

1 **Effect of a biological additive on nitrogen losses from pig slurry during storage**

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8 **Abbreviation list**

9 Nitrogen (N), Total solids (TS), volatile solids (VS), Total Kjeldahl Nitrogen (TKN), Total Ammonia

10 Nitrogen (TAN), Control slurries not treated (C), Slurry treated with additive (T), Slurry treated with

11 an extra dosage of the additive prior to external storage (E)

12

13 **Core ideas**

14 A new biological additive with denitrification enhancement capability was tested

15 The additive increased the total solids reduction during six month storage of the slurry

16 The total and ammonia nitrogen losses were not affected by the additive

17 The additive promoted stabilization of slurry but did not reduce **N content**

18

19 **Abstract**

20 Additives applied to animal manure slurries can affect both the chemical composition and biological
21 processes of slurries during storage, with possible improvement of their management and reduction
22 of environmental problems. Some new formulations are marketed claiming a nitrogen (N) removal
23 effect due to denitrification, with the consequence of a reduced N content in the manure after storage.

24 This study evaluated the effects of one of these commercial additives (BACTYcomplex®, COMAS,
25 Bovolenta, Padua, Italy) on slurry characteristics and N losses at a commercial piggery. The additive
26 was applied to four different sectors of the piggery, each with an independent under-floor slurry pit;
27 four other sectors served as controls without treatment. Pits were emptied every four weeks and the
28 manure was analyzed for total and ammonia N and total and volatile solids. Slurry samples from the
29 last month of the on-farm assessment were removed and stored thermostatically in vessels external to
30 the piggery. A sub-sample of slurry that was treated with the additive at the piggery was treated with
31 an additional dose of additive at the beginning of long-term storage. The additive did not change the
32 composition of the slurry during in-house storage (four weeks duration). During the 155 days of
33 external thermostatic storage, the total solids content of treated slurry was reduced by 18% compared
34 to control slurry, but the N content and composition of treated slurry was unaffected. The additive
35 had a positive effect in accelerating the stabilization of the slurry, but did not modify N losses.

36 **Keywords:** Slurry additive, manure management, nitrogen

37

38

39 **Introduction**

40 The intensification of agriculture and especially livestock activity has significantly increased
41 production, but at the same time has modified the equilibrium of traditional farms that were once
42 well-integrated with cultivated areas. Intensification has concentrated production activities both
43 within farms and on a regional scale. Considering the imbalance between limited production areas
44 and the excessive livestock loading, the role of animal manure as an organic amendment and
45 fertilizer has diminished and manure has taken the connotation of a waste product (Burton and Turner
46 2003). A consequence of the concentration of livestock activities is a higher environmental impact
47 due to manure management. One of the impacts derives from emissions to air, particularly ammonia
48 volatilization and losses of greenhouse gases such as nitrous oxide, methane and carbon dioxide
49 (Petersen et al. 2009); these emissions take place from livestock facilities, from manure storage tanks
50 and following land spreading. Manure application can also trigger other pollution phenomena, mainly
51 related to phosphorus runoff and N leaching (Rotz et al. 2011). Odor emissions also characterize
52 manure management, poor manure management can be responsible for disease transmission or health
53 problems (Blanes-Vidal et al. 2009).

54 Many management solutions and types of treatment have been proposed to reduce the environmental
55 impact of livestock manure. Among the most common treatments are solid-liquid separation (Hjorth
56 et al. 2010), biological N removal in aerobic conditions (Beline et al. 2007) and anaerobic digestion
57 (Burton and Turner 2003). The technologies required for these types of treatment have high cost and
58 require specific knowledge for proper operation. Another approach is the addition of chemical or
59 microbial additives to manure managed as a slurry; these aim to affect certain slurry properties, often
60 by inhibiting or stimulating a particular microbiological process (Sommer et al. 2013).

61 There are various types of additives that act on several processes simultaneously; among these
62 additives are those that affect both the chemical composition and biological processes of slurries,
63 especially in relation to the N content. McCrory and Hobbs (2001) classified additives that reduce

64 ammonia emissions into five categories: acidifying additives, adsorbents, urease inhibitors, saponins
65 from Mohave Yucca (*yucca schidigera*) and digestive-biological additives. While most of these
66 additives have a documented effect on slurries, the digestive-biological additives have given
67 controversial results. They consist of microorganisms and nutrients that can increase the degradation
68 of organic matter that has passed through animals undigested, and can enhance the reduction of
69 odorous substances and conversion of inorganic N to its organic form (Joint Research Center 2013).
70 Van der Stelt et al. (2007) evaluated several digestive additives designed to reduce ammonia
71 emissions from dairy slurry. These included Agri-mest® (designed to increase the amount of energy
72 available for anaerobic fermentation of manure by microorganisms), Effective Micro-organism®
73 (consisting of lactic acid bacteria, yeast and smaller numbers of other types of organisms) and Euro
74 Mest-mix® (consisting of a pH buffer and clay minerals together with unidentified supplements to
75 increase the activities of microorganisms). In general no reduction in ammonia emissions was
76 obtained with these products.

77 In contrast to additives designed to reduce ammonia emissions, the microbial additive (Sporzyme®)
78 tested by Zhu et al. (2006) in concert with aeration treatment, was intended to reduce the content of
79 nutrients from liquid swine manure. The results indicated that aerobic treatment reduced total
80 Kjeldahl nitrogen (TKN), total ammonia nitrogen (TAN) and total soluble phosphorus by
81 approximately 42%, 56%, and 72%, respectively. The reduction of TKN was found to be mainly
82 attributed to the reduction of ammonia because its share of TKN was remarkably reduced at the end
83 of the test. Although Sporzyme® significantly increased the quantity of aerobic microorganisms in
84 the manure, no advantage of its use could be identified, and the nutrient reduction in swine manure
85 was due only to aeration treatment.

86 Wheeler et al. (2011) tested 22 additives that included microbial digestion products, oxidizing agents
87 and chemicals, disinfectants, odor masking agents and adsorbents. Some additives reduced ammonia
88 emissions, others increased the emissions and others had no significant impact on ammonia

89 emissions. These contradictory effects appeared to be due to differences in pH and whether an
90 additive inhibited microbial activity by toxicity or provided a substrate (often a carbon source) that
91 microbes used to increase biomass, hence, consuming N in the process.

92 Similarly Andersson (1994) compared different additives (Add A; Penac-G®; Kemira No. 2; Kemira
93 No. 5; Kemira No. 15; Fly ash; Stalosan®) to verify their efficacy in reducing ammonia emission
94 from cow slurry. Add A, manufactured by the company Biosolv, is a microbial consortium of
95 anaerobic bacteria; the others are chemical additives, especially calcium salts. Kemira No. 2 and
96 Stalosan®, both of which were based on superphosphate, reduced the ammonia emission compared
97 with the emission from the untreated slurries. The approximate reduction was 30 %, probably due to
98 the carbonate ions present in the slurries that precipitated as calcium carbonate. The pH then
99 decreased, which resulted in a lower ammonia emission. At this significance level the treatment with
100 Add A resulted in a higher emission than from the untreated slurries. All the other slurries treated
101 with the different additives emitted ammonia at the same rate as the control (Andersson 1994).

102 Commercial digestives claim to reduce total solids by stimulating their degradation but the limited
103 investigations in this area report poor performances on the products (McCrorry and Hobbs, 2001). The
104 variable results obtained in previous experiments with additives highlight the need for a better
105 understanding of the effect of specific products when used in practical circumstances (i.e., non-
106 laboratory conditions). Recently new types of digestive additives have been marketed with additional
107 characteristics like the capability to remove N through denitrification due to the addition of anaerobic
108 bacteria. This possible effect might be a way to reduce nitrogen surplus in intensive livestock area but
109 has not been verified in practical condition.

110 The objective of the research described herein was to evaluate the effect of a commercial digestive-
111 biological additive, with expected denitrification enhancement, applied to the slurry in a commercial
112 fattening pig farm. The study assessed the modification of N and total solids contents caused by the

113 additive to the slurry during under-floor, in-house storage and during the subsequent long-term, off-
114 farm storage.

115

116 **Materials and Methods**

117 **Experimental test on fattening piggery**

118 *The fattening piggery – characteristics and monitoring*

119 Experiments were conducted at a commercial fattening piggery (approximately 5,500 head) located
120 in Pompiano (Lombardy, Italy). The animals were segregated into two identical buildings based on
121 sex. Each building was divided into four independent sectors. In building 1 (housing females), sectors
122 C2 and C4 were the controls, while sectors T1 and T3 were treated with additive. In building 2
123 (housing males), sectors C6 and C8 were the controls, while sectors T5 and T7 were treated with
124 additive.

125 The pens had fully-slatted floors equipped with a vacuum system (Joint Research Center, 2013). The
126 cumulative area of the pens in both the treatment and control sectors was identical (2843 m²). The
127 slurry removed from the pits below the floors was sent through a pipe into a reception tank (106 m³)
128 external to the buildings, from which it was pumped into the final storage tank away from the
129 buildings.

130 All pigs had the same diet composed of water, milk whey and a specific feed. The amount and
131 composition of the diet were modified during the growth cycle and were recorded on a weekly basis
132 together with the number of pigs and their expected weight for each sector. The parameters recorded
133 were: number of pigs, the mean live weight of pigs, the mean live weight increase, the amount of
134 feed distributed, the feeding typology given to the pigs, the amount of slurry produced and the slurry
135 temperature.

136

137 *Use of additive*

138 The additive used in this experiment was BACTYcomplex® (COMAS, Bovolenta, Padua, Italy). The
139 product data sheet defined it as a “complex bacterial enzyme lyophilized containing a mixture of
140 saprophytic heterotrophic aerobic and anaerobic bacteria, associated to catalytic thermostable
141 enzymes and related eutrophic compounds”. The bacterial complex was designed to trigger the
142 microbiological digestion of organic matter, both on litter in animal housing and in treatment plants,
143 even in the presence of moderate concentrations of disinfectants or antibiotics (which in anoxic
144 conditions give rise to denitrification and ammonia degradation). The specific BACTYcomplex®
145 characteristics were: moderate solubility; pH 6.25; Cellulase 2.95%; Protease 1.51%; Amylase
146 0.42%; Lipase 0.16%; and total bacteria 149.2 million Ufc g⁻¹ .

147 Addition of BACTYcomplex® was carried out following the manufacturer’s directions. Once every
148 15 days, the additive was distributed uniformly on the slatted floors of treated sectors from 2
149 December 2013 until 24 March 2014. The dosage used was 10 kg of BACTYcomplex® per 1000
150 pigs.

151 *Slurry sampling*

152 Slurry samples were collected from the reception tank on a sector-by-sector basis every four weeks,
153 on 30 December 2013; and on 27 January, 24 February and 24 March 2014. The slurry pits below the
154 control sectors C2, C4, C6 and C8 were emptied individually and sampled first, followed by the
155 treated sectors T1, T3, T5 and T7. At the time of sampling, the slurry had been treated two times with
156 the additive (15 and 30 days preceding sampling). Immediately prior to sampling, slurry contained in
157 the reception tank was mixed using a tractor-driven propeller to ensure homogeneity of the slurry.
158 Every 5–10 min during the transfer process, a 3-L sample (approximately) of slurry was taken from
159 the reception tank and placed in a large container; this was repeated 7–8 times to yield a 25-L
160 (approximately) composite sample for each pit. The operation lasted around 45 min for each sector.
161 The composite sample was thoroughly mixed, and a 2-L sub-sample was taken as a representative

162 sample of slurry for each sector. Before and after emptying each pit, the depth and temperature of the
163 slurry were measured to quantify the total volume of slurry produced between sampling events.

164 **Temperature-controlled slurry storage**

165 During the last sampling operation (24 March 2014) approximately 40 L of slurry from each control
166 sector and 80 L of slurry from each treated sector were collected as described above and transported
167 to the University of Milan experimental farm “A. Menozzi” in Landriano (PV) for long-term,
168 temperature-controlled anaerobic storage. After thorough mixing, samples arising from the treated
169 sectors were each split into two aliquots. One aliquot received additional BACTYcomplex® at a
170 dosage of 1 g per 30 L of slurry, which was equivalent to the dosage used at the pig-rearing facility.
171 Approximately 30 L of each sample were stored individually in vessels (diameter 0.336 m and height
172 0.320 m) for 155 days to give a total storage period of 6 months (including the under-floor storage),
173 which was typical for the farming system being studied. Thus, a total of 12 vessels (four containing
174 slurry from control sectors, four containing slurry from treated sectors and four containing slurry
175 from treated sectors and given an additional dose of BACTYcomplex®) were stored in a
176 temperature-controlled environment at 18°C. This value has been selected as it is the minimum air
177 temperature maintained in the fattening pig buildings in winter periods. The temperature of the slurry
178 did not differ from the air temperature.

179 During storage, the temperature of slurry was recorded at 30-min intervals using a temperature sensor
180 (TMC6-HD, Onset Computer Corporation, Bourne, MA, USA) located 0.15 m beneath the surface of
181 slurry in each vessel and connected to a data logger (HOBO U12-006, Onset Computer Corporation,
182 Bourne, MA, USA). In addition, 0.4 L samples were retrieved and analyzed from each vessel
183 monthly to monitor the change in slurry composition during storage.

184 **Chemical analysis**

185 Slurry samples collected at the pig-rearing facility were analyzed in a commercial laboratory
186 (Pioneer, Pioneer Hi-Bred Italia S.r.l. DuPont Agriculture & Nutrition, Gadesco Pieve Delmona,

187 Italy) for the following parameters: total solids (TS), volatile solids (VS), pH, total Kjeldahl nitrogen
188 (TKN), total ammonia nitrogen (TAN), phosphorus (P₂O₅) and potassium (K₂O). The slurry samples
189 obtained during thermostatic storage were analyzed in University of Milan research laboratories for
190 TKN, TAN, TS, VS and pH. Samples were analyzed according to standard procedures (APHA 1998).

191

192 **Data analysis**

193 Characteristics of slurry samples arising from treated and untreated sectors in the pig-rearing facility
194 were compared to evaluate the effect of BACTYcomplex® additive on TKN and TAN content. To
195 avoid potential bias in the comparisons due to different volumes and dilutions of slurries collected
196 from the various sectors, TKN and TAN contents were referenced to the TS content of each sample.
197 Moreover, the TAN:TKN ratio was used to assess the behavior of N contained in each sample.

198 Data were analyzed both to evaluate the effects of additive addition on TKN:TS and TAN:TS ratios
199 and changes in TAN:TKN ratios, and to investigate differences between untreated and treated slurry
200 samples during long-term thermostatic storage. Friedman's non parametric test was used for data
201 analysis because the assumptions for ANOVA tests were not verified. Statistical analyses were
202 conducted using the software package SPSS®, version 21 (International Business Machines Corp.,
203 Armonk, NY, USA).

204

205 **Results and Discussion**

206 **Experimental test on fattening piggery**

207 The information about the growth cycle of pigs, feed delivered and slurry produced over the 4-month
208 experiment at the pig-rearing facility are reported in Table 1 as mean values for all treated and all
209 control sectors. The mean live weights of pigs and the mean live weight increases were similar for all
210 sectors, for each day of sampling. The feeding typology was identical in each sector, while the
211 quantity of feed distributed was similar and without significant differences between the control and

212 treated sectors. The slurry production varied slightly from sector to sector, probably due to water
213 spilling from drinkers. For the entire experiment, slurry temperature remained stable between 19.3°C
214 and 21.8°C, but exhibited a slight tendency to increase due to the seasonal conditions.

215 Table 2 shows the mean and standard deviation of chemical parameters for slurry arising from the
216 control and treated sectors. All tested parameters except pH were at slightly higher concentrations
217 than the mean of data reported by Martinez-Suller et al. (2008), but were in the range they reported.
218 The relatively high concentrations may have been due to efficient water management in the facility,
219 leading to less slurry dilution than is typically found. Because the animals were reared on totally
220 slatted floors, no water was required for removal of manure during the growth cycle. Furthermore,
221 the slurry was taken from in-house pits beneath the slatted floors and was not diluted by natural
222 precipitation. In contrast, the slurry samples analyzed by Martinez-Suller et al. (2008) were taken
223 from uncovered, outdoor storage tanks. Millmier et al. (2000) analyzed slurry samples taken from
224 covered pits and obtained results similar to those in the present study.

225 The ratios of TKN:TS, TAN:TS and TAN:TKN in slurries from both control and treated sectors
226 increased over time (Figure 1). The trend reflected the increasing live weight of the animals, which
227 resulted in lower nutrient retention over time. Therefore, a comparison can be made only between
228 samples collected on the same date, because of the different ages of the pigs and different
229 environmental conditions that existed on the sampling dates.

230 There were no significant differences between the control and treated slurries on any sampling dates
231 and for all the ratios examined. The standard deviation expressed by the error bars showed the
232 presence of a comparable variability among samples from all sectors for all parameters.

233 The mean TKN:TS ratio of control samples was numerically lower than that of treated samples
234 throughout the experiment (Figure 1a). The TAN:TS ratio followed a similar pattern as the TKN:TS
235 ratio (Figure 1b). The TAN:TS ratio was the same for both treated and control slurry samples. The
236 negligible effect of the additive in the pits was corroborated by the lack of difference in the

237 TAN:TKN ratios among control and treated slurry samples on the four sampling dates (Figure 1c).
238 These results highlight the absence of clear effects of the additive on slurry composition and on
239 changes of the N content during the 4-week period between slurry emptying events, during which the
240 slurry remained in the under-floor pits.

241 **Composition during long-term thermostatic storage**

242 Variations in the composition of control slurry (C) and slurries treated with the recommended dose
243 (T) and double dose (E) of BACTYcomplex® during thermostatic storage are shown in Figure 2. All
244 N indices (TKN:TS, TAN:TS and TAN:TKN) showed an anticipated reduction over time. N
245 reductions were larger than the reductions in total solids, the latter being due to the degradation of
246 organic matter. These changes were confirmed by the reduction of TAN:TKN ratios. .

247 Andersson (1994) reported that some slurry additives could modify pH, and thus indirectly affect the
248 emission of ammonia. In the present experiment, the pH of control (C) samples increased by 0.9 units
249 (from pH 7.28 to 8.18), and by 1.18 units in both treated (T) and double-dosed (E) samples (from pH
250 7.21 to 8.39). However, the slightly greater increase in the pH of treated samples compared to control
251 samples did not significantly influence the reduction of N content in the treated samples.

252 The results show that there was a significant reduction in TKN:TS and TAN:TS ratios of control
253 slurries compared to the treated ones, while there were no significant differences in these ratios
254 between the two treatments (i.e., recommended dosage vs. double dosage) (Table 3). The total solids
255 contents of control samples were greater ($P=0.003$) than those of the treated slurry samples,
256 indicating that the additive increased the degradation of organic matter (Table 3). Thus, the higher N
257 concentrations at the end of the storage period might have been due to a greater reduction of solids
258 more than to a conservation of N. This possibility is confirmed by the various N measures for the
259 samples at the end of the storage (Figure 3). When referenced to the volume of the slurry, the mean
260 values of TAN, TKN and the TAN:TKN ratio were similar for both control and treated samples. On
261 the contrary, the total solids content was conserved more in the control samples than in the treated

262 samples, and as a consequence, the TAN and TKN contents as percentage of total solids content
263 decreased.

264 The effect of the BACTYcomplex® additive during slurry storage was reduction in the TS content of
265 treated slurry by about 18% compared to the TS reduction that occurred naturally in the untreated
266 slurry. The higher solids reduction in the treated slurry might have been due to a higher degradation
267 activity of the microorganisms in the additive and possibly sustained by the enzymes contained in the
268 additive. The reduction of total solid, and the consequent improvement of the handling properties of
269 slurry, obtained in this study highlights a different performance of the tested additive in comparison
270 to the poor effect obtained in other experiences (McCrorry and Hobbs, 2001; Patni, 1992; Waburton et
271 al., 1980). However, the effect on total solids did not affect the N content of treated slurry, which
272 remained similar to that in untreated slurry throughout long-term thermostatic storage. The addition
273 of a further quantity of additive (i.e., double the recommended dosage) at the beginning of
274 thermostatic storage did not affect the final N and total solid content of the slurries.

275

276 **Conclusions**

277 Under the conditions for this study, the additive BACTYcomplex® is ineffective in changing the N
278 content of pig slurry stored in-house for a period of one month, as there was no significant difference
279 between the TKN and TAN content , and the TAN:TKN ratio, of treated and untreated slurry over
280 this period. During long-term (approximately six months) thermostatic storage, the addition of
281 BACTYcomplex® can reduce the TS content of pig slurry. Thus, under the conditions of this study,
282 BACTYcomplex® can improve the degradation of organic matter in pig slurry but not modify **N**
283 **content**.

284 However, the effect of an additive such as BACTYcomplex® could depend on several factors that
285 affect microbial activity, including temperature, pH, dissolved oxygen concentration, nutrient
286 availability, and microbial resistance to potential toxins. The results obtained in this study confirm

287 the need to assess the effect of additives in applied conditions, as the additives are likely to have
288 different activities in different environments. Generalization of the results from this research should
289 be avoided, and suitable protocols should be used in further comparative studies.

290

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293

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346

347 **Figure captions**

348 Figure 1 – Mean and standard deviation (vertical bars) of the TKN:TS, TAN:TS and TAN:TKN
349 ratios for control (C) and treated (T) slurries in under-floor storage on the four sampling events.

350 Figure 2 – Mean and standard deviation (vertical bars) of the TKN:TS, TAN:TS and TAN:TKN
351 ratios of untreated (C) slurry samples, samples treated with the normal dose of additive (T) and
352 samples treated with a double dose of the additive (E), during thermostatic storage for 155 days.

353 Figure 3 – Mean values **and standard deviation (vertical bars)** of the studied parameters of
354 untreated (C) slurry samples, samples treated with the normal dose of additive (T) and samples
355 treated with a double dose of the additive (E), at the end of thermostatic storage for 155 days.

356 Ammonia Nitrogen (TAN) and Total Kjeldahl nitrogen (TKN) are expressed in g L⁻¹; Total solids
357 (TS), Volatile solids (VS) and all the relative indices are expressed as percentages.

358

359 **Tables**

360

361 Table 1 –Piggery performance during the 4-month experiment. Results are the means from four

362 treated (T) and four control (C) sectors from which slurry was sampled on the dates indicated.

Parameter		30 December 2013		27 January 2014		24 February 2014		24 March 2014	
		C	T	C	T	C	T	C	T
Pigs number	n°	2645	2770	2713	2674	2680	2682	2664	2676
Mean live weight	(kg)	96.7	97.2	120.7	120.1	141.6	141.2	158.9	158.5
Feed distributed	kg head ⁻¹ day ⁻¹	10.9	11.1	11.2	12.1	11.9	12.5	12.2	12.5
Feeding typology	-	A (†)		B (‡)		B (‡)		B - until 03/03/2014 (‡) C - from 04/03/2014 (§)	
Slurry production	L head ⁻¹ day ⁻¹	7.2	7.4	7.6	8.8	8.1	8.2	7.3	8.7
Slurry temperature	(°C)	20.3	19.3	20.4	20.0	21.1	20.5	21.8	21.0

363 (†) composition of feeding typology A: specific feed (23.8%); water (41.7%); milk whey (34.5%).

364 Composition of Specific feed: CP (14.8%); P (0.52%)

365 (‡) composition of feeding typology B: specific feed (24%); water (37%); milk whey (39%).

366 Composition of Specific feed: CP (14.2%); P (0.50%)

367 (§) composition of feeding typology C: specific feed (24%); water (37%); milk whey (39%).

368 Composition of Specific feed: CP (13%); P (0.45%)

369

370 Table 2 –Characteristics of slurry originating from four control sectors (C) and four treated sectors (T) sampled on four dates.

Sample event	Date	sector	TS (%)	VS (%)	pH	TKN (g L ⁻¹)	TAN (g L ⁻¹)	P ₂ O ₅ (g L ⁻¹)	K ₂ O (g L ⁻¹)
			mean ± (SD)						
1	30/12/2013	C	5.02 ± (0.72)	3.75 ± (0.61)	7.08 ± (0.03)	4.35 ± (0.18)	2.97 ± (0.06)	3.35 ± (0.36)	2.86 ± (0.18)
2	27/01/2014	C	4.27 ± (0.98)	3.11 ± (0.76)	7.41 ± (0.09)	4.16 ± (0.55)	2.80 ± (0.21)	2.97 ± (0.65)	2.73 ± (0.33)
3	24/02/2014	C	4.38 ± (1.21)	3.20 ± (0.91)	7.37 ± (0.11)	4.23 ± (0.78)	3.05 ± (0.48)	3.13 ± (0.87)	2.68 ± (0.46)
4	24/03/2014	C	4.62 ± (1.00)	3.25 ± (0.79)	7.28 ± (0.11)	5.02 ± (1.01)	3.62 ± (0.70)	3.04 ± (0.66)	2.71 ± (0.22)
1	30/12/2013	T	4.21 ± (0.91)	3.12 ± (0.72)	7.19 ± (0.05)	3.83 ± (0.42)	2.64 ± (0.30)	2.96 ± (0.73)	2.54 ± (0.30)
2	27/01/2014	T	4.11 ± (0.88)	2.99 ± (0.67)	7.42 ± (0.1)	4.06 ± (0.53)	2.73 ± (0.38)	2.78 ± (0.61)	2.54 ± (0.28)
3	24/02/2014	T	4.45 ± (0.76)	3.25 ± (0.57)	7.39 ± (0.11)	4.40 ± (0.45)	3.17 ± (0.29)	3.04 ± (0.49)	2.71 ± (0.36)
4	24/03/2014	T	4.06 ± (1.24)	2.86 ± (0.92)	7.21 ± (0.06)	4.63 ± (0.83)	3.41 ± (0.46)	2.71 ± (0.76)	2.45 ± (0.29)

372 Table 3 – Results of the Friedman’s test and mean values of N and solid contents and their ratio for
 373 the three types of slurries thermostatically stored for 155 d: untreated (C) slurry samples, samples
 374 treated with the normal dose of additive (T) and samples treated with a double dose of the additive
 375 (E). The values are means of four repetitions of each sample type and five sampling dates.

		C		T		E		
		mean	SD	mean	SD	mean	SD	P level
TKN	g/L	4.13	1.28	3.66	1.18	3.70	1.14	0.949
TAN	g/L	2.69	1.03	2.42	0.98	2.40	0.98	0.623
TS	%	5.09 a†	1.26	3.93 b	1.27	3.88 b	1.30	0.003
VS	% TS	62.70	6.65	6.47	6.62	60.61	7.22	0.196
TKN/TS	%	8.16 a	2.10	9.44 b	2.07	9.82 b	2.27	<0.001
TAN/TS	%	5.40 a	2.10	6.28 b	2.38	6.39 b	2.56	<0.001
TAN/TKN	%	0.64	0.15	0.65	0.15	0.63	0.15	0.128

376 † Within rows, means followed by the same letter are not significantly different. Letters are not reported when P level is
 377 higher than 0.05.