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Chapter · July 2022

DOI: 10.2166/9781789062755_0359

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doi: 10.2166/9781789062755_0359

Chapter 17

Anaerobic digestate: pollutants, ecotoxicology, and legislation

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ABSTRACT

Anaerobic digestion (AD) offers enormous potential towards a sustainable bioeconomy. AD is suitable for waste management of various sources and generates value in multiple sectors such as electricity, heating, or agriculture through biogas or fertilizer production. However, attention must be paid to cheap fertilizers such as digestates because these digested fractions contain heavy metals, (nano) microplastics, hormones, and other chemical compounds that, when accessible, can be detrimental to humans, animals, plants and the environment. Digestates originating from manure substrates should be monitored to control the spread of antimicrobial resistance genes to the environment. Digestate toxicity can be a problem for aquatic and terrestrial organisms as determined by bioassays to detect the adverse consequences of digestate applied to the soil. Anaerobic digestate must meet specified quality requirements prior to utilization in the soil or crops to prevent dangers to human health and the environment. The digestate is available in three forms: whole, liquid, and solid, and can be applied to the soil as a final disposal location if it complies with applicable regulatory standards and is classified as a 'product'.

Keywords: Anaerobic digestion, digestate, ecotoxicity, emerging contaminants, legislation

Editor(s): Vinay Kumar Tyagi, Kaoutar Aboudi and Cigdem Eskicioglu

17.1 INTRODUCTION

Anaerobic digestion (AD) can significantly contribute to paving the way towards a sustainable economy. This technology produces biogas from organic waste, which includes manure, crop residues, urban solid waste, sewage sludge, and so on (Tsapekos *et al*., 2021). The digestate is the main by-product of the AD process, which is regarded as an organic fertilizer (Gross *et al*., 2021). With appropriate management, AD can reduce greenhouse gas (GHG) emissions and water, soil, and air pollution (Jiang *et al*., 2012). Moreover, within the context of the sustainable development goals (SDG), AD makes a significant contribution to guaranteeing cheap and sustainable energy (SDG 7), while also contributing to additional objectives (SDG 6, 9, 13, 15) (Tsapekos *et al*., 2021). Several AD plants have been installed worldwide, resulting in a local surplus of its main by-product, digestate (Prapaspongsa *et al*., 2010). For instance, the European Union (EU) administration has adopted many AD facilities towards a more energy-efficient system and low-carbon emissions by utilizing biogas instead of fossil fuels (Huopana *et al*., 2013). However, the development of other energy carriers such as hydrogen or methanol is rapidly growing. Countries like India consider AD as a promising technological approach to manage biowaste within its municipalities (Gross *et al*., 2021). However, digestate planning and management are still inadequate in most cases. Another aspect is the subsidies to support this technology, for instance, in China, the current subsidies should also be based on output rather than building costs (Zheng *et al*., 2020). The digestate is a rich source of nutrients such as nitrogen, phosphorus, and potassium (Möller & Müller, 2012). It can provide economic revenues that encourage its usage as a soil conditioner and fertilizer (Prapaspongsa *et al*., 2010). However, because these digested fractions contain pathogens and heavy metals that can be harmful to humans and other organisms when bioavailable (Järup, 2003; Jomova & Valko, 2011), environmental agencies and governments must establish safety standards and monitor digestate compositions in order to value waste as a resource for end users (Peng & Pivato, 2017).

Researchers have recently focused their attention on the various pollutants found in digestates. For example, different metals, pesticides, mycotoxins (Jiang *et al*., 2018), or antimicrobial agents found in environmental matrices derived from manure digestate that enhance the abundance of antimicrobial-resistant bacteria (AMRB) and antimicrobial resistance genes (AMRGs) (Congilosi & Aga, 2021). Additionally, in the digestate derived from the AD process of urban solid waste or biosolids, fractions of (nano) microplastics have been identified to threaten ecosystems. They may represent a risk to human, animal, and plant health (Mohammad Mirsoleimani Azizi *et al*., 2021).

17.2 DIGESTATE: POLLUTANTS

The origin of the residues used in the AD process is mainly organic. These substrates come from animal and crop production, the food sector, urban–industrial waste, and sewage sludge (Baştabak & Koçar, 2020; Sen *et al*., 2016). The composition and origin of waste affect the macro and micronutrient content and the presence of certain contaminants such as heavy metals, traces of drugs, and other contaminants that might affect the quality of the digestate (Baştabak & Koçar, 2020).

17.2.1 Heavy metals

Various studies examined the feasibility of digestate recovery for agricultural purposes and found that high heavy metals content in the digestate limited its utilization (Chaher *et al.*, 2021). Moreover, heavy metals have a negative effect on aquatic ecosystems since they may enter food chains and impact higher life forms via biomagnification (Mohammadi *et al.*, 2019). In contrast, when heavy metals concentrations are significantly low, some alternatives are effective, such as hydrothermal carbonization and diluted digestate cake as a start-up seed and strengthening methane generation (Reza *et al.*, 2016). Therefore, heavy metal stability or removal is critical for recycling; the immobilization process is the most common approach due to its simplicity and economic effectiveness (Mohammadi *et al.*, 2019). A comprehensive list of heavy metals is revised, including Hg, Cd, Cr, Ni, among others. Table 17.1 summarizes the major physical–chemical features of digestates characterized in the literature. They are divided into mesophilic and thermophilic AD processes. The main substrates used are substrates derived from animal wastes such as animal by-products (ABP), animal sewage (AS), cattle slurry (CS), cattle manure (CM), chicken manure (ChM), manure (M), pig manure (PM), and pig slurry (PS). Different biomass or crop wastes such as herbaceous biomass (HB), beet leaves (BL), cereal bran (CB), corn (Co), corn silage (CoS), garden waste (GW), groats (Gr), grass silage (GS), maize silage (MS), olive oil cake (OOC), *Triticale* silages (TS), vegetable waste (VW) as well as another crops waste (OCW). The mixture of waste includes agro-industrial residues (AIR), food waste (FW), kitchen waste (KW), sewage sludge (SS), and municipal solid waste (MSW). The parameters C, N, P, S, Na, Mg, K, Ca, Fe, and Al, are expressed in percentage (%). At the same time, heavy metals (Zn, Cu, Pb, Cd, Ni, Cr, and As) are expressed in mg kg^{-1}.

Most studies reported primary and secondary nutrients useful for fertilization and application in crops. However, some studies quantified the presence of potentially toxic heavy metals such as Zn, Cu, Cd, Ni, Pb, Cr, Hg (Opatokun *et al.*, 2017; Pampillón-González *et al.*, 2017). These values depend on the substrate used and the conditions for the AD process.

17.2.2 Emerging contaminants

Contaminants in AD digestates are not only limited to heavy metals and undigested organic loads. They also include residual contaminants in a broad spectrum of biological waste, including emerging contaminants, pathogens discharged through solid or aqueous waste streams. Emerging contaminants such as pharmaceutical and personal care product residues (Chen *et al.*, 2014); endocrine disruptors, antimicrobial-resistant pathogens (Gondim-Porto *et al.*, 2016); microplastics (Mahon *et al.*, 2017), and other persistent organic compounds can be identified in digestates obtained from animal manure, MSW or SS (Longhurst *et al.*, 2019).

Table 17.1 Physical–chemical properties of different digestates.

Digestate origin[a]	Composition (dry basis)																		
	Moi [b]	C	N	P	S	Na	Mg	K	Ca	Fe	Al	Zn	Cu	Pb	Cd	Ni	Cr	As	Reference
	%											mg kg^{-1}							
Mesophilic																			
AIR+HB	–	38.5	1.21	1.82	0.29	0.13	0.85	2.83	3.92	0.19	–	–	–	–	–	–	–	–	Calamai *et al.* (2020)
AS+CM+ MS+TS+CB	–	43	1.3	0.53	0.14	0.18	0.36	1.2	1.08	0.06	0.04	35	11	–	–	1	1	–	Monlau *et al.* (2016)
BL+OCW	94		1.3	0.4		1	0.4	4.2				5	1	–	–	–	–	–	Gunnarsson *et al.* (2011)
CM+AIR	91.9	37.5	2.7	0.89		0.829	0.78	3.45	4.48			30.86	11.98	–	–	–	–	–	Alburquerque *et al.* (2012)
Co	–	40.7	2.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Dieguez-Alonso *et al.* (2018)
CS+CM	–	52.1	2.33	2.26	0.65	0.58	1.27	1.32	4.1	1.28	0.19	–	–	–	–	–	–	–	Garlapalli *et al.* (2016)
CS+GS+ CM	–	50.6	4.22		0.52	–	–	–	–	–	–	–	–	–	–	–	–	–	Rodriguez Correa *et al.* (2017)
CS	–	38	2.05		0.62	0.385	0.6206	1.25	0.98	–	–	–	–	–	–	–	–	–	Zhang *et al.* (2018)
CM	89.7	56.2	0.65	1.81				2.1				168.59	31.787	–	–	–	–	0.58	Jin and Chang (2011)
CM			0.96	1.14		0.039	0.015	0.158	0.088		0.02	–	–	–	–	–	–	–	Jin and Chang (2011)
FW	90.2	42.1	5.81	1.97	–	–	0.27	0.62	3.17	0.93				10		20	40	0.98	Opatokun *et al.* (2017)
G+OOC+ TS+ChM	–	42.5	1.4	–	0.14	–	–	–	–	–	–	–	–	–	–	–	–	–	Monlau *et al.* (2016)
AIR+HB	76.2	46.7	1.2	–	0.5	–	–	–	–	–	–	–	–	–	–	–	–	–	Miliotti *et al.* (2020)

MS+CM+GS	93.5	50.5	3.6	1.35	0.5	–	0.94	5.23	2.01	0.3	0.52	–	–	–	–	–	–	–	Zhao *et al.* (2018)
PM	89.1	43.11	0.75	1.03	–	–	–	3.2	–	–	–	189.54	149.91	–	–	–	–	2.385	Jin and Chang (2011)
PS+ABP	97.25	42	2.48	0.14				1.42	0.154	–	–	24.72	2.91	–	–	–	–	–	Alburquerque *et al.* (2012)
PS	91.2	43.6	0.69				24266		3.73	0.59		3873	1796	7.2	3.8	16.9	10.1	3.1	Pampillón-GonzáLez *et al.* (2017)
SS	83.5	32.7	5.1	4.58	1	0.16	0.32	0.28	3.02	1.58	–	–	–	–	–	–	–	–	Marin-Batista *et al.* (2020)
Thermophilic																			
CM+FW	24.5	37.8	1.09	0.59	–	–	–	0.32	–	0.88	–	–	–	–	–	–	–	–	Rodriguez Alberto *et al.* (2019)
CM+MS+GS	–	40.3	2.1	0.94	0.3	0.08	0.74	3.67	2.53	0.23	0.25	–	–	–	–	–	–	–	Cao *et al.* (2019a, 2019b)
C+CS+M+VW	–	39.8	1.23	0.79	0.29	0.08	0.47	–	2	0.10	–	–	–	–	–	–	–	–	Calamai *et al.* (2020)
KW+GW	–	34.3	1.9	0.39	0.2	0.66	0.54	1.8	4.29	0.66	0.92	–	–	–	–	–	–	–	Cao *et al.* (2019a, 2019b)
CM	–	42.6	2	0.71	0.4	0.16	0.54	2.84	1.71	0.11	0.1	–	–	–	–	–	–	–	Cao *et al.* (2019a, 2019b)
MS	–	44.1	3.2	1.2	0.3	0.3	0.7	1.6	1.3	2.3	–	–	–	–	–	–	–	–	Parmar and Ross (2019)
MS	–	46.6	1.1	–	0.08	–	–	–	–	–	–	–	–	–	–	–	–	–	Cao *et al.* (2020)
MSW	–	24.1	1.5	0.7	0.2	0.9	1.4	1.6	10.4	3.2	–	–	–	–	–	–	–	–	Parmar and Ross (2019)
VW+GW	–	29.5	2	2.6	0.3	0.5	0.8	0.7	4.3	3	–	–	–	–	–	–	–	–	Parmar and Ross (2019)

[a]**Digestate origin.** Animal by-products (ABP), animal sewage (AS), cattle slurry (CS), cattle manure (CM), chicken manure (ChM), manure (M), pig manure (PM), and pig slurry (PS), herbaceous biomass (HB), beet leaves (BL), cereal bran (CB), corn (Co), corn silage (CoS), garden waste (GW), groats (Gr), grass silage (GS), maize silage (MS), olive oil cake (OOC), *Triticale* silages (TS), vegetable waste (VW), other crops waste (OCW), agro-industrial residues (AIR), food waste (FW), kitchen waste (KW), sewage sludge (SS), and municipal solid waste (MSW).
[b]**Moi:** Moisture, expressed in percentage.

17.2.2.1 Antibiotics

Antibiotics can be introduced directly into the environment when they are excreted by grazing animals or with the application of manure or SS as organic fertilizer in agricultural fields. In the AD processes, different classes of antibiotics have been identified, such as tetracyclines, sulphonamides, fluoroquinolones, β-lactams, and macrolides (Congilosi & Aga, 2021). Many of them are managed as drugs in animal husbandry. These drugs have high excretion rates (see Table 17.2) and different mobility and persistence. These compounds can enter the environment on a regular scale with organic fertilization and contribute to the spread and development of antimicrobial resistance (AMR) and associated mobile genetic elements in soils, which are serious threats to human and animal health (Lehmann & Bloem, 2021). The potential of AD to degrade and eliminate antibiotics depends mainly on the concentration and class of antibiotic, the operating conditions of the bioreactor (mainly temperature), the type of raw material, and the source of inoculum (Massé *et al.*, 2014). Biodegradation and biosorption are two dominant mechanisms of antibiotic removal in AD (Zhou *et al.*, 2021a, 2021b). For example, tylosin, a veterinary antibiotic, was degraded through a mesophilic AD process (Hosseini Taleghani *et al.*, 2020). On the other hand, thermophilic AD has reduced the absolute abundance of antibiotic resistance genes (ARGs) (*sulII* (95%), *intI1* (95%), *tnpA* (77%)) (Wang *et al.*, 2021). A new method to improve the removal of antibiotics in an AD process refers to the addition of conductive or nanomaterials to the AD reactor (Zhou *et al.*, 2021a, 2021b). These additives show a higher removal efficiency of antibiotics than conventional AD, which is moderately effective (Zhou *et al.*, 2021a, 2021b). However, the agricultural use of digestates still represents a risk of contamination to the environment. Agricultural plots disturb the soil's microbial ecology for which an advanced AD is required or combined with

Table 17.2 Average annual veterinary antimicrobial excretion rates in cattle, poultry, and pigs.

Antimicrobial class	Manure matrix	Concentrations in manure (ppm)	Reference
Macrolides	Swine	1.6[a]	Angenent *et al.* (2008)
	Poultry	0.0090–14	Ho *et al.* (2014)
	Cattle	0.012–0.029	Wallace and Aga (2016)
Tetracyclines	Swine	0.048–354	Congilosi and Aga (2021)
	Poultry	0.14–18	Zhao *et al.* (2010)
	Cattle	0.0065–0.27	Congilosi and Aga (2021)
Sulphonamides	Swine	0.015–20 0.10–12[a]	Congilosi and Aga (2021)
	Poultry	0.010–91	Zhao *et al.* (2010)
	Cattle	0.0019–0.014	Wallace and Aga (2016)

Adapted from Congilosi and Aga (2021).
[a]Wet weight.

Table 17.3 Hormone excretion rates by different animals based on wet or dry weight manure.

Hormones	kg excreted per year[a]	Average excretion rate (%)[a]	Manure matrix	Concentration in manure (ppb)	Reference
Estrogens	48 530	90	Swine	70.1–518	Xu *et al.* (2018)
			Cattle	6.20–1416	Zheng *et al.* (2008)
Androgens	4350	40	Poultry	44.2–150	Andaluri *et al.* (2012)
			Cattle	1.30–35.9	Congilosi and Aga (2021)
				17.0–203[b]	
Progestagens	278 900	90	Cattle	Up to 196	Zheng *et al.* (2008)
			Poultry	<10.0–391	Ho *et al.* (2014)

[a]Estimated US excretion of hormones by feedlot animals in 2000 and excretion rates (Biswas *et al.* 2013).
[b]Wet weight.
Adapted from Congilosi and Aga (2021).

composting methods that are more effective for eliminating antibiotics and avoiding the entry of substrates with quantities of these drugs.

17.2.2.2 Endocrine disruptors

Hormones are administered to cattle to encourage growth and regulate the reproductive system. According to Noguera-Oviedo and Aga (2016), the levels of estrogens detected in anaerobically digested manure were found to be substantially higher than the levels known to cause endocrine alterations in aquatic and wildlife organisms (can be affected by EDC even in low concentrations, that is, subpart per billion) (Jobling *et al.*, 2006). According to Zheng *et al.* (2013), aerobic exposure improves the biodegradation of estrogens. It would be beneficial to keep the liquid manure in an aeration pond before application to the soil to decrease the introduction of estrogens into the environment; however, CAPEX and OPEX would need to be considered. Table 17.3 summarized the hormone excretion rates by different animals based on wet or dry weight manure.

17.2.2.3 Microplastics

Microplastics are particles less than 5 mm in diameter (Weithmann *et al.*, 2018), while nanoplastics are particles smaller than 0.1 μm (Meixner *et al.*, 2020). Both have become global issues, posing hazards to biota and public health. Concerns have been raised about the presence of microplastics particles in digestate when used as organic fertilizer and composting (Peng *et al.*, 2021; Weithmann *et al.*, 2018). Recently, a study showed that plastic particles remained in organic fertilizers after fermentation and composting (Keller *et al.*, 2020). Therefore, the management strategies in which the digestate is handled may affect microplastics' accumulation and size distribution (Meixner *et al.*, 2020). A common case is that the microplastics in food waste packaging materials prevent food waste from co-digesting (Peng *et al.*, 2021). Another issue is that land-based systems acquire from 4 to 23 times more microplastic than aquatic systems (Meixner *et al.*, 2020). Biodegradation of microplastics can be carried out by microorganisms such as algae, fungi, and bacteria and is an important process to deal with the plastisphere. For instance, *Brevundimonas* and *Sphingobacterium* were reported to degrade polybutylene adipate-*co*-terephthalate (PBAT) and polylactic acid (PLA) under thermophilic AD conditions (Peng *et al.*, 2021). Moreover, some physical strategies are the destruction of microplastics via UV irradiation, weathering, or tillage (Keller *et al.*, 2020). Studies on the fate of microplastics in the environment should consider spatial processes and temporal scales to understand the mechanisms better behind them.

Most of the (nano) microplastics investigated to date have been found to impair AD performance (Mohammad Mirsoleimani Azizi *et al.*, 2021). Their presence leads to several inhibition mechanisms, including (1) release of toxic additives/chemicals, (2) affecting the activities of key enzymes and functional genes (Campanale *et al.*, 2020), (3) production of reactive oxygen species, (4) damage/penetration of microbial cells, (5) alteration of protein structures in granular sludge (Prata *et al.*, 2021). The main generation sources are sludge from sewage treatment plants and urban solid waste without segregating at the source. Although the study developed by Iyare *et al.* (2020) shows that ∼70% of microplastics could be removed during preliminary and primary treatment processes in wastewater treatment plants, study limitations and transmission of (nano) microplastics when applied to soils and crops continues to be a major environmental challenge. It was previously noted that (nano) microplastics could adsorb various environmental pollutants, including antibiotics and heavy metals (Mao *et al.*, 2020; Rolsky *et al.*, 2020), or they can also serve as carriers of AMRGs (Dong *et al.*, 2021).

17.2.3 Microbiological safety

Digestates contain a high microbial load and therefore require treatment prior to the application to soil. Total coliforms associated with faecal coliforms are more prevalent in the tubular digester containing guinea-pig manure. Consequently, the high loads of these microorganisms must be reduced to ensure that the digestate can be disposed of in the environment following the permissible limits of the local legislation. Table 17.4 illustrates the treatment of three common feedstocks: pig slurry, guinea-pig manure, and cattle manure, using diverse reactor configurations.

17.2.4 Antimicrobial resistance in anaerobic digestate

Animal manure application to agricultural land is a widespread practice worldwide. The effects of these practices may be reflected at distant time scales

Table 17.4 Different types of digestate and the involved pathogens.

Substrates	Type of digestate	Pathogens × total coliforms	Faecal coliforms	Helminth eggs:	Study
Guinea-pig manure	Tubular biodigester	1.70×10^8 MPN mL^{-1}	1.70×10^7 MPN mL^{-1}	N/A	Garfí *et al.* (2011)
Cattle manure	Mesophilic tubular digester	–	1.06×10^6 CFU g^{-1} TS	24 HH 4 g^{-1} TS	Castro *et al.* (2017)
Pig slurry	Mesophilic–thermophilic digester (lagoon type)	240×10^4 CFU g^{-1} TS,	3.6×10^4 CFU g^{-1} TS	N/A	Pampillón-GonzáLez *et al.* (2017)

and depend on the load and farm size. Antimicrobials used in farming practices can directly influence the abundance of antimicrobial-resistant bacteria (ARB) and AMRGs in manures, and most importantly, the risk of persistence and spread of this threat in soils is high when applied as fertilizer in agricultural land (Marti *et al.*, 2013). Animal manure harbours AMRGs (Sun *et al.*, 2016), and despite the management through AD and composting, it is still an important challenge to solve prior utilization of digestate as a fertilizer additive in agriculture. Therefore, it is of utmost importance to monitor the prevalence and fate of ARGs in anaerobic digestates. Among the strategies to remove ARGs, pretreatment methods prior to the AD process of manures should be considered a feasible alternative to enhance the biogas yield degradation of pollutants (Congilosi & Aga, 2021). Microwave or activated carbon pretreatment has removed up to 95% of ARGs (Congilosi & Aga, 2021). As the efficiency of composting in reducing ARGs is limited, the combination with AD could lead to better removal efficiencies of ARGs (Congilosi & Aga, 2021).

17.3 DIGESTATE: ECOTOXICOLOGY

17.3.1 Aquatic/terrestrial toxicity assays

Digestate toxicity occurs mainly in aquatic and terrestrial species. Table 17.5 shows some relevant toxicity analysis results, tests, indicators, measurements, and results. Within aquatic species, they have been tested on microalgae (*Chlorella sorokiniana*, *Chlamydomonas reinhardtii*, *Raphidocelis subcapitata*), Gram-negative marine bacterium (*Vibrio fischeri*), crutaceans (*Daphnia magna* and *Artemia* sp.), or aquatic freshwater plant (*Lemna minor* L.). It should be highlighted that digestates can have a harmful effect on water bodies because while some species of microalgae are capable of using organic acids such as acetate or butyrate as a source of carbon and/or energy. Markou *et al.* (2018) demonstrate that certain digestate concentrations can inhibit microalgae species.

Table 17.5 Results of various toxicity tests from digestates with aquatic and terrestrial indicators.

Dig.Typ.[a]	Specie	Class	Assays	M. typ[b]	Exp. cond.[c]	Measurement results	Reference
Aquatic species							
AIR + M	*Chlorella sorokiniana*	Microalgae	Petri dishes	Inh	pH = 10, T = 25°C	Butyrate showed inhibitory effects at concentrations higher than 0.1 gC L^{-1} and 0.25 gC L^{-1}	Markou *et al.* (2018)
AIR + M	*Chlamydomonas reinhardtii*	Microalgae	Petri dishes	Inh	pH = 10, T = 25°C	Acetate caused inhibition at concentrations above 0.4 g L^{-1}	Markou *et al.* (2018)
OFWW.	*Vibrio fischeri*	Bioluminescent, Gram-negative marine bacterium	Ecotoxicity of the eluate	Inh	% NaCl solution	High significant toxic response due to low stability over 4 days.	Alvarenga *et al.* (2016)
OFWW	*Daphnia magna*	Small planktonic crustacean	Ecotoxicity of the eluate	Inh	t = 24 h	Toxic response significant medium–high due to its low stability over 4 days	Alvarenga *et al.* (2016)
CM	*Artemia* sp.	Small crustacean	Complete effluent toxicity	Inh	T = 20°C t = 48 h.	Concentrations of 100% of digestate inhibited of the eluate solid–liquid mobility 1:50 (v/v)	Pivato *et al.* (2016)
PS	*Raphidocelis subcapitata*	Green algae	Petri dishes	Inh	T = 4°C, pH = 7.0	Sensitive organism with an EC_{50} of 0.77% and UT of 129.87.	Tigini *et al.* (2016)
PS	*Lemna minor* L.	Aquatic freshwater plant	Petri dishes	Inh	T = 4°C, pH = 7.0	Developed fronds and biomass, with EC_{50} in the range of 1.02–1.77% and TU in 58.82–98.03.	Tigini *et al.* (2016)

Terrestrial species							
AW + AIR.	*Lepidium sativum*	Plant	Petri dishes	GI	pH > 7.5.	Significant differences between the dry mass of the seedlings between the digestates and the concentrations 20%, 10%, 1% and 0.1%.	Alburquerque *et al.* (2012)
AW + AIR.	*Lactuca sativa*	Plant	Petri dishes	GI	pH > 7.5	Significant differences between the dry mass of the seedlings between the digestates and the concentrations 20%, 10%, 1% and 0.1%.	Alburquerque *et al.* (2012)
CM	*Eisenia foetida*	Annelids	Complete effluent toxicity	Inh	$T = 25°C$, $t = 15$ min	Hormesis trend concerning relative growth and reproduction	Pivato *et al.* (2016)
WW + WiW.	*Lepidium sativum*	Plant	Petri dishes	GI, Bios	$T = 25°C$, $t = 72$ h	GI > 60%, Bios = 20%	Da Ros *et al.* (2018)
WW + WiW.	*Sinapsis alba*	Plant	Petri dishes	GI, Bios	$T = 25°C$, $t = 72$ h	GI > 60%, Bios = 30%	Da Ros *et al.* (2018)
WW + WiW	*Sorghum saccharatum*	Plant	Petri dishes	GI, Bios.	$T = 25°C$, $t = 72$ h	IG => 60% Bios = 19% at low doses (6.25%)	Da Ros *et al.* (2018)

[a]Dig.Typ: digestate type, agricultural waste (AW), agro-industrial residues (AIR), cattle manure (CM), manure (M), organic fraction of wastewater (OFWW), pig slurry (PS), wastewater (WW), wine waste (WiW).
[b]M. typ.: measurement type, inhibition (Inh), germination index (GI), biostimulation (Bios).
[c]Exp. Cond: experiment conditions.

Similarly, the tests with the *Daphina magna* indicators demonstrated inhibition due to the toxicity of unstabilized organic waste and chemically stabilized with lime but still contained a high load of degraded organic materials in its matrix (Alvarenga *et al.*, 2016). The terrestrial toxicity experiments mainly involved plant species, and the most used test is the germination index/biostimulation. According to various authors, these tests efficiently, simply, quickly, and economically determine the level of toxicity (Alburquerque *et al.*, 2012; Da Ros *et al.*, 2018). In general, these bioassays using organic waste eluates are critical for determining the adverse consequences of organic waste applied to the soil.

According to Boluda *et al.* (2011), the plant bioassay using cress (*Lepidium sativum*) or lettuce (*Lactuca sativa*) is the most popular plant used in phytotoxicity studies due to its high sensitivity. One of the most commonly used methods is the plate or disc bioassay, which is considered an efficient, simple, and economic ecotoxicological assay to assess toxicity risks derived from soil pollution.

The Petri dish-based assay is critical for identifying stable digestates for agricultural applications. The phytotoxic effects during early growth (germination) are identified as being primarily due to salinity; therefore, Na and Cl concentrations, as well as heavy metals (especially Cu and Zn), must be considered to avoid metal accumulation in the soil and salinization following the application of the digestates. On the other hand, soil invertebrates are good bio-indicators because of their continuous exposure to soil contaminants by skin contact, direct ingestion of soil particles and soil water, and food chain transfers. In this sense, earthworm bioassay using earthworm (*E. foetida*) allows the measurement of responses as life-cycle parameters such as survival, growth, and reproduction (Calisi *et al.*, 2011; Pivato *et al.*, 2016). They are regarded as a potential general biomarker that may be directly linked to organism health compared to other biological responses to pollutants. In addition, it has a high sensitivity to pollutant exposure, suggesting its possible applications as a sensitive, simple, and quick general biomarker for monitoring and assessment applications (Calisi *et al.*, 2011).

While the AD system has numerous benefits for agriculture farmers, it has demonstrated the constraints that practitioners must consider while using the digestate. Hence, Figure 17.1 illustrates the process of manure use on a farm, its collection, treatment, and application to crops. The flow distribution of the major pollutants is represented through all of the processes above, leading to environmental impacts on aquatic and terrestrial bodies. Consequently, these effects might reach the flora and fauna that inhabit these ecosystems but are not limited to these physical boundaries.

17.4 ENVIRONMENTAL IMPACTS LINKED TO DIGESTATE

Life-cycle assessment (LCA) is especially suitable for studying multiple aspects, particularly those related to the environment, such as carbon footprint or climate change impact (Morales-Polo *et al.*, 2018). The methodology is based on the ISO 14040 and ISO 14044 standards; the functional unit can

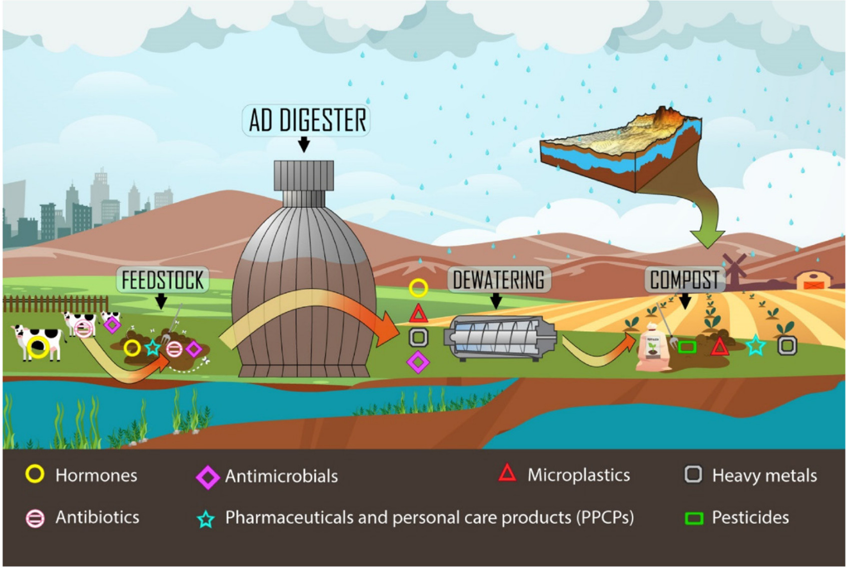

Figure 17.1 Agricultural farm with an AD digester used for the manure treatment and later used as biofertilizer. The fate of some pollutants flowing during the whole process is shown with different symbols.

be 1 kWh of generated electricity or 1 m^3 of biogas, and the software used is specialized, including OpenLCA, Simapro, and the Ecoinvent database. The most commonly evaluated categories are climate change, acidification, eutrophication, and photochemical oxidation (Zhou *et al.*, 2021a, 2021b). Its application includes waste management, AD processes, agriculture crops, and so on (Wei *et al.*, 2018). For instance, it could be implemented to compare biodigesters, measuring CO_2, SO_2, P, N, energy demand, and biodiversity loss, among other parameters (Cherubini *et al.*, 2015). Several feedstock options are represented in Figure 17.2, each of which can be introduced into the system independently or tentatively as a mixture of two or more inputs, resulting in the co-digestion of residues from animals and agriculture crops/leftovers. Additional by-products with a high market interest are identified; for example, the production of biogas with multiple applications is identified, and the production of digestate with agricultural applications and some elements in the construction field are shown. In general terms, a study of LCA is represented at least within these boundaries, though its scope can be expanded to include collection, transportation, and construction, among other stages.

The significance of post-digestion emissions and their link to AD performance has been emphasized as critical elements in reducing net GHG emissions (ca. 75%) and maximizing digestate fertilizer potential (ca. 15%); moreover, the gas-tight digestate storage with residual biogas collection is suggested (Pardo

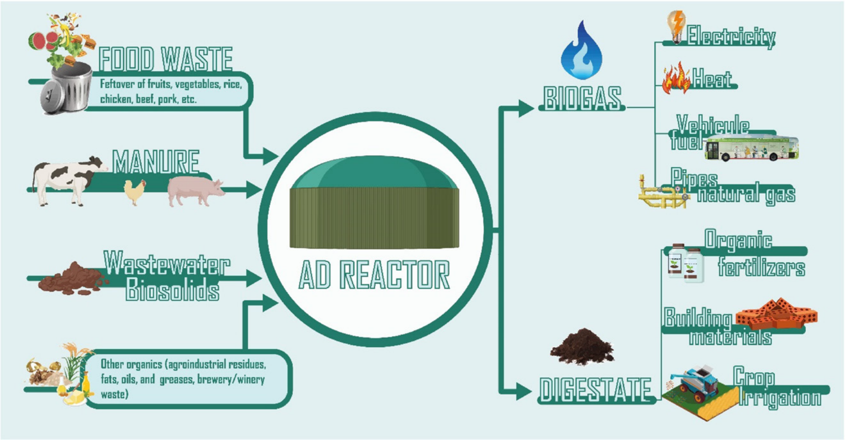

Figure 17.2 Potential inputs and outputs that an AD reactor can manage and different scales.

et al., 2017). It has been demonstrated that upgrading conventional full-scale anaerobic digesters by implementing ultrasonication, adding biochar and inorganic materials as catalysers can reduce GHGs by up to 50% (Mainardis *et al.*, 2021). Another consideration is establishing an AD system according to the local requirements; therefore, small-scale biogas plants are preferred over large AD facilities in areas with a relatively small population (Zhou *et al.*, 2021a, 2021b).

Another strategy for improving digestate management is through slurry tanks, which are a cost-effective method of mitigating the effects of terrestrial acidification and marine eutrophication; a low feed conversion rate reduces environmental impacts associated with the swine supply chain (Cherubini *et al.*, 2015). Despite numerous benefits from the AD process, some environmental impacts include soil depletion due to landfill saturation or increased ground demand for agricultural raw resources, resulting in soil pollution (Morales-Polo *et al.*, 2018).

In developing countries, appropriate management of digestates is feasible; for example, in rural areas in Egypt, fixed-dome digesters are used throughout the operation phase and less so during the construction phase (Ioannou-Ttofa *et al.*, 2021). Additionally, some experiences with cow–buffalo dung and potato waste have been developed in Pakistan. The LCA evaluated 100% cow–buffalo manure (CBM), 100% potato waste (PW), and a mixture of 75% CBM and 25% PW (CBM–PW mixture). It was determined that each 2000 kg of substrate slurry contained a climate change potential of 70, 71 and 149 kg for CBM, PW and CBM–PW mixtures, respectively (Rasheed *et al.*, 2019). A larger study examined low-cost digesters on small-scale farms in Colombia, wherein manure was used as substrate to produce biogas and digestate to replace liquefied petroleum gas

(LPG) and synthetic fertilizer, respectively. It entailed a reduction of 80% in potential environmental impacts associated with manure management, fuel and fertilizer use on farms (Garfí *et al.*, 2019). These low-cost digesters may represent a great tool to improve agricultural waste management and reduce GHG emissions.

Before developing a system architecture that balances sustainability and individual interests, all waste management and recovery parties must agree on a trade-off between the researched metrics. However, no system architecture is economically viable unless an economic analysis is conducted (Cobo *et al.*, 2018). Finally, the interaction between academia, industry and government is critical in the journey towards improved livelihood, economic income generation, and environmental impact reduction. Thus, measuring the sustainability indicators is mandatory, and LCA might continue to represent a suitable tool to better evaluate the deployment of technologies.

17.5 DIGESTATE LEGISLATION

The AD technology produces biogas and digestate; however, the latter needs to be regulated to safeguard the environment and global food chain. Table 17.6 summarizes the main digestate legislations. In the case of the United States, these are found in biosolids and the limits associated with the contaminants are regulated according to USEPA (2018). In European countries, each country has specific regulations and parameters for digestate, compost and manure. These regulations include permissible limits for discharges into soils and water bodies. The report by Saveyn and Eder (2013) groups the main guidelines by country (e.g., the SPCR 120 standard in Sweden (Sverige, 2013), and BSI-PAS-110 in the UK (British Standards Institution, 2014)).

In nations such as China, the digestate has been utilized as a feed supplement for pig, chicken, fish, and shrimp production; however, this option is constrained by national regulations and public approval (Logan & Visvanathan, 2019). Despite these advantages, certain laws restrict the market, and the Ministry of Agriculture opposes standardizing food waste treatment for safety reasons (Freese & Han, 2019).

According to Peng and Pivato (2019), certain quality criteria allow the use of the digestate as a ‘product’. These requirements are the input materials, processes and treatment techniques. The digestate is available in three forms: whole, liquid, and solid, and each fraction can be applied to the soil as a final disposal destination once they comply with the relevant regulatory standards and can be classified as a ‘product’ (Nkoa, 2014; Teglia *et al.*, 2011). Digestate quality standards include hygienic standards, impurities, degree of fermentation, odour, organic matter content, heavy metal content, and declaration parameters. The standards for heavy metals vary slightly between different countries. Among the biological parameters, the digestate used as fertilizer must ensure that the quality of the product has limited pathogens, viruses, and weed seeds (Al Seadi & Lukehurst, 2012).

Table 17.6 Main regulatory criteria for contaminants present in digestates.

Parameters	UM	USA (USEPA, 2018)	BSI PAS, UK (British Standards Institution, 2014)	EU standard[a] (European Commission, 1986; European Commission 1991; European Commission 2000)	Italy[b]	SPCR–120, Sweden (Sverige, 2013)	RAL GZ 245, Germany (Siebert, 2008)	NCh 3375 Chile (INN, 2015)
Physical–Chemical								
Odour							Free from annoying odours.	It must not present unpleasant odours
NO_3^{2-}	mg L^{-1}/ kg ha^{-1} y^{-1}			50 (170 kg N ha^{-1} y^{-1})				
TP	mg L^{-1}			10 (9–98 kg P ha^{-1} y^{-1})				
As	mg kg^{-1}	75		–				55
Cd	mg kg^{-1}	85	1.5	20–40	1.5	1	1.0–3.0	15
Cu	mg kg^{-1}	4300	200	1000–1750	230	600	100–600	667
Cr	mg kg^{-1}	3000	100	–	0.5	100	100–120	167
Hg	mg kg^{-1}	57	1	16–25	1.5	1	1–2	3
Mo	mg kg^{-1}	75		–				
Ni	mg kg^{-1}	420	50	300–400	100	50	50–100	133
Pb	mg kg^{-1}	840	200	750–1200	140	100	100–180	367
Zn	mg kg^{-1}	7500	400	2500–4000	200	800	400–800	1333

Se	mg kg^{-1}	100		–				
Fermentation degree	mg L^{-1}						<4000 mg L^{-1}, expressed as acid equivalent	<4000 mg L^{-1}, expressed as acid equivalent
Volatile fatty acids	g COD/ g VS		0.43					
Organic matter	%						>30%	>30%
Contamination with foreign matter	%		<0.5				<0.5	<0.5
Microbiology								
E. coli			CFUx g^{-1} of fresh matter	≤1.0 × 10^{3} CFU g^{-1}	≤1.0 × 10^{3} CFU g^{-1}			<1 000 MPN per g of digestate on a dry basis
Salmonella sp.			Absent in 25 g of fresh matter	Absent in 25 g of fresh matter	Absent in 25 g of fresh matter		Absent in 25 g of fresh matter	<3 MPN per g of digestate on a dry basis
Helminth eggs					-			<1 in 4 g of digestate on a dry basis
Ecotoxicity								
Weed germination			Germinating weed plants: 0/1				Less than 2 germinable weeds and germinated plant parts per L	Less than 2 weed propagules per L of digestate.

[a]EU standard: Nitrates: Council Directive 91/676/EEC, Heavy metal: Council Directive 86/278/EEC, Phosphorus. EC. Directive 2000/60/EC, and Microbiology (*E. coli* and *Salmonella* sp) Proposed end-of-waste criteria and Product Quality Requirements for compost and digestate in Saveyn and Eder (2013).
[b]Adapted from Saveyn and Eder (2013). Based on Italian Law on fertilizers (L 748/84; and: 03/98 and 217/06) for BWC/GC/SSC).

17.6 PERSPECTIVES AND CONCLUSIONS

AD offers enormous potential towards a sustainable bioeconomy. AD is suitable for waste management of various sources and generates value in multiple sectors such as electricity, heating, or agriculture through biogas or fertilizer production. However, attention must be paid to cheap fertilizer such as digestates because these digested fractions contain heavy metals, (nano) microplastics, hormones, and other chemical compounds that, when accessible, can be detrimental to humans, animals, plants and the environment. Digestates originating from manure substrates should be monitored to control the spread of antimicrobial resistance genes to the environment. Digestate toxicity can be a problem for aquatic and terrestrial organisms as determined by bioassays to detect the adverse consequences of digestate applied to the soil.

Anaerobic digestate must meet specified quality requirements prior to utilization in the soil or crops to prevent dangers to human health and the environment. The digestate is available in three forms: whole, liquid, and solid, and can be applied to the soil as a final disposal location if it complies with applicable regulatory standards and is classified as a ‘product’.

Therefore, governments must establish safety criteria and monitor digestate compositions in the waste valorization chain. There is already legislation regarding digestate management in high-income countries; however, in low-middle income countries, there is none, despite growing interest in using AD as technology for waste management. Furthermore, this chapter aimed to strengthen the importance of appropriately managing digestates in low-middle income countries. Digestates have several potential applications in agriculture, which have been transcendental in farming communities. Further studies should focus on the fate of pollutants and environmental assessment, but it should also include social life-cycle assessment (S-LCA), life-cycle costing (LCC), mass flow analysis (MFA), and exergy flow analysis (EFA).

ACKNOWLEDGEMENT

The authors thank Marco Pérez Celi for his support in the diagramming of the figures in this chapter.

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