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

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Abbreviation list

Nitrogen (N), Total solids (TS), volatile solids (VS), Total Kjeldahl Nitrogen (TKN), Total Ammonia Nitrogen (TAN), Control slurries not treated (C), Slurry treated with additive (T), Slurry treated with an extra dosage of the additive prior to external storage (E)

Core ideas

A new biological additive with denitrification enhancement capability was tested

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

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

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

Abstract

Additives applied to animal manure slurries can affect both the chemical composition and biological processes of slurries during storage, with possible improvement of their management and reduction of environmental problems. Some new formulations are marketed claiming a nitrogen (N) removal effect due to denitrification, with the consequence of a reduced N content in the manure after storage. This study evaluated the effects of one of these commercial additives (BACTYcomplex®, COMAS, Bovolenta, Padua, Italy) on slurry characteristics and N losses at a commercial piggery. The additive was applied to four different sectors of the piggery, each with an independent under-floor slurry pit; four other sectors served as controls without treatment. Pits were emptied every four weeks and the manure was analyzed for total and ammonia N and total and volatile solids. Slurry samples from the last month of the on-farm assessment were removed and stored thermostatically in vessels external to the piggery. A sub-sample of slurry that was treated with the additive at the piggery was treated with an additional dose of additive at the beginning of long-term storage. The additive did not change the composition of the slurry during in-house storage (four weeks duration). During the 155 days of external thermostatic storage, the total solids content of treated slurry was reduced by 18% compared to control slurry, but the N content and composition of treated slurry was unaffected. The additive had a positive effect in accelerating the stabilization of the slurry, but did not modify N losses.

Keywords: Slurry additive, manure management, nitrogen

Introduction

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

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

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

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

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

Wheeler et al. (2011) tested 22 additives that included microbial digestion products, oxidizing agents and chemicals, disinfectants, odor masking agents and adsorbents. Some additives reduced ammonia 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 (McCrory 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

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

Materials and Methods

Experimental test on fattening piggery

The fattening piggery – characteristics and monitoring

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

The pens had fully-slatted floors equipped with a vacuum system (Joint Research Center, 2013). The cumulative area of the pens in both the treatment and control sectors was identical (2843 m^2). The slurry removed from the pits below the floors was sent through a pipe into a reception tank (106 m^3) external to the buildings, from which it was pumped into the final storage tank away from the buildings.

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

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

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

Temperature-controlled slurry storage

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

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

Chemical analysis

Slurry samples collected at the pig-rearing facility were analyzed in a commercial laboratory (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 (P_2O_5) and potassium (K_2O). 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 (McCrory 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

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

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Figure captions

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

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

Figure 3 – Mean values **and standard deviation (vertical bars)** of the studied parameters of untreated (C) slurry samples, samples treated with the normal dose of additive (T) and samples treated with a double dose of the additive (E), at the end of thermostatic storage for 155 days. Ammonia Nitrogen (TAN) and Total Kjeldahl nitrogen (TKN) are expressed in g L^{-1}; Total solids (TS), Volatile solids (VS) and all the relative indices are expressed as percentages.

Tables

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

(†) composition of feeding typology A: specific feed (23.8%); water (41.7%); milk whey (34.5%). Composition of Specific feed: CP (14.8%); P (0.52%)

(‡) composition of feeding typology B: specific feed (24%); water (37%); milk whey (39%). Composition of Specific feed: CP (14.2%); P (0.50%)

(§) composition of feeding typology C: specific feed (24%); water (37%); milk whey (39%). Composition of Specific feed: CP (13%); P (0.45%)

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_2O_5 (g L^{-1})	K_2O (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)

371

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.