Sewage Sludge Pretreatment Strategies for Methane Recovery and Sanitization

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Deisi Cristina Tápparo, Paula Rogoviski, Rafael Favretto, Rafael Dorighello Dadamuro, Aline Frumi Camargo, Thamarys Scapini, Estêvão Brasiliense de Souza, Doris Sobral Marques Souza, Fabiane Goldschmidt Antes, Ricardo Luis Radis Steinmetz, Airton Kunz, Marta Hernández, Helen Treichel, Gislaine Fongaro, and David Rodríguez-Lázaro

D. C. Tápparo
Western Paraná State University - UNIOESTE/CCET/PGEAGRI, Cascavel, PR, Brazil

P. Rogoviski · R. D. Dadamuro · E. B. de Souza · D. S. M. Souza · G. Fongaro
Laboratory of Applied Virology, Department of Microbiology, Department of Microbiology, Immunology and Parasitology, Federal University of Santa Catarina, Florianópolis, SC, Brazil

R. Favretto
Center for Agricultural Sciences, Santa Catarina State University, Lages, Brazil

A. F. Camargo · T. Scapini · H. Treichel
Laboratory of Microbiology and Bioprocesses, Federal University of Fronteira Sul, Erechim, RS, Brazil

F. G. Antes · R. L. R. Steinmetz
Embrapa Suínos e Aves, Concórdia, SC, Brazil

A. Kunz
Western Paraná State University - UNIOESTE/CCET/PGEAGRI, Cascavel, PR, Brazil

Laboratory of Microbiology and Bioprocesses, Federal University of Fronteira Sul, Erechim, RS, Brazil

Embrapa Suínos e Aves, Concórdia, SC, Brazil

M. Hernández
Division of Microbiology, Department of Biotechnology and Food Science, Universidad de Burgos, Burgos, Spain

D. Rodríguez-Lázaro (✉)
Division of Microbiology, Department of Biotechnology and Food Science, Universidad de Burgos, Burgos, Spain

Research Centre for Emerging Pathogens and Global Health, University of Burgos, Burgos, Spain
e-mail: drlazaro@ubu.es

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Abstract

Sludge, a by-product or residue of wastewater treatment facilities, has considerably increased the generation over the years. Due to its large amount and content, organic matter, metals, and pathogens, sludge poses an environmental and health risk if not properly managed. Furthermore, stabilization and management of this residue maintain affordable costs on wastewater treatment plants (WWTPs). Anaerobic digestion (AD) is a promising technology to sludge valorization; however, it needs to be made more effective because this waste leads to low degradability and consequently low energy production. Pretreatments can be used to hydrolyze sludge and consequently improve biogas production, solid removal, and sludge quality after digestion, increasing the applicability of AD. Different technologies are being studied by physical-chemical and biological methods. This chapter addresses an overview of different technologies for pretreatment, focusing on thermal, ultrasonic, and enzymatic processes, discussing their effects on sludge properties and anaerobic digestion. Concerns related to pretreatment implementation, pathogen distribution, and directives around the world are also addressed.

Keywords

Anaerobic digestion · Thermal pretreatment · Enzymes · Ultrasonic · Pathogen

10.1 Sewage Sludge

Biological wastewater treatment processes have been widely used to treat municipal wastewater, as a result of efficient organic removal, despite the large amounts of sludge produced (Wang et al. 2017). Municipal wastewater treatment plants (WWTPs) generate sludge as a by-product of the physical and biological processes used (Appels et al. 2008). The adequate destination of biosolids is a task of great importance for growing populations and pollution reduction efforts aimed to limit the harmful by-product generation and spread (Praspaliauskas and Pedišius 2017). Nowadays, the treatment and disposal of sludge have become one of the major challenges faced by WWTPs (Xu et al. 2017), due to high costs to manage and dispose.

Sludge can be classified into primary and secondary sludge (or activated sludge). The primary wastewater treatment involves screening to remove large constituents, after by gravity sedimentation of the screened wastewater or by physical-chemical processes (i.e., coagulation, flocculation, flotation) with a solid diverted to a different stream (Elalami et al. 2019; Tyagi and Lo 2011). The residue from this process is a concentrated suspension, called primary sludge, which is further treated to become a biosolid; this step removes about 40–50% of solids in wastewater (Demirbas et al. 2017; Elalami et al. 2019). Secondary sludge is produced during biological process, consisting mainly of bacteria growing on organic and inorganic substances, extracellular polymeric substances (EPS), and recalcitrant organics from wastewater or formed during bacterial decay (Wang et al. 2017), being composed of 59–88% (w/v) organic matter, which is decomposable and produces the offensive odors and 95% is water (Tyagi and Lo 2011).

Sludges have higher pathogen concentration, such as bacteria, viruses, protozoa, and other parasitic helminths, as well as organic matter can create potential hazards to humans and animal health, needing additional treatments to ensure a product can be safely integrated back in the product chain (Neumann et al. 2016). Also, sludges are often contaminated by non-biological components such as heavy metals, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, pharmaceuticals, and pesticides, among other contaminants (Wiśniowska 2019). The WWTPs developed over the years the concern about sludge treatment, the amount of sludge increased more and more, and with that treatment technologies are improved and change together (Praspaliauskas and Pedišius 2017).

The first directive created to standardize utilization of treated sludge in agriculture or soils in European Commission dates from 1986 (86/271). The estimative directive denotes the production of 25 kg/(P.E × year) and 68 g/(P.E × day) of dry matter (DM) in 15 member states (Kelessidis and Stasinakis 2012; Milieu 2010).

There is a huge variation in values in the world: Brazil and China possess the minimum values, counting with 5.4 and 6.2 g dry matter/(P.E × day). On the other hand, the countries with maximum values are Germany, the United Kingdom, Slovenia, Finland, and the Netherlands: 66.5 g DM/(P.E × day), 67.8 g DM/(P.E × day), 77.7 g DM/(P.E × day), 78.6, and 249 g DM/(P.E × day), respectively (LeBlanc et al. 2008). The discrepancy about the established values of minimum and maximum production of sludge per capita is based on the volume treated by person and involved level of treatment. There are other influences in production per capita, such as the type of sewerage system with capability of separation of rainwater from wastewater. Large cities possess an underground system of drainage to collect wastewater, causing dilution of the volume and diminishing COD removal efficiency as well as sludge production (Mininni et al. 2015).

Some countries are developing stringent limits to directive use of sludge, as, for example, Belgium, Austria, Finland, Denmark, Germany, Slovenia, Sweden, the Netherlands, the Czech Republic (European Union 2008). Several WWTPs in Europe don't possess the technical equipment necessary to process sludge, making it suitable for other destinations. Due to the infrastructure dated from 1980s or even earlier, these plants are incapable of dovetail in new directives to dispose of landfill or via incineration (Mininni and Dentel 2013). Among this, WWTP aims to reduce sludge production turning to more feasible disposal costs via incineration or landfilling. At the same time, there are innovative processes that allow reduction of pathogens and diminish odor.

Among the contaminants there are some minerals that can be harmful when in high concentrations (mg/kg) in sludge with agricultural purposes, such as Se, Co, Zn, and Mo, that are not standardized by sludge directive (European Union 1998). Otherwise, there are concentrations allowed to potential toxic elements (PTE) in sludge with agriculture destination. Low limits are established in Finland, Latvia, Flanders, Belgium, Denmark, Sweden, the Netherland, Malta (for pH 5–6), and Carinthia (for pH 5.0–5.5).

Other countries allow limits like the sludge directive. Variation of pH in soil is utilized to determine the concentration limit of PTE in some places (Bulgaria, Spain, Portugal, Malta, and Carinthia). The amount of heavy metals is also limited during a period that can vary between 3 and 10 years in places such as Hungary, Luxembourg, Italy, France, Sweden, Finland, the Netherlands, Flanders, and Three Lander in Austria (Mininni et al. 2015). The procedures for agricultural use of sludge in Brazil are established by CONAMA Resolution 375/2006; criteria includes the determination of pathogen control (fecal coliform <3 MPN/100 g; *Salmonella* sp. absence in 10 g TS; viable helminths eggs <0.25 egg/g TS; virus <0.25 PFU g TS), bacteriological and inorganic substances, and the monitoring of 34 organic substances in sewage sludge, specifying maximum concentration maximum heavy metal contents in sludge for agriculture (as it follows: As $=$ 41, Ba $=$ 1300, Cd $=$ 39, Pb $=$ 300, Cu $=$ 1500, Cr $=$ 1000, Hg $=$ 17, Mo $=$ 50, Ni $=$ 420, Se $=$ 100, and Zn $=$ 2800 mg/kg SS (dry matter basis)) (CONAMA 2006).

The aim of these regulations is to protect the environment, and different sludge treatment and disposal are studied, as composting, landfill, land application, drying-incineration, and anaerobic digestion (AD). Anaerobic digestion is commonly used in WWTP for degradation of sludge, being transformed into methane and carbon dioxide and some smaller amounts of biosolids as the final residue. The methane generation is an attractive feature because it can be used as energy. However, how to maximize methane production has been a subject of special consideration.

10.2 Pretreatments Applied to Improve Biodegradability During Anaerobic Digestion

The AD requires strict anaerobic conditions to proceed and depends on the successive activity of a complex microbial association to transform organic material into methane (CH_4). However, hydrolysis is generally considered as rate-limiting step (Appels et al. 2008). The low efficiency of the microorganisms (hydrolysis stage) is due to sludge characteristics, mainly flocs, EPS aggregates, recalcitrant compounds of lipids and proteins, and cell walls/membrane that form strong barriers to degradation. These compounds also are responsible for increased hydraulic retention time of biodigester, once it spends more time to hydrolysis, and therefore methane production is slow (Abelleira-Pereira et al. 2015; Anjum et al. 2016).

As a result of sludge characteristics, various pretreatment methods have been developed over time to maximize biogas production. If properly designed, pretreatment process is recommended to (1) modify the physical and chemical structure of sludge, (2) solubilize organic matter, (3) increase the surface area and accelerate hydrolysis step, and (4) consequently improve methane generation (Elalami et al. 2019; Hu et al. 2019; Zhen et al. 2017). Pretreatment technology involving mechanical, chemical, physicochemical, and biological methods and their combinations have been tested in treating residual sludge.

10.2.1 Thermal Hydrolysis

Thermal pretreatment technology is a well-established, spread, and commercially implemented technology used to increase the degradability of sludge, being a process where the temperature of sludge is raised to a desired temperature to significantly increase the disintegration and solubilization of sludge solids (Pilli et al. 2015). Thermal pretreatment in the temperature range from 60 to 180 °C and is considered two types of thermal treatment process: low temperature (<120 °C) and high temperature (>120 °C). Normally, high-temperature treatment is associated with pressure in a range between 600 and 2500 kPa (Tyagi and Lo 2011; Pilli et al. 2015; Kor-Bicakci and Eskicioglu 2019).

The main advantages of thermal pretreatment includes the following: (1) increases biogas/methane yield; (2) improves sludge degradability; (3) allows increase organic loading rate, decreasing the size of biodigesters; (4) reduces sludge viscosity; (5) reduces odor and pathogens; and (6) reduces scum and foaming generation (Alfaro et al. 2014; Barber 2016; Xue et al. 2015). As other pretreatments, thermal pretreatment has some disadvantages, including increase in ammonia concentration, due to protein degradation, and costs with energy demand (Oosterhuis et al. 2014; Xue et al. 2015). Table 10.1 shows examples of thermal pretreatment effect on methane production, using different sludge types and different conditions.

The above studies of thermal pretreatment application, in general, show the increment on biogas/methane production, although in some studies this increase was not significant. The approach demonstrated that thermal pretreatment is conditioning between two variables: the exposure time temperature and pressure.

Biogas increment is linked to solubilization of organic matter (proteins, lipids, and carbohydrates) improved by higher temperatures and longer treatment times (Xue et al. 2015). As temperature increase, pretreatment is more efficient. However, temperatures above 180 °C lead to solubilization of recalcitrant and toxic organic compounds (melanoidins) reducing biodegradability (Pilli et al. 2015; Wilson and Novak 2009).

The viability of thermal pretreatment implementation in WWTPs is a crucial point, which must be analyzed, and it is necessary that energy demand of pretreatment does not exceed the biogas energy generation (Cano et al. 2015). Different pretreatment combination can be a promising alternative, generation one extra increment, and in this way contribute to viability implementation of pretreatment and consequently anaerobic digestion (Kor-Bicakci and Eskicioglu 2019).

Considering the advantages and researches over the time, the thermal pretreatment of sludge already implemented in full-scale WWTPs and is a commercial pretreatment technology, as described by Han et al. (2017), Kepp et al. (2000), Pérez-Elvira et al. (2008), and Zábranská et al. (2006), proving an increase on

Table 10.1 Biogas and/or methane production increase using thermal pretreatment method

Sludge type	Thermal pretreatment conditions	Anaerobic digestion conditions	Biogas or methane production increment	References
Activated sludge	80 °C for 6 h + mixed alkali (NaOH: $Ca(OH)_2$ molar ratios of 1: 4, 2:3, 1:1, 3:2, and 4:1)	Batch, 30 days—35 °C	Cumulative methane production increased until 308.7%, compared to the control group	Zou et al. (2020)
Activated sludge	60, 80, 100, and 120 °C for 30 min	Batch, 35 days—37 °C	Increase of methane production by 13.7%, 27.0%, 29.0%, and 29.6% when treated at 60 °C, 80 °C, 100 °C, and 120 °C, respectively	Kumar Biswal et al. (2020)
Activated sludge	130–170 °C for 30 min	Batch, 25 days—36 °C	Increase in methane potential of activated sludge (17–27%), increase in refractory sCOD in return load (3.9–8.4%), and dewaterability enhancement (12–30%)	Toutian et al. (2020)
Primary sludge	70 and 90 °C for 30 min	Batch, 12 days—36 °C	The pretreatment at 90 °C for 0.5 h was much more effective and increased the productivity of methane by 58.52% compared to untreated sewage sludge. While thermal pretreatment at 70 °C showed an improvement of only 12.70% in methane productivity	Mirmasoumi et al. (2018)
Activated sludge	70, 80, and 90 °C for 3 h/70 °C for 15 h	Batch, 20 days—35 °C	The pretreatment of 80 °C and 90 °C for 3 h showed an increase of 29.2% and 31.2%, respectively. As for the pretreatment at 70 °C for 3 h and 15 h, it showed an increase of 21.0% and 18.9% in methane production, respectively	Ruffino et al. (2015)

(continued)

Table 10.1 (continued)

Sludge type	Thermal pretreatment conditions	Anaerobic digestion conditions	Biogas or methane production increment	References
Activated sludge	170 °C 30 min, 7 bar	Continuous HRT: 12 days	Biogas production increased 40–50%	Yang et al. (2010b)
Activated sludge	70 °C for 10, 20, and 30 min 80 °C for 10, 15, and 30 min	Batch, 35 days—35 °C	Thermal pretreatment presented a methane potential similar with the untreated sludge	Ruiz-Hernando et al. (2014)
Secondary sludge	120, 150, 170, and 200 °C at 237 rpm for 1 h	Batch, 53 °C	The amount of gas produced increased with the temperature between 120 and 170 °C. However, at 200 °C, the gas production decreased 33% in comparison to 170 °C	Abe et al. (2013)
Activated sludge	1 reactor: 160 ± 1 °C and 0.55 MPa for 30 min 2 reactors: thermally pretreated at 60 ± 1 °C for 30 min with pH adjustment to 12	Semi-continuous, 92 days of operation with variation of HRT (100, 50, and 20 days)—37 °C	The methane productions and VS removals of two reactors were similar 150.22–151.02 mL methane/L/day and 22.54–23.15%, respectively	Xiao et al. (2020)
Primary and secondary sludge	60, 70, and 80 °C exposure time varied from 15 to 90 min	Semi-continuous, 175 days with variation of HRT (22 and 15 days)—37 °C	The biogas production can be increased more than 10%, and the digestion time can be shortened significantly (thermal pretreatment at 70 °C)	Liao et al. (2016)
Activated sludge	135 °C and 190 °C, for 30 min and 15 min, respectively	Semi-continuous, HRT was fixed at 20 days—35 °C	Thermal treatment allowed an increase in biogas production, around 12% for treatment at 135 °C and around 25% for a treatment at 190 °C	Bougrier et al. (2007)

(continued)

Table 10.1 (continued)

Sludge type	Thermal pretreatment conditions	Anaerobic digestion conditions	Biogas or methane production increment	References
Primary and secondary sludge	70 °C for 9, 24, and 48 h	Semi-continuous, HRT was fixed at 20 days—55 °C	Biogas yield was around 30% higher with pretreated sludge (0.28–0.30 L/g VS_{add}) when compared to raw sludge (0.22 L/g VS_{add}). Methane content in biogas was also higher after sludge pretreatment, around 69% vs. 64% with raw sludge	Ferrer et al. (2008)
Activated sludge	Continuous thermal 170 °C, HRT: 40 min, 7.6 bar followed by steam explosion	Pilot scale—CSTR (HRT 10 days)	Methane production increase until 82%	Souza et al. (2013)

biogas/methane production, reduction on hydraulic retention time, and sludge proved to have high fertilizer value.

10.2.2 Ultrasound

Ultrasound is a mechanical process of propagation of acoustic waves at a frequency higher than 16 kHz. Several phenomena are related to ultrasound depending on the applied frequency. On frequencies around 20 kHz, the most explored effect is cavitation which consist of a combined sequence of formation, growth, and collapse of microbubbles that occur is a very small interval of time (milliseconds) and realizing large amounts of energy locally. This realization of energy results in high pressure (in the range of 100–5000 bar) and temperatures (in the range of 1000–10,000 K), and these effects are observed at millions of locations in the reactor (Suslick 1990). At these conditions, •OH free radicals and H^+ ions are formed due to homolytic cleavage of water molecules. The recombination of these chemical species forms H_2O_2, a strongly oxidant compound, and therefore no selective chemical reactions can also occur in liquid media (Suslick 1990).

In sludge, the energy realized during cavitation disrupts bacterial cells by extreme shear forces, rupturing the cell wall and membranes (Bundhoo and Mohee 2018; Zou et al. 2016). The high temperature and pressure impact on physical and chemical characteristics of biomass or waste materials such as particle size, surface area, lignin, hemicellulose and cellulose content, and organic matter solubilization, among others. Sonication process for sludge treatment was studied by various researchers in laboratory and full-scale systems (Houtmeyers et al. 2014; Tyagi et al. 2014).

Dhar et al. (2012) showed the benefits of ultrasound on protein and carbohydrate solubilization on municipal waste-activated sludge. Pretreatment condition with ultrasound of 10,000 kJ/kg TSS for 10 min was used, and the temperature was maintained below 40 °C during the experiments. After pretreatment an increase in insolubilization of carbohydrate and protein of 730% and 764%, respectively, compared with the control (without the use of ultrasound) was observed. Besides, it promoted a sludge biochemical methane potential (BMP) increase of 24%. Na et al. (2007) studied the sonication and recognize a decrease in particle size of sewage sludge, due to floc disintegration, and the sludge dewater ability was improved.

The efficiency of ultrasonic disintegration is dependent on sludge characteristics, including type of sludge, primary or activated sludge, TS content and particle size, and sonification conditions (time, intensity, temperature, pH, amplitude, and power input) (Khanal et al. 2007; Tyagi et al. 2014). Based on kinetic models, ultrasonic disintegration was impacted in the order of the following: sludge pH > sludge concentration > ultrasonic intensity > ultrasonic density (Khanal et al. 2007). On the other hand, the opinion of many researchers is that the effect of ultrasonic density is supposed to be more vital than the sonication time to the acceleration of conversion of complex organics to biodegradable substrate (Pilli et al. 2011).

Numerous studies demonstrate the benefits and impact of ultrasound pretreatment on biogas/methane production using sewage sludge as substrate (Table 10.2), as well as the combination with other pretreatment types, like alkali (Bao et al. 2020; Zhang et al. 2017), low temperature (Neumann et al. 2017), and CaO_2 (Li et al. 2019).

In addition to the impacts on biogas/methane production, studies evidenced changes on the methanogenic pathway after ultrasound pretreatment. Li et al. (2018) observed through microbial diversity analysis that hydrolytic and acidification bacteria were abundant in the reactors treating waste-activated sludge. *Methanocorpusculum* and *Methanosaeta were* the alternating dominant methanogens in the anaerobic reactors, with addition of sludge after different ultrasonic treatment times. As the ultrasonic time increased, the relative abundance of *Methanocorpusculum*, which can grow by using hydrogen as substrate, increased from 55.9% (control) to 80.0%, after 40 min of ultrasound, and decreased rapidly to 5.7% of abundance after 100 min of ultrasound. However, the trend in the change of the relative abundance of *Methanosaeta* was the opposite to that of *Methanocorpusculum*. *Methanosaeta* was 27.0% in the controlled reactor but as low as 0.9% after 40 min treatment, with the maximal value of 67.7% after 100 min. The authors observed that the dominant substrate for anaerobic methanogenesis changed from hydrogen to acetic acid.

According to Pilli et al. (2011), sludge ultrasound pretreatment is one of the emerging technologies for increasing the biodegradability, but optimizing the

Table 10.2 Studies that evaluated the increase in the biogas and/or methane production in systems with ultrasound pretreatment

Sludge type	Pretreatment conditions	Anaerobic digestion conditions	Biogas or methane production increment	References
Municipal sludge	Time 5, 10, 15, 20, and 25 min. 19.1, 38.2, 57.3, 76.4, 95.5 kJ/g TS	BMP	Increase of 13%, 28%, and 35% on methane production, for respective times of 5, 10, and 15 min. However on 20 and 25 min, no increase on methane production was observed	Çelebi et al. (2020)
Mixed sewage sludge	Ultrasound-specific energy of 2000 kJ/kg TS. Thermal: 55 °C during 8 h and 70 rpm	Semi-continuous, variation of 30, 15, and 7.5 days SRT—37 °C	Sequential ultrasound-thermal pretreatment resulted in 19.1–29.9% increase in methane yield during sewage sludge anaerobic digestion	Neumann et al. (2018)
Waste-activated sludge	Frequency of 20 kHz, at different times (0, 20, 40, 60, 80, and 100 min) and at ultrasound densities of 0.5 W/mL	Semi-continuous stirred reactors (semi-CSTRs), HRT 20 days—37 °C	The gas production rate of each ultrasonic pretreated group was higher than the maximum of the control group	Li et al. (2018)
Sewage sludge	15 min in an ice bath, 20 kHz, 50 W (353 J/g TS)	BMP	Increased 34% of methane production	Mirmasoumi et al. (2018)
Activated sludge	3380 kJ/kg TS	BMP assay (35 °C)	Increment of 42% methane production and 13% VS removal	Riau et al. (2015)
Waste-activated sludge	100 W, 8 min, 96 kJ/kg TS	Semi-continuous, 37 °C, HRT 20 days	Increment of 27% biogas production	Houtmeyers et al. (2014)

methane yield (net energy yield is more than energy input) is necessary for full-scale implementation.

Xie et al. (2007) evaluated full-scale pretreatment using ultrasound for treating mixed sludge (primary and secondary sludge) and showed an increase in biogas daily production, up to 45% compared without pretreatment. Barber (2005) presented data of full-scale part-stream ultrasound pretreatment plants (Germany, Austria, Switzerland, Italy, and Japan) and showed biogas increased by 20–50% (volume/kg fed), and VS reduction improved on previous performance between 20%

and 50%. Tyagi et al. (2014) suggested one payback period of 2–3 years for a full-scale ultrasound installation.

High capital and operating costs of the ultrasonic system with high energy consumption and equipment maintenance are the main limitations of this technology (Elalami et al. 2019; Khanal et al. 2007; Tyagi et al. 2014). However, the use of ultrasound presents several advantages, like (1) no odor generation, (2) complete process automation, (3) easy maintenance, (4) potential to control filamentous bulking and foaming in the digester, (5) improved VS destruction, (6) biogas production and the quality of biosolids, (7) compact design and easy retrofit, (8) better digester stability, (9) low exposure time, and (10) a significant reduction in the size of digesters and the ultimate amount of sludge to be disposed (improved on dewater ability) (Elalami et al. 2019; Khanal et al. 2007; Tyagi et al. 2014; Pilli et al. 2011). Mass and energy balance on full-scale studies showed that 1 kW of ultrasonic energy used generates about 7 kW of electrical energy after losses (Pilli et al. 2011) which can overcome the limitations described above in a well-designed treatment plant.

10.2.3 Enzymes and Microorganisms

Biological pretreatment of sewage sludge offers an alternative to hydrolyze its structure by a cleaner and environmentally sustainable method by using enzymes and microorganisms as process catalysts. The use of biotechnologies is the focus of this type of pretreatment. In this scenario, enzymes and microorganisms encompass a multitude of possible relevant applications for the generation of bioenergy (Treichel et al. 2020; Zhen et al. 2017).

The increase in biogas production and higher volumes of gas recovery is directly related to pretreatment capable of breaking cell membranes of pathogens in order to reduce competitiveness with the microorganisms involved in the AD process and increase the availability of compounds that are used as substrates by these microorganisms (Zhen et al. 2017). In biological pretreatment processes, the approach comprises the application of enzymatic hydrolysis by the use of a single enzyme or enzyme cocktail or by the use of microorganisms or by thermophasic AD that consists of the pre-digestion of sludge in two stages of different temperatures (Bolzonella et al. 2012; Zhen et al. 2017).

Biological pretreatment offers some advantages over other treatments such as (1) no addition of chemical compounds during the process, ensuring greater environmental sustainability in the process; (2) increased biodegradation of the complex structure that makes up the sludge, releasing compounds that will serve as a substrate for microorganisms responsible for AD; (3) reduction of pathogens by cell membrane rupture; and (4) reduction in energy and thermal expenditure, enabling self-sufficiency in the process (Agabo-García et al. 2019; Treichel et al. 2020). Biological pretreatment presents some advantages in full scale (Ge et al. 2010; Recktenwald et al. 2008), but it still faces challenges. Mainly in terms of operation and optimization of the project due to the limitation by the complex hydrolysis mechanisms involved in the system that can vary with the characteristics of the biomass and negatively affect the efficiency of the process (Ding et al. 2017; Zhen et al. 2017).

The biological pretreatment based on the use of enzymes for sludge hydrolysis can be carried out by enzymatic cocktails, purified commercial enzymes, or enzymatic production in situ using microorganisms with a high production potential of the enzymes of interest (Yu et al. 2013). Proteases and glycosidases are the main enzymes used in sludge pretreatments, considering that the major components of this biomass are proteins and complex carbohydrates (Bonilla et al. 2018). Furthermore, due to the presence of other compounds in the sludge structural matrix, it is possible to apply different enzymes with different specificities such as lipases due to the presence of fatty acids and peroxidases for the oxidation of other compounds (Agabo-García et al. 2019; Elalami et al. 2019).

The application of a single enzyme in the pretreatment process can reduce the efficiency of the process due to the complexity of the sludge composition, with specificity for action on different substrates being important, as reported in the study of (Yang et al. 2010a) where the enzymatic pretreatment with a cocktail of amylases and proteases increased more than the application of each enzyme separately. This factor is related to the specificity of enzymes for different structural chains. Hydrolysis of sludge by enzymatic cocktails can be facilitated due to the synergistic action of enzymes, which may disintegrate through the action of some enzymes, the outermost matrix of the sludge. This process results in the solubilization of these compounds and exposing more internal compounds previously protected from enzymatic attack, increasing process efficiency (Yang et al. 2010a; Zhou et al. 2009).

The action of hydrolytic enzymes in the sludge occurs through the cleavage of specific substrates, releasing lower-molecular-weight products into the medium. This process causes the structure of the flakes to be reduced and proteins, peptides, and carbohydrates to be released for use by microorganisms in AD, inducing a greater biogas production (Recktenwald et al. 2008; Watson et al. 2004). Since it is already biologically active, sewage sludge has enzyme activity profiles that may vary according to the microbial population present in the environment, with enzyme activities such as α-glucosidase, β-glucosidase, alanine-aminopeptidase, esterase, dehydrogenase, proteases, phosphatases, and cellulases (Goel et al. 1998; Nybroe et al. 1992; Watson et al. 2004). The enzymatic activities present in the sludge are key elements to understand the profile of enzymes essential for greater efficiency of biological pretreatment.

The process of AD of sewage sludge depends on microorganisms' action to metabolize and stabilize the sludge. However, the microbial community can also be inserted in the process as a form of pretreatment aiming at increasing the product generated. About 50% of the organic material present in the sludge refers to proteins released during hydrolysis by proteolytic enzymes or by the action of microorganisms capable of producing these enzymes (Li et al. 2009). Strains of microorganisms such as *Penicillium* sp., *Serratia marcescens*, *Streptomyces* sp., *Rhizopus oryzae*, *Pseudomonas*, *Bacillus* sp., *Vibrio*, *Brevibacillus* sp., *Methanobrevibacter*, *Methanobacterium*, *Methanoculleus*, and *Methanocorpusculum* in addition to fungi species called white-rot fungi were studied, found in sludge, and considered to be good sludge-hydrolyzing agents, in addition to producing proteases that can increase the pretreatment yield (Ben Rebah and Miled 2013; Treichel et al. 2020; Ventorino et al. 2018).

During pretreatment using hydrolytic microorganisms, the networks of the complex chains that make up the sludge structure and the cell walls of pathogens are depolymerized and result in the release of lower-molecular-weight compounds that are easily digested as a substrate for AD (Guo et al. 2014). This process usually occurs through the excretion or intracellular enzyme production, being advantageous mainly by dispensing the continuous addition of enzymes, reducing energy, and economic expenses (Ding et al. 2017). In addition, in contact with the sludge structural complex, microorganisms produce efficient enzymatic cocktails for degrading different parts of the structure, which act synergistically and can result in efficient solubilization processes.

Another type of biological pretreatment that has been treated as viable biotechnology mainly for full-scale application is the two-stage AD process (temperature-phased anaerobic digestion (TPAD)) (Zhen et al. 2017). This process consists of a pre-hydrolysis of the sludge before AD. It applies different temperatures to the system, aiming to separate the hydrolysis + acidogenesis and acetogenesis + methanogenesis in the reactors. This process is resulting in the enrichment of different groups of microorganisms in each reactor, increasing the efficiency of solubilization of the substrates for biogas production (Bolzonella et al. 2012; Elalami et al. 2019; Schievano et al. 2012). The enrichment of specific microorganisms for each stage of digestion will maximize the system's overall reaction rate and improve the reduction in COD (Schievano et al. 2012).

One of the significant challenges of TPAD systems is associated with the high capacity to solubilize the compounds present in the sludge, which can result in the inhibition of the methanogenic phase, mainly due to the high sensitivity of this community to volatile fatty acids (Schievano et al. 2010). As it is a complex system with many different biochemical pathways, the AD system's balance between controlling the biogas production in two stages still limits the development with the high efficiency of this system. Because it can lead to substantial changes in biochemical pathways and in the formation of metabolites, strongly influencing the population and subpopulations present in the environment (Chen et al. 2008; Schievano et al. 2012).

In the single-stage AD process, the main challenge remains the slow rates of hydrolysis for complex biomass, such as sewage sludge, and biological disintegration methods that focus on using cleaner and economical technologies are increasingly being explored in recent research. As commonly reported approaches, the use of commercial enzymes and protease-producing microorganisms in batch systems followed by single-stage AD has often been reported (Table 10.3).

The biological pretreatment is relevant considering the scenario of sewage sludge recovery to produce biogas by improving sludge biodegradability through efficient technologies and ecological sustainability. The advancement of studies is based on the use of biological pretreatment, and the challenges of this technology must be solved, such as the high cost of enzyme cocktails, the reaction time of enzymes and

Table 10.3 Studies that evaluated the increase in the biogas and/or methane production in systems using enzymatic or microorganism pretreatment of sewage sludge

Sludge type	Pretreatment conditions	AD conditions	Increase of biogas or methane production	References
Sewage sludge	(a) *Bacillus licheniformis* (37 °C, 12 day, 150 rpm)	Batch, 23 days—37 °C	Increase of biogas production from 3.65 times and 5.77 times by treatment with *B. licheniformis* and proteases, respectively	Agabo-García et al. (2019)
	(b) Isolated commercial proteases (0.3% v/v)			
Activated sludge	(a) Amylase cocktail by *Bacillus subtilis*	Batch, 27 days—37 °C	Increase of biogas from 18.6%, 15.6%, and 20.2% by treatments, respectively	Yu et al. (2013)
	(b) Protease cocktail by *Aeromonas hydrophila*		Enzyme pretreatment reduces size particle sludge	
	(c) Cocktail combination			
Primary sludge	Proteases and lipases from *Bacillus amyloliquefaciens* DSM7T and *Burkholderia vietnamiensis* LMG 10929T, respectively	Batch, 30 days—37 °C	Increase of biogas production from 84.1% and methane production from 89.8%	Tongco et al. (2020)
Primary sludge and activated sludge	Commercial glycosidic enzymes (add in digester chamber at 40–65 °C)	Continuous reactor—full scale, 24 days—35 °C	Increase of biogas production by 10–20% in comparison to the reference digester	Recktenwald et al. (2008)

microorganisms to affect the hydrolysis of sludge, the efficient inactivation of pathogens, the need of a robust process with operational stability and low loss of efficiency due to biological inactivation of the microorganisms and enzymes involved, and moreover, finally, the main challenge of expanding scale for industrial applications (Treichel et al. 2020).

10.3 Pathogens and Antibiotic Resistance in Sludge and the Pre- and Post-treatment as the Controller in WWTPs

The rise of antibiotic administration to the population and animals naturally leads to its accumulation, especially in residues. Human residues are concentrated in WWTPs, being in general not efficiently treated and consequently reaching the sludge after treatment. The battle against resistant bacteria is one of the biggest world concerns of our century. In 2015 an estimate demonstrated that antibiotic resistance was responsible for more than 23,000, 25,000, and 38,000 deaths every year in the United States, the European Union, and Thailand, respectively (Berglund 2015). Developed countries face the addendum of facilitated antibiotic handling, being a concern by self-medication and lack of education about the use of antibiotics, resulting in exaggerated use (Planta 2007; Wellington et al. 2013). Beyond that, globalization carries resistant bacteria of specific regions to different areas, via travels around the world.

Classical antibiotics intervene in biochemistry and physiology of bacteria, culminating in cell death or cessation, which diminishes or stops cell replication. There are five targets of antibiotics from out to inside: bacteria cell wall, cell membrane, protein synthesis, DNA and RNA synthesis, and folic acid metabolism. The efficiency of antibiotics depends exclusively on the non-existence of these targets on the eukaryotic cells, or different compositions when there is a similarity, being relatively non-toxic, only in situations of exacerbated use. An excellent example is the β-lactam antibiotics such as cephalosporins, penicillins, and carbapenems, and their activity consists in blocking the synthesis of bacteria cell wall, which is a fundamental structure to bacteria but absent in eukaryotic cells (Wright 2010).

The selective pressure exercised in bacteria to the strong exposition to antibiotics has selected resitant microorganisms. The resistance can be acquired in horizontal dissemination, being distributed into the same species and genera by means of incorporation of dispersed plasmids on the environment. Resistance can also be reached vertically through generations of microorganisms due to mutations resulting from successive challenges with antibiotics (Martinez 2009).

A strong evidence is the comparison between bacteria in the pre-date of the antibiotic era and in our days. Nonetheless, in the dynamic nature of microorganisms, the resistance is forthcoming. Emergence of resistance is related for decades occurring in parallel between clinic cases and bacteria that produce antibiotics. In recent years, studies demonstrated that most of the non-pathogenic soil bacteria are multidrug resistant. This reinforces the difference between bacteria which evolved in an environment being challenged with small bioactive compounds and a variety of toxins plentiful. On the other hand, pathogens with more virulent forms compared to commensal bacteria have not been exposed to toxins and compounds that challenge their existence (Wright 2010).

In addition, LaPara et al. (2011) relate the rise of antibiotic resistance genes (ARG) and resistant bacteria (ARB) in effluent of wastewater, considering that classical WWTPs were not designed for removal of ARG and ARB, ever after the process of disinfection of mixed filtration due to the wastewater compile the residues of city dwellers and concentrate at WWTPs (Calero-Cáceres et al. 2014; Su et al. 2015). Characteristics of sewage sludge such as microbial diversity with high density can facilitate horizontal gene transfer (HGT) by plasmids, known as mobile genetic elements (MGE) (Gaze et al. 2011; Sentchilo et al. 2013; Zhang et al. 2011). The techniques utilized to identify the presence of ARG and ARB are quantitative PCR and metagenomic investigation (Yang et al. 2013; Zhang and Zhang 2011). The incorrect treatment of sludge can lead to the input of ARG, ARB, and antibiotics such as fluoroquinolones, macrolides, and tetracyclines into the soil (Kinney et al. 2006; Rahube et al. 2014; Sabourin et al. 2012).

Degradation of antibiotics and ARGs is related to the process applied to manure composting (Qiao et al. 2012; Sharma et al. 2009; Wang et al. 2012). Nevertheless, few studies evaluated the effect of methods of digestion of sludge, specifically tetracyclines, sulfonamides, macrolides, and resistance genes (Ma et al. 2011). The focus on sludge as mentioned is related due to the rich reservoir of ARGs and variety commonly found in sludge (Andrés et al. 2011; Rahube et al. 2014). Consequently, the post-treatment is evidenced as necessary.

In this sense, the control of pathogens is most important in WWTPs. Pretreatment methods, like ultrasound and thermal, may also impact sludge hygienization and could be used as both pretreatment and post-treatment, depending on the requirements of the WWTP (Ruiz-Hernando et al. 2014). According to studies mesophilic anaerobic digestion is inactive around 2 $\log_{10}$ of pathogens and sludge containing up to 7 $\log_{10}$ (Lizama et al. 2017).

The inactivation of three microbial indicators at 80 °C, for 30 min, behaved differently: there was a slight reduction for SSRC (spores of sulfite-reducing clostridia) (0.84 $\log_{10}$ of reduction), approximately 5 $\log_{10}$ of reduction for SOMCPH (somatic coliphage) and a high hygienization for *E. coli* (>4.01 $\log_{10}$ of reduction) (Ruiz-Hernando et al. 2014). According to Yin et al. (2016), thermal pretreatment (70 °C) is highly efficient to inactivate pathogens and the complete inactivation (approximately 6 log) of fecal coliform, *Salmonella* spp., and fecal streptococcus. The pretreatment effect was evaluated at different times (20, 40, 60, 80, 100, and 120 min) for different TS concentrations of fecal sludge (between 1% and 12%). Considering the results of ultrasound pretreatment, a reduction of pathogens was observed where the concentration of fecal coliforms and *Salmonella* spp. decreased by 4 (99.99%) and 3 (99.9%) log units, respectively, at 35,000 kJ/kg. The authors tested TS concentration without continuous stirring and did not achieve the same inactivation, so these two conditions interfere on ultrasound pathogen inactivation capability (Lizama et al. 2017). According to Kumar (2011), the pathogen concentration decreased as sonication time and frequency increased, and reduction is mainly caused by the effects of cavitation and decreased the bacterial cells showing ruptured shapes.

During ultrasonic treatment of sewage sludge, using 22 kHz, the load of *Giardia lamblia* cysts and *Cryptosporidium parvum* oocysts was reduced to non-detectable levels (control parasite density, 12–17 no./g of Cryptosporidium and 22–32 no./g of Giardia), at 15 min of sonication time for following applied amplitudes (10, 12, 14, 16 μm). The hydrodynamic shear force was considered as a factor responsible for the damaged oocyst (Graczyk et al. 2008).

Besides the treatment and pretreatment efficiency, some matrices may need addition of a post-treatment for reaching the standard established in directives for sludge use. There are some well-known options, such as the use of polishing ponds, which is common in developing countries such as India, Brazil, and China (Ali et al. 2013). This system requires large land areas, being also quite slow; however it can reduce helminth eggs and reach the discharge standards for urban wastewaters from the European Community for nitrogen (von Sperling and Mascarenhas 2005). Constructed wetlands are another example of natural post-treatment, being especially efficient on phosphorus and nitrogen removal (Ali et al. 2013). One of the advantages of this technique is the use of natural organic matter degradation processes, associated with the macrophytes' biologic filtration, being able to reduce coliforms and even viruses (Platzer et al. 2016; Stefanakis et al. 2014).

Coagulation and flocculation are other examples of a post-treatment process; however their efficacy must be increased using disinfectants such as chlorine (Jaya Prakash et al. 2007). Down-flow hanging sponge (DHS) is a reactor developed in Japan, composed of sponge cubes diagonally linked through nylon string, providing vast areas for microbiological growth under non-submerged conditions, while the effluent passage provides the nutrients for the resident microorganism development (Agrawal et al. 1997). Down-flow hanging sponges enable the recovery of dissolved methane and thus the removal of 3.5 $\log s_{10}$ of fecal contaminants (Machdar et al. 2000). Another post-treatment method for fecal contaminant removal is the use of moving bed biofilm reactors, showing a 2.3 $\log s_{10}$ removal (Tawfik et al. 2008). In these systems the predation by protozoa and metazoan along with adsorption into the media was the main inactivation mechanisms responsible for pathogen reduction.

Slow sand filtration systems show great sanitization power for anaerobic digestate treatment, being able to reduce 4 $\log s_{10}$ of fecal contaminants, reaching most of the directives for effluent reuse (Tyagi et al. 2009). There are other techniques focused on mineral element removal, involving aeration processes, variating from micro-aeration, i.e., flash aeration, to high rate aerobic methods, such as sequential batch reactors. Micro-aeration is a great option for sulfides' biological oxidation into elemental sulfur, which can be easily recovered and commercialized (Chen et al. 2010; Khan et al. 2011). These techniques can be applied for a greater effluent sanitization and thus safer agricultural use, land application, or discharge, being all feasible options. However, the most suitable sanitization option depends on the effluent and treatment plant characteristics.

10.4 Final Remarks

The WWTP's sludge is a by-product that contains a large amount of organic matters, heavy metals, and pathogens and may represent an environmental risk. In this sense, AD is a promising technology for the recovery of sludge, dependent on physical, chemical, and biological pretreatments to promote the increase in biogas production and increase the sanitary quality of the digested, aiming at valuing and recycling the final product.

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