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Review

Advancements and Innovations in Harnessing Microbial Processes for Enhanced Biogas Production from Waste Materials

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Abstract: Biogas production from waste materials has emerged as a promising avenue for sustainable energy generation, offering a dual benefit of waste management and renewable energy production. The selection and preparation of waste feedstocks, including agricultural residues, food waste, animal manure, and municipal solid wastes, are important for this process, while the microbial communities are majorly responsible for bioconversions. This review explores the role of complex microbial communities and their functions responsible for the anaerobic digestion of wastes. It covers the crucial physiological processes including hydrolysis, acidogenesis, acetogenesis, and methanogenesis, elucidating the microbial activities and metabolic pathways involved in the prospects of improving the efficiency of biogas production. This article further discusses the influence of recent progress in molecular techniques, including genomics, metagenomics, meta-transcriptomics, and stable isotope probing. These advancements have greatly improved our understanding of microbial communities and their capabilities of biogas production from waste materials. The integration of these techniques with process monitoring and control strategies has been elaborated to offer possibilities for optimizing biogas production and ensuring process stability. Microbial additives, co-digestion of diverse feedstocks, and process optimization through microbial community engineering have been discussed as effective approaches to enhance the efficiency of biogas production. This review also outlines the emerging trends and future prospects in microbial-based biogas production, including the utilization of synthetic biology tools for engineering novel microbial strains and consortia, harnessing microbiomes from extreme environments, and integrating biogas production with other biotechnological processes. While there are several reviews regarding the technical aspects of biogas production, this article stands out by offering up-to-date insights and recommendations for leveraging the potential of microbial communities, and their physiological roles for efficient biogas production. These insights emphasize the pivotal role of microbes in enhancing biogas production, ultimately contributing to the advancement of a sustainable and carbon-neutral future.

Keywords: biogas; microbial processes; circular economy; waste treatment; biological pretreatment

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1. Introduction

The demand for energy is increasing with the growing population, and to meet the needs, there is a limited amount of fossil fuel left on earth. The use of energy from fossil fuels is increasing exponentially, resulting in greenhouse gas emissions, such as carbon dioxide (CO_2), having an emission rate of 1000 kg/s [1]. Pertaining to these environmental threats associated with fossil fuel burning, researchers are now more inclined to focus on making energy from low-carbon sources using eco-friendly technology. The most common

type of these energy sources is biohydrogen, biomethane, bioethanol, biodiesel, etc., and microorganisms are the main mediators of their production [2]. Waste deposition is also a major concern, as untreated waste pollutes the ecosystem and is harmful for both the Earth and human health [3]. The production of biogas from organic waste serves two major environmental purposes simultaneously. Biogas is usually made up of 50–70% methane, 25–45% carbon dioxide, 1–5% hydrogen, and a small amount of other gases such as ammonia, hydrogen sulfide, halides, and water vapor [4]. The gases other than methane, carbon dioxide, and hydrogen are present in a very small amount and vary in different environments. The reaction is carried out in a large anaerobic bioreactor where microorganisms convert feedstock into biogas in a controlled environment. A few examples of microorganisms involved in biogas production are given in Table 1. The reaction proceeds through four steps, viz. hydrolysis, acidogenesis, acetogenesis, and methanogenesis [5]. These metabolic steps are interconnected, with one phase's byproduct becoming the substrate of the next, producing biogas and digestate as byproducts [6]. In the hydrolysis phase, the microorganism degrades complex biopolymer components into water-soluble ones, which serve as substrate for fermentative bacteria. During the fermentation stage, acidogenic bacteria convert glucose, amino acids, and lipids into organic acids, volatile fatty acids (VFAs), CO_2, and H_2. In the acetogenesis step, acetic acid and butyric acid are transformed to acetate, H_2, and CO_2 for use in the next stage. The organic acid CH_3COOH serves as substrate for methanogenic bacteria which converts CH_3COOH to CH_4 and CO_2 in the final phase [7]. Additionally, CO_2 combines with H_2 to make additional CH_4, which is facilitated by hydrogenophilic microorganisms. Acetophilic methanogenic microorganisms convert the produced acetate to CH_4 [8]. However, there are limitations pertaining to microbial processes in biogas plants, including variations in temperature, pH, acidity, and other factors that might influence microbial growth and functionality, consequently impacting the production of biogas [9]. Commonly used waste feedstocks are animal waste, lignocellulosic biomass, municipal solid waste, and industrial waste; they have inherent organic content that can be utilized as a substrate for anaerobic digestion. Lignocellulosic biowastes include a substantial sugar content, rendering them a viable and cost-effective feedstock for the production of environmentally sustainable fuels, such as biogas [10]. Lignocellulosic waste was mainly digested with animal manure, for example, wheat straw with cattle dung yields a higher production of methane (320.8 mL/gVS) among other co-digested systems. Food waste yields 460 mL/gVS methane, which is the highest among municipal wastes, and in industrial waste, the textile industry serves as the highest methane producer yielding around 200–400 mL/gVS [7]. Proper selection and preparation of these waste feedstocks by applying new strategies, such as co-digestion of more than one substrate or microbial pretreatment, results in improving yields stability [11,12]. A single microorganism may not be able to facilitate each step; and therefore, multiple anaerobic microorganisms work synergistically to produce biogas. Further, the use of non-sterile feedstock also aids in the growth of indigenous microorganisms having properties for the degradation of organic substrates along with biofuel production [13]. Syntrophic activities involve close cooperation between at least two organisms, wherein metabolic products are transferred from one organism to another. These interactions are essential for the breakdown of complex organic compounds and the conversion of intermediate products into methane [14]. The success of the process hinges on maintaining favorable conditions for the microbial community within the digester. Consequently, changes in fermentation conditions, time, and ecosystem can result in shifts in the microbial community composition within the digester. Recent advancements in molecular techniques have greatly enhanced the understanding of activities and metabolic pathways in microbial communities and their functional potentials in biogas production from waste [15]. These techniques provide insights into the composition, functional gene profiling, and metabolic pathway reconstruction of microbial communities, enabling a more comprehensive understanding of their roles in biogas production [16]. The integration of genomics and molecular techniques with process monitoring and control strategies offers new possibilities for optimizing

biogas production and ensuring process stability. An example of such a technique is stable isotope probing, in which stable isotopes of carbon or nitrogen are incorporated into the microbial population to understand the ongoing processes in the system [17]. This holistic approach allows for real-time monitoring of microbial community dynamics and metabolic activities, facilitating the implementation of timely interventions to maintain optimal process conditions and maximize biogas yields [18] (Figure 1).

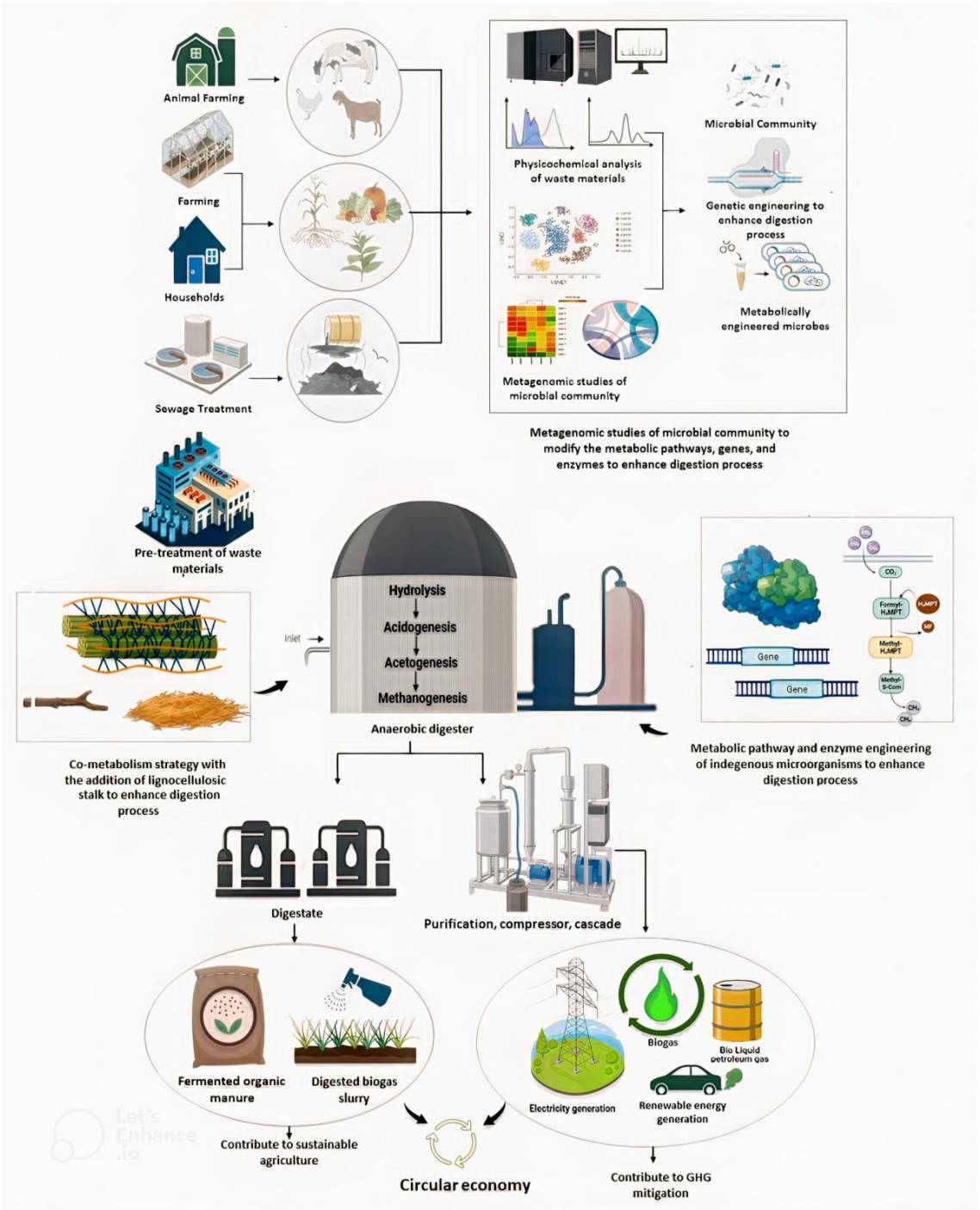

Figure 1. Illustration of Biogas production through agricultural, manure, and sewage waste materials. Several metagenomics approaches can be used to enhance the process. Produced biogas can be used as a renewable energy resource, which helps in GHG mitigation and ultimately contributes to a circular bioeconomy.

Looking ahead, there are exciting emerging trends and future prospects in microbial-based biogas production, which mainly include the utilization of synthetic biology tools for engineering novel microbial strains and consortia with enhanced biogas production capabilities, harnessing microbiomes from extreme environments to identify novel functional microbial communities, and integrating biogas production with other biotechnological processes for enhanced resource recovery and valorization [19] (Figure 1). Biogas production is an important issue for today's world, due to its environmental, energy, and economic implications. Hence, various researchers have reviewed different facets of biogas production that primarily address the technical and engineering aspects for its environmental benefits, economic viability, policy implications, and more. Industrial optimization and application, directly or indirectly, have been the focus of well-attended reviews [20–22]. Achinas et al. [21] give details about the technologies for biogas production from waste materials, while Divya et al. [22] and Mishra et al. [23] focused on technologies for enhancement and upgradation of biogas production. The legislation framework for biogas production around the globe is reviewed by Abanades et al. [9]. Waste management, which is another aspect of biogas production, has been a trending topic for research and many authors reviewed details of it around the globe [24–28]. However, more in-depth information on the role of microorganisms in biogas production, with focus on recent technological interventions in the area of microbiology, is required. Microorganisms play a pivotal role in this technology, so it is imperative to comprehend the microbial mechanisms in order to facilitate the development of biogas plants with enhanced efficiency. Ekwebelem et al. [29] and Akinsemolu et al. [30] studied the role of microorganisms in producing renewable energy for sustainable development. Ferdeș et al. [31] and Kasinath et al. [11] discussed the role of microorganisms in pretreatment processing, while Nwokolo and Enebe [32] summarized the significance of microbial community in an anaerobic bioreactor. However, a comprehensive detail of overall understanding of microbial processes, from diversity to application for this process, is required. Therefore, this review aims to fill this gap and explore the details of intricate microbial pathways, diversity, processes, genomics, and underlying biogas production from waste, and provide insights into the latest advancements and future prospects in this field. This review serves as a guide to understand the role of microorganisms in the process of biogas production via the utilization of organic wastes.

Table 1. Microorganisms and the utilized substates for biogas production and yield.

Microorganism	Substrate	Major Events during Substrate Degradation	Biogas Yield (mL/g VS)	Condition	Ref.
Acetobacteroides hydrogenigenes	Corn straw	Hydrogen gas production	258.1	Laboratory scale	[33]
Methanospirillum hungatei	Nonfat dry milk	Increase of methanogenic activity	32	Laboratory scale	[34]
Pseudobutyrivibrio xylanivorans	Brewery spent grain	Hydrolysis	261.3	Piolot scale	[35]
C. saccharolyticus	Pig slurry and sweet sorghum	Hydrogen gas production	62.5	Laboratory scale	[36]
Clostridium cellulolyticum	Wheat straw	Hydrolysis	342.5	Laboratory scale	[37]
Clostridium sp.	Cellulosic waste	Hydrolysis	168	Laboratory scale	[38]
Enterobacter cloacae	Maize silage	Hydrolysis	718.5	Laboratory scale	[39]

Table 1. *Cont.*

Microorganism	Substrate	Major Events during Substrate Degradation	Biogas Yield (mL/g VS)	Condition	Ref.
T. hermosaccharolyticum Caldanaerobacter subterraneus Thermoanaerobacter pseudethanolicus C. cellulolyticum	Corn stover and cellulose	Hydrolysis	165	Laboratory scale	[40]
Lignocellulose degrading microbial consortium	Swine manure	Hydrolysis	180	Laboratory scale	[41]
Gelria, *Anaerovorax*, *Dethiobacter*, Clostridia	Grass siliage	Hydrolysis and fatty acid metabolism	-	Laboratory scale	[42]

2. Methods

A comprehensive literature search was conducted to identify relevant studies on advancements and innovations in microbial processes for enhanced biogas production from waste materials. The search was carried out in online academic databases including PubMed, Web of Science, Scopus, and Google Scholar. The search terms used were "biogas production", "microbial processes", "waste materials", "anaerobic digestion", and "innovations." We limited the search to articles published between 2000 and 2023 to ensure the inclusion of recent advancements. However, for initial references of novel work, some previous articles were also considered. Care was taken to select the articles that focused on microbial processes for biogas production from waste materials; addressed innovations or advancements in biogas production techniques; were published in peer-reviewed journals or reputable conference proceedings; and have sufficient details of relevant information.

Upon identifying relevant articles, the data were extracted including authors' names, publication year, study objectives, waste materials used, microbial consortia employed, experimental setup, process parameters, and key findings. Studies were categorized based on the type of innovations discussed, such as pretreatment techniques, microbial enrichment strategies, co-digestion approaches, and process optimization methods. Further, no ethical considerations were applicable to this review as it involved the description and interpretation of previously published research articles.

3. Microbial Diversity and Dynamics Involved in Biogas Production

Cellulomonas sp., *Prevotella* sp., *Bacteroides* sp., *Fibrobacter* sp., *Clostridium* sp., *Propionibacterium* sp., *Defluviitoga* sp., *Methanosaeta* sp., *Methanosarcina* sp., *Methanoculleus* sp., etc. are the most common microbial genera associated with biogas production and are present in bioreactors [5]. The dominant microorganisms present in the anaerobic bioreactor are influenced by various factors such as substrate composition and availability, pH, temperature, and feedback mechanism of metabolic byproducts. Changes in these conditions can create physiological stress on the microorganisms, sometimes leading to a reduction in abundance. However, when the conditions become favorable again, these microorganisms can proliferate and become the dominant population within the bioreactor [43]. Consequently, the microbial community within the digester can change over time and with fluctuations. For example, in the initial stage of hydrolysis, bacteria such as *Porphyromonadaceae*, *Aminobacterium*, *Bacteroides*, *Fibrobacter*, *Clostridium*, *Acinetobacter*, *Paludibacter*, *Acidaminococcus*, and *Sphaerochaeta* are abundant, as these bacteria have excellent hydrolytic activity towards the feedstock [32]. Similarly, the fungi *Pichia* sp., *Aspergillus* sp., *Candida* sp., *Debaryomyces hansenii*, and Mucor dominate this stage. Fungal strains are excellent degraders of cellulose backbone; therefore, co-culture of different microorganisms is the best strategy to enhance

biogas production [44]. Towards the end of the process, methanogens such as *Methanosaeta concilii*, *Methanothrix soehngenii*, and *Methanosarcina* sp. resistant to ammonium and other harsh chemical dominate in microbial biomass [45]. *Clostridium* sp. is an important microorganism in biogas studies as it can remain viable in a wide range of temperatures, and its enzymes are also functional in both mesophilic and thermophilic conditions, but sensitive to nutrient availability [46]. The microbial diversity includes archaea, fungi, and bacteria, which are interconnected within a biogas system through various pathways, allowing for the efficient conversion of organic matter into biogas. Bacterial and archaeal populations are less steady, and their abundances change as the chemical conditions of the system change during the process of biogas production, whereas the fungal community remains stable, but their abundance remains relatively lesser [7].

Microbial diversity varies greatly in mesophilic and thermophilic tanks in two stage bioreactor plants. The abundance of microbial populations is found to be high in the mesophilic part, but its effect in methane production is negligible. In a mesophilic bioreactor the methanogenic archaea such as *Methanothrix* and *Methanospirrillum* serve as dominant genera, followed by populations from the bacterial genera *Cloacibacillus*, *Longilinea*, and *Coprothermobacter* [47]. Further, it was also reported that the age and rearing conditions of animals, whose dung is added to the bioreactor, also have an influence on microbial diversity in the bioreactor. Generally, young animals have a healthy gut microbiome and their manure, when fed to bioreactors, elevates methane production, as has been observed along with a healthy microbial community [43]. It is also proposed that the gut microbiota can be added as a starter in biogas plants where lignocellulosic material is used as feedstock [48].

The application of nanoparticles is also reported to enhance microbial activity within the bioreactor. The addition of citrate-coated Fe_3O_4 nanoparticles in sludge and crude glycerol supplied bioreactors enhanced the abundance of microorganisms from the orders Bacteroidales, *Clostridiales*, *Methanomicrobiales*, and *Methanosarcinales* with a 49.8% accumulation of methane in bioreactor [49]. In a recent study, it was found that nanoparticles are involved in the abundance and movement of mobile genetic elements in anaerobic digesters, and this movement is associated with an increase in antibiotic resistance genes within the bioreactor and is found most abundant in Proteobacteria and Firmicutes [50]. Hassaneen et al. [1] documented a substantial 185.3% increase in methane production through the incorporation of a ZnFe nanocomposite into an anaerobic bioreactor containing cattle manure. It has also been reported that the addition of nanoparticles enhances the production of enzyme cellulase and thereby hydrolysis efficiency is increased [51].

The common bacterial population in a digester is almost often associated with the substrate composition of the system. The predominant bacterial taxa in a digester fed with animal feces and municipal wastes are the Bacteroidetes and the Firmicutes [52,53]. In addition, Proteobacteria are also found in municipal solid waste digesters [53]. According to an earlier investigation, Firmicutes predominate in biogas reactors [54,55]. Bacteroidetes was the second-most prevalent phylum, accounting for 12% of the whole population. The results of earlier studies suggested that microorganisms from these phyla assist in the decomposition of cattle manure, which primarily consists of plant biomass residues, in biogas reactors [56,57]. Bacteroidetes are made up 30% of the mesophilic population and 8% of the thermophilic population, respectively, based on the relative abundance of 16S rRNA in the metagenome. The initial inoculum also affects the community dynamics; for example, the presence of *Methanolinea* sp. in the early inoculum has an impact on community dynamics, mainly by reducing the lag period in the bioreactor [58]. It is observed that the introduction of 4% food waste in sewage sludge significantly reduces the lag phase with an abundance of *Methanosaeta* sp. and *Methanosarcina* sp., while the increasing food waste concentration drastically extends this period [59]. Incorporation of activated sludge with food waste further enhances biogas production [2].

4. Effect of Feedstock Substrate on Microbial Community and Biogas Production in an Anaerobic Digester

Substrates refer to organic biomass materials supplied to the bioreactor, which can undergo enzymatic breakdown by the microorganism, resulting in the production of biogas. These materials encompass a range of sources, including agricultural waste, deceased animal tissues, animal dung, and poultry droppings. They have diverse chemical compositions, including proteins, fats, cellulose, lignin, hemicellulose, lipids, and various other chemical molecules. The presence of these chemical compounds influences their susceptibility to biodegradation and can determine microbial preferences for their utilization [7]. In addition to technical operation conditions, the type and composition of substrate have an influence on the yield of biogas production as well as the abundance of microbial communities in bioreactors [60]. In a study on methane production from different substrates, including maize silage, chicken manure, straw, swine manure, and grass silage, the predominant microorganisms associated with each substrate were examined. The results indicated that *Methanosarcinaceae*, *Synergistaceae*, and *Clostridium* sp. were the dominant methanogens observed across all fermented substrates. In straw and chicken manure digesters, the dominant microorganisms were identified as Synergistaceae, acetate-oxidizing bacteria, and *Syntrophaceae*. On an individual substrate basis, *Porphyromonadaceae* were predominantly found in maize silage, swine manure, and grass silage digesters. Straw digesters exhibited a higher abundance of *Rikenellaceae*, while the microbial community in the chicken manure digester was mainly composed of *Porphyromonadaceae* and *Ruminococcaceae*. Moreover, the 13C isotope fractionations of CH_4 and CO_2 revealed that grass silage, maize silage and swine manure have similar rates of methane production, whereas, in chicken manure- and straw-fed systems, the production is lowest. These findings indicate that the type of substrate plays a crucial role in shaping the microbial community and influencing the biogas yield in anaerobic digesters [61].

4.1. Bioaugmentation with Microbial Additives to the Feedstock

The introduction of microorganisms in anaerobic digesters primarily aims to enhance comprehension of the operational mechanisms and interrelationships within the microbial community [62]. Bioaugmentation with microbial consortium in anaerobic bioreactors increases enzymatic activity and counteracts the activity of various microbial byproducts, which acts as process inhibitors [63]. In a study conducted by Yan et al. [64], it was demonstrated that the introduction of a *Methanoculleus* sp. culture capable of tolerating ammonia resulted in a 21% increase in methane production yield from the organic fraction of municipal solid waste along with a 10% reduction in volatile fatty acids compared to the pre-bioaugmentation period. The biogas yield was observed both in single bacterium augmentation as well as in bioaugmentation with consortium. Čater et al. [35] reported that bioaugmentation with *Ruminococcus flavefaciens*, *Pseudobutyrivibrio xylanivorans*, *Fibrobacter succinogenes*, and *Clostridium cellulovorans* in an anaerobic bioreactor significantly increases the yield of methane. *Pseudobutyrivibrio xylanivorans* exhibited the highest methane production, with an increase of 17.8%. This was followed by the co-culture of *Pseudobutyrivibrio xylanivorans* and *Fibrobacter succinogenes*, which resulted in an increase in methane production of 6.9%. Additionally, the co-culture of *Clostridium cellulovorans* and *Fibrobacter succinogenes* demonstrated a methane production increase of 4.9% [35]. In another study, the introduction of *Clostridium thermocellum* as a starting culture in a bioreactor containing *Haematococcus pluvialis* microalgae biomass resulted in methane production increase ranging from 18% to 38% [65]. Fungal species can also serve this purpose, as demonstrated by the bioaugmentation of *Piromyces rhizinflata* in a bioreactor containing corn silage and cattail. This resulted in an overall enhancement in the production of methane and hydrogen, accompanied by an accelerated degradation rate of fatty acids derived from both substrates [66]. The utilization of bioaugmentation in anaerobic digesters through the introduction of microorganisms derived from manure or rumen sources is a promising approach. This method is particularly appealing due to the ease of accessibility and

the potential to utilize these microorganisms as co-substrates in the feeding process [63]. However, it should be noted that these microorganisms exhibit sensitivity to pH levels. Consequently, the synthesis of methane by *Fibrobacter* was seen to decrease as the pH range in bioreactors deviated more from 6.0 to 6.5 [67]. In another study, methanogenic sludge and rice straw were co-inoculated with rumen bacteria to improve the performance of methanogenesis and a twofold increase in the average methane yield was observed, along with an enhancement in cellulolytic activities. Moreover, they were able to alter the community structure, which was advantageous for maintaining the stability of anaerobic digestion [68]. Bioaugmentation with specific microorganisms increases the viability of important microorganisms in anaerobic bioreactors. The metabolisms of syntrophic acetate-oxidizing bacteria and hydrogenotrophic methanotrophs are closely related, and adding one affects the other's survival directly [69,70]. Thus, the addition of microbial additives in anaerobic bioreactors is an important strategy to enhance biogas production.

4.2. Co-Digestion Strategy for Biogas Production from Agricultural Waste and Animal Waste

Agricultural waste biomass often contains a higher proportion of lignocellulose, which poses challenges for its direct degradation. As a result, it is commonly co-metabolized with other waste materials to provide the microorganisms with initial sources of nutrients such as proteins, lipids, and simple sugars. This co-metabolism strategy enables more efficient breakdown of the lignocellulosic components in agricultural waste [7]. Increasing lignin content by 1% reduces methane production by 7.5 L CH_4/kg total solid [71]. Furthermore, lignocellulosic biomass with lignin content greater than 100 g/kg vs. (volatile solids) drastically reduces methane output; in addition, 15% of lignin presence in an anaerobic digester is considered as threshold [72,73]. Research was conducted to assess the methane production capacity of various constituents of maize stover, namely stem bark, stem pith, and leaves, and found to be 0.19 L/gVS, 0.21 L/gVS, and 0.19 L/gVS, respectively. The reduced methane production found in stem bark can be ascribed to its higher lignin concentration of 17.61%, which is notably higher than the lignin content of 7.16% for stem pith and 5.16% for leaves [74].

Studies have indicated that waste substrates containing high levels of proteins and lipids tend to exhibit increased methane production, whose high amount is present in animal waste. This is attributed to the metabolic breakdown of these substrates, which releases ammonium ions and long-chain fatty acids [75]. However, it has been observed that these substances can be toxic to certain bacterial communities. Interestingly, bacteria have demonstrated the ability to adapt and counteract this toxicity [76]. Moreover, these bacteria have been reported to produce methane even in the presence of these potentially harmful compounds [77]. Cattle, pig, sheep, goat, and poultry manure has been widely reported as a suitable substrate for biogas production as it has a high concentration of nutrients, organic matter, and buffering capacity [78–80]. The excellent buffering capacity of these wastes aid to counter the negative effects of volatile fatty acids. A comparative study from various literature suggests that the pig and goat dung have higher buffering capacities which supports the growth of required microbial genera and yields higher methane production in a range of 204–439 mL/gVS and 402–500 mL/gVS, respectively. Moreover, it is also reported that cattle manure yields the lowest methane production as the amount of total solid (14.5–22.7%) and volatile solid (12–72%) is more in it whereas the total water content is less, which might be due to higher lignin content in the diet of cattle [7]. In a recent study, the lignin content of various types of manure was examined. In another study, it was found that cattle manure exhibited the highest lignin content, measuring 11.5%, while pig manure had a lignin content of 8.5% and poultry manure had a lignin content of 4.2%. This higher lignin content in cattle manure was associated with inhibited methane production, as indicated by the average yield of 168 mL/gVS, while 215 mL/gVS methane was produced in pig manure, and poultry manure had the highest methane yield of 255 mL/gVS [81].

To meet the diverse nutritional needs of anaerobic microorganisms involved in substrate degradation for biogas production, a combination of substrates is commonly used. This co-digestion approach aims to enhance microbial growth and activity. For example, animal manure often has an imbalanced carbon/nitrogen ratio, which can be rectified by co-digesting it with carbon-rich substrates. The amount of protein, fat, and carbohydrate in a substrate directly influences the methane percentage in biogas. By combining substrates that are rich in these components with other substrates, process stability can be achieved, leading to improved biogas production. By adjusting the mixing ratio of corn stover and pig manure substrate, the researchers were able to achieve different carbon-to-nitrogen (C/N) ratios in the seven anaerobic reactors studied. The findings revealed that as the C/N ratio decreased, the hydrogenotrophic pathway gradually replaced the acetoclastic-methane-producing pathway [80]. This shift was accompanied by an increase in the relative abundance of hydrogenotrophic methanogens such as *Methanobacterium*, *Methanoculleus*, and *Methanobrevibacter*, particularly in response to decreased influent C/N ratios and increased total ammonia nitrogen levels. Notably, these methanogens displayed a high resistance to the toxic effects of ammonia nitrogen [82]. Under stable digestion conditions without toxic stress, *Methanosaeta* dominated the archaea community, accounting for over 50% of its abundance and resulting in higher methane yields. In contrast, in the reactors with high C/N ratios, the abundance of *Clostridium* decreased while *Methanosaeta* increased, indicating competition for substrate. High C/N ratio substrates, such as cow dung, corn straw, corn stover, and agricultural wastes, typically produce a small amount of ammonia. However, they also stimulate the rapid consumption of available nitrogen by acidogenic bacteria more than methanogenic bacteria. As a result, both high and low C/N ratios negatively affect methane yield [32]. Therefore, the study suggests that co-digestion of substrates is a viable approach to achieving an optimal carbon-to-nitrogen ratio. Recent studies are mainly based on this strategy and have produced excellent results. Zhang et al. [83] conducted a study to examine the co-digestion of corn stalk, wheat straw, and rice straw with goat manure, in general environmental conditions. The experimental results indicated that the most favorable biogas yields were obtained at mixing ratios of 30:70, 70:30, and 50:50 for the combinations of goat manure with wheat straw, goat manure with corn stalk, and goat manure with rice straw, respectively. The total yield of biogas was 12.8 L/kgVS when goat manure and wheat straw was combined proportionally by 30:70, which was 62% and 23% higher compared to the biogas yields obtained from separate digestion of goat manure and wheat straw. In a comparable manner, the co-digestion of goat manure and rice straw at a mixing ratio of 50:50 yielded a total of 15.7 L/kgVS, surpassing the yields obtained from digesting rice straw or goat manure alone by 111.28% and 51.31%, respectively. Likewise, when goat manure was co-digested with corn stalk at a ratio of 50:50, a total biogas yield of 16 L/kgVS was achieved, which was 83% and 54.44% higher than the biogas yields of corn stalk and goat manure alone, respectively. The results of this study indicate a notable enhancement in the production of biogas achieved by employing the co-digestion method. This approach effectively addresses the carbon to nitrogen imbalance commonly observed in the digestion of single substrates. The carbon to nitrogen ratio was reported to stabilize with the addition of animal waste to high lignocellulosic wastes [84]. In a recent study, it was reported that the production of biogas was increased significantly up to 441.3 L/·kg vs. when pig fat is added as supplement to the bioreactor having poultry manure [85]. Similarly, pig manure was supplied with spent coffee grounds, resulting in enhanced methane production [86]. The biomass of *Eichornia crassipes* (water hyacinth), which is one of the most evasive macrophytes, is extensively used in biogas production as co-metabolite with poultry waste, industrial effluents, cow dung, animal wastes etc. [87]. The type of condition in a reactor and the mixing proportion of the substrates also regulate the concentration of methane production; for example, when corn stover is digested with chicken manure in three physiological conditions viz. wet, semisolid, and solid state, it is observed that the solid-state condition yields highest (14.2 $L_{methane}/L_{reactor}$ volume) at a

1:1 ratio of corn stover to chicken manure. Moreover, a 3:1 ratio is optimum for wet and semisolid conditions, where yield is 218.8 mL/gVS and 208.2 mL/gVS, respectively [72].

In a comparative study, the effects of mono-digestion and co-digestion, as well as pretreatment, were investigated using wheat straw and cattle dung as substrates. The wheat straw was subjected to pretreatment using different concentrations of H_2O_2 (1%, 2%, 3%, and 4%) prior to digestion, either as a sole substrate or in combination with dung at different ratios. The mono-digestion of H_2O_2-treated wheat straw resulted in methane yields of 94.8, 108.5, 128.4, and 118.7 mL/gVS for the 1%, 2%, 3%, and 4% pretreatment, respectively. These values are significantly higher when compared to the methane yield of 84.3 mL/gVS obtained from untreated wheat straw. Moreover, a notable enhancement in methane production was detected during the anaerobic co-digestion of H_2O_2-treated straw and dung. The highest methane yield of 320.8 mL/gVS was obtained when H_2O_2-treated straw and dung were mixed in a ratio of 40:60. This value is more than the methane yield achieved through co-digestion of untreated straw and dung, which was found to be 257.6 mL/gVS. Additionally, a transition in the composition of the methanogenic community was seen during the process of co-digestion, whereby there was a shift from acetoclastic methanogens to hydrogenotrophic methanogens [88]. In such studies, cattle manure is mostly used as it is widely available because of the growing diary industry worldwide. A maximum of 386.3 NL/kg of methane is produced when the solid agricultural solid waste is pretreated with $NaHCO_3$ and enriched with cow dung [89]. In a recent study, researchers explored the process of anaerobic co-digestion involving the combination of pretreated sugarcane bagasse and cow dung. The bagasse underwent pretreatment using a solution containing NaOH as well as $Ca(OH)_2$ for a duration of one day and then it was mixed with dung at a ratio of 1:2. The experimental results revealed that the NaOH-treated bagasse exhibited the highest biogas production is 386 mL/gVS, while bagasse treated with $Ca(OH)_2$ yielded approximately 334 mL/gVS, which is higher than the untreated, pure bagasse produced around 322 mL/gVS of biogas at a temperature of 35 °C. By introducing dung to the bagasse and increasing the temperature from 35 °C to 55 °C, a noticeable rise in biogas production yield by 27 mL/gVS was observed. This increase can be achieved because of the adjustment in the carbon and nitrogen ratio, which shifted from 130:1 in pure bagasse to 29:1 when dung was added [84].

The studies from the literature have demonstrated that the simultaneous digestion of animal manure alongside agricultural residues offers several benefits, including the enhancement in biogas production as well as improved stability of the anaerobic digestion process. These advantages can be attributed to the achievement of a more balanced nutrient composition, specifically in terms of carbon and nitrogen, which is made possible through the process of co-digestion.

4.3. Biological Pretreatments of Organic Waste for Enhanced Biogas Production

At present, various biological pretreatment methods are utilized to enhance the pretreatment of organic biomass and achieve higher biogas yields. These pretreatments involve the use of microorganisms and the metabolites they produce, including bacteria, white or soft rot fungi, and actinomycetes, to degrade the resistant components of lignocellulosic materials. Generally, biological pretreatments are considered less expensive compared to other pretreatment methods. However, one drawback is that they tend to be slower in terms of the degradation process. Additionally, these pretreatments required relatively large spaces and well-maintained optimized environments to maximize their efficiency. This ensures the favorable growth and activities of the microorganisms involved. Biological pretreatment methods employing microbial consortiums primarily target the breakdown of cellulose and hemicellulose. The utilization of such microbial consortia for the biodegradation of cellulose and hemicellulose has proven to be highly efficient in pretreatment for various biotechnological applications. One significant advantage of using microbial consortia is the avoidance of regulatory issues related to feedback and metabolite repression commonly encountered with isolated strains. Additionally, biological pretreatment strategies involve

the application of hydrolytic enzymes such as cellulase and hemicellulase. In a study, cow manure was pretreated with hemicellulose digesting strain B4 in thermophilic condition. The findings revealed that mono-digestion, where only cow manure was used as the substrate, led to a significant improvement in methane production. Specifically, the methane production increased by 30%, resulting in a methane yield of 300 mL CH_4/g VS [90]. In the study conducted by Liu et al. [91], it was shown that the removal of antibiotics from the pig dung can have a significant positive influence on methane production. Recently, another strategy was employed by researchers where biological pretreatment was done by applying a biocatalyst system consisting of a microbial consortium with the capacity of antibiotics degradation. The study's findings indicate that the biocatalyst effectively decomposed antibiotics, including amoxicillin, penicillin, and cefamezine, within one hour when subjected to pretreatment. Notably, when the pretreatment was performed over a period of three days, methane production increased by a substantial 93.2% [91]. In a study by Costa et al. [92] biological pretreatment of organic poultry manure was carried out using bacteria strains including *Clostridium cellulolyticum*, *Caldicellulosiruptor saccharolyticum*, and *Clostridium thermocellum*. In the anaerobic digestion process, sewage sludge from a treatment plant was used as the inoculum. The findings indicated that the biologically pretreated manure resulted in methane production of 102 mL CH_4/gVS, showcasing a 15% improvement compared to untreated samples. In another similar study, *Bacillus* sp.C4 was used in the pretreatment of chicken feathers for 2–8 days and was inoculated in an anaerobic bioreactor having wastewater sludge. The results demonstrated a significant improvement of 292% in biogas production, yielding 430 mL CH_4/g VS [93].

White or brown rot fungi play a crucial role in the degradation of lignin, as well as cellulose and hemicellulose to a lesser extent. These fungi produce a group of extracellular enzymes known as "lignases"„ which are responsible for breaking down lignocellulosic materials [94]. White rot fungi, in particular, have the ability to degrade a wide range of persistent environmental pollutants and xenobiotics. Over time, biomass can be inoculated with these lignolytic enzymes from fungi to facilitate the degradation of lignocellulosic material [95]. Numerous studies have indicated that the incorporation of these enzymes in manure pretreatment can enhance the performance of anaerobic digestion systems. Combinations of different enzymes exhibit synergistic effects that contribute to the expansion of small passages and increased accessibility of the cell wall, enhancing overall pretreatment efficiency [96]. Various fungi are utilized in biological pretreatment, with commonly used species including *Phanerochaete*, *Trametes*, *Ceriporiopsis*, *Pleurotus*, *Ceriporia*, *Pycnoporus*, *Cyathus*, *Bjerkandera*, *Ganoderma*, *Irpex*, *Lepista*, and others belonging to genera such as *Phanerochaete*, *Sporotrichum*, *Aspergillus*, *Fusarium*, and *Penicillum*, among others [81]. In one such study, *Pleurotus ostreatus* and *Trichoderma reesei* were utilized for pre-treating straw with the objective of enhancing its decomposition and methane yield. It was found that the performance of fungal pretreatment was significantly influenced by the humidity level and incubation period. At a humidity level of 75% and an incubation period of 20 days, approximately 33.4% and 23.6% of lignin was removed, leading to a remarkable 120% and 78.3% increase in methane production by *P. ostreatus* and *T. reesei*, respectively, as compared to unprocessed rice straw [97]. Bioaugmentation with both bacterial and fungal treatments further enhanced the process. A higher biogas yield of 57% was obtained by the addition of chitinolytic bacteria to the pretreated straw of wheat and millet by *Chaetomium globosporum* in batch anaerobic fermentation. In the same study, 16% more biogas was produced in continuous culture with similar treatment to wheat straw. Further, the abundance of methanogens (mostly from the phyla Firmicutes, Bacteroidetes, and Proteobacteria) was increased in pretreated conditions [98]. A few other examples of microbial pretreatment to various feedstocks are presented in Table 2.

Table 2. The studies conducted on the application of various biological pretreatment strategies to increase biogas/biomethane production from different waste feedstocks.

Type of Waste Feed into Bioreactor	Pretreatment Strategy	Microorganism	Initial and Final Concentration of Biogas/Biomethane (mL/gVS)	Increase in Biogas/Biomethane Production (%)	Ref.
Tall Wheat Grass	Fungal	*Agropyron elongatum*		BG 120%; BM 134%	[99]
Rice Straw	Bacteria	Ligninolytic *Bacillus* sp. Co-culture	BM Control: 270 Pretreated: 528.9	BM 93.30%	[100]
Fresh leaves, dry leaves and cattle dung	Fungal	*Aspergillus terreus* and *Trichoderma viride*	BG Control: 102.6 Pretreated: 125.9 BM Control: 61.4 Pretreated: 79.8	BG 22.71%; BM 29.97%	[101]
Japanese cedar wood	Fungal	*Ceriporiopsis subvermispora*		BG 35%	[102]
Forestry waste	Fungal	*Ceriporiopsis subvermispora*		BG 270%	[103]
Hazel branches	Fungal	*Ceriporiopsis subvermispora*		BM 60%	[104]
Corn stover silage	Fungal	*Phanerochaete chrysosporium*		BM 19.6–32.6%	[105]
Paddy straw	Fungal	*Fusarium* sp.		BG 53.8%	[106]
Yard trimmings	Fungal	*Ceriporiopsis subvermispora*	BM Control: 20.5 Pretreated: 44.6	BM 54%	[107]
Rice straw	Fungal	*Pleurotus ostreatus*		BM 20%	[97]
Sisal leaf decortication residues	Fungal	Isolate CCHT-1 and *Trichoderma reesei*	BG Control: 292 Pretreated: 453	BG 30–101%	[108]
Organic waste	Fungal	*Trichoderma viride*		BM 100%	[109]
Agricultural Biomass	-	*Pleurotus ostreatus*		BM 120%	[110]
Agricultural Biomass	-	*Trichoderma reesei*		BM 78.3%	[110]
Rice straw	Fungal	*Pleurotus ostreatus*	BM Control: Pretreated: 258	BM 165%	[111]
Petroleum refinery sludge	Bacterial	*Kosakonia oryziphila*	BG Control: 0.08 Pretreated: 5.15	BG 56%	[112]
Wheat straw	Fungal	Lignin-degrading fungal culture from their natural habitat		BM 407.1%	[113]

Table 2. *Cont.*

Type of Waste Feed into Bioreactor	Pretreatment Strategy	Microorganism	Initial and Final Concentration of Biogas/Biomethane (mL/gVS)	Increase in Biogas/Biomethane Production (%)	Ref.
Sawdust waste	-	*Gymnopilus pampeanus*	BG Control: 232 Pretreated: 312 BM Control: 42.5 Pretreated: 155.2	BG 25.6% BM 72.6%	[114]
Crop waste	Fungal	*Polyporus brumalis*	BM Control: 159.6 Pretreated: 280.5	BM 75.75%	[94]
Crop waste	Fungal	*Pl. ostreatus*	BG Control: 270 Pretreated: 299 BM Control: 186 Pretreated: 212	BG 10.74% BM 13.98%	[111]
Crop waste	Fungal	*Thermoascus aurantiacus*	BG Control: 390 Pretreated: 514.9	BG 31.72%	[115]
Crop waste	Fungal	*P. chrysosporium*		BM 10.9%	[105]
Crop waste	Fungal	*C. subvermispora*	BM Control: 36.1 Pretreated: 44.6	BM 23.55%	[116]
Crop waste	MC	MC having *Clostridium straminisolvens* *Pseudoxanthomonas* *Brevibacillus* *Bordetella* *Clostridium*	BG Control: 173 Pretreated: 304 BM Control: 21 Pretreated: 79	BG 75.72% BM 276.19%	[117]
Crop waste	MC	MC having *Ochrobactrum* sp. *Coprinopsis cinereus*	BM Control: 182.7 Pretreated: 279	BM 49.04%	[118]
Crop waste	MC	MC having: *Bacillus* *Providencia* *Ochrobactrum*	BM Control: 249.3 Pretreated: 393.4	BM 61.3%	[119]
Sawdust	Fungal	*A. biennis*	BM Control: 101.5 Pretreated: 145.3	BM 47.88%	[120]
Sawdust	Fungal	*L. menziesii* *A. biennis*	BM Control: 101.3 Pretreated: 149.8	BM 43.15%	[121]
Animal/Fish waste	Fungal	*F. velutipe*	BG Control: 330.2 Pretreated: 398.1 BM Control: 125.8 Pretreated: 169.2	BG 20.56% BM 34.5%	[99]

Table 2. *Cont.*

Type of Waste Feed into Bioreactor	Pretreatment Strategy	Microorganism	Initial and Final Concentration of Biogas/Biomethane (mL/gVS)	Increase in Biogas/Biomethane Production (%)	Ref.
Animal/Fish	MC	*Bacilli* *Gammaproteobacteria* *Actinobacteria*	BG Control: 107.9 Pretreated: 150.4	BG 39.39%	[122]
Waste	Enzymatic	*Aspergillus candida*	BM Control: 68 Pretreated: 180	BM 164.71%	[123]
Animal/Fish	Enzymatic	*C. rugose* *G. candidum*	BG Control: 219.4 Pretreated: 417.4	BM 90.47%	[124]
Algae	Enzymatic	*Geotrichum rugose*	BG Control: 471 Pretreated: 626.5	BG 33%	[125]
Fruit waste	Fungal	*P. chrysosporium* *Aspergillus niger*	BG Control: 145.2 Pretreated: 308.9 BM Control: 61 Pretreated: 176	BG 112.74% BM 188.22%	[126]
Sludge	Enzymatic	*B. subtilis* *A. hydrophila*	BG Control: 207 Pretreated: 244.4	BG 18.07%	[127]
Sludge	Enzymatic	*Bacillus jerish*	BG Control: 212 Pretreated: 467	BG 120.28%	[128]
MSW	MC	MC having *Bacillus cereus*, *B. subtilis*, *Staphylococcus saprophyticus* *Staphylococcus xylosus* *P. agglomerans* *P. chrysosporium*	BM Control: 30.9 Pretreated: 81.8	BM 190.61%	[129]
MSW	MC	*B. licheniformis* and others	BG Control: 24 Pretreated: 45.3	BG 88.75%	[130]

BG—biogas; BM—biomethane; MSW—municipal sewage waste; MC—microbial consortium.

4.4. Advantages and Disadvantages of Biogas Production Enhancement Strategies

The utilization of biogas offers numerous benefits, mostly stemming from the ability to employ agricultural and industrial waste as a valuable resource in anaerobic bioreactors (Figure 2). Biomethane, once generated, finds extensive application in industrial sectors. Hydrogen fuel serves as a source of energy for automobiles, while CO_2 functions as a substrate for the production of diverse products [131]. However, there are also a number of limitations, including the fact that the process is labor-intensive and that mass production is expensive [132]. In this regard, further study and innovation in design are required to improve the efficiency of biogas generation on a large scale.

Enhancing biogas production involves optimizing the anaerobic digestion process to increase the yield of biogas from organic waste. There are several strategies to achieve this goal, each with its own advantages and disadvantages. Co-digestion of feedstock, microbial pretreatments, and bioaugmentation with anaerobic microorganisms are bio-

logical technologies to enhance biogas production [133]. The advantage of co-digestion is mixing multiple feedstocks with varying nutrient content can enhance overall biogas production and balance nutrient ratios [134], while proper feedstock mixing and balance are crucial to preventing process instability. The introduction of certain feedstocks might require additional equipment and management [135]. Bioaugmentation with specialized microorganisms or microbial consortia can enhance digestion efficiency and biogas production. However, ensuring the survival and stability of introduced microorganisms can be challenging. Over time, they might be outcompeted by indigenous microorganisms [136]. Proper waste management practices and microbial pre-treatment techniques can improve the availability and digestibility of organic matter. Advanced pretreatment methods might incur additional costs and increase energy consumption [11]. Ensuring a proper balance of carbon, nitrogen, and other nutrients in the feedstock can enhance microbial activity and biogas production. Achieving and maintaining nutrient balance might require careful feedstock selection and adjustment [137]. A holistic approach that considers a combination of these strategies is often necessary to achieve significant biogas production enhancement.

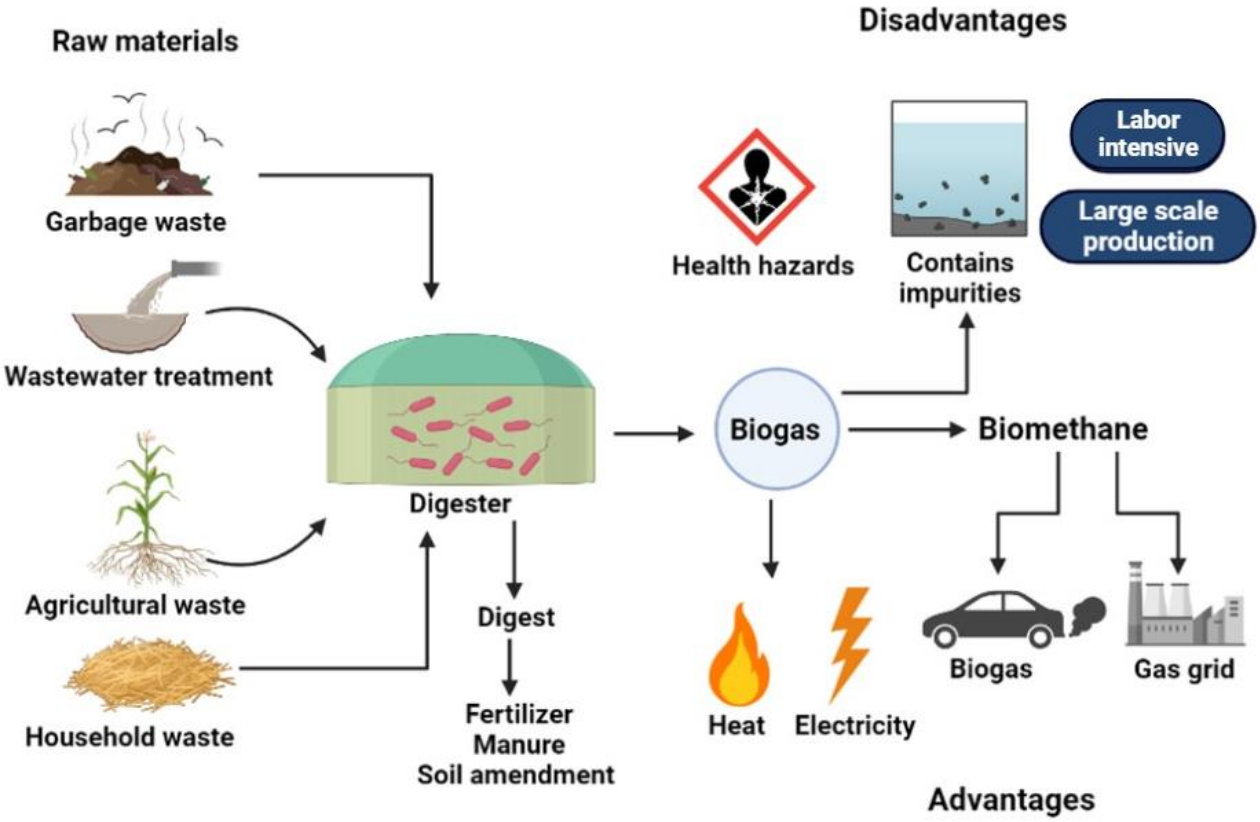

Figure 2. Advantages and disadvantages of biogas production.

5. Anaerobic Metabolic Pathways and Genes Involved in Biogas Production

Biogas systems, also known as anaerobic digestion systems, are designed to convert organic waste materials into biogas through the action of microorganisms. The production of biogas is a complex process, and these microorganisms play a crucial role in the overall efficiency and stability of the biogas production process [31]. The production of biogas in anaerobic system proceeds in four interrelated steps; these are hydrolysis, acidogenesis, acetogenesis, and methanogenesis [138]. All the steps have different requirements which are fulfilled by different groups of microorganisms. Complex organic compounds, such as cellulose and hemicellulose, are broken down by hydrolytic bacteria. These bacteria secrete enzymes that degrade these complex molecules into simpler compounds, such as sugars. The hydrolytic activity of these bacteria provides a substrate for further microbial

degradation, and this is a rate-limiting step of this process [139]. Hydrolysis is mainly facilitated by facultative anaerobes such as *Clostridium*, *Bacillus*, *Cellulomonas*, etc. [32]. *Dysgonomonas* possess metabolism for hydrolysis of lignin from plant-based wastes [140]. The addition of food waste significantly increases the abundance of *hydA* (encoding hydrogenase) and *mcrA* (encoding methyl coenzyme-M reductase) [59]. Acidogenic bacteria utilize the products of hydrolysis and ferment the simple compounds, such as sugars, to produce volatile fatty acids (VFAs). Acidogenesis serves as an important step in the breakdown of organic matter as 70% of products from the hydrolysis step are processed in this step and it provides substrates for subsequent microbial groups. Acetogenic bacteria generate acetate through the oxidation of VFAs, releasing hydrogen (H_2) and carbon dioxide (CO_2) as byproducts [141]. Microbial genera *Bacillus*, *Acidaminococcus*, *Streptococcus*, *Desulfovibrio*, and *Lactobacillus* are associated with these conversions [32]. Syntrophic bacteria establish a symbiotic relationship with methanogens and play a crucial role in the breakdown of complex compounds. They ferment organic matter, producing acetate, hydrogen, and carbon dioxide. *Syntrophaceticus*, *Syntrophomonas*, *Cloacamonas*, *Clostridium*, *Candidatus*, and *Tissierella* are some reported syntrophic bacteria capable of degrading organic polymers into its monomers, which are then utilized by methanogens for the production of methane gas [142,143]. Methanogens consume the hydrogen produced by syntrophic bacteria, maintaining a low hydrogen partial pressure and allowing fermentation to continue. Syntrophic bacteria and methanogens rely on each other to efficiently convert complex organic compounds into methane. Methanogenic archaea, known as methanogens, utilize the hydrogen and acetate produced during the preceding steps to generate methane gas (CH_4). Methanogens can be categorized into hydrogenotrophic methanogens such as *Methanobacteria* and *Methanomicrobia*, which utilize hydrogen and carbon dioxide to produce methane, and acetoclastic methanogens such as *Methanosaeta* and *Methanosarcina*, which consume acetate to generate methane [14]. The *mreA* gene plays an important role in the switching stage between hydrogenotrophic and acetoclastic methanogenesis. In a study with mutated *mreA*, its role was reported in the activation of acetate specific genes [144]. *Methanosarcinales* are the most adaptable and dominant methanogens found in large sets of literature. These methanogens are highly sensitive to environmental conditions and rely on a balanced microbial community for optimal methane production [145]. A few examples of methane producing microorganisms are *Methanosarcina mazei*, *Methanoculleus marisnigri*, *Methanothermobacter thermautotrophicus*, *Methanosarcina barkeri*, etc. [32]. About two thirds of the methane produced in biogas plants is by acetoclastic methanogens, but this pathway was reduced with an increase in ammonium content and proceeds towards the hydrogenotrophic pathway [146]. A detailed microbial metabolic pathway and the genes involved in the biogas production of an anaerobic bioreactor are presented in Figure 3.

Transcriptomic analysis identified the various genes and products: *mcr*, methyl-coenzyme M reductase; *mta*, methyltransferase; *mtr*, tetrahydromethanopterin S-methyltransferase; *mer*, 5,10-methylenetetrahydromethanopterin reductase; *frh*, coenzyme F420 hydrogenase subunit; *hmd*, 5,10-methenyltetrahydromethanopterin hydrogenase; *mch*, methenyltetrahydromethanopterin cyclohydrolase; *ftr*, formylmethanofuran-tetrahydromethanopterin N-formyltransferase; *fmd*, formylmethanofuran dehydrogenase; *fdh*, formate dehydrogenase; *cdh*, acetyl-CoA decarbonylase/synthase complex; *pta*, phosphate acetyltransferase; *ack*, acetate kinase; THMPT, tetrahydromethanopterin [64,147,148].

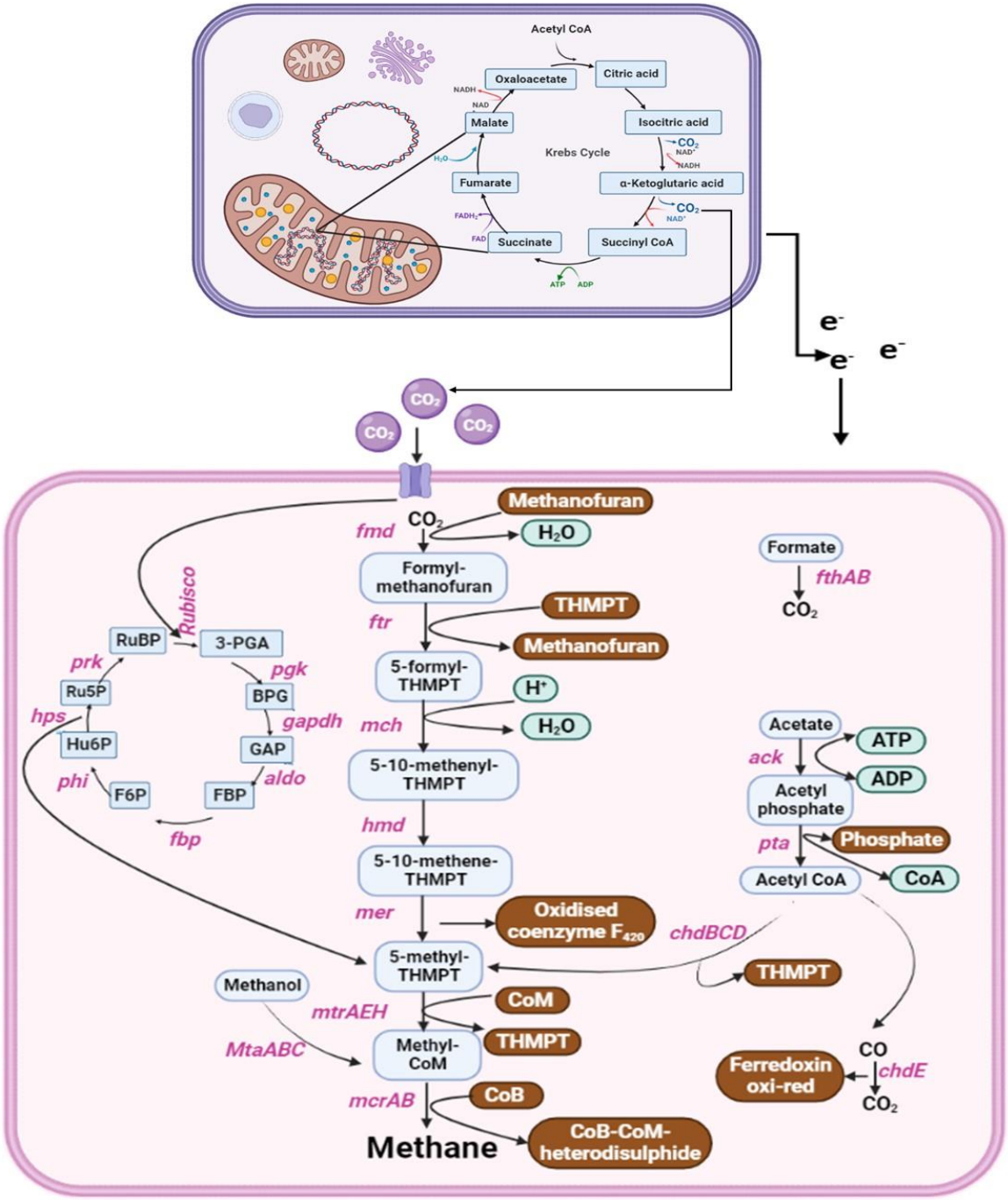

Figure 3. The pathways of acetate oxidation, acetoclastic methanogenesis (acetate conversion to methane), classical CO_2 reduction (fixation of CO_2 via formation of THPMT's as basic intermediates), and CO_2 reduction via RHP (RuBisCO−mediated reductive hexulose phosphate pathway that forms formaldehyde as an intermediate) for methane production. Through meta−transcriptomic evidence, it is demonstrated that in a methanogenic system, both classical and RuBisCO−mediated CO_2 reduction to methane are facilitated by direct interspecies electron transfer. Ru5P, ribulose−5−phosphate; RuBP, ribulose−1,5−bisphosphate; 3−PGA, 3−phosphoglycerate; BPG, 1,3−diphosphoglycerate; GAP, glyceraldehyde−3−phosphate; FBP, fructose−1,6−bisphosphate; F6P, fructose−6−phosphate; Hu6P.

6. Microbial Metagenomics, High-Throughput Sequencing, and Its Relevance to Biogas Production

The biogeochemical cycles that support all life on Earth depend heavily on microorganisms, which are pervasive in the environment [149,150]. They also serve as the foundation for a number of currently used engineered processes, such as anaerobic digestion, which converts organic feedstocks into energy-dense compounds such as alcohols, volatile fatty acids, and methane to produce renewable energy [151]. Although most of the microorganisms in these systems have not yet been grown, culture-dependent approaches have enabled the identification of important populations capable of performing particular metabolic pathways in anaerobic digestion [150]. The existing comprehension of microbial physiology pertaining to anaerobic metabolism would be deficient and potentially skewed if exclusively reliant on culture-dependent methodologies. This is because it does not explain the resource competition and interactions, which are two environmental elements that affect microbial activities and functions. Furthermore, a complex interrelating microbial network may have characteristics that are not present in any of the individual species [149,152]. Therefore, culture-independent methodologies for studying microbial communities have rapidly developed over the past decades, and this has resulted in new understandings of their structure and function in both natural habitats and manmade systems [153,154]. Anaerobic digesters are often dominated by hitherto uncharacterized bacteria, according to the use of culture-independent approaches, which have also revealed significant phylogenetic and metabolic diversity [155,156]. There should be a deeper understanding of these microbes' metabolic capacities, the degree of functional redundancy within a community, and the basic processes behind interspecies interactions in order to optimize anaerobic digestion and direct product production [157].

Maus et al. [158] conducted a study where the whole genome sequences of 22 bacterial and archaeal strains were analyzed to investigate their functions within anaerobic digestion communities. These strains included *Clostridium thermocellum* BC1, *Methanoculleus bourgensis* MS2T, *Proteiniborus* sp. DW1, *Propionispora* sp. 2/2–37, *Methanobacterium congolense* Buetzberg, *Herbinix luporum* SD1DT, *Methanobacterium formicicum* Mb9, *Methanobacterium formicicum* MFT, *Clostridium bornimense* M2/40T, *Methanobacterium* sp. Mb1, *Methanothermobacter* wolfeii SIV6, *Clostridium cellulosi* DG5, *Proteiniphilum saccharofermentans* M3/6T, *Petrimonas mucosa* ING2-E5AT, *Bacillus thermoamylovorans* 1A1, *Defluviitoga tunisiensis* L3, *Herbinix hemicellulosilytica* T3/55T, *Sporanaerobacter* sp. PP17-6a, and *Peptoniphilaceae bacterium* sp. ING2-D1G. The study focused on predicting and partially verifying the involvement of 15 bacterial strains in the hydrolysis and/or acidogenesis/acetogenesis stages of plant biomass decomposition through in vivo characterization of pure cultures. In total, 9 of the 22 bacteria were anticipated to be involved in acidogenesis and/or acetogenesis, whereas *H. hemicellulosilytica* T3/55T, *Clostridium cellulosi* DG5, *C. thermocellum* BC1, and *H. luporum* SD1DT represented cellulose degraders. The hydrogenotrophic route, which is the last link in the AD chain, was expected to be used by the seven methanogenic archaea that were studied to create CH4. An organism named *Defluviitoga tunisiensis* has been suggested as a marker for thermophilic biogas processes. A wide range of substrates can be utilized by these species converting them into metabolites that act as substrates for methanogenesis. Furthermore, Stolze et al.'s [158] fragment recruitment analysis of metagenome-assembled genomes (MAGs) demonstrated that the metagenome assembly and binning approach may also make it possible to identify and characterize previously undiscovered but numerous species with significant functional potential in the context of the anaerobic digestion process [18].

There are three main categories of biological diversity that the study of microbial diversity may shed light on: the diversity of genes within a species, the diversity of geographic ranges, and the diversity of communities, or ecology. The greatest obstacle in determining microbial diversity, however, may be the categorization of unclassified bacteria [159]. By assessing the divergence in molecular characteristics, such as nucleic acid homology, biodiversity may be assessed. The community's stability is correlated with the

system's stability, and stress within the system can cause instability and changes in the variety of the species [160]. Consequently, the analysis of diversity holds great value as it enables us to gain insights into the genetic makeup of organisms and their distribution within the community. The provided information pertains to the functional significance of the variety within the system, the distinct species types that are present, and the precise quantification of each species' abundance [159]. Recent advances in sequencing technology, referred to as high-throughput methods or next-generation sequencing (NGS), allow for the simultaneous sequencing of many DNA molecules at a cheap cost, quick turnaround time, and high resolution [161]. Large datasets are produced as a result of these qualities, which improve statistical correlation analysis [157]. The investigation of anaerobic bioreactor cultures has commonly utilized two main high-throughput techniques for 16S rRNA sequencing, namely Roche 454 and Illumina chemistry. Indeed, the utilization of high-throughput sequencing techniques enables the examination of the correlation between community composition and many operational factors, including organic load rate, temperature, ammonia concentration, feed type, etc. [162,163]. Additionally, microbial diversity and long-term operation monitoring may reveal important details about how communities work. According to Werner et al. [164], resilience is more crucial for maintaining syntrophic populations than dynamic competition. Furthermore, they showed a high correlation between substrate removal effectiveness and methanogenic activities [164]. The identification of large numbers of operational taxonomic units (OTUs) in anaerobic digestion, as opposed to the prior discovery of just 69 OTUs, is a result of the enhanced resolution of high-throughput sequencing technologies [164,165]. Additionally, this high resolution can help identify populations with low abundance and their contribution to the generation of biogas [157]. Pyrosequencing (used by the 454 Roche platform) is one of the high-throughput methods that had been frequently used to evaluate anaerobic bioreactor's community composition [166]. For larger sequencing ranges, reversible dye terminator (RDT) techniques were created [167]. RDTs can be divided into blocked and unblocked (ubRDT and bRDT, respectively) categories [167,168]. In the termination procedure (which is primarily utilized for second-generation sequencing), bRDT demonstrated greater performance, and ubRDTs were more effective in the sequence elongation outcomes [15]. The 3′-O-azidomethyl method is used by the Illumina technology, which is a second-generation sequencing methodology based on bRDT [167]. In comparison to the 454 Roche system platform, the Illumina MiSeq platform can produce 4 terabases of sequence and 2300 base–pair paired end reads each run [157].

As next-generation sequencing (NGS) technology improved in performance and efficacy, metagenomics became more significant for the analysis of microbial assemblages. By using metagenome, genome, and post-genome research techniques and using high-throughput sequencing of environmental complete community DNA and RNA, inevitable anaerobic digestion communities were clarified. Since they provide chances for their management and engineering, it is widely acknowledged that biogas-producing microbial communities are the key to process shaping and the creation of optimization techniques [169,170]. According to Yang et al. [171] the entire 16S rRNA gene or particular sections can be used as a taxonomic marker gene. Currently, 98.65% and 94.5% of the 16S rRNA gene's nucleotide sequences are recommended as taxonomic criteria for species and genera, respectively [172,173]. Amplified ribosomal DNA restriction analysis (ARDRA) of 16S rRNA gene libraries, denaturing gradient gel electrophoresis (DGGE) analysis, and terminal restriction fragment length polymorphism (T-RFLP) are several techniques that were initially developed to study the diverse and dynamic structures of microbial communities in environmental systems and to reduce the costs associated with DNA sequencing [174,175]. These methods are widely used for prior research on the microbial structures found in anaerobic bioreactor or biogas reactors [176]. Since direct DNA library sequencing was made possible by the invention of NGS systems, hundreds of samples could be read simultaneously. This led to an optimized 16S rRNA gene study. While 16S rRNA gene sequencing-based taxonomic community profiling has several benefits, it also

has some major limitations. The primers' specific properties cause biased amplification of the target region during polymerase chain reaction. Moreover, the method's resolution is constrained by its use of short reads, which might result in an underestimating of species diversity. The method of "full-length" 16S rRNA amplicon sequencing by using the PacBio single molecule, real-time technology (SMRT) can be taken into consideration to make up for resolution biases (such underestimating of species diversity) associated with sequencing of individual variable areas [177,178]. In a study by Treu et al. [52], 44.39 billion bp of sequencing data (shotgun reads) were produced by the Illumina NextSeq 500 sequencing of bulked metagenomic DNA from biogas upgrading reactor samples. The phylum Firmicutes constituted 60% of the entire population, hence exhibiting the highest degree of dominance [179]. *Bacteroidales* received the great bulk of the *Bacteroidetes*, while *Flavobacteriales* received the remainder. *Proteobacteria*, which made up 10% of the metagenome, were the third most prevalent phylum. *Gammaproteobacteria* was the most prevalent class within this phylum, followed by *Betaproteobacteria*, *Deltaproteobacteria*, and *Epsilonproteobacteria*. Of the entire community, 3% was made up of both Spirochaetes and Synergistetes. Finally, the phyla *Verrucomicrobia*, *Actinobacteria*, and *Tenericutes* were present in the microbial community at a rate of around 2%. The low prevalence of *Chloroflexi* and *Actinobacteria* indicates that the microbial community structure in biogas reactors treating industrial and agricultural wastes is very different from that in AD systems processing sludge and wastewater [52].

The implementation of large-scale, economically viable environmental shotgun sequencing initiatives was made possible by the development of second-generation ultrafast sequencing technologies such as 454 pyrosequencing. Metagenomics evolved into a flexible method for investigating the structure, gene content, and function of many autochthonous microbial communities in various settings. Ultrafast sequencing techniques are being used in an increasing number of metagenome studies [180–183]. The interpretation of metagenomic data using bioinformatics has been coordinately improved [184]. In order to anticipate coding sequences, a novel gene identification technique was recently created [185]. It makes use of the limited information present in the 250 nucleotide reads produced by 454-pyrosequencing. Additionally, the development of bioinformatics methodologies and tools for processing metagenomic data enhances understanding of the gene content and community structures of microbial consortia from various environments [15,55].

6.1. Applications of Meta-Transcriptomics in Biogas Production

Meta-transcriptomics is a powerful molecular biology technique that involves analyzing the RNA transcripts present in a microbial community. It provides insights into the active gene expression patterns of the various microorganisms in a given environment and serves as further validation of metagenomic analysis [186,187]. In the context of biogas production, meta-transcriptomics can be applied to understand and optimize the microbial processes involved in anaerobic digestion [188]. The meta-transcriptomics enables the assessment and documentation of the functional attributes of organisms with low abundance as well, along with their impact on the stability of biological processes [189]. It provides insights into the gene expression patterns of microorganisms in a biogas reactor; as such, the meta-transcriptomics study of a cellulose-rich anaerobic bioreactor reveals that *Clostridium cellulolyticum*-related bacteria carry out cellulose degradation and were dominated at 35 °C, whereas acidogenesis and acetogenesis is facilitated by *Ruminococcus*-related bacteria [190]. Meta-transcriptome data reveal the pathway details of a process. Ardèvol et al. [191] examined the meta-transcriptome of a *Spirulina*-biomass anaerobic bioreactor inoculated with haloalkaline microorganism. The study indicated continuous biogas production with 96% methane and low CO_2 and H_2S emission. The transcriptomic study showed that hydrolysis was facilitated by Bacteroidetes and *Methanocalculus* dominates the methanogenic community and follows the hydrogenotrophic pathway for methane production [191]. In a similar study, it was observed that *Methanothermobacter wolfeii* exhibited a 7% increase in methane production through the upregulation of the hydrogenotrophic pathway. The

inclusion of H_2 has been observed to enhance CO_2 fixation pathways. Notably, *Anaerobaculum hydrogeniformans* and *Defluviitoga tunisiensis* have been identified as the predominant species in this context. These species exhibit increased expression of genes related to electron transfer chains, which in turn promotes the establishment of syntrophic relationships [192]. The study conducted by Maus et al. [193] focused on a meta-transcriptomic analysis of a thermophilic biogas plant. The findings of this study revealed the involvement of three bacterial species, namely *Clostridium thermocellum*, *Clostridium stercorarium*, and *Defluviitoga tunisiensis*, in the hydrolysis of hemicellulose. This process resulted in the production of ethanol, acetate, H_2, and CO_2, which subsequently served as substrates for hydrogenotrophic and acetoclastic archaeal methanogenesis. The mRNA transcript will also serve as a marker for the evaluation of biogas production by various microbial communities in an anaerobic bioreactor [194]. Bacterial taxa belonging to the family Peptococcaceae and the order Halanaerobiales exhibited a high level of transcriptional activity in bioreactors that were supplied with chicken manure. In the same investigation, it was observed that *Firmicutes* exhibited active transcription of a wide range of genes responsible for encoding glycosyl hydrolases. Notably, several of these enzymes were found to be engaged in the hydrolysis process of lignocellulose [195]. Meta-transcriptome analyses were conducted on a thermophilic full-scale biogas production system that utilized maize silage, barley, and cattle manure as feedstock. The results revealed significant transcriptional activity according to sequence tags derived from the 16S rRNA gene. The microbial community, such as *Defluviitoga* from the Thermotogae phylum, *Methanoculleus* from the Euryarchaeota domain, *Clostridium* cluster III from the Firmicutes phylum, *Tepidanaerobacter* from the Firmicutes phylum, Anaerobaculum from the Synergistetes phylum, and *Cellulosibacter* from the Firmicutes phylum exhibited notable transcriptional activity [178]. Meta-transcriptomics can be used to monitor the health of the microbial community during anaerobic digestion. Changes in gene expression patterns can indicate stress responses, the presence of inhibitors, or shifts in community composition that might negatively impact biogas production [188]. Thus, meta-transcriptomics offers a comprehensive view of the functional aspects of microbial communities in biogas production systems.

6.2. Integration of Omics Approaches with Molecular Probing Techniques

The primary goal of DNA stable-isotope probing was to evaluate the metabolic activity of environmental microorganisms [196]. DNA-SIP (stable isotope probing) has been used successfully to detect metabolically active microorganisms in a wide variety of habitats, including soil, water, coal mine, petroleum oil fields, freshwater, marine, and anaerobic settings. The growth of microorganisms on labeled substrates allows for the insertion of stable-isotope components such as 13N or 15N into their DNA [197,198]. The combination of SIP with metagenomic sequencing is an effective method for identifying previously undetected active species within a microbial community [199]. Mosbæk et al. [200] first applied this technique in the field of biogas production. In this study, the recovery of methane production after inhibition by volatile organic substance was studied. *Methanosarcina*, *Methanoculleus*, and *Clostridium* sp. were associated with the recovery of acetate production. Further, the expression of *FTFHS* gene, which codes for formyltetrahydrofolate synthetase, an important enzyme in reductive acetogenesis, was found in all species of *Clostridium*. In a study, SIP coupled with 16S rDNA pyrotag sequencing proved that various methanogens (including *Methanosarcina thermophila*) can withstand high total ammonium nitrogen concentrations up to 916 mg/L [17]. This technique also proved that in thermophilic chemostat, the commonly followed methanogenic pathway is syntrophic oxidizing pathway [201]. Unculturable microorganisms also contribute to the degradation efficiency of that system. The outcome of DNA-SIP with 13C-propionate/acetate suggested that propionate-oxidizing bacteria were *Smithella*, *Syntrophobacter*, *Cryptanaerobacter*, and *Rhodospirillaceae*, while acetate oxidation was facilitated by unclassified *Spirochaetaceae*, *Synergistaceae*, *Elusimicrobia*, *Mesotoga*, and *Gracilibacter*; similarly, unclassified *Syntrophaceae* and *Syntrophomonas* were butyrate oxidizers [202]. With the application of this technique, it is revealed that the

virus (Caudoviricetes) can alter bacterial biogeochemical fluxes in environment, suggesting that this virus can infect non-culturable microorganisms in addition to methanogenic archaea [203]. The implementation of this integrated technique in research on the generation of biogas is anticipated to receive significant attention in research in the near future. The probing technique can be utilized to find numerous novel pathways, which will be of great assistance in gaining a better knowledge of the activities that are going on in nature.

7. Biogas Production and Circular Economy

Biogas production by microorganisms plays a crucial role in the concept of circular economy by facilitating the conversion of organic waste into a valuable energy resource. This process not only helps in waste management and the reduction of greenhouse gas emissions but also provides renewable energy and promotes sustainability. Biogas production offers an efficient solution for the management of various organic wastes, including agricultural residues, food waste, and sewage sludge. The utilization of biogas as a renewable energy source contributes to the transition from fossil fuels to more sustainable alternatives [204]. Biogas can be used for heat and electricity generation, as well as a vehicle fuel. According to a report by the International Renewable Energy Agency (IRENA), the global production of biogas in 2018 reached approximately 57 billion cubic meters, which corresponds to an estimated 3% of global natural gas consumption [205]. Additionally, an increase of 90% in electricity generation from biogas was reported for the duration 2010 to 2016 [43]. The anaerobic digestion process involved in biogas production helps mitigate greenhouse gas emissions. By converting organic waste into biogas, it prevents the release of methane, a potent greenhouse gas, during the decomposition of waste in landfills [206]. According to the Global Methane Initiative, anaerobic digestion projects can reduce methane emissions, which is not achieved in other waste management practices [207]. Biogas production also facilitates the recovery of valuable nutrients from organic waste. The byproduct of anaerobic digestion, known as digestate, is a nutrient-rich fertilizer that can be used in agriculture, closing the nutrient loop and reducing the reliance on synthetic fertilizers. According to a study, digestate from biogas plants has the potential to replace up to 30% of chemical fertilizers used in Europe [208]. The biogas sector has the potential to create employment opportunities, as is happening in commercial biogas plants such as Microb2Energy-BioPower2Gas, EcoVolt®, etc., and contribute to countries' economies [43]. The utilization of biogas helps in closing the loop on organic waste, turning it into a valuable resource and contributing to a more sustainable and circular future.

8. Microbe-Based Large-Scale Commercial Biogas Plants

Improving the performance of biogas plants while keeping the costs of CH_4 enrichment low presents a significant challenge for the widespread implementation of microbial approaches in the industry. These studies aim to find solutions that can be scaled up from laboratory-size reactors to large-scale plants while maximizing the CH_4 enrichment in biogas, considering the economic feasibility of the process. Several microbial-based large-scale plants have emerged as pioneers in the field of biogas production and upgradation. For example, Microb2Energy-BioPower2Gas, located in Allendorf, Germany, is the first commercial in situ H_2 injection biogas plant based on biological methanation. The carbon dioxide present in biogas is converted to methane by methanogenic archaea. The methanogenic bacterial population overcomes mass transfer limitations by utilizing H_2 supplied from the bottom. As a result, the CH_4 production in the produced biogas is significantly increased up to 75% [209]. Electrochaea is an ex situ biological methane upgrading plant in association with a wastewater treatment plant located in Denmark. This plant utilizes H_2 generated from electrolysis in wastewater. The biogas contains 60% CH_4 and 40% CO_2; the CO_2 is then separated from an amine scrubbing biogas upgrading process and used for biological methanation. The oxygen byproduct is recycled into the wastewater treatment process. The resulting gas from the methanation reactor contains 90–95% CH_4. The final gas composition consists of 98% CH_4, 2% H_2, 1% CO_2, and negligible water

vapor [43]. Electrogas is another in situ biomethanation plant in Denmark. The project involves the direct injection of H_2 into thermophilic reactor where agricultural waste is used as feedstock [210]. The EcoVolt® Reactor, which transforms industrial wastewater into clean water and renewable methane gas, has been made commercially available by Cambrian Innovation. A partnership between Cambrian Innovation and the US Army was recently announced in order to demonstrate BioVoltTM, which is a self-powered wastewater treatment system [43].

9. Future Prospects

9.1. Genetic Engineering of Microorganisms for Enhanced Biogas Production

Genetic engineering techniques offer the ability to modify microorganisms at the genetic level, enabling the development of strains with enhanced metabolism for biogas production. The modified strains are not only useful for the pretreatment stage but also for the mainstream and downstream processes as well. Metabolic engineering has received a lot of attention as a way to develop strains that are sturdy and efficient [211]. For this, it is important to have a full understanding of the metabolic connections of the groups of microorganisms that are involved at various stages of the process. By using high-throughput screening and high-throughput metagenomic sequencing, the important genes and pathways involved in producing biogas can be found. which is further taken into consideration for manipulation [212]. Metabolic pathway reconstruction is widely used in the degradation of environmental pollutants such as crude oil, microplastics, pesticides, etc.; therefore, its proper application in waste to energy strategy will be very promising [213]. In recent times, the field of genetic engineering has seen the emergence of new genome-editing tools, such as CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats-associated protein Cas9), TALEN (transcription activator-like effector nucleases), and ZFN (zinc-finger nucleases). These tools provide efficient means of modifying genes in microorganisms. These tools allow for targeted activation or suppression of specific gene expressions, enabling researchers to precisely manipulate the genetic makeup of microorganisms involved in any specific process [213]. The utilization of these gene-interfering tools holds significant potential in enhancing the efficiency and performance of microorganisms for biogas production. Rollin et al. [214] employed an in vitro synthetic enzymatic pathway as part of their metabolic engineering approach to enhance hydrogen production from biomass. More than 10 purified enzymes were expressed into artificial enzymatic pathways, which resulted in three times improved biogas production from glucose and xylose substrates. These findings suggest that this enzymatic pathway approach could be applied to enhance biogas production as well. Generally, the growth rate of anaerobes is less, to overcome this obstacle, the enzyme encoding genes can be recombined to a microorganism having high growth rate. By introducing genes encoding specific enzymes or metabolic pathways, microorganisms can be engineered to break down the feedstocks more effectively, leading to increased biogas production. A recombinant anaerobe has been designed that has an elevated hydrolysis property with a more thermostable enzyme and that can decrease hydraulic retention time very effectively in a biogas production unit [22]. Further, by introducing genes that confer resistance to toxins or enhance stress tolerance, microorganisms can better withstand the challenging conditions encountered during biogas production, resulting in improved overall performance [215]. Genetic engineering is still in its basal state in terms of industrial applications. Therefore, further research is needed to apply these strategies in real-time applications.

9.2. Microbial Conversion of CH_4 and CO_2 into Other Renewable Energy

Converting CH_4 and CO_2 into renewable energy sources is a challenging and complex process. Both CH_4 and CO_2 are greenhouse gases that contribute to climate change, and finding ways to utilize or transform them into valuable energy resources can help mitigate their negative impacts [216,217]. The CH_4 and CO_2 from biogas plants can be used to produce other forms of renewable energy. The CH_4 reacts with steam by a methane reform-

ing process and produces a mixture of hydrogen and carbon monoxide, known as syngas. Syngas is a versatile feedstock that can be used to produce hydrogen fuel or converted into synthetic fuels such as synthetic natural gas or liquid fuels through processes such as the Fischer–Tropsch synthesis [218]. Microorganisms (mainly *Clostridium* sp.) can further convert this syngas into acetic acid, which has wide applications in bioproduct industries [219]. Anaerobic microorganism such as *Clostridium ljungdahlii*, *C. autoethanogenum*, *C. carboxidivorans*, *Alkalibaculum bacchii*, *Oxobacter pfennigii*, *Butyribacterium methylotrophicum*, etc. can live on CO_2/CO in anaerobic bioreactors and produce industrially important bioethanol, lactate, and butyrate [219]. The symbiotic methanogens facilitate the conversion of hydrogen and CO_2, generated by the biogas plant, via the Sabatier reaction, resulting in the production of CH_4 [220,221]. This synthetic methane can be stored, transported, and used as a renewable energy source. Carbon capture and utilization involves capturing CO_2 emissions from industrial processes and power plants and then using the captured CO_2 as a feedstock for the production of valuable products such as chemicals, plastics, or even in the generation of biofuels [222]. CO_2 and H_2 can be captured by microorganisms and convert them into CH_4 and acetate [223]. Acetate can further be converted into CH_4 by acetogenic microorganisms [224]. Microbial Electrolysis Cells is another technology where microorganisms convert methane into methane-derived products and hydrogen gas [225]. It is important to note that while these processes offer potential solutions, they also come with technological, economic, and environmental challenges. Developing efficient and cost-effective conversion methods, addressing storage and transportation issues, and ensuring the overall sustainability of these approaches are key areas of research and development in the field of renewable energy and climate change mitigation.

9.3. Policy Support and Market Incentives from Government

Continued support from policies and incentives, such as feed-in tariffs, renewable energy credits, and carbon pricing mechanisms, can provide a favorable market environment for biogas production [226]. Governments and regulatory bodies play a crucial role in creating an enabling framework that encourages the development of biogas technologies, fostering investment, and facilitating market growth. Countries such as Europe, China, Malaysia, and Italy already have such provisions [205,227,228]. This type of initiative will generate interest among people, generate an income source, and provide solutions to waste management.

10. Conclusions

Biogas is the future for sustainable energy production, and microorganisms are an integral part of it. The study of microbial genomics and their diversity has provided valuable insights into the vast potential of microorganisms and their diverse metabolic capabilities. The advancements in genomic technologies, such as next-generation sequencing, have revolutionized the understanding of microbial communities and their functional potentials. These tools provided an in-depth exploration of microbial diversity which is significant in discovery of new species, gene functions, and metabolic pathways. Moreover, the integration of multi-omics approaches, including meta-transcriptomics, meta-proteomics, and metabolomics, has provided comprehensive insights into the complex functional interactions and metabolic networks within microbial communities. Further, the combination of substrates and other treatments also aids in enhanced biogas production. Further advancements in genomic technologies will continue to unravel the hidden potential of microbial communities and their applications. Additionally, the development of novel bioinformatics tools and analytical approaches will enhance our ability to interpret and integrate vast amounts of genomic data. Furthermore, synthetic biology principles should be explored for enhanced biogas production as it is giving excellent results in other applications such as bioremediation, industries, etc. This will open up new avenues for optimizing microbial processes, improving yields, and expanding the range of substrates that can be efficiently utilized. Therefore, microbial processes, genomics, and diversity are crucial areas of re-

search with wide-ranging implications. Continued exploration of microbial communities and their genomes will pave the way for innovative and sustainable solutions in fields such as environmental conservation and energy production. By harnessing the desired microorganisms, a more sustainable and bio-based economy can be developed.

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References

1. Adnan, A.I.; Ong, M.Y.; Nomanbhay, S.; Chew, K.W.; Show, P.L. Technologies for biogas upgrading to biomethane: A review. *Bioengineering* **2019**, *6*, 92. [CrossRef] [PubMed]
2. Mahapatra, S.; Kumar, D.; Singh, B.; Sachan, P.K. Biofuels and their sources of production: A review on cleaner sustainable alternative against conventional fuel, in the framework of the food and energy nexus. *Energy Nexus* **2021**, *4*, 100036. [CrossRef]
3. Hossain, M.; Haque, M. Stability analyses of municipal solid waste landfills with decomposition. *Geotech. Geol. Eng.* **2009**, *27*, 659–666. [CrossRef]
4. Fajrina, N.; Yusof, N.; Ismail, A.F.; Aziz, F.; Bilad, M.R.; Alkahtani, M. A Crucial Review on the Challenges and Recent Gas Membrane Development for Biogas Upgrading. *J. Environ. Chem. Eng.* **2023**, *11*, 110235. [CrossRef]
5. Theuerl, S.; Klang, J.; Prochnow, A. Process disturbances in agricultural biogas production—Causes, mechanisms and effects on the biogas microbiome: A review. *Energies* **2019**, *12*, 365. [CrossRef]
6. Atelge, M.; Krisa, D.; Kumar, G.; Eskicioglu, C.; Nguyen, D.D.; Chang, S.W.; Atabani, A.; Al-Muhtaseb, A.H.; Unalan, S. Biogas production from organic waste: Recent progress and perspectives. *Waste Biomass Valorization* **2020**, *11*, 1019–1040. [CrossRef]
7. Nwokolo, N.; Mukumba, P.; Obileke, K.; Enebe, M. Waste to energy: A focus on the impact of substrate type in biogas production. *Processes* **2020**, *8*, 1224. [CrossRef]
8. Anukam, A.; Mohammadi, A.; Naqvi, M.; Granström, K. A review of the chemistry of anaerobic digestion: Methods of accelerating and optimizing process efficiency. *Processes* **2019**, *7*, 504. [CrossRef]
9. Abanades, S.; Abbaspour, H.; Ahmadi, A.; Das, B.; Ehyaei, M.; Esmaeilion, F.; El Haj Assad, M.; Hajilounezhad, T.; Jamali, D.; Hmida, A. A critical review of biogas production and usage with legislations framework across the globe. *Int. J. Environ. Sci. Technol.* **2021**, *19*, 3377–3400. [CrossRef]
10. Patel, S.K.; Gupta, R.K.; Das, D.; Lee, J.-K.; Kalia, V.C. Continuous biohydrogen production from poplar biomass hydrolysate by a defined bacterial mixture immobilized on lignocellulosic materials under non-sterile conditions. *J. Clean. Prod.* **2021**, *287*, 125037. [CrossRef]
11. Kasinath, A.; Fudala-Ksiazek, S.; Szopinska, M.; Bylinski, H.; Artichowicz, W.; Remiszewska-Skwarek, A.; Luczkiewicz, A. Biomass in biogas production: Pretreatment and codigestion. *Renew. Sustain. Energy Rev.* **2021**, *150*, 111509. [CrossRef]
12. Aziz, M.M.A.; Kassim, K.A.; ElSergany, M.; Anuar, S.; Jorat, M.E.; Yaacob, H.; Ahsan, A.; Imteaz, M.A. Recent advances on palm oil mill effluent (POME) pretreatment and anaerobic reactor for sustainable biogas production. *Renew. Sustain. Energy Rev.* **2020**, *119*, 109603. [CrossRef]
13. Rajeswari, G.; Jacob, S.; Chandel, A.K.; Kumar, V. Unlocking the potential of insect and ruminant host symbionts for recycling of lignocellulosic carbon with a biorefinery approach: A review. *Microb. Cell Factories* **2021**, *20*, 107. [CrossRef]
14. Sidhu, C.; Vikram, S.; Pinnaka, A.K. Unraveling the microbial interactions and metabolic potentials in pre-and post-treated sludge from a wastewater treatment plant using metagenomic studies. *Front. Microbiol.* **2017**, *8*, 1382. [CrossRef]
15. Hashemi, S.; Hashemi, S.E.; Lien, K.M.; Lamb, J.J. Molecular microbial community analysis as an analysis tool for optimal biogas production. *Microorganisms* **2021**, *9*, 1162. [CrossRef]
16. Tian, G.; Zhang, W.; Dong, M.; Yang, B.; Zhu, R.; Yin, F.; Zhao, X.; Wang, Y.; Xiao, W.; Wang, Q. Metabolic pathway analysis based on high-throughput sequencing in a batch biogas production process. *Energy* **2017**, *139*, 571–579. [CrossRef]

17. Hao, L.; Lü, F.; Mazéas, L.; Desmond-Le Quéméner, E.; Madigou, C.; Guenne, A.; Shao, L.; Bouchez, T.; He, P. Stable isotope probing of acetate fed anaerobic batch incubations shows a partial resistance of acetoclastic methanogenesis catalyzed by Methanosarcina to sudden increase of ammonia level. *Water Res.* **2015**, *69*, 90–99. [CrossRef]
18. Maus, I.; Bremges, A.; Stolze, Y.; Hahnke, S.; Cibis, K.G.; Koeck, D.E.; Kim, Y.S.; Kreubel, J.; Hassa, J.; Wibberg, D. Genomics and prevalence of bacterial and archaeal isolates from biogas-producing microbiomes. *Biotechnol. Biofuels* **2017**, *10*, 264. [CrossRef]
19. Jagadevan, S.; Banerjee, A.; Banerjee, C.; Guria, C.; Tiwari, R.; Baweja, M.; Shukla, P. Recent developments in synthetic biology and metabolic engineering in microalgae towards biofuel production. *Biotechnol. Biofuels* **2018**, *11*, 185. [CrossRef]
20. Pöschl, M.; Ward, S.; Owende, P. Evaluation of energy efficiency of various biogas production and utilization pathways. *Appl. Energy* **2010**, *87*, 3305–3321. [CrossRef]
21. Achinas, S.; Achinas, V.; Euverink, G.J.W. A technological overview of biogas production from biowaste. *Engineering* **2017**, *3*, 299–307. [CrossRef]
22. Divya, D.; Gopinath, L.; Christy, P.M. A review on current aspects and diverse prospects for enhancing biogas production in sustainable means. *Renew. Sustain. Energy Rev.* **2015**, *42*, 690–699. [CrossRef]
23. Mishra, A.; Kumar, M.; Bolan, N.S.; Kapley, A.; Kumar, R.; Singh, L. Multidimensional approaches of biogas production and up-gradation: Opportunities and challenges. *Bioresour. Technol.* **2021**, *338*, 125514. [CrossRef]
24. Deena, S.R.; Vickram, A.; Manikandan, S.; Subbaiya, R.; Karmegam, N.; Ravindran, B.; Chang, S.W.; Awasthi, M.K. Enhanced biogas production from food waste and activated sludge using advanced techniques—A review. *Bioresour. Technol.* **2022**, *355*, 127234. [CrossRef] [PubMed]
25. Chowdhury, T.; Chowdhury, H.; Hossain, N.; Ahmed, A.; Hossen, M.S.; Chowdhury, P.; Thirugnanasambandam, M.; Saidur, R. Latest advancements on livestock waste management and biogas production: Bangladesh's perspective. *J. Clean. Prod.* **2020**, *272*, 122818. [CrossRef]
26. Yaqoob, H.; Teoh, Y.H.; Din, Z.U.; Sabah, N.U.; Jamil, M.A.; Mujtaba, M.; Abid, A. The potential of sustainable biogas production from biomass waste for power generation in Pakistan. *J. Clean. Prod.* **2021**, *307*, 127250. [CrossRef]
27. Devi, M.K.; Manikandan, S.; Oviyapriya, M.; Selvaraj, M.; Assiri, M.A.; Vickram, S.; Subbaiya, R.; Karmegam, N.; Ravindran, B.; Chang, S. Recent advances in biogas production using Agro-Industrial Waste: A comprehensive review outlook of Techno-Economic analysis. *Bioresour. Technol.* **2022**, *363*, 127871. [CrossRef]
28. Gao, Z.; Alshehri, K.; Li, Y.; Qian, H.; Sapsford, D.; Cleall, P.; Harbottle, M. Advances in biological techniques for sustainable lignocellulosic waste utilization in biogas production. *Renew. Sustain. Energy Rev.* **2022**, *170*, 112995. [CrossRef]
29. Ekwebelem, O.C.; Ofielu, E.S.; Dike, O.V.N.; Aleke, J.C.; Nwachukwu, G.A. Towards Sustainable Energy: The Requisite Role of Microorganisms in the Production of Biogas and Bioethanol. *J. Energy Res. Rev.* **2020**, *6*, 20–32. [CrossRef]
30. Akinsemolu, A.A. The role of microorganisms in achieving the sustainable development goals. *J. Clean. Prod.* **2018**, *182*, 139–155. [CrossRef]
31. Ferdeș, M.; Dincă, M.N.; Moiceanu, G.; Zăbavă, B.Ș.; Paraschiv, G. Microorganisms and enzymes used in the biological pretreatment of the substrate to enhance biogas production: A review. *Sustainability* **2020**, *12*, 7205. [CrossRef]
32. Nwokolo, N.L.; Enebe, M.C. An insight on the contributions of microbial communities and process parameters in enhancing biogas production. *Biomass Convers. Biorefinery* **2022**, 1–17. [CrossRef]
33. Zhang, J.; Guo, R.-B.; Qiu, Y.-L.; Qiao, J.-T.; Yuan, X.-Z.; Shi, X.-S.; Wang, C.-S. Bioaugmentation with an acetate-type fermentation bacterium Acetobacteroides hydrogenigenes improves methane production from corn straw. *Bioresour. Technol.* **2015**, *179*, 306–313. [CrossRef] [PubMed]
34. Tale, V.P.; Maki, J.; Zitomer, D. Bioaugmentation of overloaded anaerobic digesters restores function and archaeal community. *Water Res.* **2015**, *70*, 138–147. [CrossRef] [PubMed]
35. Čater, M.; Fanedl, L.; Malovrh, Š.; Logar, R.M. Biogas production from brewery spent grain enhanced by bioaugmentation with hydrolytic anaerobic bacteria. *Bioresour. Technol.* **2015**, *186*, 261–269. [CrossRef] [PubMed]
36. Kovács, K.L.; Ács, N.; Kovács, E.; Wirth, R.; Rákhely, G.; Strang, O.; Herbel, Z.; Bagi, Z. Improvement of biogas production by bioaugmentation. *BioMed Res. Int.* **2013**, *2013*, 482653. [CrossRef]
37. Peng, X.; Börner, R.A.; Nges, I.A.; Liu, J. Impact of bioaugmentation on biochemical methane potential for wheat straw with addition of Clostridium cellulolyticum. *Bioresour. Technol.* **2014**, *152*, 567–571. [CrossRef]
38. Martin-Ryals, A.; Schideman, L.; Li, P.; Wilkinson, H.; Wagner, R. Improving anaerobic digestion of a cellulosic waste via routine bioaugmentation with cellulolytic microorganisms. *Bioresour. Technol.* **2015**, *189*, 62–70. [CrossRef]
39. Ács, N.; Bagi, Z.; Rákhely, G.; Minárovics, J.; Nagy, K.; Kovács, K.L. Bioaugmentation of biogas production by a hydrogen-producing bacterium. *Bioresour. Technol.* **2015**, *186*, 286–293. [CrossRef]
40. Strang, O.; Ács, N.; Wirth, R.; Maróti, G.; Bagi, Z.; Rákhely, G.; Kovács, K.L. Bioaugmentation of the thermophilic anaerobic biodegradation of cellulose and corn stover. *Anaerobe* **2017**, *46*, 104–113. [CrossRef] [PubMed]
41. Tuesorn, S.; Wongwilaiwalin, S.; Champreda, V.; Leethochawalit, M.; Nopharatana, A.; Techkarnjanaruk, S.; Chaiprasert, P. Enhancement of biogas production from swine manure by a lignocellulolytic microbial consortium. *Bioresour. Technol.* **2013**, *144*, 579–586. [CrossRef] [PubMed]
42. FitzGerald, J.A.; Wall, D.M.; Jackson, S.A.; Murphy, J.D.; Dobson, A.D. Trace element supplementation is associated with increases in fermenting bacteria in biogas mono-digestion of grass silage. *Renew. Energy* **2019**, *138*, 980–986. [CrossRef]

43. Aryal, N.; Kvist, T.; Ammam, F.; Pant, D.; Ottosen, L.D. An overview of microbial biogas enrichment. *Bioresour. Technol.* **2018**, *264*, 359–369. [CrossRef] [PubMed]
44. Kazda, M.; Langer, S.; Bengelsdorf, F.R. Fungi open new possibilities for anaerobic fermentation of organic residues. *Energy Sustain. Soc.* **2014**, *4*, 6. [CrossRef]
45. Ghosh, P.; Kumar, M.; Kapoor, R.; Kumar, S.S.; Singh, L.; Vijay, V.; Vijay, V.K.; Kumar, V.; Thakur, I.S. Enhanced biogas production from municipal solid waste via co-digestion with sewage sludge and metabolic pathway analysis. *Bioresour. Technol.* **2020**, *296*, 122275. [CrossRef]
46. Heffernan, J.K.; Lai, C.-Y.; Gonzalez-Garcia, R.A.; Nielsen, L.K.; Guo, J.; Marcellin, E. Biogas upgrading using Clostridium autoethanogenum for value-added products. *Chem. Eng. J.* **2023**, *452*, 138950. [CrossRef]
47. Westerholm, M.; Crauwels, S.; Houtmeyers, S.; Meerbergen, K.; Van Geel, M.; Lievens, B.; Appels, L. Microbial community dynamics linked to enhanced substrate availability and biogas production of electrokinetically pre-treated waste activated sludge. *Bioresour. Technol.* **2016**, *218*, 761–770. [CrossRef]
48. Ozbayram, E.G.; Kleinsteuber, S.; Nikolausz, M. Biotechnological utilization of animal gut microbiota for valorization of lignocellulosic biomass. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 489–508. [CrossRef]
49. Boscaro, M.E.; Marin, D.F.C.; da Silva, D.C.; Maintinguer, S.I. Effect of Fe3O4 nanoparticles on microbial diversity and biogas production in anaerobic digestion of crude glycerol. *Biomass Bioenergy* **2022**, *160*, 106439. [CrossRef]
50. Zhang, Y.; Xu, R.; Xiang, Y.; Lu, Y.; Jia, M.; Huang, J.; Xu, Z.; Cao, J.; Xiong, W.; Yang, Z. Addition of nanoparticles increases the abundance of mobile genetic elements and changes microbial community in the sludge anaerobic digestion system. *J. Hazard. Mater.* **2021**, *405*, 124206. [CrossRef]
51. Govarthanan, M.; Manikandan, S.; Subbaiya, R.; Krishnan, R.Y.; Srinivasan, S.; Karmegam, N.; Kim, W. Emerging trends and nanotechnology advances for sustainable biogas production from lignocellulosic waste biomass: A critical review. *Fuel* **2022**, *312*, 122928. [CrossRef]
52. Treu, L.; Kougias, P.G.; Campanaro, S.; Bassani, I.; Angelidaki, I. Deeper insight into the structure of the anaerobic digestion microbial community; the biogas microbiome database is expanded with 157 new genomes. *Bioresour. Technol.* **2016**, *216*, 260–266. [CrossRef] [PubMed]
53. Bareither, C.A.; Wolfe, G.L.; McMahon, K.D.; Benson, C.H. Microbial diversity and dynamics during methane production from municipal solid waste. *Waste Manag.* **2013**, *33*, 1982–1992. [CrossRef]
54. Kröber, M.; Bekel, T.; Diaz, N.N.; Goesmann, A.; Jaenicke, S.; Krause, L.; Miller, D.; Runte, K.J.; Viehöver, P.; Pühler, A. Phylogenetic characterization of a biogas plant microbial community integrating clone library 16S-rDNA sequences and metagenome sequence data obtained by 454-pyrosequencing. *J. Biotechnol.* **2009**, *142*, 38–49. [CrossRef] [PubMed]
55. Schlüter, A.; Bekel, T.; Diaz, N.N.; Dondrup, M.; Eichenlaub, R.; Gartemann, K.-H.; Krahn, I.; Krause, L.; Krömeke, H.; Kruse, O. The metagenome of a biogas-producing microbial community of a production-scale biogas plant fermenter analysed by the 454-pyrosequencing technology. *J. Biotechnol.* **2008**, *136*, 77–90. [CrossRef] [PubMed]
56. De Francisci, D.; Kougias, P.G.; Treu, L.; Campanaro, S.; Angelidaki, I. Microbial diversity and dynamicity of biogas reactors due to radical changes of feedstock composition. *Bioresour. Technol.* **2015**, *176*, 56–64. [CrossRef]
57. Luo, G.; De Francisci, D.; Kougias, P.G.; Laura, T.; Zhu, X.; Angelidaki, I. New steady-state microbial community compositions and process performances in biogas reactors induced by temperature disturbances. *Biotechnol. Biofuels* **2015**, *8*, 3. [CrossRef] [PubMed]
58. Kang, H.-J.; Lee, S.-H.; Lim, T.-G.; Park, H.-D. Effect of microbial community structure in inoculum on the stimulation of direct interspecies electron transfer for methanogenesis. *Bioresour. Technol.* **2021**, *332*, 125100. [CrossRef]
59. Patil, S.M.; Kurade, M.B.; Basak, B.; Saha, S.; Jang, M.; Kim, S.-H.; Jeon, B.-H. Anaerobic co-digester microbiome during food waste valorization reveals Methanosaeta mediated methanogenesis with improved carbohydrate and lipid metabolism. *Bioresour. Technol.* **2021**, *332*, 125123. [CrossRef]
60. Mazurkiewicz, J. Energy and economic balance between manure stored and used as a substrate for biogas production. *Energies* **2022**, *15*, 413. [CrossRef]
61. Lv, Z.; Chen, Z.; Chen, X.; Liang, J.; Jiang, J.; Loake, G.J. Effects of various feedstocks on isotope fractionation of biogas and microbial community structure during anaerobic digestion. *Waste Manag.* **2019**, *84*, 211–219. [CrossRef] [PubMed]
62. Kim, M.; Li, D.; Choi, O.; Sang, B.-I.; Chiang, P.C.; Kim, H. Effects of supplement additives on anaerobic biogas production. *Korean J. Chem. Eng.* **2017**, *34*, 2678–2685. [CrossRef]
63. Leca, E.; Zennaro, B.; Hamelin, J.; Carrère, H.; Sambusiti, C. Use of additives to improve collective biogas plant performances: A comprehensive review. *Biotechnol. Adv.* **2023**, *65*, 108129. [CrossRef]
64. Yan, M.; Treu, L.; Campanaro, S.; Tian, H.; Zhu, X.; Khoshnevisan, B.; Tsapekos, P.; Angelidaki, I.; Fotidis, I.A. Effect of ammonia on anaerobic digestion of municipal solid waste: Inhibitory performance, bioaugmentation and microbiome functional reconstruction. *Chem. Eng. J.* **2020**, *401*, 126159. [CrossRef]
65. Aydin, S. Enhancement of microbial diversity and methane yield by bacterial bioaugmentation through the anaerobic digestion of Haematococcus pluvialis. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 5631–5637. [CrossRef] [PubMed]
66. Nkemka, V.N.; Gilroyed, B.; Yanke, J.; Gruninger, R.; Vedres, D.; McAllister, T.; Hao, X. Bioaugmentation with an anaerobic fungus in a two-stage process for biohydrogen and biogas production using corn silage and cattail. *Bioresour. Technol.* **2015**, *185*, 79–88. [CrossRef] [PubMed]

67. Fonoll, X.; Shrestha, S.; Khanal, S.K.; Dosta, J.; Mata-Alvarez, J.; Raskin, L. Understanding the anaerobic digestibility of lignocellulosic substrates using rumen content as a cosubstrate and an inoculum. *ACS EST Eng.* **2021**, *1*, 424–435. [CrossRef]
68. Dang, Y.; Holmes, D.E.; Zhao, Z.; Woodard, T.L.; Zhang, Y.; Sun, D.; Wang, L.-Y.; Nevin, K.P.; Lovley, D.R. Enhancing anaerobic digestion of complex organic waste with carbon-based conductive materials. *Bioresour. Technol.* **2016**, *220*, 516–522. [CrossRef]
69. Wang, H.; Fotidis, I.A.; Angelidaki, I. Ammonia effect on hydrogenotrophic methanogens and syntrophic acetate-oxidizing bacteria. *FEMS Microbiol. Ecol.* **2015**, *91*, fiv130. [CrossRef]
70. Westerholm, M.; Müller, B.; Singh, A.; Karlsson Lindsjö, O.; Schnürer, A. Detection of novel syntrophic acetate-oxidizing bacteria from biogas processes by continuous acetate enrichment approaches. *Microb. Biotechnol.* **2018**, *11*, 680–693. [CrossRef]
71. Xu, N.; Liu, S.; Xin, F.; Zhou, J.; Jia, H.; Xu, J.; Jiang, M.; Dong, W. Biomethane production from lignocellulose: Biomass recalcitrance and its impacts on anaerobic digestion. *Front. Bioeng. Biotechnol.* **2019**, *7*, 191. [CrossRef] [PubMed]
72. Li, Y.; Zhang, R.; Chen, C.; Liu, G.; He, Y.; Liu, X. Biogas production from co-digestion of corn stover and chicken manure under anaerobic wet, hemi-solid, and solid state conditions. *Bioresour. Technol.* **2013**, *149*, 406–412. [CrossRef] [PubMed]
73. Triolo, J.M.; Pedersen, L.; Qu, H.; Sommer, S.G. Biochemical methane potential and anaerobic biodegradability of non-herbaceous and herbaceous phytomass in biogas production. *Bioresour. Technol.* **2012**, *125*, 226–232. [CrossRef] [PubMed]
74. Xu, H.; Li, Y.; Hua, D.; Mu, H.; Zhao, Y.; Chen, G. Methane production from the anaerobic digestion of substrates from corn stover: Differences between the stem bark, stem pith, and leaves. *Sci. Total Environ.* **2019**, *694*, 133641. [CrossRef] [PubMed]
75. Rasit, N.; Idris, A.; Harun, R.; Ghani, W.A.W.A.K. Effects of lipid inhibition on biogas production of anaerobic digestion from oily effluents and sludges: An overview. *Renew. Sustain. Energy Rev.* **2015**, *45*, 351–358. [CrossRef]
76. Kalamaras, S.D.; Vasileiadis, S.; Karas, P.; Angelidaki, I.; Kotsopoulos, T.A. Microbial adaptation to high ammonia concentrations during anaerobic digestion of manure-based feedstock: Biomethanation and 16S rRNA gene sequencing. *J. Chem. Technol. Biotechnol.* **2020**, *95*, 1970–1979. [CrossRef]
77. Westerholm, M.; Moestedt, J.; Schnürer, A. Biogas production through syntrophic acetate oxidation and deliberate operating strategies for improved digester performance. *Appl. Energy* **2016**, *179*, 124–135. [CrossRef]
78. Shaibur, M.R.; Husain, H.; Arpon, S.H. Utilization of cow dung residues of biogas plant for sustainable development of a rural community. *Curr. Res. Environ. Sustain.* **2021**, *3*, 100026. [CrossRef]
79. Siddiki, S.Y.A.; Uddin, M.; Mofijur, M.; Fattah, I.; Ong, H.C.; Lam, S.S.; Kumar, P.S.; Ahmed, S. Theoretical calculation of biogas production and greenhouse gas emission reduction potential of livestock, poultry and slaughterhouse waste in Bangladesh. *J. Environ. Chem. Eng.* **2021**, *9*, 105204. [CrossRef]
80. Ning, J.; Zhou, M.; Pan, X.; Li, C.; Lv, N.; Wang, T.; Cai, G.; Wang, R.; Li, J.; Zhu, G. Simultaneous biogas and biogas slurry production from co-digestion of pig manure and corn straw: Performance optimization and microbial community shift. *Bioresour. Technol.* **2019**, *282*, 37–47. [CrossRef]
81. Orlando, M.-Q.; Borja, V.-M. Pretreatment of animal manure biomass to improve biogas production: A review. *Energies* **2020**, *13*, 3573. [CrossRef]
82. Tian, H.; Fotidis, I.A.; Mancini, E.; Treu, L.; Mahdy, A.; Ballesteros, M.; González-Fernández, C.; Angelidaki, I. Acclimation to extremely high ammonia levels in continuous biomethanation process and the associated microbial community dynamics. *Bioresour. Technol.* **2018**, *247*, 616–623. [CrossRef] [PubMed]
83. Zhang, T.; Liu, L.; Song, Z.; Ren, G.; Feng, Y.; Han, X.; Yang, G. Biogas production by co-digestion of goat manure with three crop residues. *PLoS ONE* **2013**, *8*, e66845. [CrossRef] [PubMed]
84. Kaur, M.; Verma, Y.P.; Chauhan, S. Effect of chemical pretreatment of sugarcane bagasse on biogas production. *Mater. Today Proc.* **2020**, *21*, 1937–1942. [CrossRef]
85. Buivydas, E.; Navickas, K.; Venslauskas, K.; Žalys, B.; Župerka, V.; Rubežius, M. Biogas Production Enhancement through Chicken Manure Co-Digestion with Pig Fat. *Appl. Sci.* **2022**, *12*, 4652. [CrossRef]
86. Orfanoudaki, A.; Makridakis, G.; Maragkaki, A.; Fountoulakis, M.; Kallithrakas-Kontos, N.; Manios, T. Anaerobic co-digestion of pig manure and spent coffee grounds for enhanced biogas production. *Waste Biomass Valorization* **2020**, *11*, 4613–4620. [CrossRef]
87. Adelodun, A.A.; Olajire, T.M.; Ojo, O.M. Biogas Generation from Co-Digestion Waste Systems: The Role of Water Hyacinth. In *Sustainable Rural Development Perspective and Global Challenges*; IntechOpen: London, UK, 2022.
88. Song, Z.; Zhang, C. Anaerobic codigestion of pretreated wheat straw with cattle manure and analysis of the microbial community. *Bioresour. Technol.* **2015**, *186*, 128–135. [CrossRef]
89. Almomani, F.; Bhosale, R.R. Enhancing the production of biogas through anaerobic co-digestion of agricultural waste and chemical pre-treatments. *Chemosphere* **2020**, *255*, 126805.
90. Angelidaki, I.; Ahring, B.K. Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure. *Water Sci. Technol.* **2000**, *41*, 189–194. [CrossRef]
91. Liu, M.; Ni, H.; Yang, L.; Chen, G.; Yan, X.; Leng, X.; Liu, P.; Li, X. Pretreatment of swine manure containing β-lactam antibiotics with whole-cell biocatalyst to improve biogas production. *J. Clean. Prod.* **2019**, *240*, 118070. [CrossRef]
92. Costa, J.; Barbosa, S.; Alves, M.; Sousa, D. Thermochemical pre-and biological co-treatments to improve hydrolysis and methane production from poultry litter. *Bioresour. Technol.* **2012**, *111*, 141–147. [CrossRef] [PubMed]
93. Patinvoh, R.J.; Feuk-Lagerstedt, E.; Lundin, M.; Sárvári Horváth, I.; Taherzadeh, M.J. Biological pretreatment of chicken feather and biogas production from total broth. *Appl. Biochem. Biotechnol.* **2016**, *180*, 1401–1415. [CrossRef] [PubMed]

94. Rouches, E.; Zhou, S.; Steyer, J.-P.; Carrère, H. White-Rot Fungi pretreatment of lignocellulosic biomass for anaerobic digestion: Impact of glucose supplementation. *Process Biochem.* **2016**, *51*, 1784–1792. [CrossRef]
95. Millati, R.; Syamsiah, S.; Niklasson, C.; Cahyanto, M.N.; Ludquist, K.; Taherzadeh, M.J. Biological pretreatment of lignocelluloses with white-rot fungi and its applications: A review. *BioResources* **2011**, *6*, 5224–5259.
96. Christy, P.M.; Gopinath, L.; Divya, D. A review on anaerobic decomposition and enhancement of biogas production through enzymes and microorganisms. *Renew. Sustain. Energy Rev.* **2014**, *34*, 167–173.
97. Mustafa, A.M.; Poulsen, T.G.; Sheng, K. Fungal pretreatment of rice straw with *Pleurotus ostreatus* and Trichoderma reesei to enhance methane production under solid-state anaerobic digestion. *Appl. Energy* **2016**, *180*, 661–671.
98. Yadav, M.; Vivekanand, V. Combined fungal and bacterial pretreatment of wheat and pearl millet straw for biogas production–A study from batch to continuous stirred tank reactors. *Bioresour. Technol.* **2021**, *321*, 124523.
99. Lalak, J.; Kasprzycka, A.; Martyniak, D.; Tys, J. Effect of biological pretreatment of Agropyron elongatum 'BAMAR' on biogas production by anaerobic digestion. *Bioresour. Technol.* **2016**, *200*, 194–200.
100. Shah, T.A.; Lee, C.C.; Orts, W.J.; Tabassum, R. Biological pretreatment of rice straw by ligninolytic Bacillus sp. strains for enhancing biogas production. *Environ. Prog. Sustain. Energy* **2019**, *38*, e13036.
101. Ali, S.S.; Sun, J. Physico-chemical pretreatment and fungal biotreatment for park wastes and cattle dung for biogas production. *SpringerPlus* **2015**, *4*, 712.
102. Amirta, R.; Tanabe, T.; Watanabe, T.; Honda, Y.; Kuwahara, M.; Watanabe, T. Methane fermentation of Japanese cedar wood pretreated with a white rot fungus, *Ceriporiopsis subvermispora*. *J. Biotechnol.* **2006**, *123*, 71–77. [PubMed]
103. Ge, X.; Matsumoto, T.; Keith, L.; Li, Y. Fungal pretreatment of albizia chips for enhanced biogas production by solid-state anaerobic digestion. *Energy Fuels* **2015**, *29*, 200–204. [CrossRef]
104. Liu, X.; Hiligsmann, S.; Gourdon, R.; Bayard, R. Anaerobic digestion of lignocellulosic biomasses pretreated with *Ceriporiopsis subvermispora*. *J. Environ. Manag.* **2017**, *193*, 154–162. [CrossRef]
105. Liu, S.; Li, X.; Wu, S.; He, J.; Pang, C.; Deng, Y.; Dong, R. Fungal pretreatment by *Phanerochaete chrysosporium* for enhancement of biogas production from corn stover silage. *Appl. Biochem. Biotechnol.* **2014**, *174*, 1907–1918.
106. Phutela, U.G.; Sahni, N. Effect of *Fusarium* sp. on paddy straw digestibility and biogas production. *J. Adv. Lab. Res. Biol.* **2012**, *3*, 9–12.
107. Zhao, J.; Zheng, Y.; Li, Y. Fungal pretreatment of yard trimmings for enhancement of methane yield from solid-state anaerobic digestion. *Bioresour. Technol.* **2014**, *156*, 176–181.
108. Muthangya, M.; Manoni Mshandete, A.; Kajumulo Kivaisi, A. Two-stage fungal pre-treatment for improved biogas production from sisal leaf decortication residues. *Int. J. Mol. Sci.* **2009**, *10*, 4805–4815. [CrossRef] [PubMed]
109. Mutschlechner, M.; Illmer, P.; Wagner, A.O. Biological pre-treatment: Enhancing biogas production using the highly cellulolytic fungus Trichoderma viride. *Waste Manag.* **2015**, *43*, 98–107. [PubMed]
110. Venturin, B.; Bonatto, C.; Damaceno, F.M.; Mulinari, J.; Fongaro, G.; Treichel, H. Physical, chemical, and biological substrate pretreatments to enhance biogas yield. In *Improving Biogas Production: Technological Challenges, Alternative Sources, Future Developments*; Springer: Berlin/Heidelberg, Germany, 2019; pp. 25–44.
111. Mustafa, A.M.; Poulsen, T.G.; Xia, Y.; Sheng, K. Combinations of fungal and milling pretreatments for enhancing rice straw biogas production during solid-state anaerobic digestion. *Bioresour. Technol.* **2017**, *224*, 174–182.
112. Choudhury, S.P.; Panda, S.; Haq, I.; Kalamdhad, A.S. Microbial pretreatment using Kosakonia oryziphila IH3 to enhance biogas production and hydrocarbon depletion from petroleum refinery sludge. *Renew. Energy* **2022**, *194*, 1192–1203.
113. Shah, T.; Ullah, R. Pretreatment of wheat straw with ligninolytic fungi for increased biogas productivity. *Int. J. Environ. Sci. Technol.* **2019**, *16*, 7497–7508.
114. Ali, S.S.; Abomohra, A.E.-F.; Sun, J. Effective bio-pretreatment of sawdust waste with a novel microbial consortium for enhanced biomethanation. *Bioresour. Technol.* **2017**, *238*, 425–432. [CrossRef] [PubMed]
115. Phutela, U.G.; Dar, R.A. Role of lignocellulolytic thermophilic fungus *Thermoascus aurantiacus* MTCC 375 in paddy straw digestibility and its implication in biogas production. *Afr. J. Microbiol. Res.* **2014**, *8*, 1798–1802.
116. Zhao, J. *Enhancement of Methane Production from Solid-State Anaerobic Digestion of Yard Trimmings by Biological Pretreatment*; The Ohio State University: Columbus, OH, USA, 2013.
117. Yuan, X.; Ma, L.; Wen, B.; Zhou, D.; Kuang, M.; Yang, W.; Cui, Z. Enhancing anaerobic digestion of cotton stalk by pretreatment with a microbial consortium (MC1). *Bioresour. Technol.* **2016**, *207*, 293–301. [CrossRef]
118. Wen, B.; Yuan, X.; Li, Q.X.; Liu, J.; Ren, J.; Wang, X.; Cui, Z. Comparison and evaluation of concurrent saccharification and anaerobic digestion of Napier grass after pretreatment by three microbial consortia. *Bioresour. Technol.* **2015**, *175*, 102–111. [CrossRef]
119. Poszytek, K.; Ciezkowska, M.; Sklodowska, A.; Drewniak, L. Microbial consortium with high cellulolytic activity (MCHCA) for enhanced biogas production. *Front. Microbiol.* **2016**, *7*, 324.
120. Alexandropoulou, M.; Antonopoulou, G.; Ntaikou, I.; Lyberatos, G. Fungal pretreatment of willow sawdust with Abortiporus biennis for anaerobic digestion: Impact of an external nitrogen source. *Sustainability* **2017**, *9*, 130. [CrossRef]
121. Alexandropoulou, M.; Antonopoulou, G.; Fragkou, E.; Ntaikou, I.; Lyberatos, G. Fungal pretreatment of willow sawdust and its combination with alkaline treatment for enhancing biogas production. *J. Environ. Manag.* **2017**, *203*, 704–713. [CrossRef]

122. Cai, J.; Mo, X.; Cheng, G.; Du, D. Pretreatment of piggery wastewater by a stable constructed microbial consortium for improving the methane production. *Water Sci. Technol.* **2015**, *71*, 769–775. [CrossRef]
123. Meng, Y.; Luan, F.; Yuan, H.; Chen, X.; Li, X. Enhancing anaerobic digestion performance of crude lipid in food waste by enzymatic pretreatment. *Bioresour. Technol.* **2017**, *224*, 48–55.
124. Domingues, R.D.F.; Sanches, T.; Silva, G.D.S.; Bueno, B.E.; Ribeiro, R.; Kamimura, E.S.; Neto, R.F.; Tommaso, G. Effect of enzymatic pretreatment on the anaerobic digestion of milk fat for biogas production. *Food Res. Int.* **2015**, *73*, 26–30.
125. Karray, R.; Hamza, M.; Sayadi, S. Evaluation of ultrasonic, acid, thermo-alkaline and enzymatic pre-treatments on anaerobic digestion of Ulva rigida for biogas production. *Bioresour. Technol.* **2015**, *187*, 205–213.
126. Su, H.; Tan, F.; Xu, Y. Enhancement of biogas and methanization of citrus waste via biodegradation pretreatment and subsequent optimized fermentation. *Fuel* **2016**, *181*, 843–851.
127. Yu, S.; Zhang, G.; Li, J.; Zhao, Z.; Kang, X. Effect of endogenous hydrolytic enzymes pretreatment on the anaerobic digestion of sludge. *Bioresour. Technol.* **2013**, *146*, 758–761. [PubMed]
128. Kavitha, S.; Jayashree, C.; Kumar, S.A.; Yeom, I.T.; Banu, J.R. The enhancement of anaerobic biodegradability of waste activated sludge by surfactant mediated biological pretreatment. *Bioresour. Technol.* **2014**, *168*, 159–166. [CrossRef]
129. Ge, S.; Liu, L.; Xue, Q.; Yuan, Z. Effects of exogenous aerobic bacteria on methane production and biodegradation of municipal solid waste in bioreactors. *Waste Manag.* **2016**, *55*, 93–98. [PubMed]
130. Merrylin, J.; Kumar, S.A.; Kaliappan, S.; Yeom, I.-T.; Banu, J.R. Biological pretreatment of non-flocculated sludge augments the biogas production in the anaerobic digestion of the pretreated waste activated sludge. *Environ. Technol.* **2013**, *34*, 2113–2123.
131. Bagher, A.M.; Fatemeh, G.; Saman, M.; Leili, M. Advantages and disadvantages of biogas energy. *Bull. Adv. Sci. Res.* **2015**, *1*, 132–135.
132. Khayal, O. Advantages and Limitations of Biogas Technologies. Preprint. Available online: https://www.researchgate.net/publication/336414255_ADVANTAGES_AND_LIMITATIONS_OF_BIOGAS_TECHNOLOGIES2019 (accessed on 13 August 2023).
133. Tabatabaei, M.; Aghbashlo, M.; Valijanian, E.; Panahi, H.K.S.; Nizami, A.-S.; Ghanavati, H.; Sulaiman, A.; Mirmohamadsadeghi, S.; Karimi, K. A comprehensive review on recent biological innovations to improve biogas production, part 1: Upstream strategies. *Renew. Energy* **2020**, *146*, 1204–1220.
134. Hagos, K.; Zong, J.; Li, D.; Liu, C.; Lu, X. Anaerobic co-digestion process for biogas production: Progress, challenges and perspectives. *Renew. Sustain. Energy Rev.* **2017**, *76*, 1485–1496.
135. Nielsen, H.B.; Angelidaki, I. Codigestion of manure and industrial organic waste at centralized biogas plants: Process imbalances and limitations. *Water Sci. Technol.* **2008**, *58*, 1521–1528. [CrossRef]
136. Donkor, K.O.; Gottumukkala, L.D.; Lin, R.; Murphy, J.D. A perspective on the combination of alkali pre-treatment with bioaugmentation to improve biogas production from lignocellulose biomass. *Bioresour. Technol.* **2022**, *351*, 126950.
137. Xue, S.; Wang, Y.; Lyu, X.; Zhao, N.; Song, J.; Wang, X.; Yang, G. Interactive effects of carbohydrate, lipid, protein composition and carbon/nitrogen ratio on biogas production of different food wastes. *Bioresour. Technol.* **2020**, *312*, 123566.
138. Sivamani, S.; Saikat, B.; Naveen Prasad, B.; Baalawy, A.A.S.; Al-Mashali, S.M.A. A comprehensive review on microbial technology for biogas production. In *Bioenergy Research: Revisiting Latest Development*; Springer: Berlin/Heidelberg, Germany, 2021; pp. 53–78.
139. Ma, J.; Frear, C.; Wang, Z.-w.; Yu, L.; Zhao, Q.; Li, X.; Chen, S. A simple methodology for rate-limiting step determination for anaerobic digestion of complex substrates and effect of microbial community ratio. *Bioresour. Technol.* **2013**, *134*, 391–395.
140. Kanokratana, P.; Wongwilaiwalin, S.; Mhuantong, W.; Tangphatsornruang, S.; Eurwilaichitr, L.; Champreda, V. Characterization of cellulolytic microbial consortium enriched on Napier grass using metagenomic approaches. *J. Biosci. Bioeng.* **2018**, *125*, 439–447.
141. Sarker, S.; Lamb, J.J.; Hjelme, D.R.; Lien, K.M. A review of the role of critical parameters in the design and operation of biogas production plants. *Appl. Sci.* **2019**, *9*, 1915.
142. Ozbayram, E.G.; Ince, O.; Ince, B.; Harms, H.; Kleinsteuber, S. Comparison of rumen and manure microbiomes and implications for the inoculation of anaerobic digesters. *Microorganisms* **2018**, *6*, 15. [CrossRef] [PubMed]
143. Razaviarani, V.; Buchanan, I.D. Anaerobic co-digestion of biodiesel waste glycerin with municipal wastewater sludge: Microbial community structure dynamics and reactor performance. *Bioresour. Technol.* **2015**, *182*, 8–17. [CrossRef] [PubMed]
144. Reichlen, M.J.; Vepachedu, V.R.; Murakami, K.S.; Ferry, J.G. MreA functions in the global regulation of methanogenic pathways in *Methanosarcina acetivorans*. *MBio* **2012**, *3*, e00189-12. [CrossRef] [PubMed]
145. Lendormi, T.; Jaziri, K.; Béline, F.; Le Roux, S.; Bureau, C.; Midoux, C.; Barrington, S.; Dabert, P. Methane production and microbial community acclimation of five manure inocula during psychrophilic anaerobic digestion of swine manure. *J. Clean. Prod.* **2022**, *340*, 130772.
146. Venkiteshwaran, K.; Bocher, B.; Maki, J.; Zitomer, D. Relating anaerobic digestion microbial community and process function: Supplementary issue: Water microbiology. *Microbiol. Insights* **2015**, *8*, 37–44. [CrossRef]
147. Frank, J.; Arntzen, M.Ø.; Sun, L.; Hagen, L.H.; McHardy, A.; Horn, S.J.; Eijsink, V.G.; Schnürer, A.; Pope, P.B. Novel syntrophic populations dominate an ammonia-tolerant methanogenic microbiome. *Msystems* **2016**, *1*, e00092-16. [CrossRef] [PubMed]
148. Yang, P.; Tan, G.-Y.A.; Aslam, M.; Kim, J.; Lee, P.-H. Metatranscriptomic evidence for classical and RuBisCO-mediated CO_2 reduction to methane facilitated by direct interspecies electron transfer in a methanogenic system. *Sci. Rep.* **2019**, *9*, 4116. [CrossRef]

149. Zarraonaindia, I.; Smith, D.P.; Gilbert, J.A. Beyond the genome: Community-level analysis of the microbial world. *Biol. Philos.* **2013**, *28*, 261–282.
150. Su, C.; Lei, L.; Duan, Y.; Zhang, K.-Q.; Yang, J. Culture-independent methods for studying environmental microorganisms: Methods, application, and perspective. *Appl. Microbiol. Biotechnol.* **2012**, *93*, 993–1003.
151. Amani, T.; Nosrati, M.; Sreekrishnan, T. Anaerobic digestion from the viewpoint of microbiological, chemical, and operational aspects—A review. *Environ. Rev.* **2010**, *18*, 255–278.
152. Ito, T.; Yoshiguchi, K.; Ariesyady, H.D.; Okabe, S. Identification and quantification of key microbial trophic groups of methanogenic glucose degradation in an anaerobic digester sludge. *Bioresour. Technol.* **2012**, *123*, 599–607.
153. Pervin, H.M.; Dennis, P.G.; Lim, H.J.; Tyson, G.W.; Batstone, D.J.; Bond, P.L. Drivers of microbial community composition in mesophilic and thermophilic temperature-phased anaerobic digestion pre-treatment reactors. *Water Res.* **2013**, *47*, 7098–7108.
154. Ho, D.P.; Jensen, P.D.; Batstone, D.J. Methanosarcinaceae and acetate-oxidizing pathways dominate in high-rate thermophilic anaerobic digestion of waste-activated sludge. *Appl. Environ. Microbiol.* **2013**, *79*, 6491–6500. [CrossRef] [PubMed]
155. Nelson, M.C.; Morrison, M.; Yu, Z. A meta-analysis of the microbial diversity observed in anaerobic digesters. *Bioresour. Technol.* **2011**, *102*, 3730–3739. [PubMed]
156. Sundberg, C.; Al-Soud, W.A.; Larsson, M.; Alm, E.; Yekta, S.S.; Svensson, B.H.; Sørensen, S.J.; Karlsson, A. 454 pyrosequencing analyses of bacterial and archaeal richness in 21 full-scale biogas digesters. *FEMS Microbiol. Ecol.* **2013**, *85*, 612–626. [PubMed]
157. Vanwonterghem, I.; Jensen, P.D.; Ho, D.P.; Batstone, D.J.; Tyson, G.W. Linking microbial community structure, interactions and function in anaerobic digesters using new molecular techniques. *Curr. Opin. Biotechnol.* **2014**, *27*, 55–64.
158. Stolze, Y.; Bremges, A.; Rumming, M.; Henke, C.; Maus, I.; Pühler, A.; Sczyrba, A.; Schlüter, A. Identification and genome reconstruction of abundant distinct taxa in microbiomes from one thermophilic and three mesophilic production-scale biogas plants. *Biotechnol. Biofuels* **2016**, *9*, 156. [PubMed]
159. Fakruddin, M.; Mannan, K. Methods for analyzing diversity of microbial communities in natural environments. *Ceylon J. Sci.* **2013**, *42*, 19–33. [CrossRef]
160. Yannarell, A.C.; Triplett, E.W. Geographic and environmental sources of variation in lake bacterial community composition. *Appl. Environ. Microbiol.* **2005**, *71*, 227–239. [CrossRef]
161. Churko, J.M.; Mantalas, G.L.; Snyder, M.P.; Wu, J.C. Overview of high throughput sequencing technologies to elucidate molecular pathways in cardiovascular diseases. *Circ. Res.* **2013**, *112*, 1613–1623. [CrossRef]
162. Ziganshin, A.M.; Liebetrau, J.; Pröter, J.; Kleinsteuber, S. Microbial community structure and dynamics during anaerobic digestion of various agricultural waste materials. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 5161–5174.
163. Zhang, H.; Banaszak, J.E.; Parameswaran, P.; Alder, J.; Krajmalnik-Brown, R.; Rittmann, B.E. Focused-Pulsed sludge pre-treatment increases the bacterial diversity and relative abundance of acetoclastic methanogens in a full-scale anaerobic digester. *Water Res.* **2009**, *43*, 4517–4526. [CrossRef] [PubMed]
164. Werner, J.J.; Knights, D.; Garcia, M.L.; Scalfone, N.B.; Smith, S.; Yarasheski, K.; Cummings, T.A.; Beers, A.R.; Knight, R.; Angenent, L.T. Bacterial community structures are unique and resilient in full-scale bioenergy systems. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 4158–4163.
165. Lee, S.-H.; Kang, H.-J.; Lee, Y.H.; Lee, T.J.; Han, K.; Choi, Y.; Park, H.-D. Monitoring bacterial community structure and variability in time scale in full-scale anaerobic digesters. *J. Environ. Monit.* **2012**, *14*, 1893–1905. [CrossRef]
166. Ronaghi, M.; Karamohamed, S.; Pettersson, B.; Uhlén, M.; Nyrén, P. Real-time DNA sequencing using detection of pyrophosphate release. *Anal. Biochem.* **1996**, *242*, 84–89. [CrossRef] [PubMed]
167. Ari, Ş.; Arikan, M. Next-generation sequencing: Advantages, disadvantages, and future. In *Plant Omics: Trends and Applications*; Springer: Berlin/Heidelberg, Germany, 2016; pp. 109–135.
168. Li, Z.; Bai, X.; Ruparel, H.; Kim, S.; Turro, N.J.; Ju, J. A photocleavable fluorescent nucleotide for DNA sequencing and analysis. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 414–419. [PubMed]
169. Carballa, M.; Regueiro, L.; Lema, J.M. Microbial management of anaerobic digestion: Exploiting the microbiome-functionality nexus. *Curr. Opin. Biotechnol.* **2015**, *33*, 103–111.
170. Koch, C.; Müller, S.; Harms, H.; Harnisch, F. Microbiomes in bioenergy production: From analysis to management. *Curr. Opin. Biotechnol.* **2014**, *27*, 65–72.
171. Yang, B.; Wang, Y.; Qian, P.-Y. Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. *BMC Bioinform.* **2016**, *17*, 135.
172. Kim, M.; Oh, H.-S.; Park, S.-C.; Chun, J. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int. J. Syst. Evol. Microbiol.* **2014**, *64 Pt 2*, 346–351.
173. Yarza, P.; Yilmaz, P.; Pruesse, E.; Glöckner, F.O.; Ludwig, W.; Schleifer, K.-H.; Whitman, W.B.; Euzéby, J.; Amann, R.; Rosselló-Móra, R. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nat. Rev. Microbiol.* **2014**, *12*, 635–645. [CrossRef]
174. Moyer, C.L.; Dobbs, F.C.; Karl, D.M. Estimation of diversity and community structure through restriction fragment length polymorphism distribution analysis of bacterial 16S rRNA genes from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. *Appl. Environ. Microbiol.* **1994**, *60*, 871–879. [CrossRef]

175. Muyzer, G.; De Waal, E.C.; Uitterlinden, A. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* **1993**, *59*, 695–700. [CrossRef]
176. Cabezas, A.; de Araujo, J.C.; Callejas, C.; Galès, A.; Hamelin, J.; Marone, A.; Sousa, D.Z.; Trably, E.; Etchebehere, C. How to use molecular biology tools for the study of the anaerobic digestion process? *Rev. Environ. Sci. Bio/Technol.* **2015**, *14*, 555–593.
177. Wagner, J.; Coupland, P.; Browne, H.P.; Lawley, T.D.; Francis, S.C.; Parkhill, J. Evaluation of PacBio sequencing for full-length bacterial 16S rRNA gene classification. *BMC Microbiol.* **2016**, *16*, 274. [CrossRef] [PubMed]
178. Hassa, J.; Maus, I.; Off, S.; Pühler, A.; Scherer, P.; Klocke, M.; Schlüter, A. Metagenome, metatranscriptome, and metaproteome approaches unraveled compositions and functional relationships of microbial communities residing in biogas plants. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 5045–5063. [PubMed]
179. Bassani, I.; Kougias, P.G.; Treu, L.; Angelidaki, I. Biogas upgrading via hydrogenotrophic methanogenesis in two-stage continuous stirred tank reactors at mesophilic and thermophilic conditions. *Environ. Sci. Technol.* **2015**, *49*, 12585–12593. [CrossRef]
180. Angly, F.E.; Felts, B.; Breitbart, M.; Salamon, P.; Edwards, R.A.; Carlson, C.; Chan, A.M.; Haynes, M.; Kelley, S.; Liu, H. The marine viromes of four oceanic regions. *PLoS Biol.* **2006**, *4*, e368.
181. Edwards, R.A.; Rodriguez-Brito, B.; Wegley, L.; Haynes, M.; Breitbart, M.; Peterson, D.M.; Saar, M.O.; Alexander, S.; Alexander, E.C.; Rohwer, F. Using pyrosequencing to shed light on deep mine microbial ecology. *BMC Genom.* **2006**, *7*, 57. [CrossRef]
182. Gill, S.R.; Pop, M.; DeBoy, R.T.; Eckburg, P.B.; Turnbaugh, P.J.; Samuel, B.S.; Gordon, J.I.; Relman, D.A.; Fraser-Liggett, C.M.; Nelson, K.E. Metagenomic analysis of the human distal gut microbiome. *Science* **2006**, *312*, 1355–1359.
183. Turnbaugh, P.J.; Ley, R.E.; Mahowald, M.A.; Magrini, V.; Mardis, E.R.; Gordon, J.I. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **2006**, *444*, 1027–1031. [CrossRef]
184. Raes, J.; Foerstner, K.U.; Bork, P. Get the most out of your metagenome: Computational analysis of environmental sequence data. *Curr. Opin. Microbiol.* **2007**, *10*, 490–498. [CrossRef]
185. Krause, L.; Diaz, N.N.; Edwards, R.A.; Gartemann, K.-H.; Krömeke, H.; Neuweger, H.; Pühler, A.; Runte, K.J.; Schlüter, A.; Stoye, J. Taxonomic composition and gene content of a methane-producing microbial community isolated from a biogas reactor. *J. Biotechnol.* **2008**, *136*, 91–101.
186. Bashiardes, S.; Zilberman-Schapira, G.; Elinav, E. Use of metatranscriptomics in microbiome research. *Bioinform. Biol. Insights* **2016**, *10*, BBI-S34610.
187. Wei, Y.; Wu, Y.; Zhang, L.; Zhou, Z.; Zhou, H.; Yan, X. Genome recovery and metatranscriptomic confirmation of functional acetate-oxidizing bacteria from enriched anaerobic biogas digesters. *Environ. Pollut.* **2020**, *265*, 114843. [CrossRef] [PubMed]
188. Stark, L.; Giersch, T.; Wünschiers, R. Efficiency of RNA extraction from selected bacteria in the context of biogas production and metatranscriptomics. *Anaerobe* **2014**, *29*, 85–90. [PubMed]
189. Gilbert, J.A.; Hughes, M. Gene expression profiling: Metatranscriptomics. In *High-Throughput Next Generation Sequencing: Methods and Applications*; Springer: Berlin/Heidelberg, Germany, 2011; pp. 195–205.
190. Jia, Y.; Ng, S.-K.; Lu, H.; Cai, M.; Lee, P.K. Genome-centric metatranscriptomes and ecological roles of the active microbial populations during cellulosic biomass anaerobic digestion. *Biotechnol. Biofuels* **2018**, *11*, 117.
191. Nolla-Ardèvol, V.; Strous, M.; Tegetmeyer, H.E. Anaerobic digestion of the microalga Spirulina at extreme alkaline conditions: Biogas production, metagenome, and metatranscriptome. *Front. Microbiol.* **2015**, *6*, 597. [CrossRef] [PubMed]
192. Fontana, A.; Kougias, P.G.; Treu, L.; Kovalovszki, A.; Valle, G.; Cappa, F.; Morelli, L.; Angelidaki, I.; Campanaro, S. Microbial activity response to hydrogen injection in thermophilic anaerobic digesters revealed by genome-centric metatranscriptomics. *Microbiome* **2018**, *6*, 194. [PubMed]
193. Maus, I.; Koeck, D.E.; Cibis, K.G.; Hahnke, S.; Kim, Y.S.; Langer, T.; Kreubel, J.; Erhard, M.; Bremges, A.; Off, S. Unraveling the microbiome of a thermophilic biogas plant by metagenome and metatranscriptome analysis complemented by characterization of bacterial and archaeal isolates. *Biotechnol. Biofuels* **2016**, *9*, 171.
194. Zakrzewski, M.; Goesmann, A.; Jaenicke, S.; Jünemann, S.; Eikmeyer, F.; Szczepanowski, R.; Al-Soud, W.A.; Sørensen, S.; Pühler, A.; Schlüter, A. Profiling of the metabolically active community from a production-scale biogas plant by means of high-throughput metatranscriptome sequencing. *J. Biotechnol.* **2012**, *158*, 248–258.
195. Güllert, S.; Fischer, M.A.; Turaev, D.; Noebauer, B.; Ilmberger, N.; Wemheuer, B.; Alawi, M.; Rattei, T.; Daniel, R.; Schmitz, R.A. Deep metagenome and metatranscriptome analyses of microbial communities affiliated with an industrial biogas fermenter, a cow rumen, and elephant feces reveal major differences in carbohydrate hydrolysis strategies. *Biotechnol. Biofuels* **2016**, *9*, 121.
196. Dumont, M.G.; Murrell, J.C. Stable isotope probing—Linking microbial identity to function. *Nat. Rev. Microbiol.* **2005**, *3*, 499–504.
197. Deng, Y.; Cui, X.; Dumont, M.G. Identification of active aerobic methanotrophs in plateau wetlands using DNA stable isotope probing. *FEMS Microbiol. Lett.* **2016**, *363*, fnw168.
198. Buckley, D.H.; Huangyutitham, V.; Hsu, S.-F.; Nelson, T.A. Stable isotope probing with 15N2 reveals novel noncultivated diazotrophs in soil. *Appl. Environ. Microbiol.* **2007**, *73*, 3196–3204. [CrossRef] [PubMed]
199. Chen, Y.; Murrell, J.C. When metagenomics meets stable-isotope probing: Progress and perspectives. *Trends Microbiol.* **2010**, *18*, 157–163. [PubMed]
200. Mosbæk, F.; Kjeldal, H.; Mulat, D.G.; Albertsen, M.; Ward, A.J.; Feilberg, A.; Nielsen, J.L. Identification of syntrophic acetate-oxidizing bacteria in anaerobic digesters by combined protein-based stable isotope probing and metagenomics. *ISME J.* **2016**, *10*, 2405–2418.

201. Zheng, D.; Wang, H.-Z.; Gou, M.; Nobu, M.K.; Narihiro, T.; Hu, B.; Nie, Y.; Tang, Y.-Q. Identification of novel potential acetate-oxidizing bacteria in thermophilic methanogenic chemostats by DNA stable isotope probing. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 8631–8645. [PubMed]
202. Wang, H.-Z.; Lv, X.-M.; Yi, Y.; Zheng, D.; Gou, M.; Nie, Y.; Hu, B.; Nobu, M.K.; Narihiro, T.; Tang, Y.-Q. Using DNA-based stable isotope probing to reveal novel propionate-and acetate-oxidizing bacteria in propionate-fed mesophilic anaerobic chemostats. *Sci. Rep.* **2019**, *9*, 17396.
203. Ngo, V.Q.H.; Enault, F.; Midoux, C.; Mariadassou, M.; Chapleur, O.; Mazéas, L.; Loux, V.; Bouchez, T.; Krupovic, M.; Bize, A. Diversity of novel archaeal viruses infecting methanogens discovered through coupling of stable isotope probing and metagenomics. *Environ. Microbiol.* **2022**, *24*, 4853–4868. [CrossRef] [PubMed]
204. Cheng, J. *Biomass to Renewable Energy Processes*; CRC Press: Boca Raton, FL, USA, 2017.
205. Xue, S.; Song, J.; Wang, X.; Shang, Z.; Sheng, C.; Li, C.; Zhu, Y.; Liu, J. A systematic comparison of biogas development and related policies between China and Europe and corresponding insights. *Renew. Sustain. Energy Rev.* **2020**, *117*, 109474.
206. Meyer-Aurich, A.; Schattauer, A.; Hellebrand, H.J.; Klauss, H.; Plöchl, M.; Berg, W. Impact of uncertainties on greenhouse gas mitigation potential of biogas production from agricultural resources. *Renew. Energy* **2012**, *37*, 277–284.
207. Leonard, L. Tackling Climate Change in the Global South: An Analysis of the Global Methane Initiative Multilateral Partnership. *J. Soc. Dev. Sci.* **2014**, *5*, 168–175. [CrossRef]
208. Guilayn, F.; Rouez, M.; Crest, M.; Patureau, D.; Jimenez, J. Valorization of digestates from urban or centralized biogas plants: A critical review. *Rev. Environ. Sci. Bio/Technol.* **2020**, *19*, 419–462.
209. Thema, M.; Bauer, F.; Sterner, M. Power-to-Gas: Electrolysis and methanation status review. *Renew. Sustain. Energy Rev.* **2019**, *112*, 775–787. [CrossRef]
210. Jensen, M.B.; Kofoed, M.V.W.; Fischer, K.; Voigt, N.V.; Agneessens, L.M.; Batstone, D.J.; Ottosen, L.D.M. Venturi-type injection system as a potential H_2 mass transfer technology for full-scale in situ biomethanation. *Appl. Energy* **2018**, *222*, 840–846. [CrossRef]
211. Yang, D.; Park, S.Y.; Park, Y.S.; Eun, H.; Lee, S.Y. Metabolic engineering of Escherichia coli for natural product biosynthesis. *Trends Biotechnol.* **2020**, *38*, 745–765. [CrossRef] [PubMed]
212. Choi, K.R.; Jiao, S.; Lee, S.Y. Metabolic engineering strategies toward production of biofuels. *Curr. Opin. Chem. Biol.* **2020**, *59*, 1–14. [CrossRef] [PubMed]
213. Das, N.; Das, A.; Das, S.; Bhatawadekar, V.; Pandey, P.; Choure, K.; Damare, S.; Pandey, P. Petroleum Hydrocarbon Catabolic Pathways as Targets for Metabolic Engineering Strategies for Enhanced Bioremediation of Crude-Oil-Contaminated Environments. *Fermentation* **2023**, *9*, 196. [CrossRef]
214. Rollin, J.A.; Martin del Campo, J.; Myung, S.; Sun, F.; You, C.; Bakovic, A.; Castro, R.; Chandrayan, S.K.; Wu, C.-H.; Adams, M.W. High-yield hydrogen production from biomass by in vitro metabolic engineering: Mixed sugars coutilization and kinetic modeling. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 4964–4969. [CrossRef]
215. Sorathia, H.S.; Rathod, P.P.; Sorathiya, A.S. Biogas generation and factors affecting the Bio-gas generationea review study'. Int. *J. Adv. Eng. Technol.* **2012**, *3*, e78.
216. Kosaka, F.; Liu, Y.; Chen, S.-Y.; Mochizuki, T.; Takagi, H.; Urakawa, A.; Kuramoto, K. Enhanced activity of integrated CO_2 capture and reduction to CH_4 under pressurized conditions toward atmospheric CO_2 utilization. *ACS Sustain. Chem. Eng.* **2021**, *9*, 3452–3463. [CrossRef]
217. Koytsoumpa, E.I.; Bergins, C.; Kakaras, E. The CO_2 economy: Review of CO_2 capture and reuse technologies. *J. Supercrit. Fluids* **2018**, *132*, 3–16. [CrossRef]
218. dos Santos, R.G.; Alencar, A.C. Biomass-derived syngas production via gasification process and its catalytic conversion into fuels by Fischer Tropsch synthesis: A review. *Int. J. Hydrog. Energy* **2020**, *45*, 18114–18132. [CrossRef]
219. Harahap, B.M.; Ahring, B.K. Acetate Production from Syngas Produced from Lignocellulosic Biomass Materials along with Gaseous Fermentation of the Syngas: A Review. *Microorganisms* **2023**, *11*, 995. [CrossRef] [PubMed]
220. Burkhardt, M.; Busch, G. Methanation of hydrogen and carbon dioxide. *Appl. Energy* **2013**, *111*, 74–79. [CrossRef]
221. Vogt, C.; Monai, M.; Kramer, G.J.; Weckhuysen, B.M. The renaissance of the Sabatier reaction and its applications on Earth and in space. *Nat. Catal.* **2019**, *2*, 188–197. [CrossRef]
222. Zahed, M.A.; Movahed, E.; Khodayari, A.; Zanganeh, S.; Badamaki, M. Biotechnology for carbon capture and fixation: Critical review and future directions. *J. Environ. Manag.* **2021**, *293*, 112830. [CrossRef]
223. Fu, S.; Angelidaki, I.; Zhang, Y. In situ biogas upgrading by CO_2-to-CH_4 bioconversion. *Trends Biotechnol.* **2021**, *39*, 336–347. [CrossRef] [PubMed]
224. Szuhaj, M.; Ács, N.; Tengölics, R.; Bodor, A.; Rákhely, G.; Kovács, K.L.; Bagi, Z. Conversion of H_2 and CO_2 to CH_4 and acetate in fed-batch biogas reactors by mixed biogas community: A novel route for the power-to-gas concept. *Biotechnol. Biofuels* **2016**, *9*, 102. [CrossRef] [PubMed]
225. Zhang, Y.; Angelidaki, I. Microbial electrolysis cells turning to be versatile technology: Recent advances and future challenges. *Water Res.* **2014**, *56*, 11–25. [CrossRef]
226. Mendonça, M. *Feed-in Tariffs: Accelerating the Deployment of Renewable Energy*; Routledge: London, UK, 2009.

227. Bong, C.P.C.; Ho, W.S.; Hashim, H.; Lim, J.S.; Ho, C.S.; Tan, W.S.P.; Lee, C.T. Review on the renewable energy and solid waste management policies towards biogas development in Malaysia. *Renew. Sustain. Energy Rev.* **2017**, *70*, 988–998. [CrossRef]
228. Carrosio, G. Energy production from biogas in the Italian countryside: Policies and organizational models. *Energy Policy* **2013**, *63*, 3–9. [CrossRef]