Chapter 11
Digester Slurry Management: The "One Health" Perspective

David Rodriguez-Lazaro, Aline Frumi Camargo, Thamarys Scapini, Charline Bonatto, Fernando Rosado Spilki, Maria Célia da Silva Lanna, Marta Hernández and Gislaine Fongaro

Abstract The increasing demand for food, energy and natural resources has stimulated the use of anaerobic biodigestion, aiming at the treatment of biomass derived from anthropic activities with potential for biogas production. Digestate is rich in nutrients for soil fertilization purposes, with a potential direct impact on the safety of human, animal and environmental health, within the "One Health" scope. "One Health" deals with the set of strategies applied to human and animal medicine, combined with the conservation of the environment. This chapter will address the management and recycling of digestate in agriculture, considering chemical and microbiological contaminants (pathogens) from an One Health approach.

D. Rodriguez-Lazaro (✉)
Microbiology Section, Department of Biotechnology and Food Science,
Universidad de Burgos, Burgos, Spain
e-mail: drlazaro@ubu.es

A. F. Camargo · T. Scapini · G. Fongaro
Laboratory of Microbiology and Bioprocess, Department of Environmental Science
and Technology, Federal University of Fronteira Sul, Erechim, Brazil

C. Bonatto
Postgraduate Program in Chemical Engineering, Federal University of Santa Catarina,
Florianópolis, Brazil

F. R. Spilki
Institute of Health Sciences, Feevale University, Novo Hamburgo, Brazil

M. C. da Silva Lanna
Laboratory of Microbiology and Technologic Bioprospection (LMBT),
Federal University of Ouro Preto, Minas Gerais, Brazil

M. Hernández
Instituto Tecnológico Agrario de Castilla y León, Valladolid, Spain

G. Fongaro
Laboratory of Applied Virology, Department of Microbiology, Immunology
and Parasitology (MIP), Federal University of Santa Catarina, Florianópolis, Brazil

G. Fongaro
Environmental Engineering, Universidade do Contestado, Concórdia, Santa Catarina, Brazil

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11.1 Digestate Use and Management

Anaerobic digestion produces, together with biogas, a residual material called digestate. The digestate presents a high amount of nutrients such as nitrogen (N), phosphorus (P) and potassium (K), as well as organic matter, which could be beneficial for agricultural purposes as biofertilizer (Barbanera et al. 2018). However, the digestate also presents a high moisture content and is not fully stabilized when leaving the digester, as well when applied without proper treatment into the ground, which can generate phytotoxic and odor concerns (Alburquerque et al. 2012; Arab and Mccartney 2017). For this reason, the digestate needs to be managed properly and receives specific treatment before its implementation on the ground, in order to avoid environmental problems and threats to public health (Alburquerque et al. 2012), due to the potential for emissions of ammonia and nitrate, leaching of heavy metals and the presence of pathogens (Barbanera et al. 2018).

Sanitary safety is a relevant factor that impacts on environmental, animal and human health, the three pillar of the concept "One Health"; the set of studies related to the area of human and animal medicine with the conservation and development of the environment. In this context, the concept of "Unique Health", with an innovative character, is defined as an addition of values and knowledge of human and animal health, to economize and improve environmental services, being possible through the joining of areas, professionals and institutions, according to the (WHO), Food and Agriculture Organization (FAO) and World Organization for Animal Health (OIE) (Nguyen-Viet et al. 2015).

Recycling has been the most widely used technique in the management of anaerobic digestion and its derivatives while adding value to the product and closing the cycle of matter. In Brazil, recycling is a technique which is the main priority of the National Politic of Solid Wastes (PNRS) to ensure the management of municipal solid residues (Brazil 2010). However, certain quality characteristics, stability, and hygiene must be met for the sustainable recycling of digestate in the environment (Alburquerque et al. 2012).

An option to improve the quality and stability of the solid fraction of digestate is through composting (Arab and Mccartney 2017). The composting process can be improved by direct microbial inoculation; the digestate can be applied as inoculant instead of acquiring or preparing commercial microbial cultures, being, therefore, more advantageous economically (Arab et al. 2017). The addition of digestate in windrow composting of organic municipal waste fresh and/or partially stabilized may increase the rate of reaction of the composting and decreases the time for the compound to achieve stability in 30–36% with addition of 20–40% of digestate (% ww) (Arab and Mccartney 2017). Both the composting and anaerobic digestion processes are mediated by a range of different microorganisms. Bacteria play an important role in the thermophilic and post-aeration phases and fungi are essential in the maturation phase. For this reason, the digestate should be added in the process of composting in adequate quantity, in order to ensure uniformity of microbial species. The use of 40% (wet weight basis) of digestate in the composting of municipal organic waste revealed that mixing between the two substrates (organic waste and digestate) led to a favorable condition for microbial species present (Arab et al. 2017).

Bustamante et al. (2012) studied composting by digestate (obtained from anaerobic digestion of cattle slurry and silage) and residues of grapevine pruning, as bulking agent. The results showed that the organic matter of digestate is mineralized, increasing electrical conductivity, as well as the humification index of germination during the composting, allowing the humification of organic matter in the absence of phytotoxins. The compounds reached appropriate degrees of stability and maturity, physical properties suitable for use as fertilizer for crops, and also the suppression of the phytopathogen *Fusarium oxysporum* f. sp. *melonis*. However, the salinity and the concentrations of Cu and Zn present in the composted material from digestate and various bulking agents (wheat straw, grapes, etc.), limited its application in agriculture (Bustamante et al. 2013). Similarly, the composting of solid digestate leads to the accumulation of nutrients (P, K, Mg and Ca) and heavy metals (Cd and Cr) due to the organic matter degradation during composting (Knoop et al. 2018). The digestate can replace the mineral fertilizer on the production of sida (*Sida hermaphrodita*—Malvaceae), maize (*Zea mays* L.—Poaceae) and alfalfa (*Medicago sativa* L.—Fabaceae), showing a positive effect of digestate in biomass production of plants (Barbosa et al. 2014), and the quantities of macro-element present on digestate are comparable to mineral fertilizer (Koszel and Lorencowicz 2015) and, therefore, it can be used as fertilizer for crops and food products. In addition, the use of digestate as biofertilizer in agriculture has been also evaluated by ecotoxicological tests, including direct (using plants and earthworms) and indirect tests (based on aquatic organisms and luminescent bacteria). Experiments with earthworms showed no serious negative effects for mixtures containing up to 15% (w/w referring to the dry matter) of digestate. Tests with plants did not show negative effect when lower concentrations than 20% (w/w) of digestate were applied. The indirect tests showed a LC_{50} value of 13.61% (v/v) for *Daphnia magna* and no toxicity to *Artemia* sp. and *Vibrio fischeri*. These results encourage the use of the digested as fertilizer in agriculture (Pivato et al. 2016).

However, the production of biofertilizers from digestate is hampered by legislative issues. In spite of derivatives of digestate present similar characteristics to the mineral fertilizers, the legislative framework has not encouraged the marketing of fertilizers of biological origin (Bolzonella et al. 2018). Therefore, few studies have evaluated other applications of digestate (Table 11.1).

From a bioengineering point of view, the algae and cyanobacteria could be integrated into a sewage treatment effluent, to treat both the effluent as digestate (solid and liquid), while producing products of industrial interest (Arias et al. 2017). Arias et al. (2017), for example, evaluated the use of a blend of urban and digestate

Table 11.1 Different applications of digestate presented in current literature

Origin of digestate	Fraction of the digestate utilized	Application	References
Mixture of cow manure (27% VS), cheese whey (15% VS), poultry manure (23% VS), olive pomace (2% VS) and corn silage (33% VS)	Solid	Production of an enzyme (exo- and endo-glucanase, xylanase, β-glycosidase, and laccase)	Musatti et al. (2017)
Food waste	Liquid	Production of biochar by pyrolysis	Opatokun et al. (2017)
Sewage sludge and source-segregated biodegradable waste	Liquid	Nitrogen removal of old landfill leachate	Peng et al. (2018)
Cattle slurry mixed with energy crops (maize silage and triticale silage)	Liquid	Biofertilizer	Riva et al. (2016)
Sargassum horueri	Solid	Phenol production	Wei et al. (2018)

secondary effluent as a source of nutrients to grow and select cyanobacteria from a joint consortium of microalgae (green algae of the genus *Chlorella* and *Stigeoclonium*) and cyanobacteria (cf. *Oscillatoria* sp., cf. *Aphanocapsa* sp. and *Chroococcus* sp.) on a photobioreactor. The authors reported removing an average of 96% of total ammoniacal nitrogen (TAN), 95% of dissolved reactive phosphorus $\left(\mathrm{P}-\mathrm{PO}_4^{3-}\right)$ and 91% of nitrate $\left(\mathrm{N}-\mathrm{NO}_3^-\right)$. In a similar study, *Chlorella vulgaris* was grown in liquid digestate diluted from anaerobic digestion of swine manure and maize, to reduce concentrations of nutrients and their toxicity. The results showed that a significant reduction of the toxicity (82, 88 and 100%) for the organisms tested (*R. subcapitata*, *L sativum* and *D. magna*, respectively), with a high removal efficiency (>90%) of ammonia, total nitrogen and phosphate (Franchino et al. 2016).

Barbanera et al. (2018) studied the production of bio-oil from digestate by microwave-assisted liquefaction held in polyethylene glycol (PEG) and glycerol, using sulfuric acid as a catalyst. Bio-oil yield of 59.38%, with a heating value of 28.48 MJ/kg, was obtained in optimum conditions. This result indicates the possibility of the use of digestate for production of biofuels through a process that is economically viable, whose operational time is reduced due to heating by microwave. The production of pellets and briquettes from digested pulp solid fraction (DSF) is also possible and economically feasible. The costs of production of briquettes and pellets with DSF are approximately four times smaller than the production on sawdust and the calorific power is similar (8.3–16.7 MJ/kg, depending on the moisture content) (Czekala et al. 2018). In addition, the pelleting is an

Table 11.2 Effect of stripping, drying, membranes, and vacuum evaporation in the treatment of digestate

Treatment	Effect	References
Vacuum evaporation	Condensed water can be applied as dilution water for digestion, for irrigation of crops or cleaning of floors. It can also be released into surface waters, after the adequacy to the regulations of patterns of release	Chiumenti et al. (2013)
Drying	Removes the water from the digestate through the grille. It is necessary amount of energy to total removal of water from the digestate, which corresponds to 90% of the earth. Ammonia nitrogen can be removed with steam bath or kept in the digestate if it is acidified through the addition of mineral acids	Bolzonella et al. (2018)
Stripping	Ammonia (NH_3) is stripped and physically transferred from the aqueous to the gas phase	Limoli et al. (2016)
Membranes	Liquid phase of digestate is treated in ultrafiltration (UF) and reverse osmosis (RO) systems. The concentrate is rich in both macro and micronutrients. There is reduction of initial digestate volume	Bolzonella et al. (2018) Ledda et al. (2013)

effective method to eliminate the presence of *Clostridium* spp. of digestate of milk production (Pulvirenti et al. 2015). The heat treatment can also eliminate the *Escherichia coli* present in the digestate (Solé-bundó et al. 2017).

Other studies have focused their attention for nutrient recovery of digestate through treatment technologies as the stripping, drying, membranes (Bolzonella et al. 2018) and vacuum evaporation (Chiumenti et al. 2013). The characteristics of these techniques are summarized in Table 11.2.

11.2 Unwanted Impurities and Pathogens in Digestate

The use of digestate as fertilizer is an efficient way to recycle materials and reduce the use of mineral fertilizers (Yang et al. 2017). Several raw materials are used for anaerobic digestion resulting in digestate such as animal waste, lignocellulosic waste, human waste and food waste (Al Seadi et al. 2013). The limitations of the use of the digestate are dependent on the origin and the way in which the raw material is collected, making it fundamental so that no harmful effects to the environment arise due to the quality of the material, such as pH, high organic matter content and non-material biodegradable substances such as heavy metals and antibiotics (Al Seadi et al. 2013; Yang et al. 2017). In addition, the digestate must have high quality for application as fertilizer, and therefore, the pathogens, chemical and physical impurities and pollutants must be controlled (Al Seadi et al. 2013).

11.2.1 Impurities

The addition of trace nutrients, such as iron, copper, zinc, and nickel, in anaerobic digesters is essential for the synthesis of essential coenzymes in methanogenic pathways to increase the efficiency of the anaerobic digestion of food residues. They are also added in low concentrations in the animal rations in order to increase the productivity, being frequently found in the manure (Zhang et al. 2015; Yan et al. 2018). However, when the concentrations of these compounds exceed, inducing overdoses in the digesters, can cause toxic effects on the microorganisms of the digestion process, resulting in loss of microbial resources, impairing the quality of the final digestate, increasing the difficulty of the process, and increasing the concentration of these metals in the digestate that impair its use as biofertilizer when disposed in environment (Ortner et al. 2014; Zhang et al. 2015). Bioaccumulate potential in the digestate is related to non-biodegradability of the metals, and can be found in the solid and liquid fractions, in reducible and oxidizable forms (Yan et al. 2018).

The supplementation of anaerobic digesters with small doses of heavy metals to increase the biogas production and quality is still a major challenge, facing contradictions between the increase of the economic yield and the great risk of environmental impacts due to the high load of these compounds that have carcinogenic characteristics and even in low concentrations can cause serious damage to animal health and environment (Zhang et al. 2015). Excessive levels of heavy metals (Cu, Zn, Mn, As, Cd and Pb) in the digestate have been reported in the solid and liquid fractions of a digestate that had the substrate of anaerobic digestion of pig manure (Li et al. 2018). It should be noted that the analyzes of the study in question were carried out during the stabilization period of the digestate, and the Cu, Zn, As and Pb concentrations showed a significant increase in concentrations during the period, which may have occurred due to the reduction of the volume of the digestate due to the loss of water by evaporation during storage, which caused the highest concentration of the metals in the volume of digestate. This fact is of extreme importance for the analysis of the digestate as biofertilizer, since the reduction of the amount of water in the medium concentrates the nutrients and impurities, bringing greater risks if disposed of in the environment.

The presence of antimicrobials and hormones in the digestate is linked to the therapeutic use in livestock (Bloem et al. 2017; Kemper 2008). Antibiotics act selectively against microorganisms, and when these compounds are found in the environment, the environmental microbiota can be affected, losing their activity due to low or no resistance to this type of substances (Bloem et al. 2017; Insam et al. 2015). Approximately 200,000 tons of antibiotics are used globally, only in the livestock sector, number that tends to increase (Bloem et al. 2017; Hirsch et al. 1999; Kummerer 2009). The inappropriate and excessive use of antimicrobials can cause to remain in the digestate even after the digestion process, contributing to the appearance of antimicrobial resistant bacteria (ARB). In addition, using the digestate as a fertilizer, another serious environmental problem can happen (Bloem et al. 2017;

Kemper 2008; You and Silbergeld 2014); the negative effect on the soil functions and organisms (Jechalke et al. 2014), and since the plants haven capacity to absorb these compounds (Bloem et al. 2017; Chowdhury et al. 2016; Wang et al. 2016), these compounds can be detected in the food chain (Bloem et al. 2017). Only a few studies have analyzed the elimination of antimicrobial compounds during the digestion process (Arikan et al. 2006; Cheng et al. 2018b; Ratsak et al. 2013; Spielmeyer et al. 2014), and even low concentrations are transported to the environment, which causes concern, since these antibiotics are not diluted and have low leaching capacity (Bloem et al. 2017; Cheng et al. 2018a, b).

The residual hormones in the digestate act very similar to antimicrobials and represent a significant source of pollution (Cheng et al. 2018b; Ebele et al. 2017; Speltini et al. 2011). Toxicological analysis on materials containing residues of these substances have demonstrated a risk to human health and the environment, due to a number of factors, including: endocrine disruption in the environment microbiota (Adeel et al. 2017; Ronquillo and Hernandez 2017), effects on the growth, reproduction and behavior of several species, such as fish, plants and bacteria, even in low concentrations (De Cazes et al. 2014) and even has been associated to breast and prostate cancer (Adeel et al. 2017).

The levels of ammonia present in the digestate are also essential for the possibility of subsequent application. When used as fertilizer, the greater nutrient availability is a key factor in improving soil quality (Nkoa 2013). However, when the digestate, also contains impurities, such as heavy metals, and particularly antimicrobials and hormones, the use of ammonia no longer exerts its nutritional function properly, since the synthesis of ammonia in the soil is carried out by a specific group of microorganisms sensitive to the antimicrobials and hormones (Odlare and Pell 2009; Pell et al. 1998; Risberg et al. 2017), resulting in losses of nitrogen through the volatilization of ammonia and nitrate leaching (Al Seadi et al. 2013).

In this scenario, the incorrect management of the digestate can cause serious environmental and human health problems, particularly when it also included impurities such as heavy metals, antimicrobials, hormones, among others. Researchers are looking for alternatives to remove these compounds from the final effluent of digestion process, and some current technologies including advanced oxidation, ultraviolet light and ozone, demonstrated effectiveness for the removal of antibiotics present in the digestate from swine manure (Ben et al. 2009, 2011; Qiang et al. 2006). Despite the relevance of these studies, the removal techniques are of high energy cost, besides generating secondary byproducts with polluting potential (Cheng et al. 2018b; Liu et al. 2009).

11.2.2 Pathogens

Among the potential pathogens present in digesters, enteric pathogens are the most abundant. Bacteria, as *Salmonella* and diarrheagenic types of *Escherichia coli*, *Vibrio* and *Campylobacter* are studied due to infectious potential by contaminated water and food. *E. coli*, a biomarker model of global fecal contamination, includes commensal and interactive types to the intestinal microbiota of man and animals; however, some varieties can also contain virulence determinants. Those include diarrheagenic *E. coli* such as Enteropathogenic (EPEC), Enterotoxigenic (ETEC) Enteroinvasive (EIEC), Enterohemorrhagic (EHEC) and Enteroaggregative *E. coli* (EAEC) (Al-Badaii and Shuhaimi-Othman 2015). Similarly, some protozoa can be associated, particularly those that they are waterborne pathogens, such as *Cryptosporidium* spp., *Giardia* spp. and *Ascaris* spp. They are the most resistant in the environment, against the processes of treatment and disinfection of matrices, like water, sewage, and effluent. (Centers for Disease Control and Prevention 2013; Leal et al. 2013).

Enteric viruses can be found in high concentrations in digestate from anaerobic treatment. These viruses are resistant to extreme pH, high temperatures, salinity, and natural ultraviolet (UV) radiation. They also have a rapid adsorption capacity on the solid particles dispersed in the environment, favoring their stability. Among other viral pathogens that could be present in slurry, hepatitis E virus (HEV) and rotaviruses (RVs) are remarkable due to their zoonotic potential (Delahoy et al. 2018). Hepatitis E is an acute and self-limiting viral disease with a mortality rate of less than 1%. However, in pregnant women and immunocompromised individuals, this disease may become chronic and may progress to cirrhosis of the liver, with mortality rates reported up to 25% (Meng 2010). The etiological agent belongs to the family *Hepeviridae*, genus *Orthohepevirus* and is responsible for causing outbreaks mainly in emerging countries due to poor sanitary conditions. It has recently been discovered that some genotypes of the virus are zoonotic (Park et al. 2016). Studies in industrialized countries showed a high prevalence of seropositive individuals, and sporadic cases of hepatitis E in these places were related to the consumption of game meat and mainly to pork products (viscera—mainly liver, other derivatives). The contact of humans with pigs carrying the virus is also related to a higher seroprevalence, having an impact on public health, since the pigs act as asymptomatic reservoirs. HEV Genotype 3 is often reported as a cause of hepatic illness in humans in Americas and is ubiquitous in swine populations and was reported both in swine slurry and pork byproducts (Heldt et al. 2016). RVs are members of the *Reoviridae* family (Suzuki and Hasebe 2017). Although there are vaccines to prevent the infection in humans, RV is still among the most important etiological causes of diarrhea worldwide, and the infection by new zoonotic types may not be avoided by the current immunogens (Cuffie et al. 2016). The generation of new RV types is common due to the possibility of mutation and reassortment of the 11 segments of double-stranded RNA, which makes these viruses highly variable. Animal RVs are a public health concern due to their potential for genetic exchange with human RVs and the consequent generation of viruses with enhanced zoonotic potential. Since co-infections by different animal and human RV types are a prerequisite for reassortment events, the proper management of slurry to avoid new human infections is mandatory (Delahoy et al. 2018).

It is also noteworthy in the "One Health" context that the evolution of zoonoses is highly due to antimicrobial resistance, becoming a global problem. Antimicrobials are widely used in animal farms to prevent infections and also as animal growth promoters (FAO 2015; CDC 2013). Resistance to antimicrobial drugs is characterized by the ability of microorganisms to resist the effects of a chemotherapeutic agent which it is normally susceptible to. The transmission of the antimicrobial resistance can be increased by the selective pressure due to the presence of antimicrobials in the environment, which enhances the magnitude and spread of the resistance (Haese and Silva 2004). Both antimicrobials and enteric pathogens are present in the animal and human digestates and can disseminate resistant microorganisms, as well as select antimicrobial resistance genes.

11.2.2.1 Control of Zoonotic and Resistant Pathogens

The incorrect management of animal waste can be a serious issue on human health by facilitation of the transmission of zoonotic diseases, with serious economic (losses in animal production) and environmental impacts (contamination of facilities and final products). Other environmental side effects are related to the infiltration and contamination of water and groundwater, the unpleasant odor, the potential damage to the autochthonous fauna and flora (Manyi-Loh et al. 2016). Proper management of livestock and derived slurry, the supply of adequate access to clean water and feed consumption, as well as the temperature and ventilation control systems are necessary parts of an integrated control plan to avoid the spread of zoonotic pathogens in farms (Hodgson et al. 2016). Farm sanitation and strict biosecurity measures are also needed to reduce the spreads of pathogens in animals' excreta (Staggemeier et al. 2015). Other measures like avoidance of runoff from animal housing and storage facilities are also relevant part of the process (Manyi-Loh et al. 2016).

Human and animal pathogens are usually inactivated over time due to a combination of factors such as pH, temperature, humidity, carbon content, nutrient availability, microbial antagonistic behavior, among others (Semenov et al. 2007). The natural inactivation rate is usually slow and unreliable, since the different factors inherent to environmental changes, such as seasonal ones, are not controlled. For these reasons, the storage and the treatment of human and animal excreta must be effectively carried out, since it is possible to quantify the inactivation factors as well as to control these factors (Sidhu et al. 2001). Among the classically recognized factors with potential for inactivation of enteric pathogens such as temperature, solar radiation (UV), pH variation, turbidity, organic composition of the matrix, presence of predatory microorganisms, aggregation between the microorganisms themselves or with particles, the temperature is considered the most important factor (Bertrand et al. 2012).

Functional procedures for the removal of antibiotics from digestate have been studied. Among the physical and chemical methodologies used for this purpose are chemical oxidation and biodegradation (destructive methods), adsorption and membrane techniques (nondestructive processes). The adsorption of the adsorbent on the surface of the solid (adsorbent) (Sawyer et al. 2002) is considered a potential method in the removal of different classes of antibiotics. For this purpose, aluminum oxide can be used to adsorb amoxicillin (Putra et al. 2009) or tetracycline (Chen and Huang 2010).

11.3 Final Considerations

The global demand for food as well as soil infertility and water contamination have stimulated studies aimed at the reuse of effluents, as digestate, for biofertilization purposes. However, many challenges are encountered in the safe management of this digestate, being the sanitary and agronomic aspects very relevant. It is necessary to develop strategies applied to the actual productive conditions, aiming at obtaining valued and sanitary products safe from a "One Health" perspective. To establish a global safety standard on "One Health" context, studies involving chemical and microbiological risk analysis are required, considering different exposure situations and implications for human and animal health. From the determination of contamination limits, effective and economically feasible strategies for inactivation of infectious agents that can trigger disease should be established.

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