Asian Journal of Chemistry; Vol. 23, No. 8 (2011), 3576-3580

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Spectrophotometric Determination of Biochemical Oxygen Demand in Water Sample by I_3^-- Acridine Red-Polyvinyl Alcohol System

OU SHA* and WEI-XING MA

School of chemistry and Chemical Engineering, Huaihai Institute of Technology, Lianyungang 222005, P.R. China

*Corresponding author: Tel: +86 518 85895063; E-mail: 7993259@163.com; shaou7993259@hhit.edu.cn

(*Received*: 1 November 2010; *Accepted*: 26 April 2011) AJC-9851

The use of the conventional 5-day biochemical oxygen demand (BOD_5) method in BOD determination is greatly hampered by its time-consuming sampling procedure and it is insensitive to the variation of dissolved oxygen less than 0.1 mg/L in water sample. Meanwhile the BOD_5's titration has technical difficulty in the handling of a large pool of wastewater samples within short time. This paper describes a high-throughput and sensitive method for the determination of BOD in wastewater depending on measurement of the dissolved oxygen. Based on the reaction between I_3^- and acridine red to give an ion-association complex with a characteristic absorption at 525 nm, a photometric method for determination of oxygen demand in water was proposed. Then BOD_5 could be calculated through the amount of dissolved oxygen before and after the seeding procedure determined by the proposed spectrophotometry. This biochemical oxygen demand method was found to be able to determine the BOD values of 20 waste samples within 40 min by monitoring the dissolved oxygen concentrations. Moreover, the BOD values determined by the proposed method were in good agreement with those obtained by the GB7488-87 (BOD_5) method.

Key Words: Biochemical oxygen demand, Spectrophotometry, Acridine red.

INTRODUCTION

Biochemical oxygen demand (BOD) is an important parameter of environmental indicator and it has been widely used for several decades as an index of organic pollution in industrial wastewater, effluent or natural water. BOD_5 determination is an empirical test in which standardized laboratory procedures are used to determine the relative oxygen requirements of wastewaters, effluents and polluted waters. The difference in oxygen levels between the first test and the second test, in milligrams per liter (mg/L), is the amount of BOD. The authorized assay for measuring biodegradable organic levels is the 5-day biochemical oxygen demand (BOD_5) described by the American Public Health Association Standard Methods Committee[1]. In China, the authorized assay is the water quality-5 days method for the biochemical oxygen demand (BOD_5): dilution and inoculation method (GB7488-87)[2].

To date, many rapid biosensors for BOD monitoring have been developed[3-11] and these biosensors mostly depended on the dissolved oxygen as the terminal electron acceptor for biodegradation of organic substrates. However, the variations of the dissolved oxygen level in the sample solution may cause fluctuations in the result and the accessorial instrument needed to be equipped to supply air[12,13]. On the other hand, studies suggest that the oxygen-type BOD biosensor is limited by the concentration of oxygen in water (8.7 mg/L at 25 ºC).

Though the use of the conventional 5-day biochemical oxygen demand (BOD_5) method in BOD determination is greatly hampered by its time-consuming sampling procedure and its titration technical difficulty in the handling of a large pool of wastewater samples, the BOD_5 method still has its merit of good stability and repetitiousness for measurement of life sewage and certain degradable pure organic matter. So whatever method developed for the determination of the BOD, the result got by those method must verified by the BOD_5.

In this research, a new spectrophotometric method for determination of BOD was built on the conventional BOD_5 method in water sample with the I_3^--acridine red-poly(vinyl alcohol) system was first proposed. It depended on the reaction between KIO_3 which adopted as the standard solution and excessive KI. The released I_3^- could react with acridine red to form an association complex, which has the maximum absorbance at 525 nm, if acridine red solution was added to this system after reaction between KIO_3 and KI. Then BOD_5 could be calculated through the amount of dissolved oxygen determined by I_3^--acridine red-PVA spectrophotometry between the first test of dissolved oxygen and the second test of dissolved oxygen after seeding procedure (Fig. 1).

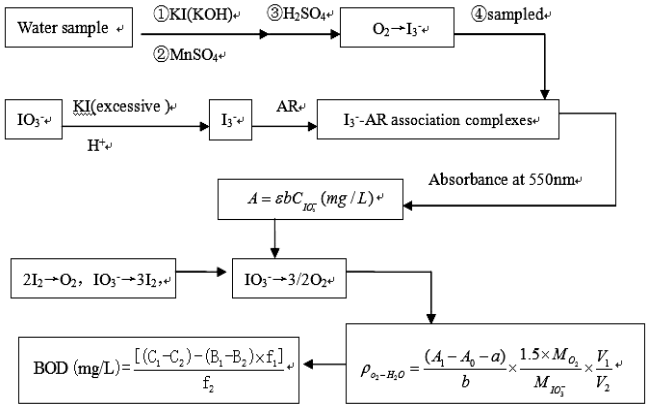

Fig. 1. Flow chart of the principle of the I_3^--acridine red-PVA spectrophotometric method

Measurements of real water samples, BOD_5 standard sample, glucose-glutamic acid solution, inoculated water, sewage were performed by the proposed method and the result also compared with those obtained by using the GB7488-87 method.

Where ρ = dissolved oxygen concentration, mg/L; A = absorbance for the water sample (against the reagent blank); V_1 = the volume of the colour comparison tube, mL; V_2 = sample volume shift from the BOD bottle, mL; a and b are slope and intercept of the equation $A = a\rho_{O_2}$ (mg/L) + b; C_1 = dissolved oxygen of diluted sample immediately after preparation, mg/L; C_2 = dissolved oxygen of diluted sample after 5d incubation at 20 ºC, mg/L; f_1 = ratio of diluted water to seed water in seed control; f_2 = ratio of water sample to seed water in seed control; B_1 = dissolved oxygen of seed control before incubation, mg/L; B_2 = dissolved oxygen of seed control after incubation mg/L.

EXPERIMENTAL

A Shimadzu UV-2501PC UV-VIS spectrophotometer equipped with a glass cell of 1 cm path length was used for the absorption spectra and the absorbance measurements.

SPX-250 constant temperature incubator (Shanghai Yuejin Medical Instruments Factory); Hach Sension-8 Benchtop dissolved oxygen meter (Hach, American); A Hach COD model reactor 45600 (Hach, American).

All reagents were of analytical reagent grade unless otherwise specified. Deionized water was used for the preparation of solutions. The effluent of sewage was adopted as inoculation solution.

Potassium iodide, 0.2 mol/L solution in distilled water; alkali potassium iodide, 0.9 mol/L solution in distilled water and NaOH was also found in this solution and its concentration was 12.5 mol/L in distilled water; acridine red, 4.4×10^{-4} mol/L; 10 g/L polyvinyl alcohol solution was prepared by dissolving 10 g polyvinyl alcohol in 1000 mL distilled water. Manganous sulfate, 0.05 mol/L solution in distilled water; H_2SO_4, 0.5 mol/L solution in distilled water; potassium iodate dissolved oxygen standard solution of 1 g/L was prepared by dissolving 0.3057 g KIO_3 in 1000 mL distilled water and before using this solution was diluted to 10 mg/L. (IO_3^- of 10 mg/L is equivalent to 2.74 mg/L O_2).

Diluted water was obtained by aerating distilled water at 20 ºC in order to make the dissolved oxygen approach to saturation. Before using 1.00 mL phosphate buffer solution, $MgSO_4$ solution, $CaCl_2$ solution, $FeCl_3$ solution were added to the aerated water respectively and mixed evenly. The diluted water was brought to pH 7.2 and the BOD_5 was less than 1 mg/L[1]. BOD_5 standard solution (institute for reference materials of state environmental protection administration, Beijing, China) was prepared with distilled water by putting 10.00 mL BOD_5 standard solution into a 250 mL flask before using this solution. Glucose-glutamic acid standard solution was prepared by dissolving 150 mg glucose and glutamic acid, respectively, which dried at 103 ºC for 1 h, in a 1000 mL flask of water and mixed evenly. Prepare fresh immediately before use.

General procedure: Collect the water samples with the Niskin bottle sampler and allow the sample to overflow the sample bottle for a while before capping with the stopper. The COD_{Cr} of the water sample should be determined. Then according to the COD_{Cr} obtained the dilution multiple could be estimated and the water samples were diluted by diluted water proportional to the dilution multiple. The amount of dissolved oxygen in the water sample was determined before and after incubation by the proposed method and then the BOD could be calculated. Meanwhile, another two samplers were full of diluted water or inoculated water by siphon as the blank test, which used to determine the dissolve oxygen as the reagent blank.

Detection method: Use a BOD bottle to collect the water sample. Add 1 mL of the 6 mol/L $MnSO_4$ solution and 1 mL of the alkali-KI solution and make sure no air is entrained into the bottle. The manganous hydroxide reacts with the dissolved oxygen to form a brown precipitate [$MnO(OH)_2$]. 1 mL of H_2SO_4 (sulphuric acid) is added to the sample and the bottles are inverted several times in order to completely redissolve the brownish/orange floc. The [$MnO(OH)_2$] immediately reacts with the H_2SO_4, liberating the number of moles of iodine exactly equivalent to the number of moles of oxygen presented in the sample. The release of iodine imparts a brown colouration to the water typical of iodine. Let the water sample stand for 5 min in the dark. Then appropriate volumes of this solution were placed in a series of 10 mL volumetric flasks, treated with 1.50 mL of 0.2 mol/L KI, 2.00 mL 0.5 mol/L H_2SO_4, 1.50 mL 4.4×10^{-4} mol L^{-1} 1.00 mL 0.05 mol/L $MnSO_4$, 1.00 mL diluted water and 0.50 mL 10 g/L poly(vinyl alcohol) solution. The solution was completed to volume with distilled water and allowed to stand for 20 min at room temperature. Then the dissolved oxygen could be got by the proposed method. The difference in oxygen levels between the first test and the second test is the amount of BOD.

RESULTS AND DISCUSSION

Under the experimental conditions, the absorption spectra of I_3^--AR-PVA system and AR-PVA system were scanned against the water blank, respectively at the wavelength range of 500-700 nm (Fig. 2). As could be seen from Fig. 2 the absorbance of I_3^--AR-PVA was decreased sharply compared with the AR-PVA system at 525 nm. So the detection wavelength was chosen at 525 nm.

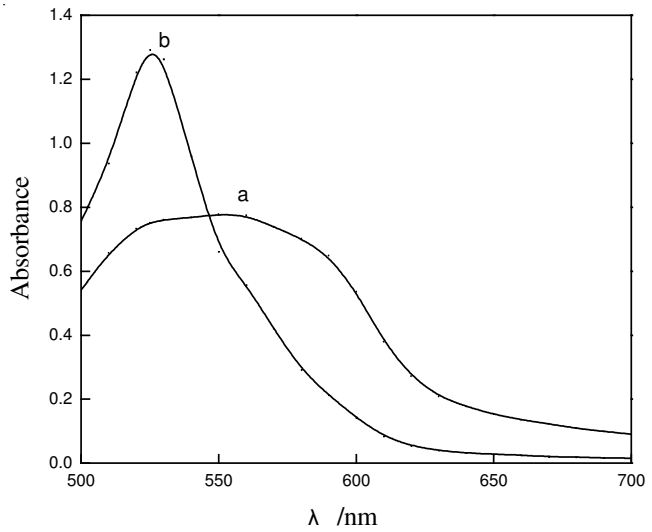

Fig. 2. Absorption spectra of ion-associated complex. (a) I_3^- + AR + PVA (against water blank); (b) acridine red + PVA (against water blank)

In addition, surfactants may shift the absorption peak of associated complexes and the shift is usually accompanied by increase in molar absorptivity. Effect of type of surfactant on spectra and sensitivity were examined. The test results showed that the higher sensitivity in poly(vinyl alcohol) solution than in other surfactant solutions when the same concentration of the iodate standard solution was added. So poly(vinyl alcohol) was selected for further studies.

Effect of reaction time: Since iodine ions could release iodine slowly in the acid medium and light irradiation, it is necessary to study the effect of the time varied with absorbance. The absorbance of the association complex was increased in the initial 10 min and then remained stable for 40 min and after that the absorbance gradually decreased. So 20 min was recommended for the further study.

Effect of pH: The reaction among the I_3^--KI-acridine red could be carried out in the acidic medium. So the effect of pH on the determination of 1.0 μg of IO_3^- was studied by measuring the absorbance of associated-complex at the range of 0.05-0.3 mol/L of concentration of H^+. The results, which were presented in Fig. 3 displayed that in PVA media the complex showed the constant and biggish absorption at 0.15-0.30 mol/L. In higher pH, the absorbance of I_3^--acridine red complex was decreased due to the less I_3^- generated by IO_3^- and I^- in the weak acid solution. So in the subsequent work, 2.00 mL 0.5 mol/L H_2SO_4 has been selected.

Effect of the amounts of poly(vinyl alcohol): Effect of amount of 10 g/L PVA solution on the complex formation reaction was tested. It was found that the absorbance leveled off with a maximum when the volume amount of 10 g/L poly(vinyl alcohol) solution was tested from 0.50-3.00 mL so 1.00 mL was selected for the further experiment.

Effect of the amounts of acridine red: With the increase of the volume of 4.4×10^{-4} mol/L acridine red solution in the system, the absorbance increased and followed by remaining almost at a maximum absorbance when 1.25-1.70 mL of 4.4×10^{-4} mol/L acridine red solution added for 1 μg IO_3^-, thus an addition of 1.50 mL acridine red solution was recommended (Fig. 4).

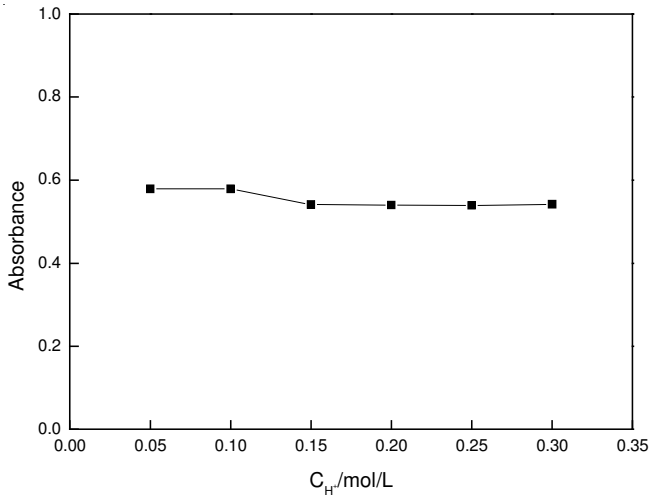

Fig. 3. Effect of concentration of H^+ on absorbance

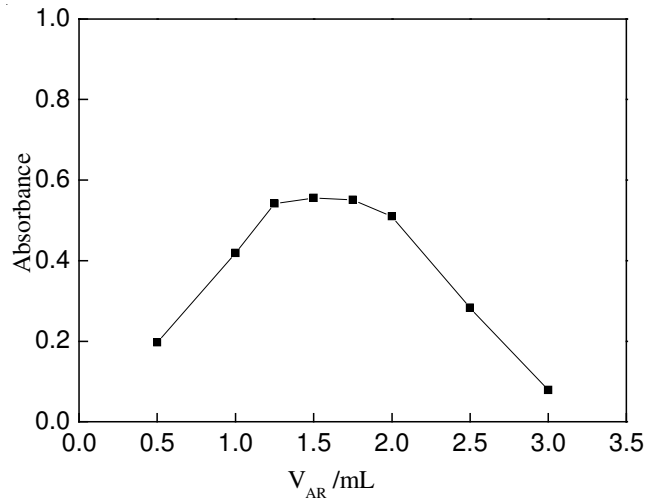

Fig. 4. Effect of volume of acridine red solution on absorbance

Effect of the dilution water and $MnSO_4$: The BOD concentration in most wastewaters exceeds the concentration of dissolved oxygen available in an air-saturated sample and because the bacterial growth requires nutrients such as nitrogen, phosphorus and trace metals, these are added to the dilution water, which is buffered to ensure that the pH of the incubated sample remains in a range suitable for bacterial growth. Before the spectrophotometric determination of dissolved oxygen, oxygen in the water sample reacts under alkaline conditions with Mn^{2+} ions forming manganese(III) hydroxide. Therefore, it is necessary to discuss the effect of amount of the dilution water and $MnSO_4$ on the I_3^--acridine red-PVA. The test results showed that the absorbance was almost constant and highest when the amount of $MnSO_4$ and dilution water was 1.00 and 1.00 mL, respectively.

Effect of interfering ions: Under the recommended conditions, the effects of various foreign ions on the determination of 2.74 mg/L of dissolved oxygen in the presence of various amounts of foreign ions were measured. The interference of foreign substrates were discussed with a relative error of less than ± 5 %, the tolerance limits for various foreign ions were as follows: F^- (500), NH_4^+, Na^+, K^+, Ca^{2+}, Mg^{2+} (300), Fe^{2+}, Al^{3+}, HPO_4^{2-}, $H_2PO_4^-$, Cl^- (100), Zn^{2+}, Ni^{2+}, Ba^{2+} (50),

Fe^{3+} (1). It was shown that most of the metal ions did not influence the determination of dissolved oxygen. Generally speaking, because the content of Fe^{3+} is negligible in the water when compared with the amount of dissolved oxygen, so the effect on the colour reaction caused by Fe^{3+} could be neglected. If NO_2^- were found in the water sample and its contents were more than 0.1 mg/L. The excessive dissolved oxygen would obtained because extra I_2 were formed from the reaction between NO_2^- and KI. In order to eliminate the interference a few drops of 50 g/L NaN_3 could be add to the water sample before the precipitation were dissolved by H_2SO_4.

Calibration curve of dissolved oxygen: The calibration curve was constructed according to the recommended procedure. Beer's law was obeyed for 0.03-1.00 mg/L of dissolved oxygen in 10 mL of solution. The determined regressive equation was $A = 0.0125 + 0.5618\rho_{O_2}$ (mg/L), the related coefficient was 0.9981 and the molar absorption coefficient was 1.803×10^4 L mol^{-1} cm^{-1}. The 3σ limit of detection was found to be 0.006 mg/L.

Water sample analysis: Collect the water samples with the Niskin bottle sampler and allow the sample to overflow the sample bottle for a while before capping with the stopper. Be careful not to allow any air bubbles to be entrapped, as it will significantly change the results. According to the COD_{Cr} obtained the dilution multiple could be estimated by the coefficient of dilution as followed 0.075, 0.15, 0.25. Then the water samples were diluted by diluted water proportional to the dilution multiple and preserved. When the dissolved oxygen in the water sample was converted to iodine, the I_3^--acridine red -PVA spectrophotometry could be used to determine the content of the dissolved oxygen.

The experimental results are shown in Tables 1 and 2. The BOD_5 mensurated of BOD_5 standard solution and the glucose-glutamic acid standard solution were within the scope of the value known. The national standard value of BOD_5 standard solution was 130 ± 7 mg/L and the prescribed value of glucose-glutamic acid standard was 180-230 mg/L. The experimental results obtained from the I_3^--acridine red-PVA spectrophotometry and GB7488-87 was in agreement with each other and the test error was in 5 %.

Conclusion

A simple, stable and highly sensitive BOD determination method built on the conventional BOD_5 method in water sample with the I_3^--acridine red-PVA system is proposed. The present method is superior to the available BOD sensor depended on the dissolved oxygen as the terminal electron acceptor for biodegradation of organic substrates. It can drastically reduce the influences of coexisting ions dissolved in the sample. Meanwhile compared with the BOD_5 the sensitivity was improved at least 100 times. The biochemical oxygen demand in different water samples such as BOD_5 standard sample, glucose-glutamic acid solution, the influent and effluent of sewage were determined by the spectro-photometric method and got a satisfactory agreement with the GB7488-87 method.

TABLE-1
DETERMINATION OF DISSOLVED OXYGEN (DO) BY GB7488-87 AND PROPOSED METHOD

Sample	Dilution times	Value of DO by GB7488-87 (mg/L)			Value of DO by proposed method (mg/L)		
		DO_1	DO_5	ΔDO	DO_1	DO_5	ΔDO
BOD_5 standard sample	20	9.56	2.77	6.79	9.48	2.89	6.59
	40	9.43	5.49	3.94	9.35	5.52	3.83
	70	9.61	7.23	2.38	9.47	7.18	2.29
Glucose-glutamic acid solution	50	9.06	4.32	4.74	9.09	4.36	4.73
	50	9.10	4.43	4.67	9.06	4.40	4.66
Influent of sewage	20	9.19	3.54	5.65	9.21	3.48	5.73
	40	8.98	6.31	2.67	9.02	6.28	2.74
Effluent of sewage	4	9.27	4.68	4.59	9.23	4.56	4.67
	8	9.05	6.56	2.49	9.01	6.63	2.38

TABLE-2
BOD_5 IN DIFFERENT WATER SAMPLES

Sample	Dilution times	Value of BOD_5 by GB7488-87 (mg/L)		Value of BOD_5 by proposed method (mg/L)	
		BOD_5	$\overline{BOD_5}$	BOD_5	$\overline{BOD_5}$
Inoculated water	–	0.48	0.48	0.39	0.39
BOD_5 standard sample	20	127	134	124	132
	40	140		138	
	70	136		134	
Glucose-glutamic acid solution	50	215	213	217	215
	50	212		214	
Diluted water	–	0.18	0.18	0.15	0.15
Influent of sewage	20	109	104	112	108
	40	100		104	
Effluent of sewage	4	17.8	18.2	18.2	18.1
	8	18.7		18.0	

ACKNOWLEDGEMENTS

We acknowledge support from the Huaihai Institute of Technology. Lian Yungang Jinzhao Water limited Company of Lianyungang of Jiangsu province of China is thanked for providing the BOD standard solution and wastewater samples.

REFERENCES

1. American Public Health Association (APHA), Standard Methods for the Examination of Waters and Wastewaters, Washington, DC, edn. 20, p. 20 (1998).
2. State Environmental Protection Administration of the People's Republic of China, Water Quality-Five Days Method for the Biochemical Oxygen demand (BOD_5): Dilution and Inoculation Method (GB 7488-87), China (1987).
3. H. Nakamura and I. Karube, *Anal. Bioanal. Chem*., **377**, 446 (2003).
4. G.J. Chee, Y. Nomura and I. Karube, *Anal. Chim. Acta*, **379**, 185 (1999).
5. G.J. Chee, Y. Nomura, K. Ikebukuro and I. Karube, *Anal. Chim. Acta*, **394**, 65 (1999).
6. G.J. Chee, Y. Nomura, K. Ikebukuro and I. Karube, *Biosens. Bioelectr*., **15**, 371 (2000).
7. C. Preininger, I. Klimant and O.S. Wolfbeis, *Anal. Chem.*, **66**, 1841 (1994).
8. J. Liu and B. Mattiasson, *Water Res*., **36**, 3786 (2002).
9. N.Y. Kwok, S. Dong, W. Lo and K.Y. Wong, *Sens. Actuators B*, **110**, 289 (2005).
10. R.M. Bukowski, R. Ciriminna, M. Pagliaro and F.V. Bright, *Anal. Chem*., **77**, 2670 (2005).
11. I. Karube, S. Mitsuda, T. Matsunaga and S. Suzuki, *J. Ferment.Technol*., **55**, 243 (1977).
12. Y. Lei, W. Chen and A. Mulchandani, *Anal. Chim. Acta*, **568**, 200 (2006).
13. Z. Du, H. Li and T. Gu, *Biotechnol. Adv*., **25**, 464 (2007).