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Review

A Review of Basic Bioinformatic Techniques for Microbial Community Analysis in an Anaerobic Digester

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Abstract: Biogas production involves various types of intricate microbial populations in an anaerobic digester (AD). To understand the anaerobic digestion system better, a broad-based study must be conducted on the microbial population. Deep understanding of the complete metagenomics including microbial structure, functional gene form, similarity/differences, and relationships between metabolic pathways and product formation, could aid in optimization and enhancement of AD processes. With advancements in technologies for metagenomic sequencing, for example, next generation sequencing and high-throughput sequencing, have revolutionized the study of microbial dynamics in anaerobic digestion. This review includes a brief introduction to the basic process of metagenomics research and includes a detailed summary of the various bioinformatics approaches, viz., total investigation of data obtained from microbial communities using bioinformatics methods to expose metagenomics characterization. This includes (1) methods of DNA isolation and sequencing, (2) investigation of anaerobic microbial communities using bioinformatics techniques, (3) application of the analysis of anaerobic microbial community and biogas production, and (4) restriction and prediction of bioinformatics analysis on microbial metagenomics. The review has been concluded, giving a summarized insight into bioinformatic tools and also promoting the future prospects of integrating humungous data with artificial intelligence and neural network software.

Keywords: microbial metagenomics; anaerobic digestion; bioinformatics techniques; metabolomics

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1. Introduction

With the increase in energy demand and rise in fuel prices in recent years, renewable sources of energy have caught much attention lately. Environmental pollution caused by burning fossil fuels is reduced by extensive use of renewable energy sources. Environment protection and reducing the energy crisis simultaneously could be attained by using and upgrading various feedstocks such as food waste, agricultural residues, solid waste, algae, etc. Feedstock-based anaerobic digesters produce biogas and are an efficient way to overcome the problems related to fossil fuel burning. Biogas containing diverse microbial communities in digesters is critical in maintaining stable activities and the efficient development of methane [1–5]. Figure 1 shows the process and microbial community involved in anaerobic digestion. Biogas engendered from anaerobically digested biological wastes by metabolism of wide-ranging anaerobically active microbes, especially bacteria and methanogenic archaea, is one of the striking energy carriers that is sustainable and

renewable and for which substantial research is the need of the hour. The monitoring methods of standard operational parameters, such as temperature, pH, gas composition, alkalinity and volatile fatty acid (VFA) concentration, has enhanced the bio-methanation process and expanded AD systems at the industrial scale [6–9]. These parameters reveal the present condition of the process, but the exact dynamics, composition, metabolism, or activity of the anaerobic system is still a mystery to be revealed. [10,11].

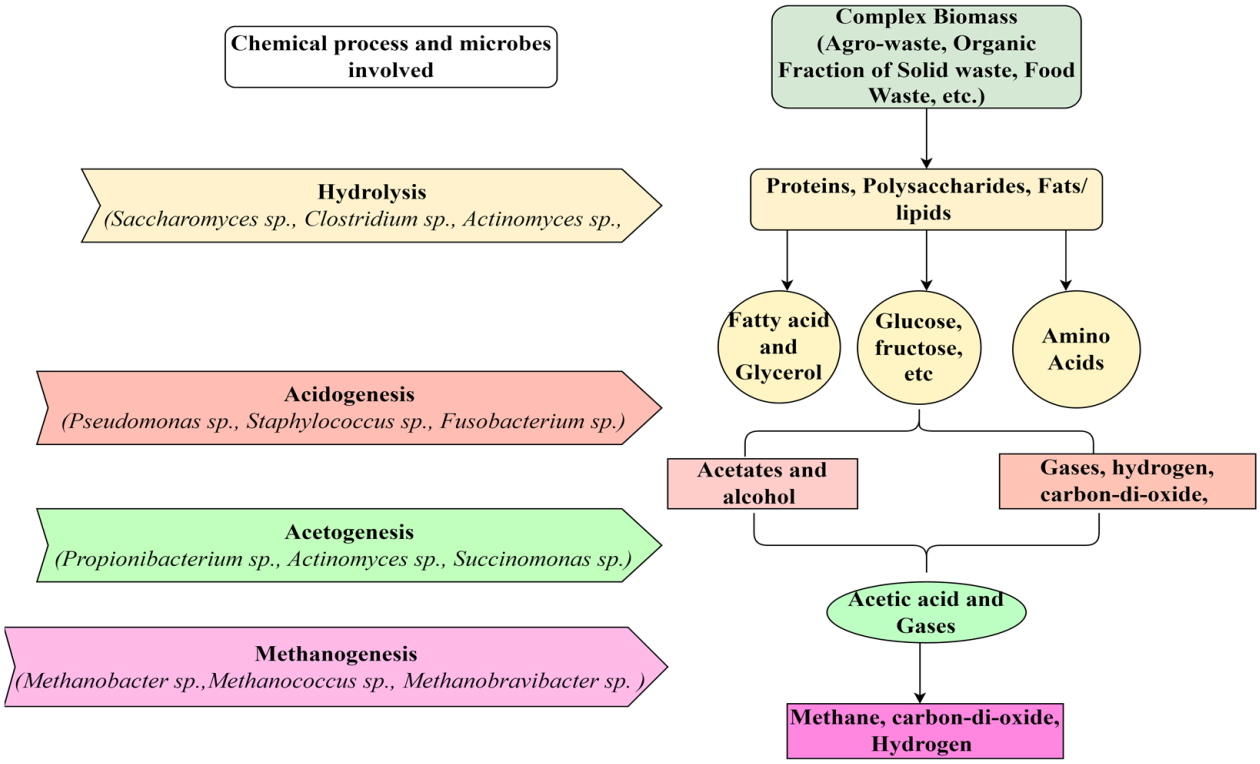

Figure 1. A schematic of the anaerobic digestion process and microbes involved.

Anaerobic digestion is divided into four phases, of which the first three phases, i.e., hydrolysis, acidogenesis, and acetogenesis, are carried out by bacterial populations and the last phase, i.e., methanogenesis, occurs by a specific group of archaea. An AD process is a complex metabolic system where different types of microbial flora interact with each other to give a different kind of end-product at different levels. This kind of interaction is called syntropic interaction with specific anaerobic functionality and has been explored using several molecular biology tools [12]. The functional group genes were targeted by designing a primer for the 16S rRNA gene. Using these primers as a phylogenetic marker, distinct functional group genes were targeted, and analyses were conducted on that basis. By means of molecular techniques it was evident that microbial flora in anaerobic digesters is unexplored, so deeper studies must be carried out using bioinformatics and molecular tools [13]. The efficiency of gas production depends on the steady operation of the anaerobic digester henceforth maintained by the intricate microbial population [1,2]. Methane production can be increased through the strategic enrichment of hydrolytic bacteria, fermentation bacteria, and advantageous methanogens through functional separation at various time points [14]. It is possible to inhibit the development of harmful metabolites and promote the production of chemicals essential for the continuity of anaerobic cell membranes with the help of micro-oxygen, which improves the overall performance of AD [15]. With respect to taxonomic arrangements, interaction systems, metabolic links, species similarity, and diversity, the microbial consortia must be evaluated so that the anaerobic digestion of the organic waste can produce a high amount of biogas. In the last ten years, the advancement of high-performance sequencing technologies and cost-reduction has rendered it possible

to use bioinformatic techniques in aerobic fermentation tanks to analyze metagenomic data in microbial communities. Many techniques from the field of bioinformatics, which are based on statistics, data mining, and artificial intelligence, such as Genovo, MG-RAST, MetaVelvet, etc, [16–18], have been used to enhance knowledge of microbial populations in anaerobic digesters working under different operational systems. Luo et al. reported that anaerobic microbial populations in biogas digesters show a strong link between functional gene form and taxonomic pattern [19]. Zhang J et al. revealed that with the help of functional genes, analysed by network-based techniques and metagenomic studies, many metabolic pathways can be estimated. Similar findings were reported by Ye L et al., who showed the overall microbial metabolic pathways of anaerobes in biogas producing digesters [20,21]. In addition, it has been found that operational parameters such as organic loading rate, type of feedstock, temperature, design of digester, alkalinity, concentration of free ammonia, and hydraulic retention time; influence both functional genes and taxonomic patterns [22–24]. Efficient analysis of the fundamental biological systems of anaerobic populations, viz., bacteria and archaea, in anaerobic bioreactors through wide-ranging sequenced data and also using these data to enhance the production of biomethane, is still a herculean task. Unavailability of accurate gene-sequencing technology, presence of very complex microbial populations in anaerobic tanks, operational errors during analysis of microbial dynamics, disintegrated analysis of microbial population data, and the high cost of metagenomic analysis, make the overall study of microbial communities a major challenge [25,26]. Metagenomic study of microbial populations is not only conducted for anaerobic digesters but also it has been conducted for numerous other habitats such as contaminated sites, sea, soil hydrocarbon contaminated sites, and biohydrogen reactors [27–30]. Many researchers have been investigating microbial populations in anaerobic digesters at different operational parameters. It has been found that although much research is being conducted in the field of metagenomics, the data generated are not consolidated and are very diverse and scattered. Therefore, a suitable review in this domain will help to build a consolidated knowledge. This review has been designed to provide overall information of the bioinformatic tools and approaches used for metagenomic studies of microbial communities in anaerobic digesters. The paper has been divided into various sections. Section 2 summarises the metagenomic studies conducted so far in anaerobic digesters with different kinds of feedstock. This section further presents methods of DNA isolation and DNA sequencing. Figure 2 shows the basic flow chart of the metagenomic analysis process. Zhang et al. [20] reported that metagenomic studies are conducted in terms of operational parameters, country of origin, and type of feedstock on which the anaerobic digester is working. Countries, for example, Singapore, UK, USA, Korea, China, Germany, Denmark, etc., have been working towards metagenomic studies of anaerobic digesters. Most of the microbial community analysis has been conducted on food waste as feedstock, followed by sludge, manure, and horticultural and agricultural residue. Moreover, mesophilic digesters are more explored than thermophilic digesters for microbial population analysis [31]. With high temperature, thermophilic AD is more advantageous than mesophilic as frothing in the digester is reduced and also due to high temperature, degradability of complex polymers is enhanced. Due to this, enhanced biomethane is obtained and organic loading rate is also increased [32–34].

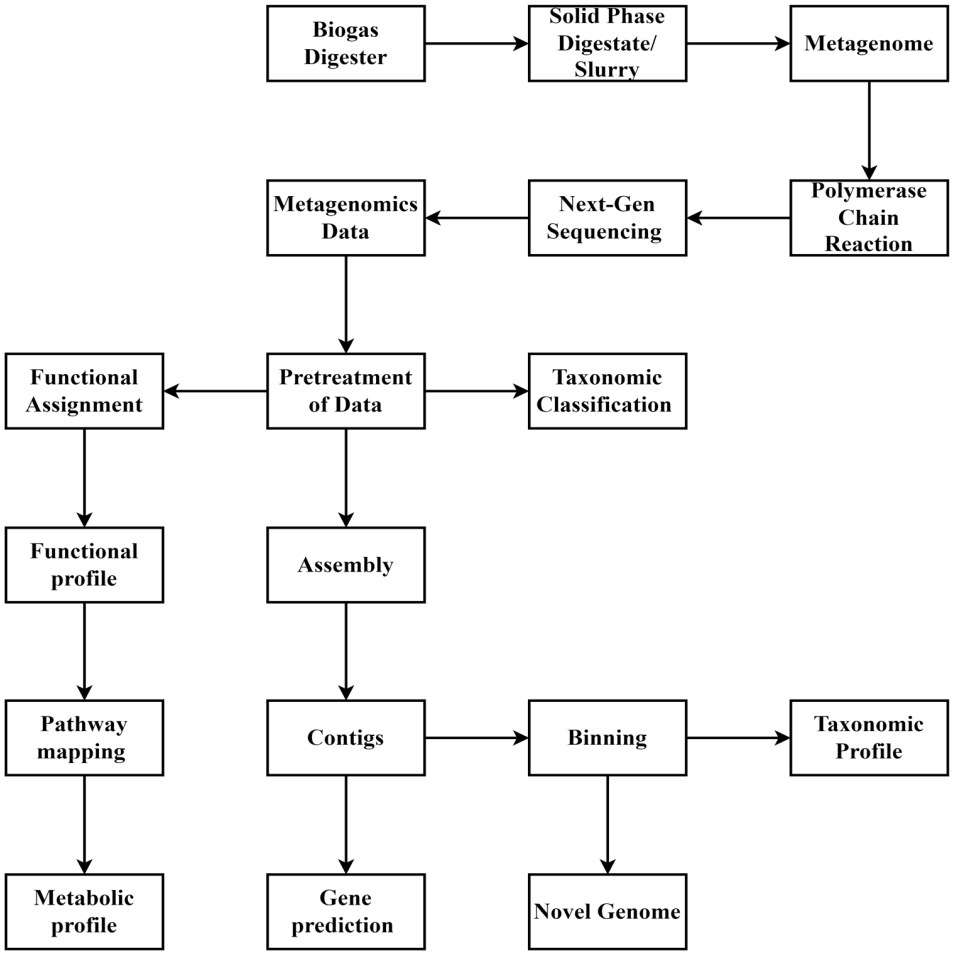

Figure 2. A basic flow chart depicting method of analysing microbial populations in AD.

2. Methods of DNA Isolation and DNA Sequencing

Isolation of DNA is the first and most important step in studying the microbial community. DNA isolation is complex process and to isolate purified DNA is a major task as it depends on many factors such as sampling method. Therefore, selection of the correct sampling method is very important and hence the designing of an experiment is conducted on the basis of sampling for DNA isolation. Repeatability of the experiment is needed to get more precise results and experimental errors are also reduced. The sampling method should be kept constant throughout the experiment to reduce errors [35]. Digestate is mostly collected at a fixed interval of days so that a complete consortium of microorganism can be analysed. The sample should be pre-treated to remove impurities that could create errors in the DNA isolation and PCR processes. Pre-treatment of the digestate should be carried out by rinsing it with phosphate buffer [36]. A general method for isolation of DNA is followed by centrifuging the digestate and collecting the supernatants. The step-by-step method of precipitation of DNA using phenol chloroform is followed. Although most of the DNA-isolation process is carried out using commercially available DNA kits, Bin dong et al. reported that the most efficient method of DNA isolation is the enzymatic disruption method. Table 1 lists the DNA extraction processes utilized by anaerobic digesters in microbial cultures. OMEGA, Precision System Science, MP Biomedicals, Intron Technology, and MOBio Laboratories are some of the leading companies that provide commercial DNA-isolation kits. After DNA isolation is completed, the quality of the DNA is checked through Agarose Gel electrophoresis. The quantity and purity of the DNA could be checked by measuring the absorbance at 260 and 280 nm, respectively, using both an UV spectrophotometer and NanoDrop [37]. Purified DNA is stored at −20 °C in

Tris-EDTA for further use. Typically, Pure DNA shows approx. 1.9 absorbance at 260 nm and 50 ng/nL concentration [38]. Microbial community analysis of anaerobic digesters on the basis of quality and quantity is conducted rapidly with the help of next-generation sequencing methods. Sun et al. reported that pyrosequencing programs such as Roche GS FLX 454 is the most frequently used platform for microbial community analysis of anaerobic digesters [39]. Some of the frequently used platforms, such as ABI SOLiD™ and reagents for ABI analysis, are platforms for short-read DNA sequencing, upon which next-generation sequencing depends. Few NGS methods are based on platforms such as the Illumina (Solexa) sequencing platform and sequencing kit (Ion PGM™ Hi-Q™) coupled with a sequencer (Ion PGM™), which is controlled by software, namely Torrent Suite™ [40]. A new generation of sensing technology uses nanopores—nano-scale holes—embedded in high-tech electronics, to perform comprehensive molecular analyses. Sequencing at the sample source using portable MinION and Flongle devices use inbuilt EPI2ME data-analysis workflows for real-time species ID and AMR profiling. However, it is used much less frequently than Illumina to analyse the microbial community of AD and is most often used as an adjunct to Illumina [41–43]. Although there are various methods of DNA sequencing, there are various errors that must be addressed. For example, by removing the error from the de-novo genome assembly, convolutions of the downstream estimations will be reduced. Many error-correction tools are under development and are being used to reduce the complexity of the metagenomic analysis process [12].

Table 1. Commonly used DNA-isolation kits.

Brand Name	Country Name	DNA Isolation Kit	Usage Percentage	Reference
Clontech	USA	PCR reaction mix	2%	[40]
Zymo Research	USA	ZR soil microbe DNA kit	2%	[44]
Felix bio-tech	USA	DNA extraction kit	2%	[45]
Intron biotechnology	Korea	I-genomic BYF DNA extraction kit	2%	[46]
Magtration System 6GC, Precision System Science	Japan	Automated nucleic acid kit	6%	[38,47,48]
Macherey-Nagel	Germany	NucleoSpin Tissue kit+NucleoSpin soil kit	6%	[49]
OMEGA	USA	E.Z.N.A Soil DNA kit	6%	[10,50]
Q-Bio gene	Australia, Carlsbad, CA, USA	Fast DNA SPIN kit for soil	25%	[51]
MP Biomedicals	Illkirch, France, Australia, Germany, USA			[52]
MoBio Laboratories	USA	MoBio PowerSoil DNA extraction kit	43%	[53,54]
-	-	CTAB (cetyltrimethylammonium bromide) based DNA extraction method	6%	[37,55–59]

3. Analysis of Anaerobic Microbial Communities Using Bioinformatic Tools

Metagenomic data are analysed through various bioinformatic tools, which are different for different steps involved in the whole procedure of metagenomic data analysis. The overall analysis of microbial populations involves different facets of analysis. First of all, raw sequences are pre-treated using several bioinformatics softwares. The pre-treated sequences are now sequentially analysed on different levels. As shown in Figure 3, the pre-treated sequences undergo different analyses. Operational taxonomic unit-clustering analysis, taxonomical compositional analysis, analysis of alpha diversity, analysis of similarity and difference in the microbial community, functional gene analysis, and statistical analysis are different attributes which require conforming techniques and platforms. Chowdhary and Kumar [29] described various software for metagenome data analysis with attached web functions and links. Similarly, Ju and Zhang [35] focused on frameworks and computational resources available for metagenomic bioinformatics analysis, covering several data-processing functions, including pre-treatment, binning, annotation, and assembly.

Nevertheless, these reported computer software and platforms [21,44] have not been fully applied to anaerobic microbial metagenomics in biogas-producing digesters as the samples were extracted from various microbial habitats such as soil, ocean, sewage, biofilms, and bioreactors, etc.

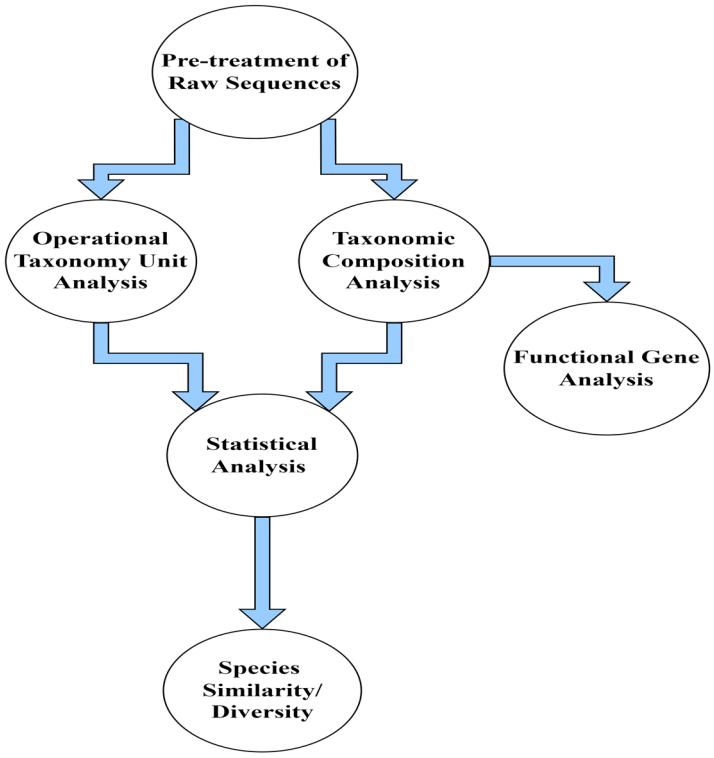

Figure 3. Basic bioinformatic approaches used for microbial community analysis.

3.1. Pre-Processing of Raw Sequences

Upon the collection of metagenome data (direct NGS reads), fresh sequence pre-treatment is quite a crucial move for achieving high-quality readings for the downstream analysis. A sequenced pre-treatment usually includes (i) removal of linkers and adapters, (ii) chimera's exclusion and its replication, and (iii) demultiplexing of barcoded samples and quality control. There are various software tools (Table 2) which have been used in last ten years for this purpose. The software tools and bioinformatics platforms include UCHIME [10,50,60], RDP tools [61], ACE Pyrotag Pipeline (APP) [52], HMMER [62], MG-RAST [63], Chimera Slayer [64], and Trimmomatic software [65]. MOTHUR and QIIME (http:/qiime.org/, accessed on 13 November 2022) are the two most-used platforms for deactivating metagenome data, while UCHIME is the most popular tool for eliminating and verifying chimaeras from raw sequences. The sequencing reads are organised by barcode and then all primers and barcodes are cleared after primary processing is complete. The barcoding is generally carried out using the Nextera XT Index Kit v.2 (Illumina, San Diego, CA, USA) [66]. Quality control must be carried out for further analysis. For example, all sequences under 150 bp are disregarded by the RDP Pyrosequencing Pipeline's quality control methodology, and samples containing more than one unknown nucleotide, forward primer mismatches, or have poor base quality ratings (Phred quality scores less than 25) require additional investigation [61]. In addition, the pre-cluster method is typically used to combine sequences with 1 bp difference.

Table 2. Different analysis type and the common software used.

Analysis Type	Name of Software	Reference
Measurement and analysis of performance, systemic metabolic processes, annotation of genomes, study of principal coordinates, statistics based on canonical correspondence, data from filtered pyrosequencing runs.	MG-RAST	[65]
Removing chimaera sequences, ensuring high quality, the assessment of variety, richness, and abundance of base coverage, study of principal coordinates, analysing Good's scope of coverage, Alignment and quality control of sequences, sample size normalisation	MOTHUR	[52]
Shortening of reads	Trimmomatic software	[62]
Shortening and aligning reads	HMMER	[67]
Identified sequence reads	ARB rRNA database	[67]
Assembling of genome	CLC Genomics workbench	[57,62,63]
Combination of end-pair reads	FLASH	[17,68]
Analysis of microbial population relation	MetaMIS	[17,65,68]
Interaction network topology analysis	Gephi	[17]
CLUSTAL_X	Iterative sequence alignments; gap editing	[17]
PAST	Illustration of beta-diversity metrics	[57,62]
mPUMA and Trinity	Assembly and processing sequences	[65]
Chimera Slayer	Chromosome complexes elimination.	[69]
ClustalW	Alignment of sequences	[49]
INFERNAL aligner	Alignment of various clean sequences	[10,49,70]
SAMS	Evaluation of the quality of sequences	[70]
GenDB genome annotation system	Long-read assembly and functional annotation	[10]
Regano	Code-sequence prediction	[60,71]
Pipeline	Aligning, trimming, and sorting sequences; analysing biodiversity; naming sequences by taxonomy	[60]
RDP (Ribosomal Database Project)	Differences in community architecture	[69]
Fast UniFrac	Illustrate co-relations between microbial structure and attributes	[69]
CANOCO	Identify causes and effects of microbial communities on reactor efficiency	[69]
XLSTAT	Comparison of taxonomies between two samples using pairwise statistics	[72]
STAMP	MG-RAST	[65]

3.2. Analysis of OTU Clustering

Various clean sequences are aligned with sequence aligners such as Py-NAST [55], INFERNAL aligner [61], MOTHUR [71], ClustalW [36], and MUSCLE [67] in conjunction with the bacterial and archaeal database SILVA [60]. Figure 4 is a model diagram for functional hits of COG. Usearch software is then used to cluster linked sequences into operating taxonomy units (OTUs) using the average adjacent clustering algorithm (Usearch-global command) [72]. Ninety-seven percent sequence similarity is typical for sequence classifiers such as RDP Bayesian classifier [61], UCLUST-RDP classifier [73], MEGA/MEGA5 MEGA 10, and MEGA X [36,60,74]. Of note, the normalization of sample size can be accomplished using a program such as MOTHUR by re-sampling the same number of readings for all samples depending on the smallest sample size. OTU-based analysis of bacterial and archaeal sequences can then be further performed. For example, utilizing the R-Venn-Diagram programme, Yun et al. [75] examined the microbial community dynamics of ammonia inhibition during its mitigation by internal dilution in high-rate anaerobic digestion of food-waste leachate. Begmatov et al. studied OTUs for taxonomic analysis by searching against the SILVA v.138 rRNA sequence database via VSEARCH v. 2.14.1 algorithm 28. Similarly, MiDAS 414, a recently developed reference database of full length

16S rRNA genes for waste-water treatment plants was used for species-level taxonomical analysis [66].

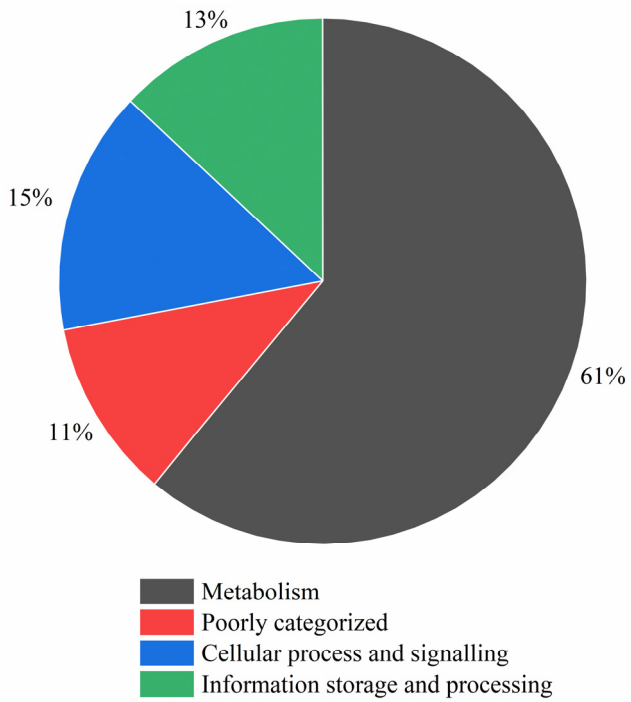

Figure 4. An example showing functional hits of COG.

3.3. Analysis of Alpha Diversity

A 97% cut-off level of OTUs was used for diversity-indices calculation using Usearch v.1127 [66]. As a rule, the Chao index characterizes the richness of a community, while the Shannon and Simpson indices characterize the diversity of a community [76]. The alpha-diversity analysis based on OTUs, which is related to the Chao1 wealth analyser, is used to evaluate the abundance of microbial species or diversity of microbial organisms in different biogas-producing systems (Chao1); the abundance-based species-richness estimator (ACE), the Shannon–Weaver diversity index (Shannon) and the Simpson diversity index (Simpson) are used for the MOTHUR program, the VEGAN library software package [77], and the RDP Pipeline [61]. Of all the indexes, there is a positive correlation between Chao1, ACE, and Shannon and microbial culture biodiversity, although Simpson has negative correlation with it. In particular, the Shannon and Simpson indices can be used to assess biodiversity, while the Chao1 and ACE indices can be specifically utilised in the microbial communities to compute the total number of species (abundance). Therefore, the greater the Good's distribution, the more the results of sequencing represent the real condition. One key benefit of utilising the presented equations to quickly estimate the biological diversity of microbial communities is that it allows for straightforward estimation, which is not the case with other diversity indices [78]. Simpson and Shannon indices combine richness and evenness into a single measure, obscuring other aspects such as the economic, ecological, and social value of each species [79]. Complex multivariate analyses such as canonical correlation analysis must be undertaken to alleviate the limits of biodiversity indexes and provide more important information on environmental variables connected with species in a microbial culture. In addition, the alpha rarefaction curve is used primarily to detect sequencing adequacy. It represents an association between sequencing numbers and species occurrence, which is typically developed using MOTHUR, QIIME, and R tools by randomly subsampling OTU sequences. The plateaus reflect adequate sequencing depth for alpha-rarefaction curves, while the monotonically rising portion is evidence of inadequate sequencing. For instance, Smith et al. [80] reported on a pilot-scale

thermophilic anaerobic digester microbial culture system handling poultry faeces and contrasted the rarefaction curves for 16 S rRNA genes and various sequence similarities. Results proved that the rarefaction curve was asymptotic with similarity levels of about 97 percent, suggesting that the sequence dataset had adequately sampled variability in this study and that adequate sequence complexity at these levels was achieved. However, the amount of OTUs at 99–100% was still increasing, which implies much more undetected diversity at this stage [80].

3.4. Metataxonomic Analysis

Compositional analysis on the basis of taxonomy is the basic information that is obtained for anaerobic microbial community analysis. First, the database is compared and categorized, then, the sequences are taxonomically classified. Several sequence databases such as EzTaxon-e database [62], RDP database [81], SILVA database [82], and GenBank NT/NR database [83] are used to filter the unknown sequences through a blast search. The confidence level is generally 80–90%. Thereafter, using software such as Bergey's taxonomy and Bayesian classifier [84,85], the resultant-matched sequences are allocated phylogenetically to the taxonomic classification. The analysis of taxonomic classification of anaerobic microbial populations is conveniently carried out on the basis of family, genus, order, class and phylum. This classification could be represented in a single multilevel species-taxonomy diagram using the software Krona [63]. Moreover, MetaPhlAn [86], PhyML [64], and CL community software [62], can also be used to find the phylogenetic composition of microbial populations. Zhang et al. [87] reported that the most dominant species were *Trichococcus* in first stage, i.e., hydrolysis, Amino bacterium in the second stage, i.e., acidogenesis, and *Levilinea* in the third stage, i.e., methanogenesis, in a self-designed three-stage anaerobic tank digesting food waste. Moreover, the dominant species of bacteria changed with a change in the feed stock found in the same anaerobic tank co-digesting horse manure and food waste. Archaeal species *Methanosarcina* and *Methanobacterium* and bacterial species *Aminobacterium* and *Proteiniphilum* were dominant species compared to mono-digesting species in the anaerobic tank [88]. Figure 5 shows a model diagram for the taxonomic hit distribution at the phylum level. The model graph shows the microbial community present in a digestate sample from an AD fed on agro-waste. In many studies, it has been found that the dominant species were altered with alterations in operating conditions such as pre-treatments [89] HRT, [47] OLR (organic loading rate) [57], temperature [90], etc, and type of feed stock. Metataxonomic analysis of a single and two-staged anaerobic digestion revealed that the species *Clostridium, Bacteroides, Desulfovibrio, Lactobacillus, Lactococcus, Longilinea, Methanosaeta* and *Syntrophus* showed changes in structure as changes in parameters such as measures of efficiency, hydrodynamics and kinetics of the performance were recorded [91]. In a recent study, a high-throughput sequencing method showed a shift in the archaeal and eubacterial population due to the inclusion of hydrogen gas in AD [92].

3.5. Study of the Similarities/Differences in Microbial Taxonomic Compositions

Based on the study of the composition of microbial taxonomy, study of the dissimilarity and similarity of different samples is carried out and the findings thus obtained can typically be reported in two ways, one being a statistical analysis plot and the other a ternary plot. Using a two-tailed Welch t-test with an alpha value of 0.05, STAMP [93] has also been used to conduct a pairwise statistical comparison of taxonomy between two samples. For example, Yu et al. [94] performed a comparative study between the relative abundance of traditional anaerobic digester (CAD) microbial communities and the "anaerobic dynamic membrane bioreactor" (ANDMBR) at the genus level, which resulted in a statistical analysis of differences. The definition of comparative excess is the fraction of sequences in a sample that are identical to a given taxon expressed as a percentage (percent). The findings suggest that the ADMBR includes a higher concentration of the genus *Methanosarcina* and a smaller volume of the genus *Methanosaeta* than the CAD in a statistically relevant way. Conversely, in the ANDMBR, *Methanosarcina* and *Methanosaeta* were more abundant as a whole than

in the CAD [94]. Microbial taxonomic compositions in the various samples can be shown via a ternary plot [95]. To create a ternary plot in R use the 'ternary plot' function in the 'ggtern' 3.3.5 package [96,97]; depending on the relative existence of the plot, only the species present in all samples are stored and imported. The ratio of each species' relative abundance to its overall abundance across all samples is used to describe the abundance of each species in each sample. Every species is distinguished by an exclusive point in the ternary sequence, and the proportions of the points correspond to the relative abundance. For example, in anaerobic granules from digesters fed with cannery wastewater, brewery wastewater, and milk wastewater, Lu [98] investigated the distribution of specific bacterial and archaeal populations using ternary plots and discovered that the majority of high-abundance bacterial phylae, such as *Chloroflexi*, *Bacteroidetes*, and *Proteobacteria*, were shared by all three types of granules. Around the same time, the phyla *Methanosaeta* occupied all cannery and brewery granules with more members of the phyla Methanobacterium found in cannery granules. The diversity and consistency of microbial communities between various anaerobic digesters can be distinguished using ternary plots. A model diagram depicting the taxonomic hit distribution is shown in Figure 6. In a metataxonomic analysis of an anaerobic digester fed with corncob, three different approaches were used to reveal that there is a noticeable change in the microbial consortium in the AD [99].

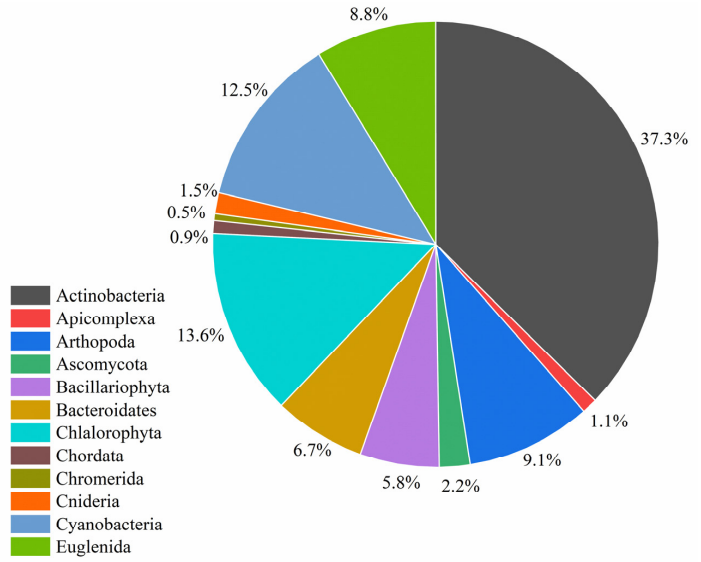

Figure 5. An example showing taxonomic hit distribution at the phylum level.

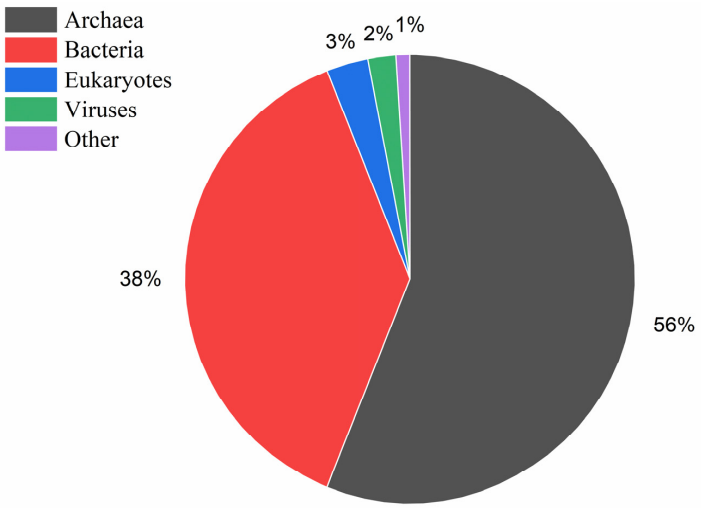

Figure 6. An example showing taxonomic hit distribution at the kingdom level.

3.6. Statistical Multivariate Analysis

Through a brief literature survey concerning anaerobic microbial community analysis, the most commonly used multivariate-analysis techniques included "main coordinate analysis" (PCoA) [56,70,100], "non-metric multi-dimensional scaling" (NMDS) [49,101,102], "main component analysis" (PCA) [52,90], "redundancy analysis" (RDA) [36,48], and "canonical correspondence analysis" (CCA) [40,58,62]. All of these multivariate methods share the same goal of displaying comparable objects in close proximity to one another and dissimilar objects at a greater distance from one another [103]. Based on the data sets and computational methods employed, these methods are typically classified as either unrestricted (principal component analysis, principal component ordination, and NMDS) or constrained (ratio dissimilarity analysis, and canonical correlation analysis) (linear versus unimodal). An important distinction between PCA and PCoA in uncontrolled ordination work is that in PCoA the starting point vector is a parallel or dissimilar distance sequence, which is distinct from the PCA starting point for data collection. Nevertheless, in the study of complicated metagenome results, PCA, PCoA [104], QIIME [56], MOTHUR [70], and MG-RAST [17] have identified an increasing number of applications. For example, PCoA was used to compare populations of specific digesters using the same method of stabilization of biosolids and compared population differences between samples using different methods of stabilization [100]. In another case, PCoA studied the changes in the bacterial community structure in response to OLR, showing that the three-fold digesters behaved similarly at each point [40]. In comparison, with various OLRs, microbial population configurations shifted significantly from stage 1 to stage 4. Although there was negligible variation in community structure between stage 1 and stage 2, the enhanced OLR significantly altered the group structure in stage 3. The decreased OLR in stage 4 moved the bacterial population structure down to those in stage 1 and 2, but not absolutely [40]. Notably, it was more challenging for PCoA to evaluate variable participation as PCoA was unable to provide a direct link between the main components and environmental variables, so PCoA was preferred over PCA when a ton of input data was lacking [78]. Another unconstrained ordination technique, nonmetric multidimensional scaling (NMDS), is commonly used to describe the complexity and resemblance of microbial population systems in anaerobic digestion across time [48,105], or in several digesters under various environmental conditions, such as specific OLRs, temperatures [36,57], and additive (e.g., zeolites [106]) using the R package vegan [107]. In general, the NMDS technique produces the high dimensionality of the data by mapping comparable datasets together and mapping dissimilar datasets with larger stepped distances [103]. On the other hand, PCA is limited to using only the "Euclidean distance matrix", but NMDS and PCoA are not limited to any particular distance matrix [108]. In general, however, NMDS is more computer-intensive than PCoA and PCA [103], resulting in a longer time needed for NMDS research. RDA and CCA are two commonly used methods for analysing metagenome data from microbial communities in restricted ordination science. These two approaches are very close, except that RDA is focused on linear models while CCA is based on unimodal interactions between organisms and climate [105]. For RDA plots a Hellinger transformation is performed, and the plots are created using vegan 2.5–7 package [97]. Next, a technique utilizing detrended communication analysis (DCA) can be conducted to help users choose which approach to follow [109]. The technique primarily involves four phases, i.e., (i) in the programme, selecting 'DCA' for indirect gradient analysis (e.g., CANOCO), (ii) choosing detrending by lines, (iii) choosing the Hill scaling for ordination-score scaling and (iv) selecting the other choices to run the study [109]. Through comparing the results of the measurements of gradient duration with 4.0 and 3.0, it is possible to quickly identify the most appropriate method. Specifically, unimodal strategies such as CCA should be used when the gradient duration value reaches 4.0; whereas the linear option is likely to be a better alternative if the value exceeds 3.0. RDA and CCA work reasonably well in the scale of 3 to 4 [109]. Because RDA and CCA are very close, RDA is given as an example by a brief case study. A CANOCO device (version 4.5, Wagenignen, The Netherlands) was used to

conduct the RDA ordination. The bacterial community structure has been demonstrated to be affected by digester performance and operational factors [36]. The arrows reflect various environmental factors (e.g., operating conditions) in a standard CCA diagram, and the longer the ray is, the greater the environmental factor's effect. A strong association between two environmental variables is seen where the angle between the environmental factors is extreme, while an obtuse angle corresponds to a negative correlation [108]. Reactor performance and operating parameters were examined, including temperature, pH, alkalinity, and volatile fatty acids, utilising CCA with XLSTAT methodologies to clarify the relationship between microbial (bacterial and archaeal) species and these factors (VFAs) [40,58,110]. The differences in sample periods among class-level microbial community composition and digester physicochemical characteristics is another great illustration of the anaerobic digestion method for the oxidation of organic industrial pollutants [111]. A Mantel test was performed using the integrated Mantel function in R, while the coordinates from a RDA plot of the material/inoculum combinations were transformed into distances using the geosphere 1.5–14 package (https://cran.r-project.org/web/packages/geosphere/geosphere.pdf, accessed on 13 November 2022) and Spearman method. The AD kinetic parameters were transformed into distances using the Euclidian method [97].

3.7. Metatranscriptomics and Application

Metagenomics data may be annotated dynamically utilizing bioinformatics frameworks or methods such as BLAST in databases (e.g., SWISSPROT [112], COG, KEGG [113]), MG-RAST (SEED) [65], GenDB [114], and IMG/M [115]) in order to obtain knowledge beyond taxonomic compositions. In particular, BLAST has been mainly used against various databases for the functional annotation of short-metagenome contiquity, while the GenDB genome-annotation software is usually responsible for the functional annotation of long-assembled contiquity [114]. The MG-RAST framework is a SEED-based program that allows users to access automatic analysis metadata [65]. IMG/M offers researchers a three-dimensional (genes, metabolites and functions) genome annotation system and comparative analysis of microbial genomes using several methods such as KEGG [116], InterPro [117], Pfam [118], and gene ontology [119]. Hybridization methods focused on microarrays are also helpful in the study of genome diversity and bacterial connectivity [120]. Although numerous bioinformatics methods for gene functional annotations have been developed, some of them, such as single genome genetic prediction tools, are not well suited for complex metagenomic data sets of anaerobic microbial populations due to heterogeneous sequence structure, length, and error [121,122]. It is clear that successful gene-classification methods (e.g., IMG/M and KEGG) can still be widely used to classify protein-coding areas, which is one of the main issues in microbial metagenomics studies. Such an example is the research by Campanaro et al. [65], which used bioinformatics methods (e.g., KEGG, COG and SEED) to model functional associations between microbial species involved in the AD process and to identify key microbial genomes that encode enzymes involved in different metabolic pathways. Microbial co-association networks include knowledge of the function of time or other external factors on the nature of population organization. Group metabolic networks, through recognizing metabolite exchanges and species-specific resource requirements, can provide a mechanistic connection between organisms [123]. In fact, MEGAN can also be used to systematically evaluate various metagenomes focused on the SEED hierarchy and KEGG pathways [68]. In reaction to minor disruptions, microbial populations may shift unexpectedly, related to changing conditions or several stable states. However, with time-varying networks, temporal variability in microbial encounters can be observed [124]. Therefore, more experiments under various operating conditions (e.g., specific substrates and temperatures) are likely to enable the anaerobic microbial genome database and related applications to be expanded in the near future through increasingly powerful tools and algorithms.

4. Application of the Analysis of Anaerobic Microbial Community on Biogas Production

Exploration in metagenomic data via bioinformatic tools has helped researchers to identify many important bacterial and archaeal species in anaerobic digester tanks [17,65,125]. Studies have also revealed many different parameters such as, changes in microbial population according to the alteration of operational parameters, methanogenesis pathways and bacterial metabolism [83,89], and performance of anaerobic digesters with respect to the microbial community [126–128]. Furthermore, technologies are being established in the field of metagenomics using new and improved bioinformatics tools, helping researchers to gain knowledge about new developments in the field of biogas production. Various developments, viz., improved and optimized AD processes, pliability and rigidity of metabolic pathways, analysis of antibiotic-resistant genes and mathematical modelling of microbial communities are summarized.

4.1. Development of Improved AD Processes

The optimization and improvisation of AD are achieved by improving various strategies such as pre-treatment of feedstock [129], optimization of operational parameters [130], design optimization of the anaerobic digester [87,88], addition of supplements such as additives [131], etc., and microbial community analyses are mostly carried out for all the alterations conducted for optimization of AD. Zhang et al. [131] reported that the process of anaerobic digestion in both pilot-scale as well as lab-scale digesters was improved by adding activated carbon as a supplement. 16 S rRNA genes were pyrosequenced to find that the dominant phyla, i.e., *Proteobacteria, Elusimicrobia and Firmicutes*, increased by 1.7–2.9 times due to supplementing the digesters with activated carbon. Another study conducted on hydrogenase and nitrate reductase at the transcript level revealed that *Blautia, Acetitomaculum* (acetogens), *Selenomonas, Wolinella* (fumarate and nitrate reducers) and *Desulfovibrio* (sulfate reducers) were the main cause for H_2 production in AD [132].

Recent research revealed that in three-staged digesters, all three stages, that is hydrolysis, acidogenesis and methanogenesis, were optimized and selectively enriched at every stage with respect to the microbial community of each stage, i.e., hydrolysing bacteria, acidogenesis bacteria, and methanogens. That study was conducted using pyrosequencing analysis, and 16 S rRNA high-throughput sequencing is yet to be performed [87,88]. In other research Suksong et al. [130] revealed that the highest methane yield was obtained at a carbon to nitrogen (C:N) ratio of 30:1, total solid (TS) content of 16%, and feedstock to inoculum (F:I) ratio of 2:1. This inference was obtained by optimizing the operational parameters such as TS, C:N ratio, and F:I ratio. The enhanced production of methane gas was due to the selectively enriched microbial community, viz., Clostridium species, Methanoculleus species, and Ruminococcus species [130]. A recent study revealed that despite producing methane at a rate of 241–247 mL/g VS, the thermal hydrolysis process-anaerobic digester (THP-AD) reactors collected around ~1999 mg/L of propionate. About 30 significant "metagenomic assembled genomes" were augmented and ~70 metagenome-assembled genomes were retrieved from that metagenomic study. A study of genome-centric metagenomics revealed the recovery of 68 metagenome-assembled genomes (MAGs), 32 of which were significantly enriched [133]. A study of the effect of carrier-based silica-lignin on anaerobic digesters showed that maximum bacterial count was noted during dehydrogenase phase [134]. The overall improvement of AD can be achieved by study of genome–centric analyses for understanding the whole microbial community. This could be accomplished by developing an assembly of whole functionality of the microbial community and assessing its coding gene annotations [135].

4.2. Development on Metabolomics Analysis

Treatment of unmanaged waste and production of biomethane gas can be accomplished with the help of highly efficient anaerobic reactions. However, the efficiency of the anaerobic digestion processes could be increased by gaining sufficient knowledge about the metabolic pathways of methanogens. Recent studies have mentioned the coupling of metagenomic sequence analysis along with radio-isotopic analysis to disclose the main metabolic pathways of dominant methanogen species present in the anaerobic tanks, digesting manure or sludge [17]. Results from the two different analysis methods have shown inconsistencies, i.e., the metabolic pathways revealed by radio-isotope analysis were different from the results determined by metagenomic analysis. Many similar studies have been carried out on networks of microbial communities and their metabolic pathways with the help of several bioinformatic tools and techniques. Likewise, research on metabolic pathways and networks of microbial communities using bioinformatics technology are available in automated AD systems. Zhang et al. [18] reported that a food-waste-digesting anaerobic digester was optimized by enriching it with activated carbon. Analysis of metabolic pathway with the help of KEGG-pathway analysis revealed that there were significant changes in the microbial community and their metabolic pathways, which was also confirmed by taxonomic tree analysis. The results of both analyses showed that the main metabolic pathway in ADs enriched with activated carbon was the metabolic pathway of propanoate which transforms propanoic acid into acetic acid. Lipid metabolism and methanogenesis pathways were also improved and it was found through the microbial community analysis that the activated carbon encouraged the growth of the species Methanosaeta and Geobacter, which form a highly populated syntrophic microbial community [18]. Jiang et al. [136] recently studied the mcrA (methyl Co-A reductase) gene and 14C-labelled sodium acetate by using a pyrosequencing technique and radiolabelling technique to find out how the relationship between dominating methanogen pathways and TAN (total ammonia nitrogen) is altered by change in syntrophic acetate oxidation and acetoclastic pathways, which further results in enhanced methane formation in AD. It was also found that the higher carbon dioxide and methane ratios (2.1–3.0) in high total ammonia nitrogen (TAN), approx. 11.1 g/kg wet weight, digesters show a maximum of 75% methane production through hydrogenotrophic pathways, whereas, only 23% methane production was through hydrogenotrophic pathways when the ratio of CO_2 and CH_4 was approx. 0.1–0.3 in low TAN (0.2 g/kg wet weight) digesters [136]. Thus, the relation between TAN and metabolic pathways could be optimized to manipulate the syntrophic and acetoclastic metabolic pathways. In another study of continuous AD, a 16 S rRNA gene-sequencing technique found that there was a huge change in the microbial community during its adjustment to an environment with a high concentration of ammonia (10 g NH^{4+}-N/L) [137]. A hydrogenotrophic species, Methanoculleus spp. and a syntrophic acetate-oxidizing bacterial species, Clostridium ultunense, were found to be growing rapidly and increasing in population at high levels of ammonia (>7 g NH^{4+}-N/L), revealing a hydrogenotrophic pathway as a major metabolic pathway [137]. A major shift from acetoclastic pathways to more flexible and complex metabolic pathways was seen at 5 g/L NH^{4+}-N concentration [138]. In another study, changes in the microbial community due to change in temperature was found. Metabolic pathways were studied in anaerobic tanks digesting activated sludge, working at thermophilic temperature range (55–65 °C) and 2–4 days of HRT (hydraulic retention time) [83]. At higher temperature, i.e., 55–60 °C, Methanosarcina species were found to be dominant as revealed by 16S rRNA pyrosequencing analysis. However, the analysis also revealed that if the temperature is raised to 65 °C, the population of Methanosarcina species is significantly reduced, resulting in a decrease in overall methane yield and deposition of VFA. Higher temperature encouraged the syntrophic acetate oxidation as found by stable-isotopic signature (δ13C) analysis. Similar results were seen when HRT was reduced to two days [52]. In a recent study, raw sequence data were subjected to quality assurance, splitting, and grouping in order to create OTUs, and then species taxonomic classification was conducted. "Canoco5" was used to conduct the analysis for redundancy. This study

revealed that feedstock breakdown sped up as a result of co-digestion, which enriched the dominating hydrolytic bacterium Defluviitoga [139]. Formation of phosphine in an anaerobic digester was investigated in a study which revealed that the bacterial species Escherichia and Ruminococcaceae were found in abundance. Two types of databases: KEGG and MetaCyc pathway revealed the close relation of phosphine synthesis [140]. Bioaugmentation in anaerobic digesters helps in recovery of VFA, and a metabolomics study revealed that during the early steps of bioaugmentation of VFA, the acetoclastic microbial population plays the major role in methanogenesis and neutralizing the pH [141]. In a recent study of a multi-staged anaerobic digester fed with food waste, an analysis was conducted on the foaming mechanism in the digester. The results of the metabolomic analysis revealed that the metabolic molecules that have higher expression, are up-regulated, and have high surface activity are the major contributors in foaming [141]. Metabolomic analysis during co-digestion of corn straw and pig manure showed the hydrogenotrophic and acetoclastic pathways working during the process [142]. Another study using beta-diversity analysis revealed that richness in species is more significant in thermophilic digesters than in mesophilic digesters [143].

4.3. Development in Identification of Antibiotic-Resistant Genes

Metagenomics methods, bioinformatic tools and techniques, and high-throughput sequencing are successfully used to identify antibiotic-resistant genes as disposal of the sludge or digestate in open environments, specially on land, may cause health risks. Lee et al. [144] presented in their study a characterization of wastes such as. sludge, food waste and manure in terms of amount of antibiotic-resistant genes. The result of the characterization revealed that comparative amounts of antibiotic-resistant genes were found to be highest in manure and the least amount was found in food waste. The results showed that the diversity and structures of antibiotic-resistant genes varies with variation in the type of substrate [144]. In a recent report, Luo et al. [126] reported a significant overall amount of antibiotic-resistant genes, 7×10^{-3} to 1.1×10^{-1} copies of ARGs/copy of 16 S rRNA DNA, in tanks digesting, industrial waste and manure. The digestate from thermophilic anaerobic digesters has a lower amount of antibiotic-resistant genes [126], which makes thermophilic digesters more dominant for removing pathogenic microorganisms [145,146]. Antibiotic-resistant genes and bacteria resistant to antibiotics were observed in ADs digesting pig manure through HTFQ PCR (high-throughput fluorescent quantitative PCR) and Illumina MiSeq sequencing [147]. Three transposons and 83 antibiotic-resistant genes were detected from the above-mentioned sequencing techniques. Also, some resistant genes such as tetracycline and Macrolide-lincosamide-streptogramin decreased after the AD processes. However, the comparatively large quantities of florfenicol, sulfa drug, aminoglycoside, chloramphenicol, and amphenicol-resistant genes were improved by 52–270 times [147]. Biogas digestate has been used as manure for farmlands for a long period of time and the presence of antibiotic-resistant genes has created an increment of the population of antibiotic-resistant bacterial strains causing contamination of cropland soil [147]. Therefore, to minimize the proliferation of antibiotic-resistant genes in farmland soil, remedial and regulatory steps must be employed. Recent research has revealed that pre-treatment of substrate, i.e., sewage sludge with microwaves, helped in reducing the antibiotic-resistant genes, which, however, increased in the AD but in nominal amounts [110]. Hence, pre-treatment with microwaves is one of the easy and potent techniques for removing antibiotic-resistant genes from bio-manure made from AD digestate. To better understand antibiotic-resistant and bacterial species, environmental metagenomics analysis techniques have been explored and developed; for example, an online open analysis portal, ARGs-OAP is one such platform which consists of a databank named SARG version 2.0 [148]. The SARG databank consists of a lot of sequence data present in ARDB, CARD, and protein databanks, namely NCBI-NR [148]. For easy and complete access to data from the ARGs-OAP databank online portal, http://smile.hku.hk/SARGs (accessed on 13 November 2022) can be browsed.

4.4. Creation of the Modelling and Optimization of Microbial Population Dynamics

Some recent trends have revealed many developments in microbial community-dynamics modelling and how these dynamics are related to operational and physiochemical parameters. For example, in order to promote rational control and intervention in microbial communities, Hanemaaijer et al. [149] determined analytical modelling methods for microbial community dynamics, ranging from methagenomics to community structure. The study was dedicated to two kind of modelling methods including a naïve coarse-grained model which could resolve the extrapolating problem from the investigational information, and the other model was a mathematical model which could assimilate prevailing physiochemical, physiological, and genomic data [149]. The first model has established unusually low consideration, although it can possibly be improved to be a more complete genomic-level stoichiometric model which can perform as mixed data integrators. The second model has been primarily considered and used because of its ability to exploit data and extrapolate power [149]. Similarly, Succurro et al. [150] gave a summary of mathematical models of microbial ecosystems found naturally and accentuated that to develop a hypothetical account of a microbial community one should choose an exact problem. A current agreement is that it is time for more progress to be made in the large theoretically defined methods, the methagenomic microbial interface simulators (e.g., MetaMIS [151]) and the mathematical amalgamation tools (e.g., MetaTopics [152]) to expand the population dynamics area of modelling from a mere explanatory example to a whole biogeochemical concept [150,153,154]. Further, Succurro and Ebenhöh [155] published a detailed review of mathematical modelling of microbial ecosystems, which determined two main divisions of mathematical approaches (differential equation models and constraint-based stoichiometric models) and presented the current purpose to the analysis of microbial communities. The benefits of these two models are determined tactics that can effectively provide a macroscopic illustration of microscopic biological systems [155]. In order to move forward, an integration modelling method has been shown to recognize growing patterns in microbial classification and their dynamics delimited by diverse spatial-temporal phenomena [155]. It was proposed that simulators and researchers would work together from the theoretical phases of the investigation plan to guarantee a precise mixing of concepts and experiments [150]. With the help of mathematical models, a common platform could be made to understand the structures of complicated bacterial communications. The results and information attained from the modelling of microbial populations help in controlling and enhancing the AD process by optimizing the microorganisms. In this regard, many significant advancements in microbial population systems alterations have been made. Digester conformation, type of feedstock, environment conditions and operating factors (e.g., temperature) are the important pushing aspects for community-structure deviations [156]. For example, in an anaerobic tank digesting pig excreta, the genus Methanocorpusculum was in the majority at pH 7.0, and at pH 6.0 and 8.0, genus Methanosarcina was dominant, showing that by controlling pH dominant species of microbial populations can be optimized [157]. Likewise, some research has been conducted to produce a feedback system which can efficiently help in detection of any malfunction in the overall system. For example, alkalinity is used as a sign or indicator for steadiness of the process in an AD [158–160]. So far these technologies have brought breakthrough advances which have helped in better understanding AD processes, however imaging the multifaceted 3-D multiple species population nevertheless creates substantial challenges in experiments, so the knowledge gained so far and utilized for optimizing and modifying existing AD processes needs to be researched further.

5. Restrictions and Predictions of Bioinformatics Analysis on Microbial Metagenomics

Analysis of the microbial community, i.e., metagenomic analysis of anaerobic digesters has been frequently explored. Most countries are working in the field of metagenomics and meta-transcriptome analysis and microbial community analysis of anaerobic digesters. However, there are many challenges that need to be tackled before bioinformatic analy-

sis becomes a general technique. Advancement in sequencing technology has made the sequencing process rapid and easy and therefore has caused some serious problems in data storage. For example, a huge amount of data, of about 600–1000 GB per run is being produced by sequencing data through Hi Seq. 2500, and similarly approx. 1,000,000 reads are produced by 454 pyrosequencing on each sequencing run, which is equal to about 0.7 GB data [146,161]. Again, raw sequences increase the sequencing data up to 10–20 times for each analysis [161]. A major challenge in the field of bioinformatics is the enormous volume of data which is being produced due to sequencing [122]. Furthermore, metagenomic research based on next-generation sequencing is still naïve. Recently sequenced metagenome data should be provided with more storage space and statistical sustenance. Therefore, to explore microbial communities of anaerobic digestion a common program or platform for the storage of enormous data must be created. Also, statistical methods, machine learning, artificial intelligence, and data mining are some of the fields from which the tools and techniques of bioinformatics have been derived for data management [162]. However, the original data of metagenomics which are generally fed into bioinformatic software differ greatly with the genomic data [162]. Genomic data analysis has been carried out using various computational approaches; however, there are some issues that need to be resolved. Humongous sequence data is an insuperable problem for assembly and binning which can be accredited to the incomplete calculating ability and explicit internal storage ability [39]. Moreover, there occurs an absurdity between precision and data managing rates because assembly and binning according to the taxonomy requires numerous hours to numerous days [161]. Therefore, more effective analysis tools should be developed that are rapid in completing analysis tasks such as the technology present in super computers and also provide more precise results. Another reason that has been found to be an obstacle in metagenomic analysis is the excessively high amount of cost of metagenomic sequencing, consequently, repeatability of experiments become less feasible, and hence it is difficult to confirm the significance of produced data [33]. Thus, to obtain more consistent and unfailing results, it is advisable to sequence in triplicate. Campanaro et al. [163] in their study stated that biasness of sequenced data of amplified 16S rRNA genes is mainly because of the improper matching of the universal primers of all the target genes and also because of lesser-known hypervariable regions [163]. The usage of several indicator genes and transcriptional studies are therefore suggested in order to improve the estimation of profusion for important taxonomic groups [163]. In fact, most bioinformatics analyses to date have relied on the contrast of sequences with reference databases. Practices for using existing databases for evaluating bioinformatics, however, may be troublesome given the potential incompleteness of datasets. Therefore, to promote reliable and efficient study of anaerobic microbial metagenomics, upgraded databases with better quality data are required. Fourth, metagenomics strategies provide a deeper insight into microbial populations, but have primarily been used for concise and informative methods to react to the original question [122]. There are still differences between research, pilot and field data, and extrapolation. In addition, there is still a substantial gap in what we can know from microbial communities through bioinformatics methods and what species and gene functions we can regulate, because the microbial environment is an extremely complex network of diverse spatio-temporal interactions between microorganisms as well as between microorganisms and the ecosystem [164]. In order to fill this void, further information should be offered on how the genetic properties produce their dynamic behaviours and the dynamic interactions between individual microorganisms. In fact, nutrient amounts and fluxes within microbial species should also be thoroughly investigated. Finally, the existing scope of mining of anaerobic microorganism metagenomics data, based primarily on clear sequence similarity searches, may not be sufficient as microbial genomes should be the basis for microbial ecology [165]. For more comprehensive data mining, it is highly recommended that methodologies focused on bioinformatics be combined with core biotechnological approaches (e.g., TRFLP, FISH, PCR-DGGE and qPCR [1,27,166]). Nevertheless, there may still be a long way to go to the ideal point where digester efficiency

can be precisely managed on the basis of anaerobic microbial community real-time analysis of methagenomics information. A large-scale precision-fermentation program using an artificial neural network is proposed to accomplish these efforts and to continue with the production of insightful solutions. With an artificial intelligence (AI) workflow, vast data (e.g., elementary feedstock materials, operating parameters, and microbial population metagenomics) derived from extensive past AD activities can be built into coevolutionary neural networks that can be further refined into useful networks such as deep Q networks using experimental data as training details. A better combination of regulatory parameters, digester sorting, and microbial population information can be proposed in a useful network with new feedstock part data to support practical AD operations. Although there are merely a few research reports [167–169] built on the disposition of AI methods over the last years, AI application in anaerobic digestion simulation, regularization, and optimization has great potential in the near future, particularly with the rapid development of novel and hybrid AI approaches. For example, a new algorithm has been created to design minimal microbial communities with required metabolic capability [170]. This algorithm will precisely enable researchers to identify minimum sets of microbial species that can collectively provide the enzymatic capability needed to synthesize the target metabolite population from a predefined selection of accessible substrates [170]. Thus, the result might be a major move towards the objective of modifying specific microbial communities for the optimization and industrial applications of anaerobic digesters.

6. Conclusions

Lately, a lot of research work has been conducted in the field of microbial community analysis and metagenomics analysis of anaerobic digesters that has helped to better understand the AD process. New techniques and tools in bioinformatics have helped in controlling and optimizing the anaerobic digestion processes in terms of feedstock, temperature, pH, HRT, VFA, TAN, etc., which has been a boon for the industries and has huge commercial potential. Despite the many applications of metagenomic analysis, there are some major issues related to this analysis such as data consistency, data storage, data usage and data managing techniques. These problems need to be assessed and tackled to further improvise and control AD processes. This review gives insights about the latest trends in metagenomic studies, newest and often used bioinformatic tools and techniques. However, the prevailing issues in these type of studies have grabbed the attention of researchers from the fields of environmental science, biotechnology, bioinformatics, computer science, and civil and chemical engineering. A lot of demand for research and development in this area is the need of hour and to strategically implement new advancements to provide molecular techniques at the commercial level. New technology in the field of database management is now being researched and technology such as artificial neural network and machine learning are finding their way into the optimization of biogas producing anaerobic tanks. Henceforth, the near future is brightening up with usage of more advanced technology in bioinformatics and to produce better yields of methane through optimally engineered anaerobic digesters.

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References

1. Ju, F.; Lau, F.; Zhang, T. Linking Microbial Community, Environmental Variables, and Methanogenesis in Anaerobic Biogas Digesters of Chemically Enhanced Primary Treatment Sludge. *Environ. Sci. Technol.* **2017**, *51*, 3982–3992. [CrossRef]
2. Wang, P.; Wang, H.; Qiu, Y.; Ren, L.; Jiang, B. Microbial Characteristics in Anaerobic Digestion Process of Food Waste for Methane Production–A Review. *Bioresour. Technol.* **2018**, *248*, 29–36. [CrossRef] [PubMed]
3. 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. [CrossRef] [PubMed]
4. Kim, E.; Lee, J.; Han, G.; Hwang, S. Comprehensive Analysis of Microbial Communities in Full-Scale Mesophilic and Thermophilic Anaerobic Digesters Treating Food Waste-Recycling Wastewater. *Bioresour. Technol.* **2018**, *259*, 442–450. [CrossRef] [PubMed]
5. Khan, S.; Lu, F.; Jiang, Q.; Jiang, C.; Kashif, M.; Shen, P. Assessment of Multiple Anaerobic Co-Digestions and Related Microbial Community of Molasses with Rice-Alcohol Wastewater. *Energies* **2020**, *13*, 4866. [CrossRef]
6. Björnsson, L.; Murto, M.; Mattiasson, B. Evaluation of Parameters for Monitoring an Anaerobic Co-Digestion Process. *Appl. Microbiol. Biotechnol.* **2001**, *54*, 844–849. [CrossRef]
7. Holm-Nielsen, J.B.; Lomborg, C.J.; Oleskowicz-Popiel, P.; Esbensen, K.H. On-Line near Infrared Monitoring of Glycerol-Boosted Anaerobic Digestion Processes: Evaluation of Process Analytical Technologies. *Biotechnol. Bioeng.* **2008**, *99*, 302–313. [CrossRef]
8. Boe, K.; Batstone, D.J.; Steyer, J.-P.; Angelidaki, I. State Indicators for Monitoring the Anaerobic Digestion Process. *Water Res.* **2010**, *44*, 5973–5980. [CrossRef]
9. Molina, F.; Ruiz-Filippi, G.; Garcia, C.; Lema, J.M.; Roca, E. Pilot-Scale Validation of a New Sensor for On-Line Analysis of Volatile Fatty Acids and Alkalinity in Anaerobic Wastewater Treatment Plants. *Environ. Eng. Sci.* **2009**, *26*, 641–649. [CrossRef]
10. Fernández, A.; Huang, S.; Seston, S.; Xing, J.; Hickey, R.; Criddle, C.; Tiedje, J. How Stable Is Stable? Function versus Community Composition. *Appl. Environ. Microbiol.* **1999**, *65*, 3697–3704. [CrossRef]
11. Li, L.; He, Q.; Ma, Y.; Wang, X.; Peng, X. Dynamics of Microbial Community in a Mesophilic Anaerobic Digester Treating Food Waste: Relationship between Community Structure and Process Stability. *Bioresour. Technol.* **2015**, *189*, 113–120. [CrossRef]
12. Hashemi, S.; Hashemi, S.; Lien, K.; Lamb, J. Molecular Microbial Community Analysis as an Analysis Tool for Optimal Biogas Production. *Microorganisms* **2021**, *9*, 1162. [CrossRef]
13. Narihiro, T.; Sekiguchi, Y. Microbial Communities in Anaerobic Digestion Processes for Waste and Wastewater Treatment: A Microbiological Update. *Curr. Opin. Biotechnol.* **2007**, *18*, 273–278. [CrossRef]
14. Zhang, L.; Kuroki, A.; Loh, K.-C.; Seok, J.K.; Dai, Y.; Tong, Y.W. Highly Efficient Anaerobic Co-Digestion of Food Waste and Horticultural Waste Using a Three-Stage Thermophilic Bioreactor: Performance Evaluation, Microbial Community Analysis, and Energy Balance Assessment. *Energy Convers. Manag.* **2020**, *223*, 113290. [CrossRef]
15. Zhen, F.; Luo, X.; Xing, T.; Sun, Y.; Kong, X.; Li, W. Performance Evaluation and Microbial Community Analysis of Microaerobic Pretreatment on Thermophilic Dry Anaerobic Digestion. *Biochem. Eng. J.* **2021**, *167*, 107873. [CrossRef]
16. Laserson, J.; Jojic, V.; Koller, D. Genovo: De Novo Assembly for Metagenomes. *J. Comput. Biol.* **2011**, *18*, 429–443. [CrossRef]
17. De Vrieze, J.; Saunders, A.M.; He, Y.; Fang, J.; Nielsen, P.H.; Verstraete, W.; Boon, N. Ammonia and Temperature Determine Potential Clustering in the Anaerobic Digestion Microbiome. *Water Res.* **2015**, *75*, 312–323. [CrossRef]
18. Rincón, B.; Borja, R.; González, J.M.; Portillo, M.C.; Sáiz-Jiménez, C. Influence of Organic Loading Rate and Hydraulic Retention Time on the Performance, Stability and Microbial Communities of One-Stage Anaerobic Digestion of Two-Phase Olive Mill Solid Residue. *Biochem. Eng. J.* **2008**, *40*, 253–261. [CrossRef]
19. Luo, G.; Fotidis, I.; Angelidaki, I. Comparative Analysis of Taxonomic, Functional, and Metabolic Patterns of Microbiomes from 14 Full-Scale Biogas Reactors by Metagenomic Sequencing and Radioisotopic Analysis. *Biotechnol. Biofuels* **2016**, *9*, 1–12. [CrossRef]
20. Zhang, J.; Mao, L.; Zhang, L.; Loh, K.-C.; Dai, Y.; Tong, Y. Metagenomic Insight into the Microbial Networks and Metabolic Mechanism in Anaerobic Digesters for Food Waste by Incorporating Activated Carbon. *Sci. Rep.* **2017**, *7*, 1–10. [CrossRef]
21. Ye, L.; Zhang, T.; Wang, T.; Fang, Z. Microbial Structures, Functions, and Metabolic Pathways in Wastewater Treatment Bior actors Revealed Using High-Throughput Sequencing. *Environ. Sci. Technol.* **2012**, *46*, 13244–13252. [CrossRef]

22. Regueiro, L.; Spirito, C.; Usack, J.; Hospodsky, D.; Werner, J.; Angenent, L. Comparing the Inhibitory Thresholds of Dairy Manure Co-Digesters after Prolonged Acclimation Periods: Part 2–Correlations between Microbiomes and Environment. *Water Res.* **2015**, *87*, 458–466. [CrossRef]
23. Genitsaris, S.; Monchy, S.; Denonfoux, J.; Ferreira, S.; Kormas, K.; Sime-Ngando, T.; Viscogliosi, E.; Christaki, U. Marine Microbial Community Structure Assessed from Combined Metagenomic Analysis and Ribosomal Amplicon Deep-Sequencing. *Mar. Biol. Res.* **2015**, *12*, 1–13. [CrossRef]
24. Krakat, N.; Anjum, R.; Demirel, B.; Schröder, P. Methodological Flaws Introduce Strong Bias into Molecular Analysis of Microbial Populations. *J. Appl. Microbiol.* **2016**, *122*, 364–377. [CrossRef]
25. Alvarez-Silva, M.C.; Álvarez-Yela, A.C.; Gómez-Cano, F.; Zambrano, M.M.; Husserl, J.; Danies, G.; Restrepo, S.; González-Barrios, A.F. Compartmentalized Metabolic Network Reconstruction of Microbial Communities to Determine the Effect of Agricultural Intervention on Soils. *PLoS ONE* **2017**, *12*, e0181826. [CrossRef]
26. Badhai, J.; Ghosh, T.S.; Das, S.K. Taxonomic and Functional Characteristics of Microbial Communities and Their Correlation with Physicochemical Properties of Four Geothermal Springs in Odisha, India. *Front. Microbiol.* **2015**, *6*, 1166. [CrossRef]
27. Kumar, G.R.; Chowdhary, N. Biotechnological and Bioinformatics Approaches for Augmentation of Biohydrogen Production: A Review. *Renew. Sustain. Energy Rev.* **2016**, *56*, 1194–1206. [CrossRef]
28. Mukherjee, A.; Chettri, B.; Langpoklakpam, J.; Basak, P.; Prasad, A.; Mukherjee, A.; Bhattacharyya, M.; Singh, A.; Chattopadhyay, D. Bioinformatic Approaches Including Predictive Metagenomic Profiling Reveal Characteristics of Bacterial Response to Petroleum Hydrocarbon Contamination in Diverse Environments. *Sci. Rep.* **2017**, *7*, 1–22. [CrossRef]
29. Zhang, L.; Loh, K.-C.; Lim, J.W.; Zhang, J. Bioinformatics Analysis of Metagenomics Data of Biogas-Producing Microbial Communities in Anaerobic Digesters: A Review. *Renew. Sustain. Energy Rev.* **2019**, *100*, 110–126. [CrossRef]
30. Suhartini, S.; Heaven, S.; Banks, C. Comparison of Mesophilic and Thermophilic Anaerobic Digestion of Sugar Beet Pulp: Performance, Dewaterability and Foam Control. *Bioresour. Technol.* **2013**, *152*, 202–211. [CrossRef]
31. Riggio, S.; Hernandez Shek, M.; Torrijos, M.; Vives, G.; Esposito, G.; van Hullebusch, E.; Steyer, J.-P.; Escudié, R. Comparison of the Mesophilic and Thermophilic Anaerobic Digestion of Spent Cow Bedding in Leach-Bed Reactors. *Bioresour. Technol.* **2017**, *234*, 466–471. [CrossRef] [PubMed]
32. Munk, B.; Guebitz, G.M.; Lebuhn, M. Influence of Nitrogen-Rich Substrates on Biogas Production and on the Methanogenic Community under Mesophilic and Thermophilic Conditions. *Anaerobe* **2017**, *46*, 146–154. [CrossRef]
33. Ju, F.; Zhang, T. Experimental Design and Bioinformatics Analysis for the Application of Metagenomics in Environmental Sciences and Biotechnology. *Environ. Sci. Technol.* **2015**, *49*, 12628–12640. [CrossRef] [PubMed]
34. Dong, B.; Yi, J.; Dai, L.; Dai, X. Evaluation of Several DNA Extraction Methods for Obtaining Total Community DNA from Anaerobic Digestion Sludge. *Procedia Environ. Sci.* **2013**, *18*, 856–863. [CrossRef]
35. Bareither, C.; Wolfe, G.; Mcmahon, K.; Benson, C. Microbial Diversity and Dynamics during Methane Production from Municipal Solid Waste. *Waste Manag.* **2013**, *33*, 1982–1992. [CrossRef]
36. Lee, S.-H.; Kang, H.-J.; Lee, Y.; Lee, T.; Han, K.; Choi, Y.; Park, H.-D. Monitoring Bacterial Community Structure and Variability in Time Scale in Full-Scale Anaerobic Digesters. *J. Environ. Monit. JEM* **2012**, *14*, 1893–1905. [CrossRef]
37. Sun, L.; Liu, T.; Müller, B.; Schnürer, A. The Microbial Community Structure in Industrial Biogas Plants Influences the Degradation Rate of Straw and Cellulose in Batch Tests. *Biotechnol. Biofuels* **2016**, *9*, 1–20. [CrossRef]
38. Han, G.; Shin, S.G.; Lee, J.; Shin, J.; Hwang, S. A Comparative Study on the Process Efficiencies and Microbial Community Structures of Six Full-Scale Wet and Semi-Dry Anaerobic Digesters Treating Food Wastes. *Bioresour. Technol.* **2017**, *245*, 869–875. [CrossRef]
39. Wei, Z.; Jin, D.; Deng, Y. Bioinformatics Tools and Applications in the Study of Environmental Microbial Metagenomics. *Microbiol. China* **2015**, *42*, 890–901. [CrossRef]
40. Chen, S.; Cheng, H.; Wyckoff, K.; He, Q. Linkages of Firmicutes and Bacteroidetes Populations to Methanogenic Process Performance. *J. Ind. Microbiol. Biotechnol.* **2016**, *43*, 771–781. [CrossRef]
41. Carabeo-Pérez, A.; Guerra-Rivera, G.; Ramos-Leal, M.; Jiménez-Hernández, J. Metagenomic Approaches: Effective Tools for Monitoring the Structure and Functionality of Microbiomes in Anaerobic Digestion Systems. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 9379–9390. [CrossRef] [PubMed]
42. Hardegen, J.; Latorre-Pérez, A.; Vilanova, C.; Günther, T.; Porcar, M.; Luschnig, O.; Simeonov, C.; Abendroth, C. Methanogenic Community Shifts during the Transition from Sewage Mono-Digestion to Co-Digestion of Grass Biomass. *Bioresour. Technol.* **2018**, *265*, 275–281. [CrossRef] [PubMed]
43. Brandt, C.; Bongcam-Rudloff, E.; Müller, B. Abundance Tracking by Long-Read Nanopore Sequencing of Complex Microbial Communities in Samples from 20 Different Biogas/Wastewater Plants. *Appl. Sci.* **2020**, *10*, 7518. [CrossRef]
44. Garcia, S.L.; Jangid, K.; Whitman, W.B.; Das, K.C. Transition of Microbial Communities during the Adaption to Anaerobic Digestion of Carrot Waste. *Bioresour. Technol.* **2011**, *102*, 7249–7256. [CrossRef] [PubMed]
45. Yu, B.; Lou, Z.; Zhang, D.; Shan, A.; Yuan, H.; Zhu, N.; Zhang, K. Variations of Organic Matters and Microbial Community in Thermophilic Anaerobic Digestion of Waste Activated Sludge with the Addition of Ferric Salts. *Bioresour. Technol.* **2015**, *179*, 291–298. [CrossRef] [PubMed]

46. Kim, S.; Bae, J.; Choi, O.; Ju, D.; Lee, J.; Sung, H.; Park, S.; Sang, B.-I.; Um, Y. A Pilot Scale Two-Stage Anaerobic Digester Treating Food Waste Leachate (FWL): Performance and Microbial Structure Analysis Using Pyrosequencing. *Process Biochem.* **2014**, *49*, 301–308. [CrossRef]
47. Cho, K.; Shin, S.G.; Kim, W.; Lee, J.; Lee, C.; Hwang, S. Microbial Community Shifts in a Farm-Scale Anaerobic Digester Treating Swine Waste: Correlations between Bacteria Communities Associated with Hydrogenotrophic Methanogens and Environmental Conditions. *Sci. Total Environ.* **2017**, *601–602*, 167–176. [CrossRef]
48. Lee, J.; Han, G.; Shin, S.G.; Koo, T.; Cho, K.; Kim, W.; Hwang, S. Seasonal Monitoring of Bacteria and Archaea in a Full-Scale Thermophilic Anaerobic Digester Treating Food Waste-Recycling Wastewater: Correlations between Microbial Community Characteristics and Process Variables. *Chem. Eng. J.* **2016**, *300*, 291–299. [CrossRef]
49. Leite, A.; Janke, L.; Harms, H.; Richnow, H.; Nikolausz, M. Lessons Learned from the Microbial Ecology Resulting from Different Inoculation Strategies for Biogas Production from Waste Products of the Bioethanol/Sugar Industry. *Biotechnol. Biofuels* **2016**, *9*, 1–16. [CrossRef]
50. Li, L.; He, Q.; Ma, Y.; Wang, X.; Peng, X. A Mesophilic Anaerobic Digester for Treating Food Waste: Process Stability and Microbial Community Analysis Using Pyrosequencing. *Microb. Cell Factories* **2016**, *15*, 1–11. [CrossRef]
51. Yu, Z.; Wen, X.; Xu, M.; Huang, X. Characteristics of Extracellular Polymeric Substances and Bacterial Communities in an Anaerobic Membrane Bioreactor Coupled with Online Ultrasound Equipment. *Bioresour. Technol.* **2012**, *117*, 333–340. [CrossRef]
52. Ho, D.; Jensen, P.; Batstone, D. Effects of Temperature and Hydraulic Retention Time on Acetotrophic Pathways and Performance in High-Rate Sludge Digestion. *Environ. Sci. Technol.* **2014**, *48*, 6468–6476. [CrossRef]
53. Lebuhn, M.; Hanreich, A.; Klocke, M.; Schlueter, A.; Bauer, C.; Pérez, C. Towards Molecular Biomarkers for Biogas Production from Lignocellulose-Rich Substrates. *Anaerobe* **2014**, *29*, 10–21. [CrossRef]
54. 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. [CrossRef] [PubMed]
55. Oosterkamp, M.; Méndez-García, C.; Kim, C.-H.; Bauer, S.; Ibáñez, A.; Zimmerman, S.; Hong, P.; Cann, I.; Mackie, R. Lignocellulose-Derived Thin Stillage Composition and Efficient Biological Treatment with a High-Rate Hybrid Anaerobic Bioreactor System. *Biotechnol. Biofuels* **2016**, *9*, 1–15. [CrossRef] [PubMed]
56. Belostotskiy, D.E.; Ziganshina, E.E.; Siniagina, M.; Boulygina, E.A.; Miluykov, V.A.; Ziganshin, A.M. Impact of the Substrate Loading Regime and Phosphoric Acid Supplementation on Performance of Biogas Reactors and Microbial Community Dynamics during Anaerobic Digestion of Chicken Wastes. *Bioresour. Technol.* **2015**, *193*, 42–52. [CrossRef] [PubMed]
57. Ziganshina, E.; Belostotskiy, D.; Ilinskaya, O.; Boulygina, E.; Grigoryeva, T.; Ziganshin, A. Effect of the Organic Loading Rate Increase and the Presence of Zeolite on Microbial Community Composition and Process Stability During Anaerobic Digestion of Chicken Wastes. *Microb. Ecol.* **2015**, *70*, 948–960. [CrossRef]
58. 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]
59. Solli, L.; Håvelsrud, O.E.; Horn, S.J.; Rike, A.G. A Metagenomic Study of the Microbial Communities in Four Parallel Biogas Reactors. *Biotechnol. Biofuels* **2014**, *7*, 146. [CrossRef]
60. Maspolim, Y.; Zhou, Y.; Guo, C.; Xiao, K.; Ng, W.J. Determination of the Archaeal and Bacterial Communities in Two-Phase and Single-Stage Anaerobic Systems by 454 Pyrosequencing. *J. Environ. Sci.* **2015**, *36*, 121–129. [CrossRef] [PubMed]
61. Cardinali-Rezende, J.; Rojas-Ojeda, P.; Nascimento, A.; Sanz, J. Proteolytic Bacterial Dominance in a Full-Scale Municipal Solid Waste Anaerobic Reactor Assessed by 454 Pyrosequencing Technology. *Chemosphere* **2016**, *146*, 519–525. [CrossRef]
62. Azizi, A.; Kim, W.; Lee, J.H. Comparison of Microbial Communities during the Anaerobic Digestion of Gracilaria under Mesophilic and Thermophilic Conditions. *World J. Microbiol. Biotechnol.* **2016**, *32*, 158. [CrossRef]
63. Wirth, R.; Kovács, E.; Maróti, G.; Bagi, Z.; Rákhely, G.; Kovacs, K. Characterization of a Biogas—Producing Microbial Community by Short-Read next Generation DNA Sequencing. *Biotechnol. Biofuels* **2012**, *5*, 41. [CrossRef] [PubMed]
64. Martínez, M.A.; Romero, H.; Perotti, N.I. Two Amplicon Sequencing Strategies Revealed Different Facets of the Prokaryotic Community Associated with the Anaerobic Treatment of Vinasses from Ethanol Distilleries. *Bioresour. Technol.* **2014**, *153*, 388–392. [CrossRef] [PubMed]
65. Campanaro, S.; Treu, L.; Kougias, P.; De Francisci, D.; Valle, G.; Angelidaki, I. Metagenomic Analysis and Functional Characterization of the Biogas Microbiome Using High Throughput Shotgun Sequencing and a Novel Binning Strategy. *Biotechnol. Biofuels* **2016**, *9*, 1–17. [CrossRef]
66. Begmatov, S.; Dorofeev, A.; Kadnikov, V.; Beletsky, A.; Pimenov, N.; Ravin, N.; Mardanov, A. The Structure of Microbial Communities of Activated Sludge of Large-Scale Wastewater Treatment Plants in the City of Moscow. *Sci. Rep.* **2022**, *12*, 3458. [CrossRef]
67. Krause, L.; Diaz, N.N.; Edwards, R.A.; Gartemann, K.-H.; Krömeke, H.; Neuweger, H.; Pühler, A.; Runte, K.J.; Schlüter, A.; Stoye, J.; et al. Taxonomic Composition and Gene Content of a Methane-Producing Microbial Community Isolated from a Biogas Reactor. *J. Biotechnol.* **2008**, *136*, 91–101. [CrossRef]
68. Mitra, S.; Rupek, P.; Richter, D.; Urich, T.; Jack, G.; Meyer, F.; Wilke, A.; Huson, D. Functional Analysis of Metagenomes and Metatranscriptomes Using SEED and KEGG. *BMC Bioinform.* **2011**, *12* (Suppl. 1), S21. [CrossRef]
69. Shaw, G.T.-W.; Liu, A.-C.; Weng, C.-Y.; Chou, C.-Y.; Wang, D. Inferring Microbial Interactions in Thermophilic and Mesophilic Anaerobic Digestion of Hog Waste. *PLoS ONE* **2017**, *12*, e0181395. [CrossRef]

70. Guo, X.; Wang, C.; Sun, F.; Zhu, W.; Wu, W.-X. A Comparison of Microbial Characteristics between the Thermophilic and Mesophilic Anaerobic Digesters Exposed to Elevated Food Waste Loadings. *Bioresour. Technol.* **2013**, *152*, 420–428. [CrossRef]
71. Lu, X.; Rao, S.; Shen, Z.; Lee, P.K.H. Substrate Induced Emergence of Different Active Bacterial and Archaeal Assemblages during Biomethane Production. *Bioresour. Technol.* **2013**, *148*, 517–524. [CrossRef] [PubMed]
72. Wilkins, D.; Rao, S.; Lu, X.; Lee, P. Effects of Sludge Inoculum and Organic Feedstock on Active Microbial Communities and Methane Yield during Anaerobic Digestion. *Front. Microbiol.* **2015**, *6*, 1–11. [CrossRef] [PubMed]
73. Pope, P.B.; Vivekanand, V.; Eijsink, V.G.H.; Horn, S.J. Microbial Community Structure in a Biogas Digester Utilizing the Marine Energy Crop Saccharina Latissima. *3 Biotech* **2013**, *3*, 407–414. [CrossRef] [PubMed]
74. Kröber, M.; Bekel, T.; Diaz, N.N.; Goesmann, A.; Jaenicke, S.; Krause, L.; Miller, D.; Runte, K.J.; Viehöver, P.; Pühler, A.; et al. 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]
75. Yun, Y.-M.; Kim, D.-H.; Cho, S.-K.; Shin, H.-S.; Jung, K.-W.; Kim, H.-W. Mitigation of Ammonia Inhibition by Internal Dilution in High-Rate Anaerobic Digestion of Food Waste Leachate and Evidences of Microbial Community Response. *Biotechnol. Bioeng.* **2016**, *113*, 1892–1901. [CrossRef] [PubMed]
76. Yan, W.; Wang, N.; Wei, D.; Liang, C.; Chen, X.; Liu, L.; Shi, J. Bacterial Community Compositions and Nitrogen Metabolism Function in a Cattle Farm Wastewater Treatment Plant Revealed by Illumina High-Throughput Sequencing. *Environ. Sci. Pollut. Res.* **2021**, *28*, 40895–40907. [CrossRef]
77. Oksanen, J.; Kindt, R.; Legendre, P.; Hara, B.; Henry, M.; Stevens, H. The Vegan Package. 2007. Available online: https://www.researchgate.net/publication/228975085 (accessed on 22 October 2022).
78. Morris, E.K.; Caruso, T.; Buscot, F.; Fischer, M.; Hancock, C.; Maier, T.S.; Meiners, T.; Müller, C.; Obermaier, E.; Prati, D.; et al. Choosing and Using Diversity Indices: Insights for Ecological Applications from the German Biodiversity Exploratories. *Ecol. Evol.* **2014**, *4*, 3514–3524. [CrossRef]
79. Barrantes, G.; Sandoval, L. Conceptual and Statistical Problems Associated with the Use of Diversity Indices in Ecology. *Rev. De Biol. Trop.* **2009**, *57*, 451–460. [CrossRef]
80. Smith, A.M.; Sharma, D.; Lappin-Scott, H.; Burton, S.; Huber, D.H. Microbial Community Structure of a Pilot-Scale Thermophilic Anaerobic Digester Treating Poultry Litter. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 2321–2334. [CrossRef]
81. Rademacher, A.; Zakrzewski, M.; Schlueter, A.; Schönberg, M.; Szczepanowski, R.; Goesmann, A.; Pühler, A.; Klocke, M. Characterization of Microbial Biofilms in a Thermophilic Biogas System by High-Throughput Metagenome Sequencing. *FEMS Microbiol. Ecol.* **2011**, *79*, 785–799. [CrossRef]
82. Güllert, S.; Fischer, M.; Turaev, D.; Noebauer, B.; Ilmberger, N.; Wemheuer, B.; Alawi, M.; Rattei, T.; Daniel, R.; Schmitz, R.; et al. 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*, 1–20. [CrossRef]
83. Li, A.; Chu, Y.; Wang, X.; Ren, L.; Yu, J.; Liu, X.; Yan, J.; Zhang, L.; Wu, S.; Li, S.Z. A Pyrosequencing-Based Metagenomic Study of Methane-Producing Microbial Community in Solid-State Biogas Reactor. *Biotechnol. Biofuels* **2013**, *6*, 3. [CrossRef] [PubMed]
84. Wang, Q.; Garrity, G.; Tiedje, J.; Cole, J.R. Naive Bayesian Classifier for Rapid Assignment of RRNA Sequences into the New Bacterial Taxonomy. *Appl. Environ. Microbiol.* **2007**, *73*, 5264–5267. [CrossRef] [PubMed]
85. Xie, Z.; Wang, Z.; Wang, Q.; Zhu, C.; Wu, Z. An Anaerobic Dynamic Membrane Bioreactor (AnDMBR) for Landfill Leachate Treatment: Performance and Microbial Community Identification. *Bioresour. Technol.* **2014**, *161*, 29–39. [CrossRef]
86. Xu, Z.; Hansen, M.A.; Hansen, L.H.; Jacquiod, S.; Sørensen, S.J. Bioinformatic Approaches Reveal Metagenomic Characterization of Soil Microbial Community. *PLoS ONE* **2014**, *9*, e93445. [CrossRef]
87. Zhang, J.; Loh, K.-C.; Li, W.; Lim, J.W.; Dai, Y.; Tong, Y.W. Three-Stage Anaerobic Digester for Food Waste. *Appl. Energy* **2017**, *194*, 287–295. [CrossRef]
88. Zhang, J.; Loh, K.-C.; Lee, J.; Wang, C.-H.; Dai, Y.; Tong, Y. Three-Stage Anaerobic Co-Digestion of Food Waste and Horse Manure. *Sci. Rep.* **2017**, *7*, 1269. [CrossRef]
89. Wong, M.T.; Zhang, D.; Li, J.; Hui, R.; Tun, H.; Brar, M.; Park, T.; Chen, Y.; Leung, F. Towards a Metagenomic Understanding on Enhanced Biomethane Production from Waste Activated Sludge after PH 10 Pretreatment. *Biotechnol. Biofuels* **2013**, *6*, 38. [CrossRef]
90. 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. [CrossRef]
91. Fernando Herrera Adarme, O.; Eduardo Lobo Baêta, B.; Cardoso Torres, M.; Camilo Otalora Tapiero, F.; Vinicius Alves Gurgel, L.; de Queiroz Silva, S.; Francisco de Aquino, S. Biogas Production by Anaerobic Co-Digestion of Sugarcane Biorefinery Byproducts: Comparative Analyses of Performance and Microbial Community in Novel Single-and Two-Stage Systems. *Bioresour. Technol.* **2022**, *354*, 127185. [CrossRef]
92. Martínez, E.J.; Sotres, A.; Arenas, C.B.; Blanco, D.; Martínez, O.; Gómez, X. Improving Anaerobic Digestion of Sewage Sludge by Hydrogen Addition: Analysis of Microbial Populations and Process Performance. *Energies* **2019**, *12*, 1228. [CrossRef]
93. Parks, D.H.; Tyson, G.W.; Hugenholtz, P.; Beiko, R.G. STAMP: Statistical Analysis of Taxonomic and Functional Profiles. *Bioinformatics* **2014**, *30*, 3123–3124. [CrossRef]

94. Yu, H.; Wang, Z.; Wu, Z.; Zhu, C. Enhanced Waste Activated Sludge Digestion Using a Submerged Anaerobic Dynamic Membrane Bioreactor: Performance, Sludge Characteristics and Microbial Community. *Sci. Rep.* **2016**, *6*, 20111. [CrossRef] [PubMed]
95. Bulgarelli, D.; Rott, M.; Schlaeppi, K.; Themaat, E.; Ahmadinejad, N.; Assenza, F.; Rauf, P.; Huettel, B.; Reinhardt, R.; Schmelzer, E.; et al. Revealing Structure and Assembly Cues for Arabidopsis Root-Inhabiting Bacterial Microbiota. *Nature* **2012**, *488*, 91–95. [CrossRef] [PubMed]
96. Hamilton, N. Ggtern: An Extension to "Ggplot2", for the Creation of Ternary Diagrams. R Package Version 2.1.4. 2016. Available online: http://CRAN.R-project.org/package=ggtern (accessed on 13 November 2022).
97. Zhuravleva, E.A.; Shekhurdina, S.V.; Kotova, I.B.; Loiko, N.G.; Popova, N.M.; Kryukov, E.; Kovalev, A.A.; Kovalev, D.A.; Litti, Y.V. Effects of Various Materials Used to Promote the Direct Interspecies Electron Transfer on Anaerobic Digestion of Low-Concentration Swine Manure. *Sci. Total Environ.* **2022**, *839*, 156073. [CrossRef]
98. Lu, Y. Microbial Ecology of Fermentative Microbes in Anaerobic Granules. Ph.D. Thesis, School of Chemical Engineering, the University of Queensland, Brisbane, Australia, 2014. [CrossRef]
99. Breton-Deval, L.; Salinas-Peralta, I.; Alarcón Aguirre, J.S.; Sulbarán-Rangel, B.; Gurubel Tun, K.J. Taxonomic Binning Approaches and Functional Characteristics of the Microbial Community during the Anaerobic Digestion of Hydrolyzed Corncob. *Energies* **2021**, *14*, 66. [CrossRef]
100. Bibby, K.; Viau, E.; Peccia, J. Pyrosequencing of the 16S RRNA Gene to Reveal Bacterial Pathogen Diversity in Biosolids. *Water Res.* **2010**, *44*, 4252–4260. [CrossRef] [PubMed]
101. Li, N.; Xue, Y.; Chen, S.; Takahashi, J.; Dai, L.; Dai, X. Methanogenic Population Dynamics Regulated by Bacterial Community Responses to Protein-Rich Organic Wastes in a High Solid Anaerobic Digester. *Chem. Eng. J.* **2017**, *317*, 444–453. [CrossRef]
102. 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] [PubMed]
103. Ramette, A. Multivariate Analyses in Microbial Ecology. *FEMS Microbiol. Ecol.* **2007**, *62*, 142–160. [CrossRef]
104. Cole's, J.R.; Wang, Q.; Fish, J.; Chai, B.; Mcgarrell, D.; Sun, Y.; Brown, C.T.; Porras-Alfaro, A.; Kuske, C.; Tiedje, J. Ribosomal DATABASE PROject: Data and Tools for High Throughput RRNA Analysis. *Nucleic Acids Res.* **2013**, *42*, D633–D642. [CrossRef]
105. Lee, J.; Hwang, B.; Koo, T.; Shin, S.G.; Kim, W.; Hwang, S. Temporal Variation in Methanogen Communities of Four Different Full-Scale Anaerobic Digesters Treating Food Waste-Recycling Wastewater. *Bioresour. Technol.* **2014**, *168*, 59–63. [CrossRef]
106. Ziganshina, E.E.; Ibragimov, E.M.; Vankov, P.Y.; Miluykov, V.A.; Ziganshin, A.M. Comparison of Anaerobic Digestion Strategies of Nitrogen-Rich Substrates: Performance of Anaerobic Reactors and Microbial Community Diversity. *Waste Manag.* **2017**, *59*, 160–171. [CrossRef] [PubMed]
107. Dixon, P. VEGAN, a Package of R Functions for Community Ecology. *J. Veg. Sci.* **2003**, *14*, 927–930. [CrossRef]
108. Paliy, O.; Shankar, V. Application of Multivariate Statistical Techniques in Microbial Ecology. *Mol. Ecol.* **2016**, *25*, 1032–1057. [CrossRef]
109. Lepš, J.; Šmilauer, P. *Multivariate Analysis of Ecological Data Using CANOCO*; Cambridge University Press: Cambridge, UK, 2003. [CrossRef]
110. Tong, J.; Liu, J.; Zheng, X.; Zhang, J.; Ni, X.; Chen, M.; Wei, Y. Fate of Antibiotic Resistance Bacteria and Genes during Enhanced Anaerobic Digestion of Sewage Sludge by Microwave Pretreatment. *Bioresour. Technol.* **2016**, *217*, 37–43. [CrossRef]
111. Alcántara-Hernández, R.; Taş, N.; Carlos, S.; Durán-Moreno, A.; Falcón, L. Microbial Dynamics in Anaerobic Digestion Reactors for Treating Organic Urban Residues during the Start-up Process. *Lett. Appl. Microbiol.* **2017**, *64*, 438–445. [CrossRef]
112. Boeckmann, B.; Bairoch, A.; Apweiler, R.; Blatter, M.-C.; Estreicher, A.; Gasteiger, E.; Martin, M.; Michoud, K.; O'Donovan, C.; Phan, I.; et al. The Swiss-Prot Protein Knowledgebase and Its Supplement TrEMBL in 2003. *Nucleic Acids Res.* **2003**, *31*, 365–370. [CrossRef]
113. Schlueter, A.; Bekel, T.; Diaz, N.; Dondrup, M.; Eichenlaub, R.; Gartemann, K.-H.; Krahn, I.; Krause, L.; Krömeke, H.; Kruse, O.; et al. The Metagenome of a Biogas-Producing Microbial Community of a Production-Scale Biogas Plant Fermenter Analyzed by the 454-Pyrosequencing Technology. *J. Biotechnol.* **2008**, *136*, 77–90. [CrossRef]
114. Meyer, F.; Goesmann, A.; McHardy, A.C.; Bartels, D.; Bekel, T.; Clausen, J.; Kalinowski, J.; Linke, B.; Rupp, O.; Giegerich, R.; et al. GenDB—An Open Source Genome Annotation System for Prokaryote Genomes. *Nucleic Acids Res.* **2003**, *31*, 2187–2195. [CrossRef]
115. Markowitz, V.; Chen, I.-M.; Chu, K.; Szeto, E.; Palaniappan, K.; Pillay, M.; Ratner, A.; Huang, J.; Pagani, I.; Tringe, S.; et al. IMG/M 4 Version of the Integrated Metagenome Comparative Analysis System. *Nucleic Acids Res.* **2013**, *42*, D568–D573. [CrossRef] [PubMed]
116. Kanehisa, M.; Araki, M.; Goto, S.; Hattori, M.; Hirakawa, M.; Itoh, M.; Katayama, T.; Kawashima, S.; Okuda, S.; Tokimatsu, T.; et al. KEGG for Linking Genomes to Life and the Environment. *Nucleic Acids Res.* **2008**, *36*, D480–D484. [CrossRef] [PubMed]
117. Hunter, S.; Apweiler, R.; Attwood, T.K.; Bairoch, A.; Bateman, A.; Binns, D.; Bork, P.; Das, U.; Daugherty, L.; Duquenne, L.; et al. InterPro: The Integrative Protein Signature Database. *Nucleic Acids Res.* **2009**, *37* (Suppl. 1), D211–D215. [CrossRef]
118. Finn, R.D.; Bateman, A.; Clements, J.; Coggill, P.; Eberhardt, R.Y.; Eddy, S.R.; Heger, A.; Hetherington, K.; Holm, L.; Mistry, J.; et al. Pfam: The Protein Families Database. *Nucleic Acids Res.* **2014**, *42*, D222–D230. [CrossRef] [PubMed]
119. Consortium, G.O. The Gene Ontology (GO) Database and Informatics Resource. *Nucleic Acids Res.* **2004**, *32* (Suppl. 1), D258–D261. [CrossRef] [PubMed]

120. Zhou, J. Microarrays for Bacterial Detection and Microbial Community Analysis. *Curr. Opin. Microbiol.* **2003**, *6*, 288–294. [CrossRef]
121. Hoff, K.J. The Effect of Sequencing Errors on Metagenomic Gene Prediction. *BMC Genom.* **2009**, *10*, 520. [CrossRef]
122. Jünemann, S.; Kleinboelting, N.; Jaenicke, S.; Henke, C.; Hassa, J.; Nelkner, J.; Stolze, Y.; Albaum, S.; Schlueter, A.; Goesmann, A.; et al. Bioinformatics for NGS-Based Metagenomics and the Application to Biogas Research. *J. Biotechnol.* **2017**, *261*, 10–23. [CrossRef] [PubMed]
123. Cardona, C.; Weisenhorn, P.; Henry, C.; Jack, G. Network-Based Metabolic Analysis and Microbial Community Modeling. *Curr. Opin. Microbiol.* **2016**, *31*, 124–131. [CrossRef]
124. Faust, K.; Lahti, L.; Gonze, D.; de Vos, W.M.; Raes, J. Metagenomics Meets Time Series Analysis: Unraveling Microbial Community Dynamics. *Curr. Opin. Microbiol.* **2015**, *25*, 56–66. [CrossRef] [PubMed]
125. 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] [PubMed]
126. Luo, G.; Li, B.; Li, L.; Zhang, T.; Angelidaki, I. Antibiotic Resistance Genes and Correlations with Microbial Community and Metal Resistance Genes in Full-Scale Biogas Reactors as Revealed by Metagenomic Analysis. *Environ. Sci. Technol.* **2017**, *51*, 4069–4080. [CrossRef] [PubMed]
127. Koo, T.; Shin, S.G.; Lee, J.; Han, G.; Kim, W.; Cho, K.; Hwang, S. Identifying Methanogen Community Structures and Their Correlations with Performance Parameters in Four Full-Scale Anaerobic Sludge Digesters. *Bioresour. Technol.* **2017**, *228*, 368–373. [CrossRef]
128. Hidaka, T.; Tsushima, I.; Tsumori, J. Comparative Analyses of Microbial Structures and Gene Copy Numbers in the Anaerobic Digestion of Various Types of Sewage Sludge. *Bioresour. Technol.* **2018**, *253*, 315–322. [CrossRef] [PubMed]
129. Zhang, J.; Li, W.; Lee, J.; Loh, K.-C.; Dai, Y.; Tong, Y.W. Enhancement of Biogas Production in Anaerobic Co-Digestion of Food Waste and Waste Activated Sludge by Biological Co-Pretreatment. *Energy* **2017**, *137*, 479–486. [CrossRef]
130. Suksong, W.; Kongjan, P.; Prasertsan, P.; Imai, T.; O-Thong, S. Optimization and Microbial Community Analysis for Production of Biogas from Solid Waste Residues of Palm Oil Mill Industry by Solid-State Anaerobic Digestion. *Bioresour. Technol.* **2016**, *214*, 166–174. [CrossRef]
131. Zhang, L.; Zhang, J.; Loh, K.-C. Activated Carbon Enhanced Anaerobic Digestion of Food Waste–Laboratory-Scale and Pilot-Scale Operation. *Waste Manag.* **2018**, *75*, 270–279. [CrossRef]
132. Greening, C.; Geier, R.; Wang, C.; Woods, L.C.; Morales, S.E.; McDonald, M.J.; Rushton-Green, R.; Morgan, X.C.; Koike, S.; Leahy, S.C.; et al. Diverse Hydrogen Production and Consumption Pathways Influence Methane Production in Ruminants. *ISME J.* **2019**, *13*, 2617–2632. [CrossRef]
133. Zhang, L.; Gong, X.; Chen, Z.; Zhou, Y. Genome-Centric Metagenomics Analysis Revealed the Metabolic Function of Abundant Microbial Communities in Thermal Hydrolysis-Assisted Thermophilic Anaerobic Digesters under Propionate Stress. *Bioresour. Technol.* **2022**, *360*, 127574. [CrossRef]
134. Pilarska, A.A.; Wolna-Maruwka, A.; Niewiadomska, A.; Pilarski, K.; Adamski, M.; Grzyb, A.; Grządziel, J.; Gałązka, A. Silica/Lignin Carrier as a Factor Increasing the Process Performance and Genetic Diversity of Microbial Communities in Laboratory-Scale Anaerobic Digesters. *Energies* **2021**, *14*, 4429. [CrossRef]
135. Kim, N.-K.; Lee, S.-H.; Kim, Y.; Park, H.-D. Current understanding and perspectives in anaerobic digestion based on genome-resolved metagenomic approaches. *Bioresour. Technol.* **2022**, *344*, 126350. [CrossRef] [PubMed]
136. Jiang, Y.; Banks, C.; Zhang, Y.; Heaven, S.; Longhurst, P. Quantifying the Percentage of Methane Formation via Acetoclastic and Syntrophic Acetate Oxidation Pathways in Anaerobic Digesters. *Waste Manag.* **2018**, *71*, 749–756. [CrossRef] [PubMed]
137. 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]
138. Yang, Z.; Wang, W.; He, Y.; Zhang, R.; Liu, G. Effect of Ammonia on Methane Production, Methanogenesis Pathway, Microbial Community and Reactor Performance under Mesophilic and Thermophilic Conditions. *Renew. Energy* **2018**, *125*, 915–925. [CrossRef]
139. Zhang, S.; Xiao, M.; Liang, C.; Chui, C.; Wang, N.; Shi, J.; Liu, L. Multivariate Insights into Enhanced Biogas Production in Thermophilic Dry Anaerobic Co-Digestion of Food Waste with Kitchen Waste or Garden Waste: Process Properties, Microbial Communities and Metagenomic Analyses. *Bioresour. Technol.* **2022**, *361*, 127684. [CrossRef]
140. Fan, Y.; Niu, X.; Zhang, D.-Q.; Lin, Z.; Fu, M.; Zhou, S. Analysis of the Characteristics of Phosphine Production by Anaerobic Digestion Based on Microbial Community Dynamics, Metabolic Pathways, and Isolation of the Phosphate-Reducing Strain. *Chemosphere* **2021**, *262*, 128213. [CrossRef]
141. Cai, G.; Zhao, L.; Wang, T.; Lv, N.; Li, J.; Ning, J.; Pan, X.; Zhu, G. Variation of Volatile Fatty Acid Oxidation and Methane Production during the Bioaugmentation of Anaerobic Digestion System: Microbial Community Analysis Revealing the Influence of Microbial Interactions on Metabolic Pathways. *Sci. Total Environ.* **2021**, *754*, 142425. [CrossRef]
142. Jin, W.; Xu, X.; Yang, F. Application of Rumen Microorganisms for Enhancing Biogas Production of Corn Straw and Livestock Manure in a Pilot-Scale Anaerobic Digestion System: Performance and Microbial Community Analysis. *Energies* **2018**, *11*, 920. [CrossRef]

143. Murillo-Roos, M.; Uribe-Lorío, L.; Fuentes-Schweizer, P.; Vidaurre-Barahona, D.; Brenes-Guillén, L.; Jiménez, I.; Arguedas, T.; Liao, W.; Uribe, L. Biogas Production and Microbial Communities of Mesophilic and Thermophilic Anaerobic Co-Digestion of Animal Manures and Food Wastes in Costa Rica. *Energies* **2022**, *15*, 3252. [CrossRef]
144. Lee, J.; Shin, S.G.; Jang, H.M.; Kim, Y.; Lee, J.; Kim, Y. Characterization of Antibiotic Resistance Genes in Representative Organic Solid Wastes: Food Waste-Recycling Wastewater, Manure, and Sewage Sludge. *Sci. Total Environ.* **2016**, *579*, 1692–1698. [CrossRef]
145. Jang, H.M.; Shin, J.; Choi, S.; Shin, S.G.; Park, K.Y.; Cho, J.; Kim, Y.M. Fate of Antibiotic Resistance Genes in Mesophilic and Thermophilic Anaerobic Digestion of Chemically Enhanced Primary Treatment (CEPT) Sludge. *Bioresour. Technol.* **2017**, *244*, 433–444. [CrossRef]
146. Oulas, A.; Pavloudi, C.; Polymenakou, P.; Pavlopoulos, G.; Papanikolaou, N.; Kotoulas, G.; Arvanitidis, C.; Iliopoulos, I. Metagenomics: Tools and Insights for Analyzing Next-Generation Sequencing Data Derived from Biodiversity Studies. *Bioinform. Biol. Insights* **2015**, *9*, 75–88. [CrossRef] [PubMed]
147. Pu, C.; Liu, H.; Ding, G.; Sun, Y.; Yu, X.; Chen, J.; Ren, J.; Gong, X. Impact of Direct Application of Biogas Slurry and Residue in Fields: In Situ Analysis of Antibiotic Resistance Genes from Pig Manure to Fields. *J. Hazard. Mater.* **2017**, *344*, 441–449. [CrossRef]
148. Yin, X.; Jiang, X.-T.; Chai, B.; Li, L.; Yang, Y.; Cole's, J.R.; Tiedje, J.; Zhang, T. ARGs-OAP v2.0 with an Expanded SARG Database and Hidden Markov Models for Enhancement Characterization and Quantification of Antibiotic Resistance Genes in Environmental Metagenomes. *Bioinformatics* **2018**, *34*, 2263–2270. [CrossRef]
149. Hanemaaijer, M.; Röling, W.; Olivier, B.; Khandelwal, R.; Teusink, B.; Bruggeman, F. Systems Modeling Approaches for Microbial Community Studies: From Metagenomics to Inference of the Community Structure. *Front. Microbiol.* **2015**, *6*, 213. [CrossRef] [PubMed]
150. Antonella, S.; Wanjiku, M.F.; Oliver, E. A Diverse Community to Study Communities: Integration of Experiments and Mathematical Models to Study Microbial Consortia. *J. Bacteriol.* **2017**, *199*, e00865-16. [CrossRef]
151. Shaw, G.; Pao, Y.-Y.; Wang, D. MetaMIS: A Metagenomic Microbial Interaction Simulator Based on Microbial Community Profiles. *BMC Bioinform.* **2016**, *17*, 1–12. [CrossRef] [PubMed]
152. Yan, J.; Chuai, G.; Qi, T.; Shao, F.; Zhou, C.; Zhu, C.; Yang, J.; Yu, Y.; Shi, C.; Kang, N.; et al. MetaTopics: An Integration Tool to Analyze Microbial Community Profile by Topic Model. *BMC Genom.* **2017**, *18*, 962. [CrossRef]
153. Hanemaaijer, M.; Olivier, B.G.; Röling, W.F.M.; Bruggeman, F.J.; Teusink, B. Model-Based Quantification of Metabolic Interactions from Dynamic Microbial-Community Data. *PLoS ONE* **2017**, *12*, e0173183. [CrossRef] [PubMed]
154. Larsen, P.; Gibbons, S.; Jack, G. Modeling Microbial Community Structure and Functional Diversity Across Time and Space. *FEMS Microbiol. Lett.* **2012**, *332*, 91–98. [CrossRef]
155. Succurro, A.; Ebenhöh, O. Review and Perspective on Mathematical Modeling of Microbial Ecosystems. *Biochem. Soc. Trans.* **2018**, *46*, BST20170265. [CrossRef]
156. Chen, Y.; Lan, S.; Wang, L.; Dong, S.; Zhou, H.; Tan, Z.; Li, X. A Review: Driving Factors and Regulation Strategies of Microbial Community Structure and Dynamics in Wastewater Treatment Systems. *Chemosphere* **2017**, *174*, 173–182. [CrossRef] [PubMed]
157. Zhou, J.; Zhang, R.; Liu, F.; Yong, X.; Wu, X.; Zheng, T.; Jiang, M.; Jia, H. Biogas Production and Microbial Community Shift through Neutral PH Control during the Anaerobic Digestion of Pig Manure. *Bioresour. Technol.* **2016**, *217*, 44–49. [CrossRef] [PubMed]
158. Argyropoulos, A. Soft Sensor Development and Process Control of Anaerobic Digestion. Ph.D. Thesis, University of Exeter, Devon, UK, 2013; p. 281. Available online: http://hdl.handle.net/10871/15068 (accessed on 13 November 2022).
159. Xue, B.; Li, Z.; Wang, X.; Xi, H.; Cheng, S.; Xiaofeng, B.; Gao, R. Online Measurement of Alkalinity in Anaerobic Co-Digestion Using Linear Regression Method. *Int. J. Agric. Biol. Eng.* **2017**, *10*, 176–183. [CrossRef]
160. Amha, Y.M.; Anwar, M.Z.; Brower, A.; Jacobsen, C.S.; Stadler, L.B.; Webster, T.M.; Smith, A.L. Inhibition of Anaerobic Digestion Processes: Applications of Molecular Tools. *Bioresour. Technol.* **2018**, *247*, 999–1014. [CrossRef]
161. Dröge, J.; McHardy, A. Taxonomic Binning of Metagenome Samples Generated by Next-Generation Sequencing Technologies. *Brief. Bioinform.* **2012**, *13*, 646–655. [CrossRef]
162. Tabish, M.; Azim, S.; Husain, M.A.; Rehman, S.; Sarwar, T.; Ishqi, H. Bioinformatics Approaches in Studying Microbial Diversity. In *Management of Microbial Resources in the Environment*; Springer: Berlin/Heidelberg, Germany, 2013; pp. 119–140. [CrossRef]
163. Campanaro, S.; Treu, L.; Kougias, P.; Zhu, X.; Angelidaki, I. Taxonomy of Anaerobic Digestion Microbiome Reveals Biases Associated with the Applied High Throughput Sequencing Strategies. *Sci. Rep.* **2018**, *8*, 1–12. [CrossRef]
164. Widder, S.; Allen, R.; Pfeiffer, T.; Curtis, T.; Wiuf, C.; Sloan, W.; Cordero, O.X.; Brown, S.; Momeni, B.; Shou, W.; et al. Challenges in Microbial Ecology: Building Predictive Understanding of Community Function and Dynamics. *ISME J.* **2016**, *0*, 2557–2568. [CrossRef]
165. Hiraoka, S.; Yang, C.; Iwasaki, W. Metagenomics and Bioinformatics in Microbial Ecology: Current Status and Beyond. *Microbes Environ.* **2016**, *31*, 204–212. [CrossRef]
166. Tolvanen, K.E.S.; Karp, M.T. Molecular Methods for Characterizing Mixed Microbial Communities in Hydrogen-Fermenting Systems. *Int. J. Hydrogen Energy* **2011**, *36*, 5280–5288. [CrossRef]
167. Palaniswamy, D.; Ramesh, G.; Sivasankaran, S.; Kathiravan, N. Optimising Biogas from Food Waste Using a Neural Network Model. *Proc. Inst. Civ. Eng.-Munic. Eng.* **2017**, *170*, 221–229. [CrossRef]
168. Nair, V.V.; Dhar, H.; Kumar, S.; Thalla, A.K.; Mukherjee, S.; Wong, J.W.C. Artificial Neural Network Based Modeling to Evaluate Methane Yield from Biogas in a Laboratory-Scale Anaerobic Bioreactor. *Bioresour. Technol.* **2016**, *217*, 90–99. [CrossRef] [PubMed]

169. Antwi, P.; Li, J.; Opoku Boadi, P.; Meng, J.; Shi, E.; Deng, K.; Bondinuba, F. Estimation of Biogas and Methane Yields in an UASB Treating Potato Starch Processing Wastewater with Backpropagation Artificial Neural Network. *Bioresour. Technol.* **2016**, *228*, 106–115. [CrossRef] [PubMed]
170. Eng, A.; Borenstein, E. An Algorithm for Designing Minimal Microbial Communities with Desired Metabolic Capacities. *Bioinformatics* **2016**, *32*, 2008–2016. [CrossRef] [PubMed]