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1	Compost mixture influence of interactive physical parameters on microbial kinetics and
2	substrate fractionation
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21 22	

23 Abstract

24 Composting is a feasible biological treatment for the recycling of wastewater sludge 25 as a soil amendment. The process can be optimized by selecting an initial compost recipe 26 with physical properties that enhance microbial activity. The present study measured the 27 microbial O₂ uptake rate (OUR) in 16 sludge and wood residue mixtures to estimate the 28 kinetics parameters of maximum growth rate $\mu_{\rm m}$ and rate of organic matter hydrolysis $K_{\rm h}$, as 29 well as the initial biodegradable organic matter fractions present. The starting mixtures 30 consisted of a wide range of moisture content (MC), waste to bulking agent (BA) ratio 31 (W/BA ratio) and BA particle size, which were placed in a laboratory respirometry apparatus 32 to measure their OUR over 4 weeks. A microbial model based on the activated sludge process 33 was used to calculate the kinetic parameters and was found to adequately reproduced OUR 34 curves over time, except for the lag phase and peak OUR, which was not represented and 35 generally over-estimated, respectively. The maximum growth rate μ_m , was found to have a quadratic relationship with MC and a negative association with BA particle size. As a result, 36 increasing MC up to 50 % and using a smaller BA particle size of 8-12 mm was seen to 37 38 maximize $\mu_{\rm m}$. The rate of hydrolysis $K_{\rm h}$ was found to have a linear association with both MC 39 and BA particle size. The model also estimated the initial readily biodegradable organic 40 matter fraction, MB_0 , and the slower biodegradable matter requiring hydrolysis, MH_0 . The 41 sum of MB_0 and MH_0 was associated with MC, W/BA ratio and the interaction between these 42 two parameters, suggesting that O₂ availability was a key factor in determining the value of 43 these two fractions. The study reinforced the idea that optimization of the physical 44 characteristics of a compost mixture requires a holistic approach.

45

46 Key words: composting, microbial kinetics, respirometry, sludge, wood residues.

47 **1. Introduction**

48 Composting the sludge produced by treatment plants receiving mainly domestic or 49 food processing wastewaters is a sustainable method of recycling carbon with minimum 50 greenhouse gas production (Amlinger et al., 2008). Composting relies mostly on the ability of microorganisms to biodegrade and stabilise the organic waste, to destroy pathogens and 51 52 produce an esthetically acceptable soil conditioner (Insam and de Bertoldi, 2007; Metcalf and 53 Eddy, 2003). Increasing urbanization and industrialization in cities resulted in the production 54 of more sludge waste requiring disposal or anaerobic digestion (Adhikari, 2005). Otherwise, 55 this sludge waste is landfilled where it is mostly transformed into CH₄ because of ambient anaerobic conditions (Rashmussen and Khalil, 1984). At the landfill site, 45 to 58 % of 56 57 organic waste on a dry mass basis is transformed into CH₄, which can be captured at a cost to 58 produce energy (Solid Waste Landfill Guidance, 1999).

59 Composting consists of 3 to 4 weeks of biodegradation in the active phase, followed 60 by several months of maturation in the curing phase (Gupta and Garg, 2008). As opposed to the curing phase which stabilizes the more resistant compounds (Haug, 1993), the microbial 61 62 activity of the active phase can be manipulated and optimized through a better understanding 63 of the interaction between the physical parameters. For the biodegradation and stabilization 64 of compost mixtures, Mohajer et al. (2009) demonstrated the interaction on O₂ uptake rate 65 (OUR) of physical parameters, namely moisture content (MC), bulking agent (BA) particle 66 size distribution and BA to waste (W) ratio.

67 The interactive effect of physical parameters on compost decomposition as measured 68 by Mohajer et al. (2009) can be further quantified by estimating microbial degradation 69 parameters. Such microbial parameters can be estimated by assuming that the compost 70 mixture OUR is similar to that of wastewater activated sludge systems decomposing BOD. 71 The traditional approach to respirometric modeling associates changes in microbial O_2 72 consumption with the growth and decay of the biomass (Spanjers et al., 1998). Past literature 73 has presented models estimating the influence on composting of parameters such as 74 temperature (Haug, 1993; Tremier et al., 2005; Richard and Walker, 2006), moisture content 75 (MC) (Richard et al., 2002) and aeration rate (Richard et al., 2006; de Guardia et al., 2008). 76 Certain physical parameters, such as MC and BA properties however, have been shown to be 77 particularly important due to their strong interdependent effect on microbial O₂ uptake (Diaz 78 et al., 2007; Mohajer et al., 2009).

79 Tremier et al. (2005) developed a kinetics model describing microbial growth rate and 80 sludge waste biodegradation. Based on the activate sludge model, the Tremier model 81 estimates kinetic parameters through the linear transformation of O2 consumption rate 82 measured during the decomposition of organic matter by a heterotrophic biomass. The 83 strength of the Tremier model lies in the fact that it considers the organic matter as split into 84 three different fractions, mainly, the readily biodegradable fraction (MB), the biodegradable 85 fraction requiring hydrolysis (MH) and the inert fraction (IM). Only a few composting models have included the hydrolysis process (Hamelers, 1993; Liwarska-Bizukojc et al., 2002), even 86 87 though it was shown to be rate-limiting (Veeken and Hamelers, 1999; Sole-Mauri et al., 88 2007). Although the Tremier model does not include all components and variables affecting 89 composting, its strength lies in its simplicity and its inclusion of a hydrolysis step.

90 Accordingly and for compost mixtures, the objective of this study was to estimate the 91 microbial kinetic parameters and organic matter biodegradation dynamics as a function of the 92 interaction between the physical properties of moisture content (MC), bulking agent to waste 93 ratio (BA/W ratio on a dry basis) and BA particle size. Consisting of slaughter house 94 wastewater sludge and wood residues recycled from a composting operation, the 95 experimental compost mixtures offered a wide range of combinations in term of physical properties. For individual compost mixtures, the O₂ uptake rate (OUR) was measured during 96 97 24 days using a respirometry apparatus (Mohajer et al., 2009). With the OUR data, the 98 Tremier model was used to estimate the maximum microbial growth rate, μ_m and then the organic matter degradation parameters, namely organic matter hydrolysis rate, K_h, and the 99 100 initial fractions of readily degradable organic matter, MB_0 and that requiring hydrolysis, 101 MH_0 .

102

103 **2. Material and Methods**

104 2.1. Conceptual approach of the model

105 The model developed by Tremier et al. (2005) assumes that the substrate has three 106 phases: a dry solid phase, an aqueous phase and a porous phase. Within this three phase 107 matrix consisting of the BA and sludge mixture, the organic matter is assumed to offer three 108 different initial fractions each with a specific biodegradation rate: the readily biodegradable 109 faction, MB_0 , the fraction requiring hydrolysis, MH_0 , and that which is inert or not degraded, 110 *MI*.

111 The first fraction, MB_0 , is already soluble and is readily used as a source of carbon 112 and energy by microorganisms. As a carbon source and depending on the biomass growth 113 yield *Y*, part of *MB* is transformed to give new biomass. The microbial population grows as a 114 function of available *MB* and its maximum growth rate and death coefficient:

$$dX/dt = X_t \times \{ \mu_m \times MB_t/(K_b + MB_t) - b \}$$
(1)

117

118 where X_t is the biomass population in mmol O₂ (kg dry matter)⁻¹; *t* is time in h⁻¹; μ_m is the 119 maximum growth rate of the biomass in h⁻¹; MB_t is the mass of readily biodegradable organic 120 matter at time *t* in mmol O₂ (kg dry matter)⁻¹; K_b is the saturation constant for *MB* set at 3 121 mmol O₂ (kg dry matter) ⁻¹; and *b* is the death coefficient of the biomass set at 0.05 h⁻¹ 122 (Tremier et al., 2005).

123 The energy needed for biomass growth is supplied through the oxidation of a fraction 124 of *MB* and the dead biomass expressed as (1-Y) *MB* and (1-f) X_t . Thus, the O₂ uptake rate 125 (OUR) can be expressed as:

126

128

 $R_{O2}(t) = X_t \times \{ (1-Y) / Y \times \mu_m \times MB_t / (K_b + MB_t) + b \times (1-f) \}$ (2)

129 where $R_{O2}(t)$ is the O₂ uptake rate (OUR) at time *t* in mmol O₂ (kg of dry matter - h)⁻¹; *Y* is the 130 yield coefficient of the biomass growth set at 0.68 (dimensionless); *f* is the fraction of dead 131 biomass contributing to *MI*, dimensionless, and *MI* is the fraction of inert organic matter in 132 mmolO₂ (kg of dry matter)⁻¹.

133 The second biodegradable fraction labeled *MH* supplies *MB* to the microorganisms 134 through enzymatic hydrolysis:

135

- $dMH/dt = X_t \times \{ -K_h \times MH_t/X_t / (MH_t/X_t + K_{mh}) \}$ (3)
- 137

where MH_t is the fraction of solid or soluble organic matter at time t which requires hydrolysis to become readily biodegradable in mmol O₂ (kg of dry matter)⁻¹; K_h is the rate of hydrolysis for the fraction MH in h⁻¹ and K_{mh} is the hydrolysis saturation constant for the ratio MH_t/X_t set at 6.5 (dimensionless).

142 The resulting mass balance for the readily biodegradable *MB* fraction can thus be 143 expressed as:

144
$$dMB/dt = X_t \times \{-1/Y \times \mu_m \times MB_t/(K_b + MB_t) + K_h \times (MH_t/X_t) / (MH_t/X_t + K_{mh}) \}$$
(4)
145

146 The hydrolysis reaction is initiated from the very beginning but becomes the rate 147 limiting process after the peak OUR, as microorganisms are left with the remaining complex 148 organic macromolecules requiring hydrolysis and governed by the rate K_h . Resistant organic matter, such as cellulose and lignin accumulated from the biodegradation of the two first fractions, are partly transformed into the third inert fraction MI. The degradation of the first two fractions also leads to the accumulation of dead biomass which, through degradation, is partly accumulated as inert matter, MI. The fraction of dead biomass contributing to MI is defined by the coefficient *f* with a value of 0.2 (Tremier et al., 2005).

154

155 2.2 Parameter Estimation

Equations 1 to 4 were used to estimate the kinetic parameters μ_{m} , K_{h} and X_{0} , and the 156 organic matter fractions of MB_0 and MH_0 at time zero (Table 1). The dynamic biological 157 model was fitted to the experimental OUR recorded through respirometry. For each trial, the 158 159 output parameters of the model were calibrated to the experimental OUR using an 160 optimization program designed by Tremier et al. (2005) for the SCILAB software (INRIA, France) based on a least squared method. Default values for Y, f, b, K_b, K_{mh} were chosen 161 based on published literature corresponding to 0.68 (dimensionless), 0.2 (dimensionless), 162 0.05 h^{-1} , 3 mmol O₂ (kg dry matter)⁻¹ and 6.5 (dimensionless), respectively (Tremier et al., 163 2005). The value of the kinetic parameters μ_m and K_h and for X_0 , MB_0 and MH_0 were 164 165 optimized through fitting using linearization methods performed on the exponential rise and 166 subsequent fall of the experimental OUR curves obtained from respirometry. The linearization method corresponds to a simplified way of interpreting the respirometry curve 167 168 and the values were derived from an identification procedure based on structural identifiable 169 procedures (Dochain et al., 1995; Sperandio and Paul, 2000).

170

172

171 2.3. Substrate Material and chemical characterization

The experimental materials consisted of sewage sludge from the wastewater treatment facility of a slaughter house and as BA, green waste or twigs and branches recycled from a composting process. For consistency, a large sludge sample obtained prior to the trial was split into 10 kg sub samples and stored until use at -20°C. Table 1 characterizes the experimental materials.

The experimental materials were analyzed for dry matter (DM), organic matter (OM), total organic carbon (TOC), chemical O₂ demand (COD) and total Kjeldahl nitrogen (TKN) prior to the start of the experiment. The DM was measured by drying the wet samples at 80°C to constant weight. A higher temperature was avoided to prevent the combustion of this type of sample during drying. The OM content was measured by burning the dried ground samples 183 at 550°C for 4 h using the standard method NF U 44-160 (Afnor, 1985). The TOC was 184 measured by oxidizing the dried ground samples to CO₂ and using infrared spectrometry (SKALAR device) to measure CO₂ production according to the standardized method NF-EN-185 186 13137 (Afnor, 2001a). The sample COD was determined by the oxidation of 50 mg of dried ground sample using potassium dichromate, according to an adaptation of the standard 187 188 method described in the norm NF T 90-101 (Afnor, 2001b) for powdered samples. The TKN 189 content was quantified on 50 mg of dried ground sample by mineralization with a strong acid 190 medium (98% sulphuric acid), followed by steam distillation and titrimetric determination, an 191 adaptation of the standard method NF ISO 11261 (Afnor, 1995) for powdered samples.

192

193 2.4. Respirometric Measurement Method

194 Mohajer et al. (2009) describes the respirometric apparatus used in this study to 195 measure OUR resulting from the biodegradation of the waste mixtures (Figure 1). It consisted 196 of six cylindrical 10 L airtight reactors made of stainless-steel and submersed in a water bath 197 controlled at 40 °C, a temperature was found to produce optimal biodegradation conditions 198 (Tremier et al., 2005). Inside the reactor, the substrate material was placed on a 3 mm mesh grid located 70 mm above the cell bottom forming a plenum receiving 65 L h⁻¹ of air via a 199 200 glass diffuser. As past studies have shown that an intermittent air supply can lead to O_2 limitations (Paletski and Young, 1995), a continuous air flow was supplied. Homogenous O2 201 202 diffusion for the trials was ensured by re-circulating part of the exhaust air back into the cells. 203 The entering and exhaust air streams were monitored for O_2 content using a paramagnetic O_2 204 gas analyser (MAIHAK Technology, Nimburger, Germany).

205 To solely test the effect of the experimental physical factors on microbial activity, the 206 apparatus controlled other environmental factors. Along with that of the sample, the 207 temperature of the inlet air was preheated at 40 °C using a copper serpentine 10 mm in 208 diameter, 2 mm in thickness and 2 m in length submerged in the water-bath. The temperature 209 of the sample was monitored by means of a Pt100 temperature probe (OMNI Instruments, 210 UK) inserted in the centre of the cell. The inflowing air was saturated with moisture by 211 bubbling through two water-filled glass bottles immersed in the water-bath. Moisture 212 condensing in the exhaust air was collected in beakers above the water-bath to prevent its 213 return into the sample cells. Once the experiment began, the samples were left inside the 214 respirometric cells until their OUR dropped to a low constant value, usually corresponding to 215 four weeks.

216

217 2.5. Experimental design and statistical analysis

218 The 16 experimental mixtures were created by combining low, intermediate and high 219 values of the three physical parameters namely MC, W/BA ratio and BA particle size (Table 220 2), determined using a response surface method experimental design. Water or dried sludge 221 were mixed with the appropriate amount of BA to obtain MCs of 20, 30, 45, 60 and 70 %, 222 although actual experimentally recorded MC varied slightly (Table 2). The waste mixtures 223 were manually mixed with BA to create W/BA ratios at 1/9.2, 1/7.9, 1/6, 1/4.1 and 1/2.1 on a 224 dry basis. Finally, different BA particle sizes of 8 to12 mm, 12 to 20 mm, 20 to 30 mm, 30 to 225 40 mm and over 40 mm were achieved by sieving through rotary screens. The C:N ratio of all 226 experimental recipes ranged between 10 and 22, which are acceptable for sludge composting 227 (Table 2).

A 3 kg (wet mass) sample of each mixture was placed in individual cells to prevent BA breakage and to minimize mixture compaction. All O₂ uptake rates (OUR) were expressed on the basis of initial mixture dry mass. Six compost recipes were tested at any one time. In total, 16 compost recipes were tested without duplication because of the wide range of incremental levels used in the combinations selected.

Using the observed experimental respirometric data, univariate linear regression was first performed to delineate the marginal relationships of the measured MC, W/BA ratio and the mean BA particle size in each range, with each of μ_{m} , K_{h} , MB_0 and MH_0 . Multivariate linear regression was subsequently performed to decipher the associations between the physical characteristics of each treatment including their interactions, upon each of the kinetic parameters and substrate matter fractions after 28 days of aeration, using the statistical software package Stata® (Statacorp LP, Texas, USA). The regression model was:

241
$$E[Y] = \beta_0 + \beta_1 \chi_1 + \beta_2 \chi_2 + \beta_3 \chi_3 + \beta_{12} \chi_1 \chi_2 + \beta_{13} \chi_1 \chi_3 + \beta_{23} \chi_2 \chi_3 + \beta_{11} \chi_1^2 + \beta_{22} \chi_2^2 + \beta_{33} \chi_3^2$$
(5)
242

where *Y* is the outcome of interest; β_0 is the intercept; β_1 , β_2 , β_3 are the linear coefficients; β_{12} , β_{13} , β_{23} are the interaction coefficients; β_{11} , β_{22} , β_{33} are the quadratic coefficients; χ_1 is the MC in %; χ_2 is the BA particle size in mm, dimensionless, and; χ_3 is the W/BA ratio. Analysis of variance (ANOVA) determined the significance of the models using the F-Test, at a confidence level of 90% (p < 0.1). Moreover, the significance of each regression model parameter (variable or interaction) was determined using a Student Test at a confidence level of 75% (p < 0.25). The regression coefficient R^2 measured the global fit of the regression model with the experimental data and the adjustment of the models was considered acceptable for R^2 greater than 55 %.

252

253 **3. Results**

254 3.1 Model validation

255 Figures 2 and 3 illustrate the OUR over time for experimental mixtures 4 and 9, 256 respectively, obtained through experimental trials and model simulations using Scilab. 257 Mixtures 4 and 9 offered the largest and smallest variations between the experimental and 258 simulation curve, respectively, obtained after optimization of the cycles to improve the fit of 259 the simulation curve. For all experimental mixtures, the general OUR curve was reproduced, 260 except for the peak which was generally slightly overestimated. Considering that the area under the curve is used to estimate MB_0 , this model overestimation introduces an error of 5 261 262 % at the very most. Furthermore, the model accurately reflected the exponential rise in OUR determining mainly the maximum growth rate μ_m of the microbial population. The 263 264 simulations however, lacked the ability to reproduce the brief lag phase observed at the beginning of the trials where microorganisms were acclimating to mixture conditions. 265 266 Nonetheless, the relatively short lag phase of less than 12 hours did not interfere with the 267 subsequent exponential rise in OUR, resulting in similar experimental and simulation curves.

After the peak, the model simulation correlated closely with the subsequent and gradual drop in sample OUR. This period is presumed to correspond mainly to the biodegradation of the more complex organic fraction requiring hydrolysis, MH_0 , resulting in a drop in OUR. As less biodegradable matter remains accessible to the microbes, the OUR recesses to lower levels (Tremier et al., 2005).

273

274 3.3. Influence of physical parameters on microbial kinetic coefficients

275 The experimental OUR curves were used to estimate the maximum growth rate and 276 hydrolysis kinetics in each of the 16 experimental recipes (Table 3). The maximum growth rate μ_m varied from 0.07 h⁻¹ achieved in mixture 6 (MC of 30 %, W/BA ratio of 1/7.9 and BA 277 particle size of 30-40 mm), to 0.20 h⁻¹ in mixture 3 (MC of 52 %, W/BA ratio of 1/6 and BA 278 particle size of 8-12 mm). The average $\mu_{\rm m}$ for all 16 experimental mixtures was 0.106 h⁻¹. 279 280 These values are somewhat lower than that found in other studies, perhaps due to the characteristic of the sludge waste. Tremier et al. (2005) found ranges between 0.13 and 0.34 281 282 h^{-1} using sewage sludge and pine bark in a 1:1 wet mass ratio biodegraded under temperatures of 20 to 70 °C. Activated sludge models suggest a typical value of 0.25 h⁻¹ at 20 °C, but they pertain to the raw volatile solids contained in municipal wastewaters. Kaiser (1996) suggested a $\mu_{\rm m}$ value of 0.2 h⁻¹ at 40 °C for the biodegradation modelling of vegetable substrates during composting.

Through regression analysis, the maximum growth rate, μ_m , was found to be significantly affected by MC, via a quadratic relationship, and BA particle size (p < 0.05) (R² = 0.63):

290

291

 $\mu_{\rm m} = 0.1126 + 0.0006 \times \chi_1 - 0.0018 \times \chi_1^2 - 0.0018 \times \chi_2 + 0.0001\chi_2^2$ (6) for $\chi_1 = (\text{MC} - 45), \%; \chi_2 = (\text{BA particle size} - 25), \text{mm}$

292

293 The nature of the quadratic association between MC and growth rate was such that 294 increased moisture levels up to 50 % were associated with larger μ_m (Figure 4). A reduction in $\mu_{\rm m}$ at levels higher than 50 %, however, resulted in MC reducing the free air space and 295 296 impeding O₂ availability. The negative association between BA particle size and μ_m , on the 297 other hand, reflected the ability of the biomass to maximize surface area attachment and 298 waste biodegradation. Found previously to affect microbial OUR (Mohajer et al., 2009), 299 W/BA ratio and its interactions with the other physical parameters did not have a significant impact on μ_m as a result of the inoculation effect of the sludge and the BA. The sludge/BA 300 301 mixture probably contained a high initial microbial population that rapidly expanded upon 302 exposure to aeration, reducing the potential impact that an optimal physical matrix could 303 provide on microbial activity.

304 Averaged for all 16 experimental recipes, the estimated rate of hydrolysis, K_h , had a mean value of 0.098 h⁻¹ over a range of 0.081 h⁻¹ in mixture 11 (MC of 52 %, W/BA ratio of 305 1/6 and BA particle sizes of over 40 mm) to 0.12 h⁻¹ in mixture 8 (MC of 62 %, W/BA ratio 306 of 1/4.1 and BA particle sizes of 12-20 mm). These values were slightly higher than those 307 suggested by Sole-Mauri et al. (2007), which ranged from 0.007 to 0.04 h⁻¹, depending on the 308 substrate, but were less than those reported by Tremier et al. (2005) at 0.1 to 0.2 h⁻¹ 309 depending on the temperature. The rate of hydrolysis was found to be significantly associated 310 to MC and BA particle size (p < 0.05) but also influenced by the interaction between W/BA 311 312 ratio and BA particle size ($R^2 = 0.56$): 313

 $314 K_{\rm h} = 0.0986 + 0.0005 \times \chi_1 - 0.0008 \times \chi_2 - 0.0101 \times \chi_2 \chi_3 (7)$

315 for $\chi_1 = (MC - 45)$, %; $\chi_2 = (BA \text{ particle size} - 25)$, mm; $\chi_3 = (W/BA \text{ ratio} -0.167)$,

dimensionless.

317 A higher MC and smaller BA particle size value tended to increase K_h . Accordingly, 318 the hydrolysis reaction is favoured by an increasing MC and a large specific surface provided 319 by smaller BA particle size (Figure 5). The importance of a small BA particle size is 320 underlined through the interaction with W/BA. Hence, if the quantity of sludge in the mixture 321 is increased, BA particle size has to decrease not to limit the hydrolysis kinetics.

322

323 3.4 Influence of physical parameters on the biodegradable organic fraction

324 The model estimated the readily biodegradable organic matter fraction, MB_0 , and the 325 fraction requiring hydrolysis, MH_0 (Table 1), available for microorganisms in the studied 326 experimental conditions. The MB_0 fraction ranged from a low of 475 mmol O₂ (kg dry matter)⁻¹ in mixture 6 with a relatively large BA particle size of 30-40 mm and a low W/BA 327 ratio and MC of 1/7.9 and 30 %, respectively, to a high of 2 085 mmol O_2 (kg dry matter)⁻¹ 328 329 for mixture 3 with a smaller particle size of 8-12 mm, higher W/BA ratio of 1/6 and an optimal MC of 50 %. A minimum MH₀ fraction of 7 950 mmol O₂ (kg dry matter)⁻¹ was 330 331 reached for mixture 1 with the lowest W/BA ratio of 1/9.2 and an intermediate value of BA 332 particle size of 20-30mm. The maximum MH_0 concentration of 18 160 mmol O₂ (kg dry matter)⁻¹ was reached with mixture 5 with a large W/BA ratio of 1/6 and a high of MC of 68 333 %. 334

The MB_0 fraction was mainly influenced by BA particle size, and the interaction between MC and W/BA ratio (p < 0.05). To a less extent, it was also influenced by individual effects of W/BA ratio (p < 0.15) and MC (p < 0.25) ($R^2 = 0.64$):

338

 $339 \qquad MB_0 = 1182 + 6.99 \times \chi_1 - 24.20 \times \chi_2 + 1861 \times \chi_3 - 275.42 \times \chi_1 \chi_3 \tag{8}$

340 for $\chi_1 = (MC-45)$, %; $\chi_2 = (BA \text{ particle size} - 25)$, mm; $\chi_3 = (W/BA \text{ ratio } -0.167)$,

- 341 dimensionless.
- 342

343 Predictably, less BA with a smaller particle size increased MB_0 since the BA is less 344 biodegradable than the sludge. Increasing MC probably allows microorganisms to better 345 reach the available MB₀. MC and W/BA ratio impacted available MB_0 through a negative 346 interactive effect where an increase in either parameter had a decreasing effect. This negative 347 crossed effect of MC and W/BA resulted from physical restrictions on the accessibility of the organic matter and oxygen to the microorganisms. Indeed, with high moisture and a high quantity of sludge versus BA, the porosity of the medium can be limited, consequently affecting the aeration of the microorganisms. On the other hand, with low moisture and a low quantity of sludge versus BA, conditions for microorganisms activity are also limited.

352 The sum of MB_0 and MH_0 is equivalent to the total amount of organic matter 353 biodegraded over the four weeks of respirometry. The total concentration ranged from a minimum of 8 912 mmol O_2 (kg dry matter)⁻¹ with mixture 1 with mid level values for both 354 MC (42 %) and BA particle size (20-30 mm) and a low W/BA ratio (1/9.2). A maximum 355 value of 19 585 mmol O_2 (kg dry matter)⁻¹ was obtained with mixture 5 with the highest MC 356 (68 %) and mid-level values for both BA particle size (20-30 mm) and W/BA ratio (1/6). The 357 358 biodegradable organic matter in the 16 mixtures comprised about 35 % of the total organic matter. As written concerning MB_0 , the Tremier model was designed to evaluate the quantity 359 360 of organic matter which can be oxidized under experimental conditions rather than the total 361 potential biodegradable matter.

The sum of the MB_0 and MH_0 fractions were associated with MC, W/BA ratio and the interaction between MC and W/BA ratio (p < 0.05) ($R^2 = 0.59$):

364

365 $(MB_0 + MH_0) = 15211 + 114 \times \chi_1 + 14208 \times \chi_3 - 1769 \times \chi_1\chi_3$ (9) 366 for $\chi_1 = (MC-45), \%; \chi_3 = (W/BA ratio -0.167), dimensionless.$

367

Increasing MC up to 50 % along with W/BA, increased the sum of MB_0 and MH_0 as a 368 369 result of a more favourable water content and a higher sludge fraction. The negative 370 interaction between the two parameters, however, altered the individual main effect (Figure 6). Coupled with larger W/BA ratio, MC above 50 % decreased the sum of MB_0 and MH_0 , 371 372 most likely due to lower O₂ diffusion as a result of less free-air-space. Thus, these results confirm the notion that the impact of MC and W/BA ratio in composting systems are 373 374 interconnected, where the level of one parameter was important to interpret the impact of the other. For MC and the W/BA ratio exceeding 50 % and 1/4.1, respectively, Mohajer et al. 375 376 (2009) also found a reduced OUR. The same conditions produced a reduction in the sum of 377 MB_0 and MH_0 also as a result of less O₂ availability. 378

379 **4. Conclusion**

With compost mixture respirometry data, this study used the microbial Tremier model adapted from the activated sludge process to obtain microbial kinetic parameters describing maximum microbial growth rate μ_m , the rate of organic matter hydrolysis K_h and the initial fractions of organic matter being readily degradable MB_0 and requiring hydrolysis MH_0 . These parameters were estimated for 16 experimental mixtures of sludge and wood residue offering various physical parameter combinations of moisture content (MC), waste to bulking agent ratio (W/BA ratio) and bulking agent (BA) particle size.

387 The model accurately simulated the OUR over time except for brief periods corresponding to the initial lag phase and peak. Differences in the estimated μ_m and K_h were 388 shown to be mostly explained by the initial physical characteristics of the experimental 389 390 mixtures and their interaction. The MC had a quadratic relationship with $\mu_{\rm m}$ and a positive 391 linear association with K_h while particle size had a negative linear association with both. 392 Bulking agent particle size in the range of 8-12 mm and MC up to 50 % yielded the highest 393 $\mu_{\rm m}$ and $K_{\rm h}$. The sum of the readily degradable fraction of organic matter and that requiring 394 hydrolysis, MB_0 and MH_0 , respectively, were impacted in an interactive manner by both MC 395 and W/BA ratio. Since the Tremier model is based on OUR, the physical parameters were found to impact the actual rather than the total potential value of MB_0 and MH_0 as the results 396 based on O₂ availability explain. The study reinforces the fact that the physical characteristics 397 398 of the initial compost mixture are optimized through a holistic approach. The interactive 399 association between MC and W/BA ratio, in particular, was important in influencing the 400 degree of biodegradable matter.

401

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482 List of symbols

- 483 b is the death coefficient of the microbial mass, h^{-1}
- 484 BA is the bulking agent
- 485 COD is the chemical oxygen demand, mg O_2 (kg of dry organic matter)⁻¹
- 486 DM is the dry matter, %
- 487 *f* is the fraction of dead biomass contributing to *MI*, dimensionless
- 488 K_b is the concentration of organic matter required to obtain half of μ_m , mmol O₂ (kg dry 489 organic matter)⁻¹
- 490 $K_{\rm mb}$ is the hydrolysis saturation constant for the ratio $MH_t/X_{\rm t}$, mmol O₂ (kg dry organic 491 matter)⁻¹
- 492 $K_{\rm h}$ is the hydrolysis rate constant for the fraction *MH* in h⁻¹
- 493 MB_0 is the initial mass of readily biodegradable organic matter at time *t*, mmol O₂ (kg dry 494 matter)⁻¹
- 495 MB_t is the mass of readily biodegradable organic matter at time t, mmol O₂ (kg dry matter)⁻¹
- 496 *MI* is the fraction of organic matter which is inert in kg (kg of dry matter)⁻¹.
- 497 MH_0 is the initial fraction of solid or soluble macromolecules at time *t* which first requires 498 hydrolysis to be reduced to the easily biodegradable matter, kg (kg of dry matter)⁻¹
- 499 MH_t is the fraction of solid or soluble macromolecules at time t which first requires
- hydrolysis to be reduced to the readily biodegradable matter, kg (kg of dry organic
 matter)⁻¹
- 502 OM is the organic matter, %
- 503 OUR is the oxygen uptake rate, mmol O_2 (kg of dry organic matter h)⁻¹
- 504 $R_{O2}(t)$ is the O₂ consumption rate at time *t*, mmol O₂ (kg of dry organic matter h)⁻¹
- 505 t is time, h^{-1}
- 506 TC is the total carbon, %
- 507 TKN is the total Kjeldahl nitrogen, mg N (kg of dry organic matter)⁻¹
- 508 W/BA ratio is the waste (agro-food sludge) to bulking agent (wood residues recycled from a
- 509 composting centre) ratio on a dry mass basis, dimensionless
- 510 X is the microbial population, mg biomass (kg dry organic matter)⁻¹
- 511 X_0 is the initial mass of microbial population at time 0, mg biomass (kg dry organic matter)⁻¹
- 512 X_t is the mass of microbial population at time t, mg biomass (kg dry organic matter)⁻¹
- 513 *Y* is the coefficient of biomass growth yield, mmol O_2 (mg biomass)⁻¹
- 514 β_0 is the regression intercept, dimensionless

- β_1 , β_2 , β_3 are the linear coefficients, dimensionless
- $\mu_{\rm m}$ is the maximum growth rate of the microbial population, h⁻¹
- χ_l is the MC in %
- χ_2 is the W/BA ratio, dimensionless
- χ_3 is the BA particle size in mm

Description of the experimental sludge and bulking agent						
	Wood residues					
	sludge	8.5 - 12	12 - 20	20 - 30	30 - 40	>40
		(mm)	(mm)	(mm)	(mm)	(mm)
Moisture content (%)	86.8	10.0	10.0	10.0	10.0	10.0
OM (% DM) _(CV %)	83(0.1)	91.0 _(0.1)	$92.1_{(0.1)}$	93.9 _(0.0)	$93.1_{(0.1)}$	89.3(0.3)
COD (g/kg DM) _(CV %)	$1,331_{(0.6)}$	1,356 _(0.1)	1 362(0.1)	1,370(0.9)	1,531 _(0.7)	1,369(0.6)
TC (g/kg DM) _(CV %)	484(0.5)	514(0.4)	508(0.9)	510(0.7)	517 _(3.2)	486(2.9)
TKN (g/kg DM) _(CV %)	46.4(1.5)	46.1(0.8)	37.1(1.0)	31.5(0.5)	34.3(0.5)	$23.8_{(0.9)}$

 Table 1

 Description of the experimental sludge and bulking agent

DM: dry matter basis; CV: coefficient of variation.

Table 2 Experimental compost recipes.

•	Nominal	Measured	Bulking	Waste/bulking	C:N ratio
Mixture	moisture	moisture	agent	agent ratio	
	content (%)	content (%)	particle size	(dry mass	
			(mm)	basis)	
1	45	41.5	20 - 30	1 / 9.2	14.4
2	60	61.6	12 – 20	1 / 7.9	13.3
3	45	52.5	8 - 12	1 / 6.0	10.4
4	45	49.0	20 - 30	1 / 2.8	13.1
5	70	68.3	20 - 30	1 / 6.0	14.1
6	30	30.3	30 - 40	1 / 7.9	17.7
7	30	35.6	12 - 20	1 / 4.1	16.2
8	60	61.7	12 - 20	1 / 4.1	11.5
9	60	56.0	30 - 40	1 / 4.1	16.5
10	45	51.4	20 - 30	1 / 6.0	14.8
11	45	53.2	> 40	1 / 6.0	21.3
12	30	31.4	12 - 20	1 / 7.9	19.3
13	20	28.5	20 - 30	1 / 6.0	15.3
14	60	61.1	30 - 40	1 / 7.9	17.6
15	45	51.0	20 - 30	1 / 6.0	15.1
16	30	33.5	30 - 40	1 / 4.1	17.6

		0			
Mixture	Maximum growth rate, $\mu_{\rm m}$ (h ⁻¹)	Hydrolysis rate constant, K _h (h ⁻¹)	<i>MB</i> ₀ (mmol O ₂ / kg DM)	MH ₀ (mmol O ₂ / kg DM)	Total MB_0 + MH_0 (mmol $O_2/$ kg DM)
1	0.1994	0.1258	959	7953	8912
2	0.1051	0.1014	1264	15342	16606
3	0.2006	0.1172	2085	13169	15254
4	0.1100	0.0873	1763	16300	18063
5	0.1119	0.1165	1429	18156	19585
6	0.0685	0.0849	475	10729	11204
7	0.0961	0.1088	1687	14381	16068
8	0.0978	0.1209	850	14179	15029
9	0.0951	0.0945	928	14710	15638
10	0.1155	0.0975	1186	16753	17939
11	0.1053	0.0813	971	12769	13740
12	0.0845	0.0847	1025	12580	13605
13	0.0805	0.0851	1068	14007	15075
14	0.0960	0.0987	1090	16699	17789
15	0.1159	0.0872	1293	15083	16376
16	0.0851	0.0774	993	14621	15614

Table 3 Microbial kinetics and substrate biodegradation components.

Note: MB_0 : initial fraction of organic matter being readily degradable; MH_0 : initial fraction of organic matter requiring hydrolysis; DM: dry matter.



Figure 1. Schematic diagram of respirometry apparatus (Tremier et al., 2005).



Figure 2. Oxygen uptake rate over time for mixture 4 (moisture content of 49 %; waste to bulking agent ratio of 1/2.8 dry mass; bulking agent particle size of 20-30mm) with the largest variation between experimental and simulation trials.



Figure 3. Oxygen uptake rate over time for mixture 9 (moisture content of 56 %; waste to bulking agent ratio of 1/4.1 dry mass; bulking agent particle size of 30-40mm) with the smallest variation between experimental and simulation trials.



Figure 4. Simulated effect of compost mixture moisture content and bulking agent particle size on the maximum microbial maximum growth, μ_m .



Fig. 5. Simulated effect of compost mixture moisture content and bulking agent particle size on the hydrolysis constant K_h for the organic matter fraction which is not readily degradable, MH_0 .



Figure 6. Simulated effect of compost mixture moisture content and waste to bulking agent (W/BA) ratio on the fraction of organic matter which is degraded during composting ($MB_0 + MH_0$).