

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

3
4 Ardavan Mohajer¹, Anne Tremier^{2,3}, Suzelle Barrington^{1,3},
5 Cecile Teglia^{2,3}, Marco Carone⁴

6 ¹Department of Bioresource Engineering, Faculty of Agricultural and Environmental
7 Sciences, Macdonald Campus of McGill University, 21 111 Lakeshore, Ste-Anne-de-
8 Bellevue, Quebec, Canada H9X 3V9

9 ²Cemagref, UR-GERE, F-35044 Rennes, France

10 ³Université Européenne de Bretagne, Rennes, France

11 ⁴Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health,
12 615 N. Wolfe St., Baltimore, MD, USA (21205)

13
14 Corresponding author: Suzelle Barrington, Ph. D., Professor,
15 Department of Bioresource Engineering, Macdonald Campus of McGill University
16 21 111 Lakeshore Road, Ste-Anne-de-Bellevue, Quebec, H9X 3V9, Canada
17 Tel.: +514 398 7776; fax: +514 398 8387.
18 E-mail: suzelle.barrington@mcgill.ca

19
20 Submitted to the journal of: Waste Management

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 μ_m and rate of organic matter hydrolysis K_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
36 quadratic relationship with MC and a negative association with BA particle size. As a result,
37 increasing MC up to 50 % and using a smaller BA particle size of 8-12 mm was seen to
38 maximize μ_m . The rate of hydrolysis K_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
51 microorganisms to biodegrade and stabilise the organic waste, to destroy pathogens and
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
56 anaerobic conditions (Rasmussen and Khalil, 1984). At the landfill site, 45 to 58 % of
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
61 the curing phase which stabilizes the more resistant compounds (Haug, 1993), the microbial
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 O₂
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 O_2 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
86 have included the hydrolysis process (Hamelers, 1993; Liwarska-Bizukojc et al., 2002), even
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
96 properties. For individual compost mixtures, the O_2 uptake rate (OUR) was measured during
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
99 organic matter degradation parameters, namely organic matter hydrolysis rate, K_h , and the
100 initial fractions of readily degradable organic matter, MB_0 , and that requiring hydrolysis,
101 MH_0 .

102 **2. Material and Methods**

103 **2.1. Conceptual approach of the model**

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

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

115

$$116 \quad dX_t/dt = X_t \times \{ \mu_m \times MB_t / (K_b + MB_t) - b \} \quad (1)$$

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

$$127 \quad R_{O_2}(t) = X_t \times \{ (1-Y) / Y \times \mu_m \times MB_t / (K_b + MB_t) + b \times (1-f) \} \quad (2)$$

128

129 where $R_{O_2}(t)$ 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

$$136 \quad dMH/dt = X_t \times \{ -K_h \times MH_t / X_t / (MH_t / X_t + K_{mh}) \} \quad (3)$$

137

138 where MH_t is the fraction of solid or soluble organic matter at time t which requires
 139 hydrolysis to become readily biodegradable in mmol O₂ (kg of dry matter)⁻¹; K_h is the rate of
 140 hydrolysis for the fraction MH in h⁻¹ and K_{mh} is the hydrolysis saturation constant for the ratio
 141 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:

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

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

149 matter, such as cellulose and lignin accumulated from the biodegradation of the two first
150 fractions, are partly transformed into the third inert fraction *MI*. The degradation of the first
151 two fractions also leads to the accumulation of dead biomass which, through degradation, is
152 partly accumulated as inert matter, *MI*. The fraction of dead biomass contributing to *MI* is
153 defined by the coefficient *f* with a value of 0.2 (Tremier et al., 2005).

154

155 2.2 Parameter Estimation

156 Equations 1 to 4 were used to estimate the kinetic parameters μ_m , K_h and X_0 , and the
157 organic matter fractions of MB_0 and MH_0 at time zero (Table 1). The dynamic biological
158 model was fitted to the experimental OUR recorded through respirometry. For each trial, the
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,
161 France) based on a least squared method. Default values for Y , f , b , K_b , K_{mh} were chosen
162 based on published literature corresponding to 0.68 (dimensionless), 0.2 (dimensionless),
163 0.05 h^{-1} , $3 \text{ mmol O}_2 (\text{kg dry matter})^{-1}$ and 6.5 (dimensionless), respectively (Tremier et al.,
164 2005). The value of the kinetic parameters μ_m and K_h and for X_0 , MB_0 and MH_0 were
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
167 linearization method corresponds to a simplified way of interpreting the respirometry curve
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

171 2.3. Substrate Material and chemical characterization

172

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

178 The experimental materials were analyzed for dry matter (DM), organic matter (OM),
179 total organic carbon (TOC), chemical O_2 demand (COD) and total Kjeldahl nitrogen (TKN)
180 prior to the start of the experiment. The DM was measured by drying the wet samples at 80°C
181 to constant weight. A higher temperature was avoided to prevent the combustion of this type
182 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
185 (SKALAR device) to measure CO₂ production according to the standardized method NF-EN-
186 13137 (Afnor, 2001a). The sample COD was determined by the oxidation of 50 mg of dried
187 ground sample using potassium dichromate, according to an adaptation of the standard
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
199 grid located 70 mm above the cell bottom forming a plenum receiving 65 L h⁻¹ of air via a
200 glass diffuser. As past studies have shown that an intermittent air supply can lead to O₂
201 limitations (Paletski and Young, 1995), a continuous air flow was supplied. Homogenous O₂
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 O₂ content using a paramagnetic O₂
204 gas analyser (MAIHAK Technology, Nimbunger, 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 to 12 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).

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

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

240

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

242

243 where Y is the outcome of interest; β_0 is the intercept; β_1 , β_2 , β_3 are the linear coefficients;
 244 β_{12} , β_{13} , β_{23} are the interaction coefficients; β_{11} , β_{22} , β_{33} are the quadratic coefficients; χ_1 is
 245 the MC in %; χ_2 is the BA particle size in mm, dimensionless, and; χ_3 is the W/BA ratio.

246 Analysis of variance (ANOVA) determined the significance of the models using the F-Test,
 247 at a confidence level of 90% ($p < 0.1$). Moreover, the significance of each regression model
 248 parameter (variable or interaction) was determined using a Student Test at a confidence level

249 of 75% ($p < 0.25$). The regression coefficient R^2 measured the global fit of the regression
250 model with the experimental data and the adjustment of the models was considered acceptable
251 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
261 under the curve is used to estimate MB_0 , this model overestimation introduces an error of 5
262 % at the very most. Furthermore, the model accurately reflected the exponential rise in OUR
263 determining mainly the maximum growth rate μ_m of the microbial population. The
264 simulations however, lacked the ability to reproduce the brief lag phase observed at the
265 beginning of the trials where microorganisms were acclimating to mixture conditions.
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.

268 After the peak, the model simulation correlated closely with the subsequent and
269 gradual drop in sample OUR. This period is presumed to correspond mainly to the
270 biodegradation of the more complex organic fraction requiring hydrolysis, MH_0 , resulting in a
271 drop in OUR. As less biodegradable matter remains accessible to the microbes, the OUR
272 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
277 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
278 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
279 particle size of 8-12 mm). The average μ_m for all 16 experimental mixtures was 0.106 h^{-1} .
280 These values are somewhat lower than that found in other studies, perhaps due to the
281 characteristic of the sludge waste. Tremier et al. (2005) found ranges between 0.13 and 0.34
282 h^{-1} using sewage sludge and pine bark in a 1:1 wet mass ratio biodegraded under temperatures

283 of 20 to 70 °C. Activated sludge models suggest a typical value of 0.25 h⁻¹ at 20 °C, but they
 284 pertain to the raw volatile solids contained in municipal wastewaters. Kaiser (1996)
 285 suggested a μ_m value of 0.2 h⁻¹ at 40 °C for the biodegradation modelling of vegetable
 286 substrates during composting.

287 Through regression analysis, the maximum growth rate, μ_m , was found to be
 288 significantly affected by MC, via a quadratic relationship, and BA particle size ($p < 0.05$) ($R^2 =$
 289 0.63):

$$290 \quad \mu_m = 0.1126 + 0.0006 \times \chi_1 - 0.0018 \times \chi_1^2 - 0.0018 \times \chi_2 + 0.0001 \chi_2^2 \quad (6)$$

291 for $\chi_1 = (\text{MC} - 45)$, %; $\chi_2 = (\text{BA particle size} - 25)$, 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
 295 in μ_m at levels higher than 50 %, however, resulted in MC reducing the free air space and
 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
 300 impact on μ_m as a result of the inoculation effect of the sludge and the BA. The sludge/BA
 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
 305 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
 306 1/6 and BA particle sizes of over 40 mm) to 0.12 h⁻¹ in mixture 8 (MC of 62 %, W/BA ratio
 307 of 1/4.1 and BA particle sizes of 12-20 mm). These values were slightly higher than those
 308 suggested by Sole-Mauri et al. (2007), which ranged from 0.007 to 0.04 h⁻¹, depending on the
 309 substrate, but were less than those reported by Tremier et al. (2005) at 0.1 to 0.2 h⁻¹
 310 depending on the temperature. The rate of hydrolysis was found to be significantly associated
 311 to MC and BA particle size ($p < 0.05$) but also influenced by the interaction between W/BA
 312 ratio and BA particle size ($R^2 = 0.56$):

313

$$314 \quad K_h = 0.0986 + 0.0005 \times \chi_1 - 0.0008 \times \chi_2 - 0.0101 \times \chi_2 \chi_3 \quad (7)$$

315 for $\chi_1 = (\text{MC} - 45)$, %; $\chi_2 = (\text{BA particle size} - 25)$, mm; $\chi_3 = (\text{W/BA ratio} - 0.167)$,
 316 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
 327 matter)⁻¹ in mixture 6 with a relatively large BA particle size of 30-40 mm and a low W/BA
 328 ratio and MC of 1/7.9 and 30 %, respectively, to a high of 2 085 mmol O₂ (kg dry matter)⁻¹
 329 for mixture 3 with a smaller particle size of 8-12 mm, higher W/BA ratio of 1/6 and an
 330 optimal MC of 50 %. A minimum MH_0 fraction of 7 950 mmol O₂ (kg dry matter)⁻¹ was
 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
 333 matter)⁻¹ was reached with mixture 5 with a large W/BA ratio of 1/6 and a high of MC of 68
 334 %.

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

338

$$339 \quad MB_0 = 1182 + 6.99 \times \chi_1 - 24.20 \times \chi_2 + 1861 \times \chi_3 - 275.42 \times \chi_1 \chi_3 \quad (8)$$

340 for $\chi_1 = (\text{MC} - 45)$, %; $\chi_2 = (\text{BA particle size} - 25)$, mm; $\chi_3 = (\text{W/BA ratio} - 0.167)$,
 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 MB_0 . 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

348 organic matter and oxygen to the microorganisms. Indeed, with high moisture and a high
 349 quantity of sludge versus BA, the porosity of the medium can be limited, consequently
 350 affecting the aeration of the microorganisms. On the other hand, with low moisture and a low
 351 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
 354 minimum of 8 912 mmol O₂ (kg dry matter)⁻¹ with mixture 1 with mid level values for both
 355 MC (42 %) and BA particle size (20-30 mm) and a low W/BA ratio (1/9.2). A maximum
 356 value of 19 585 mmol O₂ (kg dry matter)⁻¹ was obtained with mixture 5 with the highest MC
 357 (68 %) and mid-level values for both BA particle size (20-30 mm) and W/BA ratio (1/6). The
 358 biodegradable organic matter in the 16 mixtures comprised about 35 % of the total organic
 359 matter. As written concerning MB_0 , the Tremier model was designed to evaluate the quantity
 360 of organic matter which can be oxidized under experimental conditions rather than the total
 361 potential biodegradable matter.

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

$$364 \quad (MB_0 + MH_0) = 15211 + 114 \times \chi_1 + 14208 \times \chi_3 - 1769 \times \chi_1 \chi_3 \quad (9)$$

365 for $\chi_1 = (\text{MC} - 45)$, %; $\chi_3 = (\text{W/BA ratio} - 0.167)$, dimensionless.

367
 368 Increasing MC up to 50 % along with W/BA, increased the sum of MB_0 and MH_0 as a
 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
 371 6). Coupled with larger W/BA ratio, MC above 50 % decreased the sum of MB_0 and MH_0 ,
 372 most likely due to lower O₂ diffusion as a result of less free-air-space. Thus, these results
 373 confirm the notion that the impact of MC and W/BA ratio in composting systems are
 374 interconnected, where the level of one parameter was important to interpret the impact of the
 375 other. For MC and the W/BA ratio exceeding 50 % and 1/4.1, respectively, Mohajer et al.
 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

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

387 The model accurately simulated the OUR over time except for brief periods
388 corresponding to the initial lag phase and peak. Differences in the estimated μ_m and K_h were
389 shown to be mostly explained by the initial physical characteristics of the experimental
390 mixtures and their interaction. The MC had a quadratic relationship with μ_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 μ_m and K_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
396 found to impact the actual rather than the total potential value of MB_0 and MH_0 as the results
397 based on O_2 availability explain. The study reinforces the fact that the physical characteristics
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.

402 **Acknowledgment**

403 This project was accomplished through the collaboration between Cemagref (UR GERE) and
404 McGill University (Department of Bioresource Engineering). This research is part of a larger
405 project, named ESPACE, financed by the French National Research Agency (ANR),
406 currently being carried out in partnership between Cemagref, Suez-Environment and Institut
407 de Mécanique des Fluides de Toulouse (IMFT). The Natural Science and Engineering
408 Research Council of Canada (NSERC) is also acknowledged for its financial contribution.

410 **References**

411 Adani, F., Lozzi, P., Genevini, P., 2001. Determination of biological stability by oxygen
412 uptake on municipal solid waste and derived products. *Compost Science & Utilization*
413 9, 163-178.

- 414 Adhikari, B.K. 2005. Urban Food Waste Composting. MSc Thesis, Department of
415 Bioresource Engineering, McGill University, Montreal.
- 416 AFNOR, 1985. NF U 44-160 - Amendements organiques et supports de culture -
417 Détermination de la matière organique totale - Méthode par calcination.
- 418 AFNOR, 1995. NF ISO 11261 - Qualité du sol - Dosage de l'azote total - Méthode de
419 Kjeldahl Modifiée.
- 420 AFNOR, 2001a. NF EN 13137 - Caractérisation des déchets - Dosage du carbone organique
421 total (COT) dans les déchets, boues et sédiments.
- 422 AFNOR, 2001b. NF T 90-101 - Qualité de l'eau - Détermination de la demande chimique en
423 oxygène (DCO).
- 424 Amlinger, F., Peyr, S., Cuhls, C. 2008. Green house gas emissions from composting and
425 mechanical biological treatment. *Waste Management & Research*, 26, 47-60.
- 426 Diaz, L.F., Savage, G.M., 2007. Factors that affect the process. In: Diaz, L.F., de
427 Bertoldi, M., Bidlingmaier, W., Stentiford, E. (Eds.), *Compost Science and*
428 *Technology*. Elsevier, Amsterdam, pp. 49-64.
- 429 Dochain, D., Van Rollegheem, P.A., Van Daele, M., 1995. Structural identifiability of
430 biokinetic models of activated sludge respiration. *Water Research* 29 (11), 2571-2578.
- 431 Gupta, R., Garg, V.K., 2008. Stabilization of primary sewage sludge during
432 vermicomposting. *Journal of Hazardous Materials* 153, 1023-1030.
- 433 De Guardia, A., Petiot, C., Rogeau, D., 2008. Influence of aeration rate and
434 biodegradability fractionation on composting kinetics. *Waste Management* 28, 73-84.
- 435 Haug, R.T., 1993. *The practical handbook of composting engineering*. Lewis Publishers,
436 Boca Raton, FL.
- 437 Hamelers, H.V.M., 1993. A theoretical model of composting kinetics. In: Hoitink, H.A.J.,
438 Keener, H.M. (Eds), *Science and Engineering of Composting: Design,*
439 *Environmental, Microbiological and Utilization Aspects*. Renaissance Publications,
440 Worthington, pp. 36-58.
- 441 Insam, H., de Bertoldi, M., 2007. Microbiology of the composting process. In: L.F. Diaz,
442 M. de Bertoldi, W. Bidlingmaier, E. Stentiford (ed.) *Compost Science and*
443 *Technology*. Elsevier, Amsterdam. p. 25-49.
- 444 Liang, C., Das, K.C., McClendon, R.W., 2003. The influence of temperature and moisture
445 content regimes on the aerobic microbial activity of a biosolids composting blend.
446 *Bioresource Technology* 86 (2), 131-137.
- 447 Liwarska-Bizukojc, E., Bizukojc, M., Ledakowicz, S., 2002. Kinetics of the aerobic

- 448 biological degradation of shredded municipal solid waste in liquid phase. *Water*
449 *Research* 36 (8), 2124-2132.
- 450 Metcalf and Eddy, 2003. *Wastewater Engineering: Treatment and Reuse*. The McGraw-Hill
451 Companies, Inc. New York, NY.
- 452 Mohajer, A., Tremier, A., Barrington, S., Martinez, J., Teglia, C., Carone, M., 2009.
453 Microbial oxygen uptake in sludge as influenced by compost physical parameters.
454 *Waste Management* 29 (8), 2257-2264.
- 455 Paletski, W.T., Young, J.C., 1995. Stability measurement of biosolids compost by aerobic
456 respirometry. *Compost Science and Utilization* 3, 16–24.
- 457 Rasmussen, R.A., Khalil, M.A.K., 1984. Atmospheric methane in the recent and ancient
458 atmospheres: Concentrations, trends and interhemispheric gradient. *Journal of*
459 *Geophysical Research* 89, 11599-11605.
- 460 Richard, T.L., Hamelers, H.V.M., Veeken, A., Silva, T., 2002. Moisture relationships in
461 composting processes. *Compost Science and Utilization* 10 (4), 286-302.
- 462 Richard, T.L., Walker, L.P., 2006. Modeling the temperature kinetics of aerobic solid-state
463 biodegradation. *Biotechnology Progress* 22 (1), 70-77.
- 464 Richard, T.L., Walker, L.P., Gossett, J.M., 2006. Effects of oxygen on aerobic solid-state
465 biodegradation kinetics. *Biotechnology Progress* 22, 60-69.
- 466 Sole-Mauri, F., Illa, J., Magri, A., Prenafeta-Boldu, F.X., Flotats, X., 2007. An integrated
467 biochemical and physical model for the composting process. *Bioresource Technology*
468 98 (17), 3278-3293.
- 469 *Solid Waste Landfill Guidance*, 1999. *Landfill Gas Monitoring and Mitigation*. Technical
470 Section, Division of Solid Waste Management, Tennessee.
- 471 Spanjers, H., Vanrolleghem, P.A., Olsson, G., Dold, P.L., 1998. *Respirometry in Control*
472 *of the Activated Sludge Process: Principles*. IWA Publishing, London, UK.
- 473 Sperandio, M., Paul, E., 2000. Estimation of wastewater biodegradable COD fractions by
474 combining respirometric experiments in various S₀/X₀ ratios. *Water Resource* 34 (4),
475 1233–1246.
- 476 Tremier, A., de Guardia, A., Massiani, C., Paul, E., Martel, J.L., 2005. A respirometric
477 method for characterizing the organic composition and biodegradation kinetics and
478 the temperature influence on the biodegradation kinetics, for a mixture of sludge and
479 bulking agent to be composted. *Bioresource Technology* 96, 169-180.
- 480 Veeken, A., Hamelers, B., 1999. Effect of temperature on hydrolysis rate of selected biowaste
481 components. *Bioresource Technology* 69,249-254.

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, $\text{mg O}_2 (\text{kg of dry organic matter})^{-1}$
- 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 , $\text{mmol O}_2 (\text{kg dry}$
- 489 $\text{organic matter})^{-1}$
- 490 K_{mb} is the hydrolysis saturation constant for the ratio MH_t/X_t , $\text{mmol O}_2 (\text{kg dry organic}$
- 491 $\text{matter})^{-1}$
- 492 K_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 , $\text{mmol O}_2 (\text{kg dry}$
- 494 $\text{matter})^{-1}$
- 495 MB_t is the mass of readily biodegradable organic matter at time t , $\text{mmol O}_2 (\text{kg dry matter})^{-1}$
- 496 MI is the fraction of organic matter which is inert in $\text{kg} (\text{kg of dry matter})^{-1}$.
- 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, $\text{kg} (\text{kg of dry matter})^{-1}$
- 499 MH_t is the fraction of solid or soluble macromolecules at time t which first requires
- 500 hydrolysis to be reduced to the readily biodegradable matter, $\text{kg} (\text{kg of dry organic}$
- 501 $\text{matter})^{-1}$
- 502 OM is the organic matter, %
- 503 OUR is the oxygen uptake rate, $\text{mmol O}_2 (\text{kg of dry organic matter} - \text{h})^{-1}$
- 504 $R_{O_2}(t)$ is the O_2 consumption rate at time t , $\text{mmol O}_2 (\text{kg of dry organic matter} - \text{h})^{-1}$
- 505 t is time, h^{-1}
- 506 TC is the total carbon, %
- 507 TKN is the total Kjeldahl nitrogen, $\text{mg N} (\text{kg of dry organic matter})^{-1}$
- 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, $\text{mg biomass} (\text{kg dry organic matter})^{-1}$
- 511 X_0 is the initial mass of microbial population at time 0, $\text{mg biomass} (\text{kg dry organic matter})^{-1}$
- 512 X_t is the mass of microbial population at time t , $\text{mg biomass} (\text{kg dry organic matter})^{-1}$
- 513 Y is the coefficient of biomass growth yield, $\text{mmol O}_2 (\text{mg biomass})^{-1}$
- 514 β_0 is the regression intercept, dimensionless

- 515 $\beta_1, \beta_2, \beta_3$ are the linear coefficients, dimensionless
- 516 μ_m is the maximum growth rate of the microbial population, h^{-1}
- 517 χ_1 is the MC in %
- 518 χ_2 is the W/BA ratio, dimensionless
- 519 χ_3 is the BA particle size in mm

Table 1
Description of the experimental sludge and bulking agent

	Sewage sludge	Wood residues				
		8.5 - 12 (mm)	12 - 20 (mm)	20 – 30 (mm)	30 – 40 (mm)	>40 (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)

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

Table 2
Experimental compost recipes.

Mixture	Nominal moisture content (%)	Measured moisture content (%)	Bulking agent particle size (mm)	Waste/bulking agent ratio (dry mass basis)	C:N ratio
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

Table 3
Microbial kinetics and substrate biodegradation components.

Mixture	Maximum growth rate, μ_m (h^{-1})	Hydrolysis rate constant, K_h (h^{-1})	MB_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

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

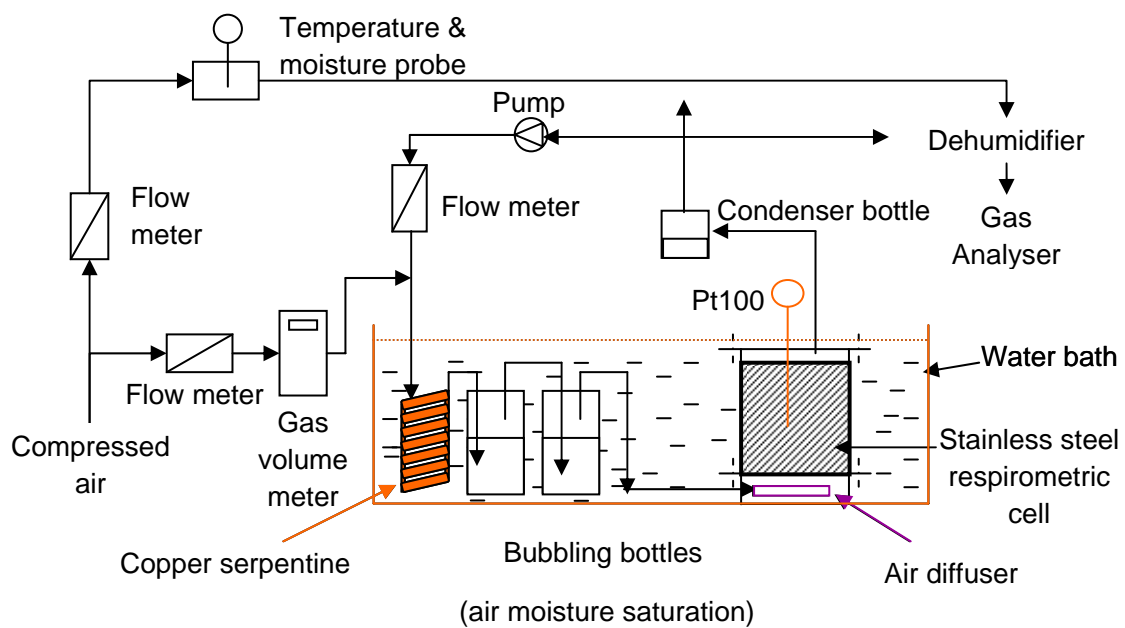


Figure 1. Schematic diagram of respirometry apparatus (Tremier et al., 2005).

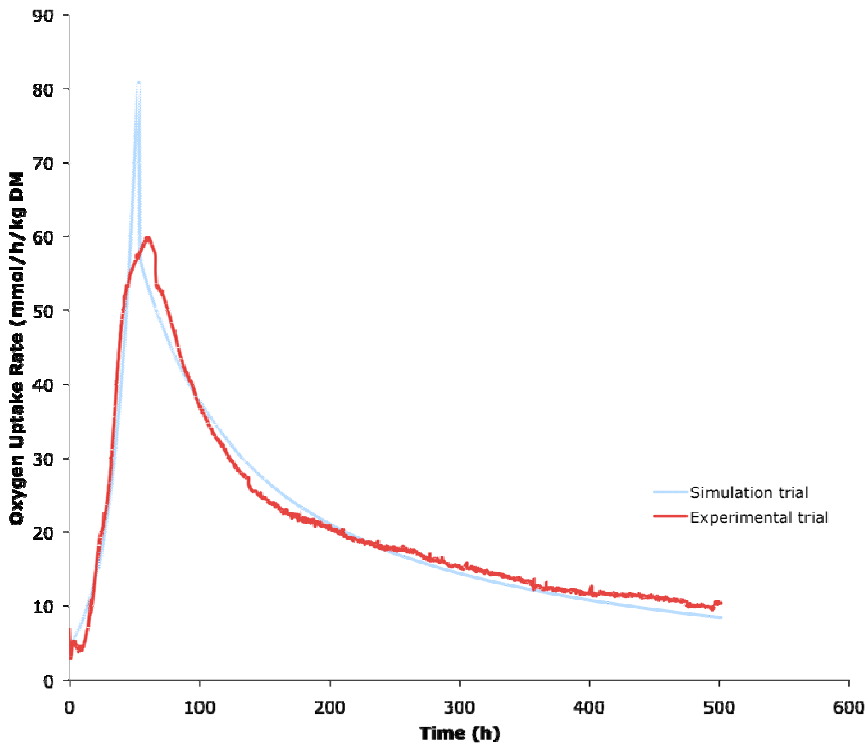


Figure 2. Oxygen uptake rate over time for mixture 4 (moisture content of 49 %; waste to bulking agent ratio of 1/2.8 dry mass; bulking agent particle size of 20-30mm) with the largest variation between experimental and simulation trials.

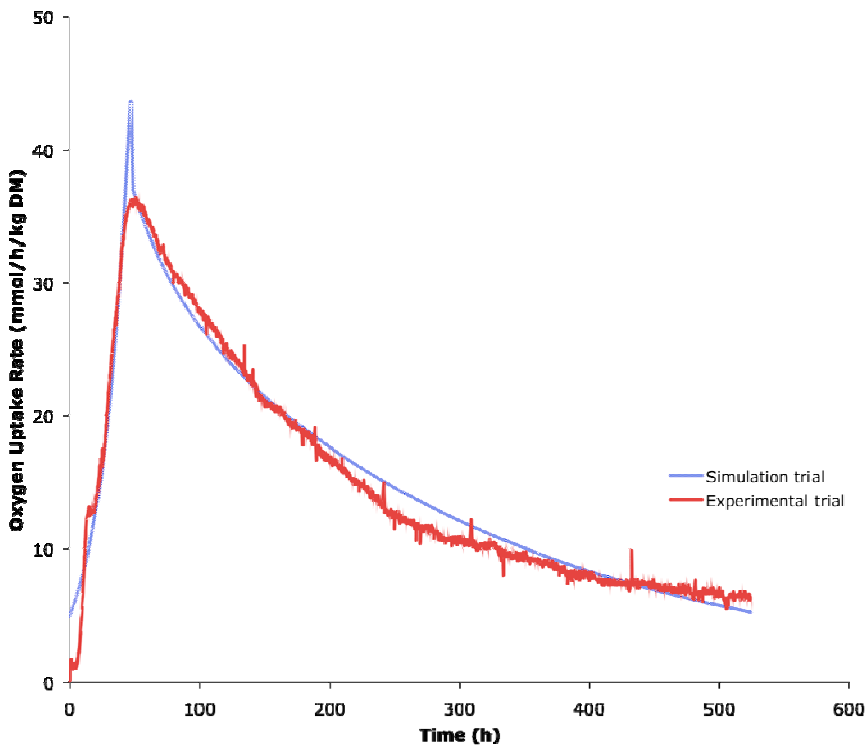


Figure 3. Oxygen uptake rate over time for mixture 9 (moisture content of 56 %; waste to bulking agent ratio of 1/4.1 dry mass; bulking agent particle size of 30-40mm) with the smallest variation between experimental and simulation trials.

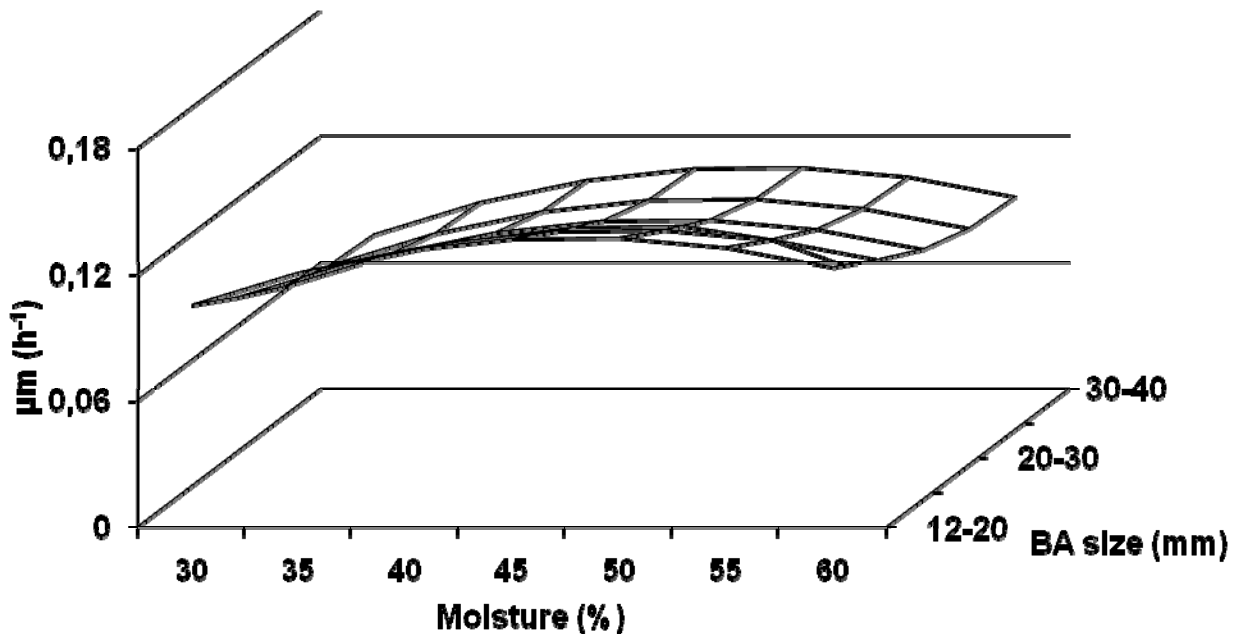


Figure 4. Simulated effect of compost mixture moisture content and bulking agent particle size on the maximum microbial maximum growth, μ_m .

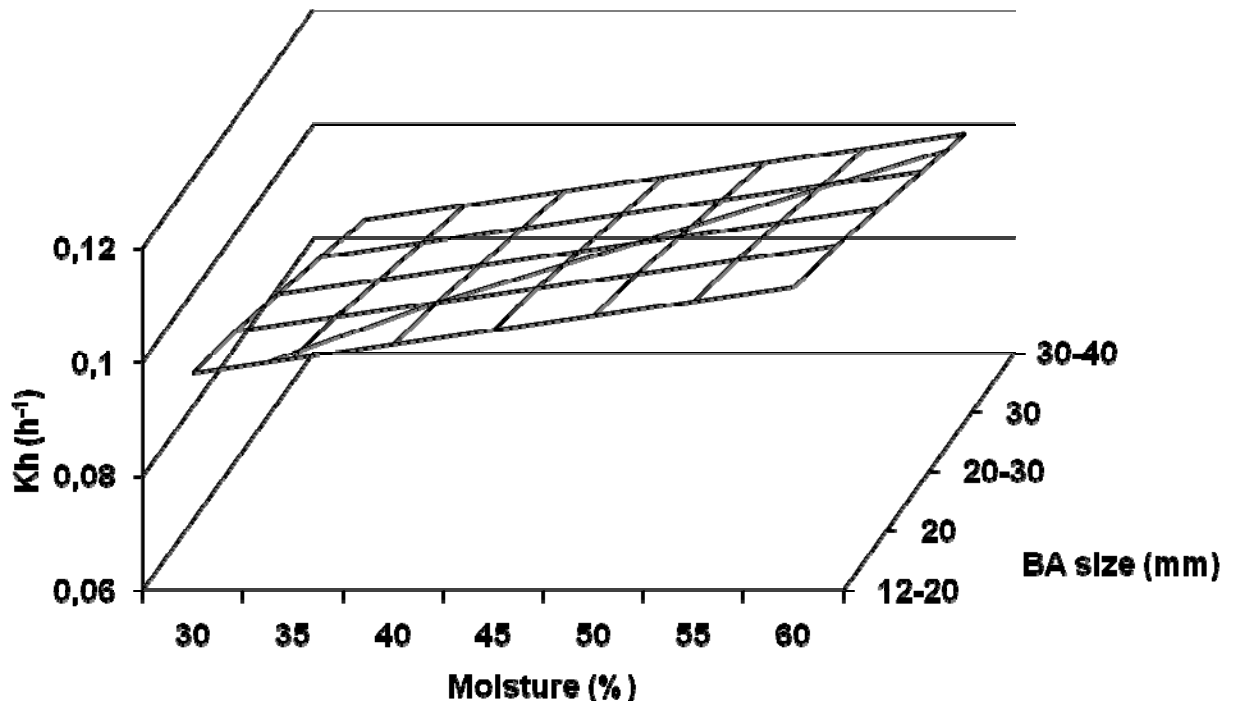


Fig. 5. Simulated effect of compost mixture moisture content and bulking agent particle size on the hydrolysis constant K_h for the organic matter fraction which is not readily degradable, MH_0 .

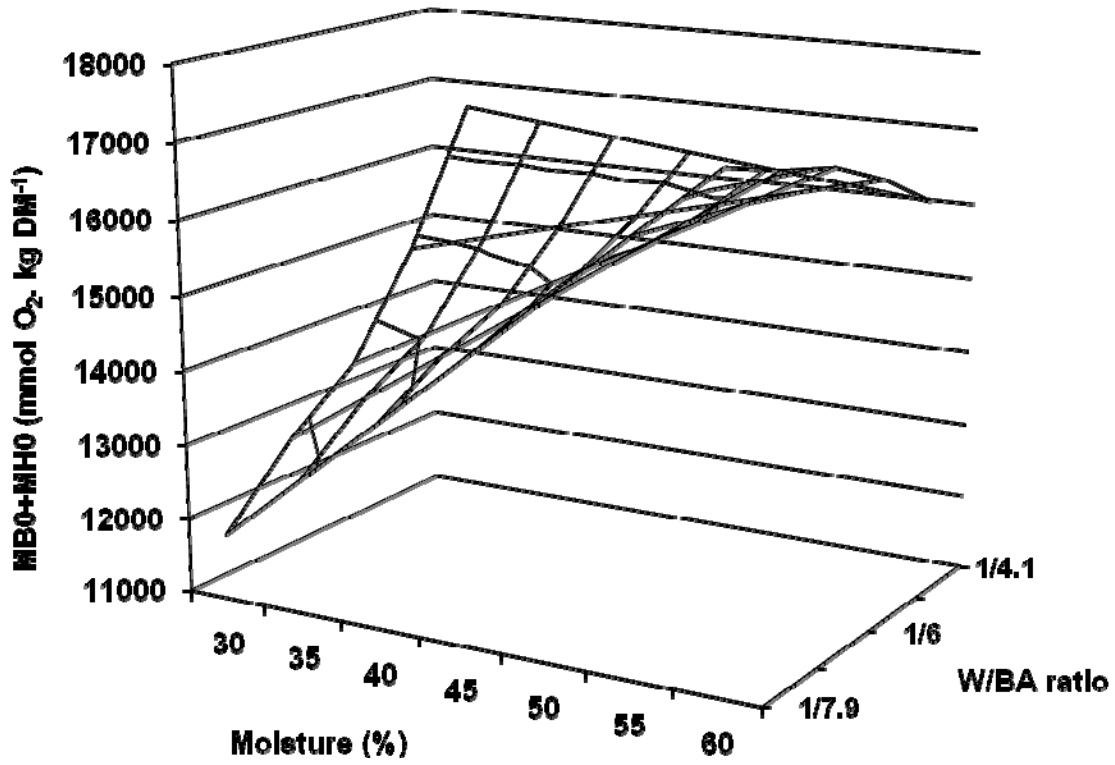


Figure 6. Simulated effect of compost mixture moisture content and waste to bulking agent (W/BA) ratio on the fraction of organic matter which is degraded during composting ($MB_0 + MH_0$).