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1 **Biodegradation activity of eight organic substrates: A correlation study of**  
2 **different test methods**

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17

18 **Abstract**

19       The biological activity of eight organic substrates was assessed using different  
20 techniques under aerobic and anaerobic environments at different scales. The used  
21 substrates included simulated fresh to actual stabilized composts. Experiments included  
22 dynamic respiration (with 30 L and 5.5 L reactors), static respiration (with 1 L reactors)  
23 and biochemical methane potential (BMP) tests (with 1.1 L flasks). The indices  
24 evaluated were: the 24-h dynamic respiration index ( $DRI_{24}$ ) and the cumulative dynamic  
25 respiration indices at 4 and 7 d ( $DCRI_4$  and  $DCRI_7$ , respectively) measured with two  
26 different methods, the maximum dynamic  $CO_2$  generation rate ( $D-CO_{2\_24}$ ) at 24h and a  
27 dynamic cumulative  $CO_2$  generation after 7 days ( $D-CO_{2\_7}$ ), the maximum static  $O_2$   
28 consumption rate in 12h and 24h ( $SRI_{12}$  and  $SRI_{24}$ ), the static cumulative  $O_2$   
29 consumptions after 4 and 7 days ( $SCRI_4$  and  $SCRI_7$ ) and the static  $CO_2$  generation after  
30 7 days ( $S-CO_2$ ) and the BMP after 30 days. The 24-h dynamic respiration index ( $DRI_{24}$ )  
31 ranged from 25 to 3000  $mg\ O_2\ kg^{-1}VSh^{-1}$  in one lab and from 150 to 3500  $mg\ O_2\ kg^{-1}$   
32  $VS\ h^{-1}$  in the other. A positive statistically significant correlation was achieved between  
33 the two types of dynamic indices. In addition, the  $CH_4$  production after 30 d showed a  
34 strong positive correlation with both  $DRI_{24}$  indices and the cumulative dynamic  
35 respiration indices at 4 and 7 d ( $DCRI_4$  and  $DCRI_7$ ), as measured in both labs. The static  
36 respiration indices did not correlate well with the dynamic respiration ones. The  
37 practical implications of the use of the biodegradation activity indices were also  
38 analysed and discussed.

39

40 *Keywords:* biochemical methane potential; compost; dynamic respiration index; organic  
41 substrate; respiration activity.

42

## 43 1. INTRODUCTION

44 A wide research on respiration activity indices has been conducted in order to  
45 characterize the biodegradation activity and stability of organic substrates [1-10].  
46 Stability usually refers to the resistance of organic matter to biodegradation [11] and/or  
47 the extent to which readily biodegradable organic matter has decomposed [12]. A high  
48 content of an easily degradable fraction means a low biological stability and vice versa  
49 [13]. Stability is commonly assessed via measurements of oxygen consumption and  
50 carbon dioxide generation [4, 12, 14-15], methane generation [15-17] as well as via  
51 temperature evolution through the self-heating test [18-20]. All above techniques can be  
52 adopted to measure the biodegradation activity of fresh to stable substrates.

53 In the current study aerobic microbial respiration activity (in which O<sub>2</sub>  
54 consumption and CO<sub>2</sub> generation are the dominant indices) and anaerobic activity (in  
55 which the CH<sub>4</sub> generation is the key index) were measured. These biological indices are  
56 essential for evaluating biomass degradation activity for compliance or process control  
57 purposes, since these methods, although in a smaller scale can reproduce the actual  
58 waste degradation process [21] and can designate treatment technologies.

59 In particular, with regard to the aerobic respiration activity, different aeration  
60 modes have been tried; static with intermittent air flow and dynamic with continuous air  
61 flow. In dynamic respiration methods, further differences exist regarding the selected  
62 airflow rate. Airflow rates between 20 and 30 ml min<sup>-1</sup> have been reported by [9, 21] in  
63 order oxygen content at the exhaust gases be above 10% (v/v). Similarly, [4]  
64 continuously adjusted the airflow rate in order to constantly maintain an oxygen content  
65 of 140 ml L<sup>-1</sup> air (14% v/v) at the reactor's outlet stream.

66 Experiments in several scales have been reported to assess static and dynamic

67 respiration indices [3-4, 8, 12, 22-23]. For example, aerobic respiration has been  
68 assessed in small 500 mL dynamic reactors [9], in 5.5 L reactors [24] and in up to 30 L  
69 reactors, such in the Di.Pro.Ve-CosTech<sup>®</sup> system [25].

70 How representatively can all these reactors measure the actual respiration activity  
71 of the material? Can they all assess the same microbial activity regardless of sample  
72 size and boundary conditions?

73 Although larger reactors are usually better in terms of sample representation, they  
74 are usually more complex during their operation, difficult and costly to be constructed.  
75 Moreover, they require large sample sizes and replication can be sometimes very  
76 limited to non-existent due to cost constraints. On the other hand, smaller reactors are  
77 easier to construct and operate and allow the experimenter to run simultaneously many  
78 replicated experiments. The Di.Pro.Ve-CosTech<sup>®</sup> system is more automated and allows  
79 the measurement of several variables at short time intervals; in addition, air flow can be  
80 controlled either through the oxygen content or through the temperature readings.  
81 However, due to economical limitations in several waste treatment facilities, plant  
82 operators are seeking less cost-benefit techniques to assess biodegradation activity and  
83 compost stability. Stability indices are used for compliance tests in Germany at a  
84 national level [26], in Italy at regional/provincial level [27] and in England and Wales  
85 [28], for both MBT materials and composts. Moreover, DRI has recently received  
86 official recognition at EU level for purposes of standardization [29] and validation [30].

87 The objective of this work refers to i) assess the biodegradation activity of 8  
88 organic substrates of different biodegradabilities in two different laboratories, using  
89 different microbial activity assessment techniques per laboratory and ii) investigate  
90 correlations among those indices. One laboratory is located at the Mediterranea

91 University of Reggio Calabria (Italy), and will be herein referred to as either UNIRC or  
92 ITA, whilst the other is located at the Democritus University of Thrace in Xanthi  
93 (Greece) and will be herein referred to as DUTH or GRE.

94

## 95 **2. MATERIALS AND METHODS**

### 96 *2.1. Substrate description and preparation*

97 Eight different organic substrates of different origin and composition were  
98 investigated in this study (Table 1). Samples were selected in such a manner so that to  
99 cover a wide range of organic matter contents and expected degradabilities.

100 HC\_1 was a mature home compost obtained from household organic waste,  
101 produced by kitchen and garden waste mixed with a limited quantity of wood  
102 combustion residues, constituted by coal and ash. Sampling for that substrate took place  
103 after one year of composting in a custom made plastic composter, by opening the top  
104 cover and grabbing randomly samples from several heights of the composter. HC\_2 was  
105 a 21-d aerobically stabilized home compost produced in Italy from a mixture of kitchen  
106 waste (60% wb) and wood chips (40% wb). OFMSWC was a compost prepared from  
107 the organic fraction of commingled municipal solid waste (OFMSW) and was obtained  
108 from a full scale mechanically and biologically treatment (MBT) facility at Reggio  
109 Calabria after 28 days of aeration. Approximately 10 kg of material was randomly  
110 sampled by the plant managers.

111 Substrates SIM\_FW1 and SIM\_FW2 were raw food wastes that were artificially  
112 prepared in both labs on-site. This was done in order to achieve an expected high  
113 degradability and to check the reproducibility of measurements between the labs with  
114 those simulated wastes. SIM\_FW1 was prepared by mixing 25% of cooked pasta, 25%

115 of white bread, 25% of chopped apples and 25% of grilled minced beef meat (on a wet  
116 weight basis, wb). SIM\_FW2 was prepared by mixing 50% of uncooked pasta, 30% of  
117 uncooked frozen fries and 20% of beef dog food (all in wb). Those ingredients were  
118 obtained from the same commercial chain that is located in Italy and Greece. The two  
119 aforementioned substrates (SIM\_FW1 and SIM\_FW2) were left for a week in a sealed  
120 plastic bag after preparation, and before the initiation of the experimental runs, so that  
121 to better simulate actual food wastes. FORW was a sample of leaves obtained from a  
122 forest floor during spring, by collecting all the available leaves from a 2 m<sup>2</sup> randomly  
123 selected area. FAMN was a fresh (1 week old) animal manure, collected from a cow  
124 breeding facility, that consisted of cow manure and straw. AMNC was obtained from  
125 the same facility, after passing through a solid-liquid separator and after stored in piles  
126 for 1 week.

127 For substrates HC\_1, OFMSWC, FORW, FAMN and AMNC almost 4 to 10 kg  
128 (wet weight) were collected, while sampling was followed by a sequential quartering  
129 process in order to ensure randomness and homogenization. In the case of simulated  
130 substrates (SIM\_FW1 and SIM\_FW2) and HC\_2, the whole amounts were used for the  
131 analysis.

132

133 *Insert Table 1*

134

135 HC\_1, HC\_2, OFMSWC and FORW were collected from Reggio Calabria (Italy),  
136 and then divided and individually sealed in two different plastic bags, containing  
137 approximately the same substrate quantity (about 4-10 kg). One of the two bags was  
138 placed in a non-insulated paper box, that was also thoroughly sealed and was shipped by

139 airmail to Xanthi (DUTH, Greece). The other bag was kept in UNIRC at room  
140 temperature until the other part reached Greece, usually after 4-5 days. As soon as  
141 samples arrived in Greece, characterization started at the same time in both labs. FAMN  
142 and AMNC were collected from a cow breeding plant near Xanthi (Greece) and were  
143 shipped to Italy following the same abovementioned procedure. Substrates SIM\_FW1  
144 and SIM\_FW2 were prepared concurrently in both laboratories, using the same raw  
145 materials and proportions, right before the initiation of the analyses.

146

## 147 *2.2. Initial characterization*

148 The preliminary characterization of the substrates was performed simultaneously  
149 at both labs using similar standard techniques. Measurements of moisture (M), volatile  
150 solids (VS), pH, water holding capacity (WHC) were performed in both labs, whilst the  
151 elemental analysis (total carbon and total nitrogen contents) was performed in DUTH  
152 only. Moisture content was measured through weight difference at 75°C till constant  
153 weight, whereas volatile solids were measured in a muffle furnace through the loss on  
154 ignition of dried and ground material at 550°C for 2 hours. Volatile solids, moisture and  
155 pH were measured according to [31] and [32]. Total carbon and total nitrogen were  
156 measured using an elemental analyzer (CE Instruments, CHNS-O Model EA-1110),  
157 according to [31] and [33].

158 Moisture, VS and pH measurements were done in 2 or up to 5 replications,  
159 whereas total C and N were done in six replications. The replications aided to check the  
160 statistical relationship between the initial parameters performed in UNIRC and DUTH  
161 using ANOVA principles.

162 The WHC was measured according to method TS 11184 [34] by placing 1000 g



163 of sample in a previously weighed wet cotton bag, which was immersed in a water  
164 container for 24 h. The bag was removed from the water and allowed to drain for 6 h  
165 and then re-weighed. Table 2 shows which properties and respiration indices (discussed  
166 in next section) were measured in each laboratory.

167 In both labs, prior to the initiation of the dynamic respiration tests, all samples  
168 were optimized for moisture content by adding, if necessary, water to reach the 75% of  
169 the corresponding WHC [34]. In addition, bulk density was adjusted, where needed, by  
170 adding a styrofoam material to maintain a value for the mixture below 450-500 kg/m<sup>3</sup>.

171

### 172 *2.3. Dynamic respiration activity tests*

#### 173 *2.3.1. Dynamic respiration index at UNIRC (Italy)*

174 The dynamic respiration activity in UNIRC was measured using a 30 L adiabatic  
175 respirometric reactor (Costech International, Cernusco sul Naviglio, Italy) according to  
176 TS 11184 method [34] and [30]. The characteristic of the Costech<sup>®</sup> reactor is that it is  
177 adiabatic and that air flow entering the reactor is continuously adjusted so that to keep  
178 the O<sub>2</sub> concentration in the outlet stream between 14% and 17% (v/v), as suggested by  
179 [5-6, 25] and [4].

180 Advantages of the Costech<sup>®</sup> system include: i) the large volume of the reactor that  
181 can help minimizing sampling errors, especially for heterogeneous materials  
182 characterized by a relatively large size of the particles, ii) the automation of oxygen and  
183 temperature measurements, and iii) the ability for continuous automatic adjustment of  
184 the air flow. The main disadvantages are related to the high investment cost and the  
185 maintenance of the apparatus. Moreover, there is a need of controlling the temperature  
186 of the inlet air, especially in case of low biological activity, since the results of the test

187 are influenced by eventual significant changes in the temperature of the air supplied to  
188 the reactor. In terms of applicability, the main issue is the availability of the substrate,  
189 each time measured, because the operational quantity needed is -at least- 3.0 kg.

190 The hourly oxygen uptake rate ( $OUR_h$ ) was calculated according to [30]:

191  $OUR_h [\text{mg O}_2 \text{ kg}^{-1} \text{ VS h}^{-1}] = Q * \Delta O_2 * V_g^{-1} * 31.98 * VS^{-1} \quad (1)$

192

193 Where:

194  $Q [\text{L h}^{-1}]$  is the airflow rate

195  $\Delta O_2 [\text{mL L}^{-1}]$  is the difference between the inlet and outlet  $O_2$  concentrations of  
196 the reactor

197  $V_g [\text{L mol}^{-1}]$  is the volume of 1 mol of gas at the inlet air temperature

198 31.98  $[\text{g mol}^{-1}]$  is the molecular weight of  $O_2$

199 VS [kg] is the initial weight of volatile solids placed in the reactor.

200

201 Oxygen content measurements were performed every one hour. The  $DRI_{24}$  was  
202 then calculated as the moving average of the 24  $OUR_h$  values, taken over the 24 h  
203 period that had the most intense biological activity. The amount of each substrate placed  
204 in the reactor ranged from 3.7 to 8.2 kg (wet weight) depending on bulk density (and  
205 availability of materials), whilst the volume occupied in the reactor was always  
206 maintained similar for all runs (approximately 15 L). The temperature in CosTech<sup>®</sup>  
207 reactors was measured via a probe placed in the centre of the biomass. One run was  
208 performed for each substrate (n=1), which is considered sufficient and representative  
209 due to the relatively large amount (> 3.5 kg) of the sample placed in the reactor [8].

210

211 2.3.2. *Dynamic respiration index at DUTH (Greece)*

212 The dynamic respiration activity in DUTH was measured in 5.5 L dynamic  
213 reactors made from plexi-glass, as described in detail by [24]. The air flowrate was  
214 adjusted with a volumetric flowmeter. Reactors were placed in a heated room at a  
215 constant temperature of  $35\pm 2^\circ\text{C}$ , but no temperature measurements within the reactor  
216 were performed. A wet sample of 500 g was placed in each reactor for each substrate.  
217 The unit air flow (UAF) rate for all eight substrates was maintained around  $12 \text{ L air kg}^{-1}$   
218  $^1\text{VS h}^{-1}$ ; thus, the actual air flow rate was adjusted accordingly from 15 to  $60 \text{ mL min}^{-1}$ .  
219 In any case, the UAF rate never exceeded  $140 \text{ mL air dry kg}^{-1} \text{ min}^{-1}$  according to [35]  
220 suggestions.

221 Measurements of the instantaneous  $\text{O}_2$  and  $\text{CO}_2$  contents at the reactors' outlet  
222 were performed manually, using a portable gas analyzer (GA-2000P, Geotechnical  
223 Instruments Ltd., U.K). The frequency of the measurement varied, from three to eight  
224 times per day, depending on the biological activity of each substrate. The most intense  
225 biological activity, characterized by high  $\text{O}_2$  consumption and high  $\text{CO}_2$  generation, was  
226 observed during the first 4 days from the initiation of the experiments. In this initial  
227 period, more frequent measurements were made to better formulate the respiration  
228 activity profile. During the last days of the experiments, the measurement frequency  
229 was decreased, since  $\text{O}_2$  consumption and  $\text{CO}_2$  generation profiles tended to stabilize.  
230 The same sampling frequency had been performed and justified in [24].

231 The  $\text{DRI}_{24}$  was calculated similarly to above [4, 9]. The maximum  $\text{CO}_2$  generation  
232 rate ( $\text{D-CO}_2_{24}$ ) was calculated as the maximum of the averages of the instantaneous  
233 carbon dioxide generation rates recorded during the same 24-hour period of higher  
234 activity. DRI measurements were done in triplicates for six substrates (OFMSWC,

235 SIM\_FW1, FORW, FAMN, AMNC and SIM\_FW2) and in duplicates for substrates  
236 HC\_1 and HC\_2. All replicates were run concurrently.

237

### 238 2.3.3. *Other dynamic respiration activity indices*

239 Other dynamic respiration indices that were calculated on the basis of the same  
240 measurements done when calculating  $DRI_{24}$ , were: cumulative dynamic  $O_2$   
241 consumption after 4 and 7 days ( $DCRI_4$  and  $DCRI_7$ , respectively); calculated as the  
242 integral below the OUR curve from day 0 to days 4 and 7, respectively. In DUTH, the  
243 maximum dynamic  $CO_2$  generation rate ( $D-CO_{2\_24}$ ) at 24h and a dynamic cumulative  
244  $CO_2$  generation after 7 days ( $D-CO_{2\_7}$ ) were also calculated.

245

### 246 2.4. *Biologic Methane Potential (BMP)*

247 BMP tests were performed in UNIRC in duplicates under mesophilic conditions.  
248 The amount of substrate used was between 1.3 to 22.8 wet g for each vessel. Tests were  
249 performed using a WTW OxiTop<sup>®</sup> AN6 (Germany) apparatus that consisted of three  
250 sets of 6 glass bottles (1.1 L volume) placed in thermostatic cabinet at  $35\pm 0.5$  °C and  
251 equipped with a manometric head that recorded pressure increases due to biogas  
252 production. Each bottle was continuously mixed by a magnetic stirrer. Biogas produced  
253 passed through a NaOH solution (3M), for  $CO_2$  adsorption and methane production was  
254 then measured by an eudiometer (water displacement method). Anaerobic inocula were  
255 collected at three different periods from the second stage of an anaerobic digester that  
256 was working in mesophilic conditions and was fed with agro-wastes (cattle manure,  
257 chicken manure, agriculture residuals and other industrial waste coming from the  
258 transformation of agriculture products such as olive and citrus). Immediately after

259 sampling, inocula were sieved (<1mm) to remove large fibrous materials (e.g. straw)  
260 and were kept under endogenous anaerobic conditions at 35°C (mesophilic inocula) for  
261 a minimum of 7-10 days to reduce blank biogas production. The BMP of the substrate  
262 was calculated (in NmL CH<sub>4</sub> g<sup>-1</sup> VS) by subtracting the methane production of the  
263 inoculum (i.e. blank biogas production) from the gross methane production. The  
264 characteristics of the inocula ranged from 21.0 to 59.0 g TS L<sup>-1</sup> with a VS content from  
265 55% to 73% (% TS). The substrate to inoculum ratio (in g VS/g VS) was always kept at  
266 around 0.4 to 0.5. The methane yield of the inocula ranged from 3% to 20% of the  
267 methane yield of the main substrates, except in the case of HC\_1 for which the methane  
268 yield was comparable to that of the inoculum.

269

#### 270 2.5. *Static Respiration Index (SRI)*

271 Static respiration indices were measured only in DUTH using a 1 L static solid  
272 phase respirometer equipped with WTW OxiTop-C<sup>®</sup> manometric heads, following the  
273 methodology described in [23] and [10]. 50 to 70 g of wet substrate were placed in each  
274 static respirometer and moisture content was adjusted to an optimal moisture content  
275 range between 50% to 60% ww. A 50 mL plastic beaker, used as an alkaline trap  
276 (containing a 2N KOH solution), was placed inside the respirometer in order to quantify  
277 the amount of C-CO<sub>2</sub> generated by the substrate. Respirometers were firmly closed and  
278 momentarily opened daily, so that the maximum pressure drop inside the vessels within  
279 two consecutive aerations would never exceed 170 hPa for any of the substrates. Based  
280 on the ideal gas law, this maximum pressure drop corresponded to a residual O<sub>2</sub> content  
281 in the vessel equal to 5% v/v, which still sustains aerobic conditions. These maximum  
282 pressure drops were observed after 24 h during the first couple of days. All

283 measurements were performed at 35°C and lasted 7 d in an incubator at the absence of  
284 light. Experiments were performed with 4 to 6 replicates per substrate.

285 The indices calculated for the static solid phase test in this study were: the  
286 maximum O<sub>2</sub> consumption rate recorded over 12h and 24h periods of highest activity  
287 (SRI<sub>12</sub>, SRI<sub>24</sub>, in mg O<sub>2</sub> kg<sup>-1</sup> VS h<sup>-1</sup>), the cumulative O<sub>2</sub> consumptions after 4 and 7 days  
288 (SCRI<sub>4</sub>, SCRI<sub>7</sub>, in g O<sub>2</sub> kg<sup>-1</sup> VS h<sup>-1</sup>) and the CO<sub>2</sub> generation after 7 days (S-CO<sub>2</sub>, in g C-  
289 CO<sub>2</sub> kg<sup>-1</sup> VS) as explained in [10].

290

291 *Insert Table 2*

292

### 293 **3. RESULTS AND DISCUSSION**

#### 294 *3.1. Substrates' characterization*

295 The initial properties of the substrates are included in Table 3, where it is shown  
296 that there was a very good agreement between the initial properties (moisture content,  
297 volatile solids and pH) of all substrates from both labs; this ensured that respirometric  
298 experiments on all materials started with identical initial properties in both countries.  
299 WHCs for half of the studied substrates were almost the same (HC\_1, FORW, AMNC,  
300 SIM\_FW2), while for the rest seemed to slightly differ. The as-received moisture  
301 contents of the eight substrates ranged from 18% (ww) for the OFMSWC, to 83% (ww)  
302 for the FAMN. Volatile solids (i.e. organic matter) ranged from a low 23% (dw) for the  
303 OFMSWC, to 96-98% (dw) for the simulated food waste substrates (SIM\_FW1 and  
304 SIM\_FW2). C/N ratios, as measured through elemental analysis, ranged from 8.2 to 40,  
305 for FAMN and HC\_2, respectively. pH values ranged from 5.1 (for SIM\_FW2, which  
306 had the higher organic matter content) to 9.5 for HC\_1 (composted kitchen waste and

307 wood ashes with a VS at 42% dw). Most of the substrates had a pH between 6 and 8,  
308 while manures (FAMN and AMNC) were alkaline.

309

310 *Insert Table 3*

311

### 312 3.2. Oxygen uptake profiles in dynamic methods

313 Table 4 presents the dynamic respiration indices and the BMP results. The profiles  
314 of the oxygen uptake rates of the eight substrates, as measured by both laboratories, are  
315 included in Figure 1. Note that the mean values are shown only in the case of the Greek  
316 tests (for which there was replication), while the errors bars in the graphs show the  
317 standard errors. Apparently, a very good replication (coefficient of variation was always  
318 less than 5%) existed for the Greek dynamic respiration tests.

319

320 *Insert Table 4 and Figure 1*

321

322 According to Figure 1, there were quite similar OUR profiles between the ITA and  
323 GRE dynamic tests for substrates HC\_1, HC\_2, SIM\_FW1, FAMN and SIM\_FW2. The  
324 largest deviation among the OURs recorded between the two labs was observed for  
325 OFMSWC; this can be also confirmed by looking at the corresponding dynamic indices  
326 of Table 4. For this substrate, the Greek index was unexpectedly high, whilst the Italian  
327  $DRI_{24}$  was close to values suggested in the past by [36], who had used similar materials  
328 and similar equipment. This contradiction with regard to OFMSWC is hard to explain  
329 and could be attributed to an inherent variability and unpredictability of biological  
330 experiments, or to a heterogeneity of the sample which was derived from MSW. It

331 might be also attributed to the fact that the temperature of OFMSWC in CosTech®  
332 remained similar to that of the ambient air (around 20 °C) throughout the run, whilst  
333 artificial mesophilic temperatures ( $\approx 35^{\circ}\text{C}$ ) were always maintained in the Greek method.

334 For FORW, a higher early peak was observed in the Greek method, although a  
335 rather stable and slightly fluctuating OUR was observed in the Italian method with a  
336 peak at the end of the run. This periodic fluctuating OUR profile in the case of the  
337 CosTech® runs was also obvious for substrates HC\_1, FORW, FAMN, AMNC,  
338 SIM\_FW2 and might be attributed to diurnal temperature variations during the  
339 experiment, since the air that was introduced into the reactor was at ambient room  
340 temperature. A similar disagreement seemed to exist in the case of FORW, in which the  
341 GRE method resulted in higher OURs compared to the ITA method. In AMNC too, the  
342 Greek method resulted in higher OURs compared to the Italian method; yet, this  
343 substrate was a rather stable one with some of the lowest OURs recorded in both  
344 methods. According to Figure 1, the OUR profiles in the Greek system were much  
345 smoother than the CosTech® OUR profiles, despite the fact that data recording was less  
346 infrequent in the former case.

347 In the CosTech® system, only 2 substrates (SIM\_FW1 and SIM\_FW2) reached  
348 temperatures up to  $45^{\circ}\text{C}$  after 1.5 days which were then reduced and maintained to  
349 between  $30^{\circ}\text{C}$  and  $40^{\circ}\text{C}$  till the end of the process. This agrees with the fact that  
350 SIM\_FW1 and SIM\_FW2 had two of the highest respiration activities. On the other  
351 hand, FAMN, which also had a high respiration activity in Italy, never reached such  
352 mesophilic temperatures. This was caused due to a malfunction of the automatic airflow  
353 adjusting system after day 8. However, the  $\text{DRI}_{24}$  values were still calculated during the  
354 first 7 days, in which the maximum biological activity was anyway observed.



355

356 *3.3. Effect of temperature and air flow on respiration activity*

357 Figure 2 presents the temperatures of all 8 substrates measured only in the Italian  
358 respiration method. The temperature in CosTech<sup>®</sup> reactors was measured via a probe  
359 placed in the centre of the biomass. In the case of the Greek method, experiments were  
360 performed in a 35±2°C temperature-controlled room, whilst the reactors cannot be  
361 considered strictly adiabatic. Since no temperature measurements were performed in the  
362 case of the Greek reactors, no temperature profile are shown.

363 According to Figure 2, only SIM\_FW1 and SIM\_FW2 reached temperatures up to  
364 45 °C, yet none reached thermophilic temperatures. Both these substrates had two of the  
365 highest respiration activities, as measured in both Italy and Greece. Yet, FAMN, which  
366 also had a high respiration activity as measured in Italy, had, surprisingly, one of the  
367 lowest temperatures among all substrates, which is difficult to explain. The internal  
368 temperatures of all other substrates (i.e. except SIM\_FW1 and SIM\_FW2) remained at  
369 ambient values between 20 to 25°C.

370 As shown in Figure 1, maximum deviations for OUR profiles are observed for  
371 OFMSWC and AMNC, which can also be confirmed through their dynamic indices  
372 (Table 4). This can be attributed to the fact that in Italian reactor, these two substrates  
373 did not exceed 25°C. Adversely, Greek dynamic reactors were always in a temperature  
374 range between 34 to 37 °C. The lack of high temperatures in the other 5 substrates might  
375 be charged to the low respiration activity of those substrates (Table 4). Moreover,  
376 difference in reactor scale is important since it might affect the temperature profile of  
377 the material.

378

379

*Insert Figure 2*

380

381        When designing solid waste treatment plants, it is also necessary to know the  
382 amount of air required to maintain optimum conditions for the degradation of organic  
383 substrates. For this reason, Figure 3 was drawn in order to study the effect of the  
384 cumulative (total) air flow that passed through the CosTech<sup>®</sup> system after 7 days, on the  
385 respiration activity. In Figure 3, only data from the Italian reactor are shown, since the  
386 flowrate in that system was continuously adjusted throughout the process. On the  
387 contrary, in the Greek dynamic method, a constant UAF rate was applied to all reactors  
388 throughout the experiments.

389

390

*Insert Figure 3*

391

392        According to Figure 3, it appears that as the total flow of air increases, the  
393 respiration activity increases too; yet it seems that a constant  $DRI_{24}$  value tends to be  
394 reached at high cumulative flows. This trend was also shown by [37] and can be  
395 explained by the fact that beyond a certain cumulative aeration threshold value, the  
396 respiration activity is maximized and stabilizes and no more excess air is necessary.  
397 Moreover, an excessive aeration could decrease microbial activity due to the reduction  
398 of the temperature of the biomass at sub-optimal levels.

399

#### 400 *3.4. Static and dynamic respiration indices*

401

402        Table 5 includes the static respiration indices and the ratios of the dynamic  
respiration (measured in the Greek lab) over the static respiration indices. As shown in

403 Table 5, the dynamic respiration indices were in general much higher than the  
404 corresponding static indices (both in terms of rate and total oxygen consumption). No  
405 consistency was also observed between the dynamic and the static test indices in the  
406 recent work of Aspray et al. [20]. After categorizing the 8 substrates in fresh  
407 (SIM\_FW1, SIM\_FW2, FAMN) and stabilized composts (HC\_1, HC\_2, OFMSWC,  
408 FORW, AMNC), it was observed that the ratio of  $DRI_{24}/SRI_{24}$ (measured in Greece) for  
409 the fresh substrates ranged from 3.3 to 11.4 as opposed to the stable substrates for which  
410 that ratio ranged from only 0.9 to 3.3. Similarly, the ratio of  $DCRI_7/SCRI_7$  ranged from  
411 18 to 40, for the fresh substrates and from 1.2 to 13, for the stable substrates. The above  
412 indicates that the static methods underestimate actual oxygen consumption, as had been  
413 also suggested by [4]. This variability in the ratios of DRI to SRI contradicts the  
414 findings of [4], who showed that the dynamic respiration rate indices ( $DRI_{24}$ ) of  
415 severalmunicipal solid waste derived organic substrates (fresh, composted and stable)  
416 were, on average, 2 times higher than the corresponding static respiration indices  
417 ( $SRI_{24}$ ). It is noted, however, that [4] had measured oxygen consumption in their static  
418 methods with an oxygen probe at 20 °C. Comparison with dynamic and static test were  
419 also conducted in the work of Godley et al. [28], who had also reported that dynamic  
420 respiration rates were at least double than the static ones. Similar results had been found  
421 by Adani et al. [6] in which all dynamic respiration rates were far higher compared to  
422 the static ones, based on a sample size of 18 organic substrates.

423

424

*Insert Table 5*

425

426 *3.5. Correlations*

427 Potential correlations among the initial physicochemical properties measured (OM  
428 content, pH, total carbon and total nitrogen) and the main biodegradation indices were  
429 calculated. The only positive correlation existed between total nitrogen content and the  
430 DRI<sub>24</sub> and CH<sub>4</sub> production after 30 d (in both cases Spearman's  $\rho=0.8$ ), as these were  
431 measured by the Italian lab. With regard to the aerobic index, this correlation can be  
432 explained by the nitrification processes that consume oxygen. On the other hand, neither  
433 total carbon nor organic matter contents correlated significantly with any of the main  
434 biodegradability indices, as also revealed in Figure 4. According to Figure 4, a clear  
435 outlier exists (OFMSWC), whilst it is observed that the substrates with VS contents  
436 above 85% (db) achieved a very wide range of respiration activities. As opposed to  
437 OFMSWC, FORW had a rather high VS content (around 80% dw) which would be  
438 expected to result to a relatively high respiration activity. However, this was not true,  
439 and the dynamic respiration indices (from both labs) were relatively low for that  
440 substrate (i.e.  $<500 \text{ mg O}_2 \text{ kg}^{-1} \text{ VS h}^{-1}$ ). This can be attributed to the likely low content  
441 of readily biodegradable substrate present in the leaves. Their high volatile solids  
442 content is most probably attributable to the high content of lignin, which is recalcitrant  
443 to fast biodegradation in aerobic conditions. From the above it appears that no clear  
444 relationship exists between the VS content of the materials and the respiration activity.  
445 Similar observations have been found out by Barrena et al. [8], in which the organic  
446 matter content did not correlate sufficiently with any of the dynamic or static respiration  
447 indices. On the contrary, Aspray et al. [20] found that the VS content correlated  
448 positively with two different dynamic methods, while no correlation was evident with  
449 the static test indices. Positive correlation between organic matter content and both O<sub>2</sub>  
450 and CO<sub>2</sub> static indices have been reported in [23] and [10]. Apparently, specific

451 constituents of the organic matter, such as sugar content (i.e. cellulose, hemicelluloses,  
452 lignin, starch), as well as the level of microbial inoculation, determine the  
453 biodegradation extents (and thus the respiration activity) regardless of total VS content.

454

455 *Insert Figure 4*

456

457 Figure 5 graphically depicts the most important correlations among the  
458 biodegradation indices measured in this work. Table 6 includes all Spearman's rank  
459 order correlation coefficients among the studied indices (only the statistically significant  
460 values at  $p < 0.05$  are shown). Note that the Spearman coefficients calculate any type of  
461 correlation and not necessarily a linear one (as in the case of the Pearson coefficients).

462

463 *Include Figure 5 and Table 6.*

464

465 According to Table 6, the  $DRI_{24}$  indices from both labs correlated between them  
466 adequately ( $\rho = 0.74$ ) with a marginally statistically significant correlation at  $p < 0.05$ .  
467 According to Figure 5a, OFMSWC appears to be an extreme value. Similarly to  $DRI_{24}$ ,  
468 the cumulative oxygen consumptions ( $DCRI_4$ ,  $DCRI_7$ ) also correlated significantly in  
469 both labs (coefficients ranged from 0.83 to 0.95), verifying the visible positive  
470 correlation trend illustrated in Figure 5b. According to Table 6, the cumulative dynamic  
471 respiration indices ( $DCRI_4$ ,  $DCRI_7$ ) also correlated well with the corresponding  $DRI_{24}$   
472 in both labs. This was also shown in [24] and practically indicates that the higher the  
473 maximum  $O_2$  consumption rate, the higher the total oxygen consumption too.

474 Table 6 also reveals a very strong correlation between the methane generation

475 after 30 days and the dynamic respiration indices of both labs. This is also evident in  
476 Figures 5c, 5d that both illustrate that positive correlation between both Greek and  
477 Italian dynamic respiration indices ( $DRI_{24}$ ) and the 30-d methane yield. This is an  
478 important finding, because it indicates that the degradation potential of an organic  
479 substrate could be similar under both aerobic and anaerobic environments, as has been  
480 shown by other researchers too [8, 15, 38]. However the values of the dynamic  
481 respiration indices measured, respectively, in the Italian laboratory, for OFMSWC, and  
482 in the Greek laboratory, for SIMFW\_2, worsened significantly this correlation.

483 According to Figures 5e and 5f, the correlation among the static and dynamic  
484 respiration indices ( $DRI_{24}$  and  $SRI_{24}$ ) was poor as was discussed in section 3.4 too.

485 The correlation between the total  $CO_2$  generation and the total oxygen  
486 consumption under dynamic conditions measured in the Greek lab was strong  
487 (correlation coefficients above 0.98), as shown in Table 6. This indicates that the moles  
488 of generated  $CO_2$  were steadily proportional to the moles of  $O_2$  consumed for all  
489 substrates. This was also noticed for the peak rates of  $CO_2$  generation ( $D-CO_{2\_24}$ ) and  
490 the peak rates of  $O_2$  consumption ( $DRI_{24}$ ) indicating that  $CO_2$  generation rate and OUR  
491 followed a parallel profile (in the dynamic tests in Greece). Moreover, respiratory  
492 quotients (RQ: moles of  $CO_2$  generated over the moles of  $O_2$  consumed) calculated for  
493 the dynamic experiments conducted in Greece, ranged between 0.7 and 1.0 (Table 4),  
494 which are reasonable values and comparable to [24].

495 Statistically significant correlation was also observed between the static  $CO_2$   
496 generation and the static  $O_2$  consumption values obtained in the Greek lab ( $\rho=0.91$ ).  
497 Therefore,  $CO_2$  generation could be also used as a biodegradation activity index,  
498 although a caution is required, since static indices are affected by temperature and  $CO_2$

499 evolution is pH-dependent due to its solubility in aqueous solutions [12]. In the case of  
500 static method RQs ranged from 1.3 to 2.3, with an average value of 1.7 (Table 5). High  
501 RQ values -using the same static method- had been also reported by Komilis et al. [10]  
502 indicating variations in the oxidation stage and the degradability of the carbon contained  
503 in each substrate.

504

### 505 *3.6. Use of biodegradation activity indices for compliance purposes*

506 According to the findings of this research, the use of both aerobic dynamic  
507 respiration and anaerobic methods is an important tool to evaluate the biological activity  
508 of fresh and composted biodegradable wastes/residues. With regard to fresh substrates,  
509 this is important knowledge for the selection of the appropriate treatment technologies.  
510 For practical purposes, and by accounting for the good correlation of the aerobic and  
511 anaerobic indices, the use of the former indices would be preferred (shorter duration  
512 than the anaerobic ones, no need of seed). Moreover, the contemporary use of aerobic  
513 and anaerobic methods allows a cross check and the detection of errors or of  
514 measurement problems (e.g. inhibition) that are frequent with this type of complicated  
515 biological measurements. This is very important when for practical reasons, as in the  
516 Di.Pro.Ve-CosTech<sup>®</sup> system, replicates are difficult to perform. Therefore, the  
517 biological stability index presented in this paper, and especially the DRI<sub>24</sub> and BMP,  
518 could be adopted for process control at the level of single plant. In this case, when the  
519 indices are used only for internal purposes, the method and equipment to be adopted for  
520 the measurements could be chosen based on the cost of the equipment and on the  
521 knowledge of the plant's technical personnel.

522 However, the use of stability indices for compliance purposes (e.g. evaluation of

523 compost quality, acceptance of waste in landfill) is more complicated. In fact,  
524 compliance threshold values must be referred to a specific method as in norms in  
525 Germany and Italy [26-27] and should be set considering the uncertainty connected to  
526 this type of measurements that is higher when replicates are not provided. In this case,  
527 also, the adoption of a double compliance (both to aerobic and anaerobic threshold  
528 values) could help to ensure the reliability of the evaluation and the detection of  
529 errors/measurement problems as done in Germany and Austria. However, practical  
530 problems linked to the long duration of the tests (at least 7 days for DRI and 30 days for  
531 BMP) should be solved prior to a generalized adoption of compliance tests based on  
532 biological activity methods.

533 A reasonable possibility would be to measure the stability indices (both dynamic  
534 aerobic and anaerobic) with a frequency related to the amount of biomass treated in the  
535 plant; the evaluation of the compliance should then be done on a statistical basis (e.g.  
536 95% of the samples analysed yearly should comply with the threshold values). If the  
537 frequency of samples with respiration indices above the threshold imposed by the norms  
538 exceeds a pre-set value, some form of penalty should be provided.

539 In the case of using indices for compliance purposes, the adoption of standard and  
540 unified methods among countries, at least at a European Level, would be greatly  
541 beneficial.

542

#### 543 **4. CONCLUSIONS**

544 Results of the work indicate that although an agreement existed among the  
545 different methods for some of the substrates, differences did exist for others so that  
546 there is no unique ideal method to propose. In addition, the OUR profile was much



547 smoother in the case of the Greek dynamic reactor compared to the CosTech<sup>®</sup> system,  
548 despite the smaller recording interval in the former case. A statistically significant  
549 correlation was calculated between the dynamic respiration indices in both labs, with  
550 regard to both rates and cumulative values. However, a weak agreement was observed  
551 for one of the substrates. A strong correlation was found between the methane yield  
552 after 30 days and the 24-h based dynamic respiration rate and the cumulative dynamic  
553 respiration indices, at 4 and 7 days. The ratio of the dynamic to static respiration rates  
554 ranged from 1 to 11 and was higher in fresh materials compared to the more stable ones.  
555 The dynamic respiratory quotients were very close to 1.0, whilst the static ones had an  
556 average of 1.7. Finally, it appears that the volatile solids content should not be used as a  
557 predictor of biodegradation activity.

558

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TABLES

Table 1. Characteristics of the organic substrates

Substrate	Type	Source materials	Composting period	Curing or storage time
SIM_FW1	Raw food waste (simulated)	Apples, cooked meat, boiled pasta, bread (25% each, in wb)	-	1 week
SIM_FW2	Raw food waste (simulated)	Uncooked pasta, uncooked fries, beef based dog food (50%, 30%, 20%, in wb respectively)	-	1 week
FAMN	Fresh animal manure	Cow manure and straw	-	1 week
HC_1	Home compost	Mix of kitchen and garden waste and ash	6months	6months
HC_2	Home compost	Mix of kitchen waste and wood chips	3 weeks forced aeration	2 weeks
FORW	Forest waste	Leaves and small branches from forest floor	-	-
AMNC	Animal manure derived compost	Cow manure and straw	n/a	1 week after solid- liquid separator
OFMSWC	Compost from the organic fraction of MSW	Organic fraction of municipal solid wastes	Aeration for 28 days in a MBT plant	n/a

n/a: no available info

Table 2. Parameters and biodegradation activity indices measured in both laboratories

	<b>Greek lab (DUTH)</b>	<b>Italian lab (UNIRC)</b>	<b>References</b>
Moisture	✓	✓	
Volatile solids	✓	✓	USDA and USCC (2002); APHA and AWWA (1998)
pH	✓	✓	
WHC	✓	✓	TS 11184, UNI (2006)
Total carbon and nitrogen	✓	✗	USDA and USCC (2002); Komilis et al (2012)
<i>Dynamic method</i>			
O <sub>2</sub> consumption rate at 24 hr (DRI <sub>24</sub> )	✓	✓	Scaglia et al. (2000); Adani et al. (2001); TS 11184, UNI (2006); Ponsa et al. (2010b)
Cumulative O <sub>2</sub> consumption after 4 d (DCRI <sub>4</sub> )	✓	✓	
Cumulative O <sub>2</sub> consumption after 7 d (DCRI <sub>7</sub> )	✓	✓	
CO <sub>2</sub> generation rate at 24 hr (D-CO <sub>2</sub> _24)	✓	✗	
Cumulative CO <sub>2</sub> generation after 7 d (D-CO <sub>2</sub> _7)	✓	✗	Komilis and Kanellos (2012)
Respiratory Quotient (RQ)	✓	✗	
<i>Biologic Methane Potential</i>			
CH <sub>4</sub> generation after 30 d (BMP <sub>30</sub> )	✗	✓	Schievano et al. (2008); Calabro et al. (2015)
<i>Static method</i>			
O <sub>2</sub> consumption rate at 12 hr (SRI <sub>12</sub> )	✓	✗	
O <sub>2</sub> consumption rate at 24 hr (SRI <sub>24</sub> )	✓	✗	
Cumulative O <sub>2</sub> consumption after 4 d (SCRI <sub>4</sub> )	✓	✗	
Cumulative O <sub>2</sub> consumption after 7 d (SCRI <sub>7</sub> )	✓	✗	Komilis et al. (2011)
Cumulative CO <sub>2</sub> generation after 7 d (S-CO <sub>2</sub> )	✓	✗	
Respiratory Quotient (RQ)	✓	✗	



Table 3. Initial properties of the substrates used in the experiments\*

Substrate	Moisture (% wb)		Volatile Solids (% db)		pH		WHC (g H <sub>2</sub> O/wet kg)		C/N
	GRE (n=3)	ITA (n=1)	GRE (n=5)	ITA (n=4)	GRE (n=2)	ITA (n=1)	GRE (n=1)	ITA (n=1)	GRE (n=6)
SIM_FW1	61%±1.0%	60%	96%±0.3% <sup>B</sup>	97%±0.0% <sup>A</sup>	5.4±0.0	5.0	729	938	13.8±0.6
SIM_FW2	40%±2.0% <sup>A</sup>	39% <sup>+</sup> ±1.1% <sup>A</sup>	98%±0.0% <sup>B</sup>	95%±0.8% <sup>A</sup>	5.1±0.0	5.5	561	565	19.6±0.4
FAMN	79%±0.1%	83%	93%±0.3% <sup>B</sup>	92%±0.1% <sup>A</sup>	8.3±0.1	7.5	969	1200	8.2±0.1
HC_1	66% <sup>+</sup> ±0.4%	68%	42%±0.3% <sup>A</sup>	41%±1.3% <sup>A</sup>	8.8	9.5	798	809	24.6±1.6
HC_2	62%±0.8%	59%	93%±0.1% <sup>B</sup>	94%±0.2% <sup>A</sup>	7.2±0.1	8.2	1241	1000	39.5±2.1
FORW	74%±0.2%	71%	81%±0.4% <sup>A</sup>	82%±2.2% <sup>A</sup>	6.2±0.1	6.9	841	861	33.9±1.3
AMNC	40%±0.5% <sup>A</sup>	41% <sup>^</sup> ±0.7% <sup>A</sup>	32%±0.2% <sup>A</sup>	32%±0.8% <sup>A</sup>	8.1±0.0	8.4	814	831	12.1±0.1
OFMSWC	22%±1.4%	18%	23%±2.0% <sup>A</sup>	26%±1.2% <sup>A</sup>	7.7±0.1	8.1	633	456	21.9±2.1

\*: all values are averages ± standard errors; <sup>+</sup>: n=2; <sup>^</sup>: n=4; ww: wet weight basis; dw: dry weight basis; WHC: water holding capacity. Means, per parameter and substrate in the same row, that do not share a letter, are significantly different based on Tukey's test (at p<0.05); (for example, the VS contents of HC\_1 are statistically similar between the 2 labs, but statistically different for HC\_2).

Table 4. Results of the dynamic respiration and BMP tests\*

Substrate	DRI <sub>24</sub> (mg O <sub>2</sub> kg <sup>-1</sup> VSh <sup>-1</sup> )		DCRI <sub>4</sub> (g O <sub>2</sub> kg <sup>-1</sup> VS)		DCRI <sub>7</sub> (g O <sub>2</sub> kg <sup>-1</sup> VS)		D-CO <sub>2</sub> _24 (mg C kg <sup>-1</sup> VS h <sup>-1</sup> )	D-CO <sub>2</sub> _7 (g C kg <sup>-1</sup> VS)	RQ	BMP <sub>30</sub> (NL CH <sub>4</sub> kg <sup>-1</sup> VS)
	GRE	ITA <sup>1</sup>	GRE	ITA <sup>1</sup>	GRE	ITA <sup>1</sup>	GRE	GRE	GRE	ITA
SIM_FW1	2721 <sup>3</sup> ±111	3522	102 <sup>3</sup> ±9.6	225.4	292 <sup>3</sup> ±18.1	446.0	1010 <sup>3</sup> ±51	105 <sup>3</sup> ±12.2	1.0 <sup>3</sup> ±0.0	467 <sup>1</sup>
SIM_FW2	2921 <sup>3</sup> ±26	1902	186 <sup>3</sup> ±0.0	147.2	327 <sup>3</sup> ±11.2	247.4	1068 <sup>3</sup> ±16	106 <sup>3</sup> ±4.0	1.0 <sup>3</sup> ±0.0	246 <sup>1</sup>
FAMN	2327 <sup>3</sup> ±40	2369	192 <sup>3</sup> ±6.7	143.6	345 <sup>3</sup> ±8.9	174.1	1585 <sup>3</sup> ±29	218 <sup>3</sup> ±6.2	1.8 <sup>3</sup> ±0.0	352±6.5 <sup>2</sup>
HC_1	255 <sup>2</sup> ±43	250	20.3 <sup>2</sup> ±3.8	16.8	37.6 <sup>2</sup> ±6.2	32.9	73 <sup>2</sup> ±15	10.8 <sup>2</sup> ±1.4	0.8 <sup>2</sup> ±0.1	55±8.1 <sup>2</sup>
HC_2	1259 <sup>2</sup> ±6	949	99.7 <sup>2</sup> ±0.3	71.4	155 <sup>2</sup> ±0.9	98.3	350 <sup>2</sup> ±1	44.4 <sup>2</sup> ±0.1	0.7 <sup>2</sup> ±0.0	168 <sup>1</sup>
FORW	474 <sup>3</sup> ±8	245	35.8 <sup>3</sup> ±0.3	20.2	57.1 <sup>3</sup> ±0.6	33.7	159 <sup>3</sup> ±27	17.9 <sup>3</sup> ±3.1	0.9 <sup>3</sup> ±0.2	167±11.4 <sup>2</sup>
AMNC	696 <sup>3</sup> ±71	148	45.5 <sup>3</sup> ±1.7	8.5	88.1 <sup>3</sup> ±3.6	17.6	183 <sup>3</sup> ±27	20.9 <sup>3</sup> ±1.5	0.7 <sup>3</sup> ±0.0	163±26.2 <sup>2</sup>
OFMSWC	2385 <sup>3</sup> ±25	371	182 <sup>3</sup> ±2.3	30.4	282 <sup>3</sup> ±5.1	48.1	682 <sup>3</sup> ±13	82.9 <sup>3</sup> ±1.8	0.8 <sup>3</sup> ±0.0	291±6.6 <sup>2</sup>

\*: all values are averages ± standard errors; <sup>1</sup>: n=1; <sup>2</sup>: n=2, <sup>3</sup>:n=3; DRI<sub>24</sub>: 24-h based dynamic respiration index; DCRI<sub>4</sub>: dynamic cumulative respiration index at 4 days; DCRI<sub>7</sub>: dynamic cumulative respiration index at 7 days; D-CO<sub>2</sub>\_24: 24-h based dynamic CO<sub>2</sub> production rate; D-CO<sub>2</sub>\_7: dynamic CO<sub>2</sub> cumulative generation at 7 days; CH<sub>4</sub>\_30: methane generation after 30 days based on the BMP tests. GRE: Greece, ITA: Italy.

Table 5. Results of the static respiration indices and respiration ratios for the Greek lab \*

Substrate <sup>1</sup>	SRI <sub>24</sub> (mg O <sub>2</sub> kg <sup>-1</sup> VSh <sup>-1</sup> )	SCRI <sub>4</sub> (g O <sub>2</sub> kg <sup>-1</sup> VS)	SCRI <sub>7</sub> (g O <sub>2</sub> kg <sup>-1</sup> VS)	C-CO <sub>2</sub> (g C kg <sup>-1</sup> VS)	RQ	DRI <sub>24</sub> / SRI <sub>24</sub>	DCRI <sub>7</sub> / SCRI <sub>7</sub>
SIM_FW1	330±12	10.6±0.3	11.8±0.4	10.3±0.7	2.3±0.1	8.2	24.7
SIM_FW2	256±13	7.3±0.4	8.2±0.4	5.6±0.2	1.8±0.0	11.4	39.9
FAMN	705±32	18.2±0.9	19.3±0.9	11.3±0.3	1.6±0.1	3.3	17.9
HC_1	273±12	20.6±1.1	31.3±1.0	15.5±0.7	1.3±0.0	0.9	1.2
HC_2	434±19	11.1±0.4	11.7±0.4	6.1±0.1	1.4±0.1	2.9	13.3
FORW	400±29	20.5±1.0	22.6±0.9	10.9±0.2	1.3±0.0	1.2	2.5
AMNC	208±17	13.8±2.0	19.4±0.8	11.5±1.0	1.6±0.1	3.3	4.5
OFMSWC	759±69	23.8±0.8	25.5±0.9	20.8±0.1	2.2±0.1	3.1	11.0

\*: all values are averages ± standard errors; <sup>1</sup>: n=4 for substrates HC\_1, OFMSWC, AMNC; n=5 for substrates HC\_2, SIM\_FW2; n=6 for substrates SIM\_FW1, FORW, FAMN; SRI<sub>24</sub>: 24-h based static respiration index; SCRI<sub>4</sub>: static cumulative respiration index at 4 days; SCRI<sub>7</sub>: static cumulative respiration index at 7 days; C-CO<sub>2</sub>: static cumulative CO<sub>2</sub> generation at 7 days;

Table 6. Spearman rank-order correlation coefficients among various indices

	VS	DRI <sub>24</sub> <sup>[G]</sup>	DCRI <sub>4</sub> <sup>[G]</sup>	DCRI <sub>7</sub> <sup>[G]</sup>	D-CO <sub>2</sub> _24 <sup>[G]</sup>	D-CO <sub>2</sub> _7 <sup>[G]</sup>	SRI <sub>12</sub> <sup>[G]</sup>	SRI <sub>24</sub> <sup>[G]</sup>	SCRI <sub>4</sub> <sup>[G]</sup>	SCRI <sub>7</sub> <sup>[G]</sup>	S-CO <sub>2</sub> _7 <sup>[G]</sup>	DRI <sub>24</sub> <sup>[I]</sup>	DCRI <sub>4</sub> <sup>[I]</sup>	DCRI <sub>7</sub> <sup>[I]</sup>
<b>DRI<sub>24</sub><sup>[G]</sup></b>	n/s													
<b>DCRI<sub>4</sub><sup>[G]</sup></b>	n/s	0.83												
<b>DCRI<sub>7</sub><sup>[G]</sup></b>	n/s	0.86	0.98											
<b>D-CO<sub>2</sub>_24<sup>[G]</sup></b>	n/s	0.86	0.98	1.00										
<b>D-CO<sub>2</sub>_7<sup>[G]</sup></b>	n/s	0.86	0.98	1.00	1.00									
<b>SRI<sub>12</sub><sup>[G]</sup></b>	n/s	n/s	0.76	0.71	0.71	0.71								
<b>SRI<sub>24</sub><sup>[G]</sup></b>	n/s	n/s	n/s	n/s	n/s	n/s	0.86							
<b>SCRI<sub>4</sub><sup>[G]</sup></b>	-0.83	n/s	n/s	n/s	n/s	n/s	n/s	n/s						
<b>SCRI<sub>7</sub><sup>[G]</sup></b>	-0.81	n/s	n/s	n/s	n/s	n/s	n/s	n/s	0.93					
<b>S-CO<sub>2</sub>_7<sup>[G]</sup></b>	-0.88	n/s	n/s	n/s	n/s	n/s	n/s	n/s	0.88	0.91				
<b>DRI<sub>24</sub><sup>[I]</sup></b>	0.76	0.74	0.71	0.81	0.81	0.81	n/s	n/s	n/s	n/s	n/s			
<b>DCRI<sub>4</sub><sup>[I]</sup></b>	0.83	0.83	0.71	0.81	0.81	0.81	n/s	n/s	n/s	-0.71	n/s	0.95		
<b>DCRI<sub>7</sub><sup>[I]</sup></b>	0.83	0.83	0.71	0.81	0.81	0.81	0.62	n/s	n/s	-0.71	n/s	0.95	1.00	
<b>CH<sub>4</sub>_30<sup>[I]</sup></b>	n/s	0.83	0.83	0.88	0.88	0.88	0.74	n/s	n/s	n/s	n/s	0.83	0.81	0.81

All Spearman's rank-order correlation coefficients shown are significant at  $p < 0.05$ ; n/s: non-significant; <sup>[G]</sup>: Greek method; <sup>[I]</sup>: Italian method.

## Figure captions

**Figure 1.** Oxygen uptake rates (OUR) for the studied substrates using the dynamic respiration activity methods in Greece (GRE) and Italy (ITA).

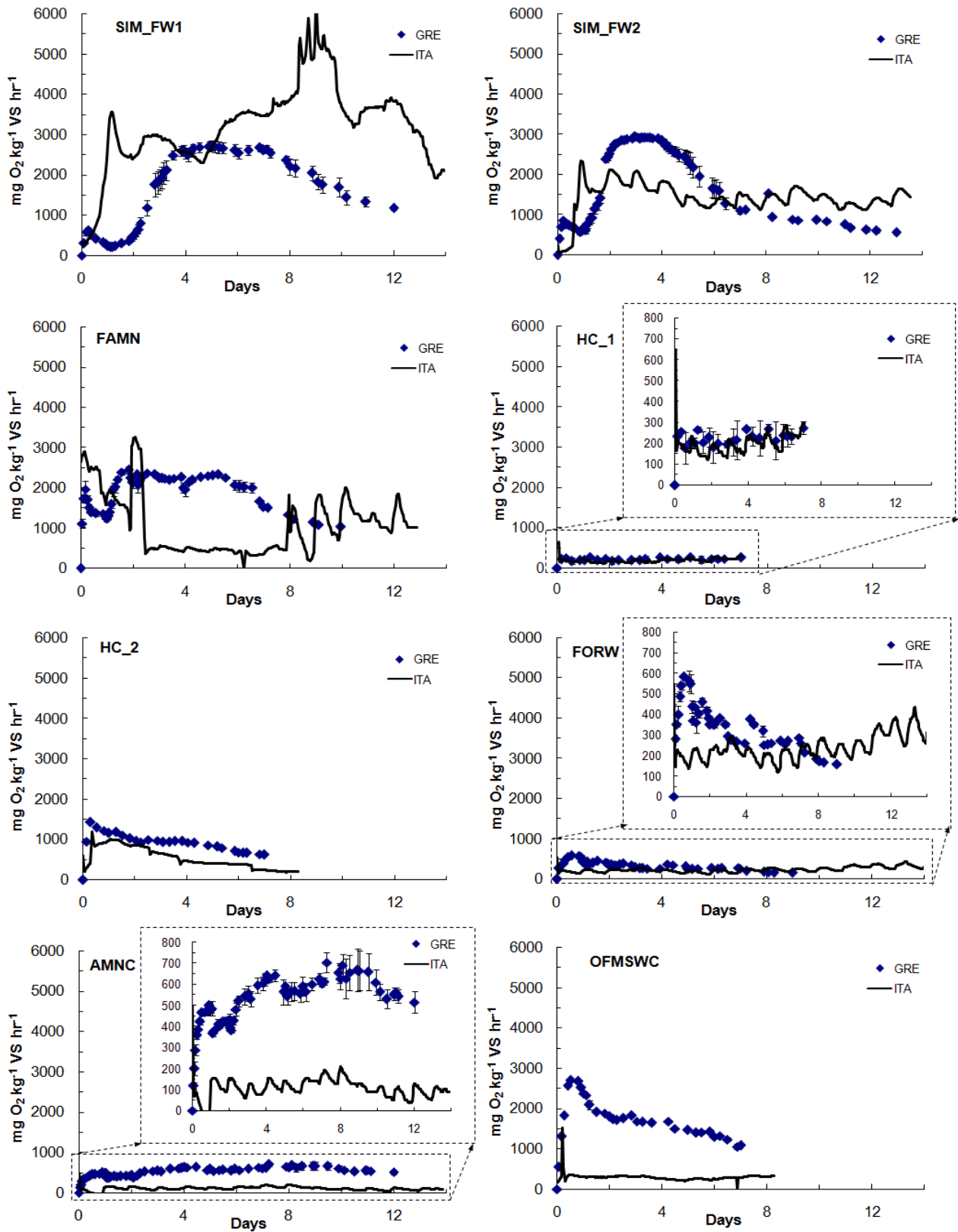
**Figure 2.** Temperature profiles of the studied substrates, in the CosTech<sup>®</sup> dynamic reactors (Italian method).

**Figure 3.** Effect of cumulative air flow on respiration activity, in the CosTech<sup>®</sup> dynamic reactors (Italian method).

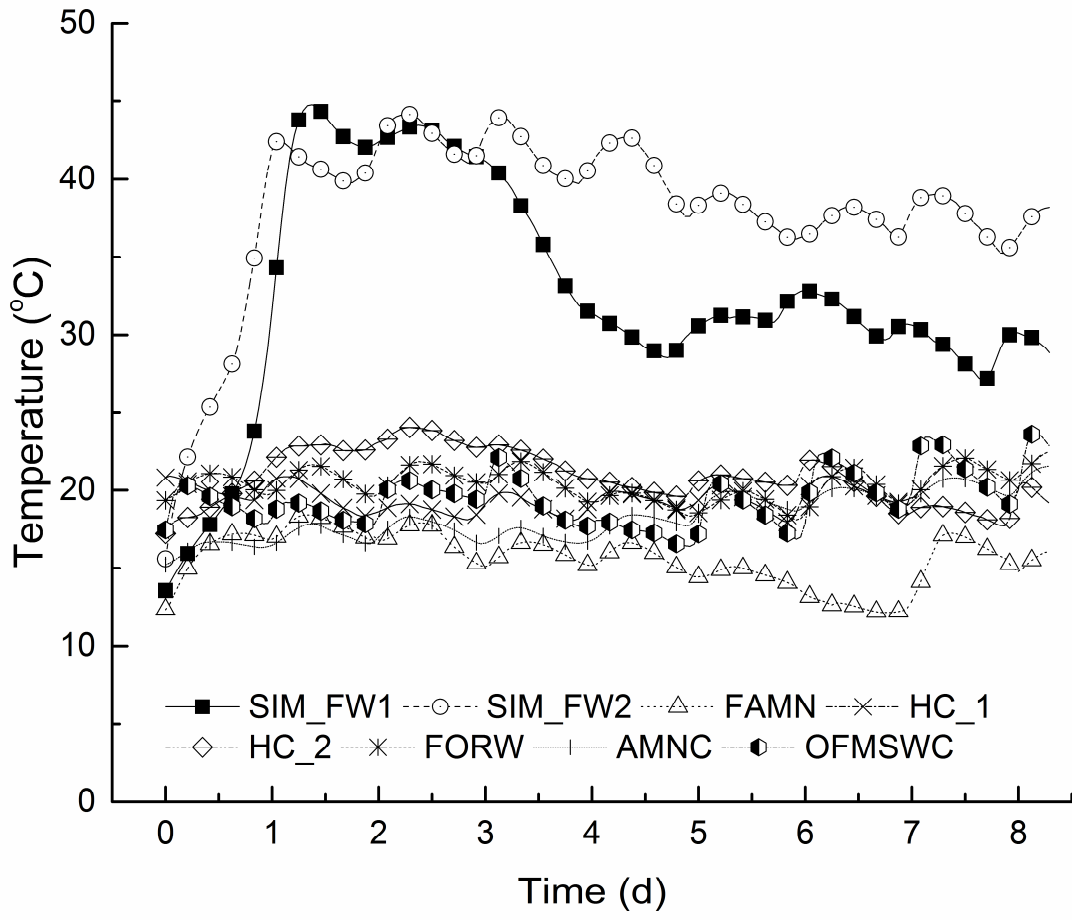
**Figure 4.** Correlation plots between the initial volatile solids content of the substrates and: (a) DRI<sub>24</sub>, (b) DCRI<sub>7</sub> measured in the Greek (GRE) and the Italian (ITA) labs.

**Figure 5.** Correlation plots between Greek (GRE) and Italian (ITA) indices; (a) and (b): dynamic indices; (c) and (d): BMP and dynamic indices; (e), (f): static and dynamic indices.

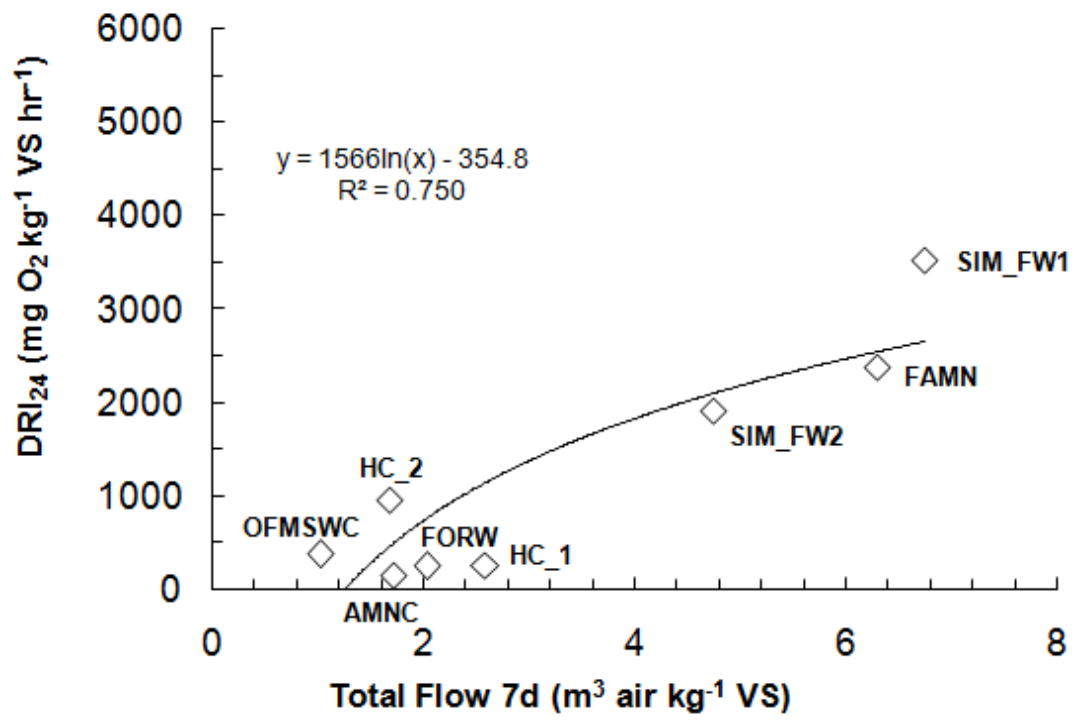
**Figure 1**



**Figure 2**

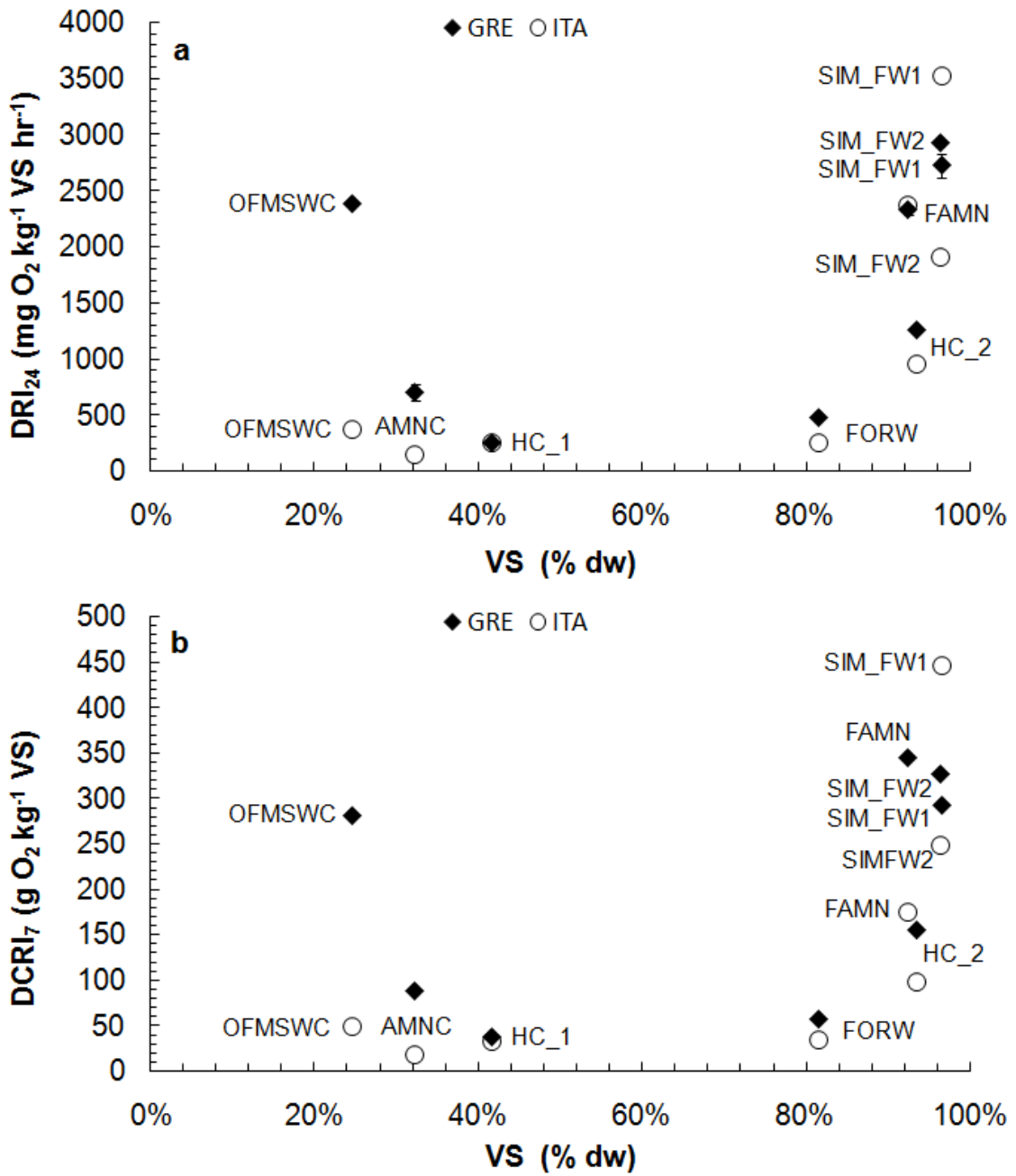


**Figure 3**





**Figure 4**



**Figure 5**

