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**Assessing Archeal Indicators of Performance by RT-qPCR Methods During Anaerobic Co-digestion of Organic Wastes**

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# UNIVERSITÀ DEGLI STUDI DI TORINO

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1 **TITLE PAGE**

2

3 **Environmental advances due to the integration of food industries and anaerobic**  
4 **digestion for biogas production: the perspectives in the Italian milk and dairy product**  
5 **branch.**

6

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18 **ABSTRACT:**

19

## 20 1 INTRODUCTION

21 Milk and dairy products are essential parts of a healthy diet. Milk is a nutrient concen-  
22 trate for humans and during the last decades various healthy improvement of its char-  
23 acteristics were carried on as lactose-free (Lasekan et al., 2011), oligosaccharides con-  
24 tents (Chichlowski et al., 2011), calcium enriched (Lewis, 2011), fatty concentration  
25 (Camfield, 2011), n-3 polyunsaturated fatty acids content (Kouba & Mourot, 2011), mi-  
26 cro-nutrients concentrations (Rooke, 2010) and probiotics and prebiotics additions  
27 (Figuroa-Gonzalez, 2011). Such changes have also economic implications. The milk  
28 and milk-derived product consists of the 10% of the food production sector in Europe.  
29 Italy is one of the first five European main producers, with a production comparable to  
30 the Netherlands (Eurostat, 2011). The Italy production and utilization on the farm  
31 showed in the last years (1999-2009) a 3% increase, constant also during the last years  
32 (Eurostat, Milk and Milk products database, 2011). This evidence, instead of the eco-  
33 nomic crisis, give us an idea about the strategic role of this food products.

34 On the other hands the introduction of standards settings (EC 2073/2005) and hazard  
35 assessment regulation (EC 852, 853 and 854/2004) during the whole production (EC  
36 178/2002) conduct to a marked transformation of the organization with the adoption  
37 of a effective food safety management systems (Ball, 2009). In Italy and in particular in  
38 some areas this approach is rapidly developed taking advantage from the traditional  
39 hygiene, veterinary and food-production capabilities.

40 Despite the diffusion of the milk and milk products, the notifications recoded by the  
41 European Rapid Alert System for Food and Feed (RASSF) on milk and milk products are  
42 at least the 2.3% of the total. The main hazard categories involved is the presence of

43 potentially pathogenic microorganisms then the presence of various contaminants:  
44 food additives, residues of veterinary products and foreign bodies (Report RASSF,  
45 2010). Italy has an invaluable food resources both in terms of raw material quality and  
46 transforming technologies and, also during the 2010, it is the first European country as  
47 typical food producing. More than 18% of the typical products, classified as DOP, IGP  
48 or STG by the European Union are dairy products (ISTAT, 2010). This data confirms the  
49 talented heredities from centuries of work but also the improvement in technologies  
50 provided modern tools to control hazards and to improve efficiency of the producing  
51 systems. Furthermore the global trade system introduced various criticisms. Among  
52 these: the business competition by producers of other parts of the world where the  
53 producing costs are lower, the changing in the transport overheads but also the major  
54 expenditure due to the energy consumption and the increasing attention to the envi-  
55 ronmental sustainability. The European regulations, especially those promulgated in  
56 the last years had the objective of reducing the environmental impact of the farming  
57 and food producing activities, also through the integration of the waste disposal and  
58 energy production. Some examples are the Council Directive 91/676/EEC and succes-  
59 sive ones, concerning the protection of waters against pollution caused by nitrates,  
60 and more recently the SEC (2011) 1154 on the Common Agricultural Policy (CAP) to-  
61 wards 2020 where it is established that "*..the overarching objective for the future CAP*  
62 *should be sustainable competitiveness to achieve an economically viable food produc-*  
63 *tion sector, in tandem with sustainable management of the EU's natural land-based re-*  
64 *sources*". The milk and milk by-products are under this last point of view a great pro-  
65 spective field (Panesar & Kennedy, 2011). In this review we analysed and discussed the

66 environmental implication of the integration between milk and milk derived produc-  
67 tion and anaerobic digestion (AD) technologies to generate biogas as energy vector to  
68 sustain the same production necessity or even an energy surplus. This discussion was  
69 conducted mainly on the Italian contest.

70

## 71 **2 THE DAIRY SECTOR IN EUROPE**

72 The vast majority of milk (over 95%) collected in the EU in 2006 came from cows,  
73 although in a number of the southern European Member States significant quantities  
74 of milk were also produced from sheep and goats. There were 2,8 million holdings in  
75 the EU-27 with dairy cows (2005). However, 1,9 million holdings only had between one  
76 and two cows, Romania and Poland accounting for 1,6 million of these small dairy units.  
77 Among those Member States the number of farms with dairy cows declined sharply  
78 between 1995 and 2005; in Italy, the number of holdings with dairy cows halved and in  
79 Spain the number fell by almost two thirds. Although the number of cows also declined  
80 in the same period, the average number of cows per holding increased, sometimes  
81 sharply (i.e. in Denmark, Greece and Portugal, see Figure 1). In contrast to the small  
82 herd sizes in Romania and Poland, the average size of a dairy herd in the United  
83 Kingdom was just under 80 head in 2005, had risen to 85 head in Denmark, and was  
84 just over 100 head, on average, in Cyprus.

85 In table1 is shown the annual milk production in various European countries (EU-27):  
86 the production of cows' milk remained approximately steady. Average milk yields  
87 across the EU-27 in 2010 were about.  $13.5 \times 10^7$  t: Germany is the country where there  
88 is higher milk production (21%), followed by France (17.2%).

89 About 90% of milk produced in the EU-27 was collected by dairies in 2006 for  
90 processing into drinking milk and a variety of milk products. The 133 million tonnes of  
91 milk delivered to dairies across the EU-27 in 2006 were principally transformed into 31  
92 million tonnes of drinking milk, 9 million tonnes of cheese, 7 million tonnes of acidified  
93 milk, 2 million tonnes of butter and 1 million tonnes of both skimmed milk powder and  
94 other milk powder. In terms of milk equivalent quantities, however, the European  
95 Commission has estimated that more milk went into the production of cheese in 2006  
96 (see Figure 3) than any other dairy product. There were about 5,400 dairies across the  
97 EU-27 (excluding Bulgaria, Cyprus and Luxembourg) in 2006, about 30% of which were  
98 in Italy and a further, combined, 30% in Greece, Spain and the United Kingdom. There  
99 were a little over 13,000 dairy products manufacturing enterprises (including dairies) in  
100 the EU-27 in 2005, employing an estimated 400,000 persons (see Table 2). One third  
101 (33.1%) of the EU-27's dairy products manufacturing enterprises were located in Italy,  
102 with a further fifth in Spain (11.5%) and France (11.2%) combined. A little over half  
103 (53.6%) of the turnover generated by the EU-27's dairy products manufacturing  
104 enterprises came from enterprises in France (20.0%), Germany (19.2%) and Italy  
105 (14.3%). Further down the chain, there were about 15,000 wholesalers specialised in  
106 dairy produce, eggs, edible oils and fats in the EU-27 in 2005, a majority of which were  
107 based in Italy (24.0%), Spain (18.8%) and Greece (12.5%). These specialist wholesalers  
108 employed a further 119,000 persons, representing 1.2% of the wholesale and  
109 commission trade workforce within the EU-27.

110 The observed transformation in favour of a big plants of farming and milk transforming  
111 produced the ability to collect a wider amount of wastes and produce refuses,

112 moreover designed a major range of investment floating assets. The economic  
113 resources allocated as develop funds in a big enterprise could distinguish easier a  
114 medium and long term ahead instead of an immediate feedback.

115 There were about 13.0 thousand enterprises across the EU-27 whose main activity was  
116 the manufacture of dairy products (NACE Group 15.5) in 2006. These enterprises  
117 employed an estimated 400.0 thousand persons, representing 8.5 % of the food,  
118 beverages and tobacco manufacturing sector's workforce. The overwhelming majority  
119 of these workers (83.9 %) were engaged in the operation of dairies and cheese making  
120 (NACE Class 15.51). The dairy products manufacturing sector generated EUR 17.7  
121 billion of value added in 2005, equivalent to 8.9 % of the value added generated by  
122 food, beverages and tobacco manufacturing activities in 2005 (Eurostat Business).

123

### 124 **3 THE DAIRY SECTOR IN ITALY**

125 According to data from 6th Census of Agriculture, in October 2010 in Italy were  
126 1,630,420 active farms and livestock. Puglia is the region with the largest number of  
127 farms (over 275 thousand), followed by Sicilia (219,000), Calabria (138,000), Campania  
128 (137,000) and Veneto (121,000). In these five regions work the 54.6% of Italian farms.  
129 46% of utilized agricultural area is concentrated in Sicilia (1,384,043 ha), Puglia  
130 (1,280,876 ha), Sardegna (1,152,756 ha) Emilia-Romagna (1,066,773 ha) and Piemonte  
131 (1,048,350 ha). The regions with the greatest number of bovine livestock are  
132 Lombardia, with 15.000 holdings and 1.5 million animals, Veneto with 13,000 holdings  
133 and 826,000 cattle and Piemonte with 13,000 holdings and 816,000 cattle. Taken  
134 together, these three regions hold approximately 55% of Italian cattle. In the last



135 decade there has been strong growth in the buffalo farming sector, with a complex of  
136 358 thousand buffaloes concentrated mainly in Campania (261,000 buffaloes, 1,406  
137 holdings) and Lazio (63,000 buffaloes, 590 holdings). The two regions hold 90.4% of the  
138 total buffaloes. Companies operating in the dairy sector are overall 2,149 (Istat, 2009),  
139 of which 411 located in Emilia-Romagna, 369 in Campania, 259 in Lombardia. In 2010  
140 were produced overall 11,207,796 tons of milk: 10,573,181 cow's milk, 432,222 sheep's  
141 milk, 177,457 buffalo's and 24,935. goat's.

142 The regions where it has the highest milk production are Lombardia (36% of total),  
143 Emilia-Romagna (19%), Veneto (9%) and Piemonte (8%). In Piemonte are raised  
144 approximately 800,000 cattle of which 155,000 are dairy cows. Milk production is  
145 about 8 million tons per year (8% of national production). Holdings with dairy cows are  
146 2,800, 69% of livestock and 78% of cows are concentrated in urban and rural areas with  
147 intensive agriculture (mainly in the plain). These are generally high or medium-sized  
148 livestock, conducted with intensive method. Dairies are 142 and employ 2,365 people.  
149 Provinces with the most cattle livestock are Cuneo, Torino and Asti.

150

#### 151 **4 DAIRY SECTOR BY-PRODUCTS**

152 (questa parte è probabilmente da sintetizzare ancora, numerose ripetizioni, non c'è  
153 molto sul latticello buttermilk... molto sul siero che può essere un po' riditto)

154 Cheese manufacturing industry generates large amounts of high strength wastewaters  
155 with different polluting characteristics (high organic matters and the disposal of the  
156 effluents may cause serious environmental pollution depending on the plant and  
157 production type (Demirel et al., 2005; Gavala et al., 1999; Kalyuzhnyi et al.,

158 1997, Montuelle et al., 1992) so that fatty matter, protein and carbohydrates constitute  
159 different percentages of the organic matter (Vidal et al., 2000)(Erdirencelebi, 2011)??.

160 A majority of dairy wastewater gets produced during cleaning operations, especially  
161 between product changes when different types of products are produced in a specific  
162 production unit and clean-up operations.(Kushwaha et al., 2011).

163 The dairy industry is one of the most polluting of industries, not only in terms of the  
164 volume of effluent generated, but also in terms of its characteristics as well. It  
165 generates about 0.2–10 liters of effluent per liter of processed milk (Vourch et al., 2008)  
166 with an average generation of about 2.5 liters of wastewater per liter of the milk  
167 processed (Ramasamy et al., 2004)(Munavalli and Saler, 2009).

168 Furthermore, the dairy industry is one of the largest sources of industrial effluents in  
169 Europe. A typical European dairy generates approximately 500 m<sup>3</sup> of waste effluent  
170 daily (Wheatley, 1990).

171 Wastewaters from the dairy industry are usually generated in an intermittent way, so  
172 the flow rates of these effluents change significantly. High seasonal variations are also  
173 encountered frequently and correlate with the volume of milk received for processing;  
174 which is typically high in summer and low in winter months (Kolarski R, 1985).

175 Moreover, since the dairy industry produces different products, such as milk, butter,  
176 yoghurt, ice-cream, various types of desserts and cheese, the characteristics of these  
177 effluents also vary greatly, depending on the type of system and the methods of  
178 operation used (Vidal et al., 2000). The use of acid and alkaline cleaners and sanitizers  
179 in the dairy industry additionally influences wastewater characteristics and typically  
180 results in a highly variable pH (Demirel, 2003).

181 Dairy wastewaters are characterized by high biological-oxygen demand (BOD) and  
182 chemical-oxygen demand (COD) concentrations, but the main contributors to the  
183 organic load of these wastes are carbohydrates, proteins, fats, nutrients, lactose, as  
184 well as detergents and sanitizing agents (USDA-SCS, 1992, Demirel et al., 2005,  
185 Farizoglu et al., 2004, Omil et al., 2003).

186 The dairy sector produces, in addition to the products of industrial processing, the fol-  
187 lowing by-products: (1) By-products such as whey and buttermilk; (2) Waste processing  
188 (cheese rinds and pulp, scrap, residues of curd, residues from cleaning); (3) Milk and  
189 cheese with the presence of contaminants (aflatoxins, residues of inhibitory substanc-  
190 es); (4) Waste arising from brushing the powdery form of cheese during ripening; (5)  
191 Effluent and process waste water washing. In particular whey is a by-product of the  
192 dairy industry in which the principal components are lactose, proteins and mineral  
193 salts (Vasala et al., 2005). Approximately 47% of the 115 million tons of whey produced  
194 world-wide every year are **disposed of** in the environment (Leite et al., 2000; Zhou and  
195 Kosaric, 1993; Siso, 1996). This represents a significant loss of resources and causes se-  
196 rious pollution problems since whey is a high strength organic pollutant with high BOD<sub>5</sub>  
197 and COD, with values of 40,000–60,000 mg/L and 50,000–80,000 mg/L, respectively  
198 (Ben-Hassan and Ghaly, 1994; Fournier et al., 1993).

199 According to data from Annual survey on milk and dairy products (Istat, 2010), whey  
200 produced in Italy in 2010 was about 8,000,000 tons. Of this, 10% was utilized for ricot-  
201 ta cheese production, 30% for animal feeding, 8% as concentrated whey and 5.5 % as  
202 whey powder. Therefore, approximately 47% of whey produced should be disposed of.

203

204 **Dalla ex tabella 4 inserire solo una frase sul 94% del bilancio tra massa organica li-**  
205 **quida in entrata ed in uscita. (RAFFA)**

206

207 The total quantity of serum (whey?) obtained in Italy was estimated at about  
208 6,092,000 tons per year (ANPA, 2001). The amount of serum (whey?) sent each year to  
209 process the ricotta is 362,000 tons (ISTAT), which are generated about 345,000 t year<sup>-1</sup>  
210 of sheet, and about 18,100 t year<sup>-1</sup> of ricotta (Table 3).

211 The major by-product of the dairy industry is whey, a greenish-yellow and turbid liquid  
212 that remains in the boiler after separation of the curd (coagulation process required for  
213 all dairy products). Of the volume of processed milk, 80-90% leaves the process as  
214 whey, that retains most of the milk fat, trace minerals, salts and vitamins (Azbar et al.,  
215 2009). Whey's composition depend on a lot of parameters such as the type of milk  
216 used, i.e. if it comes from sheep, goat, water buffaloes or cow milk, the period of the  
217 year, the farmed species and its feeding, the breed, the season of milk production, the  
218 type of cheese and techniques of production, the period of coagulation and  
219 temperature (Kavacik and Topaloglu, 2010). Depending on the mode of coagulation of  
220 the milk whey can be sweet (pH=6-7) or sour (pH<5). Acid whey has a higher ash  
221 content, especially calcium, a low serum proteins content and a lower lactose content.  
222 A summary of data obtained from literature for general properties of dairy waste  
223 effluents from full-scale operations is given in Table 4

224 **Tabella 4 nella quale confluiscono dati salient della tabelle 1,2,4,5 precedenti RAFFA**

225

226 The dairy industry, like most other agro-industries, generates residues from which

227 whey is the most important wastewater produced, with an extremely high organic load.  
228 Dairy industries all over the world generate ample amounts of whey per liter of milk  
229 processed, depending upon the processes employed, products manufactured and  
230 housekeeping exercised. World annual production of whey is estimated to be 115  
231 million tons; approximately 47 % of the produced whey is disposed into the  
232 environment (Saddoud et al., 2007). This represents a significant loss of resources and  
233 causes serious pollution problems. Particularly, for medium size cheese factories, that  
234 have growing disposal problems and cannot afford high investment costs for whey  
235 valorization technologies, physico-chemical and/or biological treatment of this effluent  
236 is imperative. When disposing whey as a wastewater into a sewage treatment plant, it  
237 has been estimated that 50 kg whey were equivalent to the waste produced by 22  
238 people every day. In other words, a cheese plant producing 50.000 kg of whey per day  
239 requires a treatment plant of about the same size like a city with a population of  
240 22.000 inhabitant equivalents (Gillies, 1974). Whey may be defined broadly as the  
241 serum or watery part of milk remaining after separation of the curd, which results from  
242 the coagulation of milk proteins by acid or proteolytic enzymes. The type and  
243 composition of whey at dairy plants mainly depends upon the processing techniques  
244 used for casein removal from liquid milk. The most often encountered type of whey  
245 originates from manufacture of cheese or certain casein cheese products, where  
246 processing is based on coagulating the casein by rennet, an industrial casein-clotting  
247 preparation containing chymosin or other casein-coagulating enzymes (Fox, Guinee,  
248 Cogan, & Mc Sweeney, 2000).  
249 The composition of cheese whey depends on a lot of parameters like the composition

250 and quality of evaluated milk, techniques of production of cheese, the amount of yeast  
251 or acid which used coagulation and their quality, the period of coagulation and  
252 temperature. A typical cheese whey contains around 6,5% of total solid and these  
253 include lactose (45–50 g/L, 68-72%), soluble proteins (6–8 g/L, 12-13%), lipids (4– 5 g/L,  
254 6-7%) and mineral salts (8–10% of dried extract). The mineral salts are comprised of  
255 NaCl and KCl (more than 50%), calcium salts (primarily phosphate) and others. Whey  
256 also contains appreciable quantities of lactic (0.5 g/L) and citric acids, non-protein  
257 nitrogen compounds (urea and uric acid) and B group vitamins (Panesar et al., 2007)  
258 (Siso, 1996). There are two kinds of whey, depending on the type of milk coagulation  
259 used, either sweet or sour whey (Table 2). Sweet whey (pH= 6,5) is obtained if milk is  
260 coagulated by proteolytic enzymes, such as chymosine and pepsine or microbial  
261 enzymes produced from **the molds** *Mucor miehei* and *Mucor pusillus*. It comes from  
262 the production of cheddar, Swiss and Italian varieties of cheese. Acid whey (pH<5)  
263 results from processes using fermentation or addition of organic or mineral acids to  
264 coagulate the casein, as in the manufacture of fresh cheese or most industrial casein  
265 (Jelen, 2003). It comes from soft cheese production, with cottage cheese as a  
266 predominant product.

267 The main components of both sweet and acid wheys, after water, are lactose  
268 (approximately 70–72% of the total solids), whey proteins (approximately 8–10%) and  
269 minerals (approximately 12–15%) (Jelen, 2003). The main differences between the two  
270 whey types are in the mineral content, acidity and composition of the whey protein  
271 fraction. The acid coagulation approach results in substantially increased acidity (final  
272 pH approximately 4.5), necessary for casein precipitation.

273 As said before because of its high organic content, cheese whey disposal constitutes a  
274 serious environmental problem, with lactose being mainly responsible for its high  
275 chemical oxygen demand values (COD= 60-80 g L<sup>-1</sup>) and a low buffer capacity  
276 (Gerardi,2003). More than 90 % of the total COD of the whey accounted for lactose,  
277 lactate, protein and fat (Mockaitis 2006).

278 Cheese production is an important part of the dairy industry in Italy. According to the  
279 report ISTAT 2011 "Annual Survey on milk and dairy products" for 2010, Italy ranks fifth  
280 in worldwide production of cheese, with about 1.2 million tons a year. Of such amount  
281 approximately 90% is attributable to smaller dairies, with a production capacity lower  
282 than 200 tonnes / year.

283 The major by-product of the dairy industry is whey, a greenish-yellow and turbid liquid  
284 that remains in the boiler after separation of the curd (coagulation process required for  
285 all dairy products). Of the volume of processed milk, 80-90% leaves the process as  
286 whey, that retains most of the milk fat, trace minerals, salts and vitamins (Azbar et al.,  
287 2009). Whey's composition depend on a lot of parameters such as the type of milk  
288 used, i.e. if it comes from sheep, goat, water buffaloes or cow milk, the period of the  
289 year, the farmed species and its feeding, the breed, the season of milk production, the  
290 type of cheese and techniques of production, the period of coagulation and  
291 temperature (Kavacik and Topaloglu, 2010). Depending on the mode of coagulation of  
292 the milk whey can be sweet (pH=6-7) or sour (pH<5). Acid whey has a higher ash  
293 content, especially calcium, a low serum proteins content and a lower lactose content.  
294 Dairy wastewaters are treated using physico-chemical and biological treatment  
295 methods. However, since the reagent costs are high and the soluble COD removal is

296 poor in physical–chemical treatment processes, biological processes are usually  
297 preferred (Vidal et al., 2000). Among biological treatment processes, treatment in  
298 ponds, activated sludge plants and anaerobic treatment are commonly employed for  
299 dairy wastewater treatment (Bangsbo-Hansen DI,1985, Demirel et al., 2005). No  
300 requirement for aeration, low amount of excess sludge production and low area  
301 demand are additional advantages of anaerobic treatment processes, in comparison to  
302 aerobic processes (Demirel et al., 2005). Proper management of cheese whey is  
303 important due to stricter legislation (Farizoglu et al., 2004) that does not permit its land  
304 disposal without prior treatment, as well as economic reasons that force its  
305 valorization (Yang et al., 2007). Because of its high organic content, cheese whey  
306 disposal constitutes a serious environmental problem, with lactose being mainly  
307 responsible for its high chemical oxygen demand (COD) values.

308

## 309 **5 ANAEROBIC DIGESTION**

310 Since the whey naturally contains lactose and biodegradable organic matter, biological  
311 treatment is a practical process. Among biological treatment processes, treatment in  
312 ponds, activated sludge plants and anaerobic treatment are commonly employed for  
313 dairy wastewater treatment, and whey in particular.

314 While the high organic content of cheese whey renders the application of conventional  
315 aerobic biological treatment costly, mainly due to the high price of oxygen  
316 supplementation, high energy consumption, large amounts of waste sludge production,  
317 organic loading limitation and sludge bulking problems, anaerobic treatment requires  
318 no oxygen supplementation and generates a significant amount of energy in the form



319 of methane gas.

320 A particular ecosystem is present in an anaerobic reactor where several groups of  
321 microorganisms work interactively in the conversion of complex organic matter into  
322 biogas. In the digestion process four stages take place: hydrolysis, acidogenesis,  
323 acetogenesis and methanogenesis (Lozano et al. 2009). The first group of micro-  
324 organisms secretes enzymes which hydrolyze polymers to monomers so particulate  
325 materials are converted into dissolved materials by the action of exoenzymes excreted  
326 by the hydrolytic fermentative bacteria such as *Bacillus* and *Pseudomonas* (Whitman et  
327 al. 2006). This group includes both obligate and facultative anaerobes, and may occur  
328 up to  $10^8$ - $10^9$  cells/ml of sewage sludge digesters. They remove the small amounts of  
329  $O_2$  present and create anaerobic conditions. Subsequently acidogenic phase includes  
330 the action of a large and diverse group of fermentative bacteria, usually belong to the  
331 clostridia group and the family Bacteroidaceae. These bacteria hydrolyze and ferment  
332 the organic materials, e.g., cellulose, starch, proteins, sugars, lipids, etc., and produce  
333 organic acids,  $CO_2$  and  $H_2$ . They were species that often form spores that surviving in  
334 adverse environment. Then acetogenic bacteria convert these monomers to  $H_2$  and  
335 volatile fatty acids. The final phase of the biogas production is carried out by  
336 aceticlastic methanogens - mainly *Methanosarcina* with high acetate level ( $>10^{-3}M$ )  
337 and *Methanosaeta* with lower acetate level - and hydrogenotrophic methanogens.  
338 Methanogenesis is considered the rate-limiting step moreover this phase is most  
339 vulnerable to temperature or pH variations and toxic chemicals (Liu and Whitman  
340 2008). Anaerobic digestion of cheese whey offers an excellent approach from an  
341 energy conservation as well as pollution control point of view. However, raw whey is

342 known to be quite problematic to be treated anaerobically, because of its low  
343 bicarbonate alkalinity, high COD concentration and its tendency to get acidified very  
344 rapidly, leading to acidification and inhibition of methanogenic activity (Garcia et al.,  
345 1991, Malaspina et al., 1996). Whey is initially hydrolyzed and converted to organic  
346 acids by acidogenic microorganisms then the degradation is followed by the  
347 Methanosarcina and methanogenic bacteria (McHugh et al., 2006).

348 During the anaerobic digestion of whey Methanosarcina barkeri and Methanotherix  
349 soehngeni were identified as the dominant acetate-utilizing methanogens, and  
350 Methanobacterium formicum was the prevalent hydrogen-utilizing methanogen  
351 (Chartrain 1986). The predominance shift to Methanosaetaceae can be observed in  
352 environments with acetate concentrations below 1 mM, indicating that in this  
353 condition the specific growth rate of Methanosaeta spp. is higher than that of  
354 Methanosarcina spp. (Yu, 2006). Whey addition is also used as biostimulation method  
355 during anaerobic digestion process to analyze the microbe population variations (Lee,  
356 2012).

357 Italy, with an annual production of 2,891 TWh of electricity from biogas, ranks third in  
358 Europe after Germany and the United Kingdom. In 2011 there were nationwide more  
359 than 500 biogas plants, with an increase of 13% over the previous year almost entirely  
360 due to agricultural and livestock biogas.

361 **Questo pezzetto da spostare sulla diffusione della digestione anaerobica**

362

## 363 **6 ANAEROBIC DIGESTION REACTORS.**

364 A number of studies have been reported in literature for the treatment of dairy

365 wastewater by anaerobic methods. Table 3 summarizes the typical operating  
366 conditions for anaerobic digesters reported conditions of anaerobic treatment of dairy  
367 wastewaters.

368 High-rate reactors such as upflow anaerobic sludge bed reactor (UASBR) (Gavala et al.,  
369 1999), hybrid UASBR (Ozturk et al., 1993), expanded granular sludge bed reactor  
370 (EGSBR) (Petruy and Lettinga, 1997) and anaerobic filters (Viraraghavan and Kikkeri,  
371 1991) have been used in the treatment of dairy wastewaters.

372 Because single stage anaerobic reactors have experienced instability or failure during  
373 the treatment of complex wastewaters, two-stage anaerobic reactors have been  
374 operated with better performance in the case of complex wastewaters and sludges  
375 (such as cheese whey), where a combination of a completely stirred tank reactor (CSTR)  
376 and upflow filter has generally been employed. The separation of the acidogenesis and  
377 methanogenesis steps in a two-stage anaerobic process could be an alternative  
378 solution for treating cheese whey (Antonopoulou et al., 2008; Demirel and Yenigun,  
379 2002; Kim et al., 2004; Zeeman et al., 1997).

380 Lipid degradation and inhibition in single-phase anaerobic systems is frequently  
381 discussed in literature, since lipids are potential inhibitors in anaerobic systems, which  
382 can often be encountered by environmental engineers and wastewater treatment plant  
383 operators. Moreover, high concentrations of suspended solids in dairy waste streams  
384 can also affect the performance of conventional anaerobic treatment processes  
385 adversely, particularly the most commonly used upflow anaerobic filters.

386 Thus, two-phase anaerobic digestion processes should be considered more often to  
387 overcome these problems that may be experienced in conventional single-phase

388 design applications, since two- phase anaerobic treatment systems are reported to  
389 produce better results with various industrial wastewaters, such as olive oil mill and  
390 food-processing effluents, which are high in suspended solids and lipids content. When  
391 two-phase anaerobic digestion processes are evaluated as a whole, it is clear that the  
392 acid phase digestion of dairy wastewaters is actually investigated in various aspects.  
393 Despite the main advantages, anaerobic digestion is not extensively used in the dairy  
394 industry, largely due to the problem of slow reaction, which requires longer HRT, and  
395 rapid acidification (Najafpour et al., 2006; Zinatizadeh et al., 2006).

396 The problem of anaerobic digestion is slow reaction. It was overcome by novel hybrid  
397 systems such as upflow anaerobic sludge fixed film bioreactor and upflow packed bed  
398 biofilters (Goblos et al., 2008).

399 (se si trova si potrebbe aggiungere qui una indicazione sulla produzione della singola  
400 digestione in termini di metano prodotto per tonnellata di material fermentato)

401

## 402 **7 ANAEROBIC WHEY DIGESTION: INIBITION**

403

### 404 Lipids

405 Dairy effluents have high levels of lipid emulsions. Lipids are potentially inhibitory  
406 compounds, which can always be encountered during anaerobic treatment of dairy  
407 wastewaters. There is little information available in literature about the anaerobic  
408 digestibility of lipids (?articoli sulla digestione scarti macelleria o altre matrici con  
409 contenuti in grassi elevati?).

410 Lipids are slowly degraded because of their limited availability in function of their low

411 solubility. The occurrence of lipids within the reactors causes severe problems for the  
412 anaerobic digestion process (Petruy and Lettinga, 1997).

413 The most frequent problems reported in literature are biomass flotation in reactors ,  
414 unavailability of the substrate for micro-organisms within biofilms, and inhibition of the  
415 methanogenesis due to the presence of intermediaries from the degradation of lipids  
416 (Leal et al., 2002). Presence of lipids in single-phase anaerobic filter treatment for dairy  
417 effluents is also a common problem, because anaerobic filters remove lipids by  
418 entrapment, without biodegradation (Hanaki et al, 1981). This may soon result in  
419 channeling and clogging, with a subsequent decrease in reactor performance. Choice  
420 of appropriate packing materials in up flow anaerobic filters also affect maximum  
421 substrate loading rates and expected treatment rates significantly. Lipids are the most  
422 resistant constituent of the complex biopolymers and are converted to methane at low  
423 levels (Rinzema et al., 1993; Yu and Fang, 2001).

424

425 Long chain fatty acid

426 During anaerobic degradation, lipid is firstly hydrolyzed to glycerol and long chain fatty  
427 acids (LCFAs), inhibitors of the methanogenesis during digestion (Kuang et al., 2006)  
428 followed by  $\beta$ -oxidation, producing acetate and hydrogen. Glycerol, a compound  
429 formed as a result of lipid hydrolysis, was found to be a non-inhibitory compound  
430 (Perle et al,1995), while, LCFAs were particularly reported to be inhibitory to  
431 methanogenic bacteria (Koster et al,1987). The inhibitory effects of lipids in anaerobic  
432 processes can mainly be correlated to the presence of LCFAs, which cause retardation  
433 in methane production (Hanaki K et al,1981). Unsaturated LCFAs seemed to have a

434 greater inhibitory effect than saturated LCFAs. Unsaturated LCFAs strongly inhibited  
435 methane production from acetate and moderately inhibited b-oxidation. Thus,  
436 unsaturated LCFAs should be saturated to prevent lipid inhibition in anaerobic  
437 processes (Komatsu et al, 1991). Difficulties experienced with the presence of lipids in  
438 anaerobic treatment processes have been previously reported in literature (Alves MM  
439 et al, 1997,2005). Pereira et al. (2002) quantified long-chain fatty acids adsorbed to  
440 anaerobic biomass; the conclusion was that these substances inhibited the  
441 acidogenesis and the acetoclastic and hydrogenotrophic methanogenesis.

442

443 Lactose

444 Lactose is the main carbohydrate in dairy wastewater and is a readily available  
445 substrate for anaerobic bacteria. Anaerobic methanation of lactose needs a  
446 cooperative biological activity from acidogens, acetogens and methanogens (Yu Jet  
447 al,1993). Anaerobic fermentation of lactose yields organic acids, namely acetate,  
448 propionate, iso- and normal-butyrate, iso- and normal valerate, caproate, lactate,  
449 formate and ethanol (Kissalita et al, 1999, 1998). Two possible carbon flow schemes  
450 were proposed for acidogenic fermentation of lactose; carbon flow from pyruvate to  
451 butyrate and lactate, both occurring in parallel (Demirel et al ,2005). The presence of  
452 high carbohydrate concentrations in synthetic dairy wastewater was found to reduce  
453 the amount of proteolytic enzymes synthesized, resulting in low levels of protein  
454 degradation (Fang et al ,2000). It was previously reported that carbohydrates could  
455 suppress the synthesis of exopeptidases, a group of enzymes facilitating protein  
456 hydrolysis (McInerney et al, 1988) .

457

458 Protein

459 Anaerobic degradation of proteins and the effects of ammonia on this mechanism  
460 were recently investigated in detail (Tommaso G et al,2003Gavala et al, 2001). Casein  
461 is the major protein in milk composition and in dairy effluents. When fed to acclimated  
462 anaerobic reactors, degradation of casein is very fast and the degradation products are  
463 non-inhibitory (Perle et al, 1995).

464

## 465 **8 CO-DIGESTION**

466 It has stated that raw cheese whey is a quite difficult substrate to treat anaerobically  
467 because of the lack of alkalinity, the high chemical oxygen demand (COD)  
468 concentration and the tendency to acidify very rapidly.

469 Because of low bicarbonate alkalinity supplemental alkalinity is required so as to avoid  
470 anaerobic process failure. This alkalinity supplementation can be minimized by using  
471 operation conditions directed at obtaining better treatment efficiency, such as using  
472 higher hydraulic residence times or dilution of the influent. Moreover when undiluted  
473 cheese whey is directly treated in anaerobic reactors, stability problems arise. It has  
474 been reported that co-digestion of whey with manure was proved to be possible  
475 without any need of chemical addition up to 50% participation of whey (by volume) to  
476 the daily feed mixture.

477 A combined treatment of different waste types like manure and cheese whey gives the  
478 possibility of treating waste, which cannot be successfully treated separately. Whey  
479 was quantitatively degraded to biogas when co-digested with diluted manure without

480 addition of any chemicals. Manure had a high content of lipids, while whey had a high  
481 content of easily biodegradable carbohydrates. Co-digestion of these two wastes is  
482 advantageous than processing each one separately.

483 Due to high organic content and biodegradability of cheese whey, the most appropriate  
484 treatment method for whey is anaerobic digestion and it can be applied to existing  
485 facilities, already used for manure digestion alone. As a result, co- digestion of cheese  
486 whey together with local agricultural residues, such as manure, is a sustainable and  
487 environmentally attractive method.

488 (se si trova si potrebbe aggiungere qui una indicazione sulla produzione della co-  
489 digestione in termini di metano prodotto per tonnellata di materiale fermentato, ideale  
490 sarebbe una tabellina che indichi rese a confronto di siero o latticello + liquami bovini  
491 oppure altre matrici organiche introdotte (materiale Silvia schede prodotte per ciascun  
492 "ipotetico cassetto")

493 Pollina articolo raffa

494

## 495 **9 ENERGY RECOVERY FROM CHEESE WHEY BY ANAEROBIC DIGESTION**

496

497 Whey contains lactose (70–80%) and soluble proteins (10–15%) which results in a high  
498 chemical oxygen demand (COD, 50–70 g /L) (Frigon et al., 2009; Yorgun et al., 2008),  
499 therefore it is the main problem of environmental pollution of cheese manufacturing  
500 process. It is estimated that the polluting potential of cheese whey is about one  
501 hundred times higher than that of domestic wastewater. Anaerobic treatment of whey  
502 is one of the most interesting alternatives to minimize this pollution problem. (Bezerra



503 [et al., 2007]. Because of its high content of protein, for centuries whey has been used  
504 as feedstock for animal feeding, particularly swine feeding, while more recently by the  
505 agrifood and pharmaceutical industries (Frigon et al., 2009). However, gradual  
506 reduction in selling price of whey, encouraged search for alternatives use of this  
507 material. Due to its high biodegradability (aerobic degradability ≈99%, anaerobic  
508 degradability 94-99%; Ergüder et al, 2001) biological treatment of whey is the most  
509 appropriate way of stabilizing and due to the high organic content the basic biological  
510 treatment process to be used can only be anaerobic digestion whereas regular  
511 treatment processes such as the activated sludge process are completely inappropriate  
512 (Gavala et al., 1999; Saddoud et al., 2007).

513 During whey fermentation most of the lactose is transformed into lactic acid, acetic  
514 acid and other VFAs. The majority of lactose (62%) was converted into VFA (5 g/l) and  
515 lactic acid (18 g/l), then transformed in methane. Also the serum proteins are readily  
516 degraded by acclimated sludge and the lipid content is not sufficient to cause inhibition.  
517 Biogas yields from whey ranging between 76 and 99% (Ergüder et al, 2001; Saddoud et  
518 al., 2007). In batch-trials carried out to assess the biogas productivity potential of some  
519 agro-industrial biomasses, Dinuccio et al., found that the most productive in terms of  
520 specific yields was whey. Its specific yields were 953 I<sub>N</sub> biogas kg<sup>-1</sup> VS and 501 I<sub>N</sub> CH<sub>4</sub> kg  
521 VS<sup>-1</sup> (possiamo usarlo per la tabella sopra!!! Non è per nulla una cattiva resa).

522 Due to its high biodegradability, high organic load, and very low bicarbonate alkalinity  
523 (50 meq l<sup>-1</sup>) (Malaspina et al., 1996), whey tends to acidify very rapidly so impairing  
524 maintenance of process . Hence, to maintain process stability, a system to control pH is  
525 necessary, requiring, in most cases, addition of some external source of alkalinity, as

526 bicarbonate, carbonate, or hydroxide (Bezerra et al., 2007).

527 A possible solution to this problem is the two-phase plant configuration that provides  
528 for the separation of the acidogenic and methanogenic steps. Due to this separation  
529 VFA production is not significant in the methanogenic reactor, so VFA concentrations  
530 are always below the inhibitory limits, allowing for the smooth running of the methane  
531 fermentation (Saddoud et al., 2007).

532 Another possible alternative is co-ferment whey together with substrates with  
533 sufficient buffering capacities like cattle manure. By mixing whey and manure you can  
534 get a substrate easier to manage. Indeed, manure can balance the low content of  
535 nitrogen and alkalinity of whey and its high content of rapidly hydrolysable substances  
536 making degradative chain more stable. Gelegenis reported, in the continuously stirred  
537 pilot reactor, methane yield of  $2.2 \text{ vm} \cdot \text{vr}^{-1} \cdot \text{d}^{-1}$  (vm: volume of methane, vr: volume of  
538 reactor) of diluted poultry manure and whey mixture. Mixtures in which whey  
539 accounts for 20-50% of the total volatile solids fed were found to be optimal (Gelegenis  
540 et al., 2007).

541 According to Kavacik et al. codigestion of cheese whey and dairy manure in continuous  
542 fermentation with HRT (Hydraulic Retention Time) of 5 days and 8 % of total solids  
543 resulted in  $0.906 \text{ vm} \cdot \text{vr}^{-1} \cdot \text{d}^{-1}$  (vm: volume of methane, vr: volume of reactor). Comino et  
544 al. investigated biogas potential of cow manure and whey biomass mix and achieved  
545  $211.4 \text{ l CH}_4 \cdot \text{kgVS}^{-1}$  and very high rates of BOD<sub>5</sub> (78%) and COD (74%) removal.

546 Center of theoretical and applied ecology of Gorizia (CETA) tested various mixtures of  
547 whey and cow manure with increasing amounts of whey (20-50 and 80%) respect to  
548 manure. The higher specific biogas yield per unit of organic matter, equivalent to 486

549 L/kg of volatile substance, were obtained using the mixture with 80% whey and 20%  
550 slurry (anche questo potrebbe essere riportato in tabella comparative rese). Biogas  
551 produced, however, presented a lower percentage of methane (60.7%) than that of  
552 mixtures with lower whey amounts (64-65% of methane). This fact can be explained by  
553 higher concentration of carbohydrates in the whey, compared to proteins or lipids, that  
554 give rise to a biogas richer in CO<sub>2</sub>. These findings showed that anaerobic digestion of  
555 whey may take place even at high concentrations of this substrate. Slurry kept stability  
556 of anaerobic process providing adequate alkalinity to maintain optimum pH values (pH  
557 6.8 to 7.2) for methanogenic bacteria. Furthermore, animal slurry, especially cattle  
558 slurry, provides microorganisms, macro- and micro-nutrients that help to establish and  
559 maintain a balanced biocenosis in the reactor by favoring optimal conditions for  
560 carrying out the process (Migliardi, 2009).

561

## 562 **10 AD IN THE EUROPEAN REGULATIONS**

563 Anaerobic digestion represents a sustainable, natural route of treatment and recycling  
564 of wastes of biological origin and a wide range of useful industrial organic by-products.  
565 Caused by a steadily increasing biowaste collection, treatment and recovery, numerous  
566 EC regulations and guidelines have been issued in this area, or are currently under  
567 development. Most of these regulations profoundly influence the technological  
568 developments and practical applications of AD. There are many legislation and  
569 regulation applying on anaerobic digestion depending on the wastes treated, the type  
570 of facilities and the use of the by-products. As a waste management facility, an  
571 anaerobic digestion site has to be run with a license and some wastes, such as animal

572 by-products, have to be treated with specific care. The application of digestate on land  
573 has to respect limits and specific regulations also apply for potentially harmful  
574 feedstock, such as sewage sludge. In favour of anaerobic digestion, the electricity  
575 produced with the biogas involves that anaerobic digestion is part of the EU policies on  
576 new renewable energy. The development of anaerobic digestion could be boost by  
577 legislation as well as good practice and sustainability. The key sets of EU legislation are:

578 • The Commission Regulation (EU) No 142/2011, implementing Regulation (EC)  
579 No 1069/2009 of the European Parliament and of the Council laying down health rules  
580 as regards animal by-products and derived products not intended for human consump-  
581 tion and implementing Council Directive 97/78/EC as regards certain samples and  
582 items exempt from veterinary checks at the border under that Directive;

583 • The new European Directive 28/2009/CE on the promotion of Electricity from  
584 RES (renewable energy sources), which includes the National Renewable Energy Action  
585 Plans.

586 Across the EU there are many different strategies for supporting the development and  
587 implementation of renewable energy as well as solving agricultural and environmental  
588 problems. These have advanced at different paces in each country.

589

## 590 **11 ITALIAN LEGISLATION CONCERNING BIOGAS PRODUCTION**

591 The Italian legislation on the management of biomass for energy recovery in AD plant is  
592 quite extensive and consists of several laws. Legislative Decree 387/2003 transposes  
593 Directive 2001/77/EC on the promotion of electricity produced from renewable energy  
594 sources. It establishes the steps of construction and management of plants; in

595 particular it provides (introduces?) a "unique authorization", for the construction and  
596 management of the plant powered from renewable sources, to be released by the  
597 region (or the institution delegated for this purpose). In compliance with the provisions  
598 of art. 12, paragraph 10 of the Legislative Decree 387/2003 in 2010 have been issued  
599 the "Guidelines for the authorization of plants powered by renewable sources" (Decree  
600 of the Ministry of Economic Development September 10, 2010). National guidelines  
601 establish the documentation to be submitted for the construction of plants powered by  
602 renewable sources depending on the size of the plant. Authorization procedures  
603 required for the different types of plant (according to the Guidelines for the  
604 authorization of plants powered by renewable sources - Decree of the Ministry of  
605 Economic Development September 10, 2010). During 2011 was issued Legislative  
606 Decree 28 that implement Directive 2009/28/EC on the promotion of energy from  
607 renewable sources. It includes and implements the goals set by Europe and translate  
608 into concrete action strategies outlined in the National Action Plan. The decree held  
609 until the end of 2012 the current incentive system for the biogas industry. The Decree  
610 makes it clear that SCIA (Segnalazione Certificata di Inizio Attività - Certified report of  
611 activity beginning) does not apply to renewable energy and introduces changes to the  
612 authorization system established by the National Guidelines, providing four new  
613 different procedural process:

- 614 • a simple communication to the City Hall
- 615 • simplified procedure for the authorization (PAS)
- 616 • Unique Authorization

617

618 In order to present the PAS the threshold power required is the same as that specified  
619 for the SCIA, even if the regions can extend this limit up to 1 MW.

620 The Decree promotes the efficient use of biogas from animal slurries and from by-  
621 products of agricultural, agrifood and farming activities as well as biogas from “short  
622 chain”. It promote also construction of plants operating in cogeneration and realization,  
623 by farmers, of biogas plants to serve the agricultural activities.

624 The definition of “short chain” is established by Decree of Agriculture and Forestry  
625 296/2010 on the traceability of biomass to produce electricity. Biomass from “short  
626 chain” comprises biomass and biogas produced within 70 km from the production of  
627 electricity (distance as the crow flies between the plant and administrative boundaries  
628 of the municipality where falls the place of production of biomass). This definition  
629 allows to get increased incentives.

630

631 **12 DESTINATION OF DIGESTATE IN RELATION TO HYGIENIC-SANITARY**  
632 **CHARACTERISTICS, AGRONOMIC QUALITY AND LEGISLATION**

633 The digestate, that is the residual matrix by anaerobic digestion treatment, can act as  
634 organic amendment and nutrient fertilizer (Adani et al., 2011 ); however its land  
635 application represents an health concern, because it may lead to transmission of  
636 pathogens to man or animals and introducing them into the environment (Bohm, 2004).  
637 Hygienic risks partly depend by the biowastes that are treated in the plant (Sahlstrom,  
638 2003). Whey is not well microbiologically characterized, so in this substrate can be  
639 supposed the presence of pathogens that may occur in milk, as *Escherichia coli*  
640 O157:H7, *Listeria monocytogenes* and *Salmonella* spp. Among biowastes used in

641 codigestion with whey, animal faeces and sewage sludge may contain a wide variety of  
642 bacteria, parasites (protozoan and helminths) and viruses (Colleran, 2000). Some  
643 pathogens (e.g. *Salmonella* and *Ascaris* eggs) can also be present in municipal solid  
644 wastes (Deportès et al., 1998). In crops and silage pathogenic enterobacteria, such as  
645 *Salmonella* and toxin-producing *E. coli*, and *L. monocytogenes* are considered  
646 hazardous (Chen et al., 2005; Sahlstrom, 2003). Anaerobic digestion is able to reduce  
647 number of microorganisms that are present in biowastes making more safety; however  
648 some pathogens may survive to treatment, so ending up in the residue that is spread  
649 on soil (Estrada et al., 2004). In particular, inactivation rates of pathogens depend on  
650 temperature, treatment time, pH, presence of volatile fatty acids or ammonia, and  
651 nutrient availability in reactor (Sahlstrom, 2003). In addition to pathogens, digestate  
652 applied as fertilizer may transport dissolved heavy metals and organic compounds to  
653 agricultural fields. Heavy metals are of particular concern for health risk, eco-toxicity  
654 and environmental accumulation (da Costa Gomez et al., 2001). Milk whey should not  
655 contain them, but for example they can be present in manure, because are associated  
656 to livestock diets and excreted with faeces (Petersen et al., 2007), and in sewage  
657 sludges, with industrial or domestic source (Appels et al., 2008). Digestate can contain  
658 organic contaminants according to the origin of the biowaste; for example agricultural  
659 wastes can contain pesticide rests, antibiotics and other medicaments, while industrial  
660 organic wastes, sewage sludges and household wastes can present different  
661 contaminants (e.g. pesticides, PAH) (da Costa Gomez et al., 2001). The organic  
662 compounds of xenobiotic origin represent a hazard due to their potential for acute  
663 toxicity, mutagenesis, carcinogenesis, teratogenesis and estrogenic effects (da Costa

664 Gomez et al., 2001). The anaerobic digestion process results in a mineralization of  
665 nitrogen contained in biowaste and in a lowering of the C/N ratio, so favoring the  
666 short-term N fertilization effect (Weiland, 2010). It has been found that N in digestate  
667 is almost as effective as the nitrogen in inorganic fertilizers, so allowing the reduced  
668 application of these (Adani et al., 2011). In addition, the presence of secondary ( $\text{Ca}^{2+}$ ,  
669  $\text{Mg}^{2+}$  and  $\text{SO}_4^{2-}$ ) and trace elements makes the digestate a complete and balanced  
670 fertilizer (Adani et al., 2011). The anaerobic digestion residue can be refined, for  
671 example, by solid-liquid separation, obtaining the liquid fraction, a high efficiency  
672 coefficient fertilizer due high content of  $\text{N-NH}_4$  and high N/P ratio, and the solid  
673 fraction, with amendment properties related to the ability of the contained organic  
674 matter to maintain the soil humus balance (Adani et al., 2011).

675 The digestate resulting from a biogas plant that uses milk whey with other biowastes,  
676 e.g. manure, activated sludges, agricultural by products, organic fraction of municipal  
677 solid wastes, falls within the European Regulations on animal byproducts and derived  
678 products not intended for human consumption (Regulation EC No 1069/2009 and its  
679 implementing Commission Regulation EU No 142/2011) and the Italian law for fertiliz-  
680 er (D.Lgs. 75/2010). Moreover Italian law on agricultural use of wastewater digestion  
681 sludges (D.Lgs. 99/1992) and European Directive on waste (2008/98/EC, acknowledged  
682 in Italy legislation with D.Lgs. 205/2010) must be considered.

683 Commission Regulation EU No 142/2011 sets the following standards for digestion res-  
684 idues: *Escherichia coli* or *Enterococcaceae* for representative samples of the digestion  
685 residues taken during or immediately after transformation at the biogas plant in order



686 to monitor the process, and *Salmonella* for representative samples taken during or on  
687 withdrawal from storage (Table 6).

688 D.Lgs. 75/2010 for fertilizer presents as standards for amendments *Salmonella* and  
689 *Escherichia coli* (Table 6). *Salmonella* is also reported by D.Lgs. 99/1992, with maximum  
690 concentration of  $10^3$  MPN/g dry matter in wastewater digestion sludges. Moreover  
691 these laws indicate the maximum contents of heavy metals (Table 6).

692 Finally Italian D.Lgs. 205/2010, defines “quality digestate” as the product obtained by  
693 anaerobic digestion of separate collected organic wastes and respecting standards of  
694 rules that to be issue by Minister of environment and land and sea protection, with  
695 Minister of agricultural, food and forest politics. Actually these quality criteria for  
696 digestate have not been fixed yet.

697

## 698 **14 CONCLUSION**

### 699 **Figure 4**

700 **Biogas:** (specific yield ~ 650 l/kg VS; Methane in biogas ~ 55% ) Comino et al., 2012

701 **50-80% whey, 50-20% cattle slurry, 0-10% other organic refuses comino 2012, gorizia**

702 **chemical oxygen demand (COD) removal efficiency of greater than 95% panesar 2011**

703 **Whey (Lactose ~ 74%, Proteins ~ 14%, La differenza tra Cattle slurry e manure è**

704 **riconcucibile principalmente alla presenza di paglia (carboidrati complessi come**

705 **cellulosa, lignina, ecc) COD mg/L di ossigeno 50943 (libro pag 117), 7% ST di cui 68%**

706 **sono volatili.**

707 **Main methanogen substrates:**

708 **Acetate ~ 80%, Hydrogen <15%**

709

710

711 The fermentable organic solids, into the cattle sludge, vary in order to different factors  
712 among which the animal species and the weight productive class, the kind of feedings  
713 of the animals and the efficiency of the transformation of the feedings, the topology of  
714 the cattle-pound, the quantity of the washing or meteoric water collected with the  
715 stools and urine.

716 54 m<sup>3</sup> liquami capo/anno (6.5% solidi di cui 80% organic) 400m<sup>3</sup> biogas/ ST circa 500 €

717 /capo

718 Vantaggio solo scarti senza colture dedicate (gratuiti) doppio vantaggio , meno  
719 impatto più produzione di energia

720 521 impianti agricoli di digestione anaerobica 58% co-digestion

721 Impatto olfattivo e riduzione emissione gas serra

722 Spore di clostridi non aumentano con utilizzo di solo liquame e sottoprodotti della  
723 lavorazione del latte, aumentano con silomails e silosorgo

724 e distrazione dell'insilato per alimentazione animale per alimentazione digestore con  
725 aumento dei prezzi

726

#### **Set-up co-digestion A**

**50% cattle slurry+ 50% whey**

**2500 m<sup>3</sup> digestor volume**

**1.6\*10<sup>6</sup> m<sup>3</sup> biogas year**

**30% 100 capi 150 litri di liquame giorno 15 tonnellate, 5500 tonnellate anno + 70%**

siero (ca. 13000 tonnellate anno)

Produzione biogas 600 m3/t SV

$18500 * 0.07 = 1295$  tonnellate SV \* 600 = 777.000 m3 biogas \* 0.35 € = 270.000€

727 **1500 m3 di digestore**

- 728 • Cenni ad altre tecnologie utili per gli scarti (produzione idrogeno, polvere di  
729 siero, farmaceutiche, ecc.) e comparazione con la tecnologia proposta
- 730 • Ritorno economico energetico per una azienda tipo partendo da un tot di scarto  
731 considerando l'ammortamento dell'investimento iniziale, in pratica riusciamo a  
732 stimare un tempo di rientro avvalendoci della bibliografia raccolta a fine cipe?  
733 Magari si potrebbe anche chiedere a qualcuno della DWA se ha competenza.
- 734 • Riusciamo a costruire una sorta di elenco vantaggi e potenzialità/svantaggi e  
735 barriere come fine della trattazione.

736

## 737 **15 ACKNOWLEDGMENT**

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742 Frabosa Soprana.

743

## 744 **16 BIBLIOGRAFY**

745

746 Per la bibliografia sarebbe bene costruire un file end-note individuale che poi può

747 essere immesso in un file complessivo. Nel testo li inseriremo con il formato corretto  
748 alla fine delle correzioni.

749

750 **Figure captions:**

751

752 **Figure 1:** Number of holdings with dairy cows and average cow number per holding  
753 during the time in Europe, 1995 and 2005. Drawn and modified from Eurostat  
754 (Eurofarm and Food: From farm to fork statistics).

755 **Figure 2:** Milk used in dairy products in the EU, 2006 (estimate) Source (EC "Milk and  
756 milk products in the European Union" - August 2006)

757 **Figure 3** Dairy products **obtained/produced/made (?)** in Italy, 2010 (quantity in metric tons x  
758 1000) (Source: Istat, Annual survey on milk and dairy products, 2010)

759 **Figure 4** Flow-diagram for the anaerobic co-digestion of cattle slurry and whey, as basic  
760 feedings in the dairy production system. Figures in brackets indicate COD fractions.

761

762 **Table captions**

763

764 **Table 1 :** Cow's milk collection in EU-27 countries in 2005 and 2011 (Source:EUROSTAT,  
765 Cows'milk collection and products obtained - annual data; publish date: 16 mar 2012)

766 (\*) Austria, Belgium, Denmark, Finland, Greece, Luxembourg, Sweden, Cyprus, Czech Republic, Estonia, Hungary,  
767 Latvia, Lithuania, Malta, Poland, Slovakia, Slovenia, Romania, Bulgaria

768

769 **Table 2:** Milk production in Italy – Details for geographical area- 2010 (quantity in  
770 metric tons) (Source: Istat, Annual survey on milk and dairy products, 2010)

771

772 **Table 3.** Estimate of the main waste of the dairy sector (Italy, ANPA, 2001).

773 Table 4 (Characteristics of dairy waste effluents (si potrebbe togliere siero e latticello, il

774 secondo non mi risulta ci sia, ed integrare quei dati nella tabella successiva di

775 descrizione dei principali sottoprodotti di scarto, per l'appunto siero e latticello) +**Table**

776 **2 Typical composition of sweet and acid whey** +Table 3: Chemical characteristics of

777 whey (CRPA, 2002+altre utilizzate per completare) **Sistema RAFFA**

778 **Table 5 Typical operating conditions for anaerobic digesters + Table 4 Data on**

779 **anaerobic treatment of cheese whey SISTEMA RAFFA**

780 **Table 6 legislazione ?? ripensare se utile una tabella**

781