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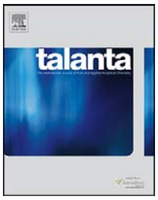

Ammonium detection by formation of colored zebra-bands in a detecting tube

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ABSTRACT

Ammonium ion was colorized by means of a diazo coupling reaction with 2-phenylphenol, where the color development reaction was conducted within 3 min by using boric acid as a catalyst. The resulting colored solution (0.5 ml) was supplied by suction to a detecting tube consisting of a nonwoven fabric test strip (2 mm wide, 1 mm thick, 150 mm long) impregnated with benzylcetyldimethylammonium chloride in a stripe pattern and enclosed in a heat-shrinkable tube. When the colored solution was supplied to the detecting tube, blue zebra-bands formed, and the ammonium concentration was determined by counting the number of zebra-bands. The detection range was 1–20 mg-N l^{-1}. Ammonium ion in actual domestic wastewater samples was successfully detected by means of this method.

1. Introduction

Various biological processes for the removal of nitrogen have been developed and employed widely for wastewater treatment to control eutrophication in closed bodies of water. These processes involve both aerobic nitrification and anoxic denitrification in various biological reactors [1–5]. Recently, the anaerobic ammonium oxidation (ANAMOX) process was developed as an alternative method for nitrogen removal [6–8].

Because processes for nitrogen removal require careful control of the reactor conditions, dissolved oxygen, pH, and redox potential must be monitored [8–10]. In addition, the performance of the removal processes is evaluated by monitoring nitrogen species including ammonium, nitrite, nitrate, and organic nitrogen. An automated analytical system can be employed in large-scale wastewater treatment plants [11,12], and ammonium is commonly detected by means of absorptometry. Recently, alternative analytical methods for ammonium have been developed [13–15]. Portable detection kits are necessary for on-site monitoring in small-scale plants. Many kinds of specially designed test kits are available for determining ammonium ion, including portable spectrophotometers, ion-selective electrodes, and spot test kits involving visual colorimetry [16]. Visual determination methods, such as visual colorimetry and test strips, are inexpensive, but they are imprecise, owing to human error and interference from suspended solids (SS).

In previous works [17–19], we reported a detection method based on the length of color bands formed in a mini-column. For detection of ammonium [19], we used a detecting tube packed with a mixture of poly(vinyl chloride) particles coated with a quaternary ammonium salt and hydrothalcite. This method had the following weaknesses: the color development reaction took 30 min, and the detection range was narrow (1–10 mg-NH_4^+-N). In addition, uniform packing of the detecting tube was important because two kinds of powdered packing materials were used. In this study, we attempted to improve on our previously developed method.

A spot test detection method must meet the following criteria: (1) rapid detection, (2) relatively hazardless reagents, (3) easy operation, (4) accuracy, (5) low potential for human error, and (6) low cost. For example, the indophenol method is commonly used [12] for ammonium detection, but the sodium nitroprusside used as a catalyst is undesirable because it is hazardous. Manganese sulfate [20] and acetone [21] are also used as alternative catalysts. Boric acid has also been examined as an alternative catalyst [22], and it is less hazardous than sodium nitroprusside. However, with all these catalysts, the color development reaction takes 10–60 min.

In this study, we focused on accelerating the color development reaction and on developing the alternative detecting tube to enable easy visual determination of ammonium. Ammonium ion was colorized by means of a diazo coupling reaction with various phenols in the presence of boric acid, and the colored solution was introduced into a detecting tube impregnated with benzylcetyldimethylammonium chloride in a stripe pattern and enclosed in a heat-shrinkable tube. Colored zebra-bands were formed in the

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detecting tube, and the ammonium concentration was determined by counting the zebra-bands. Where practical, solid reagents were used to facilitate on-site monitoring. The developed detecting tube was tested with actual wastewater samples.

2. Materials and methods

2.1. Reagents

Ammonium standard solutions were prepared from NH_4Cl at a concentration range of 0–50 mg-N l^{-1}. Powdered boric acid (Kishida Chemical Co., Osaka, Japan) was used as a catalyst for the diazo coupling reaction. Dichloroisocyanuric acid sodium salt (DCI; Tokyo Chemical Industry Co., Tokyo, Japan) was used as the chlorination reagent. An alkaline DCI solution (0.34 g of DCI in 100 ml of 2.5N NaOH) was used to study the conditions for the color development reaction. For the detection of ammonium with the detecting tube, a 1:4 DCI/NaCl mixture was used as the reagent.

We tested the following phenol coupling reagents to optimize adsorption of the colored compounds on a test strip coated with benzylcetyldimethylammonium chloride (BCDMA): phenol, *m*-cresol, salicylic acid, 3-ethylphenol, 1-naphthol, 2-isopropylphenol, 2-phenylphenol, 2-benzylphenol, and thymol (Tokyo Chemical Industry Co., Tokyo, Japan); the phenols were used as 20% (w/v) solutions in acetone/ethanol (15:85). 2-Phenylphenol showed the best performance, so for the detection of ammonia with the detecting tube, powdered 2-phenylphenol was added to the sample solution, and 1N NaOH was used as the alkaline reagent.

2.2. Effects of reagent dosages on the color formation reaction

The effects of the reagent dosages on the color formation reaction were generally examined under the following conditions: 10 ml of 5 and 50 mg-N l^{-1} standard solutions of ammonium, 0.1 g of boric acid, 0.1 g of 2-phenylphenol, 20 mg of DCI, and 5 ml of 1N NaOH. The dosage ranges for the reagents were as follows: boric acid (0.01–0.2 g), 2-phenylphenol (0.01–0.3 g), DCI (5–50 mg), and 1N NaOH (2–5 ml).

Boric acid, 2-phenylphenol, DCI and 1N NaOH were added to the sample solution in that order, and the reaction was allowed to proceed at room temperature (20–25 °C). The effects of reagent dosage were evaluated in terms of the absorbance at 670 nm (V-530 UV–vis spectrophotometer; Jasco Co., Tokyo, Japan). When the 50 mg-N l^{-1} standard solution was used, the absorbance of the final colored solution was measured after 10-fold dilution with distilled/ion-exchanged water.

We also examined the effects of the following metal ions on the color development using a 5 mg-N l^{-1} standard solution: Fe^{2+}, Fe^{3+}, Cu^{2+}, Mg^{2+}, Zn^{2+} and Ca^{2+}. The effects were evaluated on the basis of absorbance.

2.3. Construction of the detecting tube

Nonwoven fabric (Univeks SB M1: Unitika, Osaka, Japan) was used as the support for the quaternary ammonium salt. A nonwoven fabric sheet (150 mm long 50 mm wide, 1 mm thick) was covered with strips of masking tape (5 mm wide; 720, Nitto Denko Co., Osaka, Japan) such that there was a 2-mm gap between each strip. The fabric sheet was submerged in a 1-butanol solution of BCDMA, in which the BCDMA concentration was ranged from 0.2 to 1.0% (w/v). The 1-butanol in the nonwoven fabric sheet was allowed to evaporate at room temperature for 8 h. During the drying process, the BCDMA solution in the regions covered by the tape strips moved to the gaps between the strips, where BCDMA then accumulated as the 1-butanol evaporated. As a result, the sheet was impregnated with BCDMA in a striped pattern. Although other solvents such as methanol and ethanol were also examined to prepared BCDMA solution, the clearest colored zebra-bands were obtained with the test strip made from 1-butanol solution.

The tape strips were removed from the sheet, and the sheet was cut into 2-mm-wide strips. Each strip (150 mm long, 2 mm wide) was inserted into a transparent heat-shrinkable tube (2.6 mm i.d., 170 mm long; SUMITUBE-A, Sumitomo Electric Fine Polymer Inc., Osaka, Japan), and the tube was then heated in an oven at 130 °C for 5 min to obtain a detecting tube.

Fabric test strips (5 mm wide; 80 mm long) impregnated uniformly with BCDMA were also prepared from a 0.2% (w/v) solution of BCDMA in 1-butanol. The test strips were used without coverage with the heat-shrinkable tube.

2.4. Detection of ammonium with the detecting tube

Boric acid (0.1 g), 2-phenylphenol (0.1 g), and the DCI/NaCl reagent (0.1 g) were added to 10 ml of sample solution in that order, and the solution was shaken for 1.5 min. Finally, 5 ml of 1N NaOH was added, and the solution was shaken vigorously. Color development was complete within 3 min at ambient temperature (20–25 °C). Each solid reagent was added by a mini-cup made of plastic for each reagent, respectively. When the colored solutions were prepared from the 5 mg-N l^{-1} standard solution, the relative standard deviation of the absorbance was 6.07% ($N = 5$).

The colored solution was introduced into the detecting tube by suction with a disposable syringe (pulled up to 0.5 ml on the syringe scale). Introduction of the solution into the tube was complete within 40 s, and cored zebra-bands were formed immediately.

2.5. Analysis of domestic wastewater samples

Wastewater samples were obtained from the domestic wastewater treatment facility at our facility; raw wastewater and final effluent were sampled twice a day for a week. Unfiltered samples and samples filtered through a membrane filter (pore size 0.2 μm) were used to test the detecting tube method. The filtered samples were also analyzed by absorptometry according to the US standard method [12].

3. Results and discussion

3.1. Coupling reagents

The retention of the diazo-compound derived from ammonium on BCDMA is an important factor in the success of the detecting tube method. Nine phenol compounds were examined, and 20% (w/v) solutions of the phenols in acetone/ethanol (15/85) were used. Alkaline DCI solution (1 ml) and 1 ml of phenol solution were added to 10 ml of a standard solution (10 mg-N l^{-1}). In this case, boric acid was not used, because acetone acted as a catalyst. The colored solution was introduced to the test strip (80 mm long, 5 mm wide) impregnated uniformly with BCDMA by means of capillary penetration.

In the cases of 3-ethylphenol, 1-naphthol, 2-isopropylphenol, 2-phenylphenol, 2-benzylphenol, and thymol, the colored compounds were retained on the test strip and formed color bands. The colored compound derived from thymol was strongly retained, whereas the compounds derived from alkylphenols and 1-naphthol were only weakly retained. These results indicated that hydrophobic phenol compounds were desirable for color band formation, that is, compounds with an *n*-octanol/water partition coefficient ($\log P$) >2.5. 2-Phenylphenol was the best coupling reagent for color band formation.

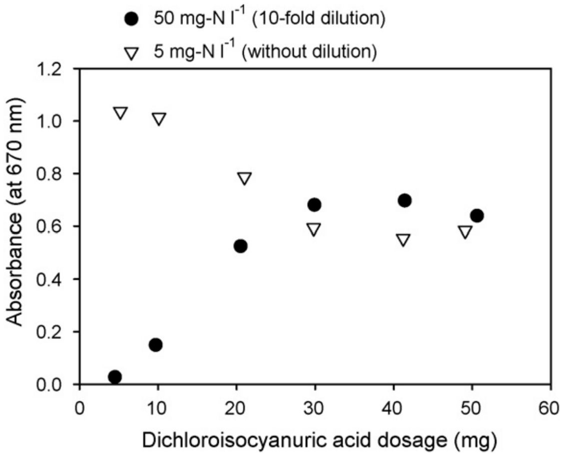

Fig. 1. Effect of dosage of dichloroisocyanuric acid on color intensity.

3.2. *Reagent dosages*

The color development reaction is strongly influenced by reaction conditions, such as chlorine concentration, pH, temperature, and so on [23], and various reaction conditions have been used for ammonium detection [24]. In this work, we optimized the reagent dosages for use with the detecting tube method.

The dosage >0.1 g of boric acid had no influence, but a dosage <0.1 g gave somewhat lower absorbance. The absorbance increased with increasing 2-phenylphenol dosage at dosages <0.2 g (data not shown). Because 2-phenylphenol did not dissolve completely in 10 ml of sample solution when the amount was >0.1 g, 0.1 g of 2-phenylphenol was used for the following experiments.

The effects of DCI dosage on absorbance are shown in Fig. 1. Opposite effects were observed for the 5 and 50 mg-N l^{-1} standard solutions. In the case of the 5 mg-N l^{-1} standard solution, excess DCI may have promoted further chlorination of the ammonium ion, to form products such as dichloramine. Although stable absorbance was obtained at DCI dosages >30 mg, the absorbance was not correlated linearly with ammonium concentration. At a dosage of 20 mg, the absorbance was correlated linearly with ammonium concentration in the range of 1–5 mg l^{-1}. Therefore, 0.1 g of a combined DCI/NaCl reagent (20% DCI) was used in the tests of the detecting tube method. The solution containing the added DCI had to be shaken for more than 1 min before addition of 1N NaOH in order to obtain stable color development.

The effects of the volume of added 1N NaOH on color development and reaction time were examined, and the results for the 50 mg-N l^{-1} standard solution are shown in Fig. 2. The absorbance was influenced by a dilution effect due to the added volume. When 5 ml of 1N NaOH was added, the color development reaction was complete within 3 min. As shown in Fig. 2, the sensitivity of detection was lower (by a factor of ~1/5) than that of standard methods [12,20,21]. However, the lower detection limit is not a disadvantage for a spot test for monitoring of wastewater.

On the basis of the above results, we chose the conditions shown in Fig. 3 to test the detecting tube method.

The effects of co-existing metal ions are shown in Table 1. Almost metal ions showed similar effects to those reported previously [19], but the effect of Cu^{2+} on absorbance decreased: from −42% to −12.6% of error. However, the disturbance with Cu^{2+} was not suppressed by the addition of EDTA.

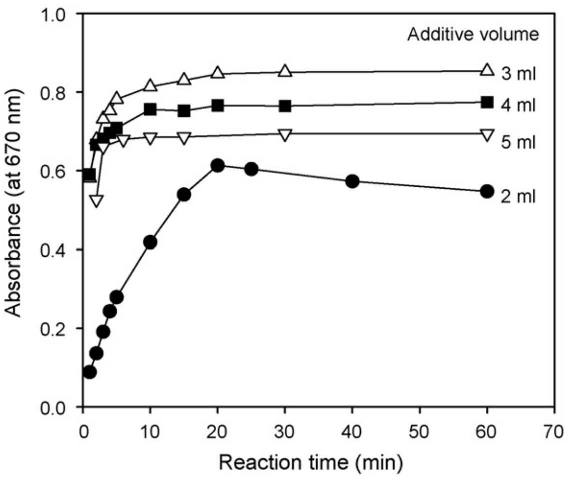

Fig. 2. Effect of additive volume of 1N NaOH solution on reaction time (NH$_4$$^+$-N: 50 mg-N l^{-1}; measurement after 10-fold dilution).

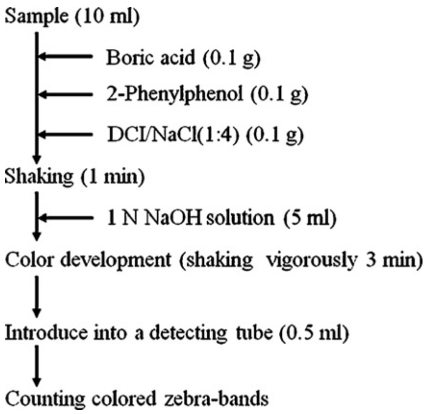

Fig. 3. Detection procedure by the detecting tube.

3.3. *Preparation of the test strip and detection by number of zebra-bands*

The effect of the BCDMA concentration in the 1-butanol solution on formation of colored zebra-bands was examined in the range of 0.2–1.0% (w/v). The colored solution prepared with standard solutions of 1–50 mg-N l^{-1} was introduced into the detecting tubes containing the test strips. Although colored zebra-bands formed in all the detecting tubes, the tube made from 0.8% (w/v) BCDMA solution gave a linear correlation between the number of zebra-bands (NZB) and the logarithm of the ammonium concentration in the range of 1–20 mg-N l^{-1} (Figs. 4 and 5). This result may have been due to multilayer adsorption of the colored compound on BCDMA in the detecting tube.

Table 1
Effects of co-existing metal ions.

Metal ion	Concentration (mg l^{-1})	Error (%)
Fe^{2+}	19.9	−7.1
Fe^{3+}	19.9	−2.3
Cu^{2+}	5.0	−12.6
Mg^{2+}	8.7	7.0
Zn^{2+}	5.0	0.9
Ca^{2+}	40.0	−2.5

NH$_4$$^+$-N: 5 mg-N l^{-1}.

Fig. 4. Zebra-bands formed in detecting tubes.

Five parallel detection experiments conducted with the detecting tube were carried out for each standard solution, and NZB in the detecting tube was counted by five people. The standard deviations (S.D.) of the NZB were smaller than 0.6, except for the 50 mg-N l^{-1} solution (S.D. = 0.67). The S.D. values are also plotted in Fig. 5. Ammonium in the concentration range of 1–10 mg-N l^{-1} was accurately detected. The upper limit of detection was 20 mg-N l^{-1}, although the accuracy was lower at this concentration, because increase of one NZB corresponded to increase of 5–10 mg-N l^{-1} at around 20 mg-N l^{-1}. The range was wider than that of the previously reported detecting tube, which involved measurement of the color band length [19]. In addition, because counting the NZB does not require any tools, ammonium detection is easier with this new method.

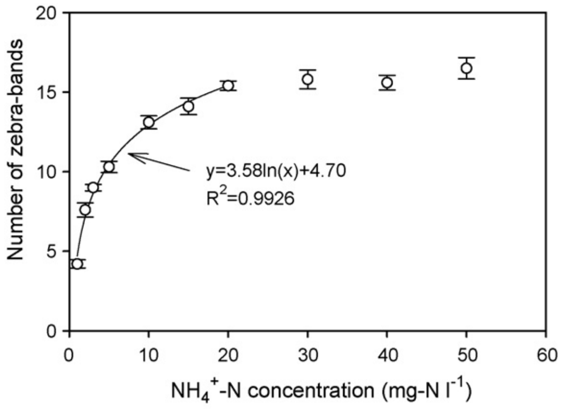

Fig. 5. Calibration curve for NH_4^+-N by the detection tube. Symbol: average; error bar: standard deviation.

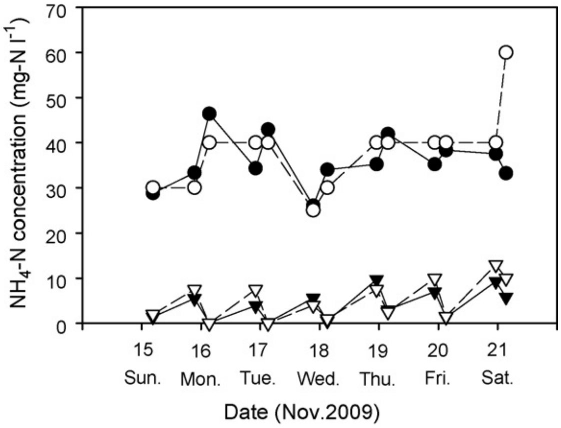

Fig. 6. Detection of NH_4^+-N in actual wastewater samples. Circle: raw wastewater (10-fold dilution); triangle: treated effluent (without dilution). Open symbol: detecting tube method; solid symbol: standard method.

3.4. Detection of ammonium in actual wastewater samples

The ammonium concentrations in actual domestic wastewater samples obtained at our facility were detected both by the detecting tube method and by absorptometry (US standard method). The raw wastewater samples contained 60–148 mg l^{-1} of suspended solids (SS) and 65.8–99.2 mg l^{-1} of dissolved chemical oxygen demand (D-COD). These raw wastewater samples were used for the detection method after 10-fold dilution with distilled/ion-exchanged water. The effluent samples contained 4.4–19.0 mg l^{-1} of SS and 0.3–22.0 mg l^{-1} of D-COD and were used without dilution.

The results for unfiltered samples are shown in Fig. 6, and the correlation factors between the results obtained with the detection tube and those obtained by absorptometry are also described. Although the detecting tube method gave only discrete value of ammonium concentration, the results corresponded well to those obtained by absorptometry. The results for the filtered samples and the supernatants were almost the same. Therefore, we concluded that wastewater samples could be analyzed without filtration by this method, although raw wastewater samples required dilution. Because of the ease of counting the NZB, the detecting tube method may be useful for on-site monitoring of ammonium in biological wastewater treatment processes.

Comparing with the detecting tube method described in our previous work [19], the proposed method in this work has the advantage of rapid color development (ca. 3 min). This was enabled by using boric acid as a catalyst, which is a less harmful reagent than nitroprusside, and boric acid was also an effective catalyst to synthesize indophenol and indonaphthol. Considering that the color development is the most time consuming process in common ammonium detection, the proposed method may be useful for absorptometry. In addition, the following advantages can be also pointed out: (1) the detecting tube to form zebra-bands was prepared more stably than the previous detecting tube in which two kinds of particulate adsorbents were packed, (2) the counting of NZB was conducted more easily than measurement of color band length, and (3) the rapid and wide range detection was enabled by employing the suction method to supply the colored solution.

4. Conclusion

We developed a new type of detecting tube in which colored zebra-bands formed, and the ammonium concentration was determined by counting the number of zebra-bands. Reagents for rapid color development were also examined. Solid-phase reagents were used, except for 1N NaOH, and this may facilitate use of this method as a spot test. Raw domestic wastewater samples were successfully analyzed by this method without filtration, but a filtered sample or the supernatant of a sample is recommended for samples containing high concentrations of SS in order to prevent plugging of the detecting tube. This method may be useful for detection of other analytes.

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