Chapter 8
Impact of Antibiotics on Biogas Production

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Abstract Besides their use in human treatments, antibiotics have been extensively used to control animal diseases and, in some countries, still used to promote animal growth in livestock industry. To attempt human diet necessities, concentrated animal feeding operations (CAFOs) are necessary, increasing antibiotics consumption and manure production. Once antibiotic active agents and its metabolites are excreted in urine and feces, these substances are present in manure and can reach the environment. Around the world, especially in rural areas, manure is the main substrate for biogas production. This chapter presents a review about fate of antibiotics, with special focus on livestock by-products, and effects during the anaerobic digestion (AD). The antibiotic interaction has two important emphases addressed: (a) inhibition on the biogas and methane production process by the presence and action of these compounds and metabolites in the digester and (b) the ability of AD to degrade the molecules of antibiotics and thereby reduce the adverse effect caused by these compounds on the environment.

Keywords Anaerobic digestion · Veterinary drugs · Inhibition

8.1 Introduction

Antibiotics were discovered in the 1920s but only introduced into medicine in the 1940s (Etebu and Arikekpar 2016) to treat or prevent diseases in human and livestock (CDDEP 2015), changing the pattern of modern way of living. With the increasing prosperity and world population growth, the demand of these substances rises constantly, where more antibiotics are necessary to human necessities as well as to attempt animal husbandry due to the growing demand for animal products (e.g., meat, milk, egg, and their products) for human consumption (Tilman et al. 2002).

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In humans, the consumption of antibiotics between 2000 and 2010 grew by 35%, from approximately 50 to 70 billion standard units, based on data from 71 countries (Van Boeckel et al. 2015). In most countries, about 20% of antibiotics are used in hospitals and other healthcare facilities, and 80% are used in the community (CDDEP 2015). In livestock, global antibiotic consumption was estimated at approximately 63,000 tons in 2010 (Van Boeckel et al. 2015), accounting for nearly two-thirds of the estimated 100,000 tons of antibiotics produced annually worldwide (Bbosa and Mwebaza 2013). At less proportion, antibiotics are also given to pets, used in food industries, as preservatives (Silva and Lidon 2016), in aquaculture for shrimp and fish production, crop growing (CDDEP 2015; O'Neill 2015), in pharmaceutical production plants, and other uses.

Considering this scenario, it is possible to differentiate the substrates that contribute to the occurrence of antibiotic compounds in the biodigester in three possible groups (Fig. 8.1): from animals, from humans, and from industry (Alexy et al. 2004). In industrial effluents, antibiotics occur in high concentration, since they are the processes that generate the drugs. In this case, biological processes for the wastewater treatment are generally ineffective, requiring additional physical and chemical process (e.g., activated carbon or advanced oxidative processes) prior to UASB reactor or anaerobic filter (Yan and Lam 2015). Similarly, it occurs in hospital effluents. On the other hand, domestic effluents have a much lower concentration of antibiotics and in this case the inhibition possibility could be substantial during the digestion of the sludge from wastewater treatment plants. However, considering the three groups of substrates, the major contribution to the interaction with antibiotics occurs in large-scale biogas plants that take advantage of animal manure to feed the anaerobic digesters. For this reason, this chapter will focus on the occurrence and interaction of veterinary drugs found in manure.

To meet the needs of animal-derived food, livestock production has become increasingly dominated by concentrated animal feeding operation (CAFO).

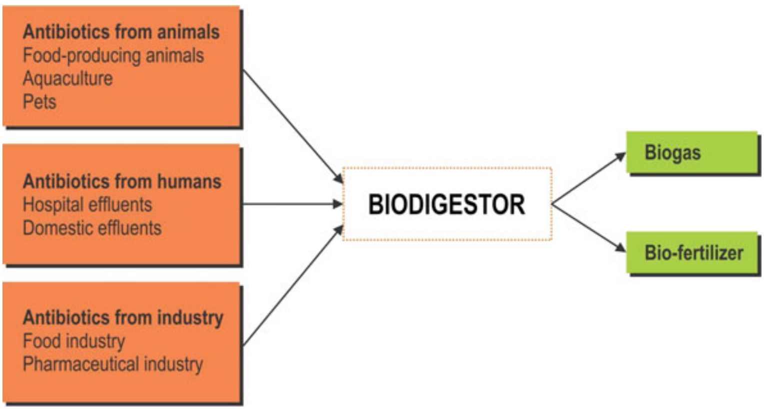

Fig. 8.1 Sources of antibiotics-containing residues which can be used as substrate in anaerobic digestion

The high population density of CAFOs in a relatively small area results in sharing of both commensal flora and pathogens, which can be conducive to rapid dissemination of infectious agents. Also, commensal bacteria found in livestock are frequently present in fresh meat products and may serve as reservoirs for resistant genes that could potentially be transferred to pathogenic organisms in humans. As a result, livestock in these environments commonly requires management strategies, which often include the use of antibiotic therapy to ensure the herd health and optimize production (Landers et al. 2012; Hao et al. 2014; O'Neill 2015).

In CAFOs, different antibiotic utilization manners are applied. Two terms are frequently used to describe the use in livestock: therapeutic and subtherapeutic. The first term, therapeutic, is used when a veterinarian prescribes a drug to treat animal with clinical signs of an illness or a condition like a respiratory infection or a skin infection. To this prescription, high doses of antibiotics for brief periods are administered to a single animal or a large group of them. The second term, subtherapeutic, is used when the antibiotics are administered in animals, which are susceptible to diseases or infection that can kill them quickly, in a preventative manner (lower doses for long periods of time), to prevent an outbreak. This definition fits also the use as growth promoters, where antibiotics are given mixed in feedstuff to improve daily weight gain and feed efficiency (CDDEP 2015; Schlüsener et al. 2003; Shi et al. 2011; Venglovsky et al. 2009; Landers et al. 2012).

The use of antibiotics in food production animals brings up an environmental preoccupation, once it is known that after administration (oral or parenteral) each antibiotic has its particular route and a significant proportion are excreted by urine or feces (17–90%, varying from compound to compound), in the unchanged or as active metabolites of the parent species (Martínez-Carballo et al. 2007; Zhou et al. 2013). Besides the occurrence of antibiotics, in CAFOs a very high manure amount (also with high concentrations of organic matter and nutrients) is produced with a worldwide estimation bigger than 9×10^9 ton annually (He et al. 2016). Thus, livestock wastes cannot be directly discarded in water bodies; therefore, they are a potential environmental contaminant on air, soil, and water resources if its destination is not correctly managed (Kunz et al. 2009). Once in the environment, it is difficult to predict how quickly antimicrobials will degrade, whether they come from animal use, human use, or manufacturing, as they are very diverse chemically. Some degrade easily, while others bind to organic matter and can persist in their active states for long periods of time (O'Neill 2015).

Because manure has been integrated as part of sustainable crop production by direct land application as biofertilizer (He et al. 2016), usually manure production is higher than the necessities for this purpose or not economically practicable by the distance between CAFOs and cropland (Seganfredo and Girotto 2004). As an alternative to manure disposal problem and recycling, multi-step advanced treatment systems were adopted enabling further effluent discharge in water bodies or water reuse. Other alternatives to reduce manure's pollution potential are composting (compost/fertilizer production) and anaerobic digestion (biogas generation). Both generate by-products with agronomic value, but they do not reduce land area needed to recycle the nutrients (Kunz et al. 2009; Scheeren et al. 2011).

It is the notorious importance of the antibiotics in both human and animal health and welfare. However, antibiotic resistance became one of the biggest threats to global health, food security, and development today (World Health Organization 2018), once infections with antibiotic-resistant bacteria may not respond to regular antibiotic treatments, resulting in increased mortality, morbidity, and social and economic costs. There are evidences that the use of antibiotics in animal food production is a source of antibiotic-resistant bacteria which are transmitted to people via occupational exposure on farms, along the food production chain, through food itself and through environmental pathways like contaminated soil and water supplies by animal production wastes (excreted material or manure-treated effluents) containing antibiotic residues (Koch et al. 2017).

Healthy animals mean a safe food supply and in turn healthy people. However, in food production animals, the use of antimicrobial drugs brings benefits and risks. These health products have important benefits such as: prevention, treatment, and control of bacterial and parasitic diseases contributing to good animal welfare and ensuring human food security and avoiding foodborne illness; protection of human against zoonosis preventing human hospitalizations and deaths; enhancement of animal production by the improvement of feed conversion ratio, animal growth, and reproductive performance; and improvement of environment by reducing manure disposal amounts and consequently the emissions of greenhouse gases. On the other hand, animal antibiotic use impacts on the development of drug-resistant pathogens, residues in food products occurrence, and also may influence on biological treatment methods of waste products (Hao et al. 2014; Sneeringer et al. 2015).

8.2 World Antibiotic Consumption in Food-Producing Animals

Antibiotics have been used to treat infections in animals for as long as they have been widely available (CDDEP 2015); however, the incorporation in animal husbandry practices became more frequent in the twentieth century. Agricultural activities represent a large proportion of the usage of antibiotics in worldwide antibiotic consumption (O'Neill 2015; Gonzalez Ronquillo and Angeles Hernandez 2017). For example, in the USA, of the antibiotics defined as medically important for humans by the US Food and Drug Administration (FDA), over 70% (and over 50% in most countries in the world) are sold for use in animals (O'Neill 2015). There is a lack of reliable information on global use of antibiotics in livestock; however, it is estimated that in 2010, more than 63,000 tons of antibiotics were used in food animals worldwide and probably will reach more than 105,000 tons by 2030. The five countries with the largest shares of global of antimicrobials used in livestock in 2010 were China, USA, Brazil, Germany, and India with approximately 15,000 ton (23%), 8500 ton (13%), 5500 ton (9%), 2000 ton (3%), and 2000 ton (3%), respectively, and the five countries with the greatest projected percentage of antimicrobial consumption by 2030 are China, USA, Brazil, India, and Mexico with approximately 33,000 ton (31%), 12,000 ton (11%), 8500 ton (8%), 4500 ton (4%), and 2500 ton (2%), respectively (Van Boeckel et al. 2015).

Looking at antimicrobial use between countries, heterogenic geographic distribution can be seen. The reasons often are: particular preferences (handling, market, etc.), national custom, practice and legislation, level of industrialization of animal production, and availability of number of authorized veterinary medicines (Jarvis et al. 2011; Gonzalez Ronquillo and Angeles Hernandez 2017). Changes in disease pattern (outbreaks) and changes in climatic condition per year also influence antibiotic consumption variations (De Briyne et al. 2014; Federation of Veterinarians of Europe—FVE 2016). An overview of classes and amounts of

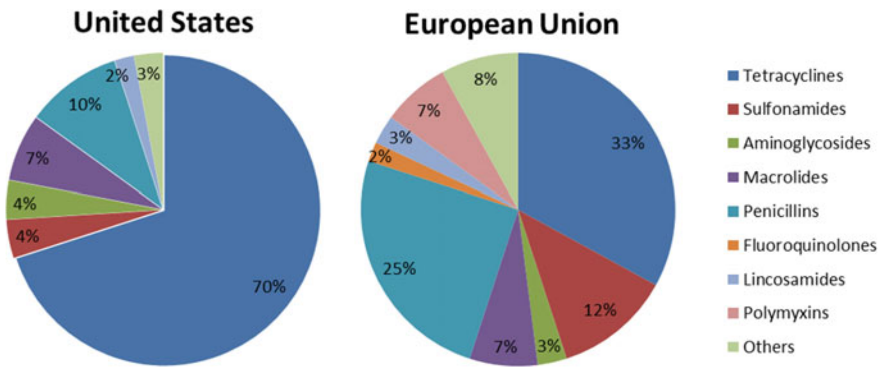

Fig. 8.2 Percentage of antimicrobial sales (by class) for food-producing animals in the USA in 2016 and European Union in 2015. *Source* ESVAC (2017); FDA (2017)

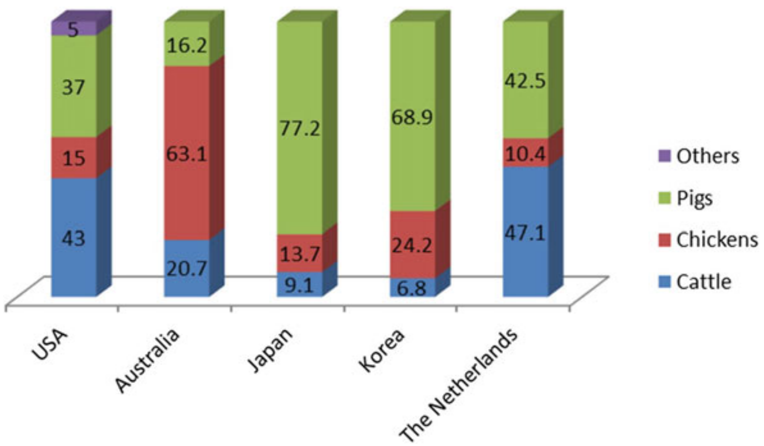

Fig. 8.3 Estimative of the intention use (percentual based on domestic sales) of medically important antimicrobials in food-producing animals. *Source* FDA (2017); Van Boeckel et al. (2015)

antimicrobials used in food-producing animals in USA and in European Union (EU) is shown in Fig. 8.2. Figure 8.3 presents an estimative of veterinary antimicrobial consumption by livestock species in food animals of five countries.

Certain antimicrobial drugs used in food-producing animals are considered "not medically important" like ionophores, polypeptides, orthosomycins, pleuromutilins, aminocoumarins, glycolipids, and quinoxalines. Ionophores, for example, are only used in veterinary medicine; thus, public health risks are much lower than medically important antimicrobials. Despite being not antimicrobial resistance target compounds, not medically important antimicrobials are widely used; for example, in USA, in 2016, tetracycline accounted for 5,866,588 kg and ionophores for 4,602,971 kg of the domestic sales (FDA 2017).

8.3 Antibiotic Used in Food-Producing Animals

Antibiotics, by definition, are chemical substances produced by microorganisms or by synthetic ways which acts by disrupting various molecular targets within bacteria and cell surface preventing growth (bacteriostatic) or initiating killing (bactericidal) (Etebu and Arikekpar 2016). There are several kinds of antibiotics, and they can be classified by different forms; however, the most common are based on their chemical structure or mechanism of action. Assuming that antimicrobial consumption in cattle, pigs, and chickens represents the majority of antimicrobial consumption in food-producing animals, Table 8.1 contains the most common groups which may be used at different times in the life cycle of these animals.

The frequently occurring diseases in food-producing animals that are likely to be treated with antibiotics are mastitis, uterine infections (metritis), joint infections, foot rot, digital dermatitis, and salmonellosis in dairy cows; enteritis, septicemia, umbilical infections and polyserositis, pneumonia, diphtheria, and foot rot in calves; erysipelas, joint infections, foot rot, mastitis, and uterine infections (metritis) in breeding sows; enteritis, septicemia, meningitis, umbilical infections, and skin infections in weaned piglets; enteritis, pneumonia, and tail bite infections in fattening pigs; enteritis, respiratory infections, septicemia, and yolk sac infection in chickens (De Briyne et al. 2014; ESVAC 2017).

8.4 Antibiotic Occurrence in Manure

Animal operations may vary widely in the administration of medicine. The occurrence in manure is dependent from the quantity administered (dosage) and the capability of excretion by the animal. Some of the factors that can influence on the manure antibiotic amount are: difference breeding performance among farms, prescription pattern for different animal types or stage of production (e.g., piglets, growing-finishing pigs, swine sows, dairy cow, and calf), susceptibility of diseases

Table 8.1 Most common group of antimicrobials used food-producing animals

Antimicrobial class	Mechanism of action[a,b,c]	Examples[d,e]
Tetracyclines	Inhibits protein synthesis	Tetracycline, chlortetracycline, oxytetracycline
Sulfonamides	Inhibits nucleic acid synthesis (folic acid metabolism)	Sulfadimethoxine, sulfamethazine
Aminoglycosides	Inhibits protein synthesis	Dihydrostreptomycin, gentamycin, Hygromycin B, Neomycin, spectinomycin
Macrolides	Inhibits protein synthesis	Erythromycin, gamithromycin, tildipirosin, tilmicosin, tulathromycin, tylosin, Tylvalosin
β-lactams		
Penicillin	Inhibits steps in bacteria cell wall synthesis	Amoxicillin, benzylpenicillin (penicillin G), ampicillin, cloxacillin
Cephalosporins		Ceftiofour, cephapirin
Fluoroquinolones	Inhibits DNA replication and transcription (DNA gyrase)	Danofloxacin, enrofloxacin
Lincosamides	Inhibits protein synthesis	Lincomycin, pirlimycin
Polymyxins	Destroys cell membrane structure or function	Polymyxin B
Streptogramins	Inhibits protein synthesis	Virginamycin
Amphenicols	Inhibits protein synthesis	Florfenicol
Diaminopyrimidines	Inhibits nucleic acid synthesis (folic acid metabolism)	Ormetoprim, trimethoprim
Polypeptides	Destroys cell membrane structure or function	Bacitracin
Orthosomycins	Inhibits protein synthesis	Avilamycin
Pleuromutilins	Inhibits protein synthesis	Tiamulin
Aminocoumarins	Inhibits DNA replication and transcription (DNA gyrase)	Novobiocin
Glycolipids	Inhibits steps in bacteria cell wall synthesis	Bambermycins
Quinoxalines	Unknown	Carbadox
Ionophores (polyether)	Modifies the permeability of cellular membranes	Lasalocid, monensin, salinomicyn, narasin, laidlomycin

[a]Etebu and Arikekpar 2016; [b]Lambert 2012; [c]Sperelakis 2012; [d]FDA 2017; [e]ESVAC 2017

according to seasons, breeding scale (in heads), relation between antibiotic price and farm income, type of animal diet, and difference between animal races (Haller et al. 2002; Jacobsen and Halling-Sørensen 2006; Pan et al. 2011; Chen et al. 2012a, b; Tong et al. 2012; Zhou et al. 2013).

For example, much higher concentration of sulfonamides and tetracyclines compounds was found in piglet manures when compared with fattening pig or sow manures (Pan et al. 2011). Some studies found higher antibiotic residues in swine manure in winter time (Chen et al. 2012b). The seasonality could be explained according to two possibilities: In winter, the animals are more susceptible to respiratory illness and need more antibiotics. On the other hand, in summer the high temperatures favor the bacteria activity that generally improve the use of drugs to combat diarrhea causes (e.g., anticoccidial) in preference to antibiotics to combat respiratory diseases. In addition, higher temperatures accelerate biodegradation and probably reduce antibiotic residues in manure at summer time. Table 8.2 shows a resume of antibiotics found in manure and livestock by-products around the world.

Table 8.2 Examples of antibiotics levels reported in livestock waste samples

Antimicrobial	Animal type	Sample	Levels	Reference
Tetracyclines				
Tetracycline	Swine	Sludge	130.6–3617.2 µg/kg	Pan et al. (2011)
	Swine	Manure	98.2 mg/kg	Chen et al. (2012a)
	Swine	Liquid manure	0.36–23 mg/kg	Martínez-Carballo et al. (2007)
Chlortetracycline	Swine	Manure	139.4 mg/kg	Chen et al. (2012a)
	Swine	Liquid manure	0.1–46 mg/kg	Martínez-Carballo et al. (2007)
	Dairy cattle	Manure	1450 µg/kg	Zhou et al. (2013)
Sulfonamides				
Sulfamethazine	Swine	Manure	3.3–24.8 mg/kg	Chen et al. (2012a)
Sulfathiazole	Swine	Manure	0.10–12.4 mg/kg	Haller et al. (2002)
Sulfamonomethoxine	Dairy cattle	Effluent	4.03 ng/L	Zhou et al. (2013)
Macrolides				
Tiamulin	Swine	Manure	0.1 mg/kg	Pan et al. (2011)
Tylosin	Swine	Wastewater	0.56–42 µg/L	Tagiri-Endo et al. (2009)
Lincosamides				
Lincomycin	Swine	Feces	0.16–17.0 mg/kg	Zhou et al. (2013)
	Dairy cattle	Effluent	700–6600 ng/L	Brown et al. (2006)
Quinolones				
Enrofloxacin	Swine	Dung	0.48–33.26 mg/kg	Zhao et al. (2010)
	Poultry	Manure	1420 mg/kg	Zhao et al. (2010)
	Poultry	Litter	30.97 mg/kg	Leal et al. (2012)

(continued)

Table 8.2 (continued)

Antimicrobial	Animal type	Sample	Levels	Reference
Norfloxacin	Swine	Dung	0.56–5.50 mg/kg	Zhao et al. (2010)
	Poultry	Manure	225 mg/kg	Zhao et al. (2010)
	Poultry	Litter	4.55 mg/kg	Leal et al. (2012)

Different analytical strategies have been employed to determine the antibiotic occurrence in wastewater and manure. Manure and wastewater samples are very complex to be analyzed, mainly because of the particulate matter, organic matter content, and various inorganic species which could cause serious matrix interferences. To eliminate or reduce interferences, usually are applied sequential liquid–liquid extraction and/or cleanup with solid phase extraction (SPE) on the sample preparation step (Hu et al. 2008). For estimation of antibiotic quantity, some authors report the use of very simple methods based on ELISA or radioimmunoassay (Aga et al. 2003). These methods, although cheap, are nonspecific and very inaccurate generally used as screening tool. For better specificity and accuracy, analysis is necessary to apply chromatographic methods like HPLC-UV, LC-MS, or LC-MS/MS (Schlüsener et al. 2003; Jacobsen and Halling-Sørensen 2006; Hu et al. 2008; Chenxi et al. 2008; Zhou et al. 2012).

8.5 Antibiotic Interaction in AD

Various antibiotics used in humans or animals have been studied asanaerobic digestion interactions agents. Studies have been focused to degrade substrates such as sewage, manure (cow and pig), and pure substances (e.g., glucose and organic acids standards) (Massé et al. 2000; Gartiser et al. 2007; Shimada et al. 2011). Interference reported in the anaerobic digestion is diverse, such as excessive foaming in the reactor, decline in biogas productivity, accumulation of organic acids, and imbalances in microbiology community (Fischer et al. 1981; Sanz et al. 1996; Shimada et al. 2011).

8.5.1 Methods for Evaluation of Antibiotic Effect in AD

Different methods are used to evaluate the antibiotic effect during anaerobic digestion, varying according to the objective of the study proposed by each author. Usually, the methods are based on a batch test, similar to a biochemical methane potential (BMP) assay, to establish comparisons at the biogas production and kinetics parameters between tests with different antibiotic concentrations. The baseline for the experimental setup is found at the standard protocol ISO 13641, Water quality—Determination of inhibition of gas production, also called anaerobic toxicity assay (ATA). This test permits to evaluate the acute inhibition effect in biogas production (International Organization for Standardization 2003).

The ISO protocol consists of incubation of the anaerobic inoculum together with a standard substrate (yeast extract and glucose) and spiked with different concentrations of the inhibitory agent to be evaluated. Cumulative biogas volume produced is measured after 3 days of incubation at 35 °C and compared to the test without addition of the inhibitor. For each test, the percent of inhibition is obtained applying Eq. 8.1. The ISO experimental setup consists in 0.1–1 L of pressure-resistant gastight closed glass test bottles, coupled at precision pressure meter for measuring total biogas production. Original equation is based on the pressure variation measured by manometric systems. Equation 8.1 presents an adaptation by equivalence of volume variation:

$$I(\%) = \left(1 - \frac{V_t}{V_c}\right) \times 100 \tag{8.1}$$

where I is the inhibition percentual, V_t is the cumulative volume produced with test material (with antibiotic), and V_c is the cumulative biogas volume produced in the control at the same time. After, as illustrated in Fig. 8.4, it is possible to plot I against the logarithm of the concentrations of test material. The inhibitory concentration (IC or EC) value could be assessed visually or by regression analysis. Alternatively, it is possible to express a correlative inhibition based on inoculum mass used in each assay (International Organization for Standardization 2003).

Usually, the IC_{10} (concentration that produces 10% of inhibitory effect on biogas production) represents the minimum quantified level of inhibition or the method limit of detection. The IC_{50} (concentration that produces 50% of inhibitory effect on biogas production) is the standard parameter to compare toxicity between different antibiotic compounds.

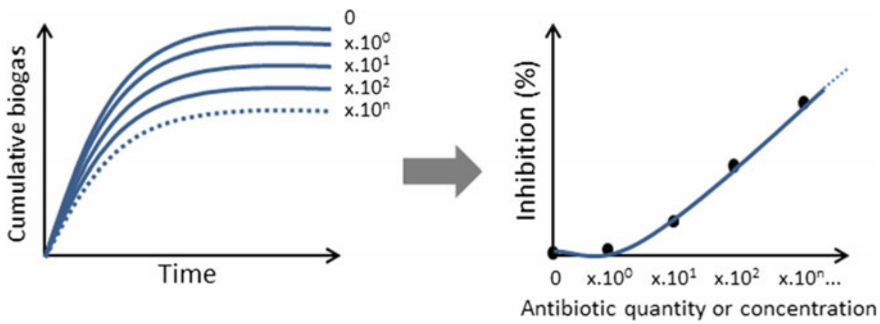

Fig. 8.4 Graphical demonstration of biogas data processing to obtain the inhibition response in ATA

Table 8.3 Suggested substrates for activity evaluation of a different trophic group anaerobic digestion inoculum

Population	Substrate
Hydrolytic	Cellulose
Acidogenic	Glucose
Proteolytic	Casein
Acetogenic	Propionic acid + n-butyric acid
Acetoclastic	Acetic acid
Hydrogenotrophic	$H_2 + CO_2$

Adapted from Angelidaki et al. (2009)

By the way, this methodology can be adapted according to the study purposed. Alternative substrates could be used to evaluate the activity or inhibition effect on the group of target microorganisms, as presented in Table 8.3 (Angelidaki et al. 2009). For example, Cetecioglu et al. (2012) used acetate to evaluate sulfamethoxazole, erythromycin, and tetracycline effect in acetoclastic activity after 5 days of digestion, using domestic anaerobic sludge as anaerobic inoculum. Other example is microcrystalline cellulose as the standard substrate applied by Steinmetz et al. (2016) to simulate a condition of co-digestion of manure and agricultural wastes. According to Angelidaki et al. (2009), each kind of substrates corresponds to stimulate the activity of different trophic groups of microorganisms from the anaerobic inoculum (Table 8.3). This means that during the ATA procedure it is possible to adapt the test conditions according to the type of substrate desired and generate results to specific conditions.

Similarly, other change in the ISO methodology could be proposed. Instead of evaluating only biogas production, it is also possible to use kinetic parameters as response variables to estimate the ATA of a substance. In this case, it is possible to evaluate if the toxic compound acts to inhibit on the adaptive phase (e.g., hydrolysis), on the biogas rate, or on the BMP.

Chronic (long-term) toxicity evaluation is also possible, but the methods are not standardized. Usually, for this case, a laboratory-scale reactor is continuous (or semi-continuous) feeding with a fixed organic loading rate and with progressive small increment of antibiotic loading (Bressan et al. 2013). The increment of antibiotic should be done by biogas productiveness stability or defined by fixed time (e.g., respecting 3 times of HRT).

8.5.2 Review of Inhibition Effect in Biogas Production Process

Inhibition during anaerobic digestion, when it occurs, has a negative impact on the generation of the products derived from the process. In addition to the reduction of the volume of biogas produced it can also reduce the contents of methane and consequently the accumulation of intermediates (e.g. organic acids). These effects in the digester are generally related to unbalance of microcosmos. The literature reports about antibiotic inhibition show variations in the toxicity level. Such variations depend on the type of chemical substance used as antibiotic, on the conditions of the ATA test, and also on the possible conditions of acclimatization of the anaerobic sludge (which could be related to the development of antibiotic resistance mechanisms from the microorganisms).

Even though ATA tests are performed using direct spike of the antibiotic before the inoculation test, there are reports confirming the inhibition of biogas production using manure from medicated animal. Turker et al. (2013) evaluated the anaerobic digestion manure from bovine medicated with oxytetracycline (concentrations of 0.8–3.4 mg/L). After mesophilic anaerobic digestion (37 °C), they found inhibition effect of 17–24% in biogas yield from manure with 0.8–3.4 mg/L of oxytetracycline, respectively. The authors also identified the reduction of *Methanomicrobiales*, *Methanobacteriales,* and *Methanosarcinaceae* and suggested as the main cause for the biogas reduction.

Sanz et al. (1996) found 20% of inhibition in methane production in digestion with 35 mg/L of gentamicin. Authors used synthetic media, a mixture of organic acids as standard substrate, and spiked different concentrations of gentamicin (5–250 mg/L). The inhibition effect was evaluated after 2 weeks of incubation and described accumulation of propionic and butyric acid in order of 10–50% based on the acid added and compared to the free antibiotic control test. In another study, Gartiser et al. (2007) found IC_{10} for biogas in gentamicin concentration range of 0.4–7.2 and IC_{50} in range of 57.2–231.8 mg/L. At this case was applied the ISO test scheme for ATA using inoculum source from a municipal sewage treatment plant and measured biogas inhibition after 7 days in mesophilic condition (35 °C).

Fischer et al. (1981) related a stress condition of swine manure anaerobic digestion process. They observed a drop of 75% on the gas production, high propionic acid content (>3000 mg/L) in the digestate and operational problems by foam in the digester headspace. The reactor stress condition was related to the possible presence of antibiotic lincomycin. Ji et al. (2013) used a luminescent fast method to evaluate the methanogens biological activity and related to the acute inhibitory effect of lincomycin in anaerobic digestion. The authors found IC_{50} level of 3.5–5.7 g/L and related synergic toxicity effect when the lincomycin was mixed with other antibiotics (amoxicillin, kanamycin, and ciprofloxacin). The authors report a strong synergism in the toxicity effect of lincomycin in the presence of metabolites from anaerobic digestion process (ethanol, acetate, propionate, and butyrate). This indicates a possibility of lincomycin present in the digestate to decrease the digester efficiency in reactors operating at stressed conditions (e.g., overload or feedstock nutrient unbalance) or any reason to promote accumulation of VFA inside the reactor. At this case, the process could be more susceptible to suffer inhibition when receiving lincomycin.

Already for the macrolide tylosin, Poels et al. (1984) did not see disturbance effect in swine waste anaerobic digestion in the presence of 1.7–16.7 mg/L.

The experiment was conducted in a 1.5 L CSTR operated at 30–33 °C. However, Sanz et al. (1996) found 20% inhibition of methane production in batch assays, using tylosin at 15 mg/L. Besides that, as a consequence they found accumulation of propionic and butyric acids in order of 10–90% added.

Shimada et al. (2008) evaluated addition of small quantities (1.67 mg/L) of tylosin in glucose-fed ASBR. They observed decrease in the rates of propionate uptake and methane production, without effects on COD removal efficiency. But, at higher concentration (167 mg/L) of tylosin added in the digester, the authors observed high disturbance in the reactor performance: decrease in the glucose uptake rate, accumulation of acetate and propionate, and drop in the COD removal efficiency. Subsequently, the authors identified that there was an imbalance between the population of fermentative bacteria and methanogens archaea, with great impact on acetoclastic methanogens. Gram-positive glucose-fermenting bacteria maintained activity with tylosin, and propionate-oxidizing syntrophic bacteria were detected less frequently after tylosin introduction. The authors postulate relation between the inhibition in propionate uptake rate and occurrence of *Syntrophobacter* (consuming propionate bacteria) sensitive to the antibiotic. Finally, the methane reduction efficiency was explained for the combination of tylosin resistance in glucose-fermenting bacteria and inhibition of propionate-oxidizing bacteria resulted in accumulation of VFA.

Studies about methane emission of swine manure in anaerobic lagoons reported methane emission tended to plateau rapidly between 20 (after 72 h) and 45% (336 h) with addition of antibiotic lincomycin and tylosin in dosage between 1 and 25 mg/L (Loftin et al. 2005). The same test was done with an inoculum sludge collected from another lagoon with less antibiotic exposure, however it was observed quicker methane emission reduction. This could be related to a higher inhibition effect and could be a consequence of a better microorganisms' adaptation in the lagoons that were in greater contact with the antibiotics.

Bressan et al. (2013) evaluated a long-term exposure (450 days) of colistin sulfate (polymyxin E) in a UASB bench-scale reactor. The UASB was inoculated using swine manure and feed with acetate as substrate. The concentration of colistin was varied from 0.1 to 100 mg/L. Authors report that methanogenesis activity showed high tolerance to colistin, not showing relevant inhibition in methane production, even at the highest concentrations tested. The highest concentration tested is much higher than the one expected in swine wastewater (generally below 1 mg/L).

In other chronic toxicity experiment, Shimada et al. (2011) developed a long-term study of the digestion of swine manure in the presence of macrolide antibiotics. They observed accumulation of acids, especially propionic acid, and verified that antibiotics directly affected the action of propionate-oxidizing syntrophic bacteria, especially of the genus *Syntrophobacter*, and indirectly inhibited *Methanosaeta*.

8.5.3 Persistence in Digestate: Factors of Fate Residual Antibiotic

It is also important to identify the risk mitigation potential for the degradation of the antibiotic compounds in the digester. Besides the necessity to elucidate the effects of the antibiotic compounds during the anaerobic digestion process, it is relevant to understand the persistence and mechanism of degradation during the anaerobic digestion. The persistence of antibiotic after digester could release contaminant on soil by fertilizer pathway or represent inhibition effect to sequential biological process. Several studies have been demonstrating an opportunity to use the anaerobic route to the removal of antibiotic residues in wastewater or animal manure.

Recently, Steinmetz et al. (2016) observed a significant reduction of tetracycline compounds in a batch test after 35 days at mesophilic digestion (37 °C). The authors were evaluating the persistence of spiked (1.3–809 mg/L) tetracycline, chlortetracycline, metacycline, and oxytetracycline, using LC-MS/MS analysis. The most part of assays show reduction of 76–98% at antibiotic concentration. Only higher concentration of tetracycline (508 mg/L) and metacycline (104 mg/L) had efficiency removal decrease. For these assays were observed 46 and 57% of antibiotic reduction, respectively.

The reduction levels of tetracycline were similar to those described by Tong et al. (2012). The author found 88.6–91.6% of tetracycline reduction and 97.7–98.2% of chlortetracycline reduction after 45 days of swine manure anaerobic digestion at mesophilic conditions (35 °C). Turker et al. (2013) reported 55–70% of oxytetracycline reduction after 30 days of anaerobic digestion, at 37 °C, in manure feed with the antibiotic.

Generally, the antibiotic persistence is dependent of synergic effect of temperature and microbiological activity. Schlüsener et al. (2006) defined a high persistence (half-life > 200 days) of the macrolide tiamulin during the swine manure storage under anaerobic condition and 20 °C. In another study, Li et al. (2018) compared antibiotic persistence in manure samples stored at 15 and 35 °C. Antibiotic reduction was more notable when digestate was stored under mesophilic conditions. Regardless of storage conditions, in cases when organic matter was further biodegraded, the residuals of antibiotics in digestate were lower. In general, more biological activity results in less antibiotic persistence after AD.

8.6 Conclusion

The presence of antibiotic substances promotes adverse reactions in biogas production. However, the toxicity degree is dependent on a broad of factors: type of antibiotic, residual concentration, temperature of the digester, and whether the microorganisms from the sludge underwent any process of acclimatization and enrichment for resistance development.

This reinforces the importance of knowing possible contaminants present in the substrate and thus predicts process changes. In some cases, the effect from antibiotic could promote synergic effect (e.g., FVA accumulation) and the anaerobic process could be more susceptible to suffer inhibition when the reactor was operated at stressed conditions. In addition, the biological effects of the combination of some drugs are still unknown.

Nevertheless, the anaerobic digester still is an important tool to treat the residual antibiotic content present in manure, for example. In this way, anaerobic digestion of livestock wastes represents an opportunity to risk mitigation potential related to intensification use of veterinary drugs in SPACs.

Despite increasing efforts to increase the rational and prudent use of antibiotics in all contexts to prevent the development of resistant bacteria, the presence of antibiotics in urban, industrial, and agricultural effluents will continue to exist.

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