





Review

The Influence of Municipal Wastewater Treatment Technologies on the Biological Stabilization of Sewage Sludge: A Systematic Review

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Abstract: Various wastewater treatment technologies are available today and biological processes are predominantly used in these technologies. Increasing wastewater treatment systems produces large amounts of sewage sludge with variable quantities and qualities, which must be properly managed. Anaerobic and aerobic digestion and composting are major strategies to treat this sludge. The main indicators of biological stabilization are volatile fatty acids (VFAs), volatile solids (VS), the carbon/nitrogen (C/N) ratio, humic substances (HS), the total organic carbon (TOC), the carbon dioxide (CO₂) evolution rate, the specific oxygen uptake rate (SOUR), and the Dewar test; however, different criteria exist for the same indicators. Although there is no consensus for defining the stability of sewage sludge (biosolids) in the research and regulations reviewed, controlling the biological degradation, vector attraction, and odor determines the biological stabilization of sewage sludge. Because pollutants and pathogens are not completely removed in biological stabilization processes, further treatments to improve the quality of biosolids and to ensure their safe use should be explored.

Keywords: biological stabilization; biosolids; organic matter; sludge management; sewage sludge; wastewater treatment



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1. Introduction

The increasing population will increase municipal wastewater (MWW) generation, and existing sanitation strategies should focus on increasing the wastewater treatment coverage by selecting appropriate treatment technologies based on local conditions [1,2]. On average, although 70% of the MWW generated in high-income countries is treated, only 38%, 28%, and 8% are treated in middle-, middle-low-, and low-income countries, respectively [3,4].

Zhang et al. [5] and Collivignarelli et al. [6] showed that the characteristics of sewage sludge, such as the solid concentration, organic matter (OM), nutrients, heavy metals, and pathogens, vary depending on the following parameters:

- (i) The characteristics of the wastewater (e.g., the biochemical oxygen demand—BOD₅, the chemical oxygen demand—COD, and the total suspended solid—TSS).
- (ii) The type of the wastewater collection system (e.g., a sanitary sewer, storm water, or combined systems).
- (iii) The type (e.g., biological or chemical) and stage (i.e., primary, secondary, or mixed) of the wastewater treatment process from which the sludge originates.
- (iv) The sludge stabilization processes (e.g., anaerobic and aerobic digestion, composting, and chemical and thermal treatment).

- (v) The operation conditions, wastewater treatment, and sludge stabilization processes (e.g., the temperature and the sludge retention time).

According to UN-Habitat [7] and Wijesekara et al. [8], the worldwide annual production of sewage sludge was estimated to be 100 million tons (Mt) in 2017 and is projected to reach 175 Mt by 2050.

The final disposal is an important aspect in sewage sludge management because it can affect the environment (e.g., greenhouse gas emissions or the accumulation of heavy metals in soil), economy (e.g., transportation costs and the area requirement in the final disposal), and society (e.g., public acceptance, land occupation, and public health) [8,9] differently.

For promoting the safe management of sewage sludge, the impact of the treatment's operating conditions, the properties of the MWW on the sludge's characteristics, and the efficiency of the stabilization processes according to the regulations of each country or region must be evaluated to identify the potential use of sludge [10,11].

The stabilization degree of the sewage sludge achieved can be identified by specific bacteria promoting the biodegradability of OM (biological stabilization), the chemical oxidation of OM (chemical stabilization), and the effect of heat stabilizing the volatile fraction (thermal stabilization) [12–14]. Biological processes constitute the most used strategy for sludge stabilization worldwide [15]. About the microbiological and parasitological quality, further treatments (hygienization) reduce the presence of pathogens in biosolids to ensure safe practices for reusing biosolids in agriculture [16,17]. Currently, several studies have revealed the detection of particles of SARS-CoV-2 in MWW and sewage sludge during the pandemic (COVID-19) [18,19].

The terms sewage sludge and biosolids are often used interchangeably [20]. Worldwide, the term biosolids indicates a stabilized sewage sludge, which achieves this condition by one or more treatments and meets the regulations for beneficial use [6,12]. The circular economy approach prioritizes implementing biosolids-reuse strategies, such as agricultural use, which promotes the replacement or reduction in using chemical fertilizers, resulting in economic and environmental benefits [21–23]. Reusing biosolids in agriculture is the most used disposal option in some countries such as the United Kingdom (79%), Spain (64%), Australia (55%), and the United States (36%) [24,25].

In Latin America, interest in this topic and the potential use of biosolids with a high agricultural vocation has been growing [26]; although systematic review articles discussing the treatment of municipal wastewater [1–3,26], processes for sludge treatment [5], and the reuse and assessment of sludge [6,11,15,20,23,25] have been published, this manuscript presents a bibliometric analysis and a comprehensive reflection on the research trends related to the technologies in municipal wastewater treatment plants (WWTPs) and their influence on the biological stabilization of sewage sludge. The different indicators and criteria are analyzed for the biological stability of the reported sludge and their relationship with sewage water treatment technologies, unveiling no consensus in defining sewage sludge (biosolids) stability or any standardized treatment process indicators.

2. Methods

Based on a bibliometric analysis (2001–2021), the sources of information were two databases, i.e., Scopus and SciELO (from international and Latin American contexts, respectively). The keywords and search equation were defined in English, Portuguese, and Spanish using boolean operators (“AND” and “OR”) [27] and were placed between the keywords to perform the search, e.g., (“municipal wastewater” OR “wastewater treatment”) AND (“sewage sludge” AND “biosolids” AND “sludge stabilization” OR “biological stabilization” OR “sludge management”) AND (“regulations” OR “organic matter” OR “vector attraction” OR “stability indices” OR “stability indicator”). Figure 1 shows the stages developed in the methodology according to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) [28].

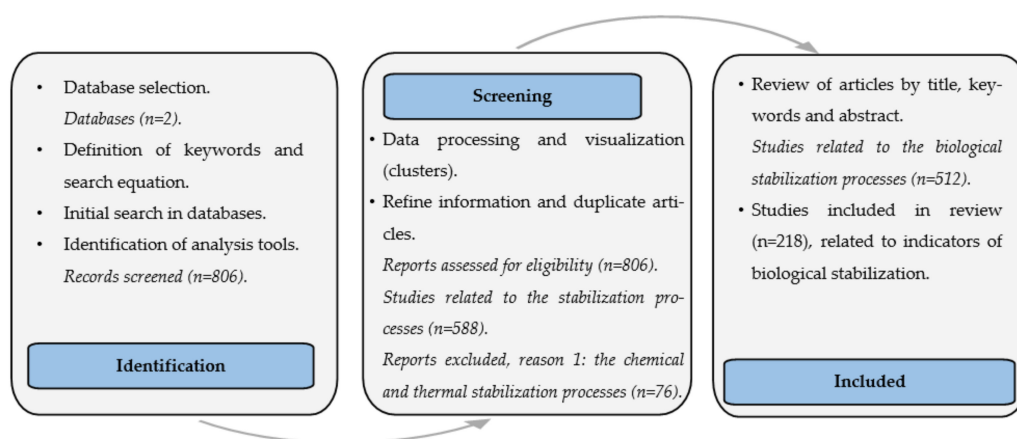


Figure 1. Stages of systematic reviews. Source: adapted from Shah et al. [28].

The information retrieved from the databases included (i) citation information, (ii) the abstract and keywords, and (iii) bibliographical information, including references to each scientific paper [29]. Subsequently, with “.RIS” files of the citations, the software Mendeley-Desktop® (version 1.19.4, Mendeley Ltd., London, UK) was used as a bibliographic manager, where the publications from the databases were unified and the duplication of the articles was identified to facilitate the organization and review of the information in the bibliometric analysis.

With the refined information, the free version of the software RefViz® (trial version 2.1.2, Omni Inc., Kennesaw, GA, USA) was used to review the content of the articles, support theories and concepts, condense the results, and feed the analyzed data; subsequently, a co-occurrence analysis was performed on the keywords, and clusters were formed that presented similarities or proximities, allowing for the compilation and review of the articles [27].

The results were visualized using the software VOSviewer® (version 1.6.18, Centre for Science and Technology Studies of Leiden University, Leiden, The Netherlands), enabling the analysis of the trends in the development of the topic under study with a minimum of 10 co-occurrences between the keywords [30].

3. Results

3.1. Bibliometric Data

The search equation identified 806 related scientific articles. Scopus found the highest number of publications (528) followed by SciELO (278). Figure 2a shows the growth trend of the publications from 2001 to 2021 and according to country. Particularly, in the last five years, 60 to 100 publications have appeared per year.

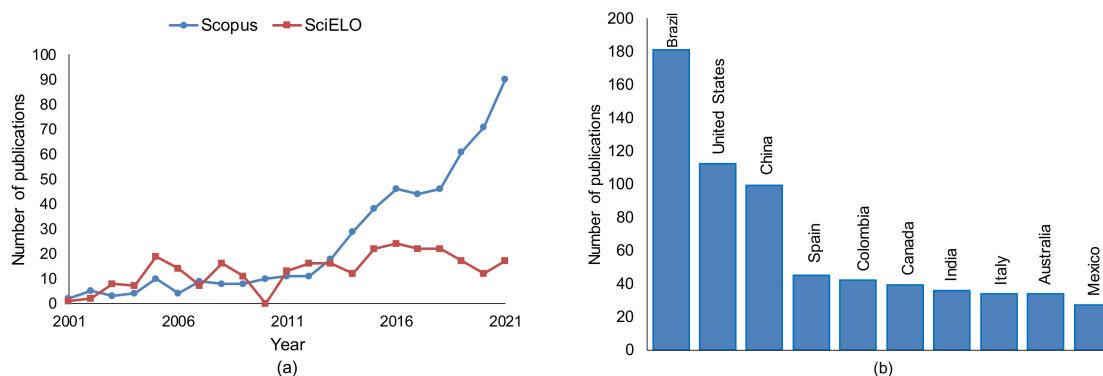


Figure 2. Number of publications (a) in databases and (b) by country.

The trend in the number of publications is related to the evolution of concepts and programs in governments and agencies, such as zero waste, reduction, reuse, and recycling in the first period (2001–2010) and the second period (2011–2021) with the inclusion of the regulations, politics, and sludge management strategies associated with Sustainable Development Goals, proper planning, environmental protection, public health risks, sustainability, recovery, a life cycle analysis, and a circular economy [6,10,11].

Brazil had the highest number of publications (28%), followed by the United States (17%) and China (15%). Figure 2b describes the top 10 countries reporting publications, adding the production per country in the databases (Scopus and Scielo) with the aim of including a Latin American context in the international analysis. In the Latin American context, Colombia ranks second after Brazil, although it only represents 6% of the publications in the period analyzed. In terms of the associated knowledge, environmental, agricultural, biological, and engineering sciences stand out at the international and Latin American levels.

Figure 3 shows the keywords related to the search equation: the main keywords are labeled in a circle, with each circle's size defining the frequency of the appearance of these words in the analyzed articles; the larger is the circle, the greater the co-occurrence. The color represents the time and the lines represent the links between the keywords. In addition, the distance between two keywords indicates the strength of the relationship; that is, the closer they are, the more connections they have [30].

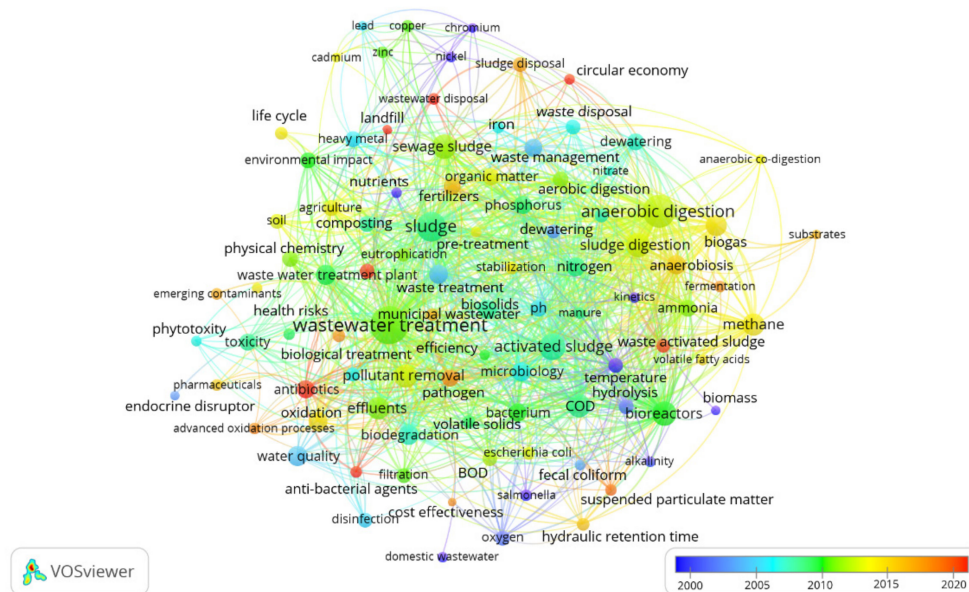


Figure 3. Cluster network obtained through the bibliometric mapping of keywords.

We found 96 keywords that group research trends into three clusters: Cluster I represents the technologies in municipal wastewater treatment plants (e.g., biological treatments, activated sludge, and bioreactors); Cluster II represents the sewage sludge characteristics (e.g., the OM, pathogens, heavy metals, and nutrients); and Cluster III represents the biological stabilization processes of sewage sludge (e.g., composting, aerobic, and anaerobic digestion). The development of each cluster is given below.

The keywords presenting the highest number of co-occurrences in descending order were wastewater treatment, anaerobic digestion, sewage sludge, activated sludge, and sludge stabilization. Regarding the research trends, some current topics are highlighted, such as a circular economy, waste activated sludge, advanced oxidation processes, organic compounds, and emerging contaminants. Furthermore, interest still arises around other issues such as wastewater and sludge final disposal, biodegradation, heavy metal, and pathogens, which is of great importance in terms of assessing the potential use of treated wastewater, sewage sludge, and biosolids.

3.2. Municipal Wastewater Treatment Plants (WWTPs)

The composition of MWW is associated with the eating habits of the population and their types of industrial, institutional, and commercial activities. The OM present in MWW constitutes proteins (40–50%), fats (5–10%), lipids (5–10%), fibers (5%), and carbohydrates (25–50%). The quantity is related to rapid population growth, urbanization, improved living conditions, and economic development [3,31].

Unit operations (physical) and processes (chemical and biological) have been identified in four stages of treatment: preliminary, primary, secondary, and tertiary or advanced [32]. Regarding the reduction in the OM, the bibliometric analysis identified that biological processes (93%) are the most studied and applied, with 63% of them associated with aerobic and 37% with anaerobic metabolism.

Figure 4 shows the leading technologies in municipal WWTPs used in different countries, which were selected based on bibliographical information published on the two databases accessed, prioritizing the countries identified in Figure 2b. Although the activated sludge system is the most widely used technology in high-income countries, in middle-income countries, stabilization ponds (SP) are the most commonly used technologies because despite the large area requirement, they have advantages such as low costs, operational simplicity, and sludge stabilization [3,26]. They are followed by an upflow anaerobic sludge blanket (UASB) and activated sludge systems. In low-income countries, primary treatment technologies predominate (e.g., septic tanks, Imhoff tanks, and primary sedimentation) [1,4].

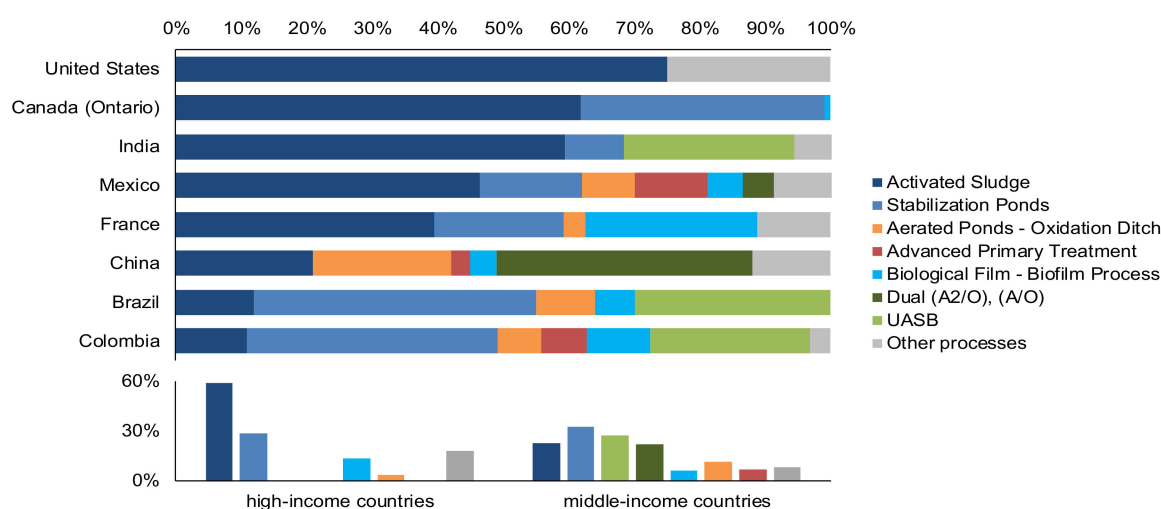


Figure 4. Main technologies in WWTPs used in different countries. A2/O: anaerobic–anoxic–aerobic; A/O: anaerobic–aerobic; UASB: upflow anaerobic sludge blanket; and other processes: primary treatment (septic tanks, Imhoff tanks, primary sedimentation, etc.). Source: adapted from Noyola et al. [1], Sato et al. [3], the World Bank [4], Pedroza et al. [13], Kaur et al. [33], Jin et al. [34], Rakedjian [35], CONAGUA [36], Zhang et al. [37], Jin et al. [38], SSPD [39], and Maltos et al. [40].

In the field of aerobic treatment, activated sludge technology has different modalities, predominantly the conventional activated sludge (CAS), sequential batch reactor (SBR), extended aeration-activated sludge (EAAS), membrane biological reactor, the elimination of the improved biological phosphorus (EBPR), and to a lesser extent, contact stabilization, bed biofilm reactor (MBBR), and oxidation ditches [35,38,40].

The UASB is the most widely used anaerobic system, particularly in middle- and low-income countries with a tropical climate, such as Brazil, India, and Colombia [1,33]. Generally, the post-treatment requirement of these systems in the case of low- and medium-load MWW for non-compliance with the discharge regulation [41,42] has led to the growth of dual technologies (mainly anaerobic followed by aerobic; A/O, A2/O) [37,40,43], which have benefits such as low energy and chemical consumption, a reduced sludge quantity

to be disposed, low equipment requirements, high operational simplicity, and sludge stabilization in the same anaerobic phase by avoiding the additional use of the biological stabilization processes of sewage sludge [2,34].

Table 1 shows a comparative analysis of the main characteristics of the biological systems (used for MWW treatment) documented in the articles and identified in 567 of the 806 publications, such as the activated sludge (e.g., CAS, EAAS, and SBR) and anaerobic treatment (e.g., UASB).

Table 1. Main characteristics of biological treatment technologies documented in articles.

Characteristics	CAS	EAAS–SBR	UASB
Kinetics of organic matter conversion	$C_nH_aO_bN_c + 5O_2$ ↓ $CO_2 + H_2O + NH_3 +$ biomass	$C_nH_aO_bN_c + 7O_2$ ↓ $CO_2 + H_2O + H^+ + NO_3^- +$ biomass	$C_nH_aO_bN_c$ ↓ $CH_4 + CO_2 + H_2O + NH_3 +$ biomass
Area requirement (m^2 /inhabitant)	0.2–0.3	0.25–0.35	0.1–0.2
Sludge retention time (SRT)	4–15 days	18–30 days	30–40 days
Hydraulic retention time (HRT)	5–14 h	18–36 h	6–14 h
Removal efficiency of COD	80–90%	90–95%	60–70%
Removal efficiency of BOD ₅	85–95%	80–98%	60–80%
Energy requirements	Reduced	High	Low to moderate
Temperature influence	Average	High	High
Biological stabilization of sludge	Low and insufficient	Sufficient	High
Complementary biological stabilization processes of sludge	Necessary	Not required	Not required
Sludge production (L/per*d)	High (8.2)	Medium (3.3–5.6)	Low (0.2–0.6)

COD: chemical oxygen demand; BOD: biological oxygen demand; CAS: activated sludge conventional; SBR: sequential biological reactor; EAAS: extended aeration activated sludge; and UASB: anaerobic upflow reactor with sludge mantle. Source: adapted from Noyola et al. [1], von Sperling et al. [41], and Chan et al. [42].

The type of treatment system or metabolism employed influences the quantity and quality of the sludge, i.e., it affects the sewage sludge stabilization, and thereby the sludge management (e.g., the costs, technologies, and usage) [42,44]. High SRT (>18 days) systems result in a low quantity of produced sludge and the development of biological stabilization processes in sewage sludge [32,43]; however, although researchers such as Cokgor et al. [45] and Fisher et al. [46] indicate that the biological stabilization of sewage sludge in EAAS or a SBR is sufficient and comparable to anaerobic processes (e.g., UASB), other researchers [47,48] indicate that the sludge produced from EAAS cannot be considered digested or stabilized because of the influence of the temperature and the SRT on the endogenous decay coefficient.

3.3. Sewage Sludge Characteristics

The annual sludge production in the three countries is as follows: (i) Brazil, with 1.5–3.0 million tons (Mt) for 188 million inhabitants and 20–40% produced from MWW treatment (43% with SP and 30% with UASB reactors); (ii) the United States, with approximately 17 Mt for 298 million inhabitants and 60–80% produced from MWW treatment (75% with CAS technology); and (iii) China, with 12 Mt for 1313 million inhabitants and 40–60% produced from MWW treatment (21% with CAS and 31% with dual systems) [7,25,49,50].

In Germany, the annual sludge production is 2.3 Mt, followed by India and South Africa (2.3 Mt and 1.0 Mt, respectively), and the United Kingdom (1.05 Mt) [25]. In Latin American countries, the sludge production is approximately 0.64 Mt in Mexico [36] and 0.37 Mt in Colombia based on the main municipal WWTPs located in Bogotá, Cali, and Medellín [39].

Historically, sewage sludge management has focused on the final disposal practices, such as incineration and landfill disposal on soil or even in the ocean [51]. However, to adopt practical sustainable solutions, the general requirements (e.g., the production and monitoring frequency), the sewage sludge's physical, chemical, microbiological, and parasitological characteristics, the environmental and operating conditions of the stabilization processes (e.g., the temperature, SRT, and efficiency), and good management practices (e.g., application rates and agricultural use) must be developed and controlled [9,52].

According to Lu et al. [53] and Kumar et al. [20], sewage sludge contains 50% carbohydrates (sugar, starch, and fiber), 20% fat, representing approximately 30–40% OM, a carbon–nitrogen ratio (C/N) of 10–20%, high levels of heavy metal ions (Cu and Zn), and a pH normally between 6.5 and 7.0. The sewage sludge contains the nutrients necessary for developing agricultural crops, such as nitrogen (N: 3–8%) and phosphorus (P: 1.5–3%) in large quantities and potassium (K: 0.1–0.7%), calcium (Ca), and magnesium (Mg) in low quantities, which increases the potential agricultural use [10,25].

In the sewage sludge, the OM content can be quantified indirectly in terms of volatile solids (VS) [14]. This variable depends on the treatment from which the sludge originates; thus, primary sludge has a higher VS content than secondary sludge originating from both aerobic and anaerobic treatment systems due to the degradation of OM in biological reactors [54,55].

Depending on aspects such as urban development, industrial activities, the characteristics of the population, and the sewerage service, sewage sludge may contain several toxic substances, such as heavy metals; thus, their presence must be analyzed because some of these metals are essential in agricultural use (such as Zn and Cu). In large proportions, metals such as Ni and Cd can have toxicity effects in soil, and the low mobility of such metals increases their concentration in the soil [25]. Other metals (i.e., As, Cr, Hg, Mo, Pb, and Se) can cause potential risks to human, animal, and plant health [44,56].

An important aspect associated with the beneficial or toxic effects of the heavy metals present in sewage sludge corresponds to pH because it influences their solubility. Particularly, acidic media can increase the solubility of heavy metals in sludge samples and make them dynamically toxic; thus, a high risk may be associated with the acidic pH range [57]. For pharmaceutical products such as disinfectants, laundry detergents, pesticides, dyes, paints, preservatives, food additives, personal care products, and organic pollutants, concern has increased owing to a risk to public health [24,58,59].

Pathogens present in sewage sludge originate from human feces and directly relate to the diet and health of the population. They can also originate from animal sources, whose excrement is disposed into the sewage system (e.g., dog and cat feces), or through vectors in the sewers, mainly rodents [25]. These microorganisms grouped in bacteria, including fecal coliforms, *Escherichia coli*, *Campylobacter*, and *Salmonella* sp. [14,60] and parasites, including helminth eggs, represent a risk to human health. Their presence is high in low- and middle-income countries [61]. Viruses, another group of pathogens present in sewage sludge, are subjected to the technical capacity of each country for their detection (e.g., enteric viruses) [62].

Recent reports have identified the presence of SARS-CoV-2 genetic material in MWW and sewage sludge and have investigated its elimination in MWW treatment systems [18]. The particles of SARS-CoV-2 in primary and secondary sludge has been identified in the MWW of the United States [63], Turkey [64], and Spain [19]. However, no epidemiological data establishing a direct relationship between sewage sludge and the risk of a SARS-CoV-2 infection are currently available [18,65].

3.4. Biological Stabilization Processes of Sewage Sludge

The sewage sludge line (Figure 5) is an essential component of municipal WWTPs, and all stages of sludge treatment can account for 40–60% of the total operating costs [66].

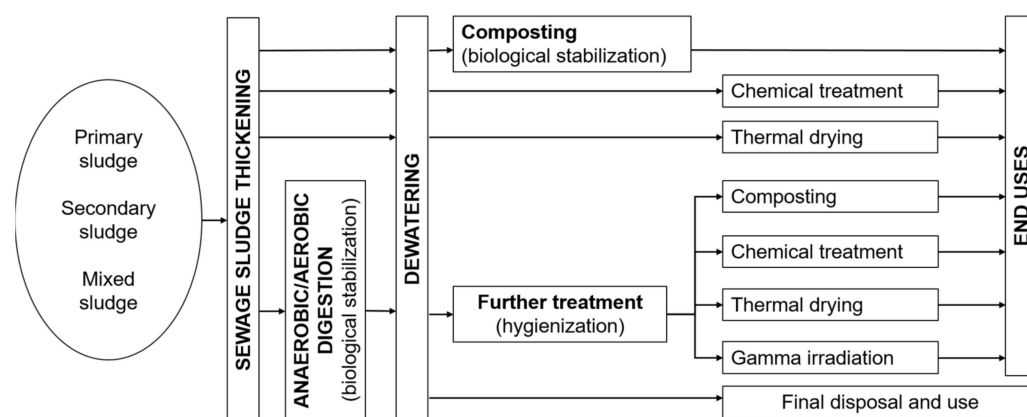


Figure 5. Sewage sludge treatment alternatives. Source: adapted from Silva-Leal et al. [16], Jin et al. [34], and the NRC [67].

The thickening reduces the water content of the sludge and increases the density and solid content. Additionally, anaerobic/aerobic digestion (biological stabilization) reduces the OM, vector attraction, and odor; dewatering reduces the remaining moisture content in sewage sludge, facilitating its transport and final disposal. Further treatment (hygienization) eliminates pathogens [6,10,12]. Table 2 summarizes the advantages and limitations of the most common biological stabilization processes and the characteristics of the material generated in each process.

Table 2. Advantages and limitations of biological stabilization processes and characteristics of the sludge generated.

Process	Advantages	Limitations	Characteristics of the Sludge Generated	Reference (s)
Anaerobic digestion (AnD)	Reduction in the biological degradation (organic matter) and attraction of vectors, pathogens, and odor. Potential to use the main gas generated (CH ₄).	High investment costs, relatively slow degradation of organic matter process, high maintenance and qualified operator requirements; the process depends on the temperature and the SRT. Limited degradation capacity of heavy metals and complex organic compounds. Excess moisture. Emission of greenhouse gases if biogas is not used as a source of renewable energy.	Requires dewatering in addition to requiring further treatment (hygienization) to eliminate pathogens and potentiate unrestricted uses in agriculture.	[5,32,50,55,68,69]
Aerobic digestion (AeD)	Rapid reduction in the biological degradation (organic matter) and attraction of vectors, pathogens, and odor.	High operating costs, odor formation, high maintenance and qualified operator requirements; the process depends on the temperature and the SRT. Limited degradation capacity of heavy metals and complex organic compounds. Excess moisture. Emission of greenhouse gases.	Requires dewatering in addition to requiring further treatment (hygienization) to eliminate pathogens and potential unrestricted uses in agriculture.	[50,55,69]

Table 2. Cont.

Process	Advantages	Limitations	Characteristics of the Sludge Generated	Reference (s)
Composting	Reduction in the biological degradation (organic matter) and attraction of vectors. Significant reduction in pathogens. Reduction in sludge volume (up to 60% in 20 days)	Complex management by the volume of sludge generated; the process depends on the temperature, lack of availability of microorganisms, and the presence of unstabilized pathogenic materials. Limited degradation capacity of heavy metals and complex organic compounds. Heavy metals are only transformed into less mobile forms. Emission of greenhouse gases.	Low moisture material; however, the sewage sludge requires dewatering before the composting process. It produces value-added products in C, N, and P for horticultural, nursery, and landscape uses.	[5,50,55,69,70]

The stabilization process primarily results from the OM degradation, which has been physically classified as soluble and particulate and biochemically classified as biodegradable and non-biodegradable. The soluble biodegradable fraction, commonly referred to as a rapidly biodegradable fraction, is related to the compounds that can be directly adsorbed for synthesizing new cellular materials, such as VFAs, simple carbohydrates, amino acids, and alcohols [43,48]. The particulate biodegradable fraction, known to be slowly biodegradable, is related to the macromolecules that must be broken down into simpler forms before being used by microorganisms [71].

According to the United States Environmental Protection Agency (USEPA) [12], the meaning of “stabilized” sludge is not used uniformly, and the reduction in the biological degradation, attraction of vectors, odor, and pathogens determines the stabilization degree of the sewage sludge. Fisher et al. [46] related stabilization to the OM, pathogens, and odor reductions in sewage sludge. In addition to the content of the OM and nutrients, it is recommended to assess the presence of inhibitory substances, such as heavy metals and pathogens, which could negatively influence the ecosystem and public health [68,72].

Selecting the biological stabilization process depends on factors such as the sewage sludge characteristics, intended use, and final disposal conditions [73]. The bibliometric analysis identified that the most commonly used biological processes are anaerobic digestion (AnD; 49%), composting (27%), and aerobic digestion (AeD; 11%). The chemical (7%) and thermal (6%) stabilization are used mainly for the hygienization process.

In the European Union, 50% of municipal WWTPs use AnD as a sewage sludge stabilization strategy, 18% use AeD, and 8% use other chemical and thermal stabilization processes (e.g., lime application and thermal drying), whereas 24% of municipal WWTPs do not perform any stabilization process [15]. In the United States, 45% of municipal WWTPs use AeD processes, 21% use AnD processes, 20% use other chemical and thermal stabilization processes, 4% use composting, and the remaining 10% do not implement a stabilization process [7,74]. In Canada (particularly Ontario), 34% and 16% of municipal WWTPs use AeD and AnD processes, respectively [38].

In countries such as Brazil, China, Colombia, and India, AnD represents 35–40% of municipal WWTPs, whereas AeD and composting are only applied to 5–10% of the total municipal WWTPs, with the remaining not implementing a stabilization process [7,33,55].

The pathogens removal occurs through various mechanisms (e.g., the external energy requirements, cellular decomposition—heterotrophic and autotrophic, endogenous respiration, the death–regeneration of microorganisms, the predation of bacteria by complex microorganisms, and cell lysis due to adverse environmental conditions—pH, toxic compounds, or temperature) [45,48]. Bacteria die in 1 to 3 months; protozoa and helminth eggs survive up to one year in sewage sludge [60] and enteric viruses and somatic phages persist

for 9 to 14 months depending on the temperature and stabilization process of the sewage sludge. Martín-Díaz et al. [62] indicate that somatic phages are a more accurate indicator of the fecal contamination load and the risk of enteric viruses.

The exposure time and temperature conditions to achieve a high reduction efficiency of bacteria and viruses are as follows: (i) for AnD, at least 15 days at 35–55 °C or 60 days at 20 °C; (ii) for AeD, 10 days at 50 °C, 40 days at 20 °C, or 60 days at 15 °C; and (iii) for composting, at least 15 days at 55 °C [50,75]. However, these processes show a low reduction efficiency (45%) of protozoa and helminths; further, complementary hygienization processes are necessary for their removal [16,23,72].

In the case of small municipal WWTPs, the main concern of operators is to reduce health and environmental risks in terms of toxicity [66]. The potential toxicity can be assessed using the seed germination index (phytotoxicity), which provides information about the impact of various hazardous substances and allows for the assessment of sensitivity to individual plant species [56]. Phytotoxicity tests are valuable tools to assess the influence of the stabilization degree on seed germination and root elongation; thus, toxicity tests are included in the directives of relevant agencies (e.g., the USEPA, OECD, and ISO) [76,77].

Although the sewage sludge converted in biosolids contains less N, P, and K than commercial fertilizers, they are considered organic fertilizers in agricultural activities owing to their contribution of OM and nutrients [53], and the land application depends on the amount of N provided, which must be transformed from its organic to inorganic form (i.e., mineralization). This practice reduces the long-term environmental pollution caused by N and P accumulation in the case of chemical fertilizer application and would help farmers by lowering the cost of agricultural inputs and increasing the income from crop production [21,22,72]. Rigby et al. [78] reported a mean mineralizable N fraction for biological stabilized sewage sludge: approximately 29.8% organic N for AnD, 47.2% for AeD, and 6.7% for compost.

Indicators Used to Evaluate the Biological Stabilization of Sewage Sludge

The effect of biological stabilization processes on the proportion of the OM, availability of nutrients, and reduction in pathogens in sewage sludge has prompted efforts to understand the efficiency of the implemented processes, thought indicators, stabilization degree according to the regulatory requirements related to the degradation of OM, control of vector attraction and odor, as well as the physical, chemical, microbiological, and parasitological characteristics of sewage sludge [11,12,22,30,69,79].

With relation to the indicators of the biological stabilization of sewage sludge, the bibliometric analysis identified 218 publications (Figure 6).

Volatile fatty acids (VFAs) and VS are the primary indicators used [80–82]. The VFAs can provide useful information on the OM content and is a control parameter mainly used in the stability process during AnD [46]; however, it is not the single indicator to describe the stabilization degree of the sewage sludge [83]. In the case of VS, the organic fraction of the total solids (TS) present in the sewage sludge also quantifies the stabilization degree and vector attraction reduction [14,48]. The performance of the stabilization process is assessed using the relationship between volatile and total solids (VS/TS) or the volatile solids reduction (VSR) using the Van Kleeck and mass balance methods. Here, the minimum threshold of VS/TS is ≤ 60 –65% and VSR are regularly used to consider the stabilized sludge of ≥ 38 –40% [12,84,85].

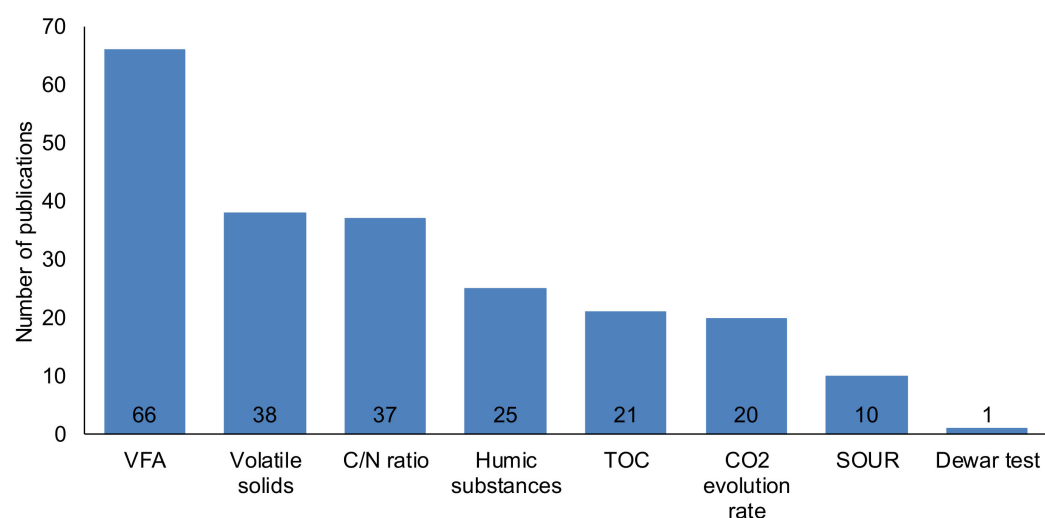


Figure 6. Main indicators of biological stabilization identified by the bibliometric analysis. VFAs: volatile fatty acids, C: carbon, N: nitrogen, TOC: total organic carbon, CO₂: carbon dioxide, and SOUR: specific oxygen absorption rate.

However, different methods and criteria exist for interpreting the same indicator (e.g., VSR) [85]. According to Özdemir et al. [48], evaluating biological stabilization based on VS represents the only indicator of endogenous decomposition, and it is far from accurate or acceptable. It does not consider the effect of different mechanisms and particulate matter fractions in sewage sludge during the stabilization period. Table 3 shows the main indicators recommended for each biological stabilization process (AnD, AeD, and composting).

Table 3. Sewage sludge biological stabilization indicators reported in literature and regulations.

Stabilization Process	Indicator	Advantages (A) and Limitations (L)	Unit	Criterion	Reference (s)
Anaerobic (AnD) and aerobic (AeD) digestion	Volatile fatty acids (VFAs) *	A: Process quality control, safe quality of the end products L: Complicated operating procedures and applicable for anaerobic digestion only	mg COD/g OM	<430	[45,86–92]
	Volatile solids (VS)	A: Simple testing methods L: Procedure control and laboratory assembly	% VS/TS	<65	[45,86,90,93]
				≤60	[45,85,86,90,94]
			% VSR	≥38	[12,45,52,86,90,93,95–100]
	Additional VS when it is anaerobically batch-digested in the laboratory (40 days at 30–37 °C) * Additional VS when it is aerobically batch-digested in the laboratory (30 days at 20 °C) **	A: Indicates process efficiency L: Complicated operating procedures	% VSR	≤15	[12,45,52,86,90,95,96,98–100]
			% VSR	≤17	[12,45,52,86,90,95,96,98–100]
	Humic substances (HS) ***	A: Indicates ecological value of end products L: Complicated operating procedures	mg/g VS	≥150	[45,81,86,90]
	Specific oxygen uptake rate (SOUR) **	A: Process quality control L: Complicated operating procedures, applicable for aerobic digestion only, and ignores the value of end products	mg O ₂ /g TS–h	≤1.5	[12,45,52,86,90,93,95–100]
			mg O ₂ /g VSS–h	≤2.5	[83,104]

Table 3. Cont.

Stabilization Process	Indicator	Advantages (A) and Limitations (L)	Unit	Criterion	Reference (s)
Composting	Volatile solids	A: Simple testing methods L: Procedure control	% VSR	≥ 50	[45,75,86,90,101–103]
	Carbon/nitrogen ratio (C/N)	A: Simple testing methods L: End-products quality control	—	<12	[45,86,87,90,105]
	Total organic carbon (TOC)	A: Simple testing methods and end-products quality control L: Need delicacy management	%	>5	[45,86,90]
	CO ₂ evolution rate	A: End-products quality control L: Unreasonable organic degradation rate and ignores the value of end products	mg CO ₂ /g OM–d	<2 (Very stable) 2–4 (Stable) >4 (Unstable)	[45,70,73,86,90]
	Specific oxygen uptake rate (SOUR)	A: Process quality control L: Complicated operating procedures, applicable for aerobic digestion only, and ignores the value of end products	mg O ₂ /g VS–d	<3 (Very stable) 3–10 (Stable) >10 (Unstable)	
	Self–heating (Dewar test)	A: Indicates ecological value of end products L: Applicable for composting only and ignores the value of end products	Dewar index (ΔT °C)	<10 (Very stable) 10–20 (Stable) >20 (Unstable)	

* Applies only to AnD, ** applies only to AeD, *** applies also to composting. TS: total solids, VS: volatile solids, VSR: reduction in volatile solids, ΔT °C: temperature difference.

In terms of the C/N, Nikaeen et al. [87] suggested that a value less than 12 reflects an advanced degree of OM stabilization. In addition to the TOC, a decreasing trend of the C/N with time indicates OM degradation and sludge stabilization. Thus, the C/N should be combined with other analytical tests and indices to characterize the quality of the final product. Because the C/N is not an appropriate stability indicator, its development during the biological stabilization process must be illustrated [68].

Humic substances (HS) and respirometric methods are alternatives to evaluate sewage sludge stabilization. The first are based on biological stabilization processes that degrade simple organic compounds (proteins, polysaccharides, lipids, etc.) and synthesize complex organic compounds (i.e., HS) [81,88].

Respirometric methods measure the biodegradable OM content and sludge stability [90]; O₂ consumption and CO₂ generation are measured as dominant indices, but the amount of CO₂ released from a biological activity is commonly used to estimate stability in co-composting processes with other organic materials [106]. Another respirometric method is SOUR in which microorganisms use O₂ while consuming OM (AeD processes). 1.5-mgO₂/g TS–h or 2.5-mgO₂/g VSS–h values indicate considerable stabilization [104,107,108]. However, it requires a procedure configuration, monitoring, and procedure, including extended experimental periods [83].

The self-heating tests (Dewar test) of the compost can be used to estimate the microbial respiration and remaining OM indirectly. The increase in temperature within the container for several days is related to the microbial activity and stability of the compost [70,106].

Table 4 presents the indicators of biological stabilization in different studies in terms of municipal WWTPs and the biological stabilization processes of sewage sludge. In 57% of the studies listed, the most related stabilization indicators were VFAs, VSR, and VS/TS in the case of stabilization through AnD. In AeD studies (23%), the stabilization indicators are mainly associated with VSR and VS/TS, and in the case of composting (the remaining 20%), the most commonly used criteria were the TOC and C/N. Indicators such as HS, SOUR, and the Dewar test were the least applied to determine biological stabilization in sewage sludge, owing to the requirement of complex procedures in the laboratory [86–88].

Moreover, the anaerobic processes, MWW treatments (e.g., UASBs), and sludge digestion (e.g., AnD) generate biologically stabilized sludge according to the indicators VFAs, VSR, VS/TS, HS, TOC, and C/N.

Additional stabilization processes are required for sewage sludge with an SRT of less than 12 days (CAS). A study in CAS systems reported SOUR values of 3–4.5 mgO₂/g VS-h, indicating unstabilized sludge, whereas the EAAS and SBR systems with an SRT of >18 days reported values very close to the stabilization of the sludge, i.e., between 0.9 and 2.0 mgO₂/g TS-h [83,107].

Tas [90] and Cokgor et al. [45] evaluated sewage sludge samples from CAS + AeD systems with similar initial loads in different climatic periods and reported the values of the VSR and TOC as 44% and 73%, respectively, in summer. In autumn, a VSR of 31% and a TOC of 43%, and in winter, a VSR of 28% and a TOC of 55% were reported. As the temperature decreased, the efficiency decreased in the VSR and TOC, which directly affected the stabilization degree.

Mei et al. [81] analyzed 16 municipal WWTPs that used AnD and composting as biological sludge stabilization processes. The VSR rates varied between 0.5% and 80.2%. The increase in the HS ranged between 19% and 81% in different cases, showing a close relationship between the stabilization processes and sewage sludge characteristics.

In addition to the analysis of the biological stability, regulations associated with the control of the vector attraction and odor of the sewage sludge are defined to determine the sludge's final disposal and use as well as to avoid the propagation of toxic compounds and pathogens in the environment. These regulations vary between countries, but in general, these set the limits for the maximum concentration of heavy metals and pathogens [51,61].

Table 4. Summary of the revised information on the indicators of biological stabilization reported in different publications.

Municipal WWTPs	Sludge Type	Stabilization Process	Operational Variables of the Process *					Indicators of Biological Stabilization							Reference (s)
			T (°C)	HRT (h)	SRT (d)	pH	VFAs (mgCOD/gVS)	VSR (%)	VS/TS (%)	C/N	HS (mg/gVS)	TOC (%)	SOUR (mgO ₂ /g TS-h)	Dewar Test (ΔT °C)	
Anaerobic	SS	NA	35–40	15	20	7.3	-	46–60	42	-	186–273	-	-	-	[81,109]
	SS	NA	54–55	15	20	7.6	-	38	51	-	146.1	-	-	-	[81,109]
UASB	SS	NA	35	24–48	18–33	8.2	160–320	55–68	60–65	9.0	-	-	-	-	[50,54,110,111]
CAS	SS	NA	-	-	4–8	-	-	-	73–87	-	-	-	3–4.5	-	[107,111]
CAS	SM	Anaerobic	12–22	-	-	7.0–7.9	-	49–52	60	6.1–17	-	13.8	-	-	[50,72,76,112]
	SS	digestion	25–50	-	5–12	8–10	140–520	52	-	-	-	-	-	-	[59,82,113]
Aerobic	SS	Composting	-	-	-	6.4–6.7	-	50–80	-	-	242–334	-	-	-	[82,114]
Anaerobic	SS	Composting	35	-	-	7.3–7.6	-	43.5	45–47	10	-	2.0	1.4–1.1	10–20	[80,87,115]
Aerobic	SS	NA	-	-	-	6.5–9.0	-	56–63	44	8.9–15	-	-	-	-	[50,70]
EAAS	SS	NA	20–25	20	18–30	7.1–7.8	-	32–40	60–70	5.4–5.9	-	-	0.9–1.5	>20	[47,48,79,107,116]
SBR	SS	NA	20	-	24–40	6.8	-	34–38	60–70	6.0	-	-	1.8–2.0	-	[76,83,117]
CAS	SM	Aerobic	20	-	18–35	-	-	26–31	65–80	-	-	-	-	-	[90,118,119]
Anaerobic	SS	digestion	35	20	-	6.8–6.9	-	-	29	-	-	-	<1.5	-	[120]
CAS	SS	Aerobic	59–61	5–15	-	7.8–8.3	-	-	25–37	-	-	-	-	-	[109]
Anaerobic	SS	digestion	35–65	20	-	6.3–6.9	-	44–24.5	62–70	-	-	-	-	-	[121]

* They correspond to the sludge stabilization process; however, when the municipal WWTPs do not use this process, the operation variables of the municipal WWTPs are reported. WWTPs: wastewater treatment plants, T: temperature, HRT: hydraulic retention time, SRT: sludge retention time, VS: volatile solids, TS: total solids, SOUR: specific oxygen absorption rate, VFAs: volatile fatty acids, VSR: reduction in volatile solids, TOC: total organic carbon, C/N: carbon/nitrogen ratio, HS: humic substances, SS: secondary sludge, SM: mixed sludge, UASB: upflow anaerobic sludge blanket, CAS: conventional activated sludge, EAAS: extended aeration activated sludge, and SBR: sequential biological reactor.

The first known regulation on the subject was the Directive 86/278–EEC of the European Community [24,122], which introduced limits for the quality of sewage sludge to protect public health. Based on this directive, each country in the region has issued a regulation that, in some cases, has provided stricter limit values and included more restrictions, mainly for pathogens and heavy metals [51,68,77]. In other countries, such as Russia, China, New Zealand, and South Africa, guidelines have also been developed to classify sewage sludge to determine its suitability for agricultural use [21,34].

In Latin America, the regulations are mainly based on the Standard for the Management and the Disposal of Sewage Sludge and Biosolids (40CFR Part 503) issued by the USEPA in 1993 [50,123].

Owing to the low pathogen removal efficiencies of the biological stabilization processes, Silva-Leal et al. [16] and Collivignarelli et al. [66] indicated that biosolids obtained from a biological stabilization process (i.e., AeD and AnD) should consider further hygienization or disinfection treatments (e.g., thermal drying and chemical treatment) to take advantage of the biosolids in restrictive uses (e.g., agriculture). Recently, Peccia et al. [63] and Ducoli et al. [17] indicated that for the safe use of biosolids, hygienization is more relevant during the COVID-19 pandemic.

4. Future Perspectives

Sludge management is key as it represents a considerable part of the yearly operative costs in municipal WWTPs. Biological stabilization processes are associated mainly with OM degradation. Likewise, from a regulatory viewpoint, as sludge stability is also related to the reduction in the attraction for vectors, the analysis of all the physical, chemical, microbiologic, and parasitological characteristics associated with the sewage sludge must be guaranteed.

Despite biological sludge stabilization being widely researched, as identified in the 512 reported articles, only 218 of them address the indicators and criteria to pinpoint the degree of stability, thus evidencing legal gaps and the lack of standardized criteria that may serve as a tool for water utility managers. Additionally, there is no consensus among researchers regarding the stabilization of sludge from aerobic systems such as EAAS and SBR. Moreover, it is important to continue researching the influence of operational and environmental factors in the degree of sludge stabilization.

Due to the increasing worldwide production of sludge and the fact that sludge is considered a unique, complex, and dynamic product, it is indispensable to characterize and follow-up on these products for their adequate reuse and/or final disposal. Its management should be including strategies framed in sustainable development and circular economy objectives, which are focused on waste reduction and reuse. Thus, future work development could be steered toward evaluating aspects such as:

- (i) How the implemented MWW treatment technology and sludge stabilization process influence the degree of stabilization and characteristics of the sewage sludge. Different stabilization indicators should be included in the sewage sludge, contributing to the safe use of sewage sludge and biosolids and minimizing the environmental impacts and public health risks.
- (ii) The verification of the need to apply complementary stabilization and hygienization processes that ensure a safe material that complies with regulations, as it is necessary depending on the characteristics of the sewage sludge.
- (iii) The agronomic potential benefits related to the proportion and availability of the nutrients present in the sewage sludge, which must be compared with that of chemical fertilizers.

5. Conclusions

The weather conditions, size and characteristics of the population, the population's economic, technical, and technological capacities, and the type of municipal wastewater (MWW) collection, transport, and treatment systems exert an important influence on the

characteristics of MWW and the quantity/quality of the sewage sludge generated. Further, the characteristics of the sludge are associated with the type of sludge stabilization and/or hygienization processes implemented.

Anaerobic technologies generally produce a stabilized sludge, whereas conventional aerobic technologies (i.e., CAS) require complementary stabilization processes. Some authors suggest that in aerobic systems with SRT values > 18 days, such as in EAAS and SBR, the sludge generated can be considered stable and would not require additional biological stabilization processes. However, the operational conditions of the system influence the sludge stabilization degree; therefore, it is recommended to verify the sewage sludge characteristics and the mechanisms of the biodegradation of organic matter using different stabilization indicators.

The main stabilization indicators are volatile fatty acids (VFAs), volatile solids (VS), the carbon/nitrogen (C/N) ratio, humic substances (HS), the total organic carbon (TOC), the carbon dioxide (CO₂) evolution rate, the specific oxygen uptake rate (SOUR), and the Dewar test. However, it is not recommended to evaluate a single stabilization indicator.

The biological stabilization processes, such as anaerobic and aerobic digestions as well as composting, are the most implemented and researched, improving the sewage sludge characteristics and potential agricultural use.

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