

Chapter 12

Closing the Loop on Biogas Plants: Recycling Digestate and Sludge on Agriculture and Microbial Risk Assessment



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Abstract Management of human and animal wastes is among the major constraints towards the sustainable development of human settlements, where we demand increasing amounts of clean water, food, and energy. The aim of most sanitation solutions is to keep waste away from the generation site, such as households or animal stalls. The misconception that wastes have no useful purpose has resulted in unsustainable systems. However, the recovery of energy and agricultural use of the organics and nutrients contained in excreta and solid waste can improve soil structure and fertility, increasing productivity, reducing the dependency of resource-demanding chemical fertilizers, and thus contributing to food security. Treatment plants for waste anaerobic biodigestion can be applied in that context, moving from “treatment” plants to become “resource recovery” plants. The recovery of biogas in those plants for energy production is highly valuable, and added value can be obtained by the recycling of the biodegradation products—accumulated sludge and digestate. Those fractions should be treated sufficiently to inactivate pathogens to a certain extent. The quantitative microbial risk assessment is an effective approach to estimate risks, which can be applied to any scenarios of recycling liquid fractions from biogas reactors in agriculture.

Keywords Waste · Risk assessment · Nutrient recycling · Pathogens · Biogas · Sludge · Digestate · QMRA

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12.1 Introduction—Risks and the Resource Recovery Concept

Management of human and animal wastes is nowadays among the major constraints towards the sustainable development of human settlements, where we demand increasing amounts of clean water, food, and energy. Water pollution is caused largely by inappropriate discharge of human and animal wastes into water bodies. It is well known that faecal sludge, either from human or animal systems, has considerably high pollution potential, and its mismanagement is a common reality in many regions, especially in low- and mid-income countries.

The aim of most sanitation solutions is to keep waste away from the generation site, such as households or animals stalls. The misconception that wastes have no useful purpose has resulted in unsustainable systems. However, the recovery of energy and agricultural use of the organics and nutrients contained in excreta can improve soil structure and fertility, increasing productivity, reducing the dependency of resource-demanding chemical fertilizers, and thus contributing to food security. These benefits can be obtained since excreta is treated sufficiently to inactivate pathogens and make it safe.

Innovations on resource recovery are urging, with special focus on integrated waste management, responding to the need of development of sustainable resilient energy and sanitation systems for areas where poor infrastructure, water scarcity, and limited energy supply restrain the capacity for economic growth. These initiatives would contribute to long-term sustainability of cities in both its urban and rural areas and climate-compatible activities for the development.

Treatment plants for waste anaerobic biodigestion can be applied in that context, moving from “treatment” plants to become “resource recovery” plants. The recovery of biogas in those plants for energy production is highly valuable, and even added value can be obtained by the organics and nutrient recycling of other degradation products of the process that consist of the accumulated sludge and the digestate outcomes from the bioreactors.

But what is the risk associated? That is always a raised question when talking about recycling of human- and animal-derived wastes, since they have a high concentration and a very diverse pathogen content of importance for public health.

Microbial risk associated with the biogas fraction is very low when compared with the liquid fractions of sludge and digestate since pathogens stay in those fractions. The objective of this chapter is to present the quantitative microbial risk assessment as an effective approach to estimate risks, which can be applied to any scenarios of recycling liquid fractions from biogas reactors, operating with human and/or animal manure in agriculture.

12.2 Risk Analysis General Concepts

Risk analysis is an effective tool used by most diverse fields including economy, business, engineering, environmental, and human health. This tool works as a systematic and preventive approach through which is possible to minimize, control, and avoid risks, as well as to aid decision-making (Haas et al. 2014).

The process of risk analysis includes risk assessment, risk management, and risk communication. Risk assessment can be qualitative or quantitative and is intended to characterize and estimate all the potential risks involved during a process. Quantitative risk assessment associates numeric values to the risk and through probabilistic calculus provides an overview of the risks (WHO 2016).

12.2.1 *Quantitative Microbial Risk Assessment (QMRA)*

Quantitative microbial risk assessment consists of the application of risk assessment principles with emphasis on microbial risk and the aim to estimate health effects associated with exposure of an individual to a pathogenic microorganism in different scenarios (Haas et al. 2014). Through the use of systematic information applied to a mathematical model, QMRA enables a preventive management of microbiological risk contamination.

QMRA methodology is supported by the World Health Organization (WHO), which describes the process and steps in “Quantitative Microbial Risk Assessment: Application for Water Safety Management”, published in 2016. The methodology is divided into four steps—hazard identification, dose–response model, exposure assessment, and risk characterization—which will be presented below.

(1) Hazard identification

Hazard identification is the first step of QMRA and has the main purpose to identify the microbial agent, all diseases associated with this pathogen, and the spectrum of human illnesses.

For this purpose, epidemiological and microbiological studies need to be accessed in order to obtain all necessary information about the microbial agent—pathogenicity, virulence, and infectivity—and about the human response to the microorganism (Haas et al. 2014).

(2) Dose–response model

According to Weir et al. (2017), the choice of an adequate dose–response model is essential for a successful analysis. The dose–response model describes the relationship between the level of microbial exposure and the probability of this exposure to affect human health.

The dose–response models are expressed by mathematical functions and are based on experimental data. Until now, some dose–response models were

developed and each of them has a parameter which best describes the microbial agent concerned (Haas et al. 2014).

The following describes the two most useful models: the exponential dose–response model and the beta-Poisson dose–response model.

Exponential dose–response model

The exponential dose–response model is characterized by the assumption that each microorganism has an independent and constant probability to survive (r) and consequently to cause an infection in a host. In other words, this model does not take into consideration some variabilities that could interfere in pathogen behaviour. Equation (12.1) describes this model.

$$Pd = 1 - \exp -r \times d \quad (12.1)$$

- P(d) risk of infection;
 d dose ingested by the individual;
 r specific parameter of each microorganism, which represent the probability to survive and infect the host.

Beta-Poisson dose–response model

The beta-Poisson model differs from the exponential model as it assumes that some variations in pathogen–host probability to survive may occur. According to Haas et al. (2014), this variation may appear due to host characteristics, such as gender, age, or immunity or due to the diversity of pathogen ability.

In mathematical terms, the parameter “ r ” of Eq. (12.1) is no longer constant and varies according to a probability distribution represented by the parameters α e β . Given this, the risk of infection is calculated using Eq. (12.2).

$$Pd = 1 - 1 + d - \alpha \quad (12.2)$$

- P(d) risk of infection;
 d dose ingested by an individual;
 α e β parameters that express the survival probability distribution of each microorganism.

(3) Exposure assessment

Exposure assessment is intended to determine the population exposed to the risk (adults, children or immunocompromised people), the routes of transmission (air, soil, water, ingestion, inhalation, contact), the exposure scenario, and the distribution of the microorganism concentration (WHO 2016; Haas et al. 2014).

In order to estimate the dose ingested by an individual, the distribution of the microorganism concentration must be known. Ideally, these values should be

determined through laboratory analysis for each case; however, the process of quantification for most pathogens can be very challenging and expensive. Therefore, in these cases the use of a comprehensive database with the most similarity as possible is recommended (Haas et al. 2014). The dose ingested by an individual is given by Eq. (12.3).

$$\text{Dose} = v \times c \quad (12.3)$$

- d* dose ingested by an individual;
- v* volume ingested per exposure;
- c* microorganism concentration.

The determination of exposure scenario enables the identification of the contact level between the individual and the microbial agent, as well as the exposure frequency. These parameters have a high influence on the risk of infection. Moreover, once established the exposure scenario it is possible to know the average ingested volume in that case. The values of volume ingested can be found in the literature, which is normally based on experimental volunteer studies (WHO 2016).

(4) Risk characterization

The risk characterization is the last step of the QMRA and consists in the integration of all data obtained on the previous steps, with the intent to estimate the probability of the risk to occur as well as its magnitude. According to Haas et al. (2014), during this characterization all the variabilities and uncertainties are taken into consideration. The uncertainties can be related to the dose–response model chosen, model parameters, ingested volumes, and microorganisms concentrations.

Usually, to conduct the characterization the Monte Carlo simulation is used—a mathematical tool, which simulates various scenarios using probability distribution and as a result shows all possible scenarios and the probability of people to get infected when exposed to pathogens present in the scenarios. The results are expressed through probability of infection, disease or death, or through disability-adjusted life years (DALY), a measure that expresses all wasted years as a consequence of health problems due to microbiological contamination (Haas et al. 2014).

12.3 Nutrients in Human and Animal Manure and Microbial Pathogen Content for Risk Assessment

In general, human and animal excreta present a large variety of primary and secondary macronutrients, which would characterize them as compound biofertilizers. Table 12.1 presents a complete characterization of excreta regarding macro- and micronutrients content.

The biofertilizers that could be produced as digestate and sludge in biogas plants, considering the percentage of macro- and micronutrients in human and animal

Table 12.1 Characterization of human and animal excreta regarding macro- and micronutrients, presented as fresh and dried human faeces, stored urine, faecal sludge from wastewater treatment plant, and swine manure digestate and sludge from anaerobic biodigesters for biogas production

Characteristics	Human faeces ¹ mg kg ⁻¹ (% db) ^a		Human urine ² mg L ⁻¹ (% db) ^a		Faecal sludge ³ (%) db) ^a	Swine manure digestate ⁴ (% db) ^a	Swine manure sludge ⁴ (% db) ^a
Organic carbon	46,620	(45) ^b	2448	(2.5)	16.7	0.14	4.32
Sulphur	2.9	0.46	510	0.5	0.23	0.02	0.6
Phosphorus (P ₂ O ₅)	14,490	2.3	530	0.5	1.25	0.012	0.06
Total nitrogen	5040	5 ^b	6834	6.8	1.83	0.24	0.28
Potassium (K ₂ O)	10,206	1.6	1824	1.8	0.02	0.05	0.09
Boron	<63	<0.01	Nd	Nd	0.01	<0.0001	<0.004
Cobalt	4.4	0.0007	<0.1	<0.0001	0.0007	–	–
Cooper	38	0.006	0.03	0.00003	0.02	<0.0001	–
Iron	1455	0.23	0.09	0.0001	1.43	0.01	0.06
Magnesium	10,275	1.6	52	0.052	0.15	0.05	1
Manganese	3213	0.51	<0.01	<0.00001	0.01	0.005	0.005
Molybdenum	<6.3	<0.001	<0.1	<0.0001	<0.0001	<0.0001	<0.0001
Nickel	2.5	0.0004	<0.1	<0.0001	21.6	<0.0001	–
Zinc	214	0.034	0.34	0.00034	0.07	0.007	0.007

^aCalculated on a dry basis (db)

^bOrganic carbon and total nitrogen measured from fresh human faeces (Magri 2013)

¹Human faeces collected from urine-diverting dry toilet (Magri 2013)

²Human urine collected from urine-diverting dry toilet (Magri 2013)

³Dry faecal sludge from anaerobic wastewater treatment plant (Kafer 2015)

⁴Digestate and sludge collected from anaerobic digester treating swine manure (Fongaro 2016)

excreta, can be compared to organic fertilizers, but are less effective in terms of concentrations when compared to chemical fertilizers.

At the same time that human and animal manure have high concentrations of carbon and nutrients, the concentrations of metals are small, which is an advantage for the reuse of excreta (Vinnerås 2002; Albiñ and Vinnerås 2007).

While manure application in agriculture has its benefits, its management, especially its storage, represents a main limiting factor, given that long storage periods can lead to nutrients and carbon losses. Castellanos-Navarrete et al. (2015) conducted a study in western Kenya regarding manure utilization in farms and indicated that poor manure management led to low nutrient cycle efficiencies, indicating that long periods of storage contribute to nitrogen and phosphorus losses by volatilization and leaching. The authors also noticed carbon losses up to 51% in

the farms monitored, which agrees with previous researches that indicate that poor storage conditions can lead to losses between 30 and 55% of carbon. However, half of the farms counteracted the carbon losses through biomass additions.

Nutrient losses by leaching can lead to a series of environmental impacts, especially on water bodies. One way of reducing this kind of impact is to apply the Ecological Recycling Agriculture (ERA), which is an organic agriculture that aims at closed nutrients cycles (Granstedt et al. 2008), and has potential to decrease nitrogen surplus in agricultural soils and its leaching due to increased nutrient efficiency (Granlund et al. 2015). Granlund et al. (2015) carried a study regarding Finnish agriculture catchments and simulated a theoretical crop rotation developed to represent ERA cultivation, with considered N fixation, mineralization, and manure as nitrogen sources. The authors observed reductions up to 33% in nitrogen losses on fields working in ERA when compared to those from conventional agriculture, which is mostly based on chemical fertilizers. Manure utilization in agriculture can also increase soil organic carbon, improve soil physical and biological properties, and lead to reduced carbon losses, especially when farmyard manure is applied (Baldivieso-Freitas et al. 2018).

The study carried by Castellanos-Navarrete et al. (2015) shows the importance of manure application in agriculture, once 43% of the total nitrogen inputs into the maize fields studied came from storage manure. Yet these amounts were insufficient to prevent major nutrient depletion in most farms analysed, it was still a considerable contribution to soil quality, especially in situations similar to those observed in the study, where at many times farmers lack resources to work and struggle to overcome difficulties. Thereby, they observed that composted manure provided the largest N inputs to the soil and similar amounts of carbon to those coming from crop residues left in the fields.

Although manure application has its advantages, Baldivieso-Freitas et al. (2018) indicate that research should consider studies about the effects of applying more stabilized organic matter, as it may be better to enhance soil quality and increase the organic matter contents in the soil. Given that, anaerobic digestion is a good way of treating animal manures in order to produce biogas, once they provide the adequate organic substrate. In addition, it is possible to mix horticultural fruit wastes with the manure, once they cannot be processed alone. Iocoli et al. (2019) carried a study in which different animal manures and onion waste were treated by anaerobic digestion, and each manure and its products of digestion and co-digestion had their fertilizing properties evaluated. The authors could notice large differences in the composition between the unprocessed wastes while the digestates had similar characteristics, which complies with the capacity of anaerobic digestion to generate more uniform products.

In this sense, it is possible to infer that the digestate is a more uniform fertilizer than the unprocessed manures. However, a high hydraulic retention time must be applied in the biodigester, in order to promote full degradation of the organic matter, as lower HRT may lead to the production of an unstable digestate, which can cause unpleasant smells, storage problems, and negative impacts on crops (Iocoli et al. 2019).

In addition to the above characteristics, excreta may contain pathogenic microorganisms and micropollutants (i.e. drugs residues and hormones). The highest concentration of pathogens is found in the faeces fractions, while the highest concentration of micropollutants in the urine fraction of excreta.

Inactivation of pathogens is recognized as a limiting factor for the reuse at any scale. Similarly, the risk posed by micropollutants in the environment, especially in the aquatic environment, is recognized as a serious environmental problem, although its consequences are still poorly understood (Carrington et al. 1991; Winker 2010; Fatta-Kassinos et al. 2011). However, from another perspective the presence of micropollutants in low concentrations is not considered a limiting factor for recycling, since it is believed that the excreta application to the soil ends up promoting an additional barrier against its direct release in the water bodies.

Enteric pathogens are excreted mainly on faeces of infected organisms, which can be human and/or animal. Some pathogens of relevance to public health are presented in Table 12.2, as well as its reservoir (e.g. human or animal faeces), the diseases each pathogen develops on human, the concentrations that are excreted per gram of faeces, the duration of the shedding of the pathogens on faeces, and the infectious dose for the disease to develop in another person. All of the presented pathogens are zoonotic, i.e. pathogens that are able to infect both animals and humans. The knowledge and collection of data on pathogen content in the biofertilizers produced from excreta are an essential step for conducting the risk analysis.

Disease propagation depends on factors such as microorganisms' survival in the environment and the required infectious dose to infect a susceptible individual. Pathogens' survival in the environment varies according to each group and species: virus, for example, are not able to multiply outside a host; however, they are able to maintain at a stable concentration or decrease over time. Protozoa are also unable to multiply in the environment, but they are highly resistant, even to most disinfectants. As for bacteria, some groups are able to multiply in the environment, while others are able to persist or decrease, depending on factors like nutrient availability and temperature (Leclerc et al. 2008). Pathogens' infectious dose represents the amount of organisms necessary to cause an infection; i.e. the lower the infectious dose, less microorganisms are necessary to cause an infection (Griffin and Tauxe 1991).

The amount of microorganisms excreted from an infected organism, as well as the duration of shedding, varies depending on the pathogen and the host. However, for most pathogens presented in Table 12.2, the amount excreted in faeces is considerably high, what changes for each pathogen is the duration of shedding, which varies from days to weeks. Furthermore, the infectious dose is lower for some pathogens, such as *Escherichia coli*, *Shigella spp.*, Rotavirus, and *Giardia intestinalis*, while higher for others. This means lower doses of these microorganisms are needed to develop an infection. These pathogens should get extra attention when conducting QMRA studies on reuse of human and animal excreta for agriculture. It is also important to evaluate zoonotic pathogens, as they are of greater importance to public and animal health.

Table 12.2 Pathogenic microorganisms present on faeces and its characteristics, sources, diseases, and infectious dose

Microorganism	Reservoir and source	Disease	Zoonotic disease	Concentration excreted or detected	Duration of shedding	Infectious dose	Reference
<i>Escherichia coli</i> O157	Human and animal faeces	Enteritis	Yes	Human: 10^8 bacteria/g faeces Calves: $10-10^7$ c.f. u./g faeces	7-13 days	<700	Ashbolt (2004), Ottosson (2003), de Roda Husman and Schets (2010), Coia (1998), Besser et al. (2001), Tuttle et al. (1999)
<i>Salmonella</i> spp.	Human and animal faeces	Salmonellosis	Yes	Human: 10^4-10^{10} bacteria/g faeces	2-7 days	10^3	Ashbolt (2004), Ottosson (2003), Blaser and Newman (1982), Haas et al. (2014)
<i>Shigella</i> spp.	Human and animal faeces	Shigellosis	Yes	Human: 10^6 bacteria/g faeces	30 days	10-100	Ashbolt (2004), Ottosson (2003), de Roda Husman and Schets (2010), Levine et al. (1973)
<i>Vibrio cholerae</i>	Human faeces and freshwater zooplankton	Cholera	Yes	Human: 10^6-10^9 bacteria/mL of rice-water stool; 10^2-10^5 bacteria/g faeces	3-5 days	Diarrhoea: 10^8-10^9 , Cholera diarrhoea: 10^{11}	Ashbolt (2004), Ottosson (2003), de Roda Husman and Schets (2010), Smith et al. (1961), Hornick et al. (1971), Dizon et al. (1967)
Hepatitis E virus	Human and animal faeces	Infectious hepatitis; miscarriage, and death	Yes	Pigs: 2.7- 6.2×10^6 genomic copies/g faeces	30 days	$10^{4.5}$	Ashbolt (2004), Ottosson (2003), Tsarev et al. (1992), Kasornkorkbua et al. (2004), Meng et al. (1998)
Rotavirus	Human and animal faeces	Enteritis	Yes	Human: 10^{10} infectious particles/g faeces Swine digestate: 7-10 GC/L	4-6 days	10-100	Ashbolt (2004), Ottosson (2003), de Roda Husman and Schets (2010), Fongaro et al. (2014)

(continued)

Table 12.2 (continued)

Microorganism	Reservoir and source	Disease	Zoonotic disease	Concentration excreted or detected	Duration of shedding	Infectious dose	Reference
<i>Cryptosporidium parvum</i>	Water, human, and other mammal faeces	Cryptosporidiosis	Yes	Human: 10^6 – 10^7 oocysts/g faeces	1–2 weeks	8–1042	Ashbolt (2004), Ottosson (2003), de Roda Husman and Schets (2010)
<i>Giardia intestinalis</i>	Water and animal faeces	Giardiasis	Yes	Cattle: $3 \cdot 10^3$ cysts/g faeces	Highest intensities 2–6 weeks (age of lambs)	10–100	Ashbolt (2004), Ottosson (2003), de Roda Husman and Schets (2010)
<i>Ascaris lumbricoides</i>	Human and pig faeces	Ascariasis	Yes	Human: 10^4 – 10^5 /g faeces	–	–	Ashbolt (2004), Ottosson (2003), Haas et al. (2014)

12.4 Inactivation of Pathogens During the Biogas Production—Anaerobic Bioreactors

The natural inactivation of pathogens is usually a slow process, and because of that, it is necessary to apply treatments that seek the disinfection in effluents and other wastes, such as human faeces, animal manures, and digestate from anaerobic biodigesters (Semenov et al. 2007; Sidhu 2001).

During the process of anaerobic biodigestion, 90% of the enteric bacteria and viruses can be inactivated (Fongaro et al. 2014). However, it is important to emphasize the importance of applying high-efficiency treatments, in order to minimize 99.9% of most pathogens, depending on the initial pathogenic load and the purpose of the recycling, keeping in mind the safety when recycling the digestate.

Methods based on alkalinity are among the main treatments used to reduce pathogens in environmental matrices, which consist of adding alkaline compounds such as ash and lime in the waste. The efficiency of this process is mainly due to the elevation of the pH that alkalinizes the cellular cytosol, as well as interferes with protein activity, inactivating microorganisms (Magri et al. 2013; Chandran et al. 2009).

As an effect of alkali treatment, non-ionized ammonia— NH_3 —is generated, which is an important biocidal agent. Therefore, wastes with high ammonia concentrations, as sludges and digestates from human and animal manure biodegradation, have good potential for ammonia sanitization. It is observed that this treatment needs to be performed outside the biogas reactor. The mechanism of pathogen inactivation mediated by NH_3 is due to the solubility of ammonium in the lipids, which facilitates its entry and diffusion in the cells, being able to act in cell destabilization, membrane destruction, and protein denaturation (Emmoth et al. 2011; Bujozek 2001). There is little research about the virucidal mechanism of NH_3 . However, it is reported that it leads to a cleavage in viral genetic material and small viral structural changes. Thus, viruses can be prevented from entering the host cell as well as from replicating (Decrey et al. 2015). In addition to NH_3 , the carbonates (CO_3^{2-}) formed as a consequence of pH and chemical equilibrium, probably due to organic matter decomposition, are also reported by their biocidal action (Magri 2013; Chandran et al. 2009).

As an example, it is possible to mention Fongaro et al. (2014) that studied pathogen inactivation based on free ammonia in swine digestate in Brazil, reaching inactivation in the order of 7 log 10 and 4 log 10 in 23 days of treatment for inactivation of enterobacteria and enteric viruses, respectively.

The use of heat in hygienic or disinfection processes is widely used, once it leads to structural protein denaturation, enzymatic inactivation, and nucleic acids denaturation, thus irreversibly preventing pathogens' replication in excreta and digestates, being widely used (Fong and Lipp 2005). Maheshwari et al. (2004) applied temperatures of 50 °C in order to inactivate adenovirus in substrata used for biodigestion and reported a significant inactivation of 4.0 log 10 decay of HAdV 5 in 10 min of exposure.

Some of the most important parameters to consider during the thermal inactivation process are the decimal reduction time (DT), which expresses the time required to reduce a logarithmic unit at the concentration of viable cells at a given temperature (T), and the thermal coefficient (z), which represents the temperature difference required for the reduction of a logarithmic unit in the DT value. They are both related to the degree of heat resistance of a microorganism in a given matrix (Wigginton et al. 2012; Pecson et al. 2007). However, it should be noted that the use of heat requires energy expenditure that could be used for other purposes.

In this context, the application of economically feasible techniques to reduce pathogens in environmental matrices, such as those generated from the biodigestion process, is extremely important for the reduction of microbiological risks in the nutrients recycling in this process.

12.5 Scenarios and Data of Risk Exposure on the Biogas Production Chain and Risk Management

In the biogas treatment plants, as well as in the sites where the recycling of excess sludge and digestate is done, there are several possible routes where microbial risk exists in different extents. Here, we focus on the risk posed by sludge and digestate during its application on land for agricultural purposes since that is considered the most sustainable use in the context of the circular economy.

The production of digestate and sludge and the possible general routes of risk exposure to human and animals are represented in the series of Figs. 12.1, 12.2, 12.3, 12.4, 12.5, 12.6.

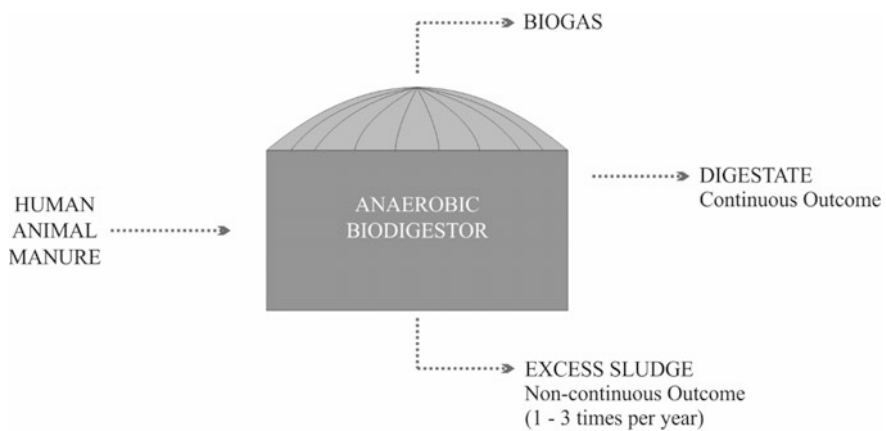


Fig. 12.1 Digestate and excess sludge formed during the anaerobic digestion and biogas generation process

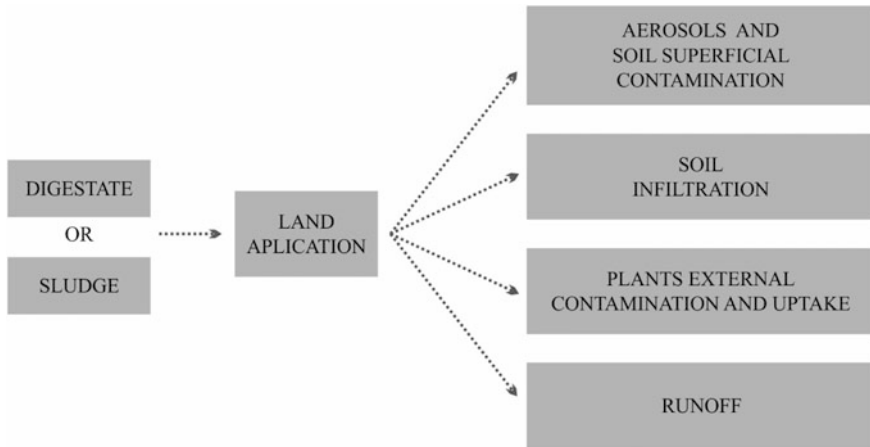


Fig. 12.2 General view of main exposure paths and sources of microbial contamination after land application of digestate or sludge

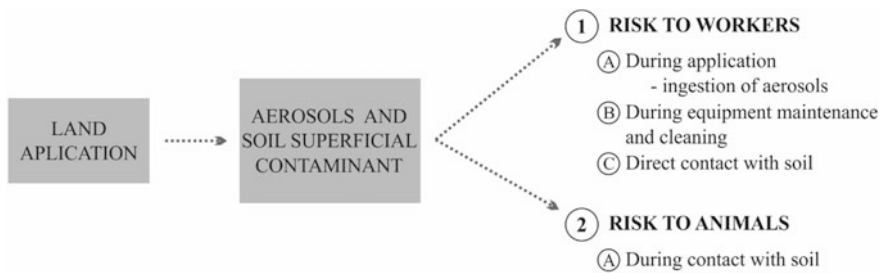


Fig. 12.3 Exposure routes related to the generation of aerosols and superficial contamination of soil during land application of digestate and/or sludge

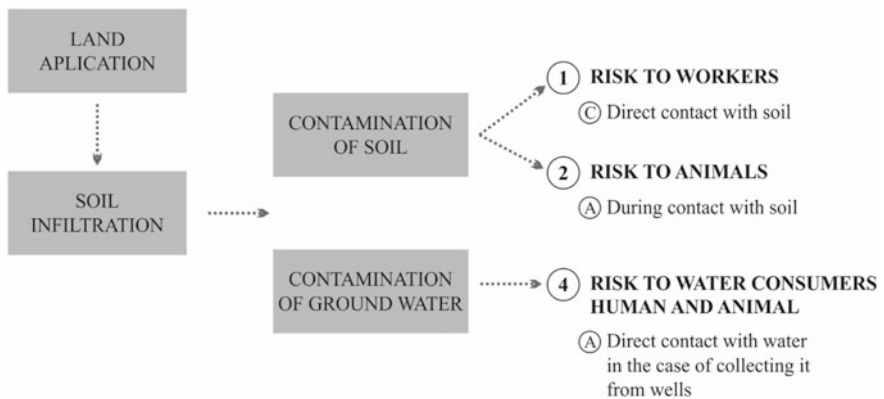


Fig. 12.4 Exposure routes related to soil infiltration of digestate and/or sludge with consequent soil and groundwater contamination

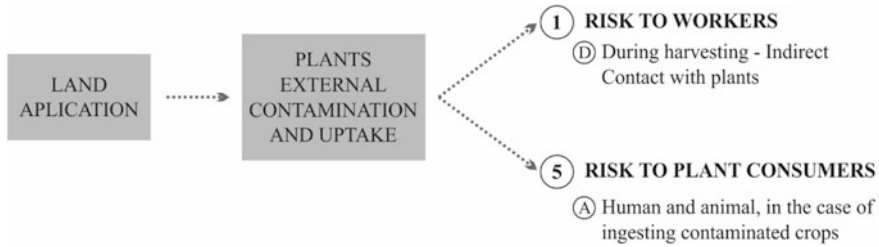


Fig. 12.5 Exposure routes related to the contamination of the external and internal parts of plants after application of digestate and/or sludge

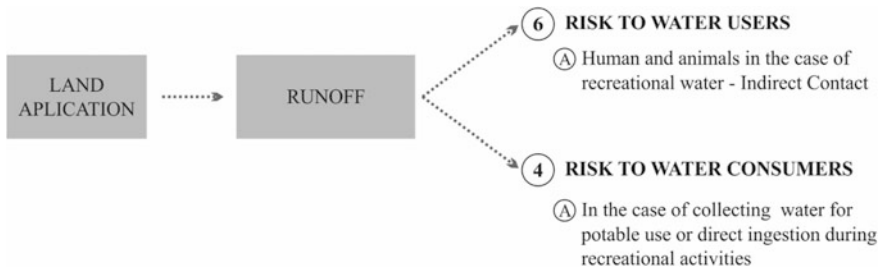


Fig. 12.6 Exposure routes related to the contamination of water bodies as a consequence of rain events promoting the runoff in the fertilized area with digestate and/or sludge

During manure application on soil, some main routes of exposure of humans and animals can be highlighted: exposure to aerosols and soil contaminated superficially (Fig. 12.3); exposure to contaminated groundwater by soil infiltration of excreta (Fig. 12.4); exposure to contaminated plants (crops) (Fig. 12.5); and exposure to superficial water contaminated by agricultural runoff (Fig. 12.6).

The ingestion of contaminated soil and aerosols is normally referred to as indirect contact and accidental ingestion. Workers (farmers in that case) accidentally ingest soil as a consequence of hands to mouth gesture (faecal–oral route), and hands can get contaminated during soil labour, harvesting crops, or still during maintenance of equipment and tools used. Julian et al. (2018) simulated (100 simulations per farmer) and predicted final *Escherichia coli* concentrations across farmers for both hands of $1.4 \pm 1.3 \log_{10} \text{CFU/cm}^2$. In this case, *E. coli* indicates the potential for a physical adhesion of enteric pathogens in hands. Comparing the concentration found for Julian et al. (2018) with the concentration of *E. coli* in human faeces of $10^8 \log_{10}$ (Table 12.2), each cm^2 was contaminated with approximately 0.0001% of *E. coli*, which might be sufficient for illness, in the case of pathogenic *E. coli*.

Moazeni et al. (2017) estimated the infection risk for farmers in contact with contaminated soil irrigated with an effluent containing 12–16 pfu/mL of

enterovirus. The probability of infection was 8.8×10^1 per person per year for farmers, which is about 2 log higher than the tolerable infection risk of 2×10^3 pppy targeted in the study.

The modelling for assessing risk can be conducted also from the opposite perspective. For instance, Kobayashi et al. (2017) targeted the tolerable burden of disease of 10^{-4} and 10^{-6} for disability-adjusted life year loss per person per year (DALYS) and calculated a threshold reduction level for norovirus gastrointestinal illness (GII) that would be required for the use of a sewage effluent for agricultural irrigation. That approach is widely used on decision-making and management processes, as well, for legislation.

Understanding the behaviour of enteric bacteria following application to soils is an important element in predicting exposure to adjacent water, and the development of regulatory guidelines to manage the risk of faecal contamination of water from soils that have received manures (Haas et al. 1999).

The chemical composition of livestock wastes and the treatment applied after the biogas reactor are some of the elements that will influence the dynamics of surviving enteric bacteria following application to soils (Topp et al. 2009).

Topp et al. (2009) compared risk for application of fresh untreated manure and treated manure for which a 3-log reduction was achieved prior to land application. In the absence of treatment, the risk of infection (expressed as a probability of risk of infection per event, as a point estimate) from *Cryptosporidium* was 1.75×10^4 and from *Campylobacter* 1.27×10^2 . In contrast when considering treated livestock waste that had a 1000-fold reduction in pathogen content, the risk from *Cryptosporidium* was 1.75×10^7 and that from *Campylobacter* was 1.27×10^5 .

Herein, we can state as an important evaluation, the multi-barrier approach for managing risk from shed microorganism. An effective multi-barrier strategy has three major components, according to Topp et al. (2009): (1) managing herd health to minimize the acquisition, potentiation, and release of zoonotic pathogens into the manure; (2) management of the manure during storage to effect a reduction in pathogen content prior to release into the broader environment; and (3) application of the material to land at a judicious rate, and under suitable land, climate, and crop conditions to minimize the off-site movement of contaminants into adjacent surface or groundwater.

The multi-barrier approach was at first considered to water supply systems, aiming to assure safe drinking water, and since then, people are beginning to shift their focus from compliance monitoring to the more holistic approach.

During the application of biofertilizer on soil, there is also a risk related to aerosols, as cited above. According to Courault et al. (2017), the risk decreases with increasing distance from the emission source and that wind speed has a great impact on atmospheric dispersion.

For the exposure route presented in Fig. 12.5, the consumption of contaminated water or plants can be understood as direct contact. The barriers applied for reducing the risk in this situation vary from cleaning vegetables before consumption to the application of water treatment processes.

Moazeni et al. (2017) estimated the risk for lettuce consumers and as result a lower level of infection and disease burden (about 10^{-3}) but higher than the guideline threshold of 10^{-4} DALY pppy (WHO).

Mok et al. (2014) evaluated the risks for both situations, eating food washed and non-washed. The median probability of infection for washers ranged from 2.94×10^{-8} to 1.51×10^{-3} , and the median probability of illness per dose ranged from 1.35×10^{-16} to 3.52×10^{-7} . For non-washers, the probabilities were one order of magnitude higher. Other important factors that can influence risk in the cited situations are: type of vegetable, type of irrigation system, temperature and type of soil, time for consumption after harvesting, and “resting” period after manure application (Mok and Hamilton 2014; Amoah et al. 2018). For instance, consumption of lettuce grown on sludge-amended soil will result in probable infections but harvest after 30 days between sludge application and harvest in the study presented by Amoah et al. (2018) gave median probability infection risks with a risk level similar to the WHO tolerable risk value (10^{-4}).

For the scenario of agricultural runoff and consequent superficial water contamination, several parameters can interfere with the risk extent. Sensitivity analysis is usually performed to investigate how variability of the outputs can be apportioned quantitatively to different sources of variability in the inputs. Clarke et al. (2017) show that the parameter of importance that affected the variance in model predictions for ingestion of contaminated river water with *E. coli* was time in the stream, which highlights the importance of residence time of bacteria in that environment. The time that bacteria stay in the stream allows their contact with environmental inactivation factors such as temperature, pH, and photolysis, which may in turn influence the growth or die-off rates. The other parameters of importance were the water intake and initial counts in surface runoff.

12.6 Final Considerations

The recovery of biogas for energy production in treatment plants for waste anaerobic biodigestion is already a reality in many countries. Conventional plants are moving from “treatment” plants to become “resource recovery” plants, and even added value can be obtained by the organics and nutrient recycling of the accumulated sludge and digestate outcomes from the bioreactors. However, for conducting a safe nutrient recycling in agriculture, the pathogen content is a limiting factor. In that context, we presented the quantitative microbial risk assessment as an effective approach to estimate risks, which can be applied to any scenarios of recycling liquid fractions from biogas reactors in agriculture.

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