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EVALUATION OF BIOLOGICAL PROCESSES' PERFORMANCES USING DIFFERENT STABILITY INDICES*

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Abstract

The EU's Circular Economy Policy Package aims to minimize waste production in accordance with the Circular Economy concept. In this respect, organic waste valorization through composting and anaerobic digestion process represents a proven solution for converting biomass in bio-based products with high fertilizing and amendment properties. In order to optimize process' performances and reuse the bio-products, biological stability index evaluation of the final products plays a key role. Several respiration tests are currently used for the determination of biological stability and the need to define homogenized regulations both at national and European level, in order to outline harmonized rules for biological stability determination, is becoming increasingly indispensable. Among these, some approaches measure respirometric activity by estimating oxygen uptake rate (SOUR and DRI test) and by recording the maximum temperature achieved by the biomass during the degradation process (Self-Heating test). Others assess the decomposition degree of organic waste by determining the residual biogas potential production (BMP test). This work aims at evaluating biological stability of different organic matrices such as compost, digestate and mixture of them in order to compare different respirometric techniques and define possible correlations between them and their suitability depending on the substrates analyzed. The results show that among the different stability tests analyzed in this work, there is a good linear correlation that allows to affirm a direct proportionality both between DRI and Self-heating test and BMP and SOUR test. The results suggest that all the respirometric methods considered in this study could be used as indicators of the biological stability degree of an organic substrate and are therefore interchangeable with each other, providing the same value of biological stability degree. Nevertheless, future studies on stability index determination could be carried out in order to confirm the results obtained from this work.

Keywords: BMP, compost, digestate, oxygen uptake rate, self-heating test.

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1. Introduction

Agriculture is an important sector for Italian economy, hence it is necessary to focus on a more responsible, efficient and sustainable use of natural resources. Fertilizers play a key role in keeping high agricultural production mainly due to the growing need to provide the soil with more nutrients - such as nitrogen (N), potassium (K) and phosphorous (P) – and organic matter. Nevertheless, its responsible use is essential to avoid water pollution problems and also for land and soil safeguard (Wilken et al., 2018).

Based on the EU action plan for Circular Economy (European Commission, 2015), waste production could be reduced by turning them into resources for new purposes (Webster, 2013). In this respect, the valorization of organic waste for bio-based product production such as compost and digestate respectively through composting and anaerobic digestion process, represents an attractive solution for a more efficient and safer recovery of resources from bio-waste (Tambone et al., 2010). Both compost and digestate are residual organic matrix with high fertilizing and organic amendment properties that can be replace the synthetic fertilizers in agriculture (Tambone et al., 2017; Tambone et al., 2019). Therefore, their use as fertilizer in agricultural system seems to be the best option for their recycling and recovery, improving soil structure and leading to a more efficient closure of organic matter and nutrients cycle (Alburquerque et al., 2012; Favoino, 2013). Moreover, the substitution of chemical fertilizers with the organic ones allows to reduce CO_2 emissions avoiding the withdrawal of energy related to the extraction and transformation of virgin materials (Centemero, 2017).

However, the fertilizers sector deals with several problems related to the access to the EU internal market due to the different national regulation on fertilizers in force. As regard the Italian legislation on fertilizers, both compost from green and lignocellulosic waste and sorted Organic Fraction of Municipal Solid Waste (OFMSW) are considered as products and can then be marketed, but this is not the case for digestate produced by anaerobic digestion process. Therefore, the digestate is not marketable unless mixed with other substrates such as those mentioned above. In order that the mixture is recognized as a fertilizer product, it must comply with the limit of 35% w/w of digestates’ dry matter presented in the mixture. To overcome this problem, the EU has proposed a Regulation with the aim of harmonizing the rules on the fertilizer market (European Commission, 2016). In order to obtain the CE marking as a fertilizer, products should satisfy defined physical, chemical and structural characteristics and must comply with biological stability threshold values, which can be assessed by means of different harmonized stability criteria.

Biological stability depends on biological activity in an organic matrix and represents the degree of decomposition of the biodegradable matter (Lasaridi and Stentiford, 1996). For this reason, its measurement is linked to the determination of biological respiration activity, which can be assess through respirometric tests (Adani et al., 2003). Amongst the available respiration tests, the most common are based on the measurement of oxygen consumption for the evaluation of biological stability (Scaglia et al., 2000; Adani et al., 2001). The determination of oxygen uptake rate is considered a reliable and simple method for the assessment of the stability index even if it required a rather complex experimental set-up (Scaglia et al., 2007). The rate of oxygen consumption can be measured directly in the process air flow or as dissolved oxygen (DO) in an aqueous solution. In the first case, the Dynamic Respirometric Index (DRI) is determined, the material is passed through by a dry air flow and oxygen concentration is measured in the exhaust air flow. In the second case, the Specific Oxygen Uptake Rate (SOUR) is detected and the oxygen concentration is estimated in an aqueous solution in which the samples is dissolved. Other methods, such as Self-Heating Test, used biomass' temperature increase during the degradation process to assess biological activity. This method is the most expeditious and suitable for daily operations (Wagland et al., 2009). In addition to these, anaerobic tests, such as Biochemical Methane Potential (BMP) test, also measure the biodegradability of an organic matrix. In this case, biodegradable organic matter is decomposed under anaerobic conditions by methanogenic bacteria and the decomposition degree is assessed by determining the residual biogas potential production (Pecorini et al., 2016).

This work estimates the performance of different composting and anaerobic digestion plants by comparing the stability indices of the final products obtained. This comparison was made in terms of stability index measured by SOUR, DRI, BMP and Self-heating test. This study aims to correlate different respiration methods and evaluate possible relationship between them and their suitability depending on the type of substrates.

2. Objectives

The main objective of this study is to analyze biological stability of different organic matrices such as compost, digestate and mixture of them in order to compare different respirometric techniques and define possible correlations between them and their suitability depending on the substrates. In this context, four different respirometric methods were proposed for the assessment of the stability index: SOUR test, DRI, Self-heating test and BMP test.

3. Outline of the work

This work is divided in two main parts:

- sample collection and processing: two organic matrices were collected from different plants (composting and anaerobic digestion plants) and mixed together in order to obtain a marketable mixture;
- evaluation of the biological stability index through several respirometric methods, comparison and analysis of the results.

4. Materials and methods

4.1. Sample collection and processing

Two different organic matrices from composting and anaerobic digestion full-scale plants located in Tuscany region were used as raw substrates for mixture combinations. Samples with different physical, chemical and structural characteristics were chosen in order to assess biological stability of matrices with variable compositions and origins. Each sample consisted of bio-waste processed materials that include composted green and lignocellulosic waste and digested wastewater sludge (WWS).

Each substrate was collected from each plant, stored and approximately 50 kg of samples were taken for pH, Total Solid (TS) and Total Volatile Solid (TVS) content analysis and for respirometric tests.

Subsequently, the matrices were manually mixed together by means of a blade in order to obtain a mixture of them as a new organic substrate. The mixing procedure was carried out in terms of weight content of each raw material.

The studied samples were as follow:

- GLWc: compost from green and lignocellulosic waste;
- WWSd: digestate from anaerobic digestion of WWS;

- WWSd+GLWc: mixture composed of 50% WWSd and 50% GLWc.

After the processing step, about 50 kg of the mixture was also sampled in order to characterize it as the raw substrates in terms of pH, TS and TVS content and stability.

TS, TVS and pH were determined in according to standard methods (APHA, 2006). Ashes and moisture contents were then obtained in accordance with TS and TVS measurements. Each analysis and respirometric test were performed in two replicates.

4.2. Respirometric tests

The assessment of respiration activity of an organic matter can be evaluated through several procedures measuring the oxygen uptake, the released heat during the degradation process and the gas production in anaerobic conditions. In this section a detailed description of the procedures and the experimental set-ups used to measure the stability index is presented.

4.2.1. Dynamic Respiration Index (DRI)

The DRI analysis was carried out using hermetically sealed stainless steel adiabatic reactors with a capacity of 30 liters developed by DIEF – University of Florence according to UNI/TS 11184:2016. The experimental set-up consists of two continuous flow aerobic respirometers in which about 20 kg of sample were placed. Before starting the analysis, the sample has to be adjusted and standardized relating to some process parameters such as humidity, density and pH (Scaglia et al., 2000). The analyzed sample was subjected to optimal insufflation conditions that are ensured by the forced injection of dry air coming from an air compressor that guarantees a constant flow through the system. The air flow is measured by two flowmeters (Aalborg Instruments) placed both at the inlet and the outlet of the system. The incoming air flow is adjusted by the flowmeter during the analysis in order to allow a value of O_2 concentration in the exhaust air greater than 14%. At the outlet of the system, special probes are set up to measure the concentration of oxygen (Zirconium oxide sensor, TEC-ZRC, Tecnosens S.p.A.) and CO_2 (GasCard NG Gascheck 10%, Edingburgh Sensors) in the exhaust air flow. An external water jacket inside which circulates water from a hot bath heated by a thermostat (FA90, FALC Instruments) is designed in order to maintain the adiabatic conditions of the system. Several thermocouples were installed to provide process air and biomass temperature measurements during the overall test. All the signal coming from the sensors are managed and acquired by a National Instrument acquisition system and processed by a software specifically developed in LabView environment. Figure 1 shows the DRI experimental set-up.

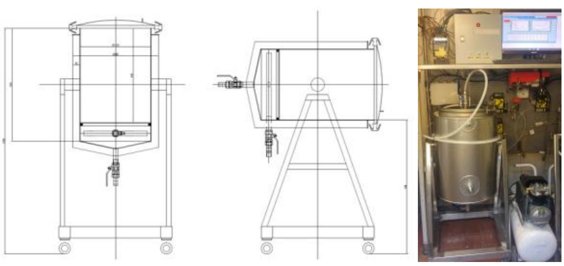


Fig. 1. DRI experimental set-up developed by DIEF – University of Florence.

Respiration index was calculate directly by the software in accordance to Adani et al. 2001. It represents the average of the indices measured during the 24 hours in which the biomass' respiration is highest and is measured as $mgO_2/kgTVS*h$.

4.2.2. Specific Oxygen Uptake Rate (SOUR)

The assessment of biological stability can be also evaluating in liquid phase. The SOUR test estimates the O_2 consumption in an aqueous suspension containing a sample both in solid and liquid form. This measurement allows to obtain two different indices: SOUR and the cumulative oxygen demand in 20 hours (OD_{20}). This method was set-up according to the methodology proposed by Lasaridi and Stentiford, 1998, with the modification of Adani et al., 2003.

Microbial respiration was evaluated by measuring DO concentration into 1-liter aqueous solution where 2.5 gr (wet weight) of sample were dissolved. The concentration measurement is guaranteed by a DO probe (Mettler Toledo, InPro6000, Optical O_2 Sensors). The flask was placed on a magnetic stirrer (Velp Scientifica, AREX Digital PRO), continuously mixed (250 rpm) and heated at 30° C for ensuring the optimal biological conditions. Since the concentration of oxygen in the solution decreases due to the biological process, it is necessary to insufflate air in the suspension in order to increase the content of oxygen available to the process. Therefore, the suspension was periodically aerated by a fish-tank air pump with intermitted aeration cycle. An automatic data acquisition and control system (LabView, National Instruments Corporation, Italy) provided the aeration/reading sequence characterized by 20 min aeration period followed by 15 min of DO measurement and acquired the signal coming from the DO probe during the overall experimental period of 20 hours. For ensuring that nutrients or pH were not limiting, phosphate buffer solution (with a pH equal to 7.2), ATU (allylthiourea) and 5 ml of nutrient solutions ($CaCl_2$, $FeCl_3$ and $MgSO_4$), made up according to the standard BOD test procedures (APHA, 2006), were added to the aqueous suspension.

The SOUR value was evaluated via DO concentration drops during the reading cycle and was calculated according to the Equation (1) (Lasaridi and Stentiford, 1998; Adani et al., 2003):

$$SOUR = \frac{|S\max|*V}{m*TS*TVS} \quad (1)$$

where: SOUR is the Specific Oxygen Uptake Rate ($mgO_2*gTVS^{-1}*h^{-1}$); |Smax| is the maximum absolute slope of oxygen consumption ($mgO_2*l^{-1}*h^{-1}$); V is the volume of the aqueous suspension (l); m is the mass of the sample (gr, wet weight); TS and TVS are the decimal fraction of dry solids and total volatile solids (dry matter) of the sample.

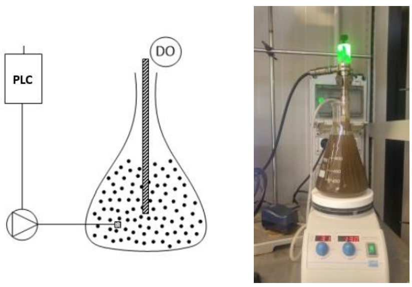


Fig. 2. SOUR experimental set-up.

4.2.3. Self-heating test

Self-heating test is an indirect measure of the biological activity based on the measurement of the temperature trend of the biomass analyzed over the experimental time. This analysis consists in placing the tested material inside an adiabatic vessel (Dewar vessel), after ensuring the optimal humidity conditions for the degradation process. Inside the sample, a thermocouple is placed to measure the biomass' temperature. There is no forced temperature control, therefore it tends to increase following the typical exothermic trend of the biological activity of degradation of the biodegradable matter. This technique is used to perform respirometric analysis on solid material and the experimental time vary from 5 to 10 days, until the biomass temperature values decreased for at least two days after the maximum temperature value reached. The biological stability is determined by evaluating the maximum temperature difference reached between the biomass and ambient temperature. Base on the difference value obtained, it is possible to define the biological stability degree of the tested substrates.

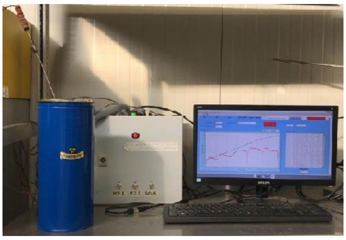


Fig. 3. Self-heating experimental set-up.

4.3. Biochemical Methane Potential (BMP) test

Anaerobic biodegradability assay was also performed in order to evaluate biogas and methane potential production of the organic materials. The analysis was carried out for 21 days according to the method proposed by Ponsà et al., 2008. The test was performed using stainless steel batch reactors developed at DIEF – University of Florence (Pecorini et al., 2012) and maintained at a constant temperature of 37° C in a water bath heated by a thermostat (FA90, FALC Instruments). Each reactor was filled in with substrate and gas production was daily estimated by measuring the pressure in the head space of the batch reactor. Using the ideal gas law, the pressure measured by a membrane pressure gauge (Model HD2304.0, Delta Ohm S.r.l., Italy) was then converted in biogas volume and Gas Sum (GS21) value measured as Nl biogas/gTVS, was estimated. In order to evaluate the methane content and determinate BMP21 value (Nl CH_4/gTVS), biogas was analyzed using an IR gas analyzer (ECOPROBE 5 – RS Dynamics).

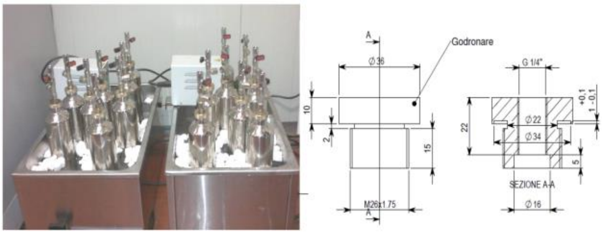


Fig. 4. BMP experimental set-up.

5. Results and discussion

5.1. Samples' characterization

Concerning TS and TVS contents measured for the analyzed samples and reported in Table 1, different results were obtained, especially for the raw samples. This variability is probably due to the different origins of the raw substrates (green-lignocellulosic waste and WWS) and the biological treatments they have undergone.

Table 1. Samples' characterization expressed in terms of average values and standard deviations.

Sample	*Origin*	*TS [% w/w]*	*TVS/TS [% w/w]*	*pH*
GLWc	Green-lignocellulosic waste	43.48±0.40	40.24±1.45	7.12±0.30
WWSd	WWS	23.35±1.37	63.33±2.28	8.09±0.46
WWSd+GLWc	50%WWSd-50%GLWc	35.91±0.29	46.97±0.48	7.30±0.10

The results shown that sample GLWc (compost from green and lignocellulosic waste) is drier than sample WWSd (digestate), with TS content equal to 43.48±0.40% and 23.35±1.37%.for GLWc and WWSd respectively.

On this basis, these two raw samples provided a mixture with different physical and structural characteristics and with a TS content of 35.91±0.29%. This variability in samples characterization allowed to assess the organic matter content, and so biological stability degree, of a wide range of organic matrices.

5.2. Biological stability results

Table 2 shows biological stability results. It should be noted that DRI is reported both in terms of stability index and maximum biomass' temperature, which was measured during the overall experimental period. Moreover, the results obtained from the SOUR test are presented both as SOUR and OD20 index. Concerning the anaerobic test, either GS21 and BMP21 for biogas and methane residual production respectively are reported for completeness.

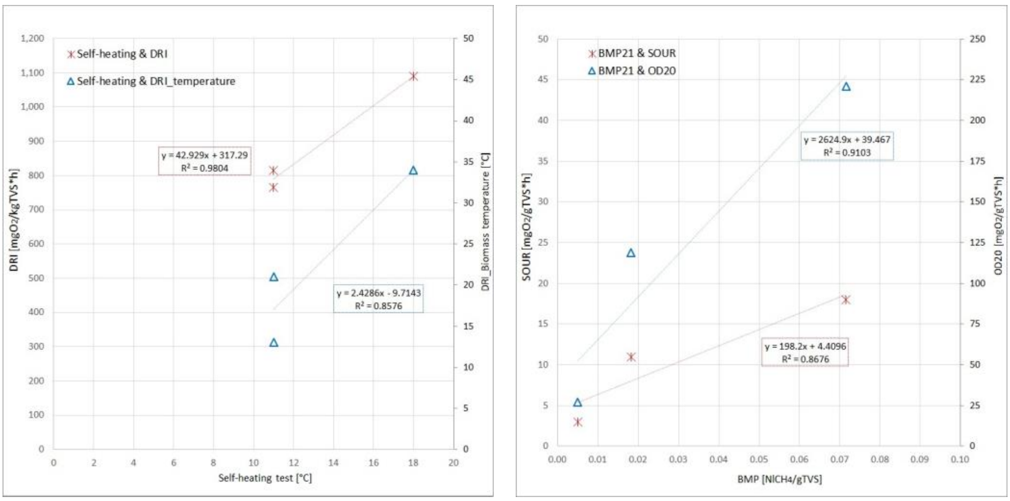
Table 2. Biological stability data expressed in terms of average values and standard deviations.

Respirometric test	*Raw samples*		*Mixture*
	GLWc	WWSd	WWSd+GLWc
*DRI [mgO$_2$/kgTVS*h]*	765±181	814±206	1,090±294
DRI_Biomass temperature [°C]	13	21	34
Self-heating test [°C]	11±1	11±3	18±2
*SOUR [mgO$_2$/gTVS*h]*	3±2	18±0	11±4
*OD20 [mgO$_2$/gTVS*h]*	27±3	221±0	119±34
GS21 [Nl biogas/gTVS]	0.01±0.01	0.14±0.00	0.04±0.00
BMP21 [Nl CH$_4$/gTVS]	0.00±0.00	0.07±0.00	0.02±0.00

All the results obtained in this study were compared with the stability threshold values corresponding to each individual method. As regard DRI index, reference is made to the limit value of Italian legislation of 1,000 mgO$_2$/kgTVS*h as this method is not recognized within the harmonized criteria for biological stability determination in the EU's fertilizers regulation. The maximum temperature measured during the experimental test can be considered as an indicator of the biological activity, in fact a high biological activity corresponds to a high process' temperature. Self-heating and residual biogas potential test are considered as stability criteria in the EU proposed regulation, therefore European threshold values are considered as reference. In order to obtain CE marking, a product must comply with the maximum temperature range of 20-30° C for Self-heating test or must meet the maximum residual biogas potential value of 0.45 Nl biogas/gTVS for anaerobic test. Finally, concerning SOUR test, there are no reference for stability determination. Therefore, literature data as considered as references. Scaglia et al., 2007 obtained SOUR values for compost and bio-stabilized products in the range of 2.6-9.0 mgO_2/kgTVS*h while Adani, et al., 2003 reported SOUR values for organic matrices between 3.0-19 mgO_2/kgTVS*h. Orzi et al., 2010 showed OD20 values between 94.0 and 264.0 mgO_2/kgTVS*h for digestate from anaerobic digestion process. It can be stated that the stability data obtained with respirometric tests are consistent with the reference limits.

5.2.1. Correlation analysis

Figure 5 shows the correlation analysis between stability data. In particular, in Figure 5a the correlation between Self-heating test & DRI and Self-heating test & DRI_Biomass temperature is presented. As it can be seen, there is a good correspondence among DRI and Self-heating values with a linear regression characterized by a R^2 value equal to 0.98. In addition to this, also the correlation between Self-heating test and the maximum biomass' temperature, reached during the DRI test, shows good results corresponding to a R^2 value of 0.87. These results demonstrate that these two tests are interchangeable each other providing the same data in terms of biological stability index. As regard Figure 5b, BMP21 & SOUR and BMP21 & OD20 are related. In both cases there is a good correlation between the data determined by the R^2 values equal to 0.91 and 0.87 for BMP21 & OD20 and BMP21 & SOUR respectively. These results mean that the amount of fresh organic matter in an organic matrix coincides with a residual biogas production that is directly proportional to the degree of decomposition of the substrate analyzed.



a) b)

Fig. 5. Correlation analysis: a) Self-heating test & DRI and Self-heating test & DRI_Biomass temperature, b) BMP21 & SOUR and BMP21 & OD20.

6. Concluding remarks

This work demonstrates the need to define homogenized regulations both at national and European level, in order to outline harmonized rules for biological stability determination. The results show that among the different stability tests analyzed in this work, there is a good linear correlation that allows to affirm a direct proportionality both between DRI and Self-heating test and BMP21 and SOUR test. In particular, the correlation among Self-heating test and DRI_Biomass temperature demonstrates that these two tests are interchangeable with each other, providing the same stability data referred to the process' temperature. The results suggest that all the respirometric methods considered in this study could be used interchangeably as indicators of the biological stability degree of an organic substrate. Nevertheless, future studies on stability index determination could be carried out in order to confirm the results obtained from this work.

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