

Comparison of Spectrophotometric and Digital Photometric Methods for Determining Chemical Oxygen Demand



ABSTRACT

Visible spectrophotometry (VS) and digital photometry (DP) for determining chemical oxygen demand (COD) were compared; the latter method involved image processing of digital photographs of analyte solutions using RGB-AIC software. Statistical analysis showed that COD values of untreated and treated (trickling filter) wastewater samples from a hog slaughterhouse were not significantly different using VS and DP methods. The COD values were not significantly different among the treated samples as well using a^* and L^* plots. Tests for accuracy and repeatability of the DP method showed acceptable results. The calculated limit of detection (LOD) for DP was 0.73 mg L^{-1} while the LOD for VS was 0.33 mg L^{-1} . The accuracy of the DP method was validated using glucose solutions of known COD values; t -tests performed at 95% confidence level showed no significant differences in COD values between (1) theory and experiment, (2) VS and DP and (3) a^* and L^* plots. These findings suggest that digital photometry is accurate and can be used as an equally accurate alternative to conventional spectrophotometry.

Key words: Chemical oxygen demand, digital photometry, image processing, visible spectrophotometry, slaughterhouse wastewater

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INTRODUCTION

Chemical oxygen demand (COD) is a water quality parameter and useful indicator of organic pollutant level in water. It is useful in evaluating the efficiency of a wastewater treatment process. It is a determinant of the oxygen equivalent of the organic matter in a sample that is susceptible to oxidation by a strong chemical oxidant (Eaton *et al.* 1995). In conventional COD methods, a known excess amount of the oxidant, usually dichromate, is added to the sample and the mixture is boiled for a definite period of time in order to oxidize COD material in the sample. The concentration of organic matter in the sample can be calculated from the amount of the remaining oxidizing agent which is determined by titration or UV-Vis spectrophotometry (Domini *et al.* 2009).

An alternative COD method using potassium permanganate was used for many years and measurements then were called oxygen consumed from permanganate rather than the oxygen demand of organic substances. The method was recommended as a standard procedure until 1965 when it was replaced by the dichromate method. In the attempt to replace the dichromate method with a more environment-friendly procedure which does not use mercury, chromium, silver or other hazardous compounds, several oxidants were used by many analysts.

These approaches were found to be unsuitable due to the difficulties in the preparation of the reagents, reagent stability, photosensitivity, low oxidation potential, poor oxidation efficiency, expense and ease of use (Boyles 1997). However, through continuous researches and experiments, analysts were able to find another suitable oxidant other than dichromate. Manganese (III) sulfate in sulfuric acid, though not widely used, was found to be a suitable reagent for chemical oxygen demand testing since it is a strong oxidant, non-carcinogenic, stable, not photosensitive, and has a desirable spectrum for colorimetric measurement. However, this new oxidant still requires one hour digestion of organic material at 150°C which can be extended which can be extended when the samples are difficult to completely oxidize (Boyles 1997; APHA 1995).

Absorption spectrophotometry is most commonly utilized for chemical analysis because of its simplicity and ease of use. However, digital photometry (DP) based on color analysis/image processing of photographs of analyte solutions has recently gained attention because of lower equipment cost as an alternative, but equally reliable method, to conventional visible spectrophotometry (VS). DP has been found to be

equally accurate and comparable to VS for measuring concentrations of soluble protein (Yanos *et al.* 2013), as well as dichromate and permanganate ions in determining the contents of Cr and Mn in steel (Vedad *et al.* 2015). It has been used for determining iron and residual chlorine in water samples (Suzuki *et al.* 2006), xylose concentration in fermentation medium (Alojado-Rubianes and del Rosario 2018), degree of fruit ripeness of papaya (Domingo *et al.* 2012) and mango (Abarra *et al.* 2018), as well as estimating the gelatinization temperature of some rice varieties (Tuano *et al.* 2018).

Color determinants were defined by the Commission Internationale de l'Eclairage (CIE) in 1931 in order to simulate the visual mechanism based on the primary colors (R, G, B) and color-matching functions. Color digital still cameras generally use an array of transistors on a CCD chip with a filter array that allows some detectors for red (R), green (G) and blue (B). The RGB color space may be used to analyze a digital color image with each color component R, G or B per pixel in the range 0 to 255 and conventionally stored using eight bits per color component. $L^*a^*b^*$ or CIELAB, which is another commonly used and CIE-specified measure of color, may be calculated from XYZ values. The latter are functions, in matrix form, of the RGB values. Some publications have provided valuable information on digital color images, color spaces/models and image processing (Kruger 2010, Kelda and Kaur 2014, Skrede 2017).

Slaughtering operations generate large volumes of wastewater from areas where animals are killed, bled and eviscerated and where carcasses are washed, chilled, trimmed or cut. As a result, wastewater contains blood, trimmed parts and other by-products. High concentrations of oxygen-demanding organic substances in the water may lead to fish kills, as well as foul odor. Thus, the release of oxygen-demanding organic substances in surface waters by municipal and industrial wastewater treatment facilities is closely monitored and restricted by environmental regulatory agencies (La Para *et al.* 2000 and Domini *et al.* 2009). This study deals with the application of digital photometry in measuring the chemical oxygen demand of slaughterhouse wastewater samples; this was done in comparison with conventional visible spectrophotometry. The COD values obtained were compared using different methods of calculation (a^* and L^* plots). The accuracy of the DP method was evaluated using glucose solutions with known COD equivalents of 350 mg L⁻¹ and 750 mg L⁻¹. The COD values of two random samples of treated and untreated wastewater from a local hog slaughterhouse were determined using both VS and DP and the COD

values obtained were used to assess the efficiency of wastewater treatment in the slaughterhouse.

MATERIALS AND METHODS

Sampling and Storage

Two random samples of treated and untreated wastewater were collected from the Municipal Slaughterhouse in Bayog, Los Baños, Laguna, Philippines. Sample A was collected at 10 AM while sample B was collected at 4 AM after several days. The wastewater samples were collected in clean plastic bottles known to be free of contamination. The samples were tested without delay and preserved by acidification to pH ≤ 2 using concentrated H₂SO₄ and stored in the freezer.

COD Determination

The sample solutions were digested using the dichromate colorimetric method (Eaton *et al.* 1995). COD values were determined by visible spectrophotometry (using Shimadzu UV Mini 1240 Spectrophotometer) and digital photometry (triplicate analysis). The accuracy of the methods was determined using glucose solutions with known COD values of 350 and 750 mg L⁻¹. Repeatability of the method was determined using the same set-up and camera settings (Figure 1).

Digital Photometric Procedure

A 12 Megapixel Samsung ES-70 Digital Camera was used to take photographs of the standard solutions and samples. The settings of the camera were fixed at f/3.5 f-stop, 1/180 second exposure time, ISO 100 speed, 5 mm focal length, center-weighted average metering mode, and no flash setting (compulsory). The photographs of the samples and standards were taken using a wooden light box (Yanos *et al.* 2013) based on the design of Suzuki and coworkers (2006). Approximately 1.0 mL of the sample was poured into the cuvette which was then positioned inside the box. At least three digital still photographs of the standard solutions and samples were taken using a 12 Megapixel Samsung ES-70 Digital Camera (Figures 2 to 4). The color photographs were analyzed using the free-access software RGB – Analysis of Image Colors (RGB-AIC) which was initially applied in the study of insects to objectively determine their colors (Byers 2006). Using this software, the RGB values of a specific area on the image was determined. Plots of a^* and L^* were constructed from the calculated RGB values in order to generate the standard curves. The RGB values of



Figure 1. The light box set up.

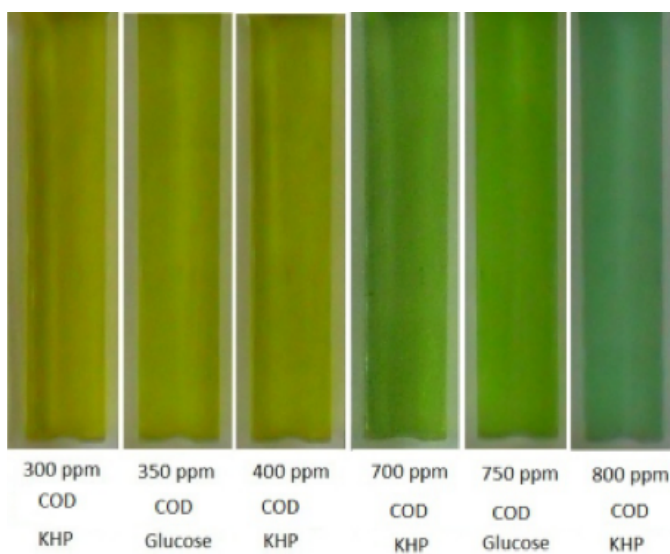


Figure 2. Photo of glucose solutions with theoretical COD of 350 and 750 ppm and KHP standards.

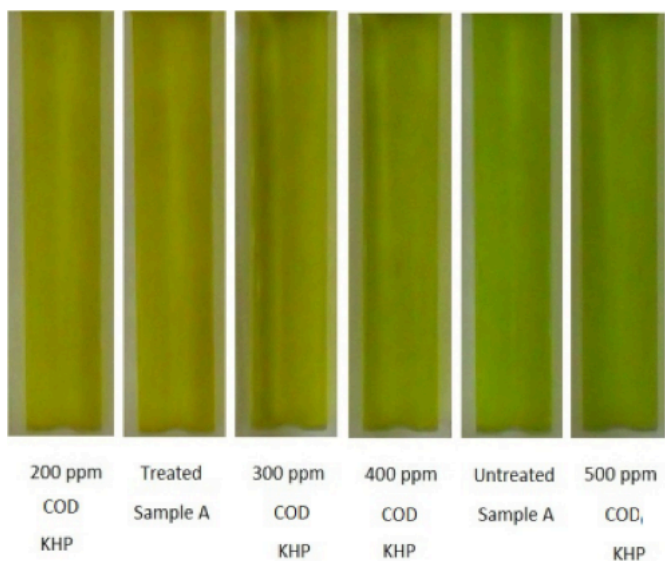


Figure 3. Photo of treated and untreated sample A and KHP standards.

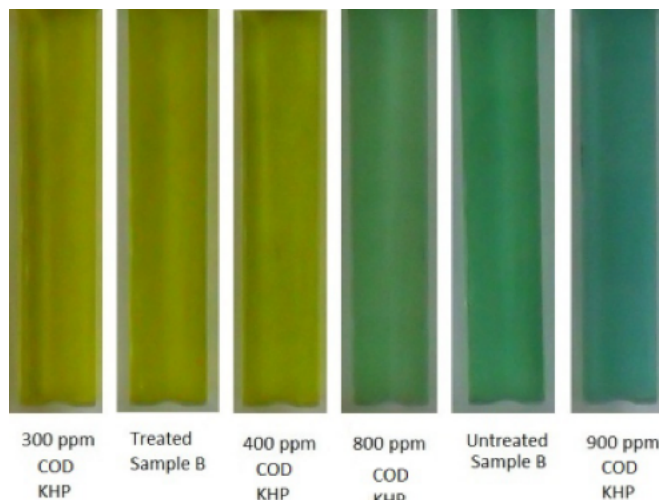


Figure 4. Photo of treated and untreated sample B and KHP standards.

the samples were then interpolated using the standard curves in order to determine sample concentrations.

Statistical Analysis

The COD values of the samples obtained using visible spectrophotometry and digital photometry were compared using t-test. T-tests were also performed in order to compare experimental COD values of the glucose solutions to the theoretical values; assess the repeatability of the method by comparing the COD values of sample B for the first and second experiments; compare the COD values obtained using a^* and L^* plots; to assess the efficiency of the treatment process by comparing the COD values of the treated and untreated samples; and compare the COD values of samples A and B. All the t-tests were performed at 95% confidence level.

RESULTS AND DISCUSSION

Generation of Standard Curves

Standard solutions of potassium hydrogen phthalate (KHP) with COD values of 0 mg L⁻¹ to 900 mg L⁻¹ were prepared in order to generate the standard curve. The KHP standards were digested by adding dichromate in sulfuric acid solution which oxidized the KHP. The amount of dichromate that was consumed to oxidize KHP was determined by measuring the absorbance of reduced chromium (Cr³⁺) at 600 nm. The absorbance of the KHP standards was then plotted against the COD value (Figure 5).

For digital photometric analysis, several photographs of the KHP standards (Figure 6) were taken. The

color of the standard solutions changed from yellow to green to bluish-green as the COD value increased. The observed color change was consistent with that predicted by the chromaticity diagram (**Figure 7**). During COD determination, the dichromate ion (Cr^{6+}) is reduced to Cr^{3+} . The wavelength for maximal absorption (λ_{max}) for the dichromate ion is 440 nm while λ_{max} for Cr^{3+} is 600 nm. The perceived color of the solution is complementary to the color that is absorbed by the dissolved species; this is shown by passing a line through the central white region (**Figure 7**).

The 600-nm orange gives its complementary color at the other end of the line (marked A) which is 488 nm (cyan or blue-green); similarly the 420-nm violet has its complementary color (yellow-green) at the end of the line (marked B). Then, by connecting the two complementary colors, the perceived color of a standard solution is

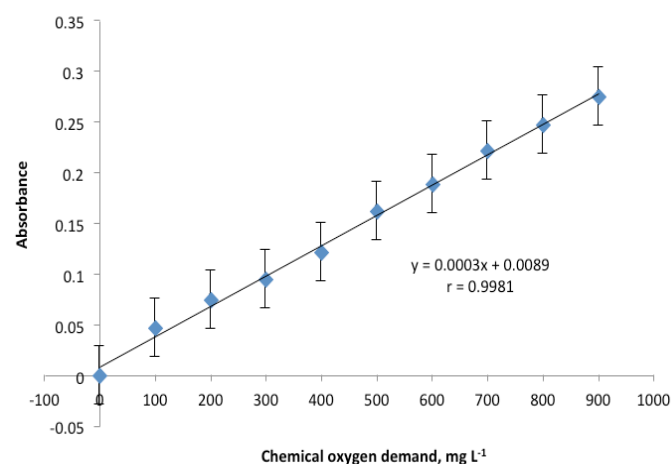


Figure 5. Standard curve for COD determination using visible spectrophotometry.

assumed to lie only on the line connecting the two complementary colors (shown as dashed line in **Figure 7**). Following the dashed line from right to left, the transition of the color is from yellow to green to cyan, which is the color transition observed in the standard solution as the COD value is increased. Furthermore, a mixture of cyan and yellow gives any intermediate color with the law of the lever being applicable. Considering color C can be produced with 'a' amount of color A and 'b' amount of color B (**Figure 7**). Applying the lever rule, the length of b becomes smaller and the length of a becomes larger as C approaches A.

The color photographs of the KHP standards were analyzed using RGB-AIC software and the RGB values were plotted against the COD values (**Figure 8**). Generally, a decrease in the R and G values was observed as the COD value increased. On the other hand, the B values are constantly zero from 0 to 500 mg L⁻¹ COD and started to increase at 600 mg L⁻¹ COD. Since the predominant colors of the solutions are yellow and green, the color is more of the R and G (red plus green is yellow), and B is a minor component.

The RGB values were then used in order to construct the a^* and L^* calibration curves (**Figure 9**). The a^* value is a measure of redness minus greenness and a negative a^* indicates a greenish solution. In this case, because the solution turns green or bluish-green as the COD value increases, the a^* value becomes more negative. On the other hand, L^* means brightness and a solution with a larger L^* value is brighter. Dilute colored solutions have greater brightness than more concentrated solutions. Therefore, values of a^* and L^* decrease with increasing COD.

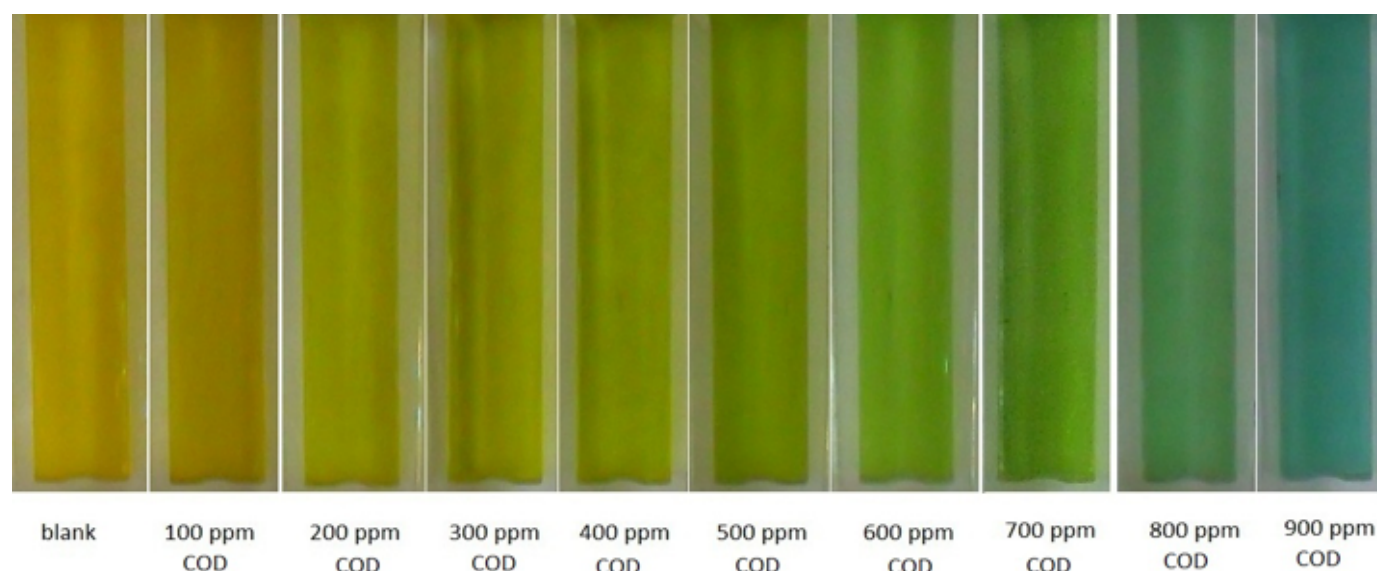


Figure 6. Sample photographs of KHP standards.

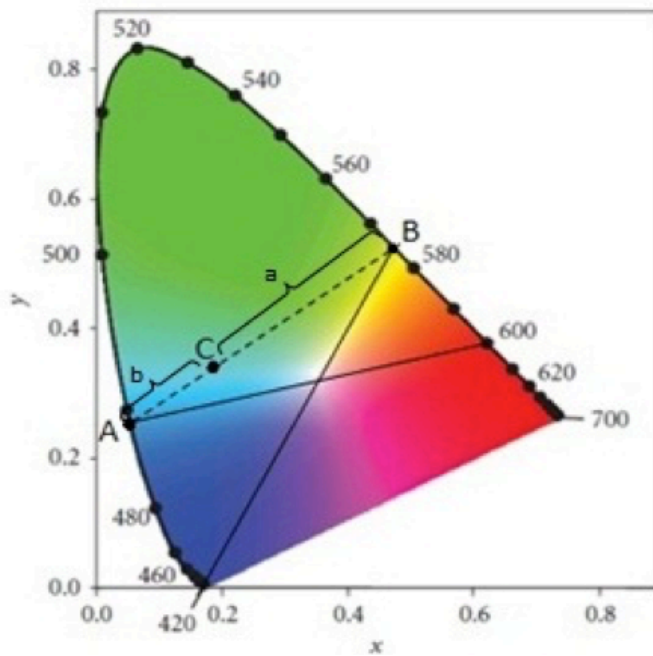


Figure 7. CIE Chromaticity diagram with the dashed line showing the perceived color of a standard solution (Brainard and Stockman 2009).

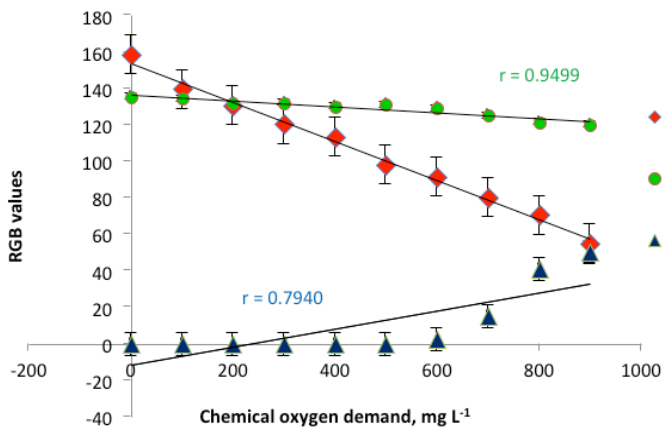


Figure 8. Plot of RGB values versus COD of KHP standards.

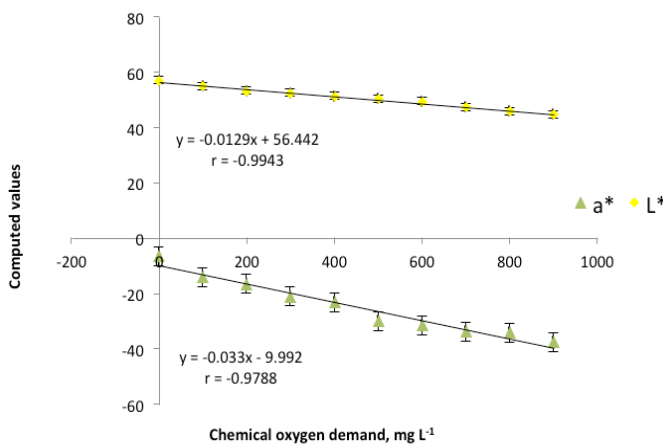


Figure 9. Plot of computed values of a^* and L^* versus COD of KHP standards.

Validation of Accuracy

The accuracy of the DP method was validated using glucose solutions with known COD values of 350 and 750 mg L⁻¹. The experimental COD values of the solutions obtained using DP and VS were compared with the theoretical COD. There was no significant difference between the COD values for both glucose solutions (**Table 1**). In general, VS gave the lowest error (0.15 % error for 350 mg L⁻¹ and 0.18 % for 750 mg L⁻¹) followed by L* plot and a^* plot, respectively. This indicates that DP can be used as an alternative to VS for accurate COD determination. Between the two methods of calculation used, L* plot gave the closer COD value to the spectrophotometric method.

Limit of Detection

The limit of detection (LOD) values for VS and DP were determined from extrapolated linear plots of absorbance and R, respectively, versus COD (**Figures 5 and 8**). They were calculated by dividing the standard error of the regression line (after multiplication by three) by the slope of the line (Vedad *et al.* 2015). Calculated values of the slope for VS and DP data are shown inside the plot areas (**Figures 5 and 8**). Values of LOD (in mg L⁻¹) for VS and DP were 0.33 and 0.73, respectively.

COD Analysis of Wastewater Samples from Slaughterhouse

Two random samples of treated and untreated wastewater from a hog slaughterhouse were examined. Sample A was collected at 10 AM in the morning, i.e. six hours after slaughter. The COD values of the treated sample were in the range 208.1 - 214 mg L⁻¹ while the values for the untreated sample ranged between 467 and 485.1 mg L⁻¹ (**Table 2**).

No significant differences between DP and VS results were observed between the COD values for both treated and untreated wastewater A samples. Also, no significant difference was observed between the COD values of the untreated and treated samples using a^* and L* plots. In order to assess the efficiency of the slaughterhouse treatment process, the COD values of the treated and untreated samples were compared. There were significant differences between the COD values of the treated and untreated samples. Furthermore, the treatment process was found not to be efficient. Ideally, treated samples should have low COD that are safe enough to be discharged into bodies of water. However, the COD values of the treated samples were found to

Table 1. Chemical oxygen demand of glucose solutions.

Theoretical COD, mg L ⁻¹	Experimental COD, mg L ⁻¹		
	Digital Photometry		Visible Spectrophotometry
	a* plot	L* plot	
350	374 ± 14 ^a	362.5 ± 9.3 ^a	350.5 ± 8.4 ^a
750	766.7 ± 9.2 ^a	763 ± 11 ^a	749 ± 10 ^a

^aMean value of three trials ± standard deviation. Values with different superscripts in the same row are significantly different (p<0.05).

Table 2. Summary of the calculated COD values of the wastewater samples.

Wastewater samples		Experimental COD, mg L ⁻¹		
		Digital Photometry		Visible Spectrophotometry
		a* plot	L* plot	
Sample A (10AM)	Treated	213 ± 14 ^{a,α}	208.1 ± 9.3 ^{a,α}	214 ± 17 ^{a,α}
	Untreated	485.1 ± 9.2 ^{a,β}	481 ± 11 ^{a,β}	467 ± 17 ^{a,β}
Sample B (4AM)	Treated	347 ± 24 ^{a,γ}	342 ± 28 ^{a,γ}	338 ± 15 ^{a,γ}
	Untreated	815 ± 13 ^{a,δ}	805 ± 11 ^{a,δ}	811 ± 12 ^{a,δ}

^{a, α - δ}Mean value of three trials ± standard deviation.

^aValues in the same row with different Latin letters are significantly different (p<0.05).

^{α - δ}Values in the same column with different Greek letters are significantly different (p<0.05).

be high and did not meet the standard (150 mg L⁻¹) set by the Environmental Protection Agency (EPA). The wastewater treatment process of the slaughterhouse was able to reduce the COD of the raw wastewater by only 54 to 60% (Table 3). In addition, statistical analysis did not show significant differences between the results of wastewater treatment for both A and B samples.

Test for Repeatability

The digital photometric determination of COD values for B samples was repeated on the same day in order to determine the repeatability of the method using the same set-up and camera settings. The results of the second experiment were compared with those of the first experiment (Table 4). No significant differences in the COD values of the treated and untreated samples were observed for the first and second experiments (0.02% difference) using the L* plot, which was the most precise method.

Table 3. COD reduction of raw wastewater sample.

Method		Reduction of COD, %	
		Sample A	Sample B
Digital Photometry	a* plot	56.1 ± 2.1 ^{a,α}	57.4 ± 3.6 ^{a,α}
	L* plot	56.8 ± 2.3 ^{a,α}	57.4 ± 3.8 ^{a,α}
Visible Spectrophotometry		54.1 ± 1.9 ^{a,α}	58.3 ± 2.5 ^{a,α}

^{a, α - δ}Mean value of three trials ± standard deviation.

^aValues in the same row with different Latin letters are significantly different (p<0.05).

^{α - δ}Values in the same column with different Greek letters are significantly different (p<0.05).

Table 4. Assessment of the repeatability of digital photometry using wastewater samples.

Sample	Plot	COD, mg L ⁻¹	
		Experiment 1	Experiment 2
Treated	a*	347 ± 24 ^a	366 ± 14 ^a
	L*	342 ± 28 ^a	369.0 ± 2.1 ^a
Untreated	a*	815 ± 13 ^a	807.36 ± 18 ^a
	L*	805 ± 11 ^a	805 ± 20 ^a

^a Mean value of three trials ± standard deviation. Values with different superscripts in the same row are significantly different (p<0.05).

CONCLUSION

Digital photometry (DP), which involves taking photographs of developed solutions followed by color image analysis using free-access computer software, was investigated as a alternative to conventional visible spectrophotometry (VS) for COD analysis. The accuracy of the DP method was validated; t-tests performed at 95% confidence level showed no significant differences in COD values between (1) theory and experiment, (2) using VS and DP and (3) using a* and L* plots. These findings suggest that DP is accurate and can be used as an equally accurate alternative to VS. The repeatability of the DP method was assessed and the limit of detection was determined. There was no significant differences between the COD values of sample B for the first and second experiments with an LOD at 0.73 mg L⁻¹.

The present study has shown the potential widespread application of digital photometry, which uses

an inexpensive digital camera and free access software for image processing, in determining chemical oxygen demand (COD). This would enable laboratories with limited equipment budgets to perform COD determination. Furthermore, this method would allow record keeping of data in terms of color images, as well as prospects for computerized COD monitoring of waste effluents.

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