Comparison of various methods for the determination of total N in liquid effluents

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Abstract. A high temperature catalytic oxidation method (HTCO) was used to determine total dissolved nitrogen in different liquid effluents produced by agro-industry. This technique consists of catalysed combustion of the sample by direct injection in a furnace at 900 °C. Results obtained by HTCO were then compared with those determined by the Kjeldahl digestion method (TKN) and the dry combustion method (DCM) for freeze-dried effluents. The results obtained by HTCO within a concentration range of 10 to 800 mg N L^{-1}, were in good agreement with those obtained by TKN. Comparison with DCM method revealed large discrepancies which were probably due to the poor precision of this method and to the ammoniac-N losses occurring during the freeze-drying of samples.

Keywords. Total nitrogen – waste water – Kjeldahl method – dry combustion method – high temperature catalytic oxidation.

Introduction

In the North of France, large amounts of effluents produced by the agro-industries are spread on cultivated areas. The determination of total nitrogen in these effluents is essential to manage the amounts of organic N spread on fields and to respect the French or European regulations. Two groups of methods have been used for the determination of total nitrogen in liquids:

i) the wet chemical analysis methods. The most famous is the Kjeldahl digestion method (TKN) which has been used for a long time, with numerous variants, in which the $N-NH_4^+$ is determined by colorimetry or by titration [1,2]. The other method which is often used consists of persulfate oxidation in alkaline media under pressure at 120 °C followed by the determination of $N-NO_3^-$ by colorimetry [3,4,5]. One last, less frequently used method, which has given some good results, is the photo-oxidation UV method at 90 °C followed by colorimetric determination of the dissolved inorganic nitrogen [6, 3]. This method was shown by these authors to be valuable for marine research and gave better precision than the persulfate method.

ii) Oxidative combustion methods to determine total dissolved nitrogen. These have principally been used by the institutes of marine sciences for low ranges of concentrations (5 to 700 µg N L^{-1}), and in petroleum chemistry. Different variants have been used, one of which is the dry combustion method (DCM) which determines N in the freeze-dried sample using an elemental analyser based on the Dumas' method [7]. N is analysed by N_2 thermal conductivity or chemiluminescence detection to obtain a better

sensitivity [8]. Main interest of this method is to determine low N concentrations by concentrating N of the sample. In fact, N determination by direct injection of liquid sample in the furnace of an elemental analyser do not provide accurate results for very small N amounts.

The high temperature oxidation method (HTO) used by Drushel [9], Walsh [3] and Merriam *et al.* [5] seemed more suitably adapted to concentrations below 10 mg N L^{-1}, and in some cases gave better recoveries than the photo oxidation UV method for amino acids and urea [3]. However, HTO methods have tended to be replaced by the high temperature catalytic oxidation method (HTCO) tested by Kunkel and Gelderman [10], Hansell [11], Lopez-Veneroni and Cifuentes [12] and Merriam *et al.* [5]. These authors showed that recoveries were further improved, sensitivity was higher and the response curve was more linear than those obtained by HTO. At present, several kinds of apparatus are proposed for the measurement of dissolved nitrogen by HTCO using detection by chemiluminescence: DN 1900 (Dohrmann, Cincinnati USA), TOCOR 101 (Mahiac, Germany), MULTI N/C (Jena Analytic, Jena, Germany) and TN 3000 (Euroglas, Delf, The Netherlands) and others.

Until now, the reference method adopted in France and for environmental applications, has been the Kjeldahl method [13] for determination of the total nitrogen in liquids. Nevertheless, HTCO could provide a valuable alternative to TKN as it is more sensitive (1 to 10 ng N), faster (*i.e.* a determination requires 3-6 minutes compared to several hours with wet chemistry), and less hazardous (*i.e.* no handling of dangerous boiling sulphuric acid or environmentally unsafe catalyst).

Our objective was therefore to evaluate the HTCO method for a large panel of agro-industrial effluents by comparison with the reference method (TKN).

Materials and methods

Sample collection

The liquid effluents were collected directly from the large settling tanks of different agro-industrial companies in Northeast France (Tab. I). For each effuent, dry matter in suspension (DMS), pH, inorganic and total dissolved N were determined (Tab. I). The mineral nitrogen (NO_2^-, NO_3^- and NH_4^+) were measured by continuous flow colorimetry (TRAACS 2000, Bran & Luebbe, Norderstedt, Germany). The nitrate and nitrite were measured using an adaptation of the method proposed by Kamphake *et al*. [14] and the ammonium by a method derived from that of Krom [15]. Those samples which contained more than 1 g L^{-1} DMS were centrifuged for 15 min with a 7000 RPM speed, as N in the solid phase was not taken into account in the comparisons. Finally, all the samples were stored at –20 °C before analysis.

High temperature catalytic oxidation method (HTCO)

Total N determinations were performed using a TN 3000 apparatus (Euroglas, Delf, The Netherlands) consisting of a horizontal furnace separated into 2 temperature zones, a chemiluminescence N detector, a data recorder and an automated liquid sampler (Fig. 1). 50 µL samples were intro-
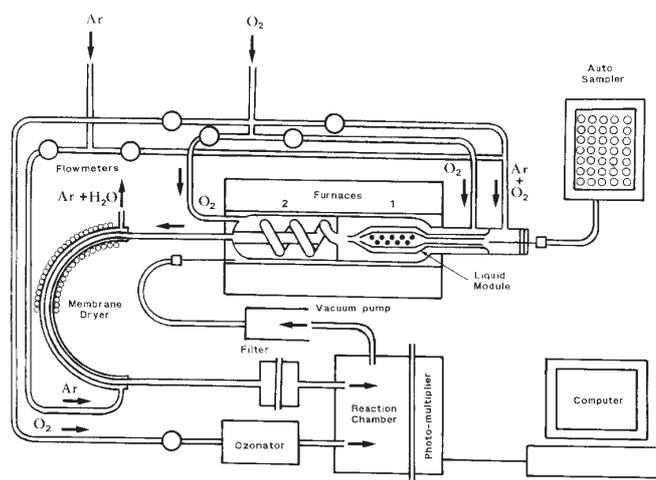

Figure 1. Schematic diagram of the total nitrogen analyser TN 3000 (redrawn from Euroglas Analytical Instruments).

duced by direct injection into a quartz liquid module (Fig. 2) inserted in the quartz combustion tube. The liquid module was packed with 11 g of catalyst (0.5 % Pt on alumina support) and a small amount of quartz wool was placed on top of the catalyst. The furnace temperature was kept at 900 ± 5 °C. Medical grade O_2 (99.6 %) was used for combustion (140 mL min^{-1}), ozone generation (180 mL min^{-1}), extra injection (55 mL min^{-1}), and technical grade Ar (99.998 %) was used as carrier gas (160 mL min^{-1}).

Table I. Origin of effluents and total N concentrations obtained by the different methods.

| Type of effluents | Origin | Sample code | DMS[1] g L^{-1} | pH | Inorganic N | | Total dissolved N mg N L^{-1} | | |
					NO_3^-	NH_4^+	HTCO	TKN	DCM
Liquid phase of sludge	Distillery (sugar beet)	B4	35.40	8.4	0.60	589	652	628	60.6
Washing and process water	Distillery (sugar beet)	E1	0.14	6.1	0.14	15.6	51.8	53.3	19.0
Process water	Glucose industry	E2	2.02	7.5	0.21	174	187	158	12.3
Process water	Alfalfa dehydration	E3	0.25	6.3	10.6	85.5	158	155	76.6
Process water	Alfalfa dehydration	E4	2.01	6.6	0.04	87.5	108	110	20.0
Settled water	Sugar industry (sugar beet)	E5	19.03	6.3	0.99	49.6	75.0	75.4	16.4
Liquid phase of muddy water	Sugar industry (sugar beet)	E6	362.9	6.1	0.11	0.0	17.8	22.9	10.5
Washing and process water	Distillery (sugar beet)	E7	0.26	6.7	3.39	13.3	64.1	67.3	50.8
Process water	Starch industry (potatoes)	E8	16.10	6.1	0.22	190	287	312	125
Diluted molasses	Potato starch industry	E9	331.0	5.1	11.6	30.3	501	540	539
Washing water	Sugar industry (sugar beet)	E10	48.63	5.8	5.50	23.1	68.1	67.3	59.6
Plonk	Distillery (sugar)	E11	1.42	3.2	16.9	1.60	452	448	499.1
Washing and process water	Distillery (sugar beet)	E12	11.19	3.3	37.7	8.62	700	715	813.4
Process water	Sugar industry (sugar beet)	E13	0.06	6.8	6.26	7.31	21.6	20.1	12.3
Process water	Sugar industry (sugar beet)	E14	2.78	4.6	4.45	14.1	33.8	35.7	38.5
Plonk without yeast	Distillery (sugar beet)	E15	1.17	3.4	28.3	19.4	492	513	588
Washing and process water	Distillery (fruits)	E16	10.97	3.4	0.45	25.0	289	272	330

[1] Dry Matter in Suspension
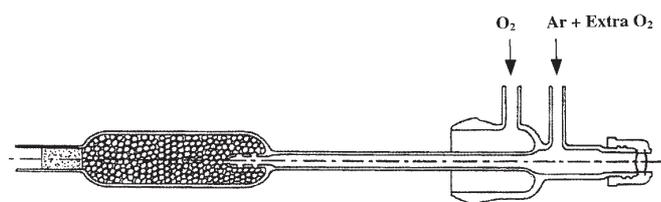
Figure 2. Direct injection liquid module used for effluent analysis (from Euroglas Analytical Instruments).

A vacuum pump was connected to the exit of the permeation tube to obtain a constant flow into the system.

$$\text{Organic N in the compound} \xrightarrow[900\,°C]{O_2} NO$$
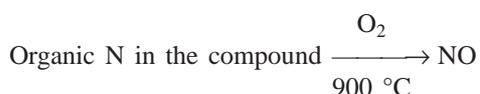
The dried NO gas was then mixed with ozone to form excited NO_2.

$$NO + O_3 \rightarrow NO_2{}^* + O_2$$
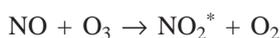
The light energy released during the return of the nitrogen dioxide to ground state was detected by a photomultiplier and was directly proportional to the mass of bound nitrogen in the sample.

$$NO_2{}^* \rightarrow NO_2 + hn$$

A mixture of 5 different standards in a range of 0 to 25 mg N L^{-1} was used to calibrate the apparatus: *i.e.* 30 % KNO_3, 20 % $(NH_4)_2SO_4$, 20 % $(NH_2)_2CO$, 15 % $C_6H_{13}NO_2$, (leucin) and 15 % C_8H_9NO (acetalinide). The concentrations of standards and samples were determined 3 times, each injection cycle taking 4 min. Samples in which the total N concentration was 25 mg N L^{-1} or more were diluted with deionised water.

Kjeldahl method (TKN)

Total nitrogen determinations (3 replicates) were performed using the modified Kjeldahl method described by Bremner and Shaw [1] and a 2012 digestion unit (Tecator, Höganäs, Sweden) equipped with a programmable controller. Samples of 5 to 30 mL, depending on the N concentration measured with the HTCO method, were introduced into the digestion tubes, to ensure sufficient sensitivity.

Before the digestion, nitrates present in the samples were reduced using the technique proposed by Guiraud and Fardeau [16] in which the nitrates were reduced for 48 hours at 120 °C in the presence of 5 M H_2SO_4 (addition of 1.4 or 8.3 mL of 96 % H_2SO_4 per sample) and reduced iron (Merck ref. 3819; approximately 0.7 g Fe per sample). For the

digestion, 14 mL or 7 mL of 96 % H_2SO_4 (depending on the H_2SO_4 volume added for $NO_3{}^-$ reduction) were introduced into the tubes with a $^1/_2$ Kjeldahl tablet (Merck ref. 15348). The digestion temperature was then slowly increased to avoid liquid projections (50 °C hour^{-1}) from 125 °C up to a final temperature of 375 °C which was maintained for 2 hours. Ammonium nitrogen present in the digests was then steam distilled after addition of excess 12 M NaOH [17]. The NH_3 formed was then trapped by 0.05 M H_2SO_4 and determined by back titration using a 719 titrator (Metrohm, Herisau, Switzerland).

N recovery was checked by comparison with the analyses of the standards mixture (0.5 g L^{-1}) together with blanks (no nitrogen) as previously described.

Dry combustion method (DCM)

9 mL of sample (3 replicates) were introduced into a 25 mL glass vial, together with 2 mL of 0.10 M Na_2SO_4 reagent (Merck ref. 106649). The vials were covered with a piece of Nylon material (500 µm mesh) and stored at –40 °C. 24 hours later, samples were freeze-dried for 36 hours in a Lyovac GT2 freeze-dryer (AMSCO/FINN-AQUA, Hürth Germany) and then stored in a desiccator until analysis.

Total N in solid residues was then determined with an NA-1500 instrument (Carlo-Erba, Milan, Italy) based on the method proposed by Dumas [7] and described by Bremner [17]. The organic compound is completely oxidized by flash combustion in presence of O_2 gas. Using He as carrier gas, the combustion products carried first in an oxydation furnace (Cr_2O_3, Co_2O_3) at 1020 °C and then in a reduction furnace (Cu) at 750 °C; N_2 and CO_2 formed are then separated in a chromatographic column and detected by a thermal conductivity detector. Standards and blanks were also considered and analysed with the previous procedure. For samples of salt, 20 mg maximum were analysed to avoid combustion problems. The standards were the same as before, except for the range of concentrations which was from 0.125 to 1.0 g L^{-1}.

Results and discussion

For the HTCO method, a better fit was obtained for the calibration curve (results not shown) using a quadratic rather than a linear adjustment (r^2 ranged between 0.9992 and 0.9996 for 6 standards × 3 replicates). The relative standard deviations (RSD) for the standards were commonly between 0.5 and 2 % (mean RSD = 1.2 %), except for the blanks (RSD > 15 %). The average RSD for effluent samples ranged from 0.7 to 11.1 % (average RSD = 3.2 %), the high values probably being due to the heterogeneity of the effluents. The detection limit was approximately 0.10 mg L^{-1} and was experimentaly determined with an injection volume of 100 µL of standards (concentrations between 0.05 and 0.5 mg N L^{-1}). On the other hand, a slight memory effect of

about 1 % could generally be measured when 2 blanks were analysed immediately after a 15 mg L^{-1} standard.

The average RSD for samples was 5.0 % for the TKN method, with a minimum and maximum of 0.5 and 20.1 % respectively, the high values being obtained for low N concentrations ($\approx$ 20 mg N L^{-1}).

Finally, the average RSD was 2.9 % for results obtained by DCM, with a minimum and maximum of 0.3 % and 11.5 % respectively. Concerning this method, the detection limit measured with the NA-1500 was near 1 µg N and the accuracy was at best 10 % for the low N quantities detected (10 to 20 µg N). In spite of the Na$_2$SO$_4$ purity reagent chosen, the N concentrations in the blanks were high (2.68 ± 0.51 mg L^{-1}). They were probably due to contaminations between the samples which occured during the freeze-drying. According to the calibration with standards (Fig. 3) the DCM method underestimated the concentrations by 16.3 % on average with a minimum and maximum of 11.1 and 20 %.

The results obtained with the HTCO and TKN methods were in good agreement (Fig. 4). Similarly, Kunkel *et al.* [10] who compared TKN and HTCO methods using pure chemicals or petroleum products found that their modified chemiluminescence method was at least as accurate as the Kjeldahl method, while the HTCO method was more efficient. Elsewhere, Merriam *et al.* [5] showed that HTCO method gave accurate results, for concentrations between 0.5 and 110 mg L^{-1} of total N in throughfall solutions, comparable to those obtained with the persulfate digestion method. Figure 5 indicates that the results obtained by HTCO and DCM methods were well correlated ($r = 0.8$, $p < 0.05$). Nevertheless, for some effluents (e.g. B4, E1, E2, E3, E4 and E5) very different concentrations were obtained by the two methods, with DCM severely under-estimating the N concentration. Loss of nitrogen may in fact occur during freeze-drying of the samples. This was confirmed by analysis of the water trapped in the condenser of the freeze-dryer which contained 37 mg N L^{-1}, mainly as ammonia. These losses were directly correlated ($r = 0.91$, $p < 0.01$) to the pH of the effluent (Fig. 6). The N concentrations in samples in which the pH was over 6, were under-estimated by DCM method probably because of losses of the ammonia contained in the effluents by volatilisation during freeze-drying. They were, in contrast, over-estimated in samples in which the pH was under 6, in which case, the ammonia present in the atmosphere of the freeze-dryer could be partially trapped by the acid samples. These results contradict the findings of Donald *et al.* [18] who showed that the recoveries by DCM were satisfactory (98 % on average) and indicated neither serious losses nor contamination for several compounds, except in the case of volatile organic compounds. Nevertheless, these authors showed that the estimated error for seawater samples could attain 20.8 % due to the great variation between replicates. In our study, the estimated error was comparable but neither explained by variation between replicate samples nor by variation between replicate analysis.
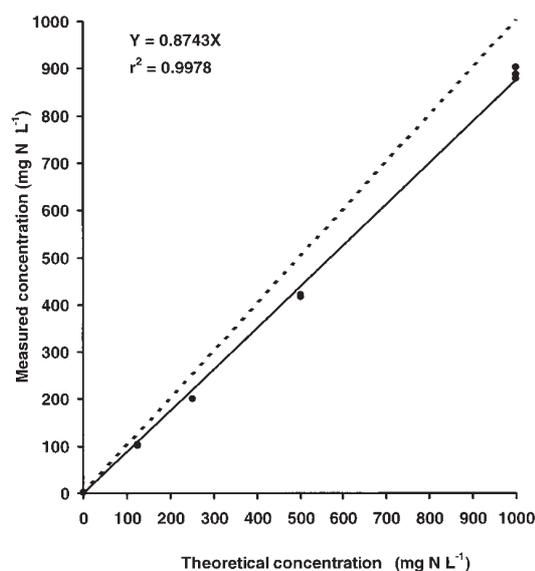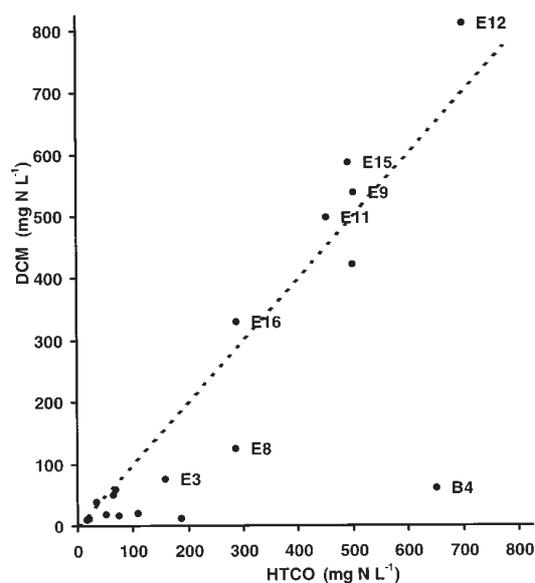

Figure 3. Comparison of theoretical total N with total N measured by DCM (broken line corresponds to $y = x$).
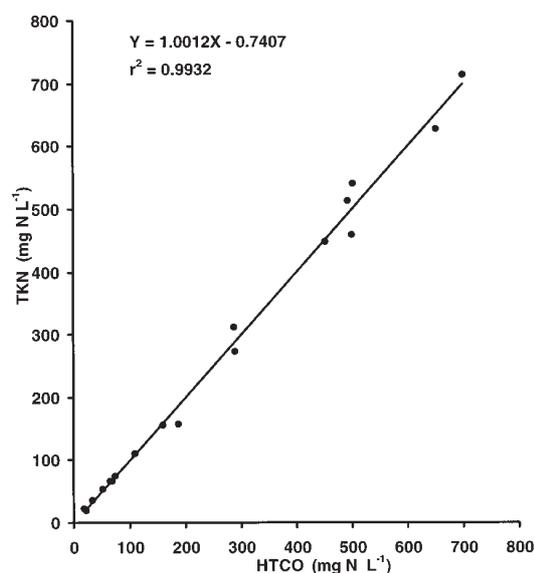

Figure 4. Comparison of total N measured by high temperature catalytic oxidation (HTCO) and Kjeldahl (TKN) methods.

Conclusion

This automated HTCO method for the determination of total nitrogen in effluent samples gave reproducible and accurate measurements applicable to a very high range of concentrations. It is a rapid and reliable analysis method and the results were well correlated with the reference Kjeldahl method. The dry combustion method (DCM) did not appear to be a reliable method for the determination of total N in

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Figure 5. Comparison of total N measured by high temperature catalytic oxidation (HTCO) and the dry combustion method (DCM) (broken line corresponds to $y = x$).
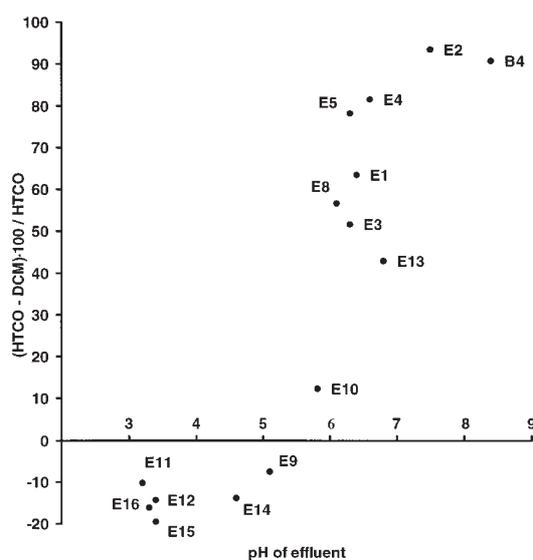
Figure 6. Relationship between the difference in concentration obtained with HTCO and DCM expressed as a % of the concentration measured by HTCO and effluent pH.

effluents, due to poor precision of the measurements and the losses or gains in ammonia occurring during freeze-drying. It will require further development or modification before it can be used for effluents containing N as ammonia.

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