method, the variances and the means are not significantly different, which implies that the presence of chloride interference does not affect the accuracy and precision of the analysis. The same observation is true for the colorimetric method. The results can be rationalized by the use of HgSO₄ in the titrimetric and colorimetric methods, which indeed helps minimize the effect of Cl⁻ interference. For the commercial kit method, on the other hand, the variances are not significantly different, implying that chloride interference does not impact the precision. However, the means are significantly different, which shows that chloride interference negatively affects the COD measurement when a high amount of Cl⁻ is present. This can be attributed to the fact that the kit is mercury-free, and excessive Cl⁻ presents significant interference.

Table 5 shows the statistical comparison of the recoveries obtained from the different COD methods for the synthetic samples without Cl⁻. As expected, since no interferents are present, there are no significant differences between the variances and the means for the three methods being compared.

Table 6 shows the statistical comparison of the recoveries obtained from the different COD methods for the synthetic samples with 1000 mg/L Cl⁻. For the standard methods (titrimetric and colorimetric), the differences in the obtained variances and means are not statistically significant. However, a comparison of the standard methods and the commercial kit methods revealed that the obtained variances and means are significantly different. The COD kit specified a permissible chloride level of <100 mg/L, while the standard methods specified a maximum allowable Cl⁻ concentration of 2000 mg/L (APHA 2017). This implies that the kit cannot measure COD as reliably as the standard methods when chlorides are present at high concentrations, which is typically true of ethanol distillery wastewater (Fito et al. 2018a; Fito et al. 2018b).

During the sample preparation with the commercial COD kit, it was observed that AgCl precipitates immediately formed upon the addition of the chloride-containing sample into the COD vial. Thus, the interference due to AgCl precipitation (R3) accounts for the considerable negative deviation in the percent recoveries. It is also possible that some of the precipitates remained suspended despite allotting ample time for the particles to settle, which led to increased absorption at 420 nm and the effective attenuation of the COD values.

Table 3. COD values obtained from the analysis of the synthetic samples.

Parameter	Titrimetric method		Colorimetric method		Commercial kit method	
	Without Cl ⁻	With Cl ⁻	Without Cl ⁻	With Cl ⁻	Without Cl ⁻	With Cl ⁻
Reference COD, mg/L	98.78	101.14	100.78		100.78	
Experimental COD, mg/L	93.86 ± 2.80	92.24 ± 4.85	94.79 ± 2.46	90.52 ± 4.44	98 ± 3	78 ± 1
Recovery, %	95.02 ± 2.84	91.21 ± 4.80	94.05 ± 2.44	89.82 ± 4.41	97.57 ± 3.19	77.39 ± 0.99
RSD, %	2.99	5.26	2.60	4.91	3.27	1.28

For all analyses, n = 3. COD and recovery values are expressed as $\bar{x} \pm s$.

Table 4. Statistical comparison of the recoveries of the synthetic samples (without Cl⁻ vs with Cl⁻) for the same method.

Method	F-test		t-test	
	P value	Conclusion	P value	Conclusion
Titrimetric	0.26	P > 0.05; No significant difference between the variances	0.30	P > 0.05; No significant difference between the means ¹
Colorimetric	0.24	P > 0.05; No significant difference between the variances	0.22	P > 0.05; No significant difference between the means ¹
Commercial kit	0.09	P > 0.05; No significant difference between the variances	0.0004	P < 0.05; There is significant difference between the means ¹

For all analyses, n = 3, df = 2, and $\alpha = 0.05$.

¹ Assuming equal variance.

Table 5. Statistical comparison of the recoveries obtained from the different methods for the synthetic samples without Cl⁻.

Method	F-test		t-test	
	P value	Conclusion	P value	Conclusion
Titrimetric vs Colorimetric	0.43	P > 0.05; No significant difference between the variances	0.68	P > 0.05; No significant difference between the means ¹
Titrimetric vs Commercial kit	0.44	P > 0.05; No significant difference between the variances	0.36	P > 0.05; No significant difference between the means ¹
Colorimetric vs Commercial kit	0.37	P > 0.05; No significant difference between the variances	0.20	P > 0.05; No significant difference between the means ¹

For all analyses, n = 3, df = 2, and $\alpha = 0.05$.

¹ Assuming equal variance.

Table 6. Statistical comparison of the recoveries obtained from the different methods for the synthetic samples with 1000 mg/L Cl⁻.

Method	F-test		t-test	
	P value	Conclusion	P value	Conclusion
Titrimetric vs Colorimetric	0.45	P > 0.05; No significant difference between the variances	0.73	P > 0.05; No significant difference between the means ¹
Titrimetric vs Commercial kit	0.041	P < 0.05; There is significant difference between the variances	0.039	P < 0.05; There is significant difference between the means ²
Colorimetric vs Commercial kit	0.048	P < 0.05; There is significant difference between the variances	0.041	P < 0.05; There is significant difference between the means ²

For all analyses, n = 3, df = 2, and $\alpha = 0.05$.

¹ Assuming equal variance.

² Assuming unequal variance.

Analysis of Wastewater Samples. To test the performance of the commercial kit in comparison to the standard methods for the analysis of real-world samples, wastewater samples from an ethanol distillery were analyzed. Table 7 shows the obtained COD levels of the mill wastewater and raw spent wash samples. The COD values are typical of wastewater samples obtained from alcohol distilleries, which are in the thousands of ppm (Chaudhari et al., 2007; Prajapati and Chaudhari, 2013; Hadavifar et al., 2016; Wagh et al., 2020). In all three methods, it can be observed that the raw spent wash samples have around twice as much COD as the mill wastewater samples. This is expected since the raw spent wash samples were obtained at a later part of the ethanol production process and thus are expected to contain more oxidizable organic species.

Table 7. COD results obtained from the analysis of the wastewater samples.

Parameter	Titrimetric method ¹		Colorimetric method ¹		Commercial kit method ²	
	Mill Wastewater	Raw Spent Wash	Mill Wastewater	Raw Spent Wash	Mill Wastewater	Raw Spent Wash
COD, $\times 10^4$ mg/L	2.43 \pm 0.05	4.98 \pm 0.17	2.13 \pm 0.04	4.73 \pm 0.09	2.88 \pm 0.15	5.58 \pm 0.82
RSD, %	2.19	3.34	1.95	1.85	6	15

For all analyses, n = 3. COD values are expressed as $\bar{x} \pm s$.

¹ DF = 100.

² DF = 500.

Tables 8 and 9 summarize the statistical comparison of the COD values obtained from the different methods for the mill wastewater and raw spent wash samples, respectively. Furthermore, the results indicate an overestimation of the COD values when the commercial kit was used (Table 10). For the mill wastewater sample, the obtained COD from the kit is 18.66% higher than the value obtained from the titrimetric method, and 35.26% higher than the value obtained from the colorimetric method. For the raw spent wash, on the other hand, the COD obtained from the kit is 16.50% higher than the value obtained from the titrimetric method, and 19.22% higher than the value obtained from the colorimetric method.

The authors hypothesize that the overestimation of the COD in the wastewater samples by the commercial kit is related to the presence of chloride in conjunction with ammonium (NH_4^+) ions. Standard methods (APHA, 2017) suggest the use of HgSO_4 in order to deal with chloride interference but is known to not completely remove it. Hence, the use of the commercial kit, which is mercury-free, for samples with high chloride concentrations can mean that Cl^- can react with the $\text{Cr}_2\text{O}_7^{2-}$ oxidant, resulting in a higher COD value (Li et al., 2017). As for the ammonium, determination was not performed in the study, but information from the literature points to the highly probable presence of ammonium in the wastewater samples. Ammonium is commonly used as a nitrogen source in large-scale ethanol fermentation (Yang et al., 2021). Thus, ammonium will likely end up in ethanol production facilities' wastewater. Furthermore, ammonium (or ammonia in some works) has been reported to be present in ethanol production wastewaters in significant amounts (Hadavifar, 2016; Hu et al., 2017; Papadopoulos et al., 2020; Ratna et al., 2021; Sankaran and Premalatha, 2018). Ammonium also interferes with COD analysis and results in positive deviations in the obtained values. A study (Kim, 1989) reveals that the interference particularly occurs in the presence of Cl^- and is not observed in its absence. Furthermore, the chloride interference intensifies at higher ammonium concentrations. Thus, as previously shown in (R4), Cl^- is oxidized by $\text{Cr}_2\text{O}_7^{2-}$ to form Cl_2 , resulting in positive deviations in the COD. In the presence of NH_4^+ , the Cl_2 can be reduced back to Cl^- (R8) (Vaidya et al., 1997). Since the commercial COD kit is mercury-free, it cannot counteract the error caused by Cl^- interference, which could have been aggravated with high ammonium concentrations.

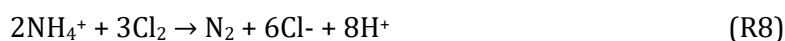


Table 8. Statistical comparison of the COD values obtained from the different methods for the mill wastewater sample.

Method	F-test		t-test	
	P value	Conclusion	P value	Conclusion
Titrimetric vs Colorimetric	0.38	P > 0.05; No significant difference between the variances	0.0016	P < 0.05; There is significant difference between the means ¹
Titrimetric vs Commercial kit	0.08	P > 0.05; No significant difference between the variances	0.01	P < 0.05; There is significant difference between the means ¹
Colorimetric vs Commercial kit	0.053	P > 0.05; No significant difference between the variances	0.002	P < 0.05; There is significant difference between the means ¹

For all analyses, n = 3, df = 2, and $\alpha = 0.05$.

¹ Assuming equal variance.

Table 9. Statistical comparison of the COD values obtained from the different methods for the raw spent wash sample.

Method	F-test		t-test	
	P value	Conclusion	P value	Conclusion
Titrimetric vs Colorimetric	0.22	P > 0.05; No significant difference between the variances	0.08	P > 0.05; No significant difference between the means ¹
Titrimetric vs Commercial kit	0.04	P < 0.05; There is significant difference between the variances	0.34	P > 0.05; No significant difference between the means ²
Colorimetric vs Commercial kit	0.01	P < 0.05; There is significant difference between the variances	0.21	P > 0.05; No significant difference between the means ²

For all analyses, n = 3, df = 2, and $\alpha = 0.05$.

¹ Assuming equal variance.

² Assuming unequal variance.

Table 10. Comparison of the difference of the COD values obtained from the different methods.

Method	% Difference	
	Mill Wastewater	Raw Spent Wash
Titrimetric vs Colorimetric ¹ , %	12.27 ± 1.71	5.17 ± 1.75
Titrimetric vs Commercial kit, %	18.66 ± 7.23	16.50 ± 9.02
Colorimetric vs Commercial kit, %	35.26 ± 8.24	19.22 ± 15.59

¹ % difference was calculated with respect to titrimetric method. Values are expressed as $\bar{x} \pm s$.

CONCLUSIONS

The commercial low-range COD test kit offers the advantages of being convenient and easy to use and being mercury-free. Despite these advantages, the extension of the test kit's application to the analysis of ethanol distillery wastewater is not suitable for three main reasons, as demonstrated in this study. First, the range of 0–150 ppm COD was too restrictive, especially since wastewater COD levels are in the thousands of ppm. This would make dilution procedures mandatory. Second, there was a significant negative deviation observed at high Cl⁻ concentration, as evident in the experimental results with the synthetic samples. Third, COD values from the test kit were notably higher than those found in the wastewater samples relative to the standard methods.

As a recommendation, it is plausible to use other commercial kits that are in the range closer to the COD values typical of ethanol distillery wastewater. However, these kits might not be mercury-free. Using a broader range could reduce or even totally eliminate the need for sample dilution before the analysis. It is also possible to adopt the closed reflux, colorimetric method of the APHA (2017), which practically works in a very similar way as the commercial kits. The method involves the minimal use of samples and reagents. Also, the amounts of K₂Cr₂O₇ and HgSO₄ can be adjusted as necessary, depending on the COD and Cl⁻ levels.

For future studies, it is suggested to assess other water quality parameters such as sulfates, sulfites, phosphates, ammonium, and other inorganics in wastewater. These will provide more valuable insights into the relationship between wastewater composition and its impact on the COD.

ACKNOWLEDGMENTS

The authors would like to acknowledge the assistance of Ms. Maria Theresa M. Escosia, Ms. Jennifer Q. Pillas, and Ms. Aimee F. Eraña of the Analytical Services Laboratory, Institute of Chemistry, UPLB. They would also like to acknowledge Engr. Bianca Nicole Catli for the acquisition of the wastewater samples, Dr. Sheryl Lozel B. Arreola for lending one of the thermoreactors used in this study, and Ms. Yvette D.C. Valenzuela for assistance in the statistical treatment of the data.

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