nutriënten management instituut nmi bv

WAGENINGEN UNIVERSITY
AGROTECHNOLOGY AND
FOOD SCIENCES

onderzoek, advisering
en voorlichting over
bemesting, veevoeding
en bodemkwaliteit

OxiTop® measuring system[1] for standardised determination of the respiration rate and N-mineralisation rate of organic matter in waste material, compost and soil

Dr. A.H.M. Veeken, V. de Wilde and Dr. H.V.M. Hamelers, Department of Environmental Technology, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands

Dr. S.W. Moolenaar and R. Postma, MSc., Nutrient Management Institute (NMI BV), P.O. Box 250,
6700 AG Wageningen, The Netherlands

[1] OxiTop® measuring system is a product of WTW, Giessen, GERMANY

postbus 250
6700 ag wageningen
haagsteeg 2-b
6708 pm wageningen
tel. (0317) 46 77 00
fax (0317) 46 77 01
e-mail nmi@nmi-agro.nl
internet www.nmi-agro.nl

Introduction

Important agricultural parameters of soil improvers are the supply of organic matter and nutrients. Also in horticulture and other fields, these parameters are of importance. Practice asks for simple, quick and cheap methods to determine the stability and N-mineralisation in composts and soils. A measuring system and related methodology are proposed for standardised determination of the stability and N-mineralisation of organic matter in various environments such as wastes, composts and soils. Our aim is to develop a standardised method for stability measurements that cannot only be applied by advanced laboratories but should also be accessible for e.g. compost producers (to monitor and optimise their composting process) and end-users (to assess compost quality). The test should be performed within 1 week and the costs should be comparable to simple wet-chemical analysis.

In literature various definitions are given for stability (and maturity) of composts. We adopt the definition given by most researchers that stability is defined as the microbial degradation rate of the organic matter under aerobic conditions (Haug, 1993). A higher stability corresponds to a lower degradation rate. The microbial degradation of the organic matter ($C_aH_bO_cN_d$) can be represented by the following equation (ignoring stoichiometric coefficients):

$$C_aH_bO_cN_d + xO_2 + yNH_4^+ \rightarrow aCO_2 + zH_2O + \Delta H + X \qquad (1)$$

where ΔH is the heat production (in kJ) and X represents biomass. De stoichiometry of the reaction depends on the type of substrate. For a typical substrate as glucose ($C_6H_{12}O_6$) these coefficients are as follows:

- Oxygen consumption: 1.07 kg O_2/kg volatile solids (VS) substrate
- Carbon dioxide production: 1.47 kg CO_2/kg VS
- Water production: 0.6 kg H_2O/kg VS
- Heat production: 16 MJ/kg VS

Eq. 1 shows that the degradation rate (or stability) of organic matter can directly be determined from its oxygen uptake rate or respiration rate (OUR), carbon dioxide production rate (CPR) or heat production rate (HPR). By definition, readily degradable substrates are consumed first and more recalcitrant substrates are retained. This implies that the stability increases in time during composting and OUR, CPR and HPR decrease in time.

Various methods are commercially available for the determination of the respiration rate (or stability) that are based on the parameters (O_2, CO_2 and ΔH) discussed above. In this we can distinguish batch and continuous systems (Page et al., 1982). Most respirometric devices are expensive, complicated and require an advanced laboratory. Therefore these systems are not suitable for non-advanced users. As we aim at the development of a simple test method, a batch system was chosen. A simple device is the OxiTop® system that will be described in the next paragraph.

Another method that is a standard method in several European countries is Rottegrad (self heating test). However, the measured temperature is not directly related to ΔH as temperature is a result of heat

production and heat removal. Moreover, the Rottegrad test is performed in solid state for which the disadvantages are discussed in the next paragraph. Without going into details, it can be concluded that this method is not standardised and can frequently lead to wrong results and interpretation (Weppen, 2002). Moreover, the method is not discriminative at high stability levels.

Besides the measurement of the directly related parameters (O_2, CO_2 and ΔH) other chemical and biological parameters have been suggested to assess the stability of organic matter. In principle, chemical parameters are preferred, as these methods are cheap and quick. Examples of chemical parameters are the C/N ratio, NO_3/NH_4 ratio, CEC, humification and dissolved COD (Hue and Liu, 1995). However, these parameters are not directly related to the stability as defined previously. Moreover, the chemical values are not absolute and highly depend on the composition of the starting materials.

Part of the substrate is mineralised (dissimilation) which produces energy and the other part is used for production of new micro-organisms (assimilation). The yield factor (amount of biomass produced per amount of substrate) depends on the type of substrate and environmental conditions (e.g. temperature, pH) and is around 0.5-0.6 for aerobic degradation of cellulose-like substrates. Moreover, the composition of biomass is fairly constant $CH_{1.8}O_{0.5}N_{0.2}$. Depending on the C/N ratio of the substrate, ammonium is produced or consumed under aerobic conditions. Based on the composition of biomass, ammonium is produced for C/N ratios lower than 10 and consumed for C/N > 10. However, biomass itself is also substrate for aerobic degradation and therefore the breakpoint will be at a C/N ratio of 20-30. In principle, the N-mineralisation rate can be determined simultaneously during the respiration test by monitoring the net development of mineral-N (NH_4+NO_3) in time.

The OxiTop® measuring system

The OxiTop® measuring system is a device that measures the drop of the gas phase in a closed system. In the case aerobic activity in the liquid or solid phase is measured, oxygen from the gas phase is consumed and carbon dioxide is produced. When the carbon dioxide in the gas phase is trapped in a basic solution, the oxygen consumption is directly related to the measured pressure drop. The test was originally developed to determine the respiration rate (e.g. BOD_5) of wastewater. Our goal is to develop a standardised method to determine the oxygen uptake rate (or respiration rate) of organic matter in solid substrates such as waste, compost and soil.

The OxiTop® system is composed of:

- Measuring (pressure) heads with screw that can be mounted on a bottle or vessel.
- A remote controller for control and registration of the measurement.

The actual reading values can be obtained at any time during the run. The OxiTop® system is easy to handle, demands low labour and is relatively inexpensive. The data are retrieved with a PC communication program and the data can further be processed, e.g. in MS Excel. More information about the system can be found on http://www.wtw.de/media_upload/13_respiration.pdf.

Standardisation of the respiration test

The oxygen uptake rate (OUR) of the solid substrate can be determined in the solid phase and the liquid phase. The advantages and disadvantages of both approaches will first be discussed (Lasaridi and Stentiford, 1998).
The true OUR of the solid substrate can only be assessed when optimal conditions are provided where the rate-limiting step is the degradation of the solid substrate itself. For this, the following conditions should be met:

1. The complete surface of the solids is accessible for aerobic degradation. This condition is only met when the solids are suspended in water.
2. Availability of macronutrients and micronutrients should not be rate-limiting (can be in cases where substrates such as or synthetic substrates such as cellulose and organic polymers are studied). A standard nutrient (macro and micro) solution can be added.
3. Microbial growth should not be rate-limiting. This means that enough NH_4 should be present to meet the N-demand for microbial growth. This can be accomplished by adding NH_4Cl to the solution.
4. Sub-optimal conditions such as low or high pH or presence of toxic compounds (VFA) should be prevented. The solution can be buffered.
5. Initial (mesophilic) aerobic activity may be insufficient to start up the aerobic degradation. An inoculum can be added.
6. Other oxygen consuming (microbial) process should be stopped. Most important is the oxidation of ammonia (nitrification) (other processes such as oxidation of sulphide and Fe(II) can be ignored). Nitrification can be suppressed by adding a nitrification inhibitor such as allylthiourea (ATU).
7. Oxygen transfer from gas phase to liquid-solid phase should not be rate limiting.

Measurement of the OUR in the solid matrix gives the following problems in relation to the above mentioned points:

- The surface of the solids is not fully accessible to degradation, as aggregates are present. The total available surface depends on the moisture content and bulk density of the substrate.
- In most cases oxygen is the rate-limiting step. Oxygen is only directly available at the outer surface of the solid matrix but has to diffuse through pores to reach the inner of the matrix. Therefore, the maximum OUR is never assessed and OUR will depend on the moisture content and density of the substrate.
- For a solid matrix, buffering, adding inoculum and adding ATU inhibitor homogeneously is problematic.

Only measurement in the liquid (suspension) state can give standardised conditions for an aerobic respiration test. Measurement of the OUR in the liquid phase gives better conditions as nutrients, buffer and inoculum can be added and the complete surface of the solids are accessible as the particles are suspended in the water phase. Moreover, respiration in liquid state results in higher respiration rates and thus shorter measuring times. The only problem can be that the oxygen transfer rate from gas to liquid phase (oxygen in the water phase should stay saturated) is smaller than the OUR. In that case not the OUR but mass transfer is measured. Also problems may occur when the adsorption rate of CO_2 in lye is slower than the CO_2 production rate from aerobic degradation. In this case, CO_2 is accumulated in the gas phase and the O_2 concentration pressure drop is no longer directly related to the pressure drop.

Development of standardised respiration test with OxiTop® system

A standardised respiration method has been developed at the department of Environmental Technology of Wageningen University. We found several limitations of the existing commercial OxiTop® system that were handled as follows:

- The cup for CO_2 adsorption in lye has to be adjusted to improve CO_2 adsorption rate.
- The prescribed lye (NaOH) is hygroscopic and clogs, in this way significantly reducing the CO_2 adsorption capacity. Therefore, NaOH has to be replaced by non-hygroscopic lye to prevent clogging.
- The solution has to be shaken (or stirred) to prevent limitations of O_2 gas-liquid transfer.
- The O_2 gas-liquid transfer rate becomes limiting at higher TS levels due to blocking of the liquid surface by the solids and increased viscosity.
- Nitrification inhibitor ATU (allylthiourea) has to be added in much higher concentrations compared to wastewater applications.
- It is advised to perform the test at constant temperature as:
  - microbial degradation rate depends on temperature.
  - temperature has an effect on the water vapour pressure which contributes to the total pressure of the gas phase

  The test can be performed at less constant temperatures but then blank solutions (without sample) have to be run parallel.

A typical pressure drop (ΔP in mbar) for aerobic degradation test is given in Figure 1. The course in ΔP can be divided in 4 regions:

1. Temperature effect on relative humidity. In case there is a difference between the temperature of the sample and the temperature of the test room, the relative humidity (water saturated air in headspace) of the gas phase will change which results in a change in pressure. This phase can be prevented or shortened by equilibrating the sample in the test room for a few hours.
2. Lag phase where microbial activity is rate-limiting. The lag phase can be shortened by adding an inoculum (or adapting the inoculum to the conditions).
3. OUR of the sample is rate-limiting. OUR of the sample is determined by linear regression of the COU during this period.
4. Oxygen is rate limiting by depletion of the gas phase. As a rule of thumb, oxygen should be higher than 10% to prevent oxygen depletion of the water phase.

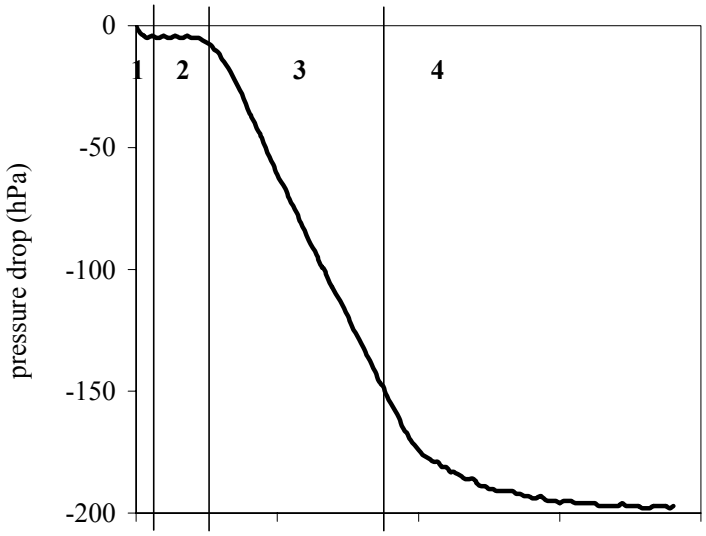

Figure 1. Typical pressure drop in time for aerobic degradation test in OxiTop (time on x-axis not defined)

Subsequently, the cumulative oxygen uptake of the sample (COU in mmol.kg^{-1} VS) can be calculated as follows:

$$COU = \frac{\Delta P}{83.14 \cdot (273.13 \cdot T)} \frac{V_{gas}}{W \cdot TS \cdot VS}$$

where ΔP is the pressure drop of the gas phase (mbar or hPa), T is the temperature of the measurement (^{o}C), V_{gas} is the volume of the gas phase (ml), W is the weight of the sample (kg) TS is the total solids content of the sample (kg.kg^{-1} wet weight) and VS is the volatile solids content of the sample (kg.kg^{-1} TS).

The temperature during the measurement has to be constant as the pressure drop is directly related to the concentration of oxygen according to the gas law (pV=nRT). The COU as calculated from Figure 1 is given in Figure 2.

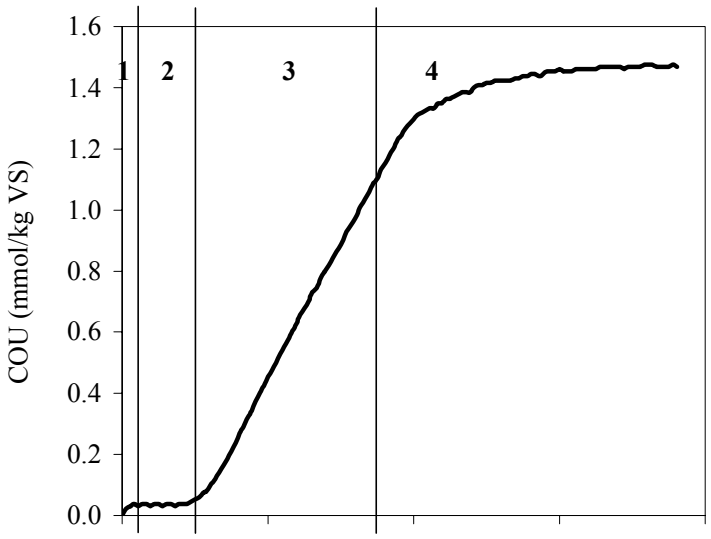

Figure 2. Typical COU curve for aerobic degradation test in OxiTop (time on x-axis not defined)

The OUR (in mmol.kg^{-1} VS.h^{-1}) of the material can now be calculated from the slope in the 3rd region according to:

$$OUR = \frac{COU(\Delta t)}{\Delta t}$$

or via linear regression of the 3rd period in e.g. MS Excel.

Comparison of standardised OxiTop respiration test with Rottegrad

The Oxitop-method and the Rottegrad-method both give an indication of stability or decomposability of organic matter. The results of both methods have been compared in a study that was carried out on behalf of the Dutch Ministry of Agriculture, Nature and Fisheries. In this study, 26 soil improvers were tested with both the Oxitop method and Rottegrad:

- compost from separately collected biowaste (fruit, vegetables and garden waste): 5 products
- compost from recycled yard and recreation waste only (greenwaste compost): 5 products
- compost from mushroom growers (finished mushroom substrate): 5 products
- peat: 4 products
- bark and bark compost: 6 products
- sewage sludge: 1 product

Figure 3 compares the results of both methods. Clearly, the Rottegrad-method is much less distinctive than the Oxitop-test. Whereas the Rottegrad measurements for almost all soil improvers indicate class 5 (the most stable class), the Oxitop method shows a much broader range in stability. Moreover, the respiration rate method is highly discriminative at high stability levels, i.e. low respiration rates. The disadvantages and shortcomings of the Rottegrad method are discussed by Weppen (2002).

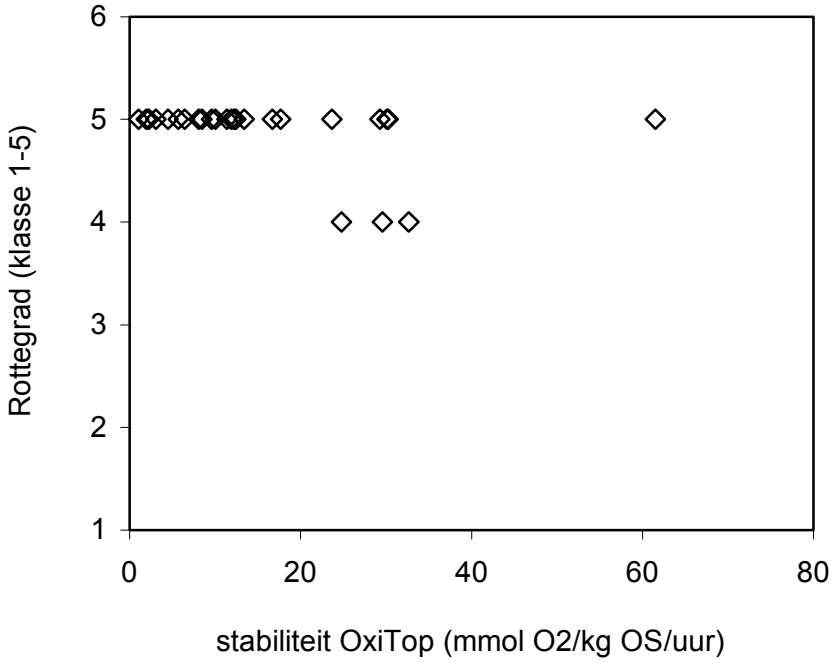

Figure 3. Comparison of stability of soil improvers as obtained from respiration rate test by OxiTop and Rottegrad

DATABASE of respiration rates for various organic matrices

Table 1 shows typical respiration rates of organic matter in different matrices as determined by the standardised OxiTop® method. The respiration rate of fresh organic matter such as biowastes or manures ranges from 50-100 mmol O_2/kg VS/h. After an initial active composting stage, the respiration rate drops to 10-15 mmol O_2/kg VS/h within 1 week and to 6-8 mmol O_2 /kg VS/h within 3 weeks. After an aerobic storage (maturation stage) of 5 months, the respiration rate drops to 3-5 mmol O_2/kg VS/h. Highly stabilised organic matter as present in peat has respiration rates <1 mmol O_2/kg VS/h. The data show that the respiration rate can be determined over a wide range. Moreover, the method is highly discriminative at low respiration levels.

Table 1 also shows the half-life of the organic matter, time after which 50% of organic matter is degraded, assuming an oxygen consumption of 1.1 kg O_2 per kg organic matter. This value gives a direct impression of the degradation rate.

Table 1. Respiration rates of various types of organic matter under standardised conditions

Type of organic matter	Respiration rate (mmol O_2/kg VS/h)	Definition	Half-life (d)
Fresh pig manure and biowaste	50-200	Very unstable	4-14
1 week composting[1]	15-25	Unstable	29-48
2 weeks composting[1]	7-15	Unstable	48-102
Fresh compost after 3-4 weeks[1]	7 ± 3	Stable	102
Matured compost after 5 months of storage[1]	3 ± 1	Very stable	239
Organic matter in soils	2-3	Very stable	239-358
Light peat	1.7 ± 0.2	Very stable	421
Dark peat	0.6 ± 0.1	Very stable	1194

[1]well-controlled composting experiments performed on laboratory scale at Dept. Environmental Technology, Wageningen University

Table 2 shows the respiration rate of commercial samples for several classes of soil improvers. The data were obtained from a study carried out on behalf of the Dutch Ministry of Agriculture, Nature and Fisheries.

Table 2. Respiration rates of various types of organic matter under standardised conditions

Type of organic matter	Respiration rate (mmol O_2/kg VS/h)
Biowaste compost[2]	17.0 ± 7.8
Green waste compost[2]	10.4 ± 3.3
Finished mushroom substrate[2]	28.1 ± 3.8
Peat[2]	2.1 ± 0.9
Bark and composted bark[2]	13.4 ± 9.6

Standard criteria for stabilised biowaste and green waste composts

Based on the respiration values from Table 1 and measurements from numerous samples for a specific type of compost, a well-founded standard for stability (maturity) can be proposed. Laboratory scale experiments show that a respiration rate of 7 mmol O_2/kg VS/h can be reached within 3 weeks of composting for manures and biowaste. In practice, a wide range of results is obtained for fresh biowaste and green waste samples. These values illustrate the functioning of a full-scale composting plant. Some samples have respiration rates around 7 mmol O_2/kg VS/h, showing properly functioning composting plants, and others have values higher than 25 mmol O_2/kg VS/h, indicating poorly operated plants. Setting up a database of respiration rates for fresh (or mature) biowaste and green waste compost samples gives a proper foundation to set up standard criteria for compost that can be used by governments.

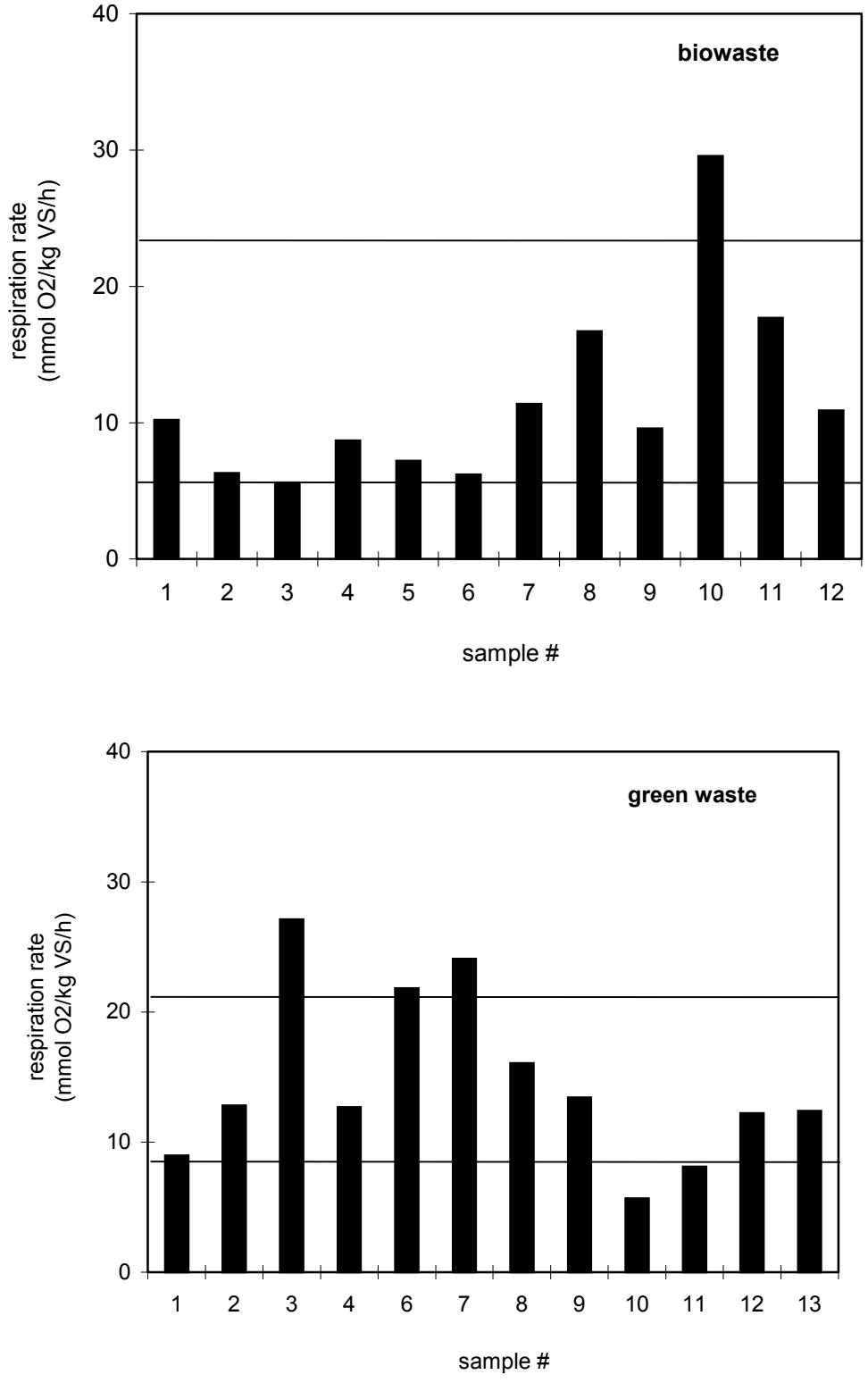

Figure 4. Respiration rates of fresh biowaste (top) and green waste (bottom) compost as obtained from composting facilities in the Netherlands (lines show average ± standard deviation)

Figure 4 shows the respiration rates of fresh composts derived from biowaste and green waste as obtained from composting facilities in the Netherlands (analysed by the standardised respiration test at Dept. Environmental Technology, Wageningen University). Based on these values and on the values of Table 1 the following criteria are proposed for biowaste and greenwaste compost:

- very unstable (compost): >30 mmol O_2/kg VS/h
- unstable (compost): 15-30 mmol O_2/kg VS/h
- stable (compost): 5-15 mmol O_2/kg VS/h
- very stable (compost): <5 mmol O_2/kg VS/h

Development of standardised method for simultaneous determination of respiration and N-mineralisation with OxiTop® system

For the respiration test, organic matter is degraded according to Eq. 1:

$$C_aH_bO_cN_d + xO_2 + yNH_4^+ \rightarrow aCO_2 + zH_2O + \Delta H + X$$

Depending on the C/N ratio of the substrate, ammonia is produced or consumed. For fresh wastes or unstable composts, dissolved substrate with high C/N ratio can be available resulting in an initial sharp consumption of ammonia followed by production of ammonia. Standard N-incubation tests are performed within 1-4 months and done in solid state by mixing the compost with a standard soil. However, it is well known that the N-mineralisation rate varies with the type of soil, moisture content and soil compaction. Moreover, organic matter of the soil itself will give a contribution to the N-mineralisation. Therefore, the results have to be corrected for N-mineralisation of the soil (blanks). The N-mineralisation is determined by the change in mineral nitrogen (NH_4+NO_3) in the soil extract (Page et al., 1982). This method is time and labour consuming. Besides that, losses of N due to denitrification can take place, which results in an underestimation of N-mineralisation.

The standardised respiration test with the OxiTop® system could be an attractive alternative method for the standard incubation test when the following conditions are met:

1. Measure mineral-N (NH_4+NO_3) without disturbing the pressure drop of the gas phase
2. Prevent losses of N by denitrification

The first point is technically solved by placing a syringe with valve through a septum in the liquid. In this way samples of the liquid can be taken without disturbing the respiration test. It should be taken into consideration that taking samples from the liquid affects the solids concentration and pressure drop in the bottle. This can be avoided by keeping the total sample volume a very small fraction of the total liquid volume (<5%).

Denitrification is a process that can hardly be prevented. In suspensions (or solid state), regardless how well aerated, there are always niches where anaerobic conditions prevail. In these niches denitrification will take place as nitrate diffuses to these areas. The only way to prevent denitrification is by preventing production of nitrate by nitrification. This can be accomplished by adding a nitrification inhibitor to the suspension. As a matter of fact, a nitrification inhibitor was already added, as oxygen consumption by nitrification would disturb the respiration test.

Another clear advantage of the N-mineralisation test in OxiTop® is the fact that it is quicker and less time and labour consuming. Samples for mineral-N are taken at regular time intervals and the net mineral-N production is plotted as function of time. Subsequently, the N-mineralisation rate is determined by linear regression from the plot.

The development of the N-mineralisation method is in progress.

DATABASE of N-mineralisation rates for various organic matrices

The results of a study that was carried out on behalf of the Dutch Ministry of Agriculture, Nature and Fisheries are summarized in Table 3. As the method has not been standardised sufficiently yet, no conclusions are drawn from these values.

Table 3. N-mineralisation determined by standardised Oxitop measurements

Soil improver	# samples	N-mineralisation (mg N/kg TS/h)
Biowaste compost[2]	5	6.6 ± 3.4
Green waste compost[2]	5	5.1 ± 2.3
Finished mushroom substrate[2]	5	38.8 ± 23.5
Peat[2]	4	5.6 ± 3.3
Bark and composted bark[2]	6	-2.9 ± 2.5

Conclusions

1. The standardised respiration rate test by Oxitop is a simple, quick, cheap, reproducible and distinctive method to determine the stability of compost samples.

2. The Rottegrad method is not reproducible and is not distinctive (also see Weppen, 2002).

3. Measuring stability with the standardised respiration rate test by Oxitop has been standardised sufficiently.

4. It is also possible to measure N-mineralisation simultaneously with the standardised respiration rate test by Oxitop. The method has not been standardised sufficiently yet.

5. The standardised respiration rate test by Oxitop can be used for product characterisation.

6. The following values, based on Oxitop measurements and on a database for composts, for judging the stability compost from biowaste and green waste are proposed:

very unstable (compost):	>30	mmol O_2/kg VS/h
unstable (compost):	15-30	mmol O_2/kg VS/h
stable (compost):	5-15	mmol O_2/kg VS/h
very stable (compost):	<5	mmol O_2/kg VS/h

References

Haug, R.T. (**1993**). *The Practical Handbook of Compost Engineering*. Lewis, Boca Raton, USA.

Hue, N.V. and J. Liu. (**1995**). *Predicting compost stability*. Compost Science & Utilization, Volume 3, page 8-15.

Lasaridi, K.E. and E.I. Stentiford. (**1998**). *A simple respirometric technique for assessing compost stability*. Water Research Volume 32, page 3717-3723.

Page, A.L., R.H. Miller and D.R. Keeny (**1982**). *Methods of Soil Analysis. Part 2. Chemical and microbiological properties*. 2nd edition, ASA/SSA Number 9 (Part 2) in the series Agronomy.

Weppen, P. (**2002**). *Determining compost maturity: evaluation of analytical properties*. Compost Science & Utilization, Volume 10, page 6-15.