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Spectrophotometric determination of hydroxylamine in biological wastewater treatment processes

B. Hu[1,2] · X. L. Tian[3] · W. N. Shi[4] · J. Q. Zhao[2,3,5] · P. Wu[1,2] · S. T. Mei[3]

Abstract Hydroxylamine, a very important intermediate in nitrification, has a direct relationship with the production of nitrous oxide in biological wastewater treatment processes. The spectrophotometric method taking ferric ammonium sulfate and 1, 10-phenanthroline as the oxidant and the chromogenic agent, respectively, was used to determine the concentration of hydroxylamine in biological wastewater treatment processes. The impacts of nitrite, nitrate, orthophosphate, calcium ion and trace elements on the method were examined. The results indicated that the spectrophotometric method can be used for the determination of hydroxylamine in biological wastewater treatment processes. The correlation was significant in the range of 0.02–1.00 mg N/L ($y = 1.5078x - 0.0132$, $R^2 = 0.9991$), and the range varied to 0.05–1.00 mg N/L when nitrite and orthophosphate presented. Nitrate, calcium ion and trace elements did not interfere with the determination of hydroxylamine nitrogen. When the concentrations of nitrite nitrogen in the samples were lower than 15.00 mg/L, nitrite had a minor interference on the method. The impacts of orthophosphate on the method were complex. When the concentrations of hydroxylamine nitrogen were higher than 0.10 mg/L, the interference of orthophosphate on the method can be ignored. However, when the concentrations of hydroxylamine nitrogen in the samples were lower than 0.10 mg/L, orthophosphate had significant impacts on the determination, and a numerical method proposed can eliminate the interference of orthophosphate. The spectrophotometric method can determine the concentration of hydroxylamine in biological wastewater treatment processes quickly and conveniently and was helpful to understand the function of NH_2OH in N_2O production in biological wastewater treatment processes.

Keywords Hydroxylamine · Determination · Interference · Nitrite · Orthophosphate

✉ J. Q. Zhao
jqzhao@chd.edu.cn

1   School of Civil Engineering, Chang'an University, The Middle Section of the South 2nd Ring Road, Xi'an 710064, Shaanxi Province, China

2   Key Laboratory of Water Supply and Sewage Engineering, Ministry of Housing and Urban-Rural Development, Chang'an University, The Middle Section of the South 2nd Ring Road, Xi'an 710064, Shaanxi Province, China

3   School of Environmental Science and Engineering, Chang'an University, The Middle Section of the South 2nd Ring Road, Xi'an 710064, Shaanxi Province, China

4   Shenyang Urban Planning Design and Research Institute, South Sanhao Street No. 1, Shenyang 110179, Liaoning Province, China

5   Key Laboratory of Environmental Protection & Pollution and Remediation of Water and Soil of Shaanxi Province, Chang'an University, The Middle Section of the South 2nd Ring Road, Xi'an 710064, Shaanxi Province, China

Introduction

Biological wastewater treatment system had been identified as a source for the increasing nitrous oxide (N_2O) in atmosphere (UNEP 2013.). In denitrification, the mechanism of N_2O production and loss is quite clear and uncontested. However, the mechanism of N_2O production

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in nitrification is very complicated. In nitrification, ammonia (NH_3) is converted to nitrate (NO_3^-) via hydroxylamine (NH_2OH) and nitrite (NO_2^-). N_2O can be produced by chemical breakdown of the unstable nitrosyl radical (NOH) (Poughon et al. 2001; Lemaire et al. 2011). Meanwhile, reduction of nitric oxide (NO) produced from the oxidation of NH_2OH can also produce N_2O (Stein 2011). Besides, the electrons released from the oxidation of NH_2OH were used to sustain ammonium oxidation and satisfy the cell's reductant needs (Arp and Stein 2003). Generally speaking, NH_2OH is an important intermediate formed in oxidation of ammonia/ammonium (NH_3/NH_4^+) to nitrite (NO_2^-) by ammonium-oxidizing bacteria (Vajrala et al. 2013), and it has a direct relationship with the production of N_2O in biological wastewater treatment processes (Kim et al. 2010).
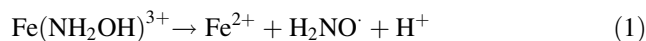
Gas chromatography method had been successively used for the determination of NH_2OH in seawater (Bulter and Gordon 1986; Gebhardt et al. 2004) and in soils (Liu et al. 2014). Due to short-live and extremely reactive properties of NH_2OH (Liu et al. 2014), determination of NH_2OH in aqueous solution or in soil with gas chromatography method is not an easy task. Many substances can result in a significant errors in the determination, such as NO_2^- (Bremner et al. 1980), transition metal ions and complexes (Brown and Drury 1967; Bengtsson 1973; Bengtsson et al. 2002), O_2 (Adhikamsetty et al. 2008), and so on. The composition of the domestic wastewater was more complex than seawater. Orthophosphate (PO_4^{3-}), NO_2^-, metal ions and organic matters were common in domestic wastewater. Consequently, determination of NH_2OH in biological domestic wastewater treatment processes was more difficult than that in seawater or in soil. Furthermore, as the lack of the quantitative relation between NH_2OH and N_2O, it caused great difficulties in understanding the mechanism of N_2O production in biological wastewater treatment process, especially in nitrification. More recently, with the development of nanotechnology, nanostructure-based electrochemical method achieved great developments in the determination of NH_2OH (Sadeghi et al. 2013; Moghaddam et al. 2014; Foroughi et al. 2014; Beitollahi et al. 2014; Mozloum-Ardakani et al. 2015; Rezaei et al. 2015). To the electrochemical method, NH_2OH cannot be oxidized at conventional electrode materials, and the electrodes used for the determination of NH_2OH must be chemically modified in order to lower the overpotentials and increase the oxidation current response (Moghaddam et al. 2014). However, the monitoring ranges of gas chromatography method and the electrochemical method were at the level of µg/L, and the two methods were not suitable for determining milligram or larger amounts of NH_2OH. Besides, the determination procedures of the two methods were also complex.

According to the above points, it was essential to construct a simple and convenient method for the determination of NH_2OH in biological wastewater treatment process. The aims of this study were: (1) to verify the feasibility of using the spectrophotometric method to determine NH_2OH in biological domestic wastewater treatment process, (2) to examine the effects of PO_4^{3-}, NO_2^-, nitrate (NO_3^-) and trace elements on the spectrophotometric method and (3) to develop a method for eliminating the interference. The research was carried out from May 2016 to January 2017 in Chang'an University, Xi'an city, Shaanxi province, China.

Materials and methods

Principles

Under acidic condition, NH_2OH can be stabilized as NH_3OH^+. The spectrophotometric method was based on oxidation of NH_2OH to N_2O by Fe(III) using ferric ammonium sulfate ($NH_4Fe(SO_4)_2$, FAS) as oxidation agent (Bengtsson et al. 2002):

$$Fe(NH_2OH)^{3+} \rightarrow Fe^{2+} + H_2NO^. + H^+ \tag{1}$$

$$Fe^{3+} + H_2NO^. \rightarrow Fe^{2+} + HNO + H^+ \tag{2}$$

$$HNO \rightarrow 0.5N_2O + 0.5H_2O \tag{3}$$

NH_2OH was determined through the formation of ferrion, a tris complex of 1, 10-phenanthroline with Fe(II). Ferrion was a red-colored octahedral complex ion and was soluble and stable in aqueous solution in the pH range of 2–9 (Adhikamsetty et al. 2008). Its molar absorption coefficient was 1.11×10^4 mol/cm at 510 nm, with no peak shift due to pH variation (Hughes et al. 1971).

Solution preparation

Stock solution of sodium nitrite (N_aNO_2): N_aNO_2 (AR) was dried in an oven at 105 °C for 24 h, and 1.2320 g N_aNO_2 was dissolved in 1000 mL deionized water. In order to preserve the solution, 2 mL $CHCl_3$ was added to the solution. 1 mL stock solution of N_aNO_2 contained 250 µg NO_2^--N.

Stock solution of hydroxylamine chloride ($NH_2OH·HCl$): $NH_2OH·HCl$ (GR) was dried in an oven at 50 °C for 24 h, and 2.4821 g $NH_2OH·HCl$ was dissolved in 1000 mL deionized water. In order to stabilize NH_2OH, 1 mL 1 mol/L hydrochloric acid solution was added to the stock solution. The solution was preserved at 4 °C in dark. 1 mL stock solution of $NH_2OH·HCl$ contained 500 µg NH_2OH-N.

Standard solution of $NH_2OH·HCl$: The standard solution of $NH_2OH·HCl$ was prepared by diluting 1 mL stock

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solution of $NH_2OH \cdot HCl$ to 200 mL before each experiment. 1 mL standard solution of $NH_2OH \cdot HCl$ contained 2.5 μg NH_2OH-N.

Stock solution of sodium phosphate (Na_3PO_4): Na_3PO_4 (GR) was dried in an oven at 110 °C for 24 h, and 0.2645 g Na_3PO_4 was dissolved in 1000 mL deionized water. 1 mL stock solution of Na_3PO_4 contained 50 μg PO_4^{3-}-P.

Standard solution of potassium nitrate (KNO_3): KNO_3 (AR) was dried in an oven at 105 °C for 24 h, and 0.7218 g KNO_3 was dissolved in 1000 mL deionized water. In order to preserve the solution, 2 mL $CHCl_3$ were added to the solution. 1 mL stock solution of KNO_3 contained 100 μg NO_3^--N.

Standard solution of calcium chloride (C_aCl_2): C_aCl_2 (GR) was dried in an oven at 105 °C for 24 h, and 0.1387 g C_aCl_2 was dissolved in 1000 mL deionized water. 1 mL standard solution of C_aCl_2 contained 50 μg C_a^{2+}.

Stock solution of trace elements: The stock solution of trace elements contained H_3BO_3, $CuCl_2$, Z_nCl_2, $(NH_4)Mo_7O_2 \cdot 4H_2O$, $MgSO_4$, $CoCl_2 \cdot 4H_2O$, $AlCl_3$ and N_iCl_2. The compositions of the solution are shown in Table 1.

1 mL concentrated hydrochloric acid was added to the solution for preservation.

Standard solution of trace elements: The standard solution of trace elements was prepared by diluting 1 mL stock solution of trace elements to 200 mL before each experiment.

Solution of ferric ammonium sulfate (FAS): 1.9287 g $NH_4F_e(SO_4)_2 \cdot 12H_2O$ (AR) was dissolved in 1000 mL 0.1 mol/L hydrochloric acid solution.

Solution of 1, 10-phenanthroline (1, 10-Phe): 1.8021 g 1,10-Phe (AR) was dissolved in 1000 mL 1 mol/L acetic acid solution.

Solution of sodium acetate and acetate (SAA): 96 mL 1.0 mol/L sodium acetate solution and 114 mL 1.0 mol/L acetic acid solution were mixed. The mixture was preserved at 4 °C in dark.

Procedure

a. Preparation of standard curve: The standard curve of NH_2OH-N was prepared in the range of 0.00–1.00 mg NH_2OH-N/L by diluting 0.00, 0.20, 0.50, 1.00, 2.00, 4.00 and 10.00 mL of the standard solution of $NH_2OH \cdot HCl$ to 25 mL.

Table 1 Compositions of the stock solution of trace elements

H_3BO_3	150 mg/L	Z_nCl_2	150 mg/L
$CuCl_2$	30 mg/L	$(NH_4)Mo_7O_2 \cdot 4H_2O$	150 mg/L
$MgSO_4$	500 mg/L	$AlCl_3$	150 mg/L
$CoCl_2 \cdot 4H_2O$	150 mg/L	N_iCl_2	150 mg/L

b. Color development: 1.00 mL SAA, 1.00 mL FAS and 1.00 mL 1, 10 Phe were added to the 25 mL samples of NH_2OH in turn. Before the addition of each reagent, the samples were thoroughly mixed.

c. Photometric measurement: 30 min after adding color reagents to the sample, the absorbance of the sample was read at 510 nm (TU1810PC, PERSEE· Beijing, China), and the absorbance of the deionized water was set at zero. The light path was 1 cm.

During determination of NH_2OH-N in other samples, the samples were treated with the same manner as the standard curve.

Experiments

Determination of the time for color development

For determining the time for color development of the spectrophotometric method, the color development time of samples containing different concentrations of NH_2OH-N was evaluated. The concentrations of NH_2OH-N in the samples were set at 0.00, 0.02 and 0.80 mg/L, respectively.

Impact of NO_2^- on the spectrophotometric method

The concentrations of NH_2OH-N in samples were set at 0.02, 0.05, 0.10, 0.20 and 0.40 mg/L. In order to examine the impact of NO_2^- on the spectrophotometric method, NO_2^- was added to the samples. In the experiment of each concentration of NH_2OH-N, the concentrations of NO_2^--N in the samples were 2.00, 5.00, 10.00 and 15.00 mg/L, respectively. Each group of the experiment was conducted in triplication.

Impact of PO_4^{3-} on the spectrophotometric method

The concentrations of NH_2OH-N in samples were set at 0.02, 0.05, 0.10, 0.20 and 0.40 mg/L. In order to examine the impact of PO_4^{3-} on the spectrophotometric method, PO_4^{3-} was added to the samples. In the experiment of each concentration of NH_2OH-N, the concentrations of PO_4^{3-}-P in the samples were 1.00, 2.00, 3.00 and 4.00 mg/L, respectively. Each group of the experiment was conducted in triplication.

Elimination of the interference of PO_4^{3-} on the spectrophotometric method

In order to eliminate the interference of PO_4^{3-} on the method, extra experiments were conducted. The concentrations of NH_2OH-N in samples were set at 0.05 and 0.10 mg/L. In the experiment of each concentration of NH_2OH-N, the concentrations of PO_4^{3-}-P in the samples

were 1.00, 1.50 2.00, 2.50, 3.00, 3.50, 4.00, 5.00, 6.00, 7.00, 8.00, 9.00 and 10.00 mg/L, respectively. Each group of experiments was conducted in triplication.

Verification

In order to verify the spectrophotometric method and the correction method proposed in this study, samples containing different concentrations of NH_2OH-N, PO_4^{3-}-P and NO_2^--N were determined by the spectrophotometric method. Meanwhile, the application of the correction method for improving the accuracy of the spectrophotometric method was evaluated. The compositions of the verification samples are shown in Table 2.

Impact of NO_3^- on the spectrophotometric method

The concentrations of NH_2OH-N in samples were set at 0.05, 0.10, 0.20 and 0.40 mg/L. In order to examine the impacts of NO_3^- on the spectrophotometric method, NO_3^- was added to the samples. In the experiment of each

Table 2 Compositions of samples for verification of the spectrophotometric method

NH_2OH-N concentrations in samples (mg/L)	PO_4^{3-}-P concentrations in samples (mg/L)	NO_2^--N concentrations in samples (mg/L)
0.06	1.40	–
	2.40	–
	3.40	–
	3.80	–
0.08	1.40	–
	2.40	–
	3.40	–
	3.80	–
0.05	3.00	2.00
	3.00	5.00
	3.00	10.00
	3.00	15.00
0.05	4.00	2.00
	4.00	5.00
	4.00	10.00
	4.00	15.00
0.10	3.00	2.00
	3.00	5.00
	3.00	10.00
	3.00	15.00
0.10	4.00	2.00
	4.00	5.00
	4.00	10.00
	4.00	15.00

concentration of NH_2OH-N, the concentrations of NO_3^--N in the samples were 6.00, 10.00, 16.00 and 24.00 mg/L, respectively. Each group of the experiment was conducted in triplication.

Impacts of other matters on the spectrophotometric method

In order to examine the impacts of C_a^{2+} and trace elements on the spectrophotometric method, the C_aCl_2 solution and the trace elements solution were added to the samples. The concentrations of NH_2OH-N in samples were set at 0.05, 0.10, 0.20 and 0.40 mg/L. The compositions of the samples are shown in Table 3. Each group of the experiment was conducted in triplication.

Results and discussion

The determination time and the standard curve

When NH_2OH-N concentrations in the samples were 0.00, 0.02 and 0.80 mg/L, the absorbance of the samples almost not varied when the time for color development was longer than 20 min (Fig. 1), especially in the range of 20–60 min. In the chromogenic process, NH_2OH was oxidized to N_2O by Fe(III), and Fe(III) was reduced to Fe(II). Then, ferrion formed by coupling 1, 10-phenanthroline with Fe(II). The reaction of NH_2OH and Fe(III) at 30 °C was completed in 3 min (Dias et al. 1979). This process was a chemical process and did not need a very long reaction time. So, the determination time of the spectrophotometric method was set at 30 min after all reagents were added.

When the concentration of NH_2OH-N was in the range of 0.02–1.00 mg/L, there was a linear relation between the concentrations of NH_2OH-N and the absorbance ($y = 1.5078x$-0.0132, $R^2 = 0.9991$) without the presence of interfering substances (Fig. 2).

The monitoring ranges of the gas chromatography method (Kock and Bange 2013) and the electrochemical method ((Sadeghi et al. 2013; Moghaddam et al. 2014; Foroughi et al. 2014; Beitollahi et al. 2014; Mozloum-Ardakani et al. 2015; Rezaei et al. 2015)) were at the level of µg/L. Compared with the gas chromatography method and the electrochemical method, the monitoring range of the spectrophotometric method was at the level of mg/L. The spectrophotometric method was more suitable for determining milligram or larger amounts of NH_2OH.

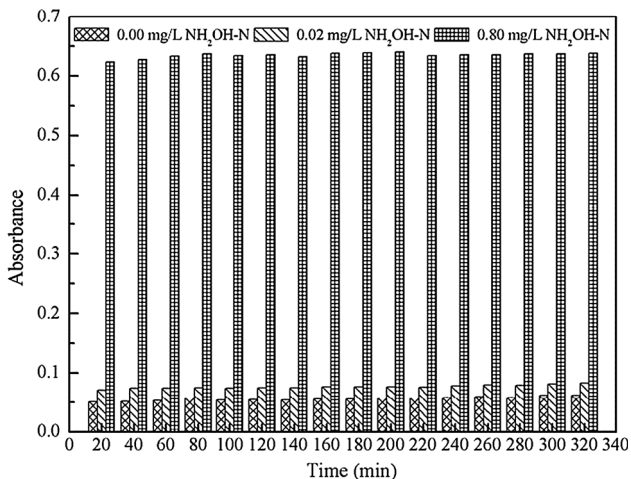### Impact of NO_2^- on the method

The determined NH_2OH-N concentrations of the samples containing NO_2^- are shown in Table 4.

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**Table 3** Compositions of samples containing C_a^{2+} and trace elements

NH_2OH-N concentrations of the samples (mg/L)	C_a^{2+} concentrations in samples (mg/L)	Volumes of the standard solution of trace elements added to the samples (mL)
0.05	2.00	1.00
	5.00	2.50
	8.00	5.00
0.10	2.00	1.00
	5.00	2.50
	8.00	5.00
0.20	2.00	1.00
	5.00	2.50
	8.00	5.00
0.40	2.00	1.00
	5.00	2.50
	8.00	5.00

When the concentrations of NH_2OH-N in the samples were 0.02 mg/L, the recoveries of NH_2OH-N were only in the range of 58.30–68.33% while NO_2^--N presented, and the spectrophotometric method cannot give satisfied determination results of NH_2OH-N. When the concentrations of NH_2OH-N in the samples were higher than 0.05 mg/L, the spectrophotometric method can give relatively accurate results of NH_2OH-N concentrations when NO_2^--N concentration was lower than 15.00 mg/L. The recoveries of NH_2OH-N were in the range of 90.49–113.58%. Compared with the method which directly determined N_2O converted from NH_2OH-N (Kock and Bange 2013), the presence of NO_2^--N only had a minor interference with the spectrophotometric method when the concentration of NH_2OH-N in the samples was higher than 0.02 mg/L.

Under acid condition, Fe(II) can react with NO_2^- as follows (Heil et al. 2016):

$$Fe^{2+} + NO_2^- + 2H^+ \rightarrow Fe^{3+} + NO + H_2O \tag{4}$$
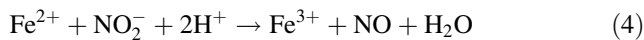
However, from the color development process, Fe(II) coupled with 1, 10-phenanthroline to form ferrion instead of reacting with NO_2^-. The presence of NO_2^- did not affect the formation of ferrion.

It was observed that the recoveries of NH_2OH-N were decreased with the increasing NO_2^--N concentration, especially when the concentration of NH_2OH-N was higher than 0.02 mg/L. This phenomenon indicated other reactions related to NH_2OH and NO_2^- occurred in the systems resulting in the decrease in the recoveries of NH_2OH-N. As mentioned before, NH_2OH is an extremely reactive matter. Although, it can be stabilized under acid condition, NH_2OH can react with NO_2^- to form hyponitrous acid ($H_2N_2O_2$) which can decompose to N_2O and water rapidly (Bothner-By and Friedman 1952):

$$NH_2OH + HNO_2 \rightarrow H_2N_2O_2 + H_2O \tag{5}$$

$$H_2N_2O_2 \rightarrow N_2O + H_2O \tag{6}$$
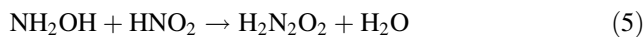
Based on the results obtained in our study, the above reactions maybe happen slowly. However, they were not the dominant process; otherwise, the recoveries of NH_2OH-N of all samples would be much lower than the obtained values. Nevertheless, the accuracy of the spectrophotometric method was obviously affected when the concentration of NH_2OH-N was at a very low level (e.g., 0.02 mg/L). It was recommended that when using the spectrophotometric method to determine the concentration of NH_2OH-N in samples containing NO_2^-, the determined concentration of NH_2OH-N higher than 0.05 mg/L was considered to be effective.

Fig. 1 Time for color development of samples containing different concentrations of NH_2OH-N
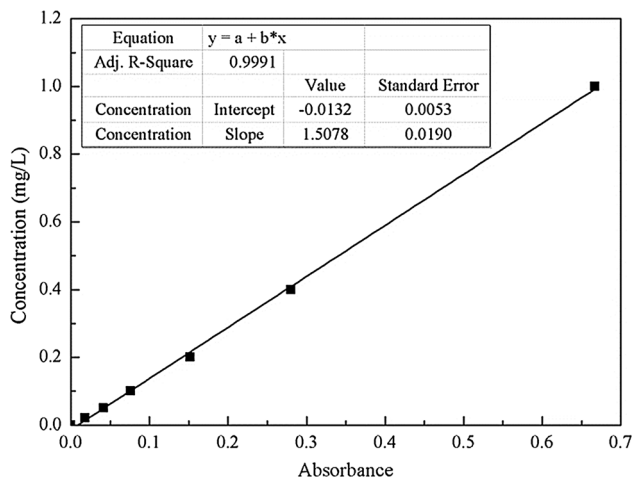
Fig. 2 Standard curve of the spectrophotometric method for determination of NH_2OH-N

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Table 4 Determined NH_2OH-N concentrations of the samples containing different NO_2^--N concentrations

NH_2OH-N concentrations in samples (mg/L)	NO_2^--N concentrations in samples (mg/L)	The determined NH_2OH-N concentrations of the samples (mg/L) ($n = 3$)	The recovery of NH_2OH-N (%)[a]
0.02	2.00	0.014 ± 0.0009	68.33
	5.00	0.012 ± 0.0009	60.80
	10.00	0.012 ± 0.0009	60.80
	15.00	0.012 ± 0.0009	58.30
0.05	2.00	0.052 ± 0.0015	103.53
	5.00	0.051 ± 0.0023	101.52
	10.00	0.048 ± 0.0009	95.51
	15.00	0.045 ± 0.0009	90.49
0.10	2.00	0.113 ± 0.0017	112.92
	5.00	0.111 ± 0.0031	111.42
	10.00	0.108 ± 0.0048	108.41
	15.00	0.105 ± 0.0061	104.90
0.20	2.00	0.227 ± 0.0048	113.36
	5.00	0.224 ± 0.0063	111.85
	10.00	0.220 ± 0.0030	110.10
	15.00	0.216 ± 0.0038	108.10
0.40	2.00	0.454 ± 0.0038	113.58
	5.00	0.448 ± 0.0076	112.07
	10.00	0.442 ± 0.0061	110.57
	15.00	0.439 ± 0.0083	109.82

[a] The recovery of NH_2OH-N was calculated based on the data before rounding

Table 5 Determined NH_2OH-N concentrations of the samples containing different PO_4^{3-}-P concentrations

NH_2OH-N concentrations in samples (mg/L)	PO_4^{3-}-P concentrations in samples (mg/L)	The determined NH_2OH-N concentrations (mg/L) ($n = 3$)	The recoveries of NH_2OH-N (%)[a]
0.02	1.00	0.013 ± 0.0009	65.82
	2.00	0.009 ± 0.0009	43.26
	3.00	0.007 ± 0.0009	35.74
	4.00	0.005 ± 0.0000	25.71
0.05	1.00	0.046 ± 0.0015	91.50
	2.00	0.043 ± 0.0022	86.48
	3.00	0.039 ± 0.0009	78.46
	4.00	0.037 ± 0.0015	73.45
0.10	1.00	0.103 ± 0.0040	102.90
	2.00	0.097 ± 0.0000	96.88
	3.00	0.091 ± 0.0038	91.37
	4.00	0.086 ± 0.0040	86.35
0.20	1.00	0.211 ± 0.0069	105.59
	2.00	0.204 ± 0.0066	101.83
	3.00	0.199 ± 0.0125	99.32
	4.00	0.190 ± 0.0046	94.81
0.40	1.00	0.423 ± 0.0128	105.68
	2.00	0.406 ± 0.0023	101.42
	3.00	0.399 ± 0.0086	99.67
	4.00	0.394 ± 0.0140	98.54

[a] The recoveries of NH_2OH-N were calculated based on the data before rounding

Impact of PO_4^{3-} on the spectrophotometric method

The determined NH_2OH-N concentrations of the samples containing PO_4^{3-} are shown in Table 5.

It was observed that the recoveries of NH_2OH-N were decreased with the increasing of PO_4^{3-}-P concentrations. When the concentrations of NH_2OH-N in the samples were higher than 0.20 mg/L, PO_4^{3-}-P almost had no impacts on NH_2OH-N determination by the spectrophotometric method. When the concentration of NH_2OH-N in the samples was 0.10 mg/L, the existence of PO_4^{3-}-P had minor impacts on NH_2OH-N determination by the spectrophotometric method. The recoveries of NH_2OH-N were in the range of 86.35–102.90%. However, when the concentrations of NH_2OH-N in the samples were lower than 0.05 mg/L, there were big errors between the actual concentrations and the determined concentrations of NH_2OH-N. The recoveries of NH_2OH-N were only in the range of 25.71–91.50%. The errors caused by PO_4^{3-}-P were increased with the increasing PO_4^{3-}-P concentrations. Overall, when the concentrations of NH_2OH-N in the samples were lower than 0.10 mg/L, the existence of PO_4^{3-} interfered with the determination of the spectrophotometric method.

In the spectrophotometric method, NH_2OH was oxidized to N_2O by Fe(III) in FAS; meanwhile, Fe(III) was reduced to Fe(II). When samples contained PO_4^{3-}, Fe(III) and Fe(II) reacted with PO_4^{3-} and formed precipitate (Galal-Gorchev and Stumm 1963; Ghassemi and Rcht 1971). In the spectrophotometric method, the formation of the precipitate was prevented by the low pH value (~ 4.5). However, due to the reaction between Fe(III)/Fe(II) and PO_4^{3-}, when the concentration of NH_2OH-N in the samples was lower than 0.10 mg/L, the accuracy of the spectrophotometric method was affected. 0.10 mg/L of NH_2OH-N in samples seemed to be the threshold concentration for the spectrophotometric method.

Numerical method for eliminating the interference of PO_4^{3-}

In order to eliminate the impact of PO_4^{3-} on the spectrophotometric method, the interferences of different PO_4^{3-}-P concentrations on the method were examined

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when the concentrations of NH_2OH-N were 0.05 and 0.10 mg/L, respectively.

When the concentrations of NH_2OH-N were 0.05 and 0.10 mg/L, the determined NH_2OH-N concentrations were decreased with the increasing PO_4^{3-}-P concentrations (Fig. 3). When the concentration of PO_4^{3-}-P in the samples was 10.00 mg/L, the recoveries of NH_2OH-N were only 42.37 and 63.80%, while the concentrations of NH_2OH-N were 0.05 and 0.10 mg/L, respectively. PO_4^{3-} seriously interfered with the spectrophotometric method (Table 6).

In order to eliminate the interference of PO_4^{3-}, a numerical method was proposed. Steps of the numerical method were as follows:
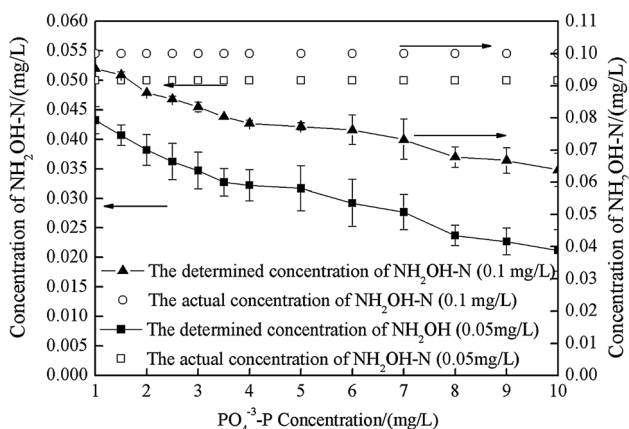
Step 1 was the normalization. The recoveries of NH_2OH-N in the samples containing different concentrations of PO_4^{3-}-P were divided with the corresponding concentrations of PO_4^{3-}-P.

Step 2 was the fitting. The concentrations of PO_4^{3-}-P and the normalized recoveries of NH_2OH-N were set as the X and Y axes, respectively, for plotting; then, the power function was used to fit the data (Fig. 1 in Supplementary Materials). The mean values of the scaling factor and the exponent of the power function under six experiments were 0.9166 ± 0.0514 ($n = 6$) and -1.1715 ± 0.0448 ($n = 6$). Consequently, the correction function for the following step was determined as:

$$y = 0.9166x^{-1.1715} \tag{7}$$

where x represented the PO_4^{3-}-P concentration in samples, mg/L, and y represented the normalized recovery of NH_2OH-N under different PO_4^{3-}-P concentrations.

Step 3 was the correction. The correction procedure included three sub-steps: (1) concentrations of PO_4^{3-}-P and NH_2OH-N in samples were determined. The PO_4^{3-}-P concentration was determined according to the standard method (APHA 1998). Based on the determined PO_4^{3-}-P

Table 6 Determined NH_2OH-N concentrations of samples containing different PO_4^{3-}-P concentrations

NH_2OH-N concentrations in samples (mg/L)	PO_4^{3-}-P concentrations in samples (mg/L)	The determined NH_2OH-N concentrations (mg/L) ($n = 3$)	The recoveries of NH_2OH-N (%)
0.05	1.00	0.043 ± 0.0023	86.48
	1.50	0.041 ± 0.0017	81.47
	2.00	0.038 ± 0.0026	76.46
	2.50	0.036 ± 0.0031	72.45
	3.00	0.035 ± 0.0031	69.44
	3.50	0.033 ± 0.0023	65.43
	4.00	0.032 ± 0.0026	64.43
	5.00	0.032 ± 0.0038	63.42
	6.00	0.029 ± 0.0040	58.41
	7.00	0.028 ± 0.0030	55.40
	8.00	0.024 ± 0.0017	47.38
	9.00	0.023 ± 0.0023	45.38
	10.00	0.021 ± 0.0035	42.37
0.10	1.00	0.095 ± 0.0000	95.38
	1.50	0.093 ± 0.0009	93.37
	2.00	0.088 ± 0.0000	87.86
	2.50	0.086 ± 0.0009	85.85
	3.00	0.083 ± 0.0015	83.35
	3.50	0.080 ± 0.0000	80.34
	4.00	0.078 ± 0.0009	78.33
	5.00	0.077 ± 0.0011	77.36
	6.00	0.076 ± 0.0046	76.33
	7.00	0.073 ± 0.0063	73.32
	8.00	0.068 ± 0.0031	67.81
	9.00	0.067 ± 0.0040	66.80
	10.00	0.064 ± 0.0052	63.80

concentration, the normalized recovery of NH_2OH-N can be ascertained via Eq. (7); (2) the normalized recovery was revised by multiplying the determined PO_4^{3-}-P concentration; (3) the determined NH_2OH-N concentration was divided by the normalized recovery in sub-step (2). As a result, the NH_2OH-N concentration in sample can be determined eventually.

Verification of the correction method

In order to verify the availability of the correction method proposed in this study, two conditions were verified. The first condition was samples contained PO_4^{3-}-P except for NH_2OH-N, and the second condition was samples contained PO_4^{3-}-P, NO_2^--N and NH_2OH-N. The determined NH_2OH-N concentrations in samples before and after correction are shown in Table 7.

Fig. 3 Determined NH_2OH-N concentrations in samples containing different PO_4^{3-}-P concentrations

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Table 7 Determined NH_2OH-N concentrations in samples before and after the correction

NH_2OH-N concentrations in samples (mg/L)	PO_4^{3-}-P concentrations in samples (mg/L)	NO_2^--N concentrations in samples (mg/L)	The determined concentrations of NH_2OH-N (mg/L)	The recovery before the correction (%)	The correction concentrations of NH_2OH-N (mg/L)	The recovery after correction (%)
0.06	1.40	–	0.055	91.67	0.064	105.95
	2.40	–	0.050	83.33	0.063	105.64
	3.40	–	0.046	76.67	0.062	103.18
	3.80	–	0.046	76.67	0.063	105.16
0.08	1.40	–	0.079	98.75	0.091	114.13
	2.40	–	0.071	88.75	0.090	112.51
	3.40	–	0.065	81.25	0.087	109.34
	3.80	–	0.065	81.25	0.089	111.45
0.05	3.00	2.00	0.040	80.00	0.053	105.37
	3.00	5.00	0.035	70.00	0.046	92.20
	3.00	10.00	0.038	76.00	0.050	100.11
	3.00	15.00	0.032	64.00	0.042	84.30
0.05	4.00	2.00	0.035	70.00	0.048	96.87
	4.00	5.00	0.029	58.00	0.040	80.26
	4.00	10.00	0.029	58.00	0.040	80.26
	4.00	15.00	0.028	56.00	0.039	77.49
0.10	3.00	2.00	0.097	97.00	0.128	127.77
	3.00	5.00	0.094	94.00	0.124	123.82
	3.00	10.00	0.089	89.00	0.117	117.23
	3.00	15.00	0.082	82.00	0.108	108.01
0.10	4.00	2.00	0.094	94.00	0.130	130.08
	4.00	5.00	0.086	86.00	0.119	119.01
	4.00	10.00	0.083	83.00	0.115	114.86
	4.00	15.00	0.077	77.00	0.107	106.55

When samples only contained PO_4^{3-}-P except for NH_2OH-N, the numerical correction for the results obtained by the spectrophotometric method was very effective. The accuracy of the spectrophotometric method was improved obviously after the numerical correction. When the NH_2OH-N concentration in the sample was 0.06 mg/L and the PO_4^{3-}-P concentrations varied in the range of 1.40–3.80 mg/L, the determined NH_2OH-N concentrations were in the range of 0.046–0.055 mg/L, and the recoveries were in the range of 76.67–91.67%. After correction by the numerical method, the determined NH_2OH-N concentrations were in the range of 0.062–0.064 mg/L, and the recoveries were in the range of 103.18–105.95%. The similar results were achieved when the concentration of NH_2OH-N in the samples was 0.08 mg/L. However, when PO_4^{3-} and NO_2^- simultaneously existed in the samples containing NH_2OH-N, the situation was more complicated.

When the NH_2OH-N concentration was 0.10 mg/L, and the PO_4^{3-}-P concentrations were 3.00 and 4.00 mg/L, respectively, the recoveries of NH_2OH-N obtained by the spectrophotometric method were higher than 83.00% when the concentration of NO_2^--N in the sample was lower than 10.00 mg/L. After correction by the numerical method, the recoveries were improved to an extremely high level, and unfortunately, the errors of the spectrophotometric method were also improved. Under these situations, the spectrophotometric method can be used to determine the concentration of NH_2OH-N directly.

When the NH_2OH-N and PO_4^{3-}-P concentrations were 0.05 mg/L and 3.00 mg/L, respectively, the recoveries of NH_2OH-N obtained by the spectrophotometric method were in the range of 64.00–80.00% when the NO_2^--N concentrations varied in the range of 2.00–15.00 mg/L. After correction by the numerical method, the recoveries of NH_2OH-N were improved to 84.30–105.37%. When the NH_2OH-N and PO_4^{3-}-P concentrations were 0.05 mg/L and 4.00 mg/L, respectively, the recoveries of NH_2OH-N obtained by the spectrophotometric method were in the range of 56.00–70.00% when the NO_2^--N concentrations varied in the range of 2.00–15.00 mg/L. After correction by the numerical method, the recoveries of NH_2OH-N were

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improved to 77.49–96.87%. Under these conditions, the recoveries of NH_2OH-N obtained by the spectrophotometric method were improved obviously by the numerical method proposed in this study.

Compared with the gas chromatography method (Gebhardt et al. 2004; Kock and Bange 2013) and other spectrophotometric method for NH_2OH determination (Kolasa and Wardencki 1974; Dias et al. 1979), the method proposed in this study was suitable for determining milligram amounts of NH_2OH-N in aqueous solution. There was a linear relation between the concentrations of NH_2OH-N and the absorbance, while the concentration of NH_2OH-N was in the range of 0.02–1.00 mg/L. When NO_2^- and PO_4^{3-} presented in samples, the linear range varied to 0.05–1.00 mg/L. The side reactions of dissolved NO_2^- can result in significant errors in the NH_2OH determination by the gas chromatography method (Kock and Bange 2013). However, when NO_2^--N concentration was lower than 15 mg/L, the spectrophotometric method can obtain relatively accurate results. The presence of NO_2^- did not cause significant errors for the NH_2OH determination. The presence of PO_4^{3-} can result in a significant error in the NH_2OH determination when the NH_2OH-N concentration was lower than 0.10 mg/L. The numerical method proposed in this study can improve the accuracy of the spectrophotometric method significantly.

However, each method had its applicable scope, and the spectrophotometric method was no exception. If the concentration of NH_2OH-N in samples was extremely low, and the concentrations of NO_2^--N and PO_4^{3-}-P in samples were high, the spectrophotometric method was probably not a good choice for the determination of NH_2OH-N. It was noteworthy that if the concentration of NH_2OH-N in samples was high enough (e.g., 0.4 mg/L), NO_2^- and PO_4^{3-} did not interfere with the spectrophotometric method.

Impacts of other matters on the spectrophotometric method

The impacts of NO_3^-, C_a^{2+} and trace elements are shown in Table 1 and Table 2 in Supplementary Materials.

When the NO_3^--N concentration varied in the range of 6.00–24.00 mg/L, the recovery of NH_2OH-N under four conditions was in the range of 104.40–111.85%. When the C_a^{2+} concentration varied in the range of 2.00–8.00 mg/L and volume of the standard solution of trace elements added to the samples varied in the range of 1.00–5.00 mL, the recoveries of NH_2OH-N under the four conditions were in the range of 98.31–112.55%. NO_3^-, C_a^{2+} and trace elements did not interfere with the determination of NH_2OH-N by the spectrophotometric method.

Conclusion

The spectrophotometric method taking ferric ammonium sulfate and 1, 10-phenanthroline as the oxidant and the chromogenic agent, respectively, can be used to determine the NH_2OH-N concentration in biological wastewater treatment processes quickly and conveniently.

1. When the concentrations of NH_2OH-N in the samples were higher than 0.05 mg/L, the spectrophotometric method can give relatively accurate results when NO_2^--N concentration was lower than 15.00 mg/L.
2. NO_3^-, C_a^{2+} and trace elements did not interfere with the method.
3. The impact of PO_4^{3-} on the method was complicated. The interference of PO_4^{3-} on the method can be eliminated by the numerical method proposed in this study.

The determination method proposed was helpful to understand the quantitative relationship between NH_2OH and N_2O emission and reveal the function of NH_2OH in N_2O production in biological wastewater treatment processes.

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