Absorbance Based Model for Determination of Biochemical Oxygen Demand

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SK and JV designed the study. Authors US, VS and SB performed the experiments, managed mathematical modelling and literature searches. Authors US and VS wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: The paper presents the development of mathematical model for the spectrophotometric determination of BOD along with its validation and an illustrative example of its application for sewage water treatment plant.
Place and Duration of Study: The concept of determination of BOD using spectrophotometric absorbance and mathematical modeling of absorbance to measure BOD was developed at the Department of Chemistry and Department of Electrical

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Methodology: The wavelength of 440 nm, selected from the linear zone of response spectrum has been used as the base for measuring absorbance. The performance of the proposed spectrophotometric method is assessed by repeated measurement of DO for standard glucose-glutamic acid (GGA) solution and its comparison with DO values obtained by Winkler's titrimetric method. Mathematical models of different orders were developed and analyzed for goodness of fit. A linear model was found to be the best in order to obtain the DO values whereas a quadratic model found to be suitable for the estimation of BOD.

Results: The results depict a good correlation in the variation of absorbance values with DO values obtained from Winkler's titrimetric method. Application of the proposed method for the measurement of biochemical oxygen demand (BOD) in waste water samples acquired from the solid waste treatment plant has been included as an illustrative example.

Conclusion: The quadratic model has been found to be the best model for estimating the depletion of DO after incubation in terms of change in absorbance from day 1 and day 5 for BOD modelling on the basis of analysis of SSE, RMSE, R-square and the polynomial coefficient of the model. The major advantage of the proposed approach is that the results do not depend on the identification of single end point and observer subjectivity.

Keywords: Spectrophotometric method; absorbance; mathematical modeling; BOD; GGA.

1. INTRODUCTION

Dissolved oxygen is one of the best indicators of the health of water ecosystem. Natural water requires 5-6 ppm dissolve oxygen (DO) to support diverse ecosystem. Decrease in DO level is usually an indication of an influx of certain organic pollutants such as animal waste, algae growth, influx of waste water etc., which consume DO in the process of their decomposition by aerobic bacteria. The biochemical oxygen demand (BOD) reflects the difference between the physical concentration of dissolved oxygen in water (i.e. theoretical concentration if there were no living organism) and the actual concentration of dissolved oxygen in water over a period of 5 days. The acceptance of the five day BOD is caused by the fact that during the first 5 days there is a decomposition of 60-70% of the organic substance. Determination of biodegradable organic substance in waste water has significant impact on environmental pollution control. Therefore measurement of BOD is one way to keep a check on the level of water pollution. The conventional method for measuring BOD is the Winkler's iodometric titration. It is based on the fact that the oxygen present in a water sample rapidly oxidizes the dispersed divalent manganous hydroxide to its higher valency, which precipitates as a brown hydrated oxide, after addition of alkaline iodide. Upon acidification, manganese reverts to divalent state and liberates iodine equivalent to the original DO content from KI. The liberated iodine is titrated against standard thiosulphate [1,2]. Although Winkler's method is labour consuming and suffers from observers' subjectivity, its unquestionable high accuracy and precision for measurement of oxygen concentration in water matrices have established it as the standard for the validation of any new methodology and instrumentation. Field testing of water samples for DO and BOD determination has necessitated the development of instrumentation based methods. Several research groups have proposed spectrophotometric technique [3-9] as an improvement of end point detection in Winkler method, where sample handling is minimized, making the analysis easier for an unskilled person. Despite several advantages, the spectrophotometric methods received limited favor of researches, due to the inconsistency of measuring

wavelength and the absence of justification for the selected value of wavelength [9]. Yang et al. [10] have reported simultaneous determination of chemical oxygen demand (COD) and BOD in waste water by Near-IR Spectrometry. Use of eukaryote *Saccharomyces cerevisiae* [11] and a yeast species [12] has been reported for absorbance based BOD determination. Fluorescence based methods [13,14] have also been reported for DO and BOD determination. Sharma et al. [15] have reviewed the modeling methods for BOD measurement and have developed a spectrophotometric absorbance based linear model for determination of instant DO. The present paper reports the optimization of the spectrophotometric conditions and the development of suitable absorbance based mathematical model to estimate BOD in water samples.

2. EXPERIMENTALS

2.1 Instrumentation

UV-Vis Spectrophotometer (model DR 5000, HACH, USA) and BOD Incubator (model PSI, Pooja Scientifics, New Delhi was used in present study. Glass cuvette (20 mL) from HACH, USA providing a path length of 2.54 cm was used for present study. MATLAB® 7.0.0 (Release 2010a) software supplied by M/s Mathworks Inc. was used for the mathematical modeling.

2.2 Reagents

All chemicals and reagents used were of A.R. grade from E. Merck, India. Double distilled water (DDW) was used throughout the experiment and the following reagents as per standard practice [1] were used in present study.

  - R_1: Manganese sulphate solution (40 g $MnSO_4.2H_2O$/ 100 mL DDW).
  - R_2: Alkaline iodide azide Regent (70 g KOH+15 g KI/ 100 mL DDW; 1 g NaN_3 dissolved in minimum amount of water was added to alkaline iodide solution before making up).
  - R_3: Standard sodium thiosulphate (0.025N) was prepared by diluting 0.1 N stock solution (14.41 g $Na_2S_2O_3.5H_2O$+0.2 g solid NaOH / 500 mL DDW). The solution was standardized with standard potassium dichromate solution using starch as indicator (paste of soluble starch powder, 2 g/100 mL boiling water). Conc. H_2SO_4 was used for acidification.
  - R_4: Dilution Water: 1 mL of each; phosphate buffer of pH 7.2 (4.25 g KH_2PO_4, 10.5875g K_2HPO_4, 16.75 g $Na_2HPO_4.7H_2O$ and 0.85 g NH_4Cl /500 mL), magnesium sulphate (11.25 g $MgSO_4.7H_2O$ / 500 mL), calcium chloride (13.75 g anhydrous $CaCl_2$ / 500 mL)and ferric chloride (0.125 g $FeCl_3.6H_2O$ / 500 mL), mix thoroughly and dilute to 1 L with distilled water Dilution water is prepared freshly and was shaken vigorously for 5 minutes (to aerate the dilution water). The temperature of dilution water was brought to 20ºC before use.
  - R_5: 150 ppm Glucose-glutamic acid (GGA) solution (75 mg glucose + 75 mg glutamic acid / 500 mL DDW) was prepared freshly immediately before use. Standard 150 ppm GGA is considered as 100% for BOD calculations.

2.3 Sample Preparation and Methodology

The 5 day BOD test was conducted using standard GGA as well as sewage samples collected from solid waste treatment plant (STP) Salawas, Jodhpur at different stages.

Different concentrations of GGA in the range 0.5% (0.75 ppm) to 5% (7.5 ppm) were prepared by adding appropriate volume of standard GGA (R_5) to dilution water (R_4). Sewage samples were also diluted with dilution water (R_4). All test samples (GGA/sewage water) were prepared in a 300 mL glass stoppered BOD bottles maintaining the water seal. Two sets of bottles were prepared for every dilution for estimating DO_1 (i.e. day 1) and DO_5 (i.e. day 5). For DO_1, to the first set of sample bottles, 2 mL of R_1 was added with the help of pipette followed by 2 mL of R_2 in such a way that the tip of pipette remains dipped below the liquid surface while adding the reagents. The bottle was stoppered immediately; the extra overflowed solution was drained. The solution is mixed well by inverting the bottle 3-4 times. A brown colour precipitate was formed. The solution was allowed to stand for 5 minutes to complete the reaction followed by impinging 2 mL of conc. H_2SO_4 and mixing after replacing the stopper till the precipitate dissolved in the solution. An intense yellow coloured solution was obtained. The second sets of sample bottles were incubated at $(20\pm1)ºC$ for 5 days and were used for determination of DO_5 following the same procedure described above. An average of triplicate analysis was considered for all experiments.

2.4 Calibration of Spectrophotometric Method for Do Determination

For spectrophotometric measurements, Winkler's reagent (2 mL each of R_1 and R_2, followed by 2 mL of conc. H_2SO_4 was introduced in 300 mL sample solution (standard GGA solution/ sewage water). Resulting yellow coloured solution was siphoned directly into glass cuvettes and absorbance was measured immediately. Absorption spectrum of different sewage water samples and standard GGA solution was recorded in the wavelength range 350 to 500 nm to select the optimum wavelength for further studies. Simultaneously 200 ml of reaction mixture was titrated against reagent R_3 following the Winkler's DO determination method for comparing the proposed method where the titrant reading is the value of DO in mg/L (since, 1 mL 0.025 N sodium thiosulphate = 0.2 mg of O_2). The BOD is calculated by substituting the DO values in the Eq. (1).

$$BOD = [(D_1 - D_5)-(B_1 - B_5)*100]/ c \qquad (1)$$

Where D_1 = DO values of sample immediately after preparation, mg/L
D_5 = DO values of day 5 after incubation, mg/L
B_1 = DO of blank solution before incubation, mg/L
B_5 = DO of blank solution on day 5 after incubation, mg/L
c = % of sample / GGA concentration

2.5 Modelling of Data

Data analysis to correlate the absorbance with DO as determined by Winkler's method, was done by using the data modelling functions available in the data visualization software MATLAB® 7.0.0 (R2010a). This software provides a curve fitting toolbox [16a] which is an interactive environment to draw curves to fit the sample data. The function cftool opens curve fitting toolbox with predictor data (x data) and the response data (y data). Polynomial curve fitting of different order equations were done and analysed for day 1, day 5 and difference of absorbance on day1 and day 5. The acceptability of the fitted resultant curve was determined by calculating goodness of fit (Gof) structure [16b]. Gof structure has fields namely: sum of square of residualsdue to errors (SSE), R-square, the coefficient of multiple determination and root mean square (RMSE). These statistical parameters are calculated respectively by using the following equations:

$$SSE = \sum_{i=1}^{n} w_i (y_i - \hat{y}_i)^2 \tag{2}$$

$$R\text{-square} = \frac{\sum_{i=1}^{n} w_i (\hat{y}_i - \bar{y})^2}{\sum_{i=1}^{n} w_i (y_i - \bar{y})^2} \tag{3}$$

$$RMSE = \sqrt{MSE} = \frac{SSE}{v} \tag{4}$$

Where, w_i is the weight associated with the ith difference value, y_i is the measured value, $\hat{y}_i$ is the fitted value and $\bar{y}$ represents the mean value.

SSE closer to 0 indicates that the model has a smaller random error component, and that the fit will be more useful for prediction. R-square can take on any value between 0 and 1, with a value closer to 1 indicating that a greater proportion of variance is accounted for by the model. RMSE and MSE values closer to 0 indicate a fit that is more useful for prediction. A curve with low RMSE value is chosen as the best fitting curve.

The MATLAB function used for fitting of polynomials is polyfit (x,y,n) [16c] and that for its evaluation at the selected test points is polyval [p,x,s] [16d] . These functions can be invoked as:

   [p,s] = polyfit (x,y,n) and [y',delta] = polyval [p,x,s]

Where, n= degree of polynomial
   p= n+1 polynomial coefficients
   S= structure for estimating errors
   x= predictor data
   y= response data
   y'= value of polynomial p evaluated at x
   Delta = estimate of standard deviation of error in predicting x by p(x).

The function polyfit fits the data in a least square sense as shown in equation (5).

$$DO = p(x) = \sum_{i=0}^{n} P_i x^{n-i} \tag{5}$$

The polyfit function also returns a structure S which is used with polyval function to predict error estimates. The polyval (p, x) returns the value of the polynomial of degree n evaluated at x. The coefficients in p are least square estimates as computed by polyfit. The error in the input data is considered to be independent normal and has constant variance.

The matrix R of Pearson correlation coefficients, C(i,j) is calculated by invoking the function corrcoef (x) on the predictor data as given by equation (6)

$$R(i,j) = \frac{C(i,j)}{\sqrt{C(i,i)C(j,j)}} \tag{6}$$

Since the rows represent the predictor data and the column represent response data, the diagonal elements are equal to 1 for good correlation.

3. RESULTS AND DISCUSSION

3.1 Selection of Optimal Wavelength for Spectrophotometric Absorbance

For selection of optimal wavelength, spectrophotometric analysis of standard GGA solution was carried out in the wavelength range 350 nm to 500 nm after incorporating the suitable reagents (section 2.3). Absorption spectrum shows maxima around 380 nm±20 nm. The wavelength of maximum absorbance, 380 nm, was successfully used for instant DO determination [15], but was not suitable for BOD determination. This necessitated the exploration of the linear behavior range beyond the maxima of the spectrum, which lies between 410 nm and 470 nm for different concentrations of standard GGA solutions. (Fig. 1) shows absorption spectrum and linear range of 2% of 150 ppm standard GGA solution. The intense absorption of ultra violet and visible light result from the iodine in solution, which is partly free and partly combined with the residual iodide as triiodide complex, having different spectral sensitivities. However, the molar absorptivity of the molecular iodine and triiodide ion are similar at 450-460 nm [6,9]. In line with these reported findings, the wavelength for the present study was selected to be 440 nm to conform to the linearity standards for instrument design [17].
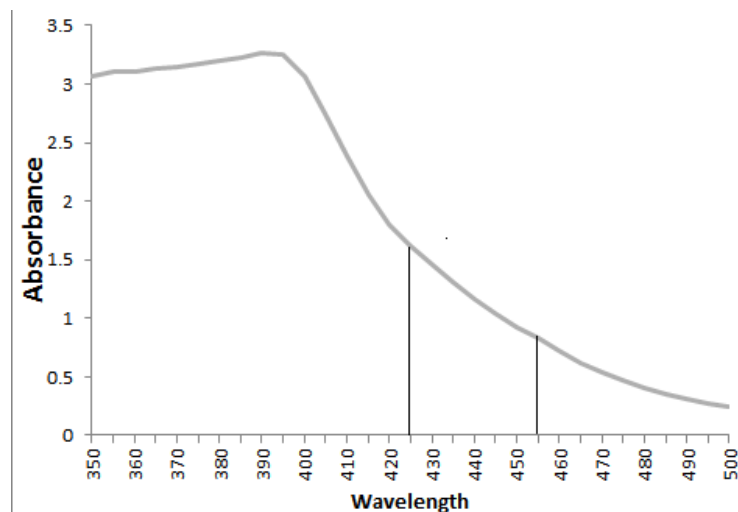

Fig. 1. UV- VIS absorption spectra of GGA solution

3.2 Calibration with GGA

(Table 1) shows the comparative data for DO as determined by Winkler's method (D) and absorbance (A) at different concentrations of GGA on day 1 (D_1 /A_1), i.e. immediately after sample preparation and day 5 (D_5 /A_5) i.e. after incubation period. A similar trend was observed in absorbance values and Winkler's DO values at different concentration (Figs. 2a, b), indicating the possibility of correlating the absorbance values with that of the Winkler's DO values. It is notable that the DO values and absorbance values for different GGA samples do not change significantly on the first day since concentration range is small to effect significant change DO values at initial stage. However, after incubation for five days, a significant decrease of absorption values and depletion in Winkler's DO was observed with increase of GGA concentration. The calculation of BOD requires the difference of initial DO

values (DO_1) and DO values after incubation period(DO_5), necessitating the modelling of absorbance $[(A_1- A_5)-(B_1-B_5)]$ in terms of Winkler's DO $=[(D_1- D_5)-(B_1-B_5)]$ after blank correction as described in the next section.
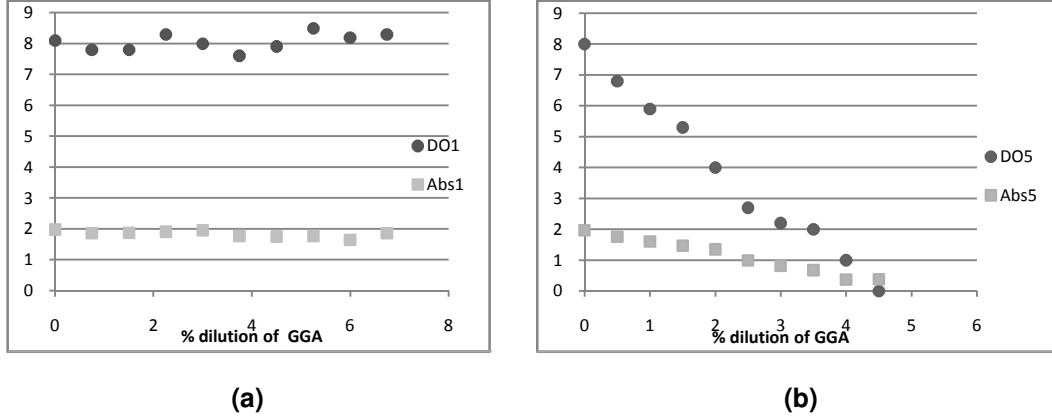

(a)

(b)

Fig. 2. Variation of absorbance at 440 nm and Winkler's DO values on day 1(a) and day 5 (b)

Table 1. Winkler's DO and absorbance at 440 nm for different dilutions of std. GGA solution

Sample (150 ppm GGA)		Winkler's DO, mg/L			Absorbance			(DO_1'– DO_5') as modelled
Blank	0	B_1= 8.1	B_5= 8.0	(B_1-B_5)= 0.1	AB_1= 1.978	AB_5= 1.965	(AB_1-AB_5)= 0.013	
ppm	% conc	DO_1	DO_5	$y=[(D_1- D_5)-(B_1-B_5)]$	A_1	A_5	$x=[(A_1- A_5)-(AB_1-AB_5)]$	
0.75	0.5	7.8	6.8	0.9	1.872	1.761	0.098	0.96
1.05	1.0	7.8	5.9	1.8	1.829	1.602	0.264	1.84
2.50	1.5	8.3	5.3	2.9	1.907	1.465	0.429	2.72
3.00	2.0	8.0	4.0	3.9	1.781	1.349	0.603	3.65
3.75	2.5	7.6	2.7	4.8	1.860	0.994	0.766	4.52
4.50	3.0	7.9	2.2	5.6	1.751	0.818	0.920	5.35
5.25	3.5	8.5	2.0	6.4	1.782	0.673	1.086	6.23
6.00	4.0	8.2	1.0	7.1	1.851	0.369	1.271	7.22
6.75	4.5	8.3	0.8	7.4	1.870	0.375	1.482	8.34
7.50	5.0	8.4	0.4	7.9	1.929	0.012	1.671	9.35

3.3 Modelling of Data

Tables 2, 3 reveal the analyses of the goodness of fit parameters for different order models for DO and absorbance data for day 1 and day 5 respectively along with the polynomial coefficients P_i.

It can be seen from Table that for day 1, the best polynomial fitting parameters (least RMSE with reasonable R-square) are obtained for the linear model equation that correlates the absorbance and DO. The final model equation for DO_1 is hence obtained, as given in equation (7).

Table 2. GoF parameters for DO and absorbance data for day 1

Model	SSE	R-square	RMSE	Polynomial coefficients				
				P_1	P_2	P_3	P_4	P_5
Linear	0.790	0.032	0.296	-0.4779	8.956			
Quadratic	0.749	0.082	0.306	6.431	-23.89	30.19		
Cubic	0.747	0.085	0.327	-15.14	89.02	-173.8	120.7	
4th degree	0.685	0.161	0.338	-1532	1.11×10^{-4}	-3.02×10^{-4}	3.64×10^{-4}	-1.64×10^{-4}

Table 3. GoF parameters for DO and absorbance data for day 5

Model	SSE	R-square	RMSE	Polynomial coefficients				
				P_1	P_2	P_3	P_4	P_5
Linear	2.934	0.957	0.571	3.99	-0.57			
Quadratic	0.403	0.994	0.224	1.50	0.97	0.39		
Cubic	0.402	0.994	0.240	0.03	1.41	1.04	0.38	
4th degree	0.386	0.994	0.254	-0.42	1.70	-0.67	1.86	0.33

$$DO_1 = -0.48x + 8.9 \tag{7}$$

The polynomial fitting parameters for day 5 indicate that the quadratic model best correlates the absorbance and DO on the basis of least RMSE with reasonable R-square. Finally, the model equation of DO_5 is hence obtained, as shown in equation (8).

$$DO_5 = 1.5x^2 + 0.97x + 0.39 \tag{8}$$

The practical application of the developed model was carried out for the estimation of BOD where depletion of DO, over incubation period of five days (DO_1- DO_5) is used. Hence, modelling was applied to difference of DO (DO_1- DO_5) versus change of absorbance values for incubation period ($Abs_1 - Abs_5$).

Results included in (Table 4) shows progressive decrease in RMSE and SSE values as the model order increases and R-square value is very close to unity for all the higher order models. The model best fitted for depletion of DO over the period of 5 days is quadratic equation, as shown in (Fig. 3) and expressed as equation (9).

Table 4. GoF parameters for DO_1-DO_5 vs $AB_1 - AB_5$

Model	SSE	R-square	RMSE	Polynomial Coefficients			
				P_1	P_2	P_3	P_4
Linear	0.489	0.995	0.233	5.34	0.43		
Quadratic	0.125	0.999	0.125	-0.73	6.53	0.15	
Cubic	0.075	0.999	0.103	0.64	-2.32	7.54	0.05
4th degree	0.056	0.999	0.097	0.91	-2.41	0.89	6.43

$$DO_1 - DO_5 = -0.73x^2 + 6.53x + 0.15 \tag{9}$$

The linear model can also be used to calculate the BOD, whenever computational burden is a prime concern. It can be seen that the RMSE for this model is acceptably small and R-square is also quite close to unity.
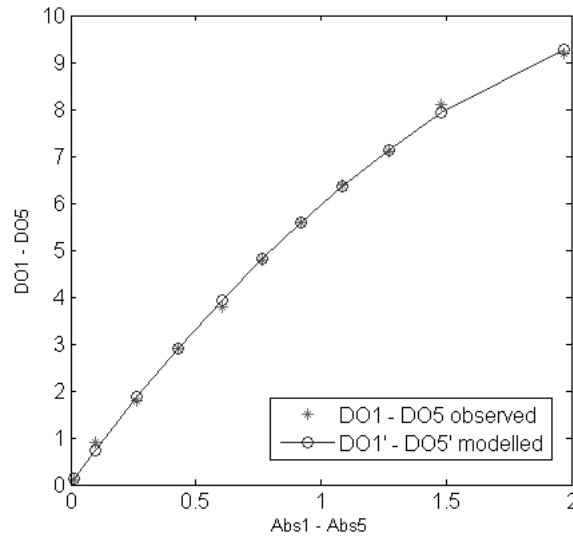
Fig. 3. Modelling for depletion in DO with respect to change in absorbance at 440 nm

The BOD values as obtained by spectroscopic method proposed in present study and Winkler's classical method are included in (Table 5) for comparison. It can be seen that the BOD determined from the spectroscopic method agrees well with the corresponding values of Winkler's method. Further it can be noted that Winkler's method is suitable for BOD determination when the minimum DO depletion during incubation period is 2.0 mg/L and the residual DO is 1.0 mg/L due to the visual detection of end point where as the proposed method may prove to be better option in such cases.

Table 5. Comparison of BOD of GGA solution values obtained from proposed spectroscopic method and Winkler's method

Concentration of GGA		BOD (ppm)	
ppm	**% dilution of stock**	**Winkler's method**	**Proposed spectroscopic method**
0.75	0.5	180.00	191.52
1.05	1.0	180.00	184.37
2.50	1.5	193.33	181.62
3.00	2.0	195.00	182.66
3.75	2.5	192.00	180.93
4.50	3.0	186.67	178.17
5.25	3.5	182.86	178.04
6.00	4.0	177.50	180.47
6.75	4.5	-	185.44
7.50	5.0	-	187.08

3.4 Application of Proposed Spectroscopic Method for Determination of BOD in Waste Water Samples

In order to check the validity of the proposed optical method and modelling for estimating BOD, wastewater sample from Solid-waste Treatment Plant, Salawas, Jodhpur were analyzed at different stages of treatment plant viz. inlet chamber, primary clarifier tank, and the outlet chambers and compared with Winkler's method. The BOD values as obtained by

Winkler's method and the proposed absorbance based model for experimental data obtained from waste water samples of inlet chamber, primary clarifying tank and outlet chamber respectively are included in (Table 6).

Fig. 4 shows the BOD values as obtained by Winkler's method and the proposed absorbance based method in inlet chamber, primary clarifying tank and outlet chamber respectively for the experimental data of (Table 6).
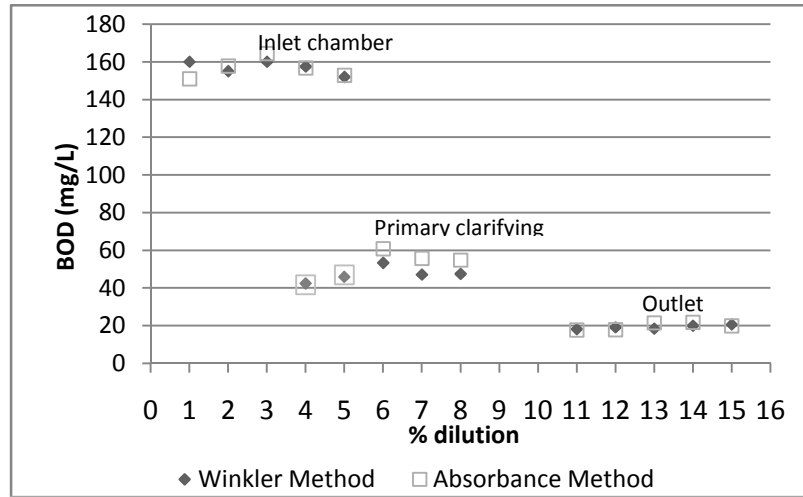

Fig. 4. BOD values of waste water samples from different stages of treatment plant

Table 6. BOD of different dilution of waste water samples

% Conc	Winkler's method				Proposed method				
	D_1	D_5	D_1-D_5	BOD	A_1	A_5	A_1-A_5	$DO_1' - DO_5'$	BOD
Inlet chamber									
0	9	8.9	0.1		2.154	1.961	0.193		
1	8.7	7	1.6	160.0	1.921	1.701	0.203	1.51	151.00
2	8.7	5.5	3.1	155.0	1.859	1.332	0.510	3.16	157.77
3	8.1	3.2	4.8	160.0	1.695	0.848	0.830	4.93	164.40
4	7.9	1.5	6.3	157.5	1.640	0.532	1.091	6.27	156.76
5	7.7	0	7.6	152.0	1.400	0.002	1.381	7.64	152.81
Primary clarifying tank									
0	8.7	8.6	0.1		1.971	1.959	0.012		
4	8.6	6.8	1.7	42.5	1.673	1.400	0.261	1.67	41.74
5	8.1	5.7	2.3	46.0	1.539	1.154	0.373	2.35	46.98
6	7.8	4.5	3.2	53.3	1.484	0.875	0.597	3.65	60.89
7	7.5	4.1	3.3	47.1	1.376	0.723	0.641	3.90	55.73
8	7.2	3.3	3.8	47.5	1.268	0.527	0.729	4.39	54.84
Outlet chamber									
0	8.1	8	0.1		1.648	1.637	0.011		
11	8	5.9	2.0	18.2	1.627	1.309	0.307	1.95	17.74
12	7.9	5.5	2.3	19.2	1.639	1.287	0.341	2.16	17.97
13	8.1	5.6	2.4	18.5	1.657	1.196	0.450	2.81	21.58
14	7.8	4.9	2.8	20.0	1.595	1.091	0.493	3.06	21.83
15	7.7	4.5	3.1	20.7	1.420	0.927	0.482	2.99	19.95

4. CONCLUSION

The spectrophotometric absorbance based mathematical model for determination of BOD proposed in this paper, has been developed and verified with the help of field experiments. The wavelength of 440 nm, selected from the linear zone of response curve was used as the base for measuring absorbance. The DO values were determined from the absorbance values measured at this wavelength for different concentrations of GGA and a linear model was fitted. The output of the model was compared with the DO values obtained by the Winkler's method for performance assessment. A similar in trend is observed in DO values and absorbance value is the basis for developing the mathematical model. The approach considered the depletion of DO after incubation in terms of change in absorbance from day 1 and day 5. The quadratic model has been found to be the best model for the difference modelling on the basis of analysis of SSE, RMSE, R-square and the polynomial coefficient of the model. However, the linear model may also be used for simplicity and ease of implementation in practical applications, as the difference between the goodness of fit parameters between the linear and the quadratic models is within acceptable limits.The major advantage of the proposed approach is that the experimental error in this measurement is lesser as compared to the Winkler method as the results do not depend on the identification of single end point and observer subjectivity. This has opened the frontiers for the development a simple optical methods and models for determination of BOD which has the merits of Winkler's method at the same time circumvents the human subjectivity of end point reliability. Finally, an illustrative example has been presented to depict the process of determination of BOD from the measurement of DO for variety of wastewater samples. The model exhibited reasonably good performance.

Presently, research is in progress to implement the proposed modelling approach for designing an optometric device [17] to measure the instant dissolved oxygen of a sample in terms of absorbance of given sample and subsequently to estimate the BOD.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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