

Non-destructive quantification of water gradient in sludge composting with Magnetic Resonance Imaging

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2	Non-Destructive Quantification of Water Gradient in Sludge Composting with Magnetic
3	Resonance Imaging
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19 Abstract

20 Sludge from a slaughterhouse wastewater plant, and mixtures of bulking agent (crushed wood 21 pallet) and sludge were studied by Nuclear Magnetic Resonance (NMR). The NMR spin-spin 22 relaxation (T₂) and spin-lattice relaxation (T₁) signals for sludge, wet crushed wood pallet and 23 mixtures of sludge and bulking agent were decomposed into three relaxation time 24 components. Each relaxation time component was explained by a non-homogeneous water 25 distribution on a microscopic length scale and by the porosity of the material. For all samples, 26 the T₂ relaxation time value of each component was directly related to the dry matter content. 27 The addition of wet crushed wood to sludge induced a decrease in the relaxation time, 28 explained by water transfer between the sludge and the wood.

Magnetic Resonance Imaging (MRI) and respirometric measurements were performed on sludge and wood mixtures. MR images of the mixtures were successfully obtained at different biodegradation states. Based on specific NMR measurements in an identified area located in the MRI cells, the results showed that grey levels of MR images reflected dry matter content. This preliminary study showed that MRI would be a powerful tool to measure water distribution in sludge and bulking agent mixtures and highlights the potential of this technique to increase the understanding of sludge composting.

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Keywords: NMR, MRI, relaxation, compost, biodegradability, moisture content, bulking
agent, sludge

41 **1. Introduction**

42 Treatment of wastewater is an important issue in western countries and research is currently 43 being undertaken to investigate the elimination or re-use of residual sludge at better cost, 44 including composting of wastewater sludge. In order to do so, sludge needs to be mixed with 45 a bulking agent which creates a porous matrix. Proportions of sludge and bulking agent are 46 chosen so that sludge covers the bulking agent with a thin layer, and then micro-organisms in 47 the sludge are sufficiently aerated to consume the available biodegradable organic matter. 48 During sludge composting treatment, the active biodegradation takes place in three to four 49 weeks, followed by several months of maturation (Gupta, 2008). Selection of an initial waste 50 recipe with optimal physical and biochemical parameters is essential to create an environment 51 conducive to high microbial growth and activity, and then to shorten and improve the 52 biodegradation in the active phase (Haug, 1993; Agnew, 2003). Some of the important 53 physical parameters for this waste recipe are water content, air porosity and specific surface 54 area.

55 Research on composting is often focused on overall understanding of the biodegradation 56 phenomena, i.e. on a macro-scale. All the parameters of biodegradation such as oxygen 57 consumption, bacteria activity, porosity and moisture content are averaged, and little is known 58 regarding the influence of local behaviour of the physical parameters on overall 59 biodegradation. Since solid wastes are very heterogeneous, better understanding of water 60 content, porosity and specific surface spatial distribution is needed, and Magnetic Resonance 61 Imaging (MRI) could help to fill this gap. Indeed, most of the common techniques to measure 62 physical parameters can not be applied along the composting process because they are 63 invasive or destructive. Moisture measurements need sampling and drying. Classic porosity 64 measurement implies to fill the porous volume with a liquid (Annan, 1998), which is destructive for the sample. Pycnometry is a non destructive method but results directly depend 65

on the applied pressure leading to the measurement of total air space (TAS) or only free air 66 space (FAS) (Su, 2006). Measurement of the specific surface is not easier. The direct 67 measurement technique through gaseous absorption (BET) used by Palmowski et al. 68 (Palmowski, 2003) can only be applied after drying the waste. On the contrary, MRI is non-69 70 destructive and non-invasive and thus makes it possible to follow the evolution of samples 71 over time. MRI is well known for its medical applications, but it has been used in other fields 72 including the wood industry (Muller, 2002), study of bacterial chemotaxis (Sherwood, 2003; 73 Olson, 2004), chemical engineering (Mantle, 2003), civil engineering (Faure, 2005), fluid 74 mechanics (Bonn, 2008), pharmaceutical science (Richardson, 2005) and food science (Hills, 1998; Butz, 2005). In an MRI experiment, the volume in which the signal is collected is 75 76 called a voxel, and its typical order of magnitude is the cubic millimetre. Voxel intensity is a 77 function of local proton density and of their respective spin-lattice (T_1) and spin-spin (T_2) 78 relaxation times. Proton density is proportional to the amount of material containing protons. 79 In most materials, water is the main constituent related to proton density. Consequently, the 80 grey level intensity of a voxel may be related to water content or air content (*i.e.* porosity). 81 Indeed when the voxel size is larger than the pore size the porosity is directly related to the 82 intensity of the MRI signal: the greater the quantity of gas, the lower the signal. This method 83 has been used in food science to quantify the distribution of porosity during proving of dough 84 (Grenier, 2003; Lucas, 2005). In the extreme case where the pore volume is much larger than 85 the voxel size, the pore size can be measured. For example, air cavities have been detected 86 and quantified in food products such as cheese (Mariette, 2004; 2006). In addition to the 87 porous fraction, the specific surface area can be estimated if the resolution of the MR images 88 is good enough.

Spin-lattice (T_1) and spin-spin (T_2) relaxation times are related to water content and also to material microstructure and to molecular structure (Brownstein, 1979; Belton, 1987; Hills,

91 1989b; a; Denisov, 2002). Several authors have used the T₂ and T₁ relaxation times as tracers
92 of water content for the investigation of draining processes (Mariette, 2006) and rehydration
93 processes (Ziegler, 2003).

The aim of this study was to evaluate the potential of the MRI technique in sludge composting to quantify the water distribution. The first part of this report focuses on the description of the nuclear magnetic resonance (NMR) signals in sludge and in mixtures of sludge and bulking agent and their sensitivity to water content. The second part describes the preliminary results of MRI images from sludge and bulking agent mixture. The potential for determination of water content and measurement of local porosity by MRI is discussed.

100

101 **2. Materials and Methods**

102 2.1 Sludge and bulking agent

The sludge came from the biological activated sludge treatment of the wastewater from a slaughter-house. Iron chloride and a polymeric flocculent (PRAESTOL K133) were added to the sludge to induce flocculation and, after a decantation step, the sludge was dewatered using a press band. The collected sludge was stored frozen at -20°C in 1kg batches. Sludge was unfrozen before each experiment, initially in a refrigerator at 4°C and then at room temperature.

The dry matter (DM, measured by drying at 105°C until constant weight) in the sludge was 13.6% of total weight, with approximately 80% organic matter to DM. The exact quantity of living bacteria present in the sludge was unknown. Flocs of bacteria were observed using optical microscopy (data not shown). Sludges with higher dry matter content were prepared using a low pressure oven (34 Mb and 30°C). Mixtures of sludge and bulking agent were prepared by hand mixing the sludge with crushed wood pallet as bulking agent. The size of the wood chips considered was 8 to 12 mm. Wood was stored after drying at 80°C. Different samples were then studied by NMR, i.e. the sludge without bulking agent, mixtures of wood and sludge, and a rehydrated wood sample (DM 45%). Since there is water transfer between sludge and dried wood after mixing, the saturated wood sample was prepared to mimic the contribution of the re-hydrated wood fraction in the mixture.

A combined experiment using MRI and respirometry was set up. Two large respirometric cells were prepared with 4.5 kg of sludge (wet) mixed with 2.5 kg of wood (dry mass ratio $1/4.1 \text{ g}_{\text{DM}}/\text{g}_{\text{DM}}$). The moisture content in both cells was approximately 56%. MRI measurements were performed at day 1, day 3 and day 27 of biodegradation. Samples were taken from these cells for NMR measurement and moisture analysis at 27 days.

125

126 2.2 Respirometry

127 Respirometry is a useful tool to assess microbial activity (Iannotti, 1994; Adani, 2001; Gea, 128 2004). The respirometric apparatus used in this study consisted of a 10 L airtight cylindrical 129 reactor made of glass. Three to four kilos of precisely weighed material were introduced into 130 the respirometric reactor on a 3 mm round mesh grid placed 70 mm above the cell bottom. An 131 entering air flow rate of 65 L/h (compressed air) was applied throughout the experiments. 132 Homogeneity of aeration of the entire sample was provided by means of the rapid 133 recirculation of the exhaust gas in the cell (360 L/h). The entering and exhaust air streams 134 were monitored for O₂ content using a paramagnetic O₂ gas analyser (MAHAIK Technology, 135 Germany).

The apparatus was monitored for the main environmental factors influencing microbial activity, i.e. temperature and moisture. As shown on **Figure 1** the temperature of samples was held constant by placing each respirometric cell in a water-bath at 40°C, which is optimal for biodegradation activity (Tremier, 2005). The inlet air was preheated to 40°C using a copper tubular coil with a diameter of 10 mm, thickness of 2 mm and length of 2 m, submerged in the 141 water-bath. The sample temperatures were monitored by means of a Pt100 temperature probe 142 (OMNI Instrument, UK) inserted in the centre of the sample. For the moisture control, the 143 inflowing air was saturated by bubbling through two water-filled glass bottles immersed in 144 the water-bath. Moisture condensing in the exhaust air was collected in beakers above the 145 water-bath to prevent its return into the sample cell and its entrance into the gas analyser.

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149 2.3 NMR measurements

150 All NMR measurements were performed with a 10 MHz Minispec Mq10 (Bruker, 151 Wissembourg, France). Typical 90° and 180° pulse durations were 14 µs and 32 µs, 152 respectively, with an 8 dB pulse attenuation. The temperature was fixed at 20°C. Spin-Spin 153 relaxation time (T₂) measurements were performed using a Carr Purcell Meiboom and Gill 154 (CPMG) sequence (Meiboom, 1958). Three thousand consecutive echoes were recorded with 155 a time spacing of 0.1 ms between the 90° and the first 180° pulse. Recycle delay and number 156 of scans were 2 s and 8 s, respectively. Spin-Lattice relaxation time (T_1) measurements were 157 performed using a saturation recovery (SR) sequence. Times between the two 90° pulses were 158 varied one hundred times from 5 ms to 2000 ms. The NMR signal was acquired 28 µs after 159 the second 90° pulse in order to detect only the longest relaxation components, *i.e.*, the water 160 proton relaxation. Recycle delay and number of scans were 2 s and 8 s, respectively.

161

162 2.5 Calculation of NMR parameters

163 Relaxation curves were fitted using two different methods, the Maximum Entropy Method
164 (MEM) (Mariette, 1996), which provides a continuous distribution of relaxation components,
165 and the Levenberg-Marquardt algorithm, which allows discrete solution for the fitting (Table

166 Curve, Jandel) (Marquardt, 1963). Moreover, no assumptions regarding the number of 167 relaxation times are necessary with MEM compared to the Levenberg-Marquardt algorithm 168 method.

169 For the spin-spin relaxation decay signal, the equation used for fitting according to the170 Levenberg-Marquardt method was:

171
$$S(t)_{CPMG} = I_1 e^{-\frac{t}{T_{21}}} + I_2 e^{-\frac{t}{T_{22}}} + I_3 e^{-\frac{t}{T_{23}}} + c$$
 Eq.1

where $S(t)_{CPMG}$ is the intensity of the total relaxation signal (a.u.), t is the time of the relaxation process (ms), T_{2i} the spin-spin relaxation time of the component *i* (ms) and I_i the associated signal (a.u.). A constant c was added into the model in order to take into account change in the decay baseline.

176

Spin-Lattice relaxation curves were fitted with the Levenberg-Marquardt algorithm with thefollowing equation:

179
$$S(t)_{SR} = I_1(1 - \alpha e^{-\frac{t}{T_{11}}}) + I_2(1 - \alpha e^{-\frac{t}{T_{12}}}) + I_3(1 - \alpha e^{-\frac{t}{T_{13}}})$$
 Eq.2

180 where $S(t)_{SR}$ is the intensity of the total relaxation signal (a.u.), t is the time of the relaxation 181 process (ms), T_{1i} the spin-lattice relaxation time of the component *i* (ms) and I_i the associated 182 signal (a.u.). A constant α was introduced to correct for imperfections in the 90° pulse.

183

184 2.6 MRI measurements

A 1.5 Tesla whole body MRI scanner (Magneton Avento Siemens, Erlangen, Germany) was used. The MR images were acquired with the standard spin echo sequence. Forty-five slices of 2 mm were recorded, the distance between the centres of two consecutive slices being 4 mm. The field of view was 220 mm x 220 mm for 256 x 256 pixel sampling. Echo time (T_E), repetition time (T_R) and pixel bandwidth were 8 ms, 1000 ms and 395 Hz/pixel, respectively. 190 The grey level intensity S_{ES} (a.u) within a voxel with a standard spin-echo sequence was given 191 by the equation:

192
$$S_{ES} = I\left(1 - \beta e^{-\frac{T_R}{T_1}}\right) \times e^{-\frac{T_E}{T_2}}$$
 Eq.3

with I the intensity related to the relaxation times T_1 and T_2 of the protons in the voxel. β is a parameter introduced to correct for imperfections in the 90° pulse. This equation assumed a single proton component with a single spin-spin relaxation and a single spin-lattice relaxation. In the case of multi-exponential behaviour, the grey level intensity would be a combination of the different relaxing components for both T_1 and T_2 .

198

199 **3. Results and discussion**

200 3.1 NMR

201 Figure 2 shows typical CPMG (a) and SR (b) signals for two wood and sludge mixtures with 202 dry matter of 24% and 70%. CPMG signal intensities decreased throughout the acquisition 203 time t, according to equation 1. An increase in signal intensity was obtained for SR as 204 expected from equation 2. The effects of water content on NMR parameters can be described 205 from the two CPMG signals. Firstly, since the initial signal intensity was proportional to the 206 sample proton content, and because water is one of the most abundant protonic molecules in 207 our system, this explains the reduction in signal intensity from 3.7 to 2.2 a.u. observed 208 between the two samples. Secondly, the water relaxation time is highly sensitive to water 209 content. Indeed, T₂ relaxation was directly related to molecular mobility, i.e., the slower the 210 molecular mobility the shorter the relaxation time (Belton, 1990). The relaxation time of a 211 small molecule is thus always higher than the relaxation time of a large molecule. Moreover, 212 the exchange between water protons and exchangeable protons from the molecule contained 213 in the dry matter has to be taken into account for water. This exchange, known as chemical

exchange, is one of the most efficient mechanisms of water relaxation (Belton, 1987; Hills, 1989b; a; Belton, 1990; Denisov, 2002). Consequently, water relaxation is always sensitive to water content and its value decreases with water content, explaining the more rapid decrease in the CPMG signal observed for the driest sample. The same mechanisms are also involved in the T₁ relaxation from the Saturation Recovery NMR sequence. The intensity measured at 2 s was lower for the driest sample and the recovery of the signal (shorter T₁) was faster for the driest sample.

221 The spin-spin relaxation distributions (T_2) from MEM obtained from fitting the CPMG curve 222 are presented in Figure 3. For all samples, two or three peaks were extracted from the signal 223 according to the sample dry matter. Two mechanisms can be proposed to explain such multi-224 exponential relaxation behaviour. The first is based on the assumption of the chemical 225 composition of the sample. For example, in the case of the water and fat mixture in an 226 emulsion, a specific exponential can be attributed to each phase, *i.e.* water and fat (Duval, 227 2006). However, since our samples were mainly composed of water, this hypothesis did not 228 hold. The second mechanism is based on the sample microstructure which may induce non-229 homogeneous water distribution on a microscopic length scale. Indeed, when water exchange 230 by diffusion between different compartments is slow compared to the relaxation time value, 231 the averaging effect is not sufficient to yield a single relaxation time value, and several peaks 232 are observed for the water relaxation. This multi-exponential relaxation for water has already 233 been reported for many samples which exhibited restriction to the water diffusion. For 234 example three peaks have been reported from the relaxation decay curve of vegetable tissues. 235 These peaks were attributed to water in the vacuole, in the cytoplasm and in the cell wall, respectively (Snaar, 1992; Van Dusschoten, 1995; Duval, 2005; Musse, 2009). This 236 237 mechanism therefore seems the most relevant to explain the multi-exponential behaviour of 238 the relaxation.

239 For the sludge, depending on the dry matter, two or three peaks were extracted from the signal 240 (Figure 3-a). Their T₂ values covered a range from 5 to 60 ms. The sludge was composed of 241 flocculated bacteria which induced heterogeneity in the water distribution. Indeed, water was 242 always present in bacteria, in and around the flocs. Since water protons are thus in three 243 different compartments, it is tempting to associate each relaxation time with a water compartment. Granular sludges have already been studied by NMR (Lens, 1997; Lens, 1999), 244 245 and a relaxing agent was used to lower the proton relaxation times in the solution. Relaxation 246 times of 10-15 ms and 30 ms were attributed to methanogenic (Lens, 1999) and sulfidogenic 247 bacteria (Lens, 1997), respectively. Water in the granular matrix had a relaxation time of 40-248 100 ms (Lens, 1999). According to these results, the first peak could be related to the bacteria 249 and the second to the water around the bacteria (Figure 3-a). However, a water proton from 250 the solution could well contribute to the first or the second peak. Since no peak was found 251 with a value close to the free water value (1.5-3 s), this means that there were some molecules 252 in the sludge solution that lowered the overall relaxation of the proton of this compartment. 253 The polymer added for the flocculation could explain the decrease in the water relaxation 254 time, but the effect of the iron chloride (used to dehydrate the sludge) has also to be taken into account. 255

256 Wood relaxation is well documented in the literature (Hsi, 1977; Menon, 1987; Araujo, 1992; 257 Araujo, 1994; Hartley, 1994; Xu, 1996). Three main relaxation times were found related to 258 water in cell wall fibrils (fast T₂), water in the ray and late wood tracheid lumens (medium T₂) 259 and water in early wood tracheid lumens (fast T₂). Above the fiber saturation point (FSP), 260 wood relaxation times are relatively constant over a wide range of water content (Menon, 261 1987). Below FSB, the relaxation times are a function of water content. The wood used in this 262 study had a similar relaxation time distribution (Figure 3-b) as that reported for Western Red 263 Cedar sapwood (Menon, 1987). At a water content of 55%, the mean relaxation times were 10

ms, 35 ms and 115 ms. The last two peaks were broad. Wood relaxation is sensitive to cell size and wood species. Since the wood used in this study was heterogeneous in origin, this would explain the peak shapes.

267 In a wood and sludge mixture there was water equilibrium between the two. After mixing, the 268 sludge tended to loose water and the wood tended to gain water. Three typical relaxation 269 distributions of wood and sludge mixtures are presented in Figure 3-c. The dry matter 270 mixtures were 24%, 50% and 71%, respectively. Two or three peaks could be seen, the first 271 being narrow (in the range of 0-10 ms), and the second peak broad, the range becoming larger 272 with the increase in water content. Eventually, two broad peaks could be seen for the wettest 273 samples. For the driest samples, the wood FSP was most probably not reached since no 274 relaxation times were found above 40 ms. When the samples became wetter, the wood water 275 content must have been above the FSP. Water filled the wood cells and then the relaxation 276 time distribution could become broader. One important result in this study was that most of the T₂ relaxation distributions for wood and sludge were in the same range. Thus it was not 277 278 possible to distinguish specific NMR signals from the sludge and the wood in the wood-279 sludge relaxation distribution.

280

281 Using the Levenberg-Marquardt algorithm to fit the T₂ relaxation decay curves, the smallest 282 residues were obtained with a three component fitting equation (equation 1). The three T_2 283 values were consistent with the T₂ distribution discussed above. Relaxation rates ($R_2 = 1/T_2$) 284 for the three components are plotted on Figure 4 for sludge samples (a) and for sludge and 285 wood mixtures (b). For all samples, a constant decrease was observed with the increase in 286 water mass over dry matter mass. Moreover, the variation in relaxation rate was the same for 287 the three components. This demonstrates that all fractions were in equilibrium and were 288 affected by the dehydration process in the same way, despite the micro heterogeneity in the water distribution in the sample. It should be noted that the same variation was observed for sludge dehydrated by air drying and by exchange with dry wood. These results confirm that the main effect on the water relaxation rate in the two samples was the dry matter content.

292 Spin-lattice relaxation times (T_1) were measured for wood and sludge mixtures. As for the 293 spin-spin relaxation curves, the spin-lattice relaxation curves were fitted with MEM and the 294 Levenberg-Marquardt algorithm (equation 2). The MEM distributions were composed of two 295 or three peaks (Figure 5-a), the first peak being narrow (range 5-20 ms), the other peaks being 296 broad (ranging from 15 ms to 250 ms). The Levenberg-Marquardt algorithm had the smallest 297 residues for the three components. The difference between the two fitting methods obtained 298 for some mixtures (two components from MEM and three from the discrete method) is 299 explained by the wide distribution of the second peak. Indeed, this effect is known to induce 300 discrepancies between the fitting methods (Mariette, 1996). The second peak obtained by 301 MEM was decomposed into two discrete T_1 values with the discrete method. As for R_2 , a 302 linear relationship in a log-log plot was observed between the spin-lattice relaxation rate R₁ 303 and the water mass over dry matter mass (Figure 5-b). Moreover, this relationship was 304 obtained for the three components. Only the T₂ sensitivity to water content will be considered 305 for the following MRI measurement.

306

307 3.2 MRI

Two experimental cells were scanned with the whole body MRI scanner at different periods of biodegradation. In the mean time, the cells were monitored for biodegradation through respirometry measurements (Figure 6). Indeed, oxygen consumption is a good indicator of sludge biodegradation. The respirometric profiles obtained, that were similar for the two cells, are typical. The oxygen uptake rate increased quickly due to biodegradation of the most easily biodegradable substrate. Then, when all this matter had been degraded, a break in the exponential growth was observed. The oxygen consumption rate then decreased until all the
biodegradable substrates had completely disappeared. During this decrease, the slowly
biodegradable organic matter was hydrolysed before being consumed by micro-organisms.
After 27 days, consumption rate returned to ground state and respirometric study could then
be stopped (Tremier, 2005; Berthe, 2007).

319 Both respirometric cells were thus scanned at D0 before biodegradation, then cell 1 at D3 320 after the biodegradation peak, and finally cell 2 at D27 when biodegradation was low and 321 constant. Examples of MR images at D0, D3 and D27 are presented in Figure 7. Images (a) 322 and (b) and images (c) and (d) were taken at the same location in cell 1 and in cell 2, 323 respectively. At D0, cell 1 (Figure 7-a) and cell 2 (Figure 7-c) images were similar: no 324 differences between the MR images for the two cells could be seen. The wet pieces of wood 325 covered with sludge were clearly visible. The black areas were the air space between the 326 bulking agents. The contrast in the image will allow to implement image segmentation method to compute the inter porosity and the specific surface area. For larger pieces of wood, 327 328 the edges of the wood tended to be more intense than the centres, showing that the water had 329 not yet fully hydrated the centres of the wood. NMR relaxation experiments showed that this 330 process required at least 10h (data not shown). After 3 days, the edge effect was less visible 331 for cell 1 and the wood chips were even better defined (Figure 7-b). MR images of cell 2 332 taken after 27 days were highly contrasted. The MR image (Figure 7-d) showed two distinct 333 areas. Very low signal intensity was detected at the bottom of the image, compared with a 334 high level signal intensity at the top. This was due to the fact that cell 2 became highly 335 heterogeneous in water content in 27 days. The low intensity area had dry matter content of 336 around 60-70%. The intensity of the mixture signal was too low to be fully detected with 337 MRI. The other area had a dry matter content of around 25%, which demonstrated water 338 transfer in the cell. At D3, the water content in cell 1 also began to be inhomogeneous, but to

a lesser extent. The average dry matter content was 44%, two percent less than at D0. The
contrast between D3 and D27 was thus due to the difference in dry matter content.

341 MRI images were obtained with a spin echo sequence, the grey level intensities being a 342 function of both relaxation times (T_1, T_2) and the amount of water (see eq.3). Since the 343 repetition time, T_R , was 1 s and the largest spin-lattice relaxation time (T_1) found for the

344 wettest wood and sludge mixture was 250 ms, then in equation 3, $\left(1 - \beta e^{-\frac{T_R}{T_1}}\right) \sim 1$. Thus, the

345 MR images were not T_1 weighted. Equation 3 can be rewritten for the three components as:

346
$$S_{ES} = I_1 e^{-\frac{T_E}{T_{21}}} + I_2 e^{-\frac{T_E}{T_{22}}} + I_3 e^{-\frac{T_E}{T_{23}}}$$
 Eq.4

347

Since the intensity of each component $I_{i}\xspace$ and the three $T_{2}\xspace$ relaxation times were strongly 348 349 dependent on the water content of the sample, the global signal intensity should consequently 350 also be directly related to water content in the mixture. This direct relationship was checked 351 by taking samples from cell 1 at D3 and from cell 2 at D27 at different heights in the cells 352 where MR images showed inhomogeneity. NMR and moisture content measurements were 353 performed on these samples. Signal intensities at 8 ms were extracted from the NMR CPMG 354 experiments and plotted against dry matter (Figure 8). As expected, the intensities decreased 355 with dry mater content. Despite the biological activity during the 27 days of storage, no 356 biodegradation effect was observed. The results provide evidence that the effect of biological 357 activity on the NMR signal of wood and sludge mixtures is negligible with regard to dry 358 matter content. The decay was non-linear. Indeed, with an 8 ms echo time, the multi-359 exponential behaviour introduced a weighting from the different components. We 360 demonstrated that the grey levels of the MRI images were directly related to dry matter 361 content when the water content was above 40-30%. Below this value the signal was too low 362 to be detected with this MRI protocol.

363 MRI has been used previously to monitor wood decay by fungus (Muller, 2002): indeed 364 biological activity means a local increase in water content, which leads to a local change in relaxation times and intensities. In this experiment, respirometry showed that biodegradation 365 366 occurred and both the NMR measurements and the MR images showed changes in the dry 367 matter content. MR images gave 3D representation of the heterogeneity of dry matter content 368 in the cells after 27 days of biodegradation (Figure 9). Heterogeneity was both vertical and 369 horizontal. Biodegradation may contribute to the heterogeneity of the dry matter, but the main 370 cause seems to be related to the cell design. The dried area is above the tube where air flows 371 into the cell. The air rate must be too fast and then has a drying effect. The humid area is 372 located under the tube where air flows out of the cell (Figure 2). Even with the water trap, 373 some water seems to condense in this tube and then drops to the wood and sludge mixture. 374 Thus, these MR images cannot be taken as proof of biodegradation activity. Nevertheless, 375 they will be used to improve the design of the respiratory cell.

376 **4. Conclusion**

377 We have shown that the NMR water relaxation for wood and sludge exhibited a multi-378 exponential relaxation time behaviour which can be explained by the microstructure of the 379 matrix. Moreover, a high dry matter content effect on the relaxation was observed for sludge 380 samples and for sludge and wood mixtures. This effect was the same whatever the relaxation 381 time component, which confirmed that the dry matter content had the main effect on the NMR 382 parameters. Wood and sludge mixtures were scanned successfully by MRI using a spin echo 383 sequence. The grey level intensities were correlated with the dry matter content, thus 384 demonstrating the feasibility of MRI to quantify the water distribution in respiratory cells. 385 Moreover, image resolution was good enough for image analysis. Since voxel size was much 386 smaller than pore volume, porosity and specific surface area should be available.

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393

391 Figure legends

392 Figure 1 : Diagram of respirometric apparatus

Figure 2 : Typical CPMG (a) and SR (b) decay curves for two ratios of sludge and wood with
70 % (■) and 24 % (♦) dry matter.

396

Figure 3 : Spin-spin relaxation time (T₂) distributions. Adjustments were made with the MEM routine. (a) Sludge samples with 15%, 25% and 52% dry matter content (b) Wet wood with dry mater of 45% (c) Mixtures of sludge and wood with 24.2%, 50.2% and 70.8% dry matter content. One used the CPMG sequence (3000 echoes, with $\tau = 0.1$ ms, RD = 2 s, NS = 8).

401

Figure 4 : Three component spin-spin relaxation rates $(1/T_2=R_2)$ for (a) sludge samples and (b) sludge and wood mixtures at various water over DM mass ratios. First component (\blacklozenge), second component (\blacksquare) and third component (\blacklozenge). One used the CPMG sequence (3000 echoes, with $\tau = 0.1$ ms, RD = 2 s, NS = 8). Dashed lines $\propto (m_W/m_{DM})^{-1}$ are to assist reading.

Figure 5 : Spin-lattice relaxation time (T₁) distributions for mixtures of sludge and wood with 24.2% and 70.1% dry matter content (a). Adjustments were made with the MEM routine. Three component spin-lattice relaxation rates (R₁) for sludge and wood mixtures at various water over DM mass ratios (b). First component (\blacklozenge), second component (\blacksquare) and third component (\blacktriangle).One used the SR sequence (100 points with τ_1 =5 ms, τ_{100} = 2000 ms, RD = 2 s, NS = 8). Dashed lines $\propto (m_W/m_{DM})^{-1}$ to assist reading.

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Figure 6 : Oxygen uptake rate over time for the two respirometric cells used in the combinedMRI-Respirometry set up.

Figure 7 : MRI images of respirometric cells 1 and 2 taken with a spin echo sequence ($T_E = 8$ 417 418 ms, $T_R = 1000$ ms, FOV = 220 mm x 220 mm, thickness 2mm, sampling 256x256): Cell 1 at 419 D0 (a) and D3 (b), Cell 2 at D0 (c) and D27 (d).MR images were representative of the area 3 420 cm below the centre of the airtight cylindrical reactor. 421 422 Figure 8 : Spin-spin relaxation intensities normalised by sample mass extracted from CPMG 423 decay curves at 8 ms compared to dry matter content of sludge and wood mixtures. Samples 424 were taken from the leftover mixture at D0 and at D3 and D27 from the respirometric cell 1 and cell 2, respectively. One used the CPMG sequence (3000 echoes, with tau=0.1ms, 425 426 RD=2s, NS=8). 427

428 Figure 9: MRI images of respirometric cell 2 at D27, taken with a spin echo sequence (TE =

429 8 ms, TR = 1000 ms, FOV = 220 mm x 220 mm, thickness 2mm, sampling 256x256).

- 430 Selected images were separated by 3.6 cm. They are labelled from the bottom (slice 1) to the
- 431 top (slice 45) of the cell.

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Figure 4



















