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

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(Article begins on next page)

TITLE PAGE

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

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ABSTRACT:

1 INTRODUCTION

Milk and dairy products are essential parts of a healthy diet. Milk is a nutrient concentrate for humans and during the last decades various healthy improvement of its characteristics were carried on as lactose-free (Lasekan et al., 2011), oligosaccharides contents (Chichlowski et al., 2011), calcium enriched (Lewis, 2011), fatty concentration (Camfield, 2011), n-3 polyunsaturated fatty acids content (Kouba & Mourot, 2011), micro-nutrients concentrations (Rooke, 2010) and probiotics and prebiotics addictions (Figueroa-Gonzalez, 2011). Such changes have also economic implications. The milk and milk-derived product consists of the 10% of the food production sector in Europe. Italy is one of the first five European main producers, with a production comparable to the Netherlands (Eurostat, 2011). The Italy production and utilization on the farm showed in the last years (1999-2009) a 3% increase, constant also during the last years (Eurostat, Milk and Milk products database, 2011). This evidence, instead of the economic crisis, give us an idea about the strategic role of this food products.

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

Despite the diffusion of the milk and milk products, the notifications recoded by the European Rapid Alert System for Food and Feed (RASSF) on milk and milk products are at least the 2.3% of the total. The main hazard categories involved is the presence of potentially pathogenic microorganisms then the presence of various contaminants: food additives, residues of veterinary products and foreign bodies (Report RASSF, 2010). Italy has an invaluable food resources both in terms of raw material quality and transforming technologies and, also during the 2010, it is the first European country as typical food producing. More than 18% of the typical products, classified as DOP, IGP or STG by the European Union are dairy products (ISTAT, 2010). This data confirms the talented heredities from centuries of work but also the improvement in technologies provided modern tools to control hazards and to improve efficiency of the producing systems. Furthermore the global trade system introduced various criticisms. Among these: the business competition by producers of other parts of the world where the producing costs are lower, the changing in the transport overheads but also the major expenditure due to the energy consumption and the increasing attention to the environmental sustainability. The European regulations, especially those promulgated in the last years had the objective of reducing the environmental impact of the farming and food producing activities, also through the integration of the waste disposal and energy production. Some examples are the Council Directive 91/676/EEC and successive ones, concerning the protection of waters against pollution caused by nitrates, and more recently the SEC (2011) 1154 on the Common Agricultural Policy (CAP) towards 2020 where it is established that "..*the overarching objective for the future CAP should be sustainable competitiveness to achieve an economically viable food production sector, in tandem with sustainable management of the EU's natural land-based resources*". The milk and milk by-products are under this last point of view a great prospective field (Panesar & kennedy, 2011). In this review we analysed and discussed the environmental implication of the integration between milk and milk derived production and anaerobic digestion (AD) technologies to generate biogas as energy vector to sustain the same production necessity or even an energy surplus. This discussion was conducted mainly on the Italian contest.

2 THE DAIRY SECTOR IN EUROPE

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

In table1 is shown the annual milk production in various European countries (EU-27): the production of cows' milk remained approximately steady. Average milk yields across the EU-27 in 2010 were about. $13.5x10^7$ t: Germany is the country where there is higher milk production (21%), followed by France (17.2%).

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

The observed transformation in favour of a big plants of farming and milk transforming produced the ability to collect a wider amount of wastes and produce refuses, moreover designed a major range of investment floating assets. The economic resources allocated as develop funds in a big enterprise could distinguish easier a medium and long term ahead instead of an immediate feedback.

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

3 THE DAIRY SECTOR IN ITALY

According to data from 6th Census of Agriculture, in October 2010 in Italy were 1,630,420 active farms and livestock. Puglia is the region with the largest number of farms (over 275 thousand), followed by Sicilia (219,000), Calabria (138,000), Campania (137,000) and Veneto (121,000). In these five regions work the 54.6% of Italian farms. 46% of utilized agricultural area is concentrated in Sicilia (1,384,043 ha), Puglia (1,280,876 ha), Sardegna (1,152,756 ha) Emilia-Romagna (1,066,773 ha) and Piemonte (1,048,350 ha). The regions with the greatest number of bovine livestock are Lombardia, with 15.000 holdings and 1.5 million animals, Veneto with 13,000 holdings and 826,000 cattle and Piemonte with 13,000 holdings and 816,000 cattle. Taken together, these three regions hold approximately 55% of Italian cattle. In the last decade there has been strong growth in the buffalo farming sector, with a complex of 358 thousand buffaloes concentrated mainly in Campania (261,000 buffaloes, 1,406 holdings) and Lazio (63,000 buffaloes, 590 holdings). The two regions hold 90.4% of the total buffaloes. Companies operating in the dairy sector are overall 2,149 (Istat, 2009), of which 411 located in Emilia-Romagna, 369 in Campania, 259 in Lombardia. In 2010 were produced overall 11,207,796 tons of milk: 10,573,181 cow's milk, 432,222 sheep's milk, 177,457 buffalo's and 24,935. goat's.

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

4 DAIRY SECTOR BY-PRODUCTS

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

Cheese manufacturing industry generates large amounts of high strength wastewaters with different polluting characteristics (high organic matters and the disposal of the effluents may cause serious environmental pollution depending on the plant and production type (Demirel et al., 2005; Gavala et al., 1999; Kalyuzhnyi et al., 1997,Montuelle et al., 1992) so that fatty matter, protein and carbohydrates constitute different percentages of the organic matter (Vidal et al., 2000)(Erdirencelebi, 2011)??.

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

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

Furthermore, the dairy industry is one of the largest sources of industrial effluents in Europe. A typical European dairy generates approximately 500 m^3 of waste effluent daily (Wheatley,1990).

Wastewaters from the dairy industry are usually generated in an intermittent way, so the flow rates of these effluents change significantly. High seasonal variations are also encountered frequently and correlate with the volume of milk received for processing; which is typically high in summer and low in winter months (Kolarski R, 1985). Moreover, since the dairy industry produces different products, such as milk, butter, yoghurt, ice-cream, various types of desserts and cheese, the characteristics of these effluents also vary greatly, depending on the type of system and the methods of operation used (Vidal et al., 2000). The use of acid and alkaline cleaners and sanitizers in the dairy industry additionally influences wastewater characteristics and typically results in a highly variable pH (Demirel, 2003).

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

The dairy sector produces, in addition to the products of industrial processing, the following by-products: (1) By-products such as whey and buttermilk; (2) Waste processing (cheese rinds and pulp, scrap, residues of curd, residues from cleaning); (3) Milk and cheese with the presence of contaminants (aflatoxins, residues of inhibitory substances); (4) Waste arising from brushing the powdery form of cheese during ripening; (5) Effluent and process waste water washing. In particular whey is a by-product of the dairy industry in which the principal components are lactose, proteins and mineral salts (Vasala et al., 2005). Approximately 47% of the 115 million tons of whey produced world-wide every year are disposed of in the environment (Leite et al., 2000; Zhou and Kosaric, 1993; Siso, 1996). This represents a significant loss of resources and causes serious pollution problems since whey is a high strength organic pollutant with high BOD_5 and COD, with values of 40,000–60,000 mg/L and 50,000–80,000 mg/L, respectively (Ben-Hassan and Ghaly, 1994; Fournier et al., 1993).

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

Dalla ex tabella 4 inserire solo una frase sul 94% del bilancio tra massa organica liquida in entrata ed in uscita. (RAFFA)

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

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

A summary of data obtained from literature for general properties of dairy waste effluents from full-scale operations is given in Table 4

Tabella 4 nella quale confluiscono dati salient della tabelle 1,2,4,5 precendenti RAFFA

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

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

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

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

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

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

Dairy wastewaters are treated using physico-chemical and biological treatment methods. However, since the reagent costs are high and the soluble COD removal is poor in physical–chemical treatment processes, biological processes are usually preferred (Vidal et al., 2000). Among biological treatment processes, treatment in ponds, activated sludge plants and anaerobic treatment are commonly employed for dairy wastewater treatment (Bangsbo-Hansen DI,1985,Demirel et al., 2005). No requirement for aeration, low amount of excess sludge production and low area demand are additional advantages of anaerobic treatment processes, in comparison to aerobic processes (Demirel et al., 2005). Proper management of cheese whey is important due to stricter legislation (Farizoglu et al., 2004) that does not permit its land disposal without prior treatment, as well as economic reasons that force its valorization (Yang et al., 2007). Because of its high organic content, cheese whey disposal constitutes a serious environmental problem, with lactose being mainly responsible for its high chemcial oxygen demand (COD) values.

5 ANAEROBIC DIGESTION

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

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

A particular ecosystem is present in an anaerobic reactor where several groups of microorganisms work interactively in the conversion of complex organic matter into biogas. In the digestion process four stages take place: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Lozano et al. 2009). The first group of micro-organisms secretes enzymes which hydrolyze polymers to monomers so particulate materials are converted into dissolved materials by the action of exoenzymes excreted by the hydrolitic fermentative bacteria such as Bacillus and Pseudomonas (Whitman et al. 2006). This group includes both obligate and facultative anaerobes, and may occur up to 108-109 cells/ml of sewage sludge digesters. They remove the small amounts of O_2 present and create anaerobic conditions. Subsequently acidogenic phase includes the action of a large and diverse group of fermentative bacteria, usually belong to the clostridia group and the family Bacteroidaceaea. These bacteria hydrolyze and ferment the organic materials, e.g., cellulose, starch, proteins, sugars, lipids, etc., and produce organic acids, CO_2 and H_2. They were species that often form spores that surviving in adverse environment. Then acetogenic bacteria convert these monomers to H_2 and volatile fatty acids. The final phase of the biogas production is carried out by aceticlastic methanogens - mainly Methanosarcina with high acetate level (>10-3M) and Methanosaeta with lower acetate level - and hydrogenotrophic methanogens. Methanogenesis is considered the rate-limiting step moreover this phase is most vulnerable to temperature or pH variations and toxic chemicals (Liu and Whitman 2008). Anaerobic digestion of cheese whey offers an excellent approach from an energy conservation as well as pollution control point of view. However, raw whey is known to be quite problematic to be treated anaerobically, because of its low bicarbonate alkalinity, high COD concentration and its tendency to get acidified very rapidly, leading to acidification and inhibition of methanogenic activity (Garcia et al., 1991,Malaspina et al., 1996). Whey is initially hydrolyzed and converted to organic acids by acidogenic microorganisms then the degradation is followed by the Methanosarcina and methanogenic bacteria (McHugh et al., 2006).

During the anaerobic digestion of whey Methanosarcina barkeri and Methanothrix soehngenii were identified as the dominant acetate-utilizing methanogens, and Methanobaterium formicicum was the prevalent hydrogen-utilizing methanogen (Chartrain 1986). The predominance shift to Methanosaetaceae can be observed in environments with acetate concentrations below 1 mM, indicating that in this condition the specific growth rate of Methanosaeta spp. is higher than that of Methanosarcina spp. (Yu, 2006). Whey addiction is also used as biostimulation method during anaerobic digestion process to analyze the microbe population variations (Lee, 2012).

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

Questo pezzetto da spostare sulla diffusione della digestione anaerobica

6 ANAEROBIC DIGESTION REACTORS.

A number of studies have been reported in literature for the treatment of dairy wastewater by anaerobic methods. Table 3 summarizes the typical operating conditions for anaerobic digesters reported conditions of anaerobic treatment of dairy wastewaters.

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

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

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

Thus, two-phase anaerobic digestion processes should be considered more often to overcome these problems that may be experienced in conventional single-phase design applications, since two- phase anaerobic treatment systems are reported to produce better results with various industrial wastewaters, such as olive oil mill and food-processing effluents, which are high in suspended solids and lipids content. When two-phase anaerobic digestion processes are evaluated as a whole, it is clear that the acid phase digestion of dairy wastewaters is actually investigated in various aspects. Despite the main advantages, anaerobic digestion is not extensively used in the dairy industry, largely due to the problem of slow reaction, which requires longer HRT, and rapid acidification (Najafpour et al., 2006; Zinatizadeh et al., 2006).

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

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

7 ANAEROBIC WHEY DIGESTION: INIBITION

Lipids

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

Lipids are slowly degraded because of their limited availability in function of their low solubility. The occurrence of lipids within the reactors causes severe problems for the anaerobic digestion process (Petruy and Lettinga, 1997).

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

Long chain fatty acid

During anaerobic degradation, lipid is firstly hydrolyzed to glycerol and long chain fatty acids (LCFAs), inhibitors of the methanogenesis during digestion (Kuang et al., 2006) followed by b-oxidation, producing acetate and hydrogen. Glycerol, a compound formed as a result of lipid hydrolysis, was found to be a non-inhibitory compound (Perle et al,1995), while, LCFAs were particularly reported to be inhibitory to methanogenic bacteria (Koster et al,1987). The inhibitory effects of lipids in anaerobic processes can mainly be correlated to the presence of LCFAs, which cause retardation in methane production (Hanaki K et al,1981). Unsaturated LCFAs seemed to have a greater inhibitory effect than saturated LCFAs. Unsaturated LCFAs strongly inhibited methane production from acetate and moderately inhibited b-oxidation. Thus, unsaturated LCFAs should be saturated to prevent lipid inhibition in anaerobic processes (Komatsu et al, 1991). Difficulties experienced with the presence of lipids in anaerobic treatment processes have been previously reported in literature (Alves MM et al, 1997,2005). Pereira et al. (2002) quantified long-chain fatty acids adsorbed to anaerobic biomass; the conclusion was that these substances inhibited the acidogenesis and the acetoclastic and hydrogenotrophic methanogenesis.

Lactose

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

Protein

Anaerobic degradation of proteins and the effects of ammonia on this mechanism were recently investigated in detail (Tommasso G et al,2003Gavala et al, 2001). Casein is the major protein in milk composition and in dairy effluents. When fed to acclimated anaerobic reactors, degradation of casein is very fast and the degradation products are non-inhibitory 8Perle et al, 1995).

8 CO-DIGESTION

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

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

A combined treatment of different waste types like manure and cheese whey gives the possibility of treating waste, which cannot be successfully treated separately. Whey was quantitatively degraded to biogas when co-digested with diluted manure without addition of any chemicals. Manure had a high content of lipids, while whey had a high content of easily biodegradable carbohydrates. Co-digestion of these two wastes is advantageous than processing each one separately.

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

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

Pollina articolo raffa

9 ENERGY RECOVERY FROM CHEESE WHEY BY ANAEROBIC DIGESTION

Whey contains lactose (70–80%) and soluble proteins (10–15%) which results in a high chemical oxygen demand (COD, 50–70 g /L) (Frigon et al., 2009; Yorgun et al., 2008), therefore it is the main problem of environmental pollution of cheese manufacturing process. It is estimated that the polluting potential of cheese whey is about one hundred times higher than that of domestic wastewater. Anaerobic treatment of whey is one of the most interesting alternatives to minimize this pollution problem. (Bezerra et al., 2007). Because of its high content of protein, for centuries whey has been used as feedstock for animal feeding, particularly swine feeding, while more recently by the agrifood and pharmaceutical industries (Frigon et al., 2009). However, gradual reduction in selling price of whey, encouraged search for alternatives use of this material. Due to its high biodegradability (aerobic degradability ≈99%, anaerobic degradability 94-99%; Ergüder et al, 2001) biological treatment of whey is the most appropriate way of stabilizing and due to the high organic content the basic biological treatment process to be used can only be anaerobic digestion whereas regular treatment processes such as the activated sludge process are completely inappropriate (Gavala et al., 1999; Saddoud et al., 2007).

During whey fermentation most of the lactose is transformed into lactic acid, acetic acid and other VFAs. The majority of lactose (62%) was converted into VFA (5 g/l) and lactic acid (18 g/l), then transformed in methane. Also the serum proteins are readily degraded by acclimated sludge and the lipid content is not sufficient to cause inhibition. Biogas yields from whey ranging between 76 and 99% (Ergüder et al, 2001; Saddoud et al., 2007). In batch-trials carried out to assess the biogas productivity potential of some agro-industrial biomasses, Dinuccio et al,. found that the most productive in terms of specific yields was whey. Its specific yields were 953 l_N biogas kg^{-1} VS and 501 l_N CH_4 kg VS^{-1} (possiamo usarlo per la tabella sopra!!! Non è per nulla una cattiva resa).

Due to its high biodegradability, high organic load, and very low bicarbonate alkalinity (50 meq l^{-1}) (Malaspina et al., 1996), whey tends to acidify very rapidly so impairing maintenance of process . Hence, to maintain process stability, a system to control pH is necessary, requiring, in most cases, addition of some external source of alkalinity, as bicarbonate, carbonate, or hydroxide (Bezerra et al., 2007).

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

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

According to Kavacik et al. codigestion of cheese whey and dairy manure in continuous fermentation with HRT (Hydraulic Retention Time) of 5 days and 8 % of total solids resulted in 0.906 $vm \cdot vr^{-1} \cdot d^{-1}$(vm: volume of methane, vr: volume of reactor). Comino et al. investigated biogas potential of cow manure and whey biomass mix and achieved 211.4 l $CH_4 \cdot kgVS^{-1}$ and very high rates of BOD_5 (78%) and COD (74%) removal.

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

L/kg of volatile substance, were obtained using the mixture with 80% whey and 20% slurry (anche questo potrebbe essere riportato in tabella comparative rese). Biogas produced, however, presented a lower percentage of methane (60.7%) than that of mixtures with lower whey amounts (64-65% of methane). This fact can be explained by higher concentration of carbohydrates in the whey, compared to proteins or lipids, that give rise to a biogas richer in CO2. These findings showed that anaerobic digestion of whey may take place even at high concentrations of this substrate. Slurry kept stability of anaerobic process providing adequate alkalinity to maintain optimum pH values (pH 6.8 to 7.2) for methanogenic bacteria. Furthermore, animal slurry, especially cattle slurry, provides microorganisms, macro- and micro-nutrients that help to establish and maintain a balanced biocenosis in the reactor by favoring optimal conditions for carrying out the process (Migliardi, 2009).

10 AD IN THE EUROPEAN REGULATIONS

Anaerobic digestion represents a sustainable, natural route of treatment and recycling of wastes of biological origin and a wide range of useful industrial organic by-products. Caused by a steadily increasing biowaste collection, treatment and recovery, numerous EC regulations and guidelines have been issued in this area, or are currently under development. Most of these regulations profoundly influence the technological developments and practical applications of AD. There are many legislation and regulation applying on anaerobic digestion depending on the wastes treated, the type of facilities and the use of the by-products. As a waste management facility, an anaerobic digestion site has to be run with a license and some wastes, such as animal by-products, have to be treated with specific care. The application of digestate on land has to respect limits and specific regulations also apply for potentially harmful feedstock, such as sewage sludge. In favour of anaerobic digestion, the electricity produced with the biogas involves that anaerobic digestion is part of the EU policies on new renewable energy. The development of anaerobic digestion could be boost by legislation as well as good practice and sustainability. The key sets of EU legislation are:

- The Commission Regulation (EU) No 142/2011, implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive;
- The new European Directive 28/2009/CE on the promotion of Electricity from RES (renewable energy sources), which includes the National Renewable Energy Action Plans.

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

11 ITALIAN LEGISLATION CONCERNING BIOGAS PRODUCTION

The Italian legislation on the management of biomass for energy recovery in AD plant is quite extensive and consists of several laws. Legislative Decree 387/2003 transposes Directive 2001/77/EC on the promotion of electricity produced from renewable energy sources. It establishes the steps of construction and management of plants; in particular it provides (introduces?) a "unique authorization", for the construction and management of the plant powered from renewable sources, to be released by the region (or the institution delegated for this purpose). In compliance with the provisions of art. 12, paragraph 10 of the Legislative Decree 387/2003 in 2010 have been issued the "Guidelines for the authorization of plants powered by renewable sources" (Decree of the Ministry of Economic Development September 10, 2010). National guidelines establish the documentation to be submitted for the construction of plants powered by renewable sources depending on the size of the plant. Authorization procedures required for the different types of plant (according to the Guidelines for the authorization of plants powered by renewable sources - Decree of the Ministry of Economic Development September 10, 2010). During 2011 was issued Legislative Decree 28 that implement Directive 2009/28/EC on the promotion of energy from renewable sources. It includes and implements the goals set by Europe and translate into concrete action strategies outlined in the National Action Plan. The decree held until the end of 2012 the current incentive system for the biogas industry. The Decree makes it clear that SCIA (Segnalazione Certificata di Inizio Attività - Certified report of activity beginning) does not apply to renewable energy and introduces changes to the authorization system established by the National Guidelines, providing four new different procedural process:

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

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

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

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

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

The digestate, that is the residual matrix by anaerobic digestion treatment, can act as organic amendment and nutrient fertilizer (Adani et al., 2011); however its land application represents an health concern, because it may lead to transmission of pathogens to man or animals and introducing them into the environment (Bohm, 2004). Hygienic risks partly depend by the biowastes that are treated in the plant (Sahlstrom, 2003). Whey is not well microbiologically characterized, so in this substrate can be supposed the presence of pathogens that may occur in milk, as *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp. Among biowastes used in codigestion with whey, animal faeces and sewage sludge may contain a wide variety of bacteria, parasites (protozoan and helminths) and viruses (Colleran, 2000). Some pathogens (e.g. *Salmonella* and *Ascaris* eggs) can also be present in municipal solid wastes (Deportès et al., 1998). In crops and silage pathogenic enterobacteria, such as *Salmonella* and toxin-producing *E. coli*, and *L. monocytogenes* are considered hazardous (Chen et al., 2005; Sahlstrom, 2003). Anaerobic digestion is able to reduce number of microorganisms that are present in biowastes making more safety; however some pathogens may survive to treatment, so ending up in the residue that is spread on soil (Estrada et al., 2004). In particular, inactivation rates of pathogens depend on temperature, treatment time, pH, presence of volatile fatty acids or ammonia, and nutrient availability in reactor (Sahlstrom, 2003). In addition to pathogens, digestate applied as fertilizer may transport dissolved heavy metals and organic compounds to agricultural fields. Heavy metals are of particular concern for health risk, eco-toxicity and environmental accumulation (da Costa Gomez et al., 2001). Milk whey should not contain them, but for example they can be present in manure, because are associated to livestock diets and excreted with faeces (Petersen et al., 2007), and in sewage sludges, with industrial or domestic source (Appels et al., 2008). Digestate can contain organic contaminants according to the origin of the biowaste; for example agricultural wastes can contain pesticide rests, antibiotics and other medicaments, while industrial organic wastes, sewage sludges and household wastes can present different contaminants (e.g. pesticides, PAH) (da Costa Gomez et al., 2001). The organic compounds of xenobiotic origin represent a hazard due to their potential for acute toxicity, mutagenesis, carcinogenesis, teratogenesis and estrogenic effects (da Costa

Gomez et al., 2001). The anaerobic digestion process results in a mineralization of nitrogen contained in biowaste and in a lowering of the C/N ratio, so favoring the short-term N fertilization effect (Weiland, 2010). It has been found that N in digestate is almost as effective as the nitrogen in inorganic fertilizers, so allowing the reduced application of these (Adani et al., 2011). In addition, the presence of secondary (Ca^{2+}, Mg^{2+} and SO_4^{2-}) and trace elements makes the digestate a complete and balanced fertilizer (Adani et al., 2011). The anaerobic digestion residue can be refined, for example, by solid-liquid separation, obtaining the liquid fraction, a high efficiency coefficient fertilizer due high content of $N\text{-}NH_4$ and high N/P ratio, and the solid fraction, with amendment properties related to the ability of the contained organic matter to maintain the soil humus balance (Adani et al., 2011).

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

Commission Regulation EU No 142/2011 sets the following standards for digestion residues: *Escherichia coli* or *Enterococcaceae* for representative samples of the digestion residues taken during or immediately after transformation at the biogas plant in order to monitor the process, and *Salmonella* for representative samples taken during or on withdrawal from storage (Table 6).

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

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

14 CONCLUSION

Figure 4

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

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

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

Whey (Lactose ~ 74%, Proteins ~ 14%, La differenza tra Cattle slurry e manure è riconducibile principalmente alla presenza di paglia (carboidrati complessi come cellulosa, lignina, ecc) COD mg/L di ossigeno 50943 (libro pag 117), 7% ST di cui 68% sono volatili.

Main methanogen substrates:

Acetate ~ 80%, Hydrogen <15%

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

54 m3 liquami capo/anno (6.5% solidi di cui 80% organic) 400m3 biogas/ ST circa 500 € /capo

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

521 impianti agricoli di digestione anaerobica 58% co-digestion

Impatto olfattivo e riduzione emissione gas serra

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

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

Set-up co-digestion A

50% cattle slurry+ 50% whey

2500 m^3 digestor volume

1.6*10^6 m^3 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€

1500 m3 di digestore

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

15 ACKNOWLEDGMENT

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16 BIBLIOGRAFY

Per la bibliografia sarebbe bene costruire un file end-note individuale che poi può essere immesso in un file complessivo. Nel testo li inseriremo con il formato corretto alla fine delle correzioni.

Figure captions:

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

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

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

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

Table captions

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

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

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

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

Table 4 (Characteristics of dairy waste effluents (si potrebbe togliere siero e latticello, il secondo non mi risulta ci sia, ed integrare quei dati nella tabella successiva di descrizione dei principali sottoprodotti di scarto, per l'appunto siero e latticello) +**Table 2 Typical composition of sweet and acid whey +**Table 3: Chemical characteristics of whey (CRPA, 2002+altre utilizzate per completare) **Sistema RAFFA**

Table 5 Typical operating conditions for anaerobic digesters + Table 4 Data on anaerobic treatment of cheese whey SISTEMA RAFFA

Table 6 legislazione ?? ripensare se utile una tabella