Revista ION | e-ISSN: 2145-8480 ISSN-L: 0120-100X | DOI: https://doi.org/10.18273/revion.v37n2-2024001

Organic supports: a low-cost alternative to enhance anaerobic digestion?

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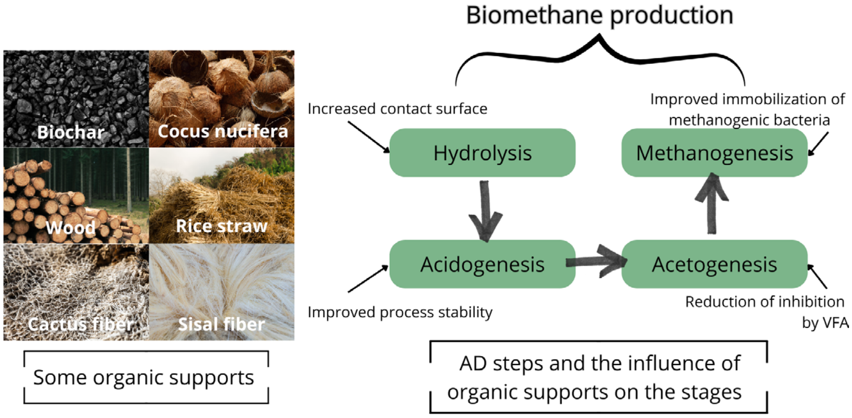
Fecha recepción: 1 de abril de 2024
Fecha aceptación: 25 de julio 2024

Graphical abstract



Abstract

Organic supports in Anaerobic Digestion facilitate the attachment of microorganisms to the surface of these media, thereby enhancing biogas production; however, the information available in the literature is limited. This article is a compilation of research focused on using organic supports in anaerobic processes published over the past 18 years, highlighting the challenges encountered during anaerobic biodegradation and the limitations of conventional approaches; in this regard, this review concentrates on the influence of organic supports on the microbiology and biochemistry of the anaerobic process. Current trends in using organic supports and their advantages for improving biogas efficiency and quality are also presented.

Keywords: *Anerobic digestion; Biofilm; Organic supports; Inorganic supports; Organic waste; Psychrofilic conditions; Biochar; Anaerobic microorganisms; Biogas production; Waste valorization; Methanogenesis; Methane yield.*

Cita: Garay Jácome MP, Parra Suárez RA, Jaimes Estévez J, Castro Molano LP, Escalante Hernández H. Soportes orgánicos: ¿Una alternativa de bajo costo para mejorar el proceso de digestión anaeróbica?. rev. ion. 2024;37(2):7-24. doi:10.18273/revion.v37n2-2024001

Soportes orgánicos: ¿Una alternativa de bajo costo para mejorar el proceso de digestión anaeróbica?

Resumen

Los soportes orgánicos en la Digestión Anaeróbica promueven la adherencia de los microorganismos en la superficie de estos medios y a su vez, mejoran la producción de biogás; sin embargo, la información reportada en la literatura es limitada. Este artículo es una compilación de investigaciones enfocadas al uso de soportes orgánicos en el proceso anaeróbico publicadas en los últimos 18 años; destacando los desafíos que se presentan durante la biodegradación anaerobia y las limitaciones de los enfoques convencionales. Esta revisión bibliográfica se enfocó en la influencia de los soportes orgánicos sobre la microbiología y bioquímica del proceso anaeróbico. Se presentan las actuales tendencias del uso de soportes orgánicos y sus ventajas en la eficiencia y calidad del biogás.

Palabras clave: *Digestión anaeróbica; Biopelícula; Soporte orgánicos; Soportes inorgánicos; Residuos orgánicos; Psicrofilia; Biocarbón; Microorganismos anaeróbicos; Producción de biogás; Valorización de residuos; Metanogénesis; Rendimiento de metano.*

Suporte orgânico: uma alternativa de baixo custo para melhorar a digestão anaeróbica?

Resumo

Os suportes orgânicos na Digestão Anaeróbica facilitam a fixação de microrganismos à superfície desses meios, melhorando assim a produção de biogás; no entanto, as informações disponíveis na literatura são limitadas. Este artigo é uma compilação de pesquisas focadas no uso de suportes orgânicos em processos anaeróbicos publicadas nos últimos 18 anos, destacando os desafios encontrados durante a biodegradação anaeróbica e as limitações das abordagens convencionais; nesse sentido, esta revisão se concentra na influência dos suportes orgânicos na microbiologia e bioquímica do processo anaeróbico. As tendências atuais no uso de suportes orgânicos e suas vantagens para melhorar a eficiência e a qualidade do biogás também são apresentadas.

Palavras-chave: *Digestão anaeróbica; Biofilme; Suportes orgânicos; Suportes inorgânicos; Resíduos orgânicos; Psicrofilia; Biocarvão; Microrganismos anaeróbicos; Produção de biogás; Valorização de resíduos; Metanogênese; Rendimento de metano.*

Introduction

Globally, nearly 50 % of the population belongs to developing countries. In these countries, where a large part of the geographical distribution is rural, energy requirements are supplied by biomass in about 35 % of cases [1]; specifically, according to the 2022 report by the Promigas Foundation, which revealed the Multidimensional Energy Poverty Index (IMPE), approximately 18.5 % of the Colombian population lives in rural areas that still do not have access to natural gas service [2]. This situation leads to using propane gas and firewood as substitutes, causing health problems for users, negative environmental impacts, and economic and technical issues; a viable alternative to mitigate these complications is anaerobic digestion (AD) [3]. AD is a microbiological process that, through different metabolic stages (hydrolysis, acidogenesis, acetogenesis, and methanogenesis), breaks down organic waste and generates energy in the form of biogas, which has a considerable calorific value (6.56 kWh/m³) equivalent to a proper heat of 3.3 kWh/m³ [4]; for AD to proceed correctly, conditions such as the concentration of fed nutrients, the source of inoculum, and the temperature must be appropriate [5]. The latter considerably affects the metabolic process because, under psychrophilic conditions (temperatures below 20 ± 2 °C), the rates of chemical and biological reactions are slow compared to those obtained under optimal temperature conditions (37 ± 2 °C) [6].

A proposal to improve AD at low temperatures is the immobilization of microbial cells through the addition of organic support materials [7,8]; these types of supports allow the adherence of the microbial consortium, increasing the interaction between the microorganisms and the substrate to be treated [9]. Most studies using organic supports in AD focus on improving biogas production yields; a representative example is Jang *et al.* [10], who reported an increase in methane (CH_4) content of 27.65 % by adding biochar as a biofilm carrier. Research has demonstrated the importance of using organic supports in the anaerobic process; therefore, this article presents a systematic literature review on the use of organic supports based on their effect on (i) the metabolic stages and microbiology of the process (under different temperature conditions) and (ii) biogas production yield and AD stability. Finally, a roadmap is proposed for studying AD using organic supports to improve the process.

Methodology

The collected information was tabulated to determine the effect of the support on microbial communities in the AD process (Supplementary Material S1) Based on a literature search, information was categorized concerning anaerobic digestion processes using organic supports (n=25) published between 2005 and 2023, with a notable increase in 2016 (n=21), reflecting the growing interest in the topic. These articles were published in various databases, with Scopus as the database with the most research articles in the area of interest; additionally, the impact of supports on the stability of the anaerobic digestion process was analyzed, considering the content of volatile fatty acids (VFAs) and the removal of organic matter (OM), as well as biogas production and quality, specifically methane content.

Results

Effect of the Use of Organic Supports on the Microbiology of the Anaerobic Digestion Process.

Literature Review. Figure 1 shows the results obtained according to the number of publications per country from the literature review on using supports in anaerobic digestion. Overall, a significant contribution to the research topic is observed from countries such as Colombia, followed by Brazil and Spain, with 23, 18, and 15 publications, respectively. This may be due to factors related to waste management and demographic distribution; for example, in Colombia, approximately 32,580 tons/day of solid waste are generated from residential, commercial, and institutional activities [11], in addition to the growth in research and development infrastructure by universities and research centers with technologies that facilitate high-quality research. Brazil has the largest territory in Latin America, producing a significant amount of waste annually, highlighting the need for technologies for its treatment. Spain generates around 453 kg/person/year of waste, mainly composed of organic waste (45.14 %), which must be managed to promote a sustainable economy that allows compliance with European legal obligations [12]. Hence, there is a global need to manage emerging waste through anaerobic digestion [13].

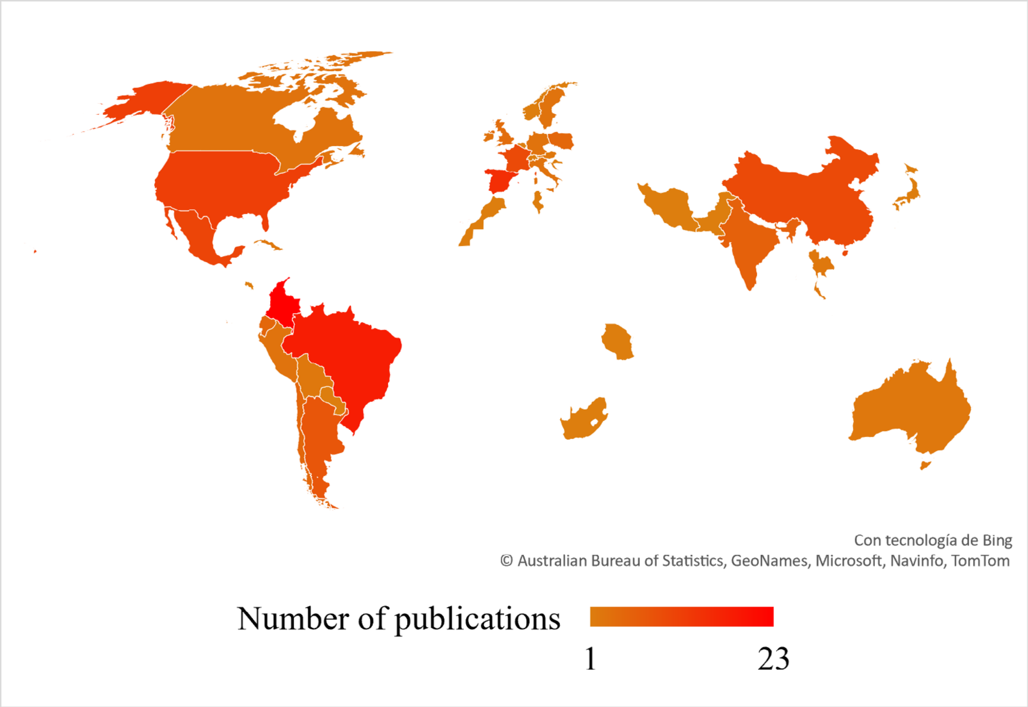


Figure 1. Number of publications related to the AD process using support by country. n = 179.

According to the results, a consistent trend is observed in the number of publications per year (Figure 2). In 2016, the highest number of publications was reached with 21 documents, distributed in Scopus (6), Dialnet (8), Scielo (2), and Nature (5); this significant increase is related to the need to develop more effective methodologies in the anaerobic digestion process using organic supports that significantly improve the efficiency of this technology. As shown in Figure 2, the bibliographic database with the most contributions is Scopus, with 72 publications, followed by Dialnet, with 54 documents.

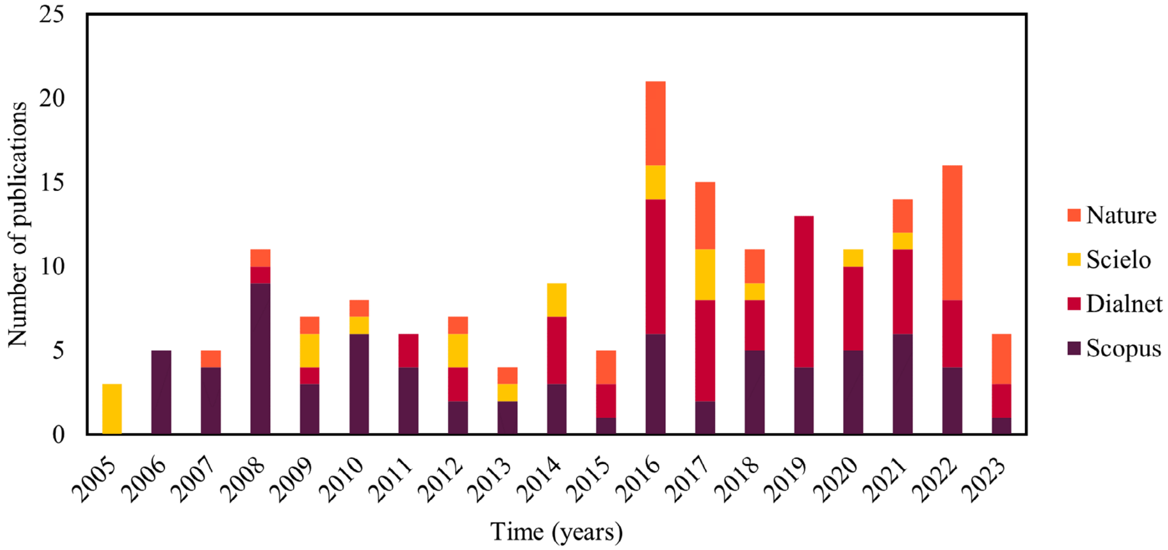


Figure 2. Statistics of published works (2007-2022) related to AD using supports. n=179.

It is known that the nature of the support material influences AD. Figure 3 shows the percentage of publications concerning the type of support material used. Due to their defined shape and durability, commercial artificial carriers made of polyethylene [14], polypropylene [8], and polyurethane are used. Other notable inorganic supports include gravel [15], pumice stone [7,14,16], porous glass beads [7], and zeolite [17]. However, some of these supports are polluting and costly [15]. Notable examples of organic supports are biochars [14,15,18-25], sisal fiber [7,16], grape stalk [16,26], and wheat straw [8,27]. Organic materials as biofilm carriers are abundant in the agroindustry and, in some cases, are considered solid waste that can be utilized. Thus, organic support materials are essential in waste management, providing a low-cost alternative to improving AD. Therefore, this study focuses on the use of organic supports.

It is essential to clarify that of the 179 selected publications, 52 % report the formation of biofilms without the use of supports, and the remaining percentage is distributed among studies implementing organic and inorganic supports. Hence, 25 documents related to the topic of anaerobic digestion using the organic supports were selected. Studies that did not employ organic supports were discarded.

General Overview of the Influence of Organic Supports on the Anaerobic Digestion Process.
Table 1 presents the investigations used to develop the discussion of this research, synthesizing the most relevant aspects of the documents consulted in the literature review (Supplementary Material S1); the selected parameters included the type of support, its concentration, the substrate, the type of reactor, the inoculum, and the temperature. The concentration of material added as support significantly depends on the operational parameters of both the influent and the volume to be treated in the AD process; however, no apparent effect of this parameter has been observed due to the lack of consistent reports from numerous authors. Additionally, it was observed that there is no consensus on the effect of support concentration on AD among studies addressing this variable; the substrates used include municipal and industrial wastes such as sludge and wastewater, agricultural wastes such as animal manure, food waste, and crop residues, and organic fractions of urban solid waste, which are readily biodegradable and have considerable potential for CH_4 production. Regarding the source of inoculum, anaerobic sludge from reactors previously treated with the same substrates is commonly used.

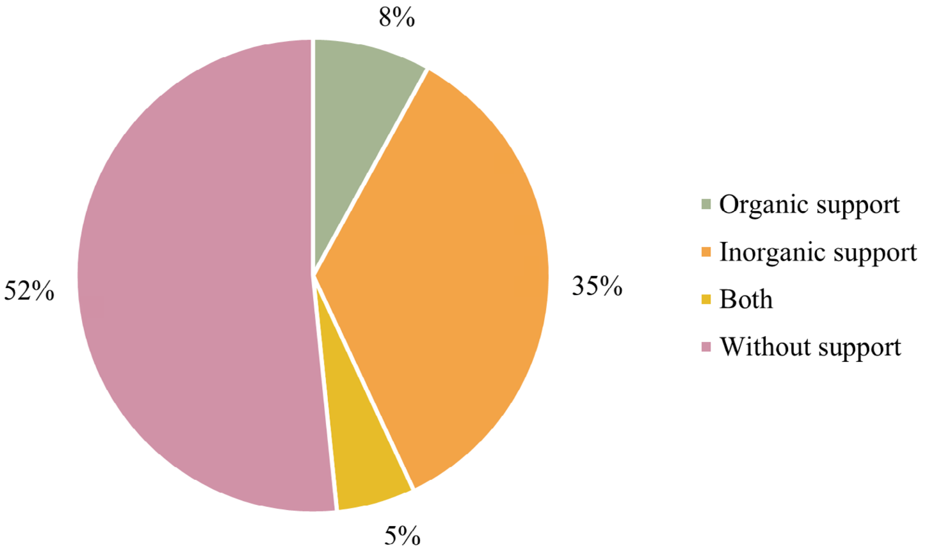


Figure 3. Publications based on the type of support material used. n=179.

Table 1. Summary of selected studies on the influence of support on the microbiology of the Anaerobic Digestion process.

Support Type	Support Concentration	Substrate	Inoculum	Temperature	Reactor Type	Results and Comments	Ref.
Garden waste	N/E	Food waste	Digested sludge from a Wastewater Treatment Plant	36 °C	Semi-continuous reactor V= 500 L	Higher biogas yield. Delay in system acidification.	[30]
Grape waste biochar	N/R	Cattle manure	N/E	24 °C	Batch	The biochar obtained from torrefaction is effective as a support.	[31]
Rice straw biochar	7.1 g L^{-1}	Cattle manure	N/E	41 °C	Batch V= 2000 L	↓ lag phase. Increased biogas yield. Improved buffering capacity of the AD process.	[32]
- Magnetic biochar -Polyurethane foam - Gravel	2.480 kg m^{-3} 23 kg m^{-3} 2.820 kg m^{-3}	Diluted and undiluted wastewater from coffee processing	Sewage sludge from an UASB reactor	Ambient: 6.4 - 32.9°C	Upflow Fixed Bed Anaerobic Reactor V= 139.5 L	Biochar showed better potential as a support material for the removal of phenolic compounds from ARC, with maximum removal efficiency of 92%.	[15]
Activated sludge-based activated carbon doped with nitrogen Fe_3O_4/N-SBAC	5 g L^{-1}	Wastewater from coal gasification plant	Anaerobic sludge from a wastewater treatment plant	37 ± 1°C	Laboratory-scale UASB reactor V= 2.8 L	↑ CH_4 production rate. Reduction in wastewater toxicity. Fe_3O_4/N-SBAC promoted the formation of larger, more stable sludge flocs.	[24]
- PVC - Cocus nucifera	N/R	Standard substrate (Glucose, ammonium chloride, potassium bicarbonate, monopotassium phosphate, and ethamide)	Greywater	24 - 26°C	Multi-chamber anaerobic biofilm reactor (AnBR) V= 10 L	↑ OLR and ↓ TRH. ↑ DQO removal.	[33]
Biochar derived from cattle manure (M-BC)	0 g L^{-1} 1 g L^{-1} 10 g L^{-1}	Manure	Tarleton Lake sediment	20°C 35°C 55°C	N/E V= 0.28 L	↓ lag phase. ↓ total AGV and propionic acid ↓ concentration. ↑ nutrient potential in digestate. ↑ alkalinity and ↑ CH_4 production. ↑ resistance to inhibitory compounds. ↑ microbial activity. Porosity and surface area of biochar facilitate biofilm formation.	[10]
Vermicompost Vermicompost biochar	N/R	Kitchen waste	Anaerobic sludge	35 °C	Batch V= 1 L	↑ buffering capacity of acids. Inhibition of AGV accumulation by less than 5%.	[23]

Support Type	Support Concentration	Substrate	Inoculum	Temperature	Reactor Type	Results and Comments	Ref.
Biochar	N/R	Chlorine-free water and fresh, liquid bovine rumen	Wastewater from grease trap	37 °C	Packed bed anaerobic reactor	↓ TRH from 3.1 d to 1.8 d. Improvement in the content of methanogenic microbial communities. ↑ soluble N in digestate.	[20]
Grape stalks	1 kg	Ethanol	Synthetic wastewater from vineyard wineries	35 °C	Fixed bed anaerobic reactor V= 16.7 L	↑ DQO removal efficiency. ↓ AGV concentration. Grape stalks act as support and a secondary carbon source.	[26]
Magnetic biochar	20 kg m^{-3}	N/R	Anaerobic sludge from laboratory-scale reactors	42 °C	Batch laboratory-scale reactor “glass syringes” V=0.1 L	General reduction in NH_4-N in digestate, attributable to microbial cell growth. Anaerobic biofilm grows homogeneously.	[21]
Corn stover biochar	1.82 - 3.64 g/g TS of sludge	N/E	Wastewater sludge	35 - 55°C	Batch V= 600 mL	↑ alkalinity and mitigates NH_3 inhibition in the digester. ↑ CO_2 sequestration and improvement in CH_4 yield. Digestate enriched with nutrients like K, N, and P with high potential for soil applications.	[22]
- Biochar BEC - Biochar ESI - Biochar Klin	0.5 g mL^{-1}	Ethanol	Laboratory culture microbial consortium	30 °C 37 °C	N/E	Stimulation of direct interspecies electron transfer (DIET) improving CH_4 production.	[19]
- Wheat Straw - Sunflower stalk - Grape stalk - Cactus fiber - Loofah fiber - Cypress cones	N/R	Winery wastewater supplemented with NH_4Cl and NaH_2PO_4	Industrial sludge from a sugar factory	35 °C	Fixed bed anaerobic reactor Semi-continuous V=16.7 L	↓ lag phase. Adequate methanogenic activity of biofilm. Lower operational cost using lignocellulosic biomass as biofilm support. ↓ AGV	[8]
Native cassava starch (Polymer)	N/R	Mixture of ground organic matter, chlorine-free water	Urban organic waste	52 °C	Glass bioreactors V=0.8 L	Under test conditions, the assay shows adequate levels (CH_4 greater than 55% and final pH close to 7.7) favoring the development of methanogenic bacteria.	[34]
- Sisal fiber - Pumice Stone - Nile perch scales	145 kg m^{-3} 271 kg m^{-3} 200 kg m^{-3}	Fish solid waste	Anaerobic sludge from wastewater	27 – 35°C	Upflow packed bed bioreactor with recirculation V= 3 L	↓ DQO due to lag phase. ↑ CH_4 production. ↑ acid buffering capacity.	[16]

Support Type	Support Concentration	Substrate	Inoculum	Temperature	Reactor Type	Results and Comments	Ref.
Porous ceramic cubes (CCs) - GAC	N/R	Olive mill wastewater (OMW)	Olive mill wastewater inoculated with high-density biomass	25 °C 35 °C 55 °C	Compact bed biofilm reactors (PBBRs) V=2.5 L	Significant polyphenol removal, particularly with GAC. ↓ DQO much greater removal with GAC vs. CCs. CCs had higher AGV concentrations than GAC reactors. ↑ methanogenic activity with GAC.	[18]
- Polyurethane foam - Synthetic pumice stone - Charcoal - Low-density polyethylene	N/R	Domestic wastewater	Anaerobic sludge from a poultry wastewater treatment reactor	N/R	Sequencing Batch Anaerobic Biofilm Reactor (AnSBBR) V=7.2 L	Compared to the organic supports used, polyurethane showed better cell immobilization favoring microbial adaptation.	[14]
- Coconut fiber - Wood - Nylon	N/R	Vinasse	Anaerobic reactor sludge and cattle manure slurry	37 °C	Upflow fixed column reactor V= 3 L	↑ DQO removal. ↑ biogas yield.	[28]
Wheat straw bed	N/R	Undiluted mixture of fresh beet leaves and ensiled grass	Municipal waste sludge Digested cattle manure	33 °C 35 °C	Laboratory-scale plexiglass column reactor V=4.75 L Pilot-scale insulated steel column reactors V= 390 L	↑ mass transfer level. Accelerates and ensures the feeding phase. Microorganisms retained in the bed quickly adapted.	[27]
- Sisal (agave species) - Pumice Stone - Porous glass beads	N/R	Sisal leaf tissue waste and synthetic medium	Sludge from a mesophilic digester	35 °C 37 °C	Upflow packed bed bioreactor with recirculation V=2 L	Of the supports tested, sisal fiber showed the highest CH_4 production (2.6 L/L/day) and ↓ DQO around 80%. Microorganisms on sisal waste fiber support maintained high AGV concentrations with ↑ OLR without severe operational issues.	[7]
- GAC - Tezontle	N/R	Domestic wastewater	Sludge from a UASB reactor treating domestic wastewater	35 °C	Jacketed upflow biofilters V= 9.4 L	↑ DQO removed by 80%. ↑ CH_4 production yield. ↑ organic matter removal capacity using GAC.	[35]

Most studies are conducted in batch reactors at the laboratory scale due to their simplicity of construction and operation, allowing the adjustment of operational parameters for their implementation at the pilot scale. The diversity in the design of reactors used focuses mainly on optimizing mixing, responding to high organic loads, and reducing the risk of inhibition by toxic substances [28]; for example, fluidized bed digesters facilitate the retention and increase of microbial cell growth due to their trapping capacity [29]. Fixed bed anaerobic reactors (AFBR) show good potential for wastewater treatment, allowing high solid retention times, translating into high system efficiency and stability, with low hydraulic retention times that reduce operational costs [8]; it should be noted that many anaerobic reactors are inoculated in batch operation, i.e., the support material is contacted with active inoculum sludge within the reactor. Some authors report that the contact time of the inoculum-support system favors biofilm growth in batch operation. However, the contact time is an empirical variable that can last from days to months, and in most reviewed articles, data were not reported (Supplementary Material S1); however, Bertin *et al.* [18], reported an adaptation time of 35 to 40 days before adding wastewater from olive milling to the AD process, using GAC as biofilm support.

Physical Characteristics of Supports and Their Influence on Anaerobic Digestion

Below are some physical characteristics of supports, such as density, specific surface area (particle size), and porosity, which directly influence DA.

Density. The amount of feed and packing density are important factors that affect the DA process's mass transfer and operational efficiency [36]. Svensson *et al.* [27], investigated the use of wheat straw as a support in a single-stage reactor, both at laboratory and pilot scale, finding that a high packing density produces excessive AGV formation (total AGVs with a peak of 13 g L^{-1}), which inhibits methanogens in the process; additionally, they demonstrated that maintaining the initial bed density between 60 and 100 g L^{-1} allows wheat straw to function as a biofilm support and particle filter.

Specific Surface Area. Particle size is a physical property that directly affects DA. Lü *et al.* [37], evaluated the influence of different particle sizes of biochar (2-5 mm, 0.5-1 mm, 0.075 mm - 0.150 mm) on microbial distribution during glucose DA under ammonium stress (NH_4); they demonstrated that bacteria could access fine particles more efficiently than coarse particles. Similarly, Linville *et al.* [38], investigated the influence of particle size and biochar concentration (derived from a nutshell) in the DA of food waste under mesophilic and thermophilic conditions; their study showed that smaller particle sizes led to more excellent CO_2 removal, increasing from 51 % for a 500 µm size to 61 % for finer particle sizes (125 - 137 µm). The authors attribute this behavior to the increased specific surface area when operating with smaller particles.

Porosity. Various support materials have been investigated in porous and non-porous configurations to improve the biomethanization process in bioreactors [13]. Acharya *et al.* [28], reported that the predominance of organisms in the biofilm is influenced by the porosity and surface area of the support material; therefore, biofilm formation occurs quickly on porous materials like coconut fiber and charcoal compared to non-porous nylon fibers. The authors justified that the retention of microbes by porous materials enabled the functioning of the bioreactor packed with coconut fiber, with a high OLR and reduced HRT of 31 kg COD m^3 d^{-1} and 6 d, respectively. In another study, Jang *et al.* [10], showed that the porous structure of biochar derived from manure can contribute to direct interspecies electron transfer (DIET) or hydrogen (H_2) transfer between syntrophic bacteria and methanogens.

Moreover, S. Wang *et al.* [32], observed that the use of biochar as a support in DA provides good adsorption performance for small particles or colloids; this facilitates the pores of the biochar becoming abundant sites for microorganisms, improving digestion efficiency. The biochar's capacity to absorb these particles and provide an adequate habitat for essential microorganisms not only optimizes microbial activity but also contributes to improved stability and productivity of the DA process; it is necessary to note that many authors do not report information about the physical properties of the support, biofilm formation time, or the effect on microbial communities in the DA process (Supplementary Material S1). Therefore, there is an evident opportunity for research to identify the impact of each of the above-mentioned properties.

Implementation of the Use of Supports in Anaerobic Digestion under Different Temperature Conditions. Temperature is a highly influential factor in the AD process, as it limits or accelerates the metabolic processes of microorganisms, conditioning their survival and biological interactions [39] and affecting process stability and methane yield [40]. Figure 4 presents a chronological scheme of publications based on operating temperature (psychrophilic range <25 °C, mesophilic range 25<T<40 °C, and thermophilic range >40 °C); there is a clear trend in operating temperature towards the mesophilic range because, at temperatures close to 35 °C, AD is efficient [4], operation is more stable, and less energy is required for mechanical mixing or agitation [40]. However, there is a limited number of studies on psychrophilia, [10,15,18,33] due to operational, environmental, physicochemical, and microbiological issues in cold climates (between 5 and 20 ºC) [41].

Authors studying the AD process in ranges above mesophilic (37-45 °C) report that these temperatures improve conditions for the development and growth of methanogenic bacteria and the reaction rate of hydrolysis and acidogenesis is faster; for example, Mumme *et al.* [42], studied the effect of adding biochar as support on biogas production under thermophilic conditions. Compared to the AD process without support addition, methane yield and biogas production improved by 31 and 46 %, respectively. On the other hand, Mshandete *et al.* [7], studied the use of sisal compared to pumice stone and porous glass beads, concluding that the bioreactor performance with sisal fiber waste as a biofilm carrier was higher (2.6 L L^{-1} biodigestor d-1) in mesophilia.

Jang *et al.* [10], investigated the effect of M-BC addition on methane production under psychrophilic conditions (20 °C) implementing organic supports in AD, reporting that the addition of biochar reduced the concentration of total volatile fatty acids and propionic acid (413.50 mg COD L^{-1}); however, the accumulated methane is considerably higher with biochar addition under thermophilic and mesophilic conditions compared to psychrophilic, with increases of 11.02, 7.31, and 1.18 % respectively compared to the control. Bertin *et al.* [18], studied acidogenic anaerobic digestion based on immobilized cells of wastewater from olive milling using GAC as a biofilm support, finding a notable effect of temperature in the experiment carried out at 25 °C in the reduction of methanogenesis with an increase in AGV conversion. In both examples, biochar was used as a biofilm support, and improvements in methane production could be attributed to the physical properties of the material, which stimulate microbial activity in AD.

Considering the above, it has been demonstrated that organic supports improve biogas production in AD under all temperature conditions. However, most research reported in the literature focuses on mesophilic conditions. Therefore, due to the limited information available on the effect of organic supports in the AD process under psychrophilic conditions, there is an evident need to investigate the improvement of technology at temperatures below 25 °C and optimize operating parameters by implementing organic supports.

Effect of Organic Supports on the Microbiology of Anaerobic Digestion. The stability of AD requires a symbiotic balance between the trophic levels of the central metabolic groups of bacteria (acid-forming bacteria, obligate hydrogen-producing acetogens) and archaea (methanogens) [43]. The microorganisms involved in the AD process work in series or groups to degrade organic matter through successive stages, each triggering the next [39] ; that is, they symbiotically depend on each other in terms of metabolite consumption and production and are also conditioned by physical and chemical factors (temperature, pH, MO load) that influence their proper development [20].

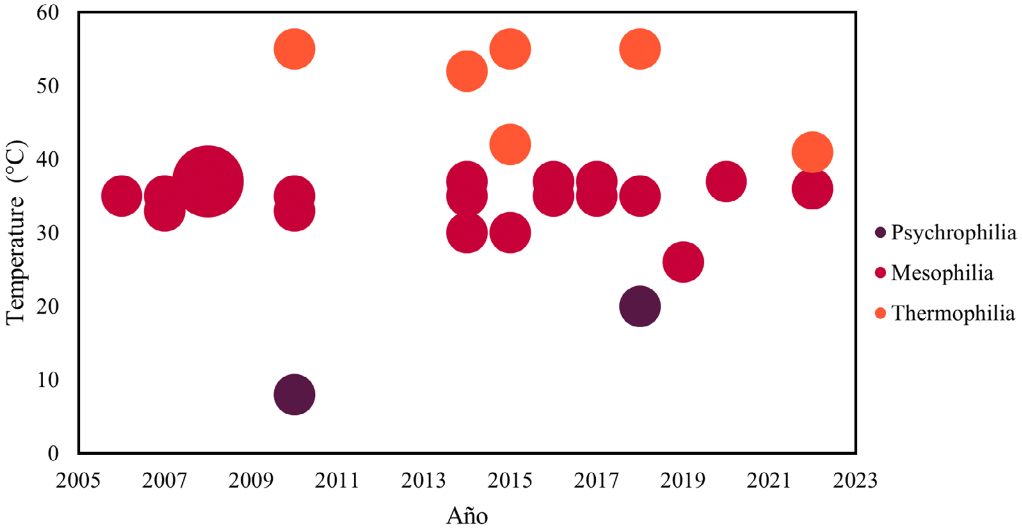


Figure 4. Chronological Scheme of Publications Based on Operating Temperature. n=25.

Organic supports can play a crucial role in improving the AD process by promoting microbial growth, providing better habitat and necessary nutrients to anaerobic microorganisms, facilitating bioelectric connections between cells, enhancing enzymatic activity, and buffering the capacity of inhibitory compounds (such as VFA and NH4), resulting in a more balanced formation and utilization of VFA, faster production of H_2 and CH_4, shorter lag phase, higher CH_4 content, and better digestate quality [18-20,24,33].

A representative example is Zhyang *et al.* [24], who concluded that Fe_3O_4/N-SBAC promoted microbial growth and enzymatic activity supported by microbiological analysis, suggesting that the presence of the support resulted in increased microbial population and diversity. The authors state that the presence of Fe_3O_4/N-SBAC increased the abundance of microorganisms such as Proteobacteria (26.5 %), which is one of the critical consumers of long-chain VFAs; the proportion of Chloroflexi (16.33 %), some bacteria in its phylum being potential partners in interspecies electron transport; and Petrimonas (6.0 %), Longilinea (3.1 %), and Ornatilinea (2.4 %), which are related to the degradation of inhibitory compounds like phenol.

Furthermore, other authors also suggest that biochar is an excellent packing material to support the growth and retention of biofilms rich in well-balanced methanogenic microbial communities; the dominant population was Methanobacterium (hydrogenotrophic methanogens), with relative abundances ranging between 19.3 and 31.1 %. Biochar samples also contained a variety of other populations, including genera of some acetogenic species like Sporanaerobacter (2.5 - 4.3 %) and Syntrophomonas (8.5 - 12.3 %) and fermentative bacteria of the genus Escherichia (4.2 - 5.1 %) and Aminobacterium (6.9 - 8.8 %) [20].

The literature reports that support materials are a suitable medium for forming biofilms that favor the development of certain species of microorganisms; for example, Borth *et al.* [30] analyzed the microbial communities present in garden waste used as support and found a higher presence of methanogenic archaea, specifically *Methanospirillum, Methanobacterium, Methanobrevibacter, and Methanoculleus,* compared to the reactor where such support material was not used. These findings suggest that using support materials can improve the efficiency of the anaerobic digestion process by promoting a favorable environment for the growth and activity of critical microorganisms in methane production.

One of the most significant effects on the microbiology of AD when using organic supports is the reduction of the adaptation or lag phase. Jang *et al.* [10], reported that the effect of M-BC addition shortened the lag phase in AD at all evaluated operating temperatures (25, 35 and 55 °C) for a concentration of 0 g - 10 g of M-BC L^{-1} under psychrophilic conditions, the lag phase decreases from 10.81 to 9.26 d, in mesophilia from 2.08 to 1.52 d, and finally in thermophilia from 3.94 to 2.98 d. Additionally, it is observed that the concentration of M-BC and the lag phase present an inversely proportional relationship; the higher the biochar concentration, the shorter the lag phase. Conversely, Kassuwi *et al.* [16], observed that using fish scales as a biofilm carrier under mesophilic conditions, the adaptation phase extends, even causing decreases in the percentages of organic matter removal (soluble COD removal between 22 and 40 %); this is attributed to the delay caused by microbial acclimation to the support due to its low affinity with the inoculum.

Three main aspects can be highlighted in the effect of the support on AD: i) support compatible with the inoculum allows microbial cell adhesion, improving cell concentration and contact between biomass and substrate; additionally, adaptation phases are shortened, and the development of methanogenic microorganisms is promoted. ii) The reviewed studies indicate that higher support concentration, smaller particle size, and greater porosity improve bioprocess; moreover, depending on particle size, retaining microorganisms on the support surface is possible. iii) As is known, temperature directly affects AD reaction rates; using organic support in mesophilic improves methanogenesis, but this effect is unknown at low temperatures.

Evaluation of the Use of Organic Supports on Process Stability and Biogas Production Yield.

Incidence of Support on Anaerobic Digestion Stability. VFAs represent the organic matter readily accessible for biodegradation by certain microorganisms; these compounds (acids between 2 and 6 carbons) are a direct indicator of process stability: concentrations above 1.5 and 4 g L^{-1} for continuous and batch experiments, respectively, cause a pH drop in the medium and process inhibition [44]. In the investigations reported in Table 1, it was identified that when organic supports are used, VFA concentrations are below the previously indicated ranges (Supplementary Material S2), which would suggest that these supports have a buffering capacity justified by the alkalinity contribution and possible adsorption of inhibitory compounds.

The addition of biochar improves VFA generation and consumption in acetogenesis and methanogenesis, respectively; moreover, the system's pH remains stable as the imbalance between rapid acidification and slow methanogenesis is avoided, improving process stability [23]; for example, Jang *et al.* [10], reported that biochar potentially alleviates VFA accumulation and improves their degradation rate, resulting in a relatively lower VFA concentration during AD than those without biochar. This suggests that methanogenesis for AD without biochar was insufficient and that the alkaline nature of M-BC plays a vital role in influencing methane production and yield. A similar effect occurs when using sisal fibers as support, where even with an increase in OLR (up to 24.9 g COD L^{-1} d^{-1}), VFA degradation efficiency was over 50 %. The authors suggest that this is likely because sisal has an indigenous population of already adapted degrading microorganisms that increased with the gradual feeding of propionic acid [7]; regarding supports with high lignin concentrations, it is possible to mention that they are difficult to biodegrade, thus having a reduced contribution to the VFA concentration at the process exit [23]. Likewise, this type of support improves organic matter removal percentages, as seen in Figure 5; using carriers with high lignin concentrations (>20%) results in higher removal percentages: 94 % for sunflower stalks, 92 % for grape stalks, and 90 % for cypress cones. However, the support's biochemical characteristics (lignin and hemicellulose content) can affect reactor efficiency due to organic overloads and be considered a second substrate [8].

As a particular case, Mijaylova-Nacheva *et al.* [35], compared COD removal efficiency using GAC and a porous stone, tezontle; these authors found that after 40 days, a COD removal close to 80 % was achieved using GAC, while tezontle required 145 days, which is attributed to the adsorption capacity of these materials. Similarly, these supports increased organic load up to 1.7 kg m^{-3} d^{-1} for tezontle and 22.8 kg m^{-3} d^{-1} for GAC; additionally, these supports improve methane production and biomass retention and counteract the effects of inhibitory compounds.

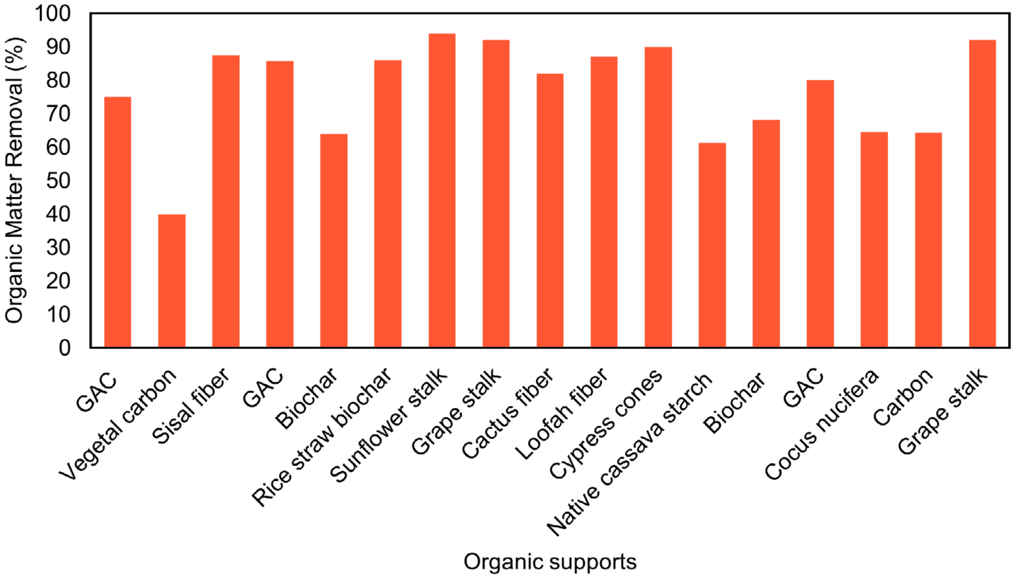


Figure 5. Percentage of Organic Matter Removal in Different Investigations Using Organic Supports in AD.

Incidence of Support on Biogas Production Yields and Quality. Organic support materials are a low-cost alternative to ensure the stability of methanogenesis to produce biogas with a high methane content [14,18,28]. Mshandete *et al.* [7], studied the effect of particle size on biogas yield with sisal fiber residues as support. The results showed that reducing the particle size (<2 mm) increased methane yield by 23 % compared to untreated fibers [45]; for their part, Bertin *et al.* [18], studied the effect of packing material on process performance using CCs and GAC. Reactors loaded with GAC produced high methane yields (close to 0.35 L d CH_4 produced per COD removed); at the same time, experiments with CCs showed low methanogenic activity (methane production did not exceed 0.2 L d CH4 COD^{-1} removed). Acharya *et al.* [28], evaluated methane production using coconut fiber as a support material; this fiber showed higher biogas production with high methane yield, 7.25 m^3 m^{-3} d^{-1} and 4 m^3 m^{-3} d^{-1}, respectively. The authors attribute this to the large surface area and high porosity of coconut fiber, which allows more excellent retention of microorganisms that favor the biomethanation process in the reactor.

Regarding porous supports, Zhyang *et al.* [24], found that CH_4 production could be related to the stimulating effect of dopant agents such as Fe3O4/N present in activated carbon (Fe_3O_4/N-SBAC); adding this type of support favored CH_4 percentage by decreasing CO_2 content. The CH_4 and CO_2 proportion in the reactor with support was 57.6 and 36.2 %, while in the control, it was 49.8 and 43.5 %; this is attributed to the fact that Fe_3O_4/N-SBAC significantly improves the CH_4 production rate due to the presence of carbonaceous material promoting microbial accumulation. On the other hand, Shen *et al.* [22], improved biogas quality by adding corn residue biochar; there was an increase in CH4 content by up to 42.4 %, and CO_2 removal was over 85 % compared to the control digester. An additional explanation for this behavior is that biochar provides alkalinity to the system, improving internal conditions for methane production; furthermore, the physical properties of biochar, such as particle size and surface area, enhance the development of methanogenic microorganisms.

Considering the previously mentioned physical properties, it is observed that using a support material (lignocellulosic biomass or biochar) helps mitigate substrate-induced instability in the AD process and improve biogas production in the digester; however, some support properties (alkalinity, ion exchange, and surface morphology) and species transfer mechanisms (DIET) need to be studied to identify optimal conditions that improve AD application.

Alternative to Improve the Anaerobic Digestion Process Using an Organic Support

Case Study Information. As previously discussed, AD is a technology that can be improved using organic support; the selected case study was the municipality of Cáchira (Norte de Santander). The relief of Cáchira determines a wide diversity of climates, ranging from 5 to 27 °C with an average temperature of 17 °C [46].

The municipality is located at an altitude of 2,025 m.a.s.l. and its mountainous physiography makes it a hard-to-access area; therefore, its population does not have coverage of the national home gas network; the economy of the municipality is based on agriculture, forestry, and livestock production [47]. According to the 2018 Municipal Agricultural Evaluations reported by the Ministry of Agriculture and Rural Development [48], the fastest-growing economic activity was forestry and wood extraction (70 %); however, the most relevant activity in the region is the dairy industry, which produces about 22,816 Liters of milk per day [48]; much of the milk is used in cheese production, resulting in a residue known as whey, which is neither managed nor valorized and represents about 90 % of the raw material used. The municipality has approximately 3130 cows producing the mentioned milk volume [48]. Cattle generate around 8 kg of manure/100 kg of weight per head daily [4], whose improper disposal can cause environmental problems such as foul odors, vector attraction, greenhouse gas emissions, and water source contamination, among others [49].

Based on the described scenario, there is a need to manage and utilize the residues (whey and cattle manure) through the AD process to mitigate the energy deficit. It is important to note that AD of cattle manure does not yield high biogas (approximately 0.32 m^3 biogas kg^{-1} VS) due to the low presence of macromolecules like lipids and proteins; similarly, whey digestion presents inhibition problems due to VFA accumulation from carbohydrate, lipid, and protein fermentation [50]. Currently, the municipality has an 8 m^3 biodigester fed with cattle manure and whey; about 2 m^3 of biogas d^{-1} (1.2 m^3 CH_4 d^{-1}) is generated daily, representing a digester yield of 0.25 m^3 biogas m^{-3} digester d^{-1}, which could be improved considering the average yields for biodigesters of around 0.3 m^3 biogas m^{-3} digester [51].

Strategy for Improving the Anaerobic Digestion Process. According to the literature review, the use of supports generally and significantly favors the AD process; given the conditions of the case study, such as temperature and the waste generated in the region, selecting a support that enhances the co-digestion of whey in psychrophilia is necessary. A viable alternative to address the previous scenario is using organic supports. Below are some essential factors for selecting the support to use.

Type of substrate to be treated. Adding fibrous or granular support, such as lignocellulosic biomass (BL) and biochar, is of great interest to researchers to improve methane production and the operational stability of the process; when treating an acidic substrate like whey, it is essential to consider the contribution of VFA from BL, as their accumulation leads to a more significant decrease in pH, causing inhibition in the methanogenic stage. Biochar, on the other hand, allows for the development of a biofilm that improves the retention of methanogens and can lead to increased methane production [13]. Additionally, the properties of biochar influence the performance of AD by increasing the system's buffering capacity due to its alkaline nature, mitigating possible inhibitors, and improving the quality of the biogas [10,15,35]; it also presents economic and environmental advantages compared to conventional solutions in AD processes [22].

Availability of support. Considering the availability of waste that can be used as a biofilm carrier in the municipality of Cáchira, Norte de Santander, to implement the AD process, a viable alternative is to use wood waste from forestry and wood extraction, given the 70 % growth in this economic activity by 2018 according to MinAgricultura [48]; according to the Corporación Nacional de Investigación y Fomento Forestal (CONIF), the central zone of the Norte de Santander, which includes the municipality of Cáchira, has a potential area for commercial forest crops of 278,302 Ha, where the cultivation of the Pinus patula (Pine) species is prioritized [47]

Physical properties of the support. As mentioned earlier, physical properties such as particle size and the concentration of the support directly influence the performance of the process; for biochar, Lü *et al.* [37], determined that a particle size of 2 to 5 mm increased methane production, and Sunyoto *et al.* [52] showed that adding biochar above 16.6 g/l resulted in low cumulative CH_4 production. Some authors indicate that pretreating lignocellulosic waste, such as thermochemical conversion, improves the physical properties of the support [10,20,52]; for example, Zabaniotou *et al.* [53], reported that biochars produced at high temperatures (>800 °C) have a higher proportion of micropores (50 – 78 %), which are directly related to surface area, attributing a high adsorption capacity.

Site temperature. Through the literature review, it is possible to mention that psychrophilia has not been extensively studied, leaving gaps in the knowledge of the support's influence during the process; of the few studies using supports with T<20 °C, Jang *et al.* [10], stand out, reporting improvements in methane production using biochar derived from cattle manure. The results showed an improvement in cumulative methane, with a 1.18 % increase for the digester with biochar addition compared to the control.

Based on the above factors, pine wood biochar is the best alternative as a support material for the case study. Therefore, it is proposed to feed the biodigester with this material at a concentration between 10 and 16.6 g L^{-1}, as data reported by Jang *et al.* [10], and Sunyoto *et al.* [52], show that adding biochar at these concentrations yields optimal results in AD; this alternative is expected to improve the bioprocess, mainly in its performance. It is worth mentioning that experimental tests at the laboratory scale are necessary to previously understand the effects of using this support under established conditions.

Final Recommendations for Implementing Biochar in the Anaerobic Digestion Process. Future research could study the optimization of biochar production's economic and environmental yields and its integration into the AD process; biochar production is closely related to its performance as a support, as the production method can affect characteristics such as particle size, porosity, and surface area. The chemical properties of biochar can significantly influence the efficiency of AD; a biochar with a high fixed carbon content provides a stable structure that supports microorganisms, stabilizing the pH and adsorbing toxic compounds. The pH of biochar influences the reactor balance and can maintain an optimal environment for anaerobic microorganisms. Additionally, the porous structure of biochar facilitates substrate adsorption and microorganism retention; based on this, it is essential to understand better the control of production conditions, dosage, and recovery of biochar, as well as the optimal values of these variables, to enhance the performance of the support and, in turn, the AD process.

Although experimental work related to using biochar as a support in anaerobic digestion has increased in recent years, there are still many research gaps; based on this, it is recommended that future research evaluate the interactions of the support with microorganisms, feeding rate, reuse, and other maintenance conditions during the AD process. Moreover, it is also important to focus research on a technical, economic, and environmental analysis of the integration of biochar in Anaerobic Digestion.

Conclusions

Using organic supports in anaerobic digestion presents a viable and economical alternative to improve the process; these supports reduce the lag phase and hydraulic retention time, enhance biomass retention, especially of methanogenic microorganisms, increasing the efficiency of the bioprocess and the quality of the biogas. They also contribute to process stability, demonstrating high efficiencies in removing organic matter and inhibitory compounds. Integrating a thermochemical process and anaerobic co-digestion for waste valorization is suggested in the context of specific climatic conditions and available resources; however, additional environmental, economic, and technical research is necessary to optimize process performance and biogas quality.

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