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Article

Liquid-phase respiration activity assays to assess organic waste stability: A comparison of two tests

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Abstract: The stability of twenty seven composts and organic substrates (including raw, less stable and stable materials) was assessed using two different liquid phase tests were carried out. One of the tests was introduced in 1998 and was based on the calculation of a Specific Oxygen Uptake Rate (SOUR). The newly introduced liquid phase test presented here is simpler to set-up and to perform that the older liquid phase test. It is based on the quantification of oxygen consumption in the headspace of a BOD bottle that contains the liquid-solid solution. The results indicate that a marginal correlation does exist between the main indices calculated from both tests. The correlation was stronger for the indices calculated for stable-processed organics than for the raw (unprocessed materials). The SOUR ranged from 1520 to 3650mg O_2/kg VS-h for the raw materials and from 110 to 1150 mg O_2/kg VS-h for the processed materials, respectively. The corresponding stability rate related index ($LSRI_{24}$) of the new liquid phase test introduced here ranged from 240 to 1180 mg O_2/dry kg-h for the raw materials and from 64 to 792 mg O_2/dry kg-h for the processed ones.

Keywords: manometric tests; liquid phase test; SOUR; stability; organic substrates; respiration activity; respirometry

1. Introduction

According to the waste management hierarchy, landfilling of untreated wastes is the least preferable environmental option under the framework of sustainability. Existing legislation in European Union aims both to reduce the biodegradable wastes that are disposed of to landfills as well as to establish recycling systems, separate collection and mechanical and biological pre-treatment [1]. Composting is one of the classic biological treatment techniques applied worldwide to stabilize the biodegradable fraction of solid wastes. The composting end-product should be a stable and sanitized organic material, that contains carbon of low degradability, that could be applied to agricultural or arid land.

Stability can be defined as the extent to which readily biodegradable organic matter has decomposed [2]. This term is usually related to the resistance of organic matter against extensive degradation or against major microbiological activity [3]. The more stable an organic material is, the more resistant to microbial attack it is. Stability is typically assessed through respirometric techniques being a function of the microbial respiration activity. Microbial respiration activity is quantified either by monitoring the oxygen consumption or by measuring carbon dioxide production during a specific period of time. A wide research on respiration activity indices has been conducted over the years to quantify the extend of stability, and several analytical methods and indices have been proposed. Stability is differentiated from maturity, the latter expressing the effect of compost to plant or seed growth. Both stability and maturity are important compost quality parameters and should be assessed and reported together for all composted substrates.

Several respiration indices and methods have been proposed for assessing and evaluating compost stability; most of them are classified as static or dynamic methods [6, 8, 10, 11, 14, 15]. The above classification depends on whether there exists a continuous supply of air (dynamic) or not (static) into the experiment vessel. In dynamic respiration methods, oxygen uptake rate and carbon

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dioxide generation rates are determined by measuring the difference in O_2 and CO_2 contents in the air in the inlet and outlet reactor streams [4-8]. On the other hand, in static methods, sealed reactors of different size and volume are used and oxygen consumption is calculated through manometric principles [9-11], via the use of the ideal gas law, or by simply placing oxygen sensors within the reactors [9, 12, 13].

An alternative categorization of stability methods is into solid phase and liquid phase ones, depending on the state of the substrate which is undergoing analysis. In the solid phase methods, the substrate is placed as received into the reactor, without any previous pretreatment, but with an optimal moisture content to allow an active microbial degradation [7, 8, 14]. Sometimes, only small quantities of water or nutrients are added to the sample to optimize the degradation.

In liquid phase methods, solid substrates are being diluted into an aquatic solution (typically deionized laboratory grade water that contains nutrients) to form a "slurry". Oxygen consumption is measured via dissolved oxygen probes [2]. Due to the state of the sample, liquid tests are considered as static-based methods, since air does not pass continuously through the reactor [15, 16].

One of the pioneering liquid phase compost stability assessment techniques was proposed by Lasaridi and Stentiford [2]. They had proposed, then, a specific oxygen uptake rate (SOUR) to assess stability. The aforementioned test (herein referred to as the "SOUR test") required a controlled temperature water bath, an air pump, a dissolved oxygen (DO) probe and a data logging system. The duration of the experiment was approximately 30 h (in some case up to 60 h), while two aeration sequences were used depending on the biodegradability of each sample. Oxygen content was measured directly in the aqueous phase via oxygen probes, like in the old-fashioned BOD_5 tests applied to wastewater. Two respiration indices had been proposed by Lasaridi and Stentiford [2]; a maximum oxygen consumption rate (SOUR) and a cumulative oxygen demand for the first 20h (OD_{20}). Adani et al. [15] examined different organic substrates by adding a second cumulative index at 12h (OD_{12}) and by comparing SOUR, OD_{20} and OD_{12} with the corresponding indices derived from static and dynamic solid phase ests. According to Adani et al. [15] both static and dynamic solid phase indices correlated positively with the OD_{12}, OD_{20} and SOUR. Scaglia et al. [17] validated the method by measuring (among others) SOUR, OD_{12} and OD_{20} for six different organic samples, in three different laboratories. As stated by Scaglia et al. [17], the cumulative indices OD_{12} and OD_{20} were better to characterize biological stability, as opposed to SOUR, which was a "rate" index. Moreover, Stentiford [18] considers that specific oxygen uptake rate (as a liquid phase based index) is focusing not to reproduce the field conditions within a composting process but to evaluate what effect composting would have on a raw material, in a similar manner to the classic BOD_5 test used in wastewater.

Composting is actually a solid-state process in which transfer phenomena and air limitations are dominant factors that can affect the whole process. In the liquid phase methods, the water phase limits oxygen diffusion and transfer, since O_2 needs to first dissolve into the water and then to interact with the biomass attached onto the solid matrix. Thus, O_2 transfer is limited by the water saturation concentration. On the other hand, the solid phase stability tests are considered to simulate more accurately field conditions compared to the liquid-phase ones [19] since an oxygen transfer limitation is not expected, because the solid matrix comes in direct contact with the oxygen in the air. Adani et al. [15] also identified a significant dependence of SOUR on the water-soluble organic fraction of the samples.

A drawback of the liquid phase methods is the low amount (typically 3 to 8g) of sample required in the experiments [19]. To overcome this, several replications (ideally >=3) should be performed in liquid phase stability assessment methods, usually more than the solid-phase ones. Despite the above, liquid phase stability assessment techniques are occasionally preferred by researchers due to the need for use of small amounts of sample.

The assay described by Lasaridi and Stentiford [2] required a DO probe, that is used less frequently nowadays to measure BOD_5 in wastewater. This is because manometric based oxygen consumption tests have completely replaced the classic DO probe-based tests when measuring BOD_5 in wastewater. Thus, there is a need for an easier and faster to use liquid phase test that applies the manometric principles that are commonly applied in other compost stability tests (e.g. AT_4). A

manometric liquid phase method could be an alternative for use in real facilities since: a) it requires standard equipment and apparatus that is common in a typical wastewater laboratory; b) it is an easy and simple technique with a relatively low cost; c) it is based on the standard method of biochemical oxygen demand (BOD) determination, as used in typical wastewater analysis; d) it needs a shorter testing time (< 3 d) compared to the typical solid phase dynamic tests (5-7 d); e) it is a bench scale method. The oxygen transfer limitations of the liquid phase tests are compensated by the fact that those stability tests have a relative value, even though they do not simulate field conditions as accurately as the solid phase tests.

The objectives of this study were:

(i) to develop a new, simple to use, liquid-phase respirometric method using manometric principles, and

(ii) to compare the indices calculated by this new test with the indices calculated by the SOUR method that had been proposed by [2] in 1998 .

To achieve those objectives, we used 27 raw and processed organic substrates of variable origin and biodegradability. The respiration acitivities of all substrates were calculated using with both tests.

2. Materials and Methods

2.1. Substrates

Twenty seven (27) organic substrates from variable origin were used in this study so that to obtain a wide range of degradabilities and organic matter contents (see Table 1). The substrates included raw, short-term and long-term processed materials and were classified into two major categories here: "*Raw*" and "*Processed*" materials. In the latter case, some type of direct (e.g, composting) or indirect treatment process (i.e. storage) had been already applied.

The sampling procedure was performed using a sequential quartering process, followed by obtaining a grab sample from the final quarter. The final sample obtained was 2.5-3.0 wet kg. Immediately after sampling, organic substrates were stored at -20^{o}C. One day before analysis, samples were thawed at room temperature.

2.1.1. Raw Substrates

Five (5) raw organic substrates were evaluated. These were simulated food wastes (*R-FW1, R-FW2, R-FW3*), a dewatered sludge sample obtained from a nearby wastewater treatment facility (*R-DSL*) and a material (leaves, branches) obtained from a forest floor (*R-FOR*).

R-FW1, *R-FW2* and *R-FW3* were artificially prepared raw food wastes, with differences in the proportions of each ingredient. Simulated food wastes were selected to achieve substrates with high level of degradability, and for reasons of better homogeneity. *R-FW1* was prepared by mixing cooked pasta, white bread, chopped apples and grilled minced beef meat (25% each, on a wet weight basis, wb). *R-FW2* was simulated by mixing cooked meat and white bread in 50%-50% wb. *R-FW3* was prepared by mixing 50% of uncooked pasta, 30% of uncooked frozen fries and 20% of beef based dog food (wb). These three aforementioned substrates were sealed in a plastic bag after their preparation and remained in ambient temperature for a week to better simulate actual food wastes conditions.

R-DSL was a dewatered sludge derived from a nearby wastewater treatment plant (WWTP), where the thickened aerated sludge was dewatered via a filter-press. Sampling of the dewatered sludge was done as soon as the sludge was passed through the filter-press. *R-FORW* was a sample of leaves and small branches obtained from a forest floor during spring season, by collecting all the available material from a randomly selected 2 m^{2} surface.

2.1.2. Processed Substrates

Twenty-two (22) processed substrates were studied, some of which had already undergone some type of a treatment process, namely in a Mechanical Biological Treatment (MBT) plant for

municipal solid waste, in small-scale treatment facilities, after home composting or commercial composts. The processed substrates also included four cow manure derived composts (*P-MAN1, 2, 3, 4*), four MSW derived composts (*P-MSWC1, 2, P-OFMSW, P-MSWB*), two vermicomposts (*P-VERC1, 2*), and a sea-weeds derived compost (*P-SWC*) which is marketed as a commercial product. Moreover, eleven home composts samples were also collected and studied (*P-HC1 to P-HC11*), with a minimum of 6 months processing.

P-MAN1 was a fresh -cow derived- manure, stored in a in static pile for one month, which was obtained from a cattle farm. *P-MAN2* was a fresh animal manure, collected from a different cow breeding facility, that consisted of cow manure and straw. It was stored in an open pile for one week prior to sampling. *P-MAN3* was also a mixture of cow manure and straw. It was obtained from the same facility, as previously mentioned *P-MAN2*, after passing through a solid–liquid separator and after stored in piles for 1 week. Above materials were considered processed since they had been stored for at least one week at the time of sampling.

P-MSWC1 was a compost derived from the undersized fraction of commingled MSW. It was obtained from a full scale MBT plant after 18 h pre-treatment, followed by a 6-week negative aeration composting phase in agitated channels and a 5-6 weeks curing stage in static windrows. *P-MSWC2* was also a compost derived from undersized fraction of commingled MSW but from a different Greek MBT plant. The processing line included in-vessel active composting with pre-selection units for 6 weeks, and curing phase in open piles for at least 4 weeks. *P-OFMSW* was an end-product obtained from a full scale MBT facility which treated the organic fraction of commingled MSW, after 28 days of aeration. *P-MSWB* was the undersized fraction (<50 mm) of commingled MSW that were wrapped in bales for 12 months, in an interim storage facility.

P-VERC1 and *P-VERC2* were vermicomposts derived from mixtures of dewatered wastewater sludge and straw after composting times for around 100 and 30 days, respectively. The samples were obtained from the same vermicomposting facility. *P-SWC* was a sea weeds derived compost, mainly consisting of sea weeds (80% wb), cow manure (20% wb) and other agricultural by-products. The composting process lasted approximately two months, including curing. It was sold as a commercial end-product compost that was purchased from a local agricultural store.

P-HC1 was a home compost produced by kitchen and garden waste mixed with a wood combustion residues, such as coal and ash. Active composting phase was done in a plastic home composter bin, while sampling was conducted after one year of composting and curing. *P-HC2* was a home compost mixture of kitchen waste (60% wb) and wood chips (40% wb), which was produced after 21 days of active in-vessel composting and 14 days of open aired curing. Substrates *P-HC3* to *P-HC11* were also home composts, with composting times that varied from 6 to up to 12 months. The initial mixture consisted of daily kitchen wastes (fruits and vegetables, eggshells, coffee grounds), garden wastes (yard trimmings and grass clippings), nut shells, wood chips, ash, sawdust and straw. The composting for substrates *P-HC3* to *P-HC11* was achieved in 275 L plastic composters, while moisture addition and manual mixing of the material were performed at least once a week.

All substrates are included in Table 1.

2.2. Initial substrate characterization

Initial characterization of the substrates included measurements of moisture, organic matter, pH and elemental analysis quantification. The moisture content was measured by drying the material at 75°C until constant weight [20]. Organic matter (OM) or volatile solids (VS) content was measured in a muffle furnace, through the loss on ignition (LOI), at 550°C after 2 h [20, 21]. pH was measured in a 5:1 liquid:solid (L/S) ratio by adding deionized (DI) water to the initial wet samples [20]. For the calculation of the empirical formulas, elemental analysis was performed according to the procedure described in Komilis et al. [22]. Oxygen content was calculated indirectly after subtraction of the sum of the C, H, N contents from the organic matter content (db).

197 *2.3. Manometric liquid phase test*

198 In the newly introduced manometric liquid phase test used here, microbial respiration activity
199 (MRA) was quantified by measuring pressure drops in the headspace of a sealed bottle for 7 d under
200 controlled conditions. The WTW® apparatus that was used for the manometric measurements
201 included: BOD glass-made bottles with total operating volume of 520 mL, manometric heads
202 (OxiTop-C/B®), an infrared controller and a stirring plate.
203 The methodology adopted was based on the standardized method for determining BOD in
204 wastewater liquid samples, which is described below: approximately 8 g of wet samples were
205 diluted in 220 mL of deionized water, in each bottle. Four different nutrient solutions were added as
206 follow: 15 mL of phosphate buffer (without NH_4Cl), 5 mL of magnesium sulfate, 5 mL of calcium
207 chloride and 5 mL of ferric chloride. Moreover, 0.5 mL of allylthiourea solution (5 g/L, ATU) were
208 also added in the bottles as a nitrification inhibitor. Nutrient solutions were prepared according to
209 APHA and AWWA [21]. In the nozzle of each bottle, a rubber stopper was filled with 8 to 10 pellets
210 of potassium hydroxide (KOH) to sorb CO_2. A magnetic stirrer bar was placed in the bottom of each
211 bottle to agitate the slurry.
212 Respirometers were tightly closed with the manometric head and were placed in a pre-heated
213 incubator at 35°C for 7 days at the absence of light. Manometric heads recorded instantaneous
214 pressure values every 28 min for 7 days. Four to eleven replications (usually 8) were performed per
215 substrate simultaneously. Manual aerations were performed by removing the manometric heads
216 daily and prior to reaching a 100 mbar internal pressure into the bottle.
217 Oxygen consumptions were calculated using the principles of the ideal gas law, via the
218 pressure drops recorded and logged separately for each bottle, as described in Equation (1) [10]:

$$\Delta O_{2,t} = \frac{\Delta P_t * V_{free} * 32}{R * T * W_{sub}} \quad (1)$$

219 where: $\Delta O_{2,t}$: mass of oxygen consumed at time interval t per dry mass of the substrate (g/dry kg) or
220 per initial volatile solids mass of the substrate (g/kgVS); ΔPt: pressure decrease over the time
221 interval t (mbar); V_{free}: free air volume in the respirometer (L); *32*: molecular weight of oxygen
222 (g/mole); R: universal gas constant (83.14 L*mbar/°K-mol); T: incubation temperature (°K); W_{sub}:
223 initial dry mass (kg) or initial volatile solids mass of the substrate (kg VS) placed in the respirometer.
224 The respiration activity indices that were calculated from the manometric liquid phase method,
225 were calculated using a similar methodology to the calculation of the solid-phase static test indices,
226 as presented in [10, 11, 23]. That is, a liquid cumulative respiration index (LCRI) was calculated as
227 the sum of all ΔO_2 values found by Equation (1) after 7 days. $LCRI_7$ was expressed in g O_2/kg VS,
228 which is the total amount of O_2 consumed at 7 days. In addition, a liquid static respiration index
229 (LSRI) was also calculated over the 24 h period of highest biological activity ($LSRI_{24}$). $LSRI_{24}$ was
230 defined as the maximum average O_2 consumption rate that would occur over a 24 h period, and was
231 expressed in mg O_2/kg VS-h.
232 The biodegradability of each substrate was also expressed using a Biodegradable Fraction (BF),
233 calculated by Equation 2:

$$BF = \frac{LCRI_7}{ThOD_T} \quad (2)$$

234 where: *BF*: biodegradable decimal fraction after 7 d (BF<1); $LCRI_7$: mass of oxygen consumed after 7
235 days per dry mass of the substrate (g O_2/dry kg); ThOD_T: theoretical oxygen demand (g O_2/dry kg),
236 calculated through stoichiometry using the complete oxidation chemical equation $C_aH_bO_cN_d +$
237 $\left(\frac{4a+b-2c+3d}{4}\right) O_2 \rightarrow aCO_2 + \left(\frac{b-3d}{2}\right) H_2O + dNH_3$ and the corresponding empirical formula for each
238 substrate.

Table 1. Source materials and description of the organic substrates used

Category	Substrate	Type	Description and process applied
Raw	R-FW1	Food waste	Simulated mixture of apples, cooked meat, boiled pasta, bread (25% each, in wb).
	R-FW2	Food waste	Simulated mixture of cooked meat, bread (50% each, in wb).
	R-FW3	Food waste	Simulated mixture of uncooked pasta, uncooked fries, beef based dog food (50%, 30%, 20%, in wb).
	R-DSL	Dewatered sludge	Dewatered sludge derived from a WWTP
	R-FOR	Forest derived material	Leaves and small branches collected from a forest floor
Processed	P-MAN1	Cow manure	Fresh cow manure. Prepared in static piles for 1 month.
	P-MAN2	Cow manure and straw	Mixture of fresh cow manure and straw. Stored for 1 week in an open pile
	P-MAN3	Cow manure and straw	Mixture of fresh cow manure and straw. 1 week old, after passing through a solid-liquid separator.
	P-MAN4	Cow manure compost	Mixture of fresh com manure and straw. 10 months active composting period.
	P-MSWC1	MSW derived compost	Compost derived from commingled MSW, in a mechanical and biological aerobic pretreatment facility.
	P-MSWC2	MSW derived compost	Compost derived from screened and undersized fraction of commingled MSW, in a MBT plant.
	P-OFMSW	Organic fraction of MSW	Organic fraction of municipal solid wastes. Aeration for 28 days in a MBT plant.
	P-MSWB	MSW wrapped in bales	Commingled MSW wrapped in bales. Sampling after 1 year of storage within the bales.
	P-VERC1	Vermicompost	Mixture of dewatered wastewater sludge with straw. Vermicomposting process.
	P-VERC2	Vermicompost	Mixture of dewatered wastewater sludge with straw. Vermicomposting process.
	P-SWC	Sea weeds derived compost	Prepared from 80% sea weeds and 20% cow manure and other agricultural products
	P-HC1	Home composts	Mix of kitchen and garden waste and ash. 6 months composting and 6 months curing.
	P-HC2		Mix of kitchen waste and wood chips. 3 weeks forced aeration and 2 weeks curing.
	P-HC3		Mix of kitchen waste, garden waste, wood chips, ash, sawdust and various household organic amendments. Composting for a period between 6 to 12 months, in home composters.
	P-HC4		
	P-HC5		
	P-HC6		
	P-HC7		
	P-HC8		
	P-HC9		
	P-HC10		
	P-HC11		

2.4. SOUR liquid phase test

This liquid phase test had been initially proposed by Lasaridi and Stentiford [2], based on the typical BOD analysis used for wastewater samples. Approximately 8 g of the as received substrates were used as the initial wet mass. The same nutrient solutions (as stated in section 2.3.) were added to each flask to ensure that nutrients or pH were not limiting. The SOUR liquid test used here was set-up according to Lasaridi and Stentiford [2] with the following modifications: the flasks were placed in a temperature-controlled incubator at 35°C for 2 days at the absence of light, instead of a water bath; the intermittent aeration cycle was 15 minutes aeration (pumps in operation) followed by 30 min of no aeration.

In this test, microbial respiration activity is quantified directly by measuring dissolved oxygen (DO) into the solution. The DO probes recorded dissolved oxygen concentration with an interval of 30 sec, throughout the 48 h experimental period. Three to eight replicates per substrate were carried out. Two indices were calculated after processing the DO concentrations obtained from the data logging procedure; the specific oxygen uptake rate (SOUR) expressed as mg O_2/kg VS-h and the cumulative oxygen demand after 48 h (OD_{48}) expressed in mg O_2/kg VS.

The oxygen uptake rates (OUR) were calculated via the DO concentration drops in the slurry during the 30 min non-aeration period. This OUR value was calculated as an average of around 60 individual uptake rates, during every 30 min period of non-aeration. The specific oxygen uptake rate (SOUR) was calculated according to (3), as also suggested in [2, 15]:

$$SOUR = \frac{|S_{max}| * V}{m * DM * VS} \quad (3)$$

where: *SOUR*: specific oxygen uptake rate (mg O_2/kg VS-h); $|Smax|$: the absolute maximum oxygen consumption slope (mg O_2/L-h) during the 48h duration of the experiment; *V*: volume of the suspension (L); *m*: wet mass of the substrate (kg); *DM*: decimal fraction of dry matter (wb); *VS*: decimal fraction of volatile solids (db).

The cumulative oxygen demand after 48 h (OD_{48}) was calculated according to Lasaridi and Stentiford [2], as the area below the SOUR curve and 48 h period, using the following Equation (4):

$$OD_{48} = \frac{1}{1000} * \frac{V}{m * DM * VS} * \int_{t=0}^{t=48} |S|_t * dt \quad (4)$$

where: OD_{48}: cumulative oxygen demand after 48 h (g O_2/kg VS); $|S|_t$: rate of oxygen consumption at time t (mg O_2/L-h); the other parameters were defined in Equation (3).

3. Results and discussion

3.1. Substrates' characterization

Results for the initial characterization of the substrates are reported in Table 2, including moisture and organic matter content, pH, C/N ratio and the calculated empirical formulas. The as-received moisture contents of the nine raw materials ranged from 40% (wb) for R-MAN3, to 82% (wb) for the R-DSL. The range of the corresponding moisture content for the processed substrates was even wider; two MSW derived materials (P-MSWC1 and P-MSWC2) were below 10%, while the rest varied from 22% (wb) to almost 65%. These variations over the moisture content are attributed not only to the different origin of each substrate (raw or processed) but also to the variety of process applied (static piles, enclosed composting systems), duration and curing or not of the final end-products.

During the composting process, the organic matter content decreased steadily due to the reduction of available carbon sources and easily biodegradable organic fraction. Depending on the selected composting process, availability of easily degradable carbon and duration of the three main degradation phases (mesophilic, thermophilic and curing), organic matter reduction rates may vary for substrates of different origin and composition. As shown in Table 2, initial volatile solids for the majority of raw substrates were above 80 % (db). Food waste (*R-FW1*, *R-FW2* and *R-FW3*) volatile

284 solids content ranged from 95% to 98% (db) while in two raw manures the corresponding values
285 were around 31% (db). As for the processed substrates most of their organic matter content was
286 below 50%, while only two of them (*P-VERMC1* and *P-HC2*) had relatively high values of 57% and
287 93%. However, organic matter content measured as volatile solids should not be used itself as an
288 index or predictor of biodegradation activity [11], since a limit value cannot be fixed for expressing
289 compost stability [24]. Moreover, volatile solids (as measured at 550°C) do not coincide with the
290 biodegradable organic matter since they can contain recalcitrant to biodegradation carbon,
291 depending on the substrate.

292 **Table 2.** Initial properties of the 27 organic substrates used in the study

Substrate[1]	Moisture (% wb)[+]	Volatile Solids (% db)[-]	pH[^]	C/N[*]	Empirical formula[*]
R-FW1	61% ± 1.7%	96% ± 0.6%	5.4± 0.0	13.8 ± 1.6	$C_{16}H_{34.8}NO_{10.6}$
R-FW2	49% ± 0.2%	95% ± 2.8%	6.0 ± 0.0	7.7 ± 1.6	$C_{8.7}H_{20.6}NO_{4.9}$
R-FW3	40% ± 3.6%	98% ± 0.1%	5.1 ± 0.0	19.6 ± 1.1	$C_{22.9}H_{49.4}NO_{24.9}$
R-DSL	82% ± 0.2%	69% ± 0.1%	n/a	8.2 ± 0.2	$C_{9.6}H_{18.4}NO_{4.2}$
R-FOR	73% ± 0.4%	81% ± 0.9%	6.2 ± 0.2	33.9 ± 3.1	$C_{39.5}H_{81}NO_{35.1}$
P-MAN1	41% ± 1.3%	30% ± 1.6%	n/a	15.0 ± 0.7	$C_{17.5}H_{14.2}NO_{11.5}$
P-MAN2	79% ± 0.2%	93% ± 0.6%	8.3 ± 0.1	8.2 ± 0.2	$C_{9.6}H_{18.4}NO_{8.9}$
P-MAN3	40% ± 0.8%	32% ± 0.3%	8.1 ± 0.0	11.3 ± 0.9	$C_{13}H_{24.6}NO_{5.5}$
P-MAN4	41% ± 0.2%	34% ± 1.0%	n/a	7.6 ± 0.8	$C_{8.8}H_{11.8}NO_{10.8}$
P-MSWC1	8% ± 0.2%	49% ± 1.1%	n/a	14.6 ± 2.0	$C_{17}H_{23.5}NO_{8.7}$
P-MSWC2	6% ± 0.0%	45% ± 1.5%	n/a	10.7 ± 1.0	$C_{12.4}H_{21.6}NO_{10.8}$
P-OFMSW	22% ± 2.4%	23% ± 4.3%	7.7 ± 0.1	21.9 ± 5.2	$C_{24.4}H_{22.5}NO_{2.3}$
P-MSWB	44% ± 1.9%	56% ± 1.1%	n/a	45.2 ± 4.9	$C_{51.9}H_{88}NO_{35.9}$
P-VERC1	46% ± 0.5%	41% ± 1.1%	n/a	14.8 ± 1.0	$C_{17.2}H_{33.1}NO_{13.8}$
P-VERC2	42% ± 0.5%	57% ± 0.2%	n/a	8.6 ± 0.2	$C_{10}H_{16.9}NO_{6.3}$
P-SWC	25% ± 0.2%	12% ± 0.0%	n/a	50.9 ± 10.1	$C_{57.5}H_{17.9}NO_{11.9}$
P-HC1	65% ± 0.4%	42% ± 0.6%	8.8 ± 0.1	24.6 ± 3.9	$C_{28}H_{23.9}NO_{12}$
P-HC2	62% ± 1.4%	93% ± 0.3%	7.2 ± 0.1	39.5 ± 5.3	$C_{45.3}H_{68.8}NO_{26.6}$
P-HC3	51% ± 0.2%	26% ± 1.7%	9.4 ± 0.0	14.3 ± 1.7	$C_{17.7}H_{24.1}N_{1.1}O$
P-HC4	54% ± 1.0%	37% ± 0.3%	9.2 ± 0.0	15.0 ± 2.0	$C_{17.4}H_{22.4}NO_{9.4}$
P-HC5	45% ± 0.1%	20% ± 0.8%	9.7 ± 0.0	18.4 ± 4.5	$C_{21}H_{17.5}NO_{11.1}$
P-HC6	44% ± 0.7%	22% ± 1.3%	9.5 ± 0.0	14.4 ± 3.1	$C_{21.8}H_{26.4}N_{1.4}O$
P-HC7	39% ± 1.7%	26% ± 1.1%	8.4 ± 0.1	12.2 ± 1.9	$C_{14.1}H_{19.7}NO_{5.8}$
P-HC8	35% ± 3.4%	33% ± 0.9%	9.4 ± 0.0	14.7 ± 4.3	$C_{16.5}H_{25.4}NO_{7}$
P-HC9	24% ± 0.2%	13% ± 0.5%	8.4 ± 0.0	11.8 ± 1.5	$C_{13.7}H_{14.7}NO_{3.7}$
P-HC10	51% ± 0.1%	26% ± 0.4%	7.3 ± 0.0	10.1 ± 1.1	$C_{11.7}H_{16.1}NO_{6.1}$
P-HC11	26% ± 0.9%	22% ± 0.9%	7.9 ± 0.1	15.1 ± 2.5	$C_{17.1}H_{27.8}NO_{3.7}$

293 All values are averages ± standard deviations; *wb*: wet weight basis; *db*: dry weight basis; [+]: n=3; [-]: n=5; [^]:
294 n=2; [*]: n=6; *n/a*: not available; [1]: R- indicates raw substrate and P- indicates processed substrate.
295
296 In this study, pH ranged from 5.1 to 8.3 for the raw materials, while all the processed substrates
297 had pH above 7.2. Both ranges of the initial and final composting samples are in agreement with
298 findings of previously published research [3, 16, 23, 25, 26]. End products that were sampled from
299 home composters (*P-HC2* to *P-HC11*) had a pH between 7.3 and 9.7.
300 The initial C/N ratio of the studied substrates, as measured through elemental analysis, found
301 to vary for both categories. For the raw materials, total C/N ratio was between 7.7 and 34, while for

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the processed ones ranged from 7.6 to 25 (with the exception of three values of 40, 45 and 51). In this context, Puyuelo et al. [27] had measured total C/N ratios for MSW, for the organic fraction of MSW and for raw sludge equal to 34, 22 and 6.3, respectively. These values are comparable to the C/N ratios of substrates *P-MSWB* (45.2), *P-OFMSW* (21.9) and *R-DSL* (8.2).

3.2. Manometric liquid phase indices

Typical cumulative oxygen consumption profiles from the manometric liquid phase test are included in Figure 1. Solid lines represent the seven replicates for a raw (R-FW3) and the eight replicates for a processed (P-HC9) substrate. Oxygen consumption for raw materials (e.g. R-FW3) starts immediately after the initiation of the experiment, without any lag phase. Raw substrates had higher O_2consumption rates during the first 2 days and lower rates thereafter until 7th day. On the other hand, in the processed substrates (e.g. P-HC9) the consumption curveswere flatterand lower than the raw materials. The same graphical trend for different substrates has been also found in other studies that dealt with solid phase static stability methods using manometric principles [10, 28].

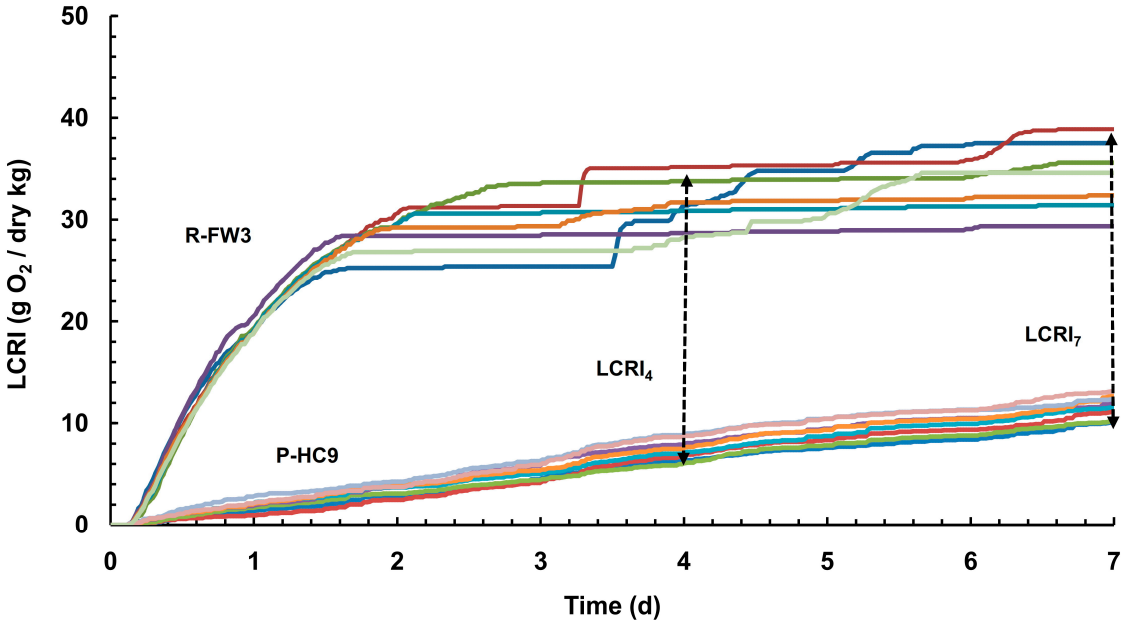

Figure 1. Cumulative oxygen consumption profiles of raw (R-FW3) and processed (P-HC9) substrates for the manometric liquid phase test.

Figure 2 depicts the derived respirometric indices of manometric liquid phase test for all the raw and processed substrates. Fig. 2i shows all the $LCRI_7$ values of the studied substrates, expressed on a per dry kg basis (orange bars) and on a per kg volatile solids basis (grey bars). Maximum $LCRI_7$ values for raw materials were around 60 g O_2/dry kg, whilst all the processed substrates were below 35 g O_2/dry kg. Differentiations between the cumulative indices when expressed in a per dry basis or per volatile solids basis, especially in the processed substrates, may be attributed to the different easily biodegradable organic fraction contents per substrate. Organic matter is not differentiated into readily and not readily biodegradable fraction here.

The same trend is evident in Figure 2ii, which depicts the oxygen consumption rate over 24 h rate ($LSRI_{24}$) of the manometric test, expressed both in a per dry kg basis (orange bars) and in a per organic matter kg basis (grey bars). The highest $LSRI_{24}$ values were attributed to the dewatered sludge (1179 mg O_2/dry kg-h or 1714 mg O_2/kg VS-h) and the food waste (897 mg O_2/dry kg-h or 920 mg O_2/kg VS-h). On the other hand, most of the processed substrates had values below 200 mg O_2/dry kg-h.

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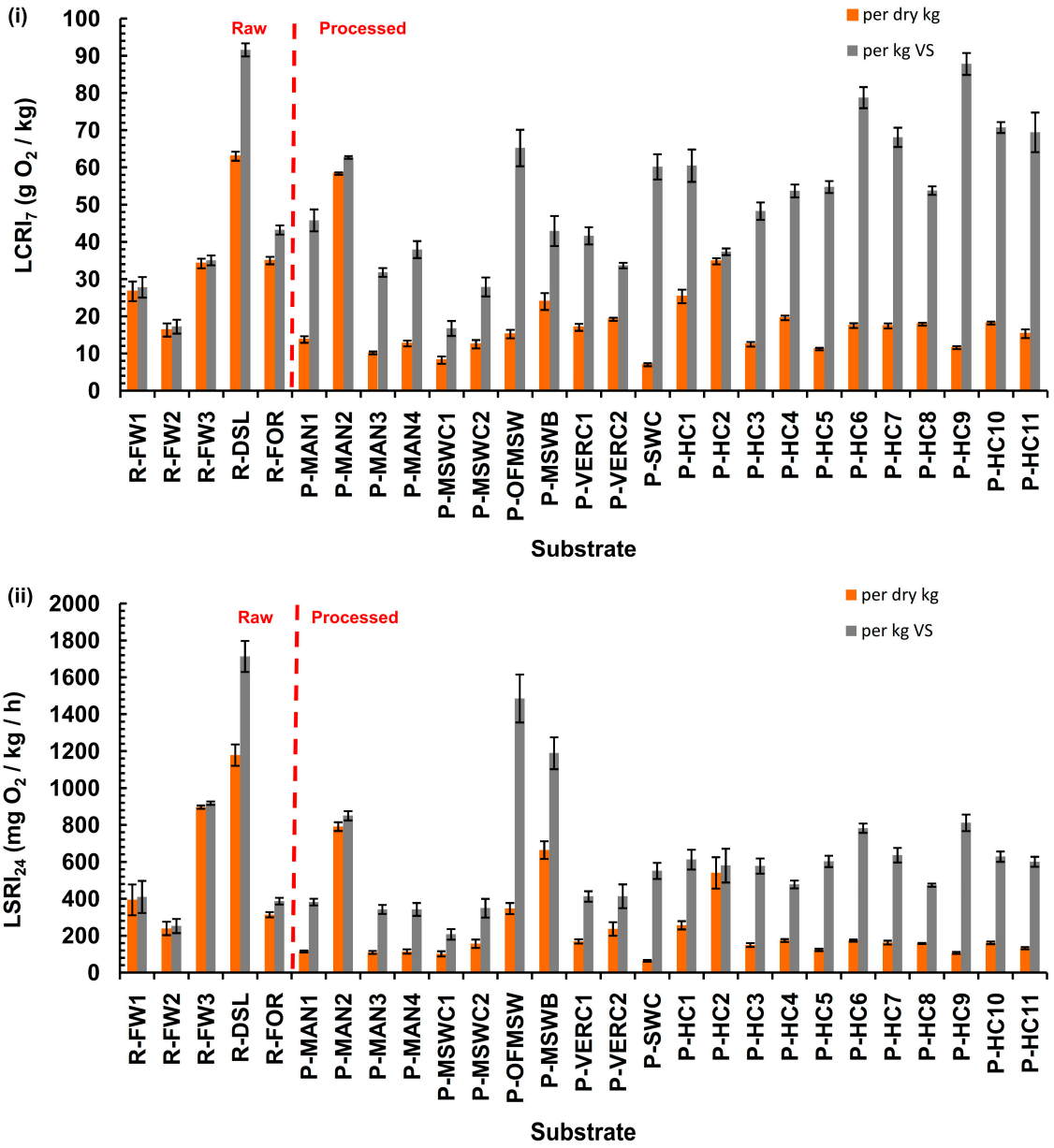

Figure 2. Respirometric indices derived from the manometric liquid phase test for raw and processed substrates; (i) cumulative oxygen consumption index after 7 days ($LCRI_7$), and (ii) maximum oxygen consumption rate over a 24h period ($LSRI_{24}$). In each graph indices are expressed both per dry kg and per organic matter basis.

The biodegradability of studied substrates was also expressed using the Biodegradable Fraction (BF), as calculated by Equation (2). BF values varied from 0.009 (0.9%) to 0.035 (3.5%) for the raw substrates, while the range for the processed substrates was slightly higher, namely from 0.01 (1%) to 0.05 (5%). These relatively low BF values can be attributed to the fact that the denominator of the ratio (theoretical oxygen demand; $ThOD_T$), is calculated based on the notion that all total carbon measured via elemental analysis is biodegradable and eventually mineralizable to CO_2. This is definitely not the case for any organic substrate. On the contrary, the nominator of the BF ($LCRI_7$) is based on actual testing conditions, and thus indirectly accounts for the biodegradable carbon that consumes oxygen. The difference in the ranges of BF between the raw and processed materials indicates that the latter have a higher percentage of easily degradable carbon since a microbial degradation has been already initiated, leading to an initial hydrolysis of the raw organic. This microbial hydrolysis generates readily degradable organic compounds of lower molecular weight (i.e. VFAs, monosaccharides) that can be easily further degraded. This microbial degradation can

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take place regardless of whether the (pre)processing is a targeted treatment or a result of simple storage.

3.3. SOUR test indices

Respirometric indices for the liquid SOUR test are shown in Figure 3.

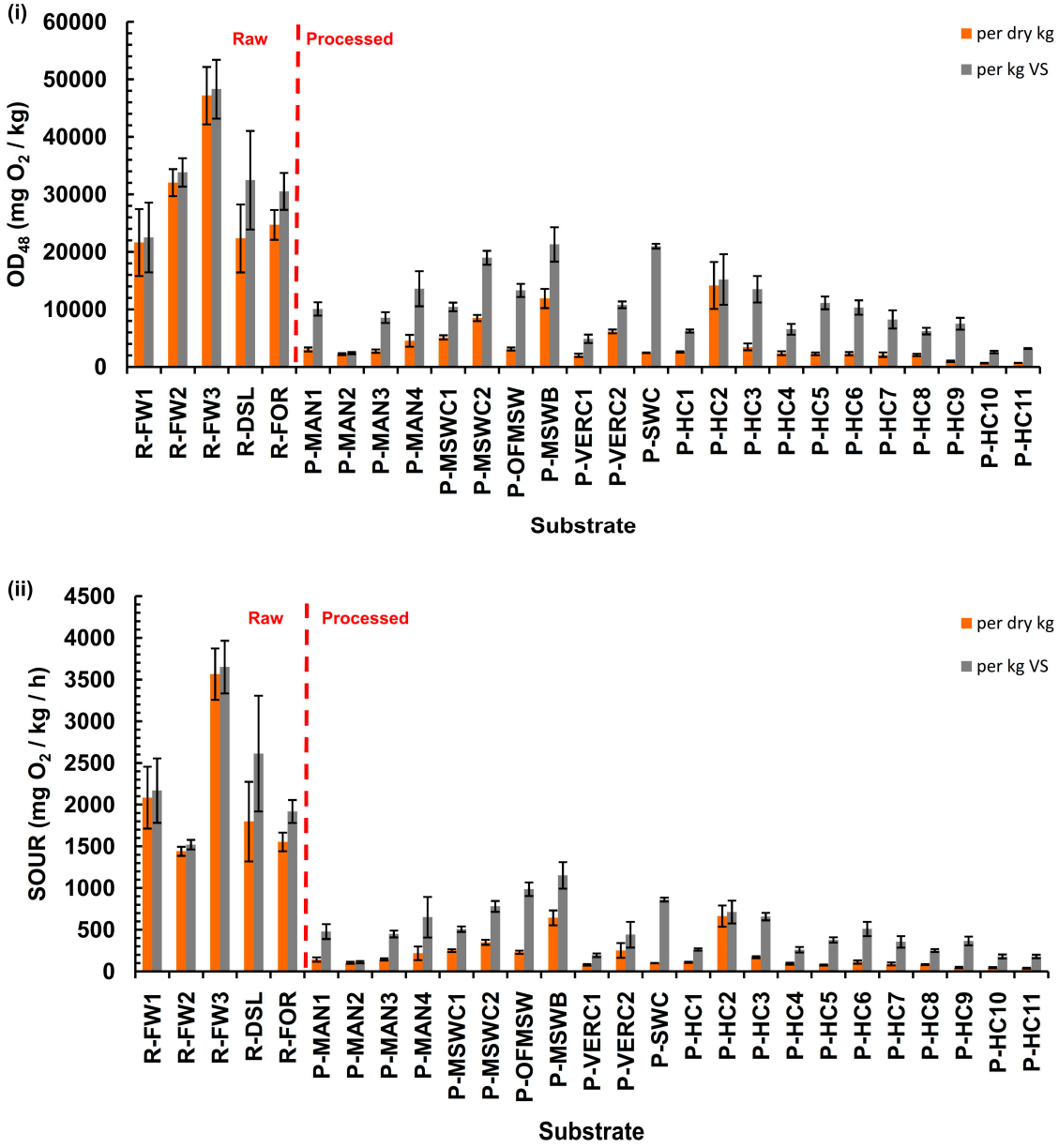

Figure 3. Respirometric indices of SOUR test for raw and processed substrates; (i) total oxygen demand after 48 hours (OD_{48}), and (ii) maximum oxygen uptake rate (SOUR). In each graph indices are expressed both per dry kg and per organic matter basis.

The cumulative oxygen demand index after 48h (OD_{48}) for all the studied substrates is shown in Figure 3i. Figure 3ii shows the corresponding peak rate indices over the whole duration of the test. All the processed substrates had SOURs below approximately 1000 mg O_2/kg VS-h. This was a limit that hadbeen initially proposed by Lasaridi and Stentiford [2] to differentiate "*well matured, biosolids composts*" from other less stable material, based on measurements of sludge composts obtained from turned windrows. In the study of Adani et al. [15], the SOUR of the end-products was higher and varied from 2940 to 8300 mg O_2/kg VS-h, while substrates that had been sampled at the middle of the process had SOUR between 11000 and 12400 mg O_2/kg VS-h. Scaglia et al. [17] used substrates

372 derived from household wastes and the organic fraction of MSW, reporting high degree of biological
373 stability for SOUR values around 2600 mg O_2/kg VS-h. The limit that was proposed by Adani et al.
374 [15] and Scaglia et al. [17] for materials of medium stability was 7000 mg O_2/kg VS-h, which was
375 derived from the corresponding dynamic respiration index (DRI) stability limit of 1000 mg O_2/kg
376 VS-h. However, this limit value was based only on MSW and household derived samples.
377 In our work, the five raw substrates had SOURs from around 1500 to 3700 mg O_2/kg VS-h, being
378 below the limit of 7000 mg O_2/kg VS-h which classifies a substrate as "*medium processed*".

379 *3.4. Correlation analysis*

380 A statistically significant linear Pearson correlation coefficient (r=0.63) was calculated between
381 $LCRI_7$ and $LSRI_{24}$ (n=184). This has been also demonstrated by Evangelou et al. [28], using the same
382 indices but with a static solid-phase respirometer. That is, as the total O_2 consumption increases, the
383 maximum rate of O_2 consumption increases too. A statistically significant Pearson correlation
384 coefficient (r=0.89) was also calculated between OD_{48} and SOUR (n=151), which is also in agreement
385 with the findings of Scaglia et al. [17]. This statistically significant correlation suggests that both
386 SOUR and OD_{48} can be used as indicators for the assesment of biological stability. The same
387 information is revealed with regard to the manometric test, since both the O_2 consumption and the
388 rates are highly correlated.
389 Figure 4 graphically depicts the correlations among the biodegradation indices for each of the
390 two liquid tests. Correlations were checked among rates (Fig 4i) or among cumulative values (Figure
391 4ii) separately for the raw and processed substrates. As demonstrated, a graphical positive
392 correlation trend exists only for the rate indices, namely the SOUR and the $LSRI_{24}$ (Figure 4i). On the
393 other hand, no significant correlation was found between the cumulative indices of the two tests
394 (Figure 4ii).

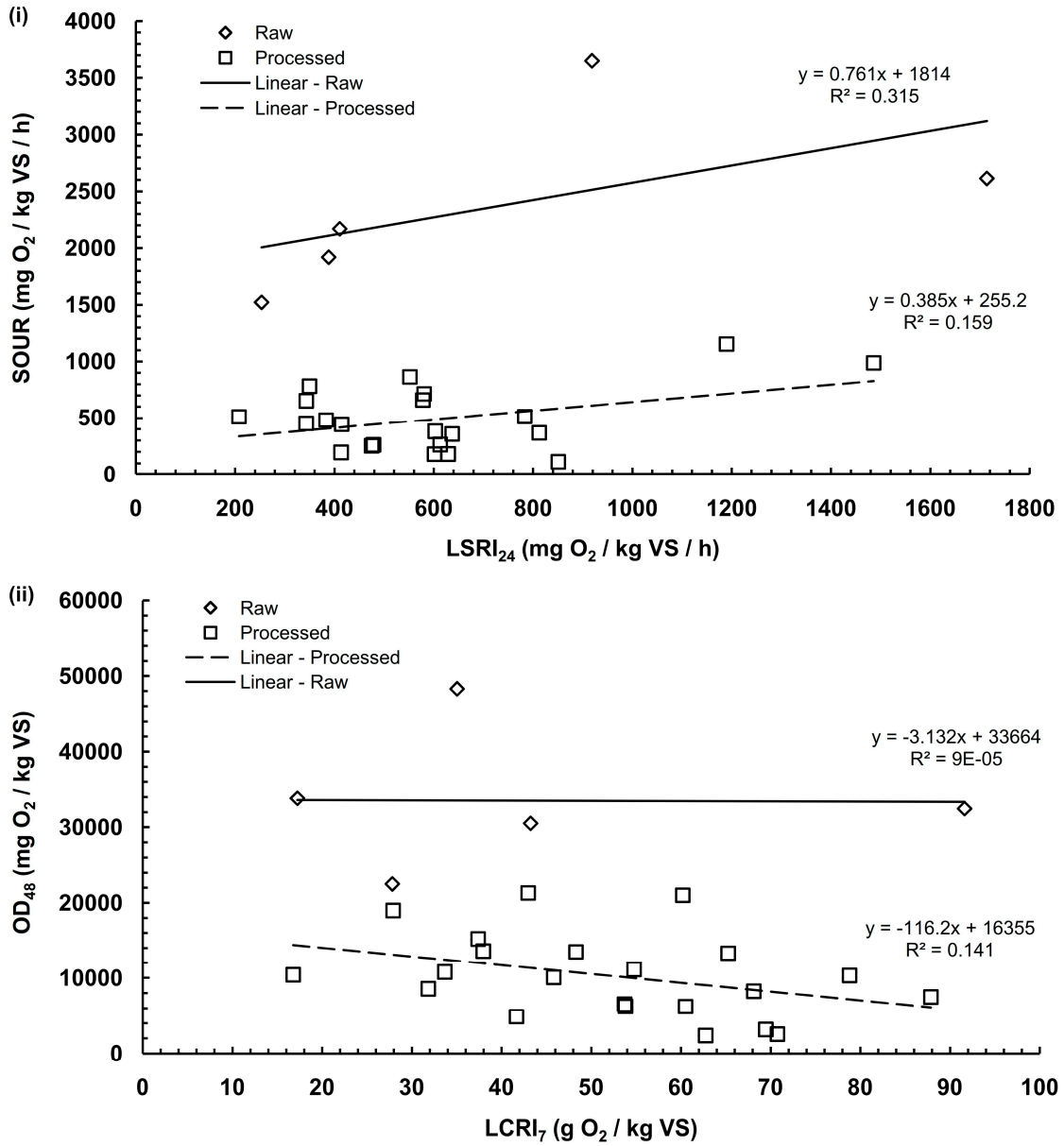

Figure 4. Correlation plots between indices of the two tests: (i) SOUR (SOUR test rate) vs $LSRI_{24}$ (manometric test rate); (ii) OD_{48} (SOUR test cumulative index) vs $LCRI_7$ (manometric test cumulative index).

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Table 3 includes all the statistically significant (at $p<0.05$) Pearson correlation coefficients among the four respiration indices. Statistical analysis revealed that the only significant correlation was achieved between the oxygen consumption rates (SOUR and $LSRI_{24}$) of the raw substrates, with a calculated coefficient of r = 0.459 (despite the small amount of the substrates being n=5). On the contrary, both SOUR test's indices (OD_{48} and SOUR) found to be correlated negatively with $LCRI_7$ (with Pearson's coefficients equal to -0.360 and -0.326, respectively).

Table 3. Linear Pearson's correlation coefficients among all respiration activity indices for raw and processed substrates

	Raw substrates		
	$LCRI_7$	**$LSRI_{24}$**	**OD_{48}**
$LSRI_{24}$	0.830		
OD_{48}	n/s	n/s	
SOUR	n/s	0.459	0.603

	Processed substrates		
	$LCRI_7$	**$LSRI_{24}$**	**OD_{48}**
$LSRI_{24}$	0.617		
OD_{48}	-0.360	n/s	
SOUR	-0.326	n/s	0.857

All Pearson's correlation coefficients shown are significant at $p < 0.05$; correlations were based on a total sample size of n = 154 to 181; n/s: non-significant

$LCRI_7$: cumulative oxygen consumption index at 7 days; $LSRI_{24}$: maximum oxygen consumption rate over the 24 h period of highest biological activity; OD_{48}: cumulative oxygen demand after 48 h; SOUR: specific oxygen uptake rate.

A correlation was calculated using the average values of all indices per material used and the initial chemical characteristics of all substrates (VS, C,N). The sample size in this case was n=27. The Spearman correlation coefficients (ϱ) were chosen here since they can reveal any type of correlation and not only the linear one, as in the case of Pearson coefficient. The Spearman's ϱ among $LCRI_7$-VS, $LSRI_{24}$-VS, OD_{48}-VS and SOUR-VS ranged from 0.67 to 0.76 and were all statistically significant at $p<0.05$. Despite the above statistical finding, the graphs below reveal that those correlations are not very strong in the high values of VS (see Figure 6). For example, the graph of OD_{48} and VS reveals that there were at least 5 samples with a VS content of more than 90% db that had corresponding OD_{48} values between 200 to 48000 mgO_2/kg VS. This is not an indication of a strong correlation despite the fact that the corresponding Spearman correlation coefficient was 0.723 and statstically significant at $p<0.05$ for that pair of variables. Other researchers [6, 29] have also not found significant corellations between volatile solids and static respiration indices. For example, Bayard et al. [30] found that neither organic matter content nor cellulose and hemicellulose contents were correlated to the results of bioassays that assessed biodegradability via respiration activity.That is, organic matter content alone cannot be an adequate predictor of respiration activity, since it can contain both readily degradable and non-degradable organic matter. This is in agreement with the findings of Evangelou et al. [11] too, who had used solid-phase static respiration activity tests.

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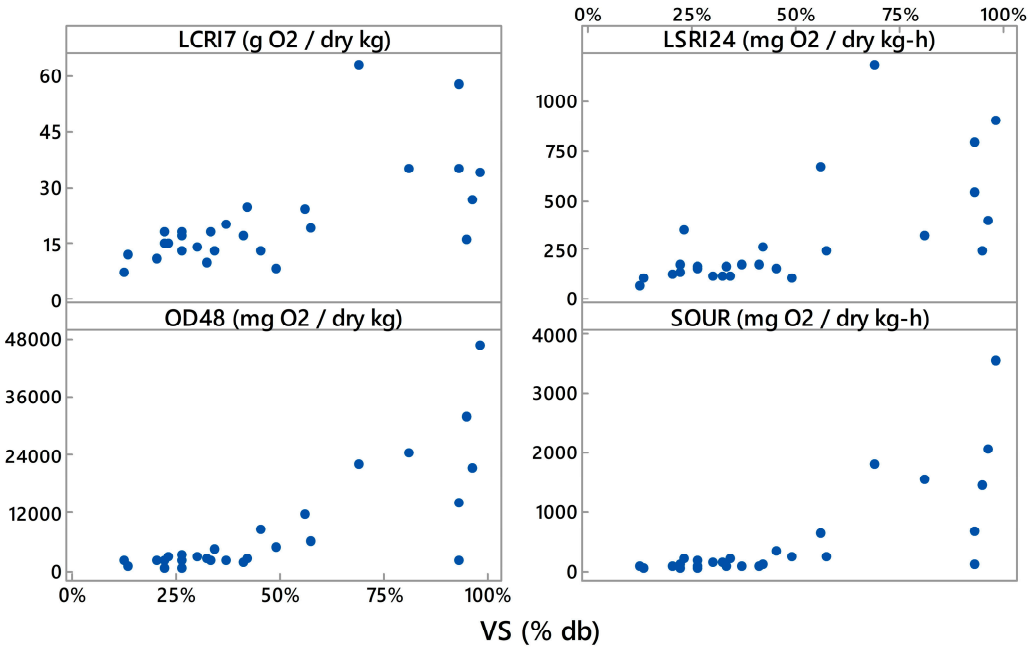

Figure 5. Correlation plots between the initial volatile solids content and respiration activity indices for both liquid-phase tests ($LCRI_7$, $LSRI_{24}$, OD_{48}, SOUR).

3.7. Statistical differences among substrate groups and types

Using one-way ANOVA, statistical differences were investigated among the characteristics of the individual substrates, between the two groups of substrates (raw, processed) and among different types (home composts, MSW derived composts, maures, etc). The results are presented in Table 4. Analysis showed that all four respiration indices were statistically different between raw and processed substrates (expressed either on a per dry basis or per organic matter basis). The only exception was $LSRI_{24}$ (when expressed in a per kg VS basis) for the manometric test, which was statisticallysimilar for both the raw and the processed substrates (i.e. mean values were 623 and 571 mg/kg VS-h, respectively). On the other hand, the $LCRI_7$ was statistically different between the Raw and Processed substrates (32.9 and 17.6 g/dry kg, respectively), based on 35 and 149 replicates for each test, respectively. This finding enhances the intial ad-hoc grouping of the 27 substrates into raw and processed.

On the other hand, when the 27 substrates were categorized per source material, most respiration indices were statistically similar. For example, home composts, manures, MSW derived materials and seaweed compost were statistically similar with regard to the four indices of the two tests. Statistical similarities were found also for the categories of food wastes, sludges and forest debris with regard to our newly introduced manometric test indices ($LCRI_7$ and $LSRI_{24}$).

455 **Table 4.** Statistical differencesbetween groups of substrates (Raw; Processed) and among types of substrates (MSW derived, home composts, etc)

		Liquid manometric test				Liquid SOUR test			
		$LCRI_7$ (g/dry kg)	**$LSRI_{24}$ (mg/dry kg-h)**	**$LCRI_7$ (g/kg VS)**	**$LSRI_{24}$ (mg/kg VS-h)**	**OD_{48} (g/dry kg)**	**SOUR (mg/dry kg-h)**	**OD_{48} (g/kg VS)**	**SOUR (mg/kg VS-h)**
		per dry kg		*per kg VS*		*per dry kg*		*per kg VS*	
Groups	Raw	32.9^{A}±13.7	529^{A}±350 (*n=35*)	39.3^{B}±21.8	622^{A}±473	31.1^{A}± 12.6	2026^{A}±975 (*n=28*)	35.0^{A}±12.5	2294^{A}±1013
	Processed	17.6^{B}±10.5	208^{B}± 82.3 (*n=149*)	52.6^{A}±19.2	571^{A}±256	3.35^{B}±0.57	156^{B}±59.9 (*n=123*)	9.27^{B}±5.77	433^{B}±276
Types	Home composts	18.0^{CD}±6.55	189^{B}±129 (*n=83*)	62.6^{A}±15.7	618^{AB}±147	2.75^{D}±1.85	125^{D}±63.7 (*n=77*)	8.15^{E}±5.18	369^{D}±215
	Manures	21.6^{BCD}±9.25	253^{B}±81.5 (*n=29*)	43.1^{BC}±12.7	459^{B}±214	2.96^{D}±1.24	145^{D}±79.1 (*n=25*)	8.01^{DE}±4.90	395^{D}±284
	MSW	13.6^{D}±6.30	258^{B}±59.0 (*n=22*)	32.2^{CD}±17.5	612^{AB}±220	6.99^{CD}±3.13	339^{D}±146 (*n=15*)	15. 9^{C}±4.87	799^{CD}±257
	Food wastes	25.7^{ABC}±9.01	516^{A}±317 (*n=20*)	26.7^{D}±9.09	533^{AB}±322	36.4^{A}±12.7	2319^{A}±1122 (*n=16*)	37.9^{A}±12.8	2401^{A}±1130
	Sludges	32.2^{AB}±21.5	511^{A}±172 (*n=13*)	53.3^{AB}±26.9	814^{A}±139	13.2^{C}±2.55	982^{BC}±176 (*n=8*)	20.2^{BC}±7.45	1466^{BC}±531
	Forest waste	35.0^{A}±3.32	314^{AB}±48.3 (*n=11*)	43.2^{BCD}±4.10	388^{B}±59.6	24.7^{B}±7.35	1553^{B}±315 (*n=8*)	30.5^{AB}±9.08	1919^{AB}±389
	Seaweed	7.02^{D}±0.97	64.4^{B}±12.4 (*n=6*)	60.2^{AB}±8.35	552^{AB}±106	2.45^{CD}±0.07	101^{CD}±3.83 (*n=2*)	21.0^{BCDE}±0.58	863^{BCD}±32.8

456 Means ± standard deviations; Means that do not share the same letter per column are statistically different at $p < 0.05$; Statistical comparisons are performed per column,
457 between groups of substrates and between types of substrates
458

4. Conclusions

A newly introduced manometric liquid phase respirometric activity test to assess organic waste stability is presented here and compared to an already established liquid phase test (SOUR test). The conclusions from this work are:

1. A significant correlation among the respiration indices of both tests was found for the raw substrates only (n=5). On the other hand, no significant correlation was found for the processed substrates (n=22).
2. The limits of the SOUR that had been proposed in the past (1000 mg O_2/kg VS-h for matured composts) are verified here too, since almost all processed materials had SOUR values below that limit.
3. The newly introduced liquid phase test can be used as an alternative test to assess organic waste stability. The 24 h oxygen consumption rates ($LSRI_{24}$) for the raw and processed substrates ranged from 240 to 1180 mg O_2/dry kg-h (250 to 1700 mg O_2/kg VS-h) and from 64 to 792 mg O_2/dry kg-h (210 to 1480 mg O_2/kg VS-h), respectively. A clearer differentiation between fresh and more stabilized substrates is thus achieved when the above index is expressed on a per dry matter basis than on a per VS basis.
4. The respiration activity indices calculated here were significantly lower in the processed substrates compared to the raw ones. On the other hand, no statistical differences were found between substrates when grouped by source material (food waste, manures, MSW, etc.).

A comparison of the liquid phase indices with respiration activity indices derived from solid-phase tests is necessary to further investigate the potential of adopting this newly introduced liquid phase test in a wider scale.

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Conflicts of Interest: The authors declare no conflict of interest.

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