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INTERFERENCES IN NITROGEN DETERMINATION METHODS
Part 3. Determination of Organic Nitrogen in The Presence of Nitrate
KERANCUAN DALAM BEBERAPA METODE PENETAPAN NITROGEN
Bagian 3. Penetapan Organik Nitrogen Pada Sampel Yang Mengandung Nitrate

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Summary

Dalam bagian ke-3 ini, penelitian diarahkan untuk mengetahui kemungkinan adanya pengaruh reaksi kimia yang tidak diinginkan dalam penetapan kadar nitrogen terhadap sampel yang mengandung organik N dan nitrate (NO_3), Penetapan NO_3 dilakukan dengan metode MgO, auto-analyzer dan Devarda. Penetapan organik N dilakukan dengan metode Kjeldahl dan total N dengan metode persulfat. Metode tersebut dilakukan untuk mengetahui perolehan kembali nitrogen dari beberapa sampel yang merupakan campuran senyawa organik N dengan NO_3 yang telah diketahui kadar nitrogennya.

Hasil penelitian menunjukkan perolehan kembali nitrogen yang lebih kecil atau lebih besar dari kandungan nitrogen yang sebenarnya. Hal tersebut menunjukkan kecenderungan kuat adanya pengaruh reaksi kimia yang tidak diharapkan. Perolehan kembali NO_3 dengan metode Devarda sangat rendah, hal tersebut disebabkan oleh beberapa kemungkinan yaitu menurunnya kapasitas reduksi NO_3 menjadi NO_2 dan kemudian menjadi NH_4^+ oleh karena pengaruh Cu_2^+, PO_4^{3-} atau Mg_2^+ yang digunakan dalam prosedur analisis tersebut. Selain itu diduga kehilangan NO_3 sudah terjadi sebelum analisis dilakukan, yaitu oleh karena reaksi NO_3 dengan senyawa organik menjadi N_2 gas dan dalam kasus tertentu menjadi organik N. Analisis NO_3 dengan metode auto-analyzer memberikan perolehan kembali NO_3 yang cukup tinggi.

Metode Kjeldahl memberikan perolehan kembali nitrogen yang rendah untuk senyawa yang mengandung amine dan sangat rendah untuk senyawa azo. Hal tersebut disebabkan karakteristik yang lebih dengan adanya NO_3 serta kondisi destilasi yang tinggi (400°C). Metode persulfat relati sangat tinggi dalam perolehan kembali nitrogen dibandingkan dengan metode Kjeldahl, namun dalam beberapa kasus memungkinkan perolehan kembali yang terlampau tinggi.

Reaksi nitrasi atau reaksi nitrat dengan phenol/naphtol terlihat jelas dengan perubahan status NO_3 menjadi organik N pada penetapan nitrogen dengan metode Kjeldahl dan persulfat untuk senyawa campuran α-naphtol dan 1-4-naphtoquinone dengan NO_3.

I. INTRODUCTION

The end product of the aerobic nitrification process, nitrate, is present in quite large amount in environmental samples and is linked with the problem of the eutrophication of surface water. It is known that in soils containing a lot of organic matter significant amount of NO_3 is withdrawn from the nitrification process (Boudot and Chone, 1985). At a much lesser extent, this is also the case for normal agricultural soils (Azhar *et al*, 1989). It is not known whether a similar reaction might take place between NO_3^- and typical constituents of soil organic matter. There are, however, indications pointing to the possibility of such a reaction: it is well established that organic compounds often interfere with colorimetric methods for the determination of NO_3^- (Norwitz, 1979), while it has been reported that nitrate was fixed by hydrolyzed lignin in a Chernozem soil.

In this paper the reactions between NO_3^- and organic compounds during incubation and analysis were investigated and the reliability of commonly used laboratory methods for the determination of N for samples containing those two components was assessed.

II. MATERIALS AND METHODS

A. *Methods of nitrogen determination*

Solution containing both organic compounds and NO_3^- were subjected to five N-determination procedures. Mineral N was determined by two steam distillation methods, i.e. the MgO and the Devarda method and one automated colorimetric determination, in which nitrate was first reduced to nitrate on a Cd-column (Norwitz,

1979). Two acid digestion methods were used for the determination of resp. organic and total N, i.e. a macro-Kjeldahl and the persulphate method.

B. *Reaction between NO_3^- and organic compounds*

Solutions buffered at pH 7.0 and pH 5.5 were prepared. KNO_3—N was added to a level of 50 mg l^{-1}. The solutions were shaken for three days on a rotary shaker, where after N was determined in threefold.

The % recovery of N as well as the standard deviations were calculated. For mineral N. the recovery was expressed as a % of the NO_3—N added, for Kjeldahl N as a % of both total and organic N and persulphate N as a % of total N.

III. RESULTS AND DISCUSSION

Table 1 gives the averages and the standard deviations of the % N- recovery for the various methods.

Only in two cases, i.e. for adenine (pH 5,5) and aminophenol (pH 7.0), a significant amount of NH_4^+—N appeared have been formed. For the reference compounds as such, no NH_4^+ was recovered upon Mg0 distillation. Therefore, this phenomenon cannot be contributed to the alkali-labile character of these compounds. The results of the analysis of NO_3^-—N by the different methods are realigned in Fig. 1 and 2. For the auto-analyzer method, the recovery of NO_3—N is ranged from 70% to 100% and no significant differences could be noticed between the two pH values. Diaminonaphthalene seemed to be an exception to this pattern. The recovery of NO_3^-—N is higher for the auto-analyzer method in all cases but one, i.e. diaminonaphthalene and NO_3^-.

The recovery of NO_3^- with the Devarda method in pure KNO_3 solution was over 90%. In a buffered solution containing both NO_3^- and organic compound, NO_3^-—N recovery by the Devarda method was much lower.

In all cases, the recovery was much lower for samples incubated at pH 5.5 than for samples incubated at pH 7.0. One factor explaining the lower N-recovery as compared to the reference may well be the PO_4^{3-} content of the buffer solution. It has been reported that the reduction of NO_3^- to NO_2^- by a Cd-Cu reduction column was 40% lower in the presence of 25 uM PO_4^{3-} (Olson, 1969). All though in the auto-analyzer method, NO_3^- is reduced on a Cd-column, the samples were 100 times diluted. Hence, the actual concentration of PO_4^{3-} in the samples was far below this value. For the Devarda method, the samples were undiluted. Since the Devarda alloy consists of 50% Cu, PO_4^{3-} might have interfered with the reduction of NO_3^-, Mg_2^+ also has been reported to hamper the reduction of NO_3^- during the Devarda procedure (Chausen *et al.*, 1980). It was hypothesized that in acid solution. Mg0

Table 1. Percentage recovery of nitrogen for the various methods.

Tabel 1. Persentase perolehan kembali nitrogen untuk metode yang berbeda.

Compounds (Senyawa)		% N recovered					
		Method					
		Mg0	AA	Devarda	Kjeldahl N-org	Kjeldahl N-tot	Persulphate
Adenine + NO_3	7.0	0.6	69.7	26.3	96.8	88.3	68.2*
		0.1	1.3	0.9	1.4	1.3	2.2**
	5.5	26.4	72.9	10.1	25.2	23.1	76.8
		5.5	0.4	2.5	4.5	4.1	2.0
Alanine + NO_3	7.0	3.2	90.8	73.4	94.4	71.6	118.6
		0.5	0.5	5.6	1.1	0.9	1.1
	5.5	1.1	90.2	22.1	94.1	71.3	104.1
		0.8	0.7	5.0	4.5	0.2	1.6
Amino phenol + NO_3	7.0	23.6	73.9	30.9	80.8	58.1	94.2
		1.2	0.3	4.0	11.2	8.0	1.7
	5.5	5.0	77.2	20.7	94.1	67.6	90.1
		0.9	1.0	6.9	1.6	1.1	1.8
Benzo-triazole + NO_3	7.0	0.0	96.6	71.0	33.3	25.9	45.6
		0.0	1.0	0.8	0.2	0.2	1.3
	5.5	0.0	95.0	22.9	33.9	26.4	36.8
		0.0	0.6	0.7	0.4	0.3	1.8
Diamino-naphthalene + NO_3	7.0	0.0	48.2	60.4	76.3	59.5	103.7
		0.0	34.2	4.8	1.3	1.0	11.4
	5.5	0.0	83.6	8.7	41.3	32.2	93.8
		0.0	0.9	3.5	4.3	3.4	3.1
Glucos-amine + NO_3	7.0	0.0	95.6	68.9	104.5	58.4	100.9
		0.0	0.3	0.5	9.4	5.3	1.6
	5.5	0.0	94.8	25.9	102.7	57.4	96.9
		0.0	0.7	4.8	2.4	1.3	1.7
Naphthol + NO_3	7.0	0.1	102.1	56.6	—	24.9	105.3
		0.1	0.6	13.9	—	4.7	2.3
	5.5	0.0	102.1	13.2	—	100.6	102.9
		0.0	2.2	2.8	—	18.9	4.3
Naphtha-quinone + NO_3	7.0	0.0	75.2	19.5	—	9.3	75.5
		0.0	2.1	2.1	—	2.5	8.0
	5.5	0.0	75.9	7.0	—	16.5	42.6
		0.0	1.6	0.5	—	2.8	1.4

Remarks *(keterangan)* : *) Averages *(Rata-rata)*
**) Standard deviations *(Simpangan baku)*

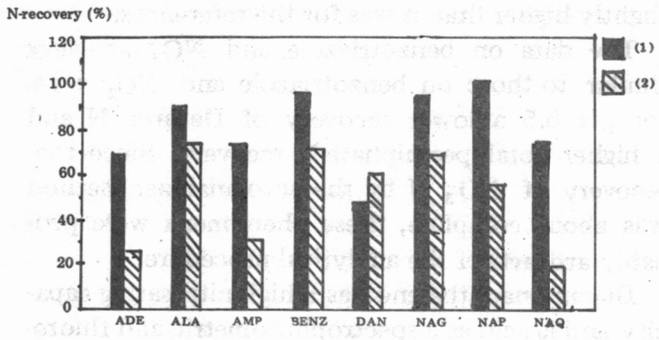

Figure 1. Sample mixtures of organic N and NO_3^- kept at pH 7.0 Recovery of NO_3^- by two different methods.

Gambar 1. Sampel yang mengandung organik N dan NO_3^- pada pH 7,0 Perolehan kembali NO_3^- dengan menggunakan dua metode yang berbeda.

(1) : Auto-analyzer
(2) : Devarda

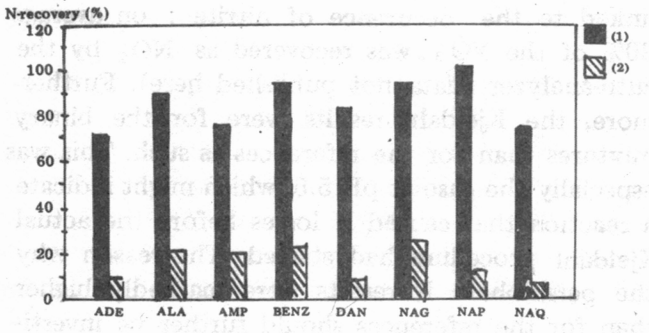

Figure 2. Sample mixtures of organic N and NO_3^- kept at pH 5.5 Recovery of NO_3^- by different methods.

Gambar 2. Sampel yang mengandung organik N dan NO_3^- pada pH 5,5 Perolehan kembali NO_3^- dengan menggunakan dua metode yang berbeda.

1) : Auto-analyzer
2) : Devarda

was partially dissolved, providing a quantity of Mg_2^+ in the solution, compromising the Devarda reduction alloy. MgO was added prior to the Devarda treatment, it was therefore possible that for the pH 5.5 buffer, the amount of MgO added was not enough to increase the pH sufficiently to prevent MgO dissolution.

The influence of the presence of NO_3^- on the recovery of total and organic N in illustrated by Fig. 3 and 4. Fig. 3 gives the Kjeldahl-N recovery rates for organic compounds in the presence of NO_3^- as well as for organic compounds as such. Three cases can be distinguished : (1). No marked influence of the presence of NO_3^- : alanine, aminophenol and benzotriazole; (2). Loss of organic N : adenine and diaminonaphthalene; (3). Increase of organic N : naphthol and naphthoquinone. The data on total Kjeldahl (Table 1) show that in one case, i.e. naphthol at pH 5.5, there was a complete reaction between NO_3^- and the organic compound: 100% of the mineral N was recovered as Kjeldahl N. When there was an influence of the NO_3^- presence, Fig 3 shows that it was most likely to occur in samples incubated at pH 5.5.

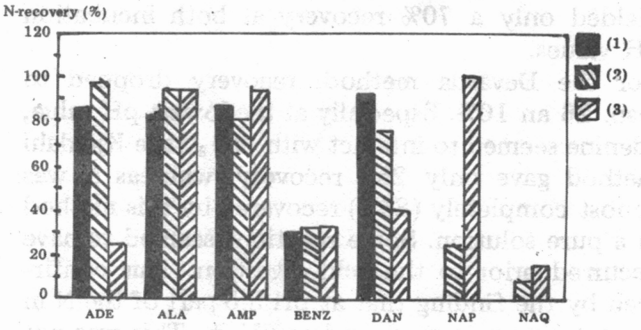

Figure 3. Determination of organic N by the Kjeldahl N method.

Gambar 3. Penetapan organik N dengan menggunakan metode Kjeldahl.

(1) : Organic compound as such
(2) : Organic compound Kept with NO_3^- at pH 7.0
(3) : Organic compound kept with NO_3^- at pH 5.5

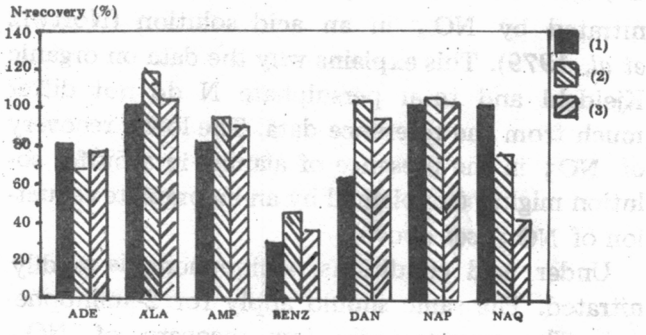

Figure 4. Determinations of total N by the persulphate method.

Gambar 4. Penetapan total N dengan menggunakan metode persulphate

(1) : Organic compound as such
(2) : Organic compound kept with NO_3^- at pH 7.0
(3) : Organic compound kept with NO_3^- at pH 5.5

Fig. 4 shows the persulphate N-recovery of the organic compounds in the presence NO_3^-, the combined N-recovery for NO_3^- and the organic compounds as determined separately. There was a clear loss of N for naphthoquinone, a clear increase of N for diaminonaphthalene and minor or no changes for the other compounds. The recovery of total N was deficient for adenine, benzotriazole, and naphthoquinone while it was about complete for the cases.

In the presence of adenine, NO_2^- was adequately recovered by the auto-analyzer method. In solutions containing adenine and NO_3^-, this method yielded only a 70% recovery at both incubation pH values.

For the Devarda method, recovery dropped to resp. 26 an 10%. Especially at the lowest pH value, adenine seemed to interact with NO_3^-: the Kjeldahl method gave only 25% recovery, whereas N was almost completely (86%) recovered by this method in a pure solution. Some reactions seemed to have occurred prior to the acid digestion. This confirmed by the finding that at pH 5.5 part of the N in the solution was recovered as NH_4^+. This was not merely an artifact of the MgO-method; in a separate set of analyses, NH_4 was also recovered by the auto-analyzer using indophenol blue method (data not published here). The reason for this phenomenon is not clear.

Except for the Devarda determinations, alanine nor NO_3^- seemed to affect to a great extent the resp. N determinations. Another amino acid, L-asparagine, was listed as a compound which was not readly nitrated by NO_3^- in an acid solution (Nortwiz *et al*, 1979). This explains why the data on organic Kjeldahl and total persulphate N do not differ much from the reference data. The lower recovery of NO_3^- in the presence of alanine in a buffer solution might be explained by an incomplete reduction of NO_3^- (see above).

Under acid conditions, aminophenol is readily nitrated. The same should apply for 2-aminophenol. The unexpectedly low recovery of NO_3^- (only 70%) by the auto-analyzer method could be due to this phenomenon. The fact the Kjeldahl recovery rates were not very much different from the data for the reference compound does not contradict this : the resulting product of an acid nitration is likely to be a nitrocompound, which is only partly recovered by the Kjeldahl method. Though for the persulphate method, recovery of amino-N and nitro-N is more or less the same, total N recovery by the persulphate method was slightly higher than it was for the references.

The data on benzotriazole and NO_3^- are very similar to those on benzotriazole and NO_2^- : i.e. for pH 5.5 a lower recovery of Devarda N and a higher total persulphate-N recovery. Since the recovery of NO_3^- N by the auto-analyzer method was about complete, these phenomena were probably artifacts of the analytical procedure.

Diaminonaphthalene has a high nitrosating capacity and is acid as a spectrophotometric and fluorometric reagent for the determination of NO_2^-. Even though there was no interference of NO_3^- on this method, it cannot be excluded that nitrosation takes place on another site of the molecule. Diaminonaphthalene indeed seemed to react with NO_3^- : the recovery of NO_3^- by the auto-analyzer method at pH 7.0 was rather poor (50%) on average, but had a large variability (34% S.D.). This was linked to the occurence of nitrite : on average 20% of the NO_3^- was recovered as NO_2^- by the auto-analyzer (data not published here). Furthermore, the Kjeldahl results were for the binary mixtures than for the references as such. This was especially the case at pH 5.5, which might indicate a reaction that caused N losses before the actual Kjeldahl procedure had started. The reason why the persulphate N results were markedly higher than for the references should further be investigated.

For N-acetylglucosamine, there was only a difference between the pH values for the Devarda method. Since no determination on NAG as such were carried out, the impact of the presence of the NO_3^- is difficult to assess.

Phenolic compounds are easily nitrated under acidic conditions (Norwitz, 1979). This reaction is actually used for the determination of NO_3^-. The end products of the nitration of naphthol are nitro- and dinitronaphtols. As the auto-analyzer data indicate, the nitration at pH 7.0 and 5.5 was either reversible or non-existent. The Kjeldahl procedure, however, recovered organic N, which was not initially present. At pH 5.5, even all NO_3^- N initially present was converted to organic N. Nitration seemed to have taken place during the acid digestion. As only a minor fraction of Nitro-N was recovered by the Kjeldahl method, it was unlikely that nitro compounds are the sole end product of this acid digestion catalyzed nitration.

Naphthoquinone too became nitrated, probably

also at neutral pH (only 75% recovery of NO_3^- N for the auto-analyzer method) and 10 to 15% of the NO_3^- N initially present was recovered as Kjeldahl -N. The reaction was probably linked to N-volatilization since there was a net-deficit of total persulphate N, which was more obvious at the lower pH.

IV. CONCLUSIONS

Determination of nitrogen in samples containing NO_3^- and organic compounds did not always give accurate results. The compounds reacted and interfered with one another before and during the N determination prcedures. This caused under or over estimation of the actual amount of N present in the samples. The following cases could be distinguished :

- Compounds containing an amino group were liable to decompose during the MgO treatment when NO_3^- as well was present in the samples;
- The auto-analyzer method gave the best recovery results for NO_3^-, the recovery with the Devarda method was much lower;
- Organic N in phenolic compounds was excessively recovered by the Kjeldahl method. Nitration is likely to have occured during the digestion.

REFERENCES *(PUSTAKA)*

Azhar, E.S., J. Vandenabeele and W. Verstraete. 1986. Nitrification mediated organic nitrogen formation in soils. Plant & Soil (94) : 383—389.

Boudot, J. and T. Chone. 1985. Internal nitrogen cycling in two humic rich acidic soils. Soil Biol. Biochem (17) : 135—142.

Clausen, C., B. Bock, G. Petersen and R. Olson. 1980. The effect of Mg on the reduction of NO_3^- in the determination of N by Devarda method. Soil Sci. Soe. Am. J. (44) 1326—1327.

Norwitz, G., J. Farrino and P. Kelider. 1979. Procedure of the determination of NO_3^- by colorimetric methods. Anal. Chem. Reta (105) : 335—351.

Olson, R. 1980. The effect of PO_4^- on the reduction of NO_3^- to NO_2^- by Cd-Cu column in the auto-analyzer method. Limnol. Oceanogr (14) : 799.