The Use of Respiration Indices in the Composting Process: A Review

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Abstract

Respiration is directly related to the metabolic activity of a microbial population. Microorganisms respire at higher rates in presence of large amounts of bioavailable organic matter while respiration rate is slower if this type of material is scarce. In the composting process respiration activity has become an important parameter for the determination of the stability of compost. It is also used for the monitoring of the composting process and it is considered an important factor for the estimation of the maturity of the material. A wide range of respirometric protocols has been reported based either on CO_2 production, O_2 uptake or heat releasing. Most common methods are those based on O_2 uptake. Respirometric assays are affected by a number of parameters including temperature, humidity and, incubation and pre-incubation conditions. Results from respirometries are generally expressed as Respiration Indices, most of them with their own units and basis. In consequence, some confusion exists when referring and comparing respiration indices. This is particularly important because current and future legislations define and measure biological stability of wastes on the basis of respiration activity of the material. This paper discusses and compares most common respiration indices currently used.

Keywords: compost stability, compost maturity, organic solid waste, respiration index, respirometry.

1. Introduction

Composting is a natural aerobic process by which microorganisms decompose organic matter into simpler nutrients. Final product, the compost, is a stable, sanitised and humus-like material.

Maturity and stability are important parameters for compost quality assessment. Maturity is a general term describing fitness of a compost for a particular end use (Brewer and Sullivan, 2001). It is commonly associated with plant-growth potential or phytotoxicity (Iannotti et al., 1993). Mature composts are ready to use; they contain negligible or acceptable concentrations of phytotoxic compounds like NH_3 or short-chain organic acids (Brewer and Sullivan, 2003).

Stability can be defined as the extent to which readily biodegradable material has decomposed. A material is considered unstable if it contains a high proportion of biodegradable matter that may sustain high microbial activity. If the material contains mainly recalcitrant or humus-like matter, it is not able to sustain microbial activity and therefore, it is considered stable. Stability is not only an important compost quality characteristic but it can also be used for process performance monitoring and comparative evaluation of different composting systems (Lasaridi and Stentiford, 1998).

Respiration is a global measure of the total microbial activity. It can provide a reliable, repeatable and scientifically sound assessment of microbial activity. For this reason, respirometry (CO_2 evolution rate and/or O_2 uptake rate) has been widely used to evaluate microbial activity and therefore, stability of a compost sample. Different respiration indices, obtained from different respirometry techniques, are currently used to determine the level of microbial activity in a sample of compost as determined by a respiration test. In general, a Respiration Index (RI) can be defined as the rate of O_2 uptake or CO_2 evolution of a sample under specific conditions. All indices use their own units and nomenclature. Besides, some of them have threshold value below of which determine if a compost is stable or not. This has produced a certain degree of confusion when referring to respirometric techniques and stability limits.

On this basis, the objective of this paper is to review and discuss the different respirometric techniques currently available and the different stability limits that have been proposed based on respiration indices. A detailed description of the analytical procedures used in the respiration measurements is also presented.

2. Methods for determining respirometric activity

As mentioned above, respirometric activity of a material can be directly determined either from the O_2 uptake or the CO_2 production. It can also be indirectly estimated from the released heat during the process. Figure 1 shows a general diagram of the general procedure to obtain the respiration index of a compost sample. The following methods have been described for the determination of the respirometric activity.

2.1 Self-heating test

This method measures the temperature increase due to the heat released from the biological and chemical activity of a compost sample. It is a handy and suitable method for every day operations. It is simple to implement and results are easy to understand. It is widely used in Europe and North America (ADAS, 2003; Brinton et al., 1995). However, it could be argued that this test cannot be directly correlated to respiration since many chemical and biochemical reactions not related to respiration are also exothermal. Moreover, biomass heating is also influenced by other factors such as porosity or moisture content. Nevertheless, Koening and Bari (2000) indirectly determined the respirometric activity of a compost sample from results obtained in a self-heating test using a bioenergy approach to estimate the heat generated along the process. Maximum respirometric activity is obtained on the basis that the generation of 14,000 J of biological heat consumes 1 g of O_2.

2.2 Methods based on CO_2 production

These methods are widely used in commercial labs. Their equipment is generally very simple and easy to use. CO_2 production is directly correlated with the aerobic respiration. Amongst the most commonly used are those that use alkaline traps to fix the CO_2. These methods include the commercial kit Solvita®, widely used for the determination of the respirometric activity and ammonia production of volumetric compost samples. There are also more complex methods based on colorimetric techniques and gas chromatography. More sophisticated methods such as microtiter plate methods (Biolog) have also been reported for the monitoring of CO_2 evolution (Campbell et al., 2003). Many authors have proposed new versions and modifications of the original methods (Brewer and Sullivan, 2003; California Compost Quality Council CCQC, 2001). The main disadvantage of these methods is that they are unable to distinguish between CO_2 produced aerobically from that produced anaerobically. Moreover, these methods assume that CO_2/O_2 ratio is always 1. However, it can vary depending on the oxidation degree of the organic carbon. On this basis, some authors argue that they cannot be used to estimate the Respiration Index (RI) of a material (Lasaridi and Stentiford, 1998). Conversely, it has been indicated that if the assay is carried out under controlled aerobic conditions, all CO_2 will be produced under aerobic respiration (ADAS, 2003). However, monitoring of CO_2 evolution presents two major drawbacks i) the solubility of CO_2 in aqueous solutions and, ii) this solubility is pH-dependent. This is particularly important when comparing respiration activities of different residues since their pH can vary over a wide range. For instance, pH of organic fraction of municipal solid wastes is often near acidic conditions (5.5-6.5), whereas pH of sewage sludge is in the alkaline range (7.5-8.5) (Gea et al., 2004). Since pKa of CO_2 is 6.37, a difference of 2-3 units in the pH of two different residues may not permit the comparison between respiration indices obtained measuring CO_2 production.

2.3 Methods based on O_2 uptake

They are the most accepted methods for the determination of the biological activity of a material (Iannotti et al., 1994; Adani et al., 2001; Adani et al., 2003; Gea et al., 2004; Barrena et al., 2005). Respirometries provide accurate information on the activity of a compost sample. Their main disadvantage is that they need more specific instrumentation and more skilled labour. Besides, equipment needs constant maintenance and frequent calibration. Different commercial equipments are currently available (Costech, Oxytop, Micro-Oxymax, etc.) however they are expensive and troublesome. The rate of O_2 uptake can be quantitatively measured using manometric or electrolytic respirometers, by measuring changes in O_2 concentrations with gas chromatography or O_2 electrodes. O_2 can be measured either directly or as dissolved O_2 in aqueous suspensions. Expression of the RI and assay conditions depend on the method used for its determination. This will be reviewed in detail later on this paper.

Methods based on O_2 uptake rate have been classified into two different classes: statics and dynamics (Adani et al., 2001). Dynamic methods are those where a continuous supply of air is used throughout the assay minimising thus O_2 diffusion limitations. This is particularly important since it is well known that biological reactions that take place within solid substrates are often limited by the O_2 transfer rate (Paletski and Young, 1995). Several authors have described the use of dynamic methods (Paletski and Young, 1995; Adani et al., 2002a; Scaglia et al., 2000; Gea et al., 2004). Static methods do not include a continuous O_2 supply during the assay. They can be performed either with solid or liquid samples (Pressel and Bidlingmaier, 1981; Usui et al., 1983; Wilson and Dalmat, 1986; Haug and Ellsworth, 1991; Iannotti et al., 1993; Lasaridi and Stentiford, 1998).

Table 1 summarises the characteristics of the main respirometry methods including the type of respirometry, assay conditions and nomenclature used. A brief description of some of them is given below.

The static respirometry proposed by Iannotti et al. (1993), measures changes in O_2 concentration in the head space of a closed flask containing a moist compost sample of known volume and mass, at known temperature and barometric pressure. The decline in O_2 concentration over time is monitored with an O_2 electrode.

In the DiProVe method proposed by Adani et al. (2001), the Dynamic Respiration Index (DRI) is determined measuring the difference in O_2 concentration (ml l^{-1}) between the inlet and outlet of an air flow passing throughout a compost reactor. DRI is calculated from the average of 12 measurements taken every 2 hours, representing 24 hours of the maximum activity during 4 days. According to the assay conditions, authors distinguish between a Real Dynamic Respiration Index (RDRI) carried out with no moisture adjustment of the sample and, Potential Dynamic Respiration Index (PDRI) for samples adjusted to optimal moisture. The Static Respiration Index (SRI) can also be estimated in the same reactor. For this case, aeration is stopped and an O_2 electrode is placed in the head-space on top of the solid material. O_2 uptake rate is calculated from the decline in O_2 concentration. Readings are made every 5 minutes during 3 hours. SRI is calculated according to Iannotti et al. (1993) requiring also the measurement of the Free Air Space (FAS). Since all measures are obtained in an adiabatic reactor respirometries are then done at the process temperature of the material at the moment of the assay.

A protocol based on the Biological Oxygen Demand (BOD), method customarily used in wastewater treatment has also been suggested (Lasaridi and Stentiford, 1998). Two indices are obtained: the Specific Oxygen Uptake Rate (SOUR) and the cumulative oxygen demand in 20 hours (OD_{20}). For the SOUR determination a dissolved O_2 probe is used to measure changes in O_2 concentration of a sample suspended in water under optimal conditions for microbial activity and O_2 uptake at a temperature of 30 ºC. OD_{20} is calculated from the integration of the oxygen uptake curve from 0 to 20 hours. The two methods can be used to determine the stability of a compost sample. However, SOUR determination is faster (Chica et al., 2003). Besides, it only needs a single reading from the curve O_2 concentration over time while the OD_{20} requires a graphical integration. DSOUR, the specific oxygen uptake rate for a solid sample is calculated as described by Iannotti et al.

(1993), but in this case the assay is performed at 30 ºC for its comparison with SOUR results.

In Europe, the Respiration Activity after 4 days (AT_4) and the Dynamic Respiration Index (DRI) are recommended in the 2nd Draft of the Working Document on the Biological Treatment of Biowaste as parameters for the estimation of the stability of compost (European Union, 2001). This Working Document was supposed to be included in a new Directive on Compost. However, the Eurpean Commission abandonded this initiative very recently y this initiative was abandoned by the European Commission (European Compost Network, 2005). Nevertheless, this document is widely used as guidelines in the design of treatment plants all over Europe.

Meanwhile other official bodies (US Department of Agriculture and US Composting Council, 2001) recommend the use of the static respirometer proposed by Iannotti et al. (1993), for the determination of compost stability.

Moreover, another significant impeding use of respiration indices will be the assessment the degree of biological stability of end-products from Combined Mechanical Biological Waste Processing Plants (MBT) (Adani et al., 2002b; Adani et al., 2004). This is important since European legislation states that only stabilised waste can be disposed in landfill according to the Landfill Directive (European Union, 1999).

3. Comparison amongst the different respirometric methods

Several studies have compared the different respirometric techniques amongst themselves and with other protocols used either for the monitoring of the composting process or for the evaluation of the stability of the end product. Some of these studies include:

- Koening and Bari (2000) compared the self-heating test with a respirometry based on O_2 consumption. They concluded that the former is a simpler, cheaper and more suitable method than the latter. Besides, since self-heating test uses a higher amount of sample (1.5 l) results are more representative of the process.
- Lasaridi et al. (2000) indicated that during the first stages of the composting process, the self-heating test is not accurate enough. Therefore, they suggest that during the 2-3 first weeks of the process, respirometries are more useful for the monitoring of the process. However, self-heating test together with germination tests are more appropriate for the determination of the stability/maturity of the end product.
- Brinton (2001) has also compared the information provided by the self-heating test with that from respirometries. The author argues that the former gives more comprehensive information about the composting process but respirometries include a bigger number of factors related with the composting process. Besides, it is considered that the self-heating test is not able to distinguish between different curing stages during the late stages of the process. This information is particularly important when final product is intended for land application. The author also emphasises that a single method should not be used.
- Butler et al., (2001) indicate that the self-heating test is more appropriate for the monitoring of the process and the determination of the stability of the material than the respirometric techniques. They observed that respirometric

values obtained from day 29 of the process did not change however, self-heating values varied until day 57.

- Brewer and Sullivan (2003) compare different respirometry methods: self-heating test, colorimetric CO_2 (Solvita®), alkaline trap and CO_2 evolution via Dräger tube method. According to the authors, all methods provide similar information, however, it is considered that self-heating test takes substantially longer to provide such information.
- Brinton et al. (1995) propose a standardised protocol for the self-heating test. They have also found a correlation between this test and the production of CO_2. This equivalency is shown in Table 2. This table shows that this test is unable to distinguish between active and very active samples.
- Lasaridi et al. (2000) consider that respirometries based on O_2 uptake are the best method for the evaluation of microbial activity during the composting process.
- The CCQC (2001) compares different respirometry techniques and concludes that measurement of O_2 uptake takes longer and requires more control and more sophisticated equipment than methods based on the measurement of CO_2 evolution.
- Adani et al. (2002a) agree with Haug (1986) indicating that methods based on the monitoring of O_2 uptake are better than those that monitor the production of CO_2 since O_2 uptake is directly related to the oxidation of organic matter. It is argued that in the case of CO_2 production, oxidation of organic matter not related to microbial respiration, may interfere with the measurement. Methods based on O_2 are not affected by this interference. Nevertheless no practical

comparison has been reported so far on the monitoring of composting following O_2 uptake and CO_2 evolution.

- According to ADAS Consulting Ltd. (2003), composting process is better monitored by a combination of the self-heating test and respirometries based on O_2 uptake. It is also mentioned that there are no references regarding the use of CO_2 measurements for the monitoring of the process although they are very useful for the determination of the stability of the material.
- Palestski and Young (1995) consider that respirometries based on O_2 uptake are the best method for the determination of the stability of a compost sample since they directly provide information about the metabolic activity of the aerobic microbial population.

From these studies, it can be seen that at present, there is no general consensus on the use of a common respirometric technique.

3.1 Comparison amongst methods based on O_2 uptake

In static solid methods, the potential O_2 uptake rate is underestimated. The actual O_2 uptake rate is lower than in dynamic and/or soluble methods, and it is the actual O_2 uptake rate that is measured. Methods using liquid suspensions do not have these problems since sample is continuously stirred, therefore, in the SOUR determination there are not O_2 transfer limitations as with solid samples. Results obtained with liquid samples are also more reproducible since for solid samples they depend on the material structure and moisture. A liquid suspension obviates limitations related to the structure and moisture of the sample and O_2 transfer limitations.

Nevertheless, liquid respirometries are limited by the small quantity of sample used for the assay (3 – 8 g). Samples from Organic Fraction of Municipal Solid Wastes (OFMSW) are highly heterogeneous mainly during the early stages of the composting process. Therefore, bigger samples are required to improve their representativeness.

Length of assays can also vary; dynamic respirometries can be made on-line (Gea et al., 2004) or take up to 2 days (Adani et al., 2003) while static assays can last up to 2 days.

The main advantage of DRI is that the assay is carried out under conditions similar to those of real scale. However, a more important advantage of DRI is that it may be used in production scale composters for the determination of the respiration index on-line, although no reference about this use has been reported so far. On the contrary, one of the main disadvantages of the SOUR index is that it does not really represent the actual conditions of the material. SOUR measurements are made in aqueous suspension where O_2 transfer limitations are avoided. However, composting does not take place in aqueous suspension thus transfer phenomena occurring during the process are different.

The SOUR index was compared with the DSOUR dry index (Lasaridi et al., 2000) for the monitoring of a composting process. Results showed that both indices were fairly similar during the curing stage. Correlation coefficient between the two parameters was 0.94 with a 0.01 significance level indicating a good correlation between them. However, DSOUR values were somewhat erratic during the initial thermophilic stage; therefore, they could not clearly represent this phase. This could be attributed to experimental errors to which the DSOUR test is more susceptible, and to the inherent limitations of respirometric tests using solid samples.

Adani et al. (2003) compared three different methods with the aim of finding their similarities. Two of the methods used solid samples, one in static conditions (SRI) and the other under dynamic conditions (DRI), while the third was carried out in liquid samples (SOUR). Results indicated that there is a good correlation amongst them and all can be used to describe the biological stability of the samples. However, they are affected by different factors that in some cases can influence the results. For instance, it seems that soluble organic matter content may affect SOUR index. Hence, depending on the material, stability estimated using this method can be different from those obtained with methods using solid samples. Authors recommend then more research on the relationship between SOUR and soluble organic matter. When the SRI and DRI are compared it was shown that former were lower. This is probably because of mass transfer limitations in O_2 diffusion in static methods while continuous supply of O_2 in dynamic methods prevents these limitations. Another possible drawback of the static method is the systematic error when measuring the Free Air Space (FAS) of the sample. This is because accurate measurement of FAS is complicated. Equipment is generally expensive and complicated to use (Agnew et al., 2003; Oppenheimer et al., 1997). Nevertheless, stability values obtained with the three methods are reliable.

DRI, SRI and Respiratory Quotient (RQ) have been used for the monitoring of the composting of different materials (Gea et al., 2004). RQ is the ratio between CO_2 produced and O_2 consumed. It is assumed that under aerobic conditions, RQ value is close to one although it depends on the biochemical composition of the material (Atkinson and Mavituna, 1983). Results indicated that DRI values where the most reliable to evaluate the microbial activity in the process. SRI was evaluated at 37 ºC and at the process temperature. It was found that during the first stages of the process SRI at 37 ºC were significantly lower than DRI probably due to O_2 diffusion limitations while at latter stages both DRI and SRI were similar. Respiratory Quotient (RQ) did not show any significant change throughout the process.

Other studies have shown that SOUR index is a good indicator of the stability of the material (Lasaridi and Stentiford, 1998). However, it cannot be used for the monitoring of the first stages of the process. Conversely, SRI and DRI are useful for both stability determination and monitoring of the process.

Equipment required for the determination of SRI described by Iannotti et al. (1993) and the US Department of Agriculture and US Composting Council (2001) is cheaper and easier to use than that required for the SRI and DRI determination proposed by Adani et al. (2003). Respiration indices obtained at conditions closer to the actual process conditions are more realistic than those obtained at more different conditions such as SOUR.

4. Respirometry techniques conditions

Respirometries should be done under conditions that allow the optimum development of microorganisms.

Respirometries can be used to determine the biological activity in a sample if the assay is performed under optimal and controlled conditions (Adani et al., 2001). A respirometry requires optimal moisture content, oxygen content, appropriate temperature and, a nutrient balance that favours microbial activity.

Microbial activity in a compost process and in consequence, in a respirometry, is affected by many different factors such as: moisture content and temperature of the sample, microbial population, nutrients equilibrium, or occurrence of toxic compounds.

4.1 Moisture content

For many authors (ADAS, 2003; US Department of Agriculture and US Composting Council, 2001; Adani et al., 2003) this is the most influential parameter in a respirometry. Palentski and Young (1995) have shown that O_2 uptake is directly related to the moisture content of a solid matrix. Reliable results require a sample with an optimal moisture content since microbial activity can be limited either in too wet samples (anaerobic conditions are favoured) or too dry (lower potential microbial activity). In general, compost samples with moisture below 35% wet weigh basis, will be biologically dormant in consequence, its respiration index will be falsely low.

Some debate exists on the way moisture content is expressed, according to the US Department of Agriculture and US Composting Council (2001) it should be referred to the water holding capacity of the material rather than based upon its total wet weight. For instance, samples with high bulk density (0.75 kg m^{-3}) and low organic matter content are generally over-saturated at moisture contents between 40-50%. Conversely, samples with low bulk density and very high water holding capacity may be too dry at these moisture levels. However, it has also been pointed out (US Department of Agriculture and US Composting Council, 2001) that appropriate moisture content should be between 70 –85% of water holding capacity which, for most samples corresponds to 40-50% moisture (wet weight basis). Moreover, over-moist samples, tightly packed in a sealed container may reach an anaerobic state unrepresentative of the sample source and therefore, are not suitable for respirometry analysis.

4.2 Temperature and microbial population

Temperature is considered a critical parameter for the determination of respiration indices since biological activity is a function of temperature (ADAS, 2003; Iannotti et al., 1993; Lasaridi et al., 2000; Mari et al., 2003; Liang et al., 2003; Cronjé et al., 2004).

There is no agreement about an optimal temperature range for the respirometry assays. Most of them are performed at a standard temperature, normally set between 30-37 ºC (Paletski and Young, 1995; Iannotti et al., 1993; Lasaridi and Stentiford, 1998; Pressel and Bidlingmaier, 1981). American procedures generally use 35 ºC as standard temperature, while in other countries a temperature of 30 ºC is used (Stentiford, 2002). It is considered that respirometries carried out at these temperatures are a good indicator of the metabolic potential of the sample once the compost is incorporated into the soil.

Stentiford (2002) carries out respirometries at 30 ºC and argues that working at higher temperatures, for instance 35 ºC, produces higher uptakes. The author proposes an equation to convert the SOUR obtained at 30 ºC to any given temperature based on empirical data:

$$SOUR_T = SOUR_{30}\Theta^{(T-30)} \quad (1)$$

Meanwhile, Cronjé et al. (2003) have related OUR to the process temperature according to:

$$OUR = 0.11 \times 1.066^{T} \quad (2)$$

where OUR is based on the volatile solid (VS) content at temperature T, expressed in centigrade degrees, and expressed in g O_2 kg^{-1} VS h^{-1}.

Mari et al. (2003) use a “thermogradient” respirometer to carry out respirometries at different temperatures. They conclude that assays at 35 ºC are good indicators of the metabolic potential of the compost. However, assays at 48.5 ºC were better indicators of the respiration activity occurring *in situ*.

Another advantage of carrying out respirometries at working temperature when in the thermophilic stage is that nitryfing bacteria are inhibited. Haug and Ellsworth (1991) working with suspended samples, recommend a temperature of 45 ºC to avoid the effect of nitrifying bacteria otherwise they suggest the use of nitrifying inhibitors.

The influence of temperature on SRI has also been studied by Barrena et al. (2005) using sludge. SRI indices of composting samples were determined at 37 ºC and at process temperature at sampling. Results indicated that both indices correlated well with temperature profile in the composter. SRI at process temperature were more representative of the metabolic activity in the composter and more sensitive to temperature and composition variations of the composting material however, they could not indicate the stability of the material at later stages of the process. Therefore, SRI at process temperature are more useful for the monitoring of the process while SRI at 37 ºC are more suitable for the determination of the stability of the material.

4.3 Preincubation requirements

When samples are moistened either because their moisture content is very low or because they have been previously dried, a pre-incubation is required to restore the metabolic equilibrium of the population. However, no uniform criteria exist about the conditions under which this should be carried out. The US Department of Agriculture and US Composting Council (2001) recommend adjusting the moisture content directly in the pile or reactor. However, in cases where this is not possible, a 24 h pre-incubation is proposed at the specified temperature of the assay. Some samples may require up to 3 days of pre-incubation at temperatures between 25 – 28 ºC. Iannotti et al. (1993) emphasise the importance of using an appropriate temperature and thus avoiding a thermal shock for thermophillic microorganisms. In consequence, samples should be incubated prior to the assays at the corresponding temperature. Recommended incubation times vary between 16 hours (Iannotti et al., 1993) and 25 hours (US Department of Agriculture and US Composting Council, 2001), although in some instances incubation times may be as long as 3 days. If short incubation times are used false respiration indices maybe obtained. Respiration indices of samples from early stages of decomposition may be too low if incubation time has not been long enough. According to Iannotti et al. (1993), respirometries should be carried out at 37 ºC. However, it has also been argued that using such a temperature may be selectively testing for organisms in the upper range of the mesophillic organisms and may not be indicative of what happen in the soil after the compost is incorporated (US Department of Agriculture and US Composting Council, 2001). Thus, a pre-incubation at 25-28 ºC and testing at 34 ºC is suggested as more representative of the actual compost metabolic activity potential.

Sometimes it is necessary to pre-incubate the samples prior to their assay, especially those that had been previously dried. Once the samples are moistened they need to be pre-incubated since there is a lag phase when the metabolic activity is re-established. This may require from 3 to 5 days. Nevertheless, more work is needed to determine optimal conditions for pre-incubations (temperature, moisture, time).

4.4 Nutrients equilibrium and occurrence of toxic compounds

Respirometry assays require an appropriate nutrients balance and the absence of toxins and other compounds that may inhibit microbial respiration.

Low microbial activity may be a consequence of lack of nutrients. For instance, sludge from paper industry has enough carbon but low levels of nitrogen for microbial growth. As a result, respiration indices could be low. Nevertheless, nitrogen and/or phosphate can be added to fulfil such deficiencies.

Conversely, problems can also arise from excess of nutrients. For instance, in samples with very high organic N content such as fish waste, this nitrogen can be transformed to produce very high levels of NH_4, (above 500 mg kg^{-1}). As a result, these samples may be colonised by saprophytic fungi (US Department of Agriculture and US Composting Council, 2001). Fungal mycelium serves as a food source for bacteria and will induce an abundant bacterial activity during incubation and upon aeration. If the presence of fungi is not diminished through incubation prior to respirometry measurements, respiration measures will indicate high O_2 uptake rates. Figure 2 shows a compost sample where fungi have proliferated during incubation.

5. Biological stability limits

Different limits have been established for the respiration indices for their use as a biological stability parameter. Table 3 shows the different limits proposed by several authors and the countries where these indices are mostly used for the determination of the stability of compost.

Several protocols, such as those proposed by the US Department of Agriculture and US Composting Council (2001) or the CCQC (2001), are based on the static model described by Iannotti et al. (1993), however nomenclature and limits used to express the indices are different from the original. Table 4 shows how SOUR nomenclature is used to define a static method with solid samples. As it can be seen, some references refer the respiration index to the amount of organic sample while others utilise the volatile solids content. The use of this nomenclature can cause some confusion if results are compared with those obtained using the method proposed by Lasaridi and Stentiford (1998).

The maturity test recommended by the CCQC (2001) differentiates between the SOUR and OUR indexes. Difference is given by the way results are expressed: SOUR is referred to the volatile solid content of the sample while OUR is referred to the total solid content.

Moreover, different limits have been proposed for the respiration indices to determine the stability of a material as described below.

In general, it can be said that some confusion exists when applying respirometry protocols probably because of lack of scientific assessment. For instance, some regulations have recommended the use of determined methods but using stability limits derived from different ones. In Italy, the UNI methods recommend to carry out the respirometry assays at 20 ºC but the proposed limits (UNI U53001080, 2005) derive from the DiProVe method, where respirometries are carried out at process temperatures (Adani et al., 2003).

Besides, the way results are expressed can also be a source of confusion. For instance, respiration indices can be determined either from maximum values or as average of measurements made over 24 hours, they can also be referred either to dry weight or to organic matter content. Table 5 shows different ways used to determine respiration indexes.

Moreover, there is no general interpretation to the biological stability of a material. In Germany and Austria, threshold values are much lower than in Italy. As shown in Table 3, the AT_4 proposed by Germany and Austria is lower, 5 mg O_2 g^{-1}VS 96 h^{-1}, than that proposed by the European Union, 10 mg O_2 g^{-1}VS h^{-1}. In Austria and Germany, compost is considered mature after 4 to 6 months process while in Italy the index is referred to a 15-30 day process.

Equivalences amongst the most commonly used indices have been proposed (Adani et al., 2003) as shown in Table 6. These equivalences have been obtained from the DRI proposed as stability threshold value in the 2nd draft of the European Union. The DRI has also been compared with the Solvita® test (Adani et al., 2003). Results indicate that a stable material according to the Solvita® would have very low DRI values, around 0.2-0.3 mg O_2 g^{-1}VS h^{-1} while the stability limit for this index is 1, therefore this test does not have enough resolution for the determination of compost stability.

6. Future trends

Although respirometry methodologies are established there are still different aspects that need more detailed investigation. Amongst them it is worth mentioning: i) the relationship between pH and CO_2 and its influence on respirometries based on CO_2 production, ii) the effect of optimising the porosity of the material on static respirometries and the comparison of results with those obtained from dynamic methods, iii) the effect of humidity when highly energetic residues are composted; that is, when temperatures above 70 ºC are reached during composting, iv) the influence of microbial population on respiration indices since respìrometries are currently performed based on microbial activity of native microbial populations; no optimum population has been considered so far, v) the effect of toxins contained in the material and, vi) a collective effort aiming at the unification of criteria in the selection of most suitable methodologies depending on the final application of the compost.

7. Conclusions

From the information found in the literature, it is evident that there is not a single respirometric method that can be used for both the monitoring of the process and the determination of the stability of a compost sample. Most appropriate method will depend on the aim of the assay. Moreover, although respirometries are routinely carried out further investigation is needed in aspects such as those mentioned above for a better understanding of the metabolic activity of a composting material and hence, how this affects the degree of stability of a compost.

Besides, it is clear that more work needs to be done to correlate all the different methods and indices that are currently used. This is particularly important since respiration indices are now considered as key parameters in the determination of the stability of a compost and hence, its quality.

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Legends to Figures

Figure 1. General diagram of the general procedure to obtain the respiration index of a compost sample.

Figure 2. Material used for respirometry assay that has been colonised by fungi: a) original, b) colonised.

NOMENCLATURE

AT_4	Respirometry Activity at 4 days
DM	Dry Matter
DRI	Dynamic Respiration Index
DSOUR	Specific Oxygen Uptake Rate for Solid sample
OD_{20}	Cumulative O_2 uptake on 20 h
OM	Organic Matter
OUR	Oxygen Uptake Rate
PDRI	Potential Dynamic Respiration Index
RI	Respiration Index
RDRI	Real Dynamic Respiration Index
SOUR	Specific Oxygen Uptake Rate
SRI	Static Respiration Index
VS	Volatile Solids

Table 1. Comparison between most commonly used Respiration Indices bases on O_2 uptake.

Index	Name	Reference	Type	Sample				Assay conditions	
				State	Weight	Sieving	Moisture	Time	Temperature
O_2 uptake	O_2 uptake	Iannoti et al., 1993	Static	Solid	60 g	< 9.5 mm	50-55% w/w	16 h incubation + 1 h assay	37 °C
SOUR	Specific O_2 Uptake Rate	Lasaridi and Stentiford, 1998	Static	Liquid	3 - 8 g	< 9.5 mm	In suspension	5 – 6 h	30 °C
OD_{20}	Cumulative O_2 uptake in 20 h		"	"	"	"	"	20 h	30 °C
DSOUR	SOUR in solid sample		"	Solid	"	"		20 h	30 °C
DRI	Dynamic Respiration Index	Adani et al., 2001	Dynamic	Solid	From few grams up to industrial scale	< 50 mm if necessary	Adjustment to 750 g kg^{-1} water holding capacity	53 h as mean 4 days maximum	Process
SRI	Static RI		Static	"	"	"	"	3 h	"
RDRI	Real DRI		Dynamic	"	"	"	No adjustment	53 h	"
PDRI	Potential DRI		"	"	"	"	Optimal moisture	53 h	"
AT_4 Sapromat	Respiration activity at 4 days	Binner and Zach, 1998	Static	Solid	50 g	< 10 mm	Saturation	4 days	20 ºC
RI_T	O_2 uptake	Barrena et al., 2005	Static	Solid	250 ml	< 10 mm	40 –55 %	4 h incubation + 1.5 h assay	Process
RI_{37}			"	"	"	"	"	18 h incubation + 1.5 h assay	37 °C

Table 2. Relationship between CO_2 techniques and self-heating test, adapted from Brinton et al., (1995) and Körner et al., (2003).

CO_2 production mg CO_2-C g^{-1} C	**Respiration rate**	**Self-heating grade equivalent**	**O_2 consumption mg $O_2 \cdot g^{-1}$ dry matter**	**Material status**
0 – 2	very slow	V	≤ 20	stable
2 – 8	moderately slow	IV – III	30-20	stable
8 – 15	medium	II – I	50-30	fresh
15 – 25	medium – high	I	80-50	fresh
> 25	high	I	> 80	raw

Table 3. Different limits recommended for the Static Respiration Index (SRI) and the Dynamic Respiration Index (DRI), adapted from Adani et al., (2002).

Static Respiration Index	**Reference**
0.5 mg O_2 g^{-1} VS h^{-1}	US Department of Agriculture and US Composting Council,1997; Iannotti et al., 1993
3 mg O_2 g^{-1} VS d^{-1}	US Department of Agriculture and US Composting Council, 2001
0.6 mg O_2 g^{-1} VS h^{-1}	Italia (Regione Veneto, I)
5 mg O_2 g^{-1} TS 96 h^{-1}	Sapromat, Austrian and German indicator (AT_4)
10 mg O_2 g^{-1} TS	AT_4 (EU, 2001)
1 mg O_2 g^{-1} VS h^{-1}	SOUR (Lasaridi and Stentiford, 1998)
Dynamic Respiration Index	
0.5 mg O_2 g^{-1} VS h^{-1}	Italy (Regione Lombardia) ;
1.0 mg O_2 g^{-1} VS h^{-1}	DRI (EU, 2001)
35 – 50 mg O_2 g^{-1} VS 96 h^{-1}	ASTM, 1996

Table 4. Different nomenclatures proposed for the Static Respiration Index (SRI).

Reference	Index	Units	Stability limit
US Department Agriculture and US Composting Council (2001)	SOUR	mg O_2 g^{-1} OM d^{-1}	< 3
CCQC Maturity Index according to the USDA and US Composting Council (2001)	SOUR	mg O_2 g^{-1} OM d^{-1}	< 3
CCQC Maturity Index according to CCQC (2001)	SOUR OUR	mg O_2 g^{-1} VS h^{-1} mg O_2 g^{-1} TS h^{-1}	< 0.5 < 0.4

Table 5. Considered values for the estimation of the different Respiration Indexes.

Index	Considered Value
SOUR, Mean Uptake	Maximum value
DRI	Average of the 24 hours of maximum biological activity
Sapromat, AT_4	Cumulative in 96 hours

Table 6. Equivalences amongst different stability limits for the most commonly used respiration indexes, adapted from Adani et al., (2003).

Index	Value
DRI* (mg O_2 g^{-1}VS h^{-1})	1.000
SRI (mg O_2 g^{-1}VS h^{-1})	0.395
SOUR (mg O_2 g^{-1}VS·h^{-1})	7.038
Sapromat® (mg O_2 g^{-1}VS 96 h^{-1})	45.39

*Used as reference value for equivalences

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Figure 1. Barrena et al. The use of respiration indices....

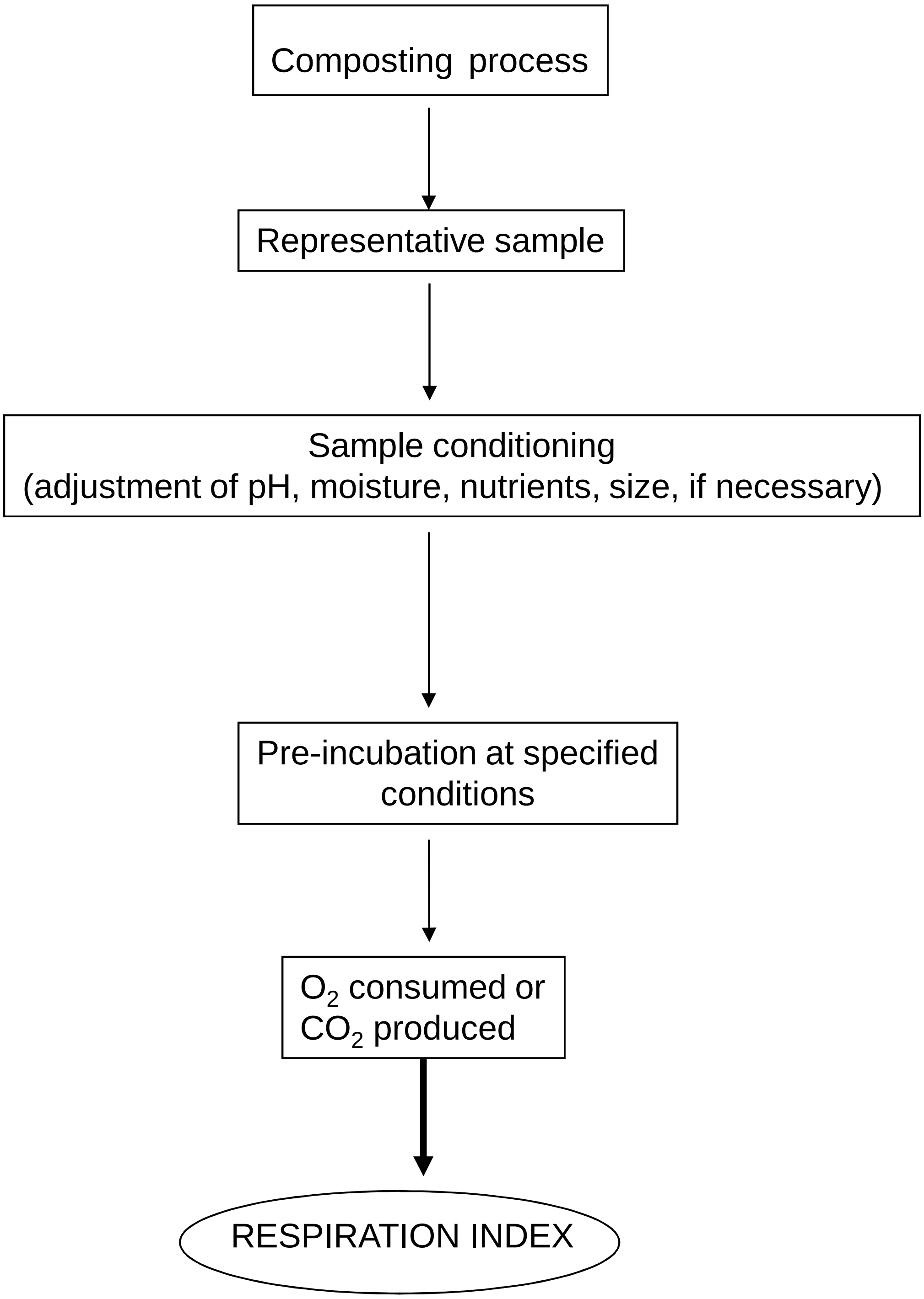

Figure 2. Barrena et al. The use of respiration indices.....

a) b)