

Review

# Kinetics and Energy Yield in Anaerobic Digestion: Effects of Substrate Composition and Fundamental Operating Conditions

Krzysztof Pilarski <sup>1</sup> and Agnieszka A. Pilarska <sup>2,\*</sup>

<sup>1</sup> Department of Biosystems Engineering, Poznań University of Life Sciences, Wojska Polskiego 50, 60-627 Poznań, Poland; krzysztof.pilarski@up.poznan.pl

<sup>2</sup> Department of Hydraulic and Sanitary Engineering, Poznań University of Life Sciences, ul. Piątkowska 94A, 60-649 Poznań, Poland

\* Correspondence: agnieszka.pilarska@up.poznan.pl; Tel.: +48-61-846-65-93

## Abstract

This review relates the kinetics of anaerobic digestion (AD) to energy outcomes, including typical ranges of methane yields and volumetric methane productivities (down to hourly  $\text{g L}^{-1} \text{h}^{-1}$  scales relevant for industrial plants). It further translates these relationships into practical control principles that support stable, high methane productivity. Evidence spans substrate selection and co-digestion with emphasis on carbon/nitrogen (C/N) balance, pretreatment strategies, and reactor operation, linking process constraints with operating parameters to identify interventions that raise performance while limiting inhibition. Improving substrate accessibility is the primary step: pretreatment and co-digestion shift limitation beyond hydrolysis and allow safe increases in organic loading. Typical mesophilic operation involves hydraulic retention times of about 10–40 days for food waste and 20–60 days for different types of livestock manure and slowly degradable energy crops, with stable performance achieved when the solids retention time (SRT) is maintained longer than the hydraulic retention time (HRT). Stability is further governed by sustaining a low hydrogen partial pressure through hydrogenotrophic methanogenesis. Temperature and pH define practicable operating ranges; meanwhile, mixing should minimise diffusion resistance without damaging biomass structure. Early-warning indicators—volatile fatty acids (VFAs)/alkalinity, the propionate/acetate ratio, specific methanogenic activity, methane ( $\text{CH}_4$ )% and gas flow—enable timely adjustment of loading, retention, buffering, mixing intensity and micronutrient supply (Ni, Co, Fe, Mo). In practice, robust operation is generally associated with VFA/alkalinity ratios below about 0.3 and  $\text{CH}_4$  contents typically in the range of 50–70% ( $v/v$ ) in biogas. The review consolidates typical feedstock characteristics and biochemical methane potential (BMP) ranges, as well as outlines common reactor types with their advantages and limitations, linking operational choices to energy yield in combined heat and power (CHP) and biomethane pathways. Reported pretreatment effects span approximately 20–100% higher methane yields; for example, 18–37% increases after mechanical size reduction, around 20–30% gains at 120–121 °C for thermal treatments, and in some cases nearly a two-fold increase for more severe thermal or combined methods. Priorities are set for adaptive control, micronutrient management, biomass-retention strategies, and standardised monitoring, providing a coherent route from kinetic understanding to dependable energy performance and explain-

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ing how substrate composition, pretreatment, operating parameters, and kinetic constraints jointly determine methane and energy yield, with particular emphasis on early-warning indicators.

**Keywords:** anaerobic digestion; kinetics; energy yield; pretreatment; organic loading rate; hydraulic retention time; specific methanogenic activity

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## 1. Introduction

Climate change is among the most serious global challenges of our time. A major driver of this phenomenon is the emission of greenhouse gases resulting from the widespread use of fossil fuels as the primary energy source. Current assessments indicate that energy-related activities are responsible for the majority of anthropogenic greenhouse gas emissions, with global energy-related CO<sub>2</sub> (carbon dioxide) releases amounting to several tens of gigatonnes per year. One of the most significant consequences is the steady rise in mean global temperatures, which affects ecosystems, sea level, the availability of water resources, food security, and human health and life. In response, energy–climate policies are increasingly focused on curbing CO<sub>2</sub> emissions. Over the last decades, international and regional frameworks have progressively introduced targets for reducing greenhouse gas emissions and increasing the share of renewable energy in the overall energy mix. Against this backdrop, renewables have become a cornerstone of the energy transition. Biomass and waste are of particular importance because—unlike solar and wind—they can provide a stable, continuous energy supply regardless of weather or location. As such, this segment of renewable energy sources (RES) has the potential to play a substantial role in global CO<sub>2</sub> mitigation and in supporting sustainable development [1].

Waste materials, such as manure and post-harvest agricultural residues, are valuable energy feedstocks because their use does not compete with food production or agricultural land use [1,2]. Although intensive and poorly regulated land exploitation by profit-driven entities can raise concerns and affect public acceptance, the environmental and energy-efficiency benefits of this technology far outweigh the potential risks when appropriately governed and monitored.

Anaerobic digestion (AD) stands out among energy technologies because it can be implemented across a wide range of scales—from small farm-based units to large industrial facilities—while delivering multiple environmental and economic outcomes. The AD process is a biological process in which organic matter is decomposed under anaerobic conditions. Unlike aerobic processes, where microorganisms utilise oxygen and produce mainly CO<sub>2</sub> and water, anaerobic digestion proceeds through four principal stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The main products of the anaerobic digestion process are biogas and digestate. The biogas typically contains about 50–70% methane (CH<sub>4</sub>) and 30–50% carbon dioxide (CO<sub>2</sub>), with trace amounts of hydrogen sulphide (H<sub>2</sub>S), ammonia (NH<sub>3</sub>), water vapour, nitrogen, oxygen, and hydrogen also present [3]. Although CO<sub>2</sub> is a natural co-product of AD, appropriate biogas upgrading and off-gas treatment (e.g., CO<sub>2</sub> removal, H<sub>2</sub>S scrubbing) limit direct emissions and enable biomethane production. The resulting digestate, rich in organic matter and plant-available nutrients, can be used as a soil improver or biofertiliser, which represents an additional environmental benefit of the AD process, provided that its storage and field application are properly managed. Environmental performance is further improved by controlled digestate management (e.g., covered storage and appropriate land application) and by minimising fugitive emissions through leak detection and gas capture/flare systems [1,2]. In practice, however, the real mitigation potential of biogas systems depends not only on the

choice of substrate and technology but also on how efficiently the process kinetics are controlled and how close plants operate to their attainable methane and energy yields.

Biogas-based technologies can deliver three key functions for sustainable development: (i) supply renewable energy carriers, (ii) enable the management of diverse organic waste fractions, and (iii) support closing material and energy loops in agricultural systems [4]. When biogas replaces fossil energy carriers, and manure is no longer stored in open systems, the wider deployment of biogas systems can reduce net greenhouse gas emissions compared with conventional manure and organic waste management, improve waste management efficiency and strengthen the resilience of agriculture to environmental pressures. These advantages make anaerobic digestion one of the most promising energy technologies for the future. At the same time, the extent to which these advantages are realised in practice depends on maintaining sufficiently high methane yields and volumetric productivity under stable operating conditions.

Despite extensive research on anaerobic digestion, there is still a lack of a coherent and operational synthesis linking substrate composition and preparation with key operational parameters (organic loading rate (OLR), HRT/SRT, temperature, pH), kinetic limitations of individual stages, and their implications for overall energy yield. The aim of this review is therefore to integrate dispersed data to identify when and why hydrolysis or subsequent stages become rate-limiting, how pretreatment and co-digestion can shift these constraints and enable safe increases in OLR while maintaining  $SRT > HRT$ , and which early-warning indicators (e.g., VFA/alkalinity ratio, propionate/acetate ratio, specific methanogenic activity) allow preventive process control before inhibition occurs. In parallel, it summarises the typical ranges of key kinetic parameters, such as methane yields, substrate conversion rates, biomass yields and volumetric methane productivities, including indicative hourly values where available. These indicators are important both for assessing how fully the potential of given substrates is utilised and for comparing the performance of different plants. They are subsequently used to relate product formation, substrate consumption and biomass growth to the operating windows and stability margins of full-scale agricultural and agro-industrial anaerobic digestion facilities. Consequently, a set of evidence-based kinetic control rules is proposed, linking feedstock selection and conditioning with reactor configuration and the stability window (pH–T), to maximise stable  $CH_4$  yield and minimise process disturbances. This perspective—from mechanisms to operational decisions—forms the guiding framework of the entire article and justifies its focus on kinetics and energy yield.

## 2. Fundamentals of Anaerobic Digestion

Anaerobic digestion is a complex biological process in which microorganisms break down the organic matter in feedstocks under oxygen-free conditions [1,3]. The reactions yield a gas mixture dominated by methane and carbon dioxide, with smaller amounts of hydrogen ( $H_2$ ) and hydrogen sulphide, among others. An anaerobic environment is essential, as some of the microorganisms involved cannot survive in the presence of oxygen [1]. AD is conducted in hermetically sealed reactors, which ensures stable process conditions and control over operating parameters. The resulting biogas is directed to storage tanks and then most commonly used in combined heat and power systems. It can also be burned directly to generate heat or upgraded to biomethane for grid injection or use as a vehicle fuel, depending on local energy demand profiles and the availability of appropriate infrastructure. After digestion, a residue (digestate) remains in the reactor. It can be separated into liquid and solid fractions. Both contain valuable mineral nutrients and may be applied as natural fertilisers in agriculture, supporting a circular economy [3].

From a microbiological and kinetic perspective, the process is carried out by functionally distinct but interacting groups of microorganisms. Hydrolytic and fermentative

bacteria (mainly within the phyla *Firmicutes* and *Bacteroidetes*) initiate polymer breakdown and convert complex biopolymers into soluble sugars, amino acids, alcohols, and volatile fatty acids (VFAs) through glycolytic and fermentative pathways [1,5]. These intermediates are further processed by syntrophic acetogenic bacteria, which oxidise higher VFAs such as propionate and butyrate to acetate, CO<sub>2</sub> and H<sub>2</sub> in close association with hydrogenotrophic partners, while methanogenic Archaea (e.g., acetoclastic genera such as *Methanosaeta*/*Methanosarcina* and hydrogenotrophic *Methanobacteriales* and *Methanomicrobiales*) convert acetate and CO<sub>2</sub>/H<sub>2</sub> into methane via acetoclastic and hydrogenotrophic methanogenesis [3]. The balance between these trophic groups underpins the four classical stages of AD (hydrolysis, acidogenesis, acetogenesis, and methanogenesis) and determines whether intermediates accumulate or are efficiently channelled towards methane. In kinetic terms, the relative growth rates and sensitivities of these guilds govern which stage becomes rate-limiting under given operating conditions. Selected reactions and their implications for overall energy yield are analysed in more detail in Section 5.

AD is important for several reasons: it stabilises organic matter, reduces odour and uncontrolled methane emissions from manure storage, and recovers nutrients. The process also produces local, renewable energy, thereby increasing the usable energy recovered per unit of manure compared with conventional storage and land application. It is estimated that in the United States alone, more than one billion tonnes of animal manure are generated annually. Owing to its availability, it is among the most common feedstocks for AD [1]. On a global scale, agricultural activities generate several billion tonnes of animal manure and other organic residues each year, while the organic fraction of municipal solid waste and food-processing by-products together amounts to hundreds of millions of tonnes annually, which illustrates the substantial technical potential for anaerobic digestion as a treatment and energy-recovery option [5]. In livestock systems, the main sources of methane emissions are enteric fermentation in ruminants and open lagoons used to stabilise slurry. Manure management is estimated to account for around 10% of global agricultural greenhouse gas emissions [6,7]. By capturing biogas and substituting fossil fuels, deploying AD in large farms can reduce net greenhouse gas emissions relative to open storage and land application of raw manure, while enabling efficient waste management and delivering green energy.

In recent years, interest in diverse feedstocks for biomethane production as a renewable energy source has increased [8–10]. Feedstock selection depends largely on local conditions—materials available on-site or nearby are preferred in practice to minimise transport costs. For example, in Poland, a biogas plant uses pig slurry from animals fed with stillage from a nearby distillery, and the generated energy is supplied back to the facility. In Finland, the potential for producing biomethane from condensate after pine wood torrefaction has been analysed, reflecting the ready availability of this resource [11].

Similar solutions are increasingly implemented in Poland. Agricultural biogas plants rely mainly on cattle slurry, agricultural residues and by-products from the agri-food industry [1,2]. The local nature of feedstocks reduces logistics costs and transport-related emissions, while supporting the development of distributed generation in line with national and EU energy–climate strategies [9]. The literature highlights that the same type of substrate may show different properties depending on geographical and technological factors [5]. For instance, cattle slurry from different countries can vary in chemical composition and methane potential depending on animal diets, soil conditions and manure collection systems [12]. Consequently, AD plant design should always account for the local characteristics of the available feedstocks. It is worth noting that, among the available feed materials, cattle slurry is one of the most widely used and studied substrates for anaerobic digestion due to its high production volume, relatively well-characterised compo-

sition and central role in agricultural systems [1,5,9]. It therefore serves as a key benchmark for investigations of AD performance and biomethane potential, both globally and within specific national contexts.

### 3. Substrates Used in Anaerobic Digestion

Anaerobic digestion is highly flexible with respect to feedstock type. In practice, almost any biodegradable organic material can serve as an input for biogas production. Examples from different sectors of the economy are listed in Table 1, and a cross-sector comparison of these substrates shows that AD feedstocks span a continuum from dilute agricultural slurries and sewage sludge to high-solids energy crops, food-processing residues, and lignocellulosic field and green wastes [1,9]. At the compositional level, these streams differ in total solids and volatile solids contents and, crucially, in the relative proportions of carbohydrates, proteins, lipids, and lignocellulosic fibre; the share of readily biodegradable versus structurally bound organic matter largely determines the amount of biogas and methane that can be produced per unit of substrate. Slurry- and manure-based streams occupy the low-solids, nutrient-rich end of this spectrum and typically provide a well-buffered, microbially active matrix that supports stable operation. By contrast, energy crops and many food-industry wastes exhibit higher volatile solids contents and, in some cases, more favourable C/N ratios, which translates into higher specific methane yields but also increases the risk of rapid acidification, foaming, or nutrient imbalance when they are used as mono-substrates [8]. Municipal biowaste, slaughterhouse residues and fats, oils and grease combine high methane potential with heterogeneous composition, contamination, hygienic constraints, or long-chain fatty acid (LCFA) inhibition, necessitating cautious dosing and, frequently, additional hygienisation or mechanical or thermal treatment. At the opposite end of the spectrum, lignocellulosic residues such as straw and mixed green waste show high total solids and C/N ratios and low inherent degradability, often requiring more intensive physico-chemical or biological pretreatment to achieve acceptable conversion [1,5]. Taken together, the patterns summarised in Table 1 indicate that it is not only the total organic load, but also the internal distribution between soluble and particulate fractions and between easily fermentable and recalcitrant components, that is responsible for the attainable biogas and methane yields of a given effluent or residue. Overall, this means that no single substrate is ideal: effective AD system design relies on combining complementary feedstocks and pretreatment strategies to balance C/N, moderate inhibition risks, and align substrate properties with the chosen reactor configuration.

Given this diversity, feedstocks for AD should be readily degradable and free from toxic compounds that could inhibit microbial activity [1,9]. In many cases, pretreatment is required to improve the accessibility of organic matter to methanogenic microbes. This need arises from kinetic and structural constraints: hydrolysis of complex polymers is frequently rate-limiting because lignin, cellulose crystallinity, and dense fibre matrices restrict enzymatic access and mass transfer. By reducing particle size and disrupting structure, pretreatment increases reactive surface area and the apparent hydrolysis rate constant, alleviating this bottleneck [1,5]. Nonetheless, co-digestion can raise process yields, but unsuitable pairings may depress performance or precipitate failure. Excess protein increases free ammonia, lipid-rich feeds yield long-chain fatty acids, and sulphur-bearing streams raise sulphide, all of which inhibit methanogenesis. From a compositional standpoint, this means that substrates rich in proteins and lipids must be combined with more carbohydrate- and fibre-rich co-substrates to dilute inhibitory compounds while still exploiting their high methane potential. These risks are mitigated through careful substrate characterisation, conservative dosing, and routine monitoring (VFA/alkalinity, TAN,

foaming), with targeted co-substrate ratios and incremental loading to keep intermediates below inhibitory thresholds [3,12].

In practical terms, one of the most widespread AD feedstocks is slurry from agricultural holdings, chiefly due to its high availability and properties conducive to stable operation. It typically offers a near-neutral pH, an established microflora and a rich nutrient profile [1]. However, its biogas yield is lower than that of many other materials because a portion of the organics has already been digested in the animal gut. In addition, slurry—particularly from dairy cattle—often contains a considerable lignocellulosic fraction originating from undigested fibre and bedding material, which is only slowly degradable under standard AD conditions and further constrains its specific methane yield [1,5]. Consequently, although the total solids content of slurry may be modest, the effective fraction that is rapidly biodegradable is limited, and this compositional feature explains the relatively low methane yield per unit of fresh mass compared with many food-industry effluents or energy crops. Despite this, slurry often acts as a base substrate that is combined with other materials to balance nutrients and stabilise the process. Furthermore, certain substrates—such as sewage sludge, slaughterhouse wastes, or the organic fraction of municipal solid waste (OFMSW)—may contain substances harmful to the microorganisms driving digestion. Such substances include, in particular, residual disinfectants and surfactants, heavy metals, pharmaceuticals, and other xenobiotic organic compounds, as well as locally elevated levels of ammonia, sulphide, or long-chain fatty acids, all of which can inhibit key biochemical steps of the AD process [1,3,5]. Consequently, these materials require appropriate cleaning or conditioning to prevent biological inhibition in the reactor. It is also necessary to limit non-biodegradable fractions so that the digester volume is used efficiently. Similarly, lignocellulosic biomass—particularly wood and materials with high lignin content—shows low anaerobic degradability. Therefore, specialised pretreatments are needed to loosen the biomass structure and increase its bioavailability to microorganisms.

**Table 1.** Example substrates used in anaerobic digestion and their key characteristics (author’s own elaboration).

Source Category	Substrate	Typical Origin	Total Solids, TS (%)	Volatile Solids VS (% of TS)	C/N Ratio (–)	Inhibition Risks/Issues	Required/Beneficial Pretreatment	References
Agriculture—cattle slurry	Cattle slurry	Dairy/beef cattle farms	7–10	70–80	8–15	Ammonia, hydrogen sulphide; low readily fermentable sugars	No pretreatment; mixing and homogenisation advised	[1,5]
Agriculture—pig slurry	Pig slurry	Pig farms	3–6	70–80	8–14	Ammonia; foaming with excess protein	Screens/grates; homogenisation	[1,2]
Agriculture—cattle manure	Cattle manure	Barns/sties with bedding	20–30	70–85	15–25	Fibres; risk of pH drop with fresh fraction	Size reduction; dilution	[1,5]
Energy crops	Maize silage	Maize plantations	28–35	90–95	25–35	Nitrogen deficiency in mono-feed	Size reduction; balanced C/N (co-digestion)	[2,5]
Crops/meadows	Grass silage	Grasslands	25–35	85–90	12–20	Lignocellulose; risk of fibrous mats and scum	Size reduction; optional enzymatic conditioning	[1,10]
Food industry	Bakery/confectionery waste	Bakeries, confectioneries	50–70	95–98	20–30	Rapid pH drop; foaming	Dilution; batch-wise dosing	[3,8]
Dairy industry	Whey	Dairies	5–7	80–90	20–30	Volatile acids; pH drop	Alkaline buffering; mixing	[5]
Wastewater treatment	Raw sewage sludge	Municipal WWTPs	2–4	60–75	6–12	Heavy metals; trace toxins	Thickening; hygienisation as required	[1,9]

Municipal waste	OFMSW (bio-waste fraction)	Source-separated biowaste	20–35	80–90	15–25	Physical contaminants (plastics, glass)	Mechanical sorting; pulper; washing	[1,8]
Meat industry	Slaughterhouse waste	Abattoirs, slaughterhouses	20–35	90–95	5–8	High nitrogen (NH <sub>3</sub> ), H <sub>2</sub> S, lipids (LCFA)	Hygienisation; cautious dosing	[3,12]
Kitchen wastes (HORECA)	Kitchen waste	Food service, catering	25–35	85–95	15–25	Physical contaminants; fats	Sorting; size reduction; dilution	[8,9]
Oil industry	Fats/oils/FOG	Grease traps	~100	~100	–	LCFA inhibition; foaming	Slow dosing; co-digestion with slurry	[5,12]
Chemical–food industry	Crude glycerol	Biodiesel/technical glycerine	85–90	85–90	–	Rapid pH drop; osmotic inhibition	Low dosing; buffering	[3,12]
Agriculture—field residues	Wheat straw	Arable fields	85–90	85–90	60–90	Lignin; low degradability	Size reduction; thermo-chemical/biological pretreatment	[1,9]
Sugar industry	Pressed beet pulp	Sugar factories	22–28	90–95	20–30	Rapid fermentation; foaming	Dilution; batch-wise dosing	[1,9]
Distillery industry	Stillage	Distilleries/ breweries	8–12	85–95	–	Volatile acids; pH drop	Buffering; co-digestion	[3,12]
Green waste management	Green waste (branches, leaves)	Municipal services	30–50	70–85	30–60	Lignin; foreign materials	Sorting; size reduction; optional hydrothermal treatment	[1,9]

Explanations: WWTPs—Wastewater Treatment Plants, HORECA—Hotels, Restaurants, Catering, FOG—Fats, Oils, Grease.

The co-digestion of different substrate types can substantially improve biogas production, provided that the nutrient balance in the fermentation system is maintained [8]. Table 2 summarises the BMP of commonly used feedstocks. Slurry- and manure-type substrates have relatively low BMP and methane yield per unit fresh mass [1,5]. At the same time, they are often associated with robust and stable digester performance. Energy crops and many food- and municipal waste-derived substrates show markedly higher BMP and methane yields. However, these feeds are more prone to overloading, foaming and process inhibition. Lipid-rich substrates such as fats, oils, and grease or crude glycerol provide the highest BMP ranges and energy densities [5,8]. They must be added in limited doses because LCFA accumulation and rapid acidification can quickly destabilise the process. Lignocellulosic residues (e.g., straw, green wastes) occupy the opposite end of the spectrum, with modest BMP and low methane yield per unit fresh mass [5,9]. In general, substrates dominated by easily fermentable carbohydrates and lipids show high specific methane yields, whereas those with a high share of structural carbohydrates and lignin exhibit low BMP despite comparable total solids contents; the composition of the effluent thus directly determines the quantity of biogas obtainable from a given mass of material. They therefore usually require pretreatment or co-digestion with more readily degradable substrates.

**Table 2.** Biochemical methane potential of selected substrates (author’s own elaboration).

Substrate	BMP (Nm <sup>3</sup> CH <sub>4</sub> /kg VS)	Methane Yield (Nm <sup>3</sup> CH <sub>4</sub> /Mg Fresh Mass)	Methane Content in Biogas (%)	Process Notes	References
Cattle slurry	220–280	10–25	55–60	Lower yields—stable base feedstock for co-digestion	[1,5]
Pig slurry	260–320	12–25	55–60	Higher energy density than cattle slurry; risk of NH <sub>3</sub>	[1,2]
Cattle manure	180–250	40–70	50–58	High TS; requires dilution/efficient mixing	[1,5]
Maize silage	300–340	90–120	52–55	High stability; nitrogen needed in mono-feed	[2,5]
Grass silage	250–320	70–100	50–55	Improved after size reduction; synergistic with slurry	[1,10,13]

Bakery/confectionery wastes	400–500	150–300	55–60	Rapid fermentation; control VFA and foam	[3,8]
Whey	350–420	10–25	55–60	Low TS—low yield per fresh mass; good for process start-up	[5]
Raw sewage sludge	150–250	5–15	60–65	Lower BMP; sanitary requirements	[1,9]
Biowaste fraction (OF-MSW)	350–500	80–180	55–60	Composition variability; thorough removal of contaminants required	[1,8,14]
Slaughterhouse wastes	400–600	120–220	60–65	Beware NH <sub>3</sub> and H <sub>2</sub> S; restricted dosing	[3,12]
Kitchen wastes (HORECA)	380–520	100–200	55–60	Variable composition; good blend with slurry	[8,9,15]
Fats/oils/FOG	700–1000	300–600	65–70	Very high BMP; LCFA risk—low dosing	[5,12]
Crude glycerol	400–600	200–350	55–60	Rapid acidification; buffering required	[5,12]
Wheat straw (raw)	150–220	20–40	50–55	Requires pretreatment; otherwise, low conversion	[5,9,16]
Pressed beet pulp	300–380	60–100	55–60	Good synergy with slurry; foam control	[1,9]
Distillery stillage (stillage)	350–450	30–60	55–60	High VFA; buffering required	[3,12]
Green wastes (mixed)	150–250	20–60	50–55	Lignin limits yield; pretreatment beneficial	[1,9,16,17]

Explanations: HORECA—Hotels, Restaurants, Catering, FOG—Fats, Oils, Grease.

The choice of feedstock depends on many factors, including local availability, planned use of the digestion products, environmental conditions, the digester technology applied, and the overall economics of the project. Because methane is the main energy carrier in biogas, differences in BMP and methane concentration directly translate into differences in specific energy yield at the plant scale. These differences, in turn, reflect the underlying chemical composition of the substrates—particularly their organic load, the balance between soluble and particulate fractions, and the relative contents of proteins, carbohydrates, and lipids—which together control both the rate and the extent of anaerobic conversion.

#### 4. Pretreatment Processes to Improve AD Performance

To date, substrate pretreatment has been used mainly for sewage sludge. There is now growing interest in applying it to organic fractions of solid wastes to raise the efficiency of anaerobic digestion. The literature commonly distinguishes four main groups of pretreatment methods—mechanical, thermal, chemical and biological—most of which have so far been tested largely at laboratory scale [9]. The suitability of each method is strongly dependent on the physical state and composition of the residue, with different approaches being preferred for fibrous high-solids materials, sewage sludge, or mixed organic wastes.

##### 4.1. Mechanical Pretreatment of Substrates

Mechanical processing employs devices such as mills (e.g., rotary), screw presses, disc shredders, or piston presses to comminute solid particles in the feed [2]. This increases the specific surface area and promotes more intense microbial growth in the subsequent stages of AD [12]. The method is particularly useful for lignocellulosic materials, which are more resistant to biological degradation [13]. Studies show that mechanical pretreatment (rotary drum pre-composting) of the organic fraction of municipal solid waste—a mixture of fruit, vegetable and yard wastes with cow dung—can increase the cumulative biogas yield from about 275 to 325 mL gVS<sup>-1</sup> at an F/M ratio of 0.5 and from 340 to 465 mL gVS<sup>-1</sup> at an F/M ratio of 1.0. This corresponds to an improvement of roughly 18–37% compared with untreated waste [14]. High-pressure homogenisation is also effective: the material is subjected to several hundred bar and then rapidly depressurised. Key advantages include technical simplicity, improved dewatering, no odour emissions and

moderate energy demand. Drawbacks include a lack of disinfection effect against pathogens and a higher risk of mechanical failures.

#### 4.2. Thermal Pretreatment of Substrates

Thermal processing is among the most advanced and best-studied pretreatment methods used in AD. Industrial deployment focuses chiefly on sewage sludge and lignocellulosic substrates [15–17]. This approach inactivates pathogens, improves dewaterability and reduces digestate viscosity, which greatly facilitates downstream handling [18–20]. Two principal temperature ranges are reported for thermal pretreatment, namely 70–121 °C and 160–180 °C [21,22]. For waste activated sludge, treatments at around 120–121 °C have been reported to increase biogas production typically by about 20–30% compared with untreated sludge, whereas more severe conditions in the range of 160–180 °C can lead to much higher enhancements, in some cases approaching a doubling of biogas production [21]. In the case of thermally pretreated olive mill solid waste, removal of phenols and furans from the liquid phase generated after pretreatment increased methane yield by approximately 42% relative to the raw liquid phase. This highlights both the potential of thermal pretreatment and the need to control inhibitory by-products [22]. Notably, some studies indicate that even mild treatment at 70 °C can effectively suppress pathogens and boost biogas output by 78% [23]. Differences in reported gains most likely reflect the diverse physicochemical properties and compositions of the wastes being treated.

#### 4.3. Chemical Pretreatment of Substrates

Chemical processing breaks down organic matter using strong reagents—primarily acids, alkalis, or oxidants. As AD typically proceeds under alkaline conditions, acid use may require subsequent pH correction by alkalinisation. For this reason, alkaline treatment is more common from both technological and economic perspectives and is regarded as more effective and operationally stable. Among frequently used alkalis, NaOH is reported as the most effective, followed by KOH, Mg(OH)<sub>2</sub>, and Ca(OH)<sub>2</sub> [24–26]. Alkalis cause particle swelling and increase the reactive surface area, improving access for anaerobic microorganisms and accelerating degradation. For instance, Lin et al. reported that NaOH pretreatment of pulp and paper sludge co-digested with monosodium glutamate waste liquor (8 g NaOH per 100 g TS) increased methane yield from approximately 0.17 to 0.32 m<sup>3</sup> CH<sub>4</sub> kg<sup>-1</sup> VS removed, i.e., to 183.5% of the untreated control [24].

#### 4.4. Biological Pretreatment of Substrates

Biological methods include both aerobic and anaerobic approaches, in which specific enzymes (e.g., peptidases, carbohydrases, lipases) or selected microbial strains are introduced into the digestion system [15,27]. This is considered environmentally friendly and enables synergistic microbiological consortia that support efficient hydrolysis of organics [28,29]. The use of *Bacillus*-based biological additives markedly enhanced anaerobic activity: in batch tests with physicochemical sludge from enhanced primary treatment, the addition of lyophilised bacilli increased methane production so that, on day 17, it was 95% higher than in the untreated control. When the bacilli were combined with a micronutrient solution containing Fe, Ni, Co, and Mo (4.5, 0.75, 0.45, and 0.09 mg g<sup>-1</sup> VS, respectively, together with 12 mg g<sup>-1</sup> VS of *Bacillus* spp.), methane production on day 17 reached 167% above the control. This treatment also resulted in about 40% lower VFA concentrations and higher volatile solids removal than in the control sludge [30]. Employing thermophilic aerobic bacteria resulted in a 2.2-fold increase in biogas production and a 21% reduction in volatile solids [31]. Compared with other pretreatments, biological methods are characterised by low energy demand; however, their wider use is limited by odour issues and the relatively small reduction in waste mass.

As summarised in Sections 4.1–4.4, reported improvements in biogas or methane yield range from several tens of per cent up to more than a two-fold increase, depending on the pretreatment method, substrate characteristics, and operating conditions. A comparative assessment of the four main pretreatment methods shows that mechanical approaches are technically simple and widely applied to fibrous feedstocks; however, they provide only a moderate increase in biogas yield and lead to considerable wear of working components. Thermal processes achieve the highest degree of solubilisation and sanitation, although their substantial energy demand may limit the overall benefit unless heat recovery or cogeneration integration is applied [16,20,23]. Chemical methods are effective in decomposing lignocellulosic materials and can markedly enhance methane yield; however, the use of costly reagents, corrosion-resistant equipment, and pH adjustment lowers their economic and environmental feasibility [13,16]. Biological pretreatment offers low energy consumption and mild operational conditions, yet its efficiency remains moderate and strongly dependent on the microbial community and process control [31]. Comparative analysis indicates that the most effective methods for organic matter disintegration are not necessarily the most energy-efficient, and their selection should reflect both substrate properties and the energy balance of a given installation [13,16]. The final choice of pretreatment method should result from a compromise between yield improvement, energy input, and operational complexity, with hybrid and sequential approaches increasingly adopted to enhance the overall energy balance and stability of the process. In commercial practice, the decisive factors include operating costs and energy consumption, together with additional benefits such as higher biogas yield, pathogen removal, reduced digestate volume, and shorter hydraulic retention time.

## 5. Biochemical Pathways and Kinetic Control in Anaerobic Digestion

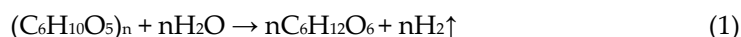
Anaerobic digestion proceeds through sequential phases, whose rate balance governs conversion and energy yield [32]. Intermediates formed upstream are substrates downstream; any imbalance in rates constrains carbon flow to methane and lowers energy yield [33]. Under steady-state conditions, the slowest phase effectively sets the overall conversion rate, and its apparent rate constant and sensitivity to operating conditions therefore become key design and control parameters. The four phases that are kinetically and energetically relevant are hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Figure 1). Each of these stages is mediated by distinct, functionally specialised microbial guilds: hydrolytic and fermentative bacteria dominate hydrolysis and acidogenesis, syntrophic acetogenic bacteria couple acetogenesis to hydrogen removal, and methanogenic archaea complete the final conversion of intermediates to methane.

The rate of one phase sets the boundary conditions for the next; accumulation of intermediates signals a kinetic mismatch and potential loss in methane productivity [34]. In practice, such imbalances are reflected in routine indicators such as VFA profiles, the VFA/alkalinity ratio, and the propionate-to-acetate ratio, which provide early warning of rate limitations at specific stages. From a microbiological perspective, shifts in these indicators reflect changes in the relative activity of the underlying microbial groups and thus provide an indirect view of which guild is currently rate-limiting. The individual phases are detailed below with emphasis on rate control and implications for energy performance.

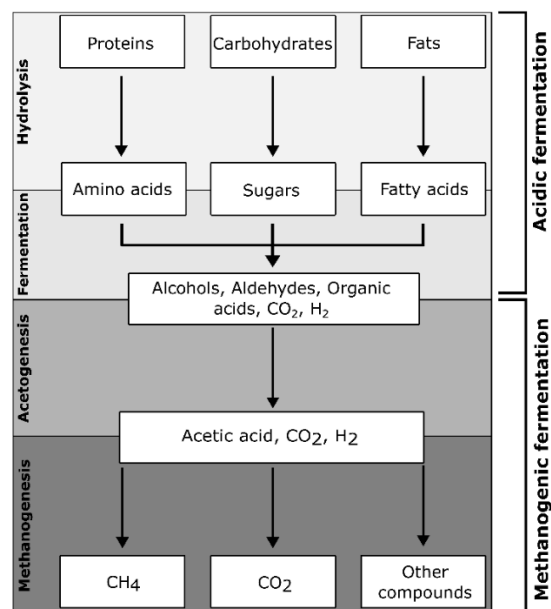
- **Hydrolysis**

This is the initial stage, in which complex polymers—such as cellulose, starch, and proteins—are cleaved by water and extracellular enzymes. The products are simple organics (monomers and oligomers), including glucose, amino acids, and fatty acids [35]. Hydrolysis is mainly carried out by hydrolytic and fermentative bacteria, including genera such as *Clostridium*, *Bacteroides*, *Ruminococcus*, and *Fibrobacter*, which secrete cellulases,

hemicellulases, and proteases capable of attacking particulate organic matter and plant-derived fibre [32,36]. The overall course of hydrolysis can be written as in Equation (1):



For lignocellulosic and structured substrates, hydrolysis often limits the overall rate; restricted accessibility lowers the effective hydrolysis constant and delays downstream methane formation. Increasing accessibility (e.g., particle-size reduction, fibre disruption) accelerates hydrolysis, shortens time to peak gas production, and raises the fraction of the biochemical methane potential realised within a given retention window [36,37]. Operationally, this is captured by higher apparent first-order hydrolysis coefficients and steeper initial slopes of cumulative methane curves in BMP and reactor tests, which translate into higher space–time methane yields at a given HRT [5]. Insufficient hydrolytic activity, or an imbalance between hydrolytic and downstream communities, is therefore often the primary cause of the low conversion of fibre-rich substrates and underutilisation of their theoretical methane potential.

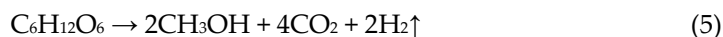


**Figure 1.** Scheme of the anaerobic digestion process (author’s own elaboration [9]).

- **Acidogenesis**

Acidogenesis rapidly generates short-chain intermediates; maintaining balance with downstream uptake prevents energy losses [38]. This phase is driven by a broad consortium of fermentative and acidogenic bacteria, including representatives of *Clostridium*, *Lactobacillus*, *Enterobacter*, and *Bacteroides*, which convert sugars, amino acids, and other hydrolysis products into volatile fatty acids (VFAs), alcohols, CO<sub>2</sub>, and H<sub>2</sub> [32,39]. Acidogenesis is typically rapid; if formation outpaces downstream uptake, VFA and alcohols accumulate, pH declines, and carbon is diverted from methane, lowering specific energy output. From a kinetic standpoint, excessive acidogenic activity relative to acetogenesis and methanogenesis manifests as rising total VFA concentrations and a shift towards propionate and butyrate, signalling that downstream rates are no longer sufficient to stabilise the system [39]. In well-balanced digesters, acidogenic and acetogenic bacteria operate at rates that match the capacity of methanogens, keeping VFAs at non-inhibitory levels and preserving the energy-conversion efficiency.

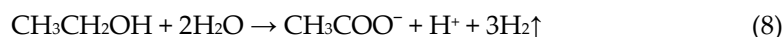
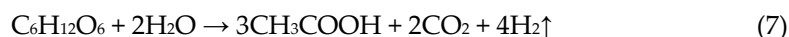
Illustrative reactions for this phase are given in Equations (2)–(5):



- **Acetogenesis**

Acetogenesis channels carbon into acetate,  $\text{CO}_2$ , and  $\text{H}_2$ ; its effectiveness depends on continuous hydrogen removal, which maintains thermodynamic favourability [39]. Because the process is syntrophic, the rate of  $\text{H}_2$  removal controls the feasible acetogenic rate and the stability of the whole chain [35,40]. Key actors at this stage are syntrophic acetogenic bacteria such as *Syntrophomonas* and *Syntrophobacter*, which oxidise VFAs (e.g., propionate and butyrate) to acetate and  $\text{H}_2$  only when hydrogenotrophic partners efficiently consume the produced  $\text{H}_2$  [32,36]. If hydrogenotrophic methanogens or other hydrogen consumers are inhibited, hydrogen partial pressure increases, the Gibbs free energy change ( $\Delta G$ ) for key acetogenic reactions becomes less favourable, and propionate and butyrate start to accumulate, indicating a kinetic bottleneck at this stage [35].

Selected reactions for acetogenesis are shown in Equations (6)–(8):



Effective hydrogen scavenging prevents VFA build-up, supports higher organic loading and protects the net energy balance by favouring acetate-centred routes. In engineering terms, the kinetic coupling between syntrophic acetogens and hydrogenotrophic methanogens means that any disturbance in one group (e.g., inhibition by ammonia or sulphide) immediately constrains the other, narrowing the safe window for organic loading rate and retention time.

- **Methanogenesis**

Methanogenesis finalises energy recovery; maintaining activity under strictly anoxic conditions preserves gas calorific value and volumetric productivity [40]. Two routes dominate: the acetoclastic and the hydrogenotrophic pathways. Acetoclastic methanogenesis is mainly performed by archaea of the genera *Methanosaeta* and *Methanosarcina*, which convert acetate to  $\text{CH}_4$  and  $\text{CO}_2$ , whereas hydrogenotrophic methanogenesis is carried out by genera such as *Methanobacterium*, *Methanospirillum*, and *Methanococcus*, which reduce  $\text{CO}_2$  with  $\text{H}_2$ . Methylotrophic methanogens (e.g., *Methanomethylovorans*) can additionally utilise methanol and methylated compounds in some systems, contributing to overall methane formation [32,35]. Their combined activity is often quantified through specific methanogenic activity (SMA) tests, which provide an operational measure of the maximum methane production rate and are widely used to assess whether the methanogenic community can sustain planned organic loading rates [36].

Illustrative reactions are listed in Equations (9)–(11):





The realised methane fraction governs usable energy in CHP or biomethane pathways; sustained methanogenic rates, coupled to low hydrogen partial pressure and adequate buffering, secure high specific methane yield and stable net energy output. In engineering terms, the maximum attainable methanogenic rate, together with the upstream hydrolysis and acetogenesis kinetics, defines the safe operating window for organic loading rate and retention time [40]. From a control perspective, maintaining a robust and diverse methanogenic community that can respond to load fluctuations is essential to prevent VFA accumulation and preserve the long-term energy efficiency of the plant. In practice, these kinetic dependencies set the bounds for loading and retention times, which ultimately determine achievable methane productivity and the net energy balance of the plant.

## 6. Reactor Design, Kinetic Regimes, and Energy Performance

The choice of AD process and reactor configuration depends on the intended application and the nature of the available substrates. In industrial practice, two main operating regimes are used—mesophilic and thermophilic—which differ primarily in fermentation temperature. High-solids substrates are typically digested under thermophilic conditions in batch systems, whereas low-solids materials are most often processed at mesophilic temperatures in flow-through configurations [41,42]. Additionally, AD processes may also be classified by moisture content and flow regime. “Wet” systems operate at total solids of about 5–15%, while “dry” (high-solids) systems typically run at total solids  $\geq 20\%$ . Wet digestion facilitates pumping and mixing of the feed owing to the use of pumps and mechanical agitators [43–45]. By contrast, dry anaerobic digestion is designed for stackable, high-solids feedstocks such as the organic fraction of municipal solid waste and source-separated biowaste, and allows operation at higher organic loading rates with reduced water demand, smaller digester footprint, and lower heating and mixing energy requirements, which makes it particularly attractive for solid waste treatment in space- and water-constrained installations [43–45].

In kinetic terms, reactor configuration therefore conditions the kinetic regime by shaping biomass retention (SRT relative to HRT), mass-transfer resistance and the parasitic energy required for mixing, pumping, and heating, which together determine the net energy yield. Mesophilic operation provides a wider stability window with lower reaction maxima, whereas thermophilic regimes accelerate kinetics and shorten the required HRT at the expense of narrower disturbance tolerance and higher thermal demand. Furthermore, wet systems reduce diffusion limitations through easier mixing but may raise circulation energy, while dry systems increase solids residence and plug-flow behaviour, improving contact at the risk of diffusion-limited hydrolysis unless mild recirculation is applied [2,16].

With respect to operating mode, the most common solution is continuous-flow operation, in which the substrate moves progressively through the successive stages of digestion and fresh feed is added at regular intervals [46]. By contrast, an alternative is batch operation, where material is loaded into the reactor in discrete charges at set time intervals. Consequently, plants operating in continuous mode usually achieve higher biogas productivity per unit of substrate, reflecting greater process stability and tighter control of fermentation conditions [47]. This supports more efficient microbial activity than batch systems.

Accordingly, continuous systems can align organic loading with realised conversion rates and maintain buffering, which sustains volumetric methane productivity and improves net energy performance. However, batch systems can deliver high specific yield

per charge, but downtime and transient VFA peaks may depress average energy output unless cycle design minimises idle periods and ensures adequate alkalinity and mixing. Overall, across configurations, designs that secure  $SRT > HRT$  and minimise diffusion resistance without excessive shear allow higher OLR without loss of methanogenic activity, which translates into greater usable energy per unit volume [9,28].

Examples of biogas reactor types in current industrial use are summarised in Table 3.

**Table 3.** Example AD reactor designs: characteristics, advantages and disadvantages, and kinetic-energy implications (author’s own elaboration).

Reactor Type	Description	Advantages	Disadvantages	Kinetic/Energy Implications	References
ASBR—Anaerobic Sequencing Batch Reactor	A single vessel performing both digestion and clarification; the substrate is fed and withdrawn periodically.	<ul style="list-style-type: none"> <li>• high biogas production efficiency,</li> <li>• flexible process control,</li> <li>• low demand for mechanical energy,</li> <li>• simple operation</li> </ul>	<ul style="list-style-type: none"> <li>• risk of clogging,</li> <li>• lower effectiveness with large working volumes</li> </ul>	Feast–famine cycling can recover SMA; average $CH_4 \cdot m^{-3} \cdot d^{-1}$ depends on cycle design and minimising idle time; low mixing energy if cycles are optimised.	[1,28,40]
CSTR—Continuous Stirred-Tank Reactor	Single- or two-stage installation in which the substrate is mixed with microorganisms continuously or intermittently.	<ul style="list-style-type: none"> <li>• stable operation,</li> <li>• ability to treat high-strength liquid wastes,</li> <li>• easy operation</li> </ul>	<ul style="list-style-type: none"> <li>• difficult to retain microorganisms in the reactor,</li> <li>• risk of system acidification</li> </ul>	Mixing lowers diffusion limits and evens pH/T, but raises mixing energy; $SRT \approx HRT$ unless biomass retention (two-stage/attached growth) keeps methanogens and supports higher OLR.	[1,2,4]
APFR—Anaerobic Plug-Flow Reactor	A long, narrow tank operating continuously without internal mixing; the substrate moves along the reactor like a “plug.”	<ul style="list-style-type: none"> <li>• low capital cost,</li> <li>• high efficiency,</li> <li>• operates under mesophilic and thermophilic conditions,</li> <li>• simple construction</li> </ul>	<ul style="list-style-type: none"> <li>• slow conversion of solid fractions,</li> <li>• requires periodic cleaning</li> </ul>	Low mixing energy and quasi-staging; mild recirculation reduces diffusion limits for fibrous feeds without losing plug-flow benefits; productivity limited by hydrolysis.	[33,43,47]
UASB—Upflow Anaerobic Sludge Blanket Reactor	A system with a granular sludge bed at the bottom that enables contact between biomass and the medium being treated.	<ul style="list-style-type: none"> <li>• low operating costs,</li> <li>• high biogas production,</li> <li>• small footprint,</li> <li>• no need for effluent recirculation</li> </ul>	<ul style="list-style-type: none"> <li>• long start-up period,</li> <li>• requires experienced operation,</li> <li>• some substrates hinder sludge granulation</li> </ul>	Very high SRT at low HRT → high space-time $CH_4$ yields with minimal mixing energy; start-up/granule stability are kinetically decisive for net energy performance.	[1,9,41]

From an economic perspective, capital expenditure is largely determined by digester volume, civil works, and gas utilisation equipment, whereas operating costs are dominated by labour, maintenance, heating and mixing energy, as well as any pre- and post-treatment steps [4,34]. In general, reactor configurations that deliver higher space-time methane yields through effective biomass retention and mass-transfer control are more favourable in terms of volumetric utilisation of the reactor. In practice, this translates into lower specific costs per unit of energy produced compared with low-rate systems of similar footprint. In essence, configuration choice is an optimisation of rate enhancement ver-

sus parasitic energy demand under site-specific constraints (substrate rheology, opportunities for heat integration and available footprint) [4,34]. In essence, configuration choice is an optimisation of rate enhancement versus parasitic energy demand under site-specific constraints (substrate rheology, opportunities for heat integration and available footprint).

Designs that combine effective biomass retention with targeted mass-transfer control provide the highest net benefits when the organic loading rate is aligned with the realised process kinetics [28]. Where feasible, heat recovery, variable-speed mixing, and incremental (stepwise) feeding of the substrate further improve methane space–time yields without eroding the overall energy balance. In recent years, advanced configurations such as anaerobic membrane bioreactors, staged CSTR–UASB or CSTR–plug–flow systems, and reactors with integrated heat-recovery loops have been implemented. These solutions aim to intensify mass transfer and biomass retention while at the same time reducing parasitic energy demand [48,49]. As a result, they further strengthen both the kinetic performance and the overall energy return of AD installations [28,41].

## 7. Factors Affecting the Anaerobic Digestion Process

Microorganisms driving AD are highly sensitive to operating conditions. The key parameters determining performance are temperature, pH, OLR, HRT and SRT, C/N, inhibitors, and the quality of mixing and recirculation (see Figure 2). In addition to these primary operational levers, the rheological properties of the sludge—governed by total solids (TS) content, particle size and floc structure—critically condition mixing efficiency, gas–liquid mass transfer and, ultimately, the achievable space–time methane productivity in anaerobic digesters [18,21,36]. Matching these parameters to the substrate and local conditions is essential for process stability, the rate of organic matter breakdown, the final biogas yield, and, consequently, the energy yield achievable in practice. Even small deviations from optimal values can reduce microbial activity and suppress methane production [50,51].



**Figure 2.** Key parameters determining the performance of anaerobic digestion (author’s own elaboration).

In view of the above, these factors operate as a coupled system: pH and temperature define the kinetic window, HRT/SRT govern biomass retention, OLR sets the pace of conversion, C/N balances growth and ammonia formation, inhibitors constrain pathway activity, and mixing resolves mass-transfer limits. When tuned together, they shift the process from constraint-limited to rate-limited operation and improve the net energy balance [52].

### 7.1. pH

pH in the digester is critical to overall stability—it directly affects microbial activity, biochemical pathways, and the final biogas yield. Different microbial groups have different pH requirements, with methanogens most active near neutrality, whereas acidogenic microflora thrives at slightly acidic values. This divergence supports designs that physically or operationally separate early acid formation from methane synthesis [53–56].

Accordingly, kinetically, pH governs enzyme activity and carbonate buffering, while also setting ammonia speciation that modulates methanogenic rates. Operational practice targets a narrow working window (6.8–7.4) with resilience up to 6.5–8.2 when alkalinity is adequate; VFA/alkalinity ratios below common alarm thresholds indicate headroom against upsets. Routine titration alkalinity and online pH–TAN checks enable early correction of emerging imbalances, protecting methanogenic rates and preserving the net energy balance. Maintaining this control prevents VFA-driven acidification, sustains specific methane production rates, and protects volumetric productivity and energy yield [57].

### 7.2. Temperature

In AD practice, two temperature windows are widely recognised [58,59]. Thermophilic operation (55–60 °C) speeds decomposition and enhances sanitation, while mesophilic operation (35–40 °C) offers broader stability and lower auxiliary energy demand [60–62]. The choice of regime reflects feedstock character, heat availability, and control objectives. From a kinetic–energetic standpoint, thermophilic set-points are warranted when the gain in space–time methane productivity demonstrably exceeds the additional thermal and mixing energy at the target OLR/HRT [63,64]. For variable or inhibition-prone feeds, mesophilic operation often maximises net energy by preserving stability and minimising downtime-related losses.

Given these considerations, temperature sets the kinetic ceiling (typical  $Q_{10} = 2$ ): higher set-points accelerate reactions and can reduce the HRT required for a given conversion, but narrow the tolerance to disturbances and raise thermal demand [63]. Thermophilic regimes therefore benefit most when integrated with heat recovery (e.g., CHP) [65], whereas mesophilic operation trades lower maxima for robust, energy-efficient stability [66,67].

### 7.3. Hydraulic Retention Time and Solids Retention Time

SRT denotes how long microorganisms and suspended organics remain in the digestion chamber, while HRT reflects the hydraulic turnover. Both parameters are pivotal for process stability and biogas productivity and must be tuned to substrate degradability and loading strategy [68,69]. In practice, designs that decouple SRT from HRT (e.g., granular sludge, carrier-based retention or membrane separation) permit higher OLR while maintaining methanogenic activity and process robustness [70]. Under mesophilic conditions, wet CSTRs treating readily degradable organic wastes such as food waste are commonly operated at HRTs of about 10–40 days, whereas mono-digestion of cattle slurry or dairy manure typically employs HRTs of approximately 20–30 days. Nitrogen-rich poultry manure and slowly degradable energy crops often require HRTs above 30 days, in some cases approaching 60 days [68,70,71].

In light of the foregoing, for sustained methanogenesis, SRT should exceed HRT so that slow-growing archaea are retained and hydrogen is scavenged before VFAs accumulate. Typical mesophilic practice spans SRT on the order of weeks, with wet CSTR HRT matched to OLR and degradability to balance utilisation and stability. Optimising this pair maximises space–time methane yield and strengthens the net energy balance at plant scale [70–76]. When co-substrates are incorporated, co-digestion of livestock manure with readily fermentable organic wastes (e.g., food waste or selected agro-industrial residues)

often permits operation at similar or slightly shorter overall HRT while maintaining or increasing specific methane yields. In contrast, co-digestion with lignocellulosic residues generally requires somewhat longer HRT if high conversion of structural carbohydrates is targeted [69,73,76]. Moreover, excessively long SRT can increase endogenous respiration and solids handling requirements, partially offsetting energy gains unless wastage and loading are actively controlled [74–76].

#### 7.4. Carbon-to-Nitrogen Ratio

C/N is one of the most influential parameters for stable AD. If C/N is too low, excess nitrogen promotes ammonia formation, and if it is too high, nitrogen becomes limiting for microbial growth. Co-digestion is a practical lever to bring mixed feeds into the desired window. Routine elemental characterisation of feeds and forward planning of blend ratios help maintain C/N within bounds before inhibitory TAN builds up [77–80].

On this basis, mechanistically, C/N governs biosynthesis versus ammonia release and shifts the process either towards inhibition at low C/N with high TAN/FA or towards nutrient limitation at high C/N. Operationally, a target range around 20:1–30:1, often near 25:1, supports steady methanogenic kinetics. Keeping C/N in range raises conversion rates and improves energy yield through higher and more stable methane productivity. In practice, simple ratio control and incremental adjustments to co-substrate dosing sustain the target C/N despite seasonal variability in feed composition [81,82].

#### 7.5. Inhibitors

As a complex, multi-guild bioprocess, AD is sensitive to inhibitory compounds. Ammonia, sulphide and long-chain fatty acids are among the most common chemical stressors; heavy metals and phenolic organics may also impair key enzymes. Transient accumulation of propionate frequently signals a kinetic imbalance upstream. Elevated TAN with rising free ammonia, dissolved sulphide above cautionary levels, and LCFA pulses from lipid-rich feeds are typical early warnings. Practical diagnosis relies on concurrent tracking of pH, VFA profiles, alkalinity, TAN/FA, and sulphide alongside gas-rate trends. Targeted responses should be initiated before thresholds are exceeded to avoid rate collapse and secondary inhibition [69,83].

As a result, ammonia toxicity increases with pH and temperature as the free-ammonia fraction rises and directly suppresses methanogens, which erodes methane rates [84]. Dissolved sulphide inhibits acetogens and methanogens and degrades gas quality, whereas LCFA adsorb to biomass and impede syntrophic  $\beta$ -oxidation [69,85]. Mitigation relies on co-substrate choice and dosing, micronutrient management, phased loading, and gas/liquid handling that limits inhibitor build-up, actions that stabilise kinetics and preserve the net energy balance. Crossing inhibitory thresholds shifts control back to upstream steps, lengthens the effective HRT, and lowers volumetric methane productivity. Restoring concentrations below inhibitory ranges re-establishes rate coupling across phases and improves specific and space–time energy yields [51,84–90].

#### 7.6. Mixing and Recirculation

Mixing and recirculation are key technological levers for efficiency and stability. Their primary function is to maintain homogeneity, equalising temperature and pH and sustaining contact between microorganisms and substrates. Insufficient mixing yields stratification and local acidification; excessive mixing may shear flocs and reduce biomass retention [91,92]. Impeller geometry (diameter-to-tank ratio, blade type, tip speed) and jet-recirculation rate should be matched to sludge rheology (TS, viscosity, gas hold-up) to secure a uniform residence–time distribution and eliminate dead zones without over-shear.

From a kinetic perspective, mixing lowers diffusion resistance and shortens the time to effective conversion at a given HRT, but incurs auxiliary energy costs and must avoid destructive shear. Plant practice typically targets modest specific mixing power in wet digesters and uses recirculation to eliminate dead zones and sludge build-up. Well-tuned mixing improves volumetric methane productivity without eroding energy performance [93–97]. Intermittent or variable-speed control—triggered by torque, biogas rate, or real-time gradients—maintains homogeneity at lower specific energy input while preserving floc integrity.

### 7.7. Organic Loading Rate

OLR is among the most important technological parameters governing AD stability and performance [57]. As the principal loading set-point, it governs the balance between acidogenesis and methanogenic uptake. Too low a value underutilises the reactor and fails to stimulate methanogens [98]. Excessive loading drives VFA accumulation and pH decline, increasing the risk of instability [99,100]. Practical set-points should be tied to substrate degradability and real-time VFA/alkalinity ratios to prevent rate–capacity mismatch.

In view of the above, effective control matches OLR to realised kinetics and buffering capacity, often by stepwise increases that allow acclimation [101]. Wet CSTRs treating manure-type feeds commonly operate in a modest load band [102]. Food-waste co-digestion and dry plug–flow systems may sustain higher loads when mass transfer and stability are preserved [103]. The online monitoring of VFAs, alkalinity, and gas-rate, coupled with incremental feeding, maintains conversion while protecting the energy balance [104].

Overall, pH and temperature define the kinetic window, HRT/SRT and mixing determine accessible rates, C/N and inhibitors constrain pathway capacity, and OLR fixes the pace of conversion. Aligning all seven factors moves AD from constraint-limited operation to stable, energy-positive performance. In practice, coordinated control that links OLR ramps to measured kinetics within the pH–alkalinity buffer delivers the highest methane space–time yields at the lowest auxiliary energy cost.

## 8. Kinetic and Thermodynamic Interrelations in Methanogenic Fermentation Under Operating Conditions

The kinetics of anaerobic digestion are governed by three interacting groups of factors: substrate composition and biodegradability; the balance between OLR and retention times (HRT/SRT); and environmental parameters that modulate enzyme activity and the phase balance [105].

From a kinetic perspective, anaerobic digestion can also be described in terms of the specific microbial growth rate ( $\mu$ , expressed as the increase in active biomass per unit biomass and time, e.g.,  $\text{d}^{-1}$ ), the specific substrate uptake rate ( $q_S$ , substrate consumed per unit biomass and time, e.g.,  $\text{g COD}_S \text{ g}^{-1} \text{ VSS d}^{-1}$ ), and the specific product formation rate ( $q_P$ , product—here methane—formed per unit biomass and time, e.g.,  $\text{L CH}_4 \text{ g}^{-1} \text{ VSS d}^{-1}$ ). These rates are complemented by yield coefficients: biomass yield on substrate ( $Y_{X/S}$ , active biomass formed per substrate consumed, e.g.,  $\text{g VSS g}^{-1} \text{ COD}_S$ ), product yield on substrate ( $Y_{P/S}$ , product formed per substrate consumed, e.g.,  $\text{L CH}_4 \text{ g}^{-1} \text{ COD}_S$ ), and product yield on biomass ( $Y_{P/X}$ , product formed per unit of active biomass, e.g.,  $\text{L CH}_4 \text{ g}^{-1} \text{ VSS}$ ) [106]. For methane, treated here as a primary metabolite under steady-state conditions,  $q_P$  is closely linked to  $\mu$  and  $Y_{P/X}$ , so that an increase in specific growth rate or in product yield on biomass directly enhances the specific methane production rate and, consequently, the volumetric methane productivity of the reactor [107]. In practice, these

parameters cannot be measured directly at full scale; however, they are reflected in routinely monitored operational indicators such as specific methanogenic activity (SMA, maximum methane production rate per unit methanogenic biomass under defined conditions, e.g.,  $\text{mL CH}_4 \text{ g}^{-1} \text{ VSS d}^{-1}$ ) [108], volatile fatty acid concentrations (VFA, usually expressed as acetic acid equivalents), the VFA/alkalinity ratio (dimensionless ratio of total VFA to buffering capacity, e.g., as  $\text{CaCO}_3$ ) [109], and the propionate/acetate ratio (molar or mass ratio of propionate to acetate), which together signal whether substrate conversion in the acidogenic and acetogenic phases is temporarily outpacing the methane-forming capacity of the system [110,111].

In the literature, these kinetic descriptors are usually reported as broad ranges that together provide an order-of-magnitude picture of substrate consumption, biomass formation and methane production in full-scale anaerobic digesters [79,106]. For mesophilic agricultural and agro-industrial plants, methane yields of roughly  $0.2\text{--}0.3 \text{ Nm}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}$  and daily volumetric productivities of about  $0.5\text{--}1.0 \text{ Nm}^3 \text{ CH}_4 \text{ m}^{-3} \text{ d}^{-1}$  are commonly cited as representative of stable operation [1,28,89]. Under such conditions, biomass yields on substrate remain comparatively low, which confirms that only a minor fraction of the degraded organic matter is incorporated into new biomass, whereas most of it is channelled into methane and carbon dioxide [106]. The reported values of specific methanogenic activity for well-acclimated sludges fall into a similar, relatively narrow band and are consistent with these volumetric productivities when realistic concentrations of active biomass in industrial reactors are taken into account [106,107].

Expressed in space–time terms, the above ranges correspond to hourly volumetric productivities in the order of  $0.02\text{--}0.04 \text{ Nm}^3 \text{ CH}_4 \text{ m}^{-3} \text{ h}^{-1}$ , which can be conveniently converted into COD units and thus into  $\text{g L}^{-1} \text{ h}^{-1}$  [79,89,96]. In practical terms, these values define a realistic performance window for industrial biogas plants: for a given substrate and organic loading rate, persistently lower hourly methane productivities suggest that one or more kinetic steps (most often hydrolysis or hydrogen-dependent acetogenesis) are limiting overall conversion, whereas attempts to operate far above this window tend to promote an accumulation of intermediates, deterioration of VFA-based control ratios, and, ultimately, the loss of process stability [79,89]. In this way, yield coefficients and hourly volumetric productivity parameters provide a coherent, literature-supported framework for linking substrate consumption, biomass growth and methane formation in industrial anaerobic digestion [28,106].

The kinetic descriptors and performance ranges outlined above are, however, ultimately shaped by the nature of the feedstock and its inherent biodegradability. A higher share of readily biodegradable fractions (simple sugars, starch, and hemicelluloses) accelerates hydrolysis and shifts the bottleneck from polymer breakdown to downstream phases (acidogenesis–acetogenesis–methanogenesis) [107,112]. In contrast, high lignin and lignocellulosic structure lower the effective hydrolysis constant, making hydrolysis rate-limiting [113,114]. Feedstock selection and balance—especially C/N—set the maximum growth rates of the microbial consortia: excess nitrogen favours free ammonia (FA, unionised  $\text{NH}_3$ ) formation and methanogen inhibition, whereas the nitrogen deficit limits biomass growth and conversion rates [115].

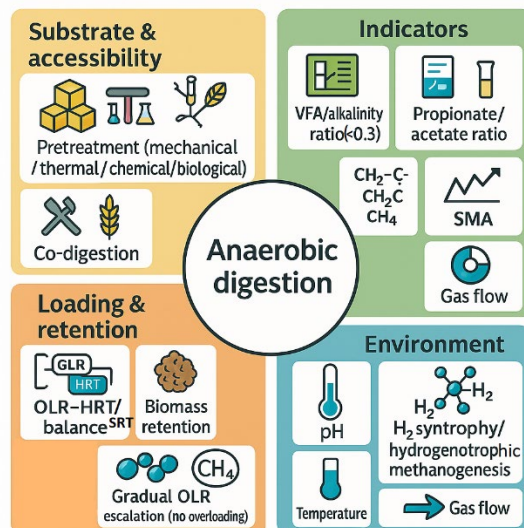
On this substrate-controlled kinetic background, process interventions are primarily aimed at modifying accessibility and stoichiometry. Within this context, pretreatment (mechanical, thermal, chemical, biological) acts as a “catalyst of accessibility”: by disrupting structure, it increases reactive surface area and access to polymers, raising the effective hydrolysis constant and allowing operation at higher OLR without overloading [116,117]. Co-digestion of nitrogen-rich with carbohydrate-rich substrates corrects C/N and reduces the likelihood of inhibitors (FA/ $\text{NH}_3$ , long-chain fatty acids, LCFA), indirectly stabilising downstream kinetics [118]. OLR is the main operational lever, but must be balanced with

HRT and SRT. Rapid OLR increases at fixed HRT promote VFA accumulation and pH decline—shifting from kinetic limitations to buffer/transport limitations [119]. If SRT approaches HRT (no biomass retention), slow-growing methanogens risk being washed out, impairing hydrogen consumption and, in turn, thermodynamically sensitive acetogenesis. A robust strategy is gradual OLR escalation while maintaining  $SRT > HRT$  (granulation, immobilisation, sludge recirculation), sustaining high active-biomass density and fast conversion without overloading [120].

Once substrate accessibility, stoichiometry, and loading have been aligned, environmental parameters tune the realised rate constants. Temperature follows Arrhenius-type behaviour: thermophilic regimes raise reaction rates and shorten HRT but narrow the stability window; mesophilic regimes provide lower maxima yet a broader stable range. pH is equally critical: methanogenesis peaks near neutrality, whereas acidogenesis favours mildly acidic conditions. This underpins two-stage systems or, in single-stage reactors, tight control of VFA/alkalinity to prevent pH drifting below the methanogenic activity window [121]. The kinetic “keystone” of AD is the syntrophic coupling between acetogens and methanogens via hydrogen. Many acetogenic conversions are unfavourable at elevated  $H_2$  partial pressures; only continuous  $H_2$  uptake by hydrogenotrophic methanogens maintains low  $pH_2$  (partial pressure of hydrogen) and renders these reactions exergonic. In practice, the rate of hydrogenotrophic methanogenesis sets the feasible rate of acetogenesis—determining whether the system accumulates propionate/butyrate or sustains rapid carbon flow to acetate and methane [122,123]. Mixing and circulation shift the boundary between diffusion- and kinetics-controlled regimes: well-designed mixing minimises boundary layers, homogenises composition and temperature, and maintains persistent substrate–biomass contact [124]. This brings observed rates closer to biological capacities rather than mass-transfer limits. Over-mixing can, however, disrupt granules and flocs, reducing effective active-biomass concentration; under-mixing produces dead zones, local pH drops, and secondary inhibition. Kinetically, the optimum minimises diffusion resistance without structural damage [125]. Micronutrient management also shapes methanogenic pathways: Ni, Co, Fe, and Mo are cofactors of key methanogenic enzymes—deficiency reduces maximum rates and prolongs adaptation, while excess heavy metals are toxic [126]. Operationally, coupling kinetics with sentinel indicators—VFA/alkalinity ratio (preferably  $< 0.3$ ), propionate/acetate ratio (rising values signal stress on  $H_2$  syntrophy), specific methanogenic activity (SMA), and trends in  $CH_4\%$  and gas flow—supports timely adjustments in OLR, HRT, buffering, mixing intensity, and micronutrient dosing before stability is lost [127].

The relationships discussed above are synthesised in a colour-coded, keyword-based schematic (see Figure 3). It condenses substrate accessibility and C/N balance (via pre-treatment and co-digestion), the OLR–HRT/SRT balance, and the temperature–pH window into one operational view. The diagram also highlights  $H_2$ -based syntrophy and the control indicators that anchor real-time optimisation.

Figure 3 provides a concise, keyword-based synthesis that links kinetic determinants with practical control. It encodes an operational rule: first enhance accessibility (pretreatment/co-digestion) to move the bottleneck beyond hydrolysis, then increase OLR only while maintaining  $SRT > HRT$  and low  $pH_2$  via hydrogenotrophic methanogenesis; the VFA/alkalinity and propionate/acetate ratios serve as early-warning indicators of imbalance. In practice, secure substrate accessibility and stoichiometric balance, tune OLR against HRT/SRT within the appropriate temperature–pH window while sustaining low  $pH_2$ , and keep indicators (VFA/alkalinity, propionate/acetate, SMA,  $CH_4\%$  and gas flow) within guard-bands to maintain a kinetically driven, resilient regime with maximised methane yield and minimal instability risk.



**Figure 3.** Colour-coded keyword map of kinetic–thermodynamic interrelations in anaerobic digestion (author’s own elaboration).

In practical terms, accessibility and stoichiometry should be secured before loading is increased. Matching OLR to HRT and SRT with sustained biomass retention preserves hydrogen uptake and keeps acetogenesis feasible. Operating under these conditions maintains a kinetics-driven regime with stable methane rates and low risk of instability.

## 9. Conclusions

Methane yield and process stability in AD depend on the coupling of phase kinetics—hydrolysis, acidogenesis, acetogenesis, and methanogenesis—with informed design and disciplined control. Performance improves when the rate-limiting step is identified and relieved, operation is kept within an appropriate pH–temperature window, and sufficient biomass is retained to support slow-growing methanogens. In this review, these relationships are illustrated using typical literature ranges of methane yields, biomass yields and volumetric—including hourly—methane productivities reported for full-scale agricultural and agro-industrial digesters.

Building on these considerations, the following six conclusions translate these principles into operational guidance across substrates, pretreatment, reactor configuration, operating factors, kinetic–thermodynamic relations, and monitoring.

1. Substrates and co-digestion. Feeds should be selected and blended to hold C/N near the optimal range and to minimise precursors of inhibition such as free ammonia, long-chain fatty acids and sulphides. Co-digestion is most effective when it first corrects stoichiometry and buffering capacity and only then enables a controlled increase in organic loading once hydrolytic constraints have been reduced.
2. Pretreatment. Mechanical, thermal, chemical, or biological pretreatments are warranted when substrate structure makes hydrolysis rate-limiting. Adoption should be contingent on a positive net balance where gains in space–time methane productivity exceed additional thermal, mixing, and reagent demands. Heat recovery and integration with cogeneration improve viability where thermal steps are applied.
3. Reactor configuration with respect to kinetics and energy. Designs that decouple solids retention from hydraulic residence—by granulation, carriers, or staged layouts—support higher loadings at stable methane rates. Mesophilic operation generally maximises net energy for variable or inhibition-prone feeds, whereas thermophilic set-points are justified when the increase in productivity outweighs thermal demand and narrower stability margins. In wet CSTRs, variable-speed agitation and adequate

buffering sustain homogeneity without excessive power draw. In dry plug-flow systems, mild recirculation improves contact and alleviates diffusion-limited hydrolysis. Across configurations, operating practice should match loading to realised kinetics and maintain SRT greater than HRT to protect methanogenesis.

4. Operating factors. pH should be controlled around 6.8–7.4 with resilience up to about 6.5–8.2, where alkalinity is sufficient. Temperature choice should reflect measured kinetic benefit against energy cost and disturbance tolerance. Mixing must homogenise the medium and lower diffusion resistance while preserving floc or granule integrity. Organic loading is best increased in steps and referenced to contemporaneous VFA and alkalinity trends to avoid a mismatch between conversion capacity and feed rate.
5. Kinetic-thermodynamic interrelations. Low hydrogen partial pressure should be maintained through active hydrogenotrophic methanogenesis so that syntrophic acetogenesis remains favourable. This requirement elevates the importance of biomass retention, conservative ramping of load and prompt control actions that prevent accumulation of inhibitory intermediates.
6. Monitoring and control. Early-warning indicators such as the VFA to alkalinity ratio, the propionate to acetate ratio, specific methanogenic activity, methane content, and gas-rate trends should trigger timely adjustments to loading, residence times, buffering, mixing intensity, and micronutrient supply. Coordinated use of these measurements shifts operation from constraint-driven to rate-controlled and delivers high, durable methane productivity.

This review synthesises the topics of substrates, pretreatment methods, reactor types, and operating factors into a coherent operational framework that links each design and process-control decision to phase-specific rate management and net energy outcomes. It formulates practical implementation principles: improving substrate accessibility before increasing loading, maintaining biomass retention so that SRT exceeds HRT, reducing diffusion resistance without destructive shear, and selecting temperature and pH within defined stability ranges. In addition, it harmonises decision criteria that support reactor design and day-to-day operation, establishing a framework that enables predictable control of kinetics and maximisation of methane yield while maintaining a positive energy balance. In comparison with previous partial reviews, this study provides new substantive and practical insights into the kinetic and energetic optimisation of anaerobic digestion systems.

A key practical conclusion is that high methane yields are not achieved through extreme adjustment of individual operating parameters. They depend on the coordinated management of substrate accessibility, stoichiometry, loading, hydrodynamics, temperature, and monitoring within biologically and thermodynamically consistent windows. Future research should link multi-step kinetic descriptions with real-time monitoring data and plant-scale energy assessments. Such integration will support advanced control, digitalisation, and the robust scale-up of AD as a reliable component of low-carbon energy systems.

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## References

1. Appels, L.; Lauwers, J.; Degreève, J.; Helsen, L.; Lievens, B.; Willems, K.; Van Impe, J.; Dewil, R. Anaerobic digestion in global bio-energy production: Potential and research challenges. *Renew. Sustain. Energy Rev.* **2011**, *15*, 4295–4301. <https://doi.org/10.1016/j.rser.2011.07.121>.
2. Pilarski, K.; Pilarska, A.A.; Kolasa-Więcek, A.; Suszanowicz, D. An Agricultural Biogas Plant as a Thermodynamic System: A Study of Efficiency in the Transformation from Primary to Secondary Energy. *Energies* **2023**, *16*, 7398. <https://doi.org/10.3390/en16217398>.
3. Kangle, K.M.; Kore, S.V.; Kore, V.S.; Kulkarni, G.S. Recent trends in anaerobic co-digestion: A review. *Univers. J. Environ. Res. Technol.* **2012**, *2*, 210–219.
4. Budzianowski, W.M.; Postawa, K. Renewable energy from biogas with reduced carbon dioxide footprint: Implications of applying different plant configurations and operating pressures. *Renew. Sustain. Energy Rev.* **2017**, *68*, 852–868. <https://doi.org/10.1016/j.rser.2016.05.076>.
5. Labatut, R.A.; Angenent, L.T.; Scott, N.R. Biochemical methane potential and biodegradability of complex organic substrates. *Bioresour. Technol.* **2011**, *102*, 2255–2264. <https://doi.org/10.1016/j.biortech.2010.10.035>.
6. Owen, J.J.; Silver, W.L. Greenhouse gas emissions from dairy manure management: A review of field-based studies. *Glob. Change Biol.* **2015**, *21*, 550–565. <https://doi.org/10.1111/gcb.12687>.
7. Ding, L.; Lu, Q.; Xie, L.; Liu, J.; Cao, W.; Shi, Z.; Li, B.; Wang, C.; Zhang, G.; Ren, S. Greenhouse gas emissions from dairy open lot and manure stockpile in northern China: A case study. *J. Air Waste Manag. Assoc.* **2016**, *66*, 267–279. <https://doi.org/10.1080/10962247.2015.1124058>.
8. Zhang, R.; El-Mashad, H.M.; Hartman, K.; Wang, F.; Liu, G.; Choate, C.; Gamble, P. Characterization of food waste as feedstock for anaerobic digestion. *Bioresour. Technol.* **2007**, *98*, 929–935. <https://doi.org/10.1016/j.biortech.2006.02.039>.
9. Gadirli, G.; Pilarska, A.A.; Dach, J.; Pilarski, K.; Kolasa-Więcek, A.; Borowiak, K. Fundamentals, Operation and Global Prospects for the Development of Biogas Plants—A Review. *Energies* **2024**, *17*, 568. <https://doi.org/10.3390/en17030568>.
10. Kandel, T.P.; Ward, A.J.; Elsgaard, L.; Møller, H.B.; Lærke, P.E. Methane yield from anaerobic digestion of festulolium and tall fescue cultivated on a fen peatland under different harvest managements. *Acta Agric. Scand. Sect. B Soil Plant Sci.* **2017**, *67*, 670–677. <https://doi.org/10.1080/09064710.2017.1326522>.
11. Doddapaneni, T.R.K.C.; Praveenkumar, R.; Tolvanen, H.; Palmroth, M.R.T.; Kontinen, J.; Rintala, J. Anaerobic batch conversion of pine wood torrefaction condensate. *Bioresour. Technol.* **2017**, *225*, 299–307. <https://doi.org/10.1016/j.biortech.2016.11.073>.
12. Pilarska, A.A.; Pilarski, K.; Kulupa, T.; Kubiak, A.; Wolna-Maruwka, A.; Niewiadomska, A.; Dach, J. Additives Improving the Efficiency of Biogas Production as an Alternative Energy Source—A Review. *Energies* **2024**, *17*, 4506. <https://doi.org/10.3390/en17174506>.
13. Amin, F.R.; Khalid, H.; Zhang, H.; Rahman, S.U.; Zhang, R.; Liu, G.; Chen, C. Pretreatment methods of lignocellulosic biomass for anaerobic digestion. *AMB Express* **2017**, *7*, 72. <https://doi.org/10.1186/s13568-017-0375-4>.
14. Ponkumar, S.; Subramani, T. Anaerobic digestion of aerobic pretreated organic waste. *Int. J. Mod. Eng. Res.* **2012**, *2*, 607–611.
15. Ariunbaatar, J.; Panico, A.; Frunzo, L.; Esposito, G.; Lens, P.N.L.; Pirozzi, F. Enhanced anaerobic digestion of food waste by thermal and ozonation pretreatment methods. *J. Environ. Manag.* **2014**, *146*, 142–149. <https://doi.org/10.1016/j.jenvman.2014.07.042>.
16. Zheng, Y.; Zhao, J.; Xu, F.; Li, Y. Pretreatment of lignocellulosic biomass for enhanced biogas production. *Prog. Energy Combust. Sci.* **2014**, *42*, 35–53. <https://doi.org/10.1016/j.pecs.2014.01.001>.
17. Pilarska, A.; Pilarski, K.; Witaszek, K.; Dukiewicz, H.; Dobrzański, K. Preliminary studies on the effect of thermal treatment of maize silage on biogas yield. *Nauka Przyr. Technol. (Sci. Nat. Technol.)* **2015**, *9*, 18. <https://doi.org/10.17306/J.NPT.2015.2.18>.
18. Val del Río, A.; Morales, N.; Isanta, E.; Mosquera-Corral, A.; Campos, J.L.; Steyer, J.P.; Carrère, H. Thermal pre-treatment of aerobic granular sludge: Impact on anaerobic biodegradability. *Water Res.* **2011**, *45*, 6011–6020. <https://doi.org/10.1016/j.watres.2011.08.050>.
19. Nayak, J.K.; Ranade, V.V. Valorisation of digestate: Characteristics, products, processes and potential. *Chem. Eng. J. Adv.* **2025**, *24*, 100887. <https://doi.org/10.1016/j.cej.2025.100887>.
20. Menardo, S.; Balsari, P.; Dinuccio, E.; Gioelli, F. Thermal pre-treatment of solid fraction from mechanically-separated raw and digested slurry to increase methane yield. *Bioresour. Technol.* **2011**, *102*, 2026–2032. <https://doi.org/10.1016/j.biortech.2010.09.067>.
21. Bougrier, C.; Delgenès, J.P.; Carrère, H. Effects of thermal treatments on five different waste activated sludge samples solubilisation, physical properties and anaerobic digestion. *Chem. Eng. J.* **2008**, *139*, 236–244. <https://doi.org/10.1016/j.cej.2007.07.099>.

22. Caroca, E.; Serrano, A.; Borja, R.; Jiménez, A.; Carvajal, A.; Braga, A.F.M.; Rodriguez-Gutierrez, G.; Feroso, F.G. Influence of phenols and furans released during thermal pretreatment of olive mill solid waste on its anaerobic digestion. *Waste Manag.* **2021**, *120*, 202–208. <https://doi.org/10.1016/j.wasman.2020.11.027>.
23. Rafique, R.; Poulsen, T.G.; Nizami, A.-S.; Asam, Z.-U.-Z.; Murphy, J.D.; Kiely, G. Effect of thermal, chemical and thermo-chemical pre-treatments to enhance methane production. *Energy* **2010**, *35*, 4556–4561. <https://doi.org/10.1016/j.energy.2010.07.011>.
24. Lin, Y.; Wang, D.; Wu, S.; Wang, C. Alkali Pretreatment Enhances Biogas Production in the Anaerobic Digestion of Pulp and Paper Sludge. *J. Hazard. Mater.* **2009**, *170*, 366–373. <https://doi.org/10.1016/j.jhazmat.2009.04.086>.
25. Sharma, A.; Thakur, M.; Bhattacharya, M.; Mandal, T.; Goswami, S. Commercial application of cellulose nano-composites—A review. *Biotechnol. Rep.* **2019**, *21*, e00316. <https://doi.org/10.1016/j.btre.2019.e00316>.
26. Machhirake, N.; Singh, D.; Yadav, B.R.; Tembhare, M.; Kumar, S. Optimizing Alkali-Pretreatment Dosage for Waste-Activated Sludge Disintegration and Enhanced Biogas Production Yield. *Environ. Res.* **2024**, *252*, 118876. <https://doi.org/10.1016/j.envres.2024.118876>.
27. Vinuthana, V.H.; Govindaraj, O.; Subramaniam, S.; Gnanachitra, M.; Uthandi, S. Harnessing lignocellulosic biomass: Insights into pre-treatment strategies and hydrolytic enzyme production. *Ind. Crops Prod.* **2025**, *229*, 120986. <https://doi.org/10.1016/j.indcrop.2025.120986>.
28. Gupta, P.; Singh, R.S.; Sachan, A.; Vidyarthi, A.S.; Gupta, A. A re-appraisal on intensification of biogas production. *Renew. Sustain. Energy Rev.* **2012**, *16*, 4908–4916. <https://doi.org/10.1016/j.rser.2012.05.005>.
29. Zheng, C.-C.; Gao, L.; Sun, H.; Zhao, X.-Y.; Gao, Z.-Q.; Liu, J.; Guo, W. Advancements in enzymatic reaction-mediated microbial transformation. *Heliyon* **2024**, *10*, e38187. <https://doi.org/10.1016/j.heliyon.2024.e38187>.
30. Noyola, A.; Tinajero, A. Effect of biological additives and micronutrients on the anaerobic digestion of physicochemical sludge. *Water Sci. Technol.* **2005**, *52*, 275–281. <https://doi.org/10.2166/wst.2005.0528>.
31. Hasegawa, S.; Shiota, N.; Katsura, K.; Akashi, A. Solubilization of organic sludge by thermophilic aerobic bacteria as a pretreatment for anaerobic digestion. *Water Sci. Technol.* **2000**, *41*, 163–169. <https://doi.org/10.2166/wst.2000.0068>.
32. Cao, J.; Zhang, C.; Li, X.; Wang, X.; Dai, X.; Xu, Y. Microbial Community and Metabolic Pathways in Anaerobic Digestion of Organic Solid Wastes: Progress, Challenges and Prospects. *Fermentation* **2025**, *11*, 457. <https://doi.org/10.3390/fermentation11080457>.
33. Karagiannidis, A.; Perkoulidis, G. A multi-criteria ranking of different technologies for the anaerobic digestion for energy recovery of the organic fraction of municipal solid wastes. *Bioresour. Technol.* **2009**, *100*, 2355–2360. <https://doi.org/10.1016/j.biortech.2008.11.033>.
34. Iavi-Borazjani, S.A.; da Cruz Tarelho, L.A.; Capela, M.I. Biohythane production via anaerobic digestion process: Fundamentals, scale-up challenges, and techno-economic and environmental aspects. *Environ. Sci. Pollut. Res.* **2024**, *31*, 49935–49984. <https://doi.org/10.1007/s11356-024-34471-8>.
35. Thauer, R.K.; Kaster, A.-K.; Seedorf, H.; Buckel, W.; Hedderich, R. Methanogenic *Archaea*: Ecologically relevant differences in energy conservation. *Nat. Rev. Microbiol.* **2008**, *6*, 579–591. <https://doi.org/10.1038/nrmicro1931>.
36. Nayeri, D.; Mohammadi, P.; Bashardoust, P.; Eshtiaghi, N. A comprehensive review on the recent development of anaerobic sludge digestions: Performance, mechanism, operational factors, and future challenges. *Results Eng.* **2024**, *22*, 102292. <https://doi.org/10.1016/j.rineng.2024.102292>.
37. Pilarska, A.A.; Wolna-Maruwka, A.; Pilarski, K. Kraft Lignin Grafted with Polyvinylpyrrolidone as a Novel Microbial Carrier in Biogas Production. *Energies* **2018**, *11*, 3246. <https://doi.org/10.3390/en11123246>.
38. Lee, H.-G.; Dulany, Z. Biogas and Hydrogen Production from Waste Biomass via Dark Fermentation Evaluating VFAs, COD, and HRT for Process Optimization. *Biomass* **2025**, *5*, 57. <https://doi.org/10.3390/biomass5030057>.
39. Detman, A.; Bucha, M.; Treu, L.; Chojnacka, A.; Pleśniak, Ł.; Salamon, A.; Łupikasza, E.; Gromadka, R.; Gawor, J.; Gromadka, A.; et al. Evaluation of acidogenesis products' effect on biogas production performed with metagenomics and isotopic approaches. *Biotechnol. Biofuels* **2021**, *14*, 125. <https://doi.org/10.1186/s13068-021-01968-0>.
40. Borja, R. Biogas Production. In *Comprehensive Biotechnology*, 2nd ed.; Moo-Young, M., Ed.; Academic Press: Burlington, MA, USA, 2011; pp. 785–798. <https://doi.org/10.1016/B978-0-08-088504-9.00126-4>.
41. Zieliński, M.; Kazmierowicz, J.; Dębowski, M. Advantages and Limitations of Anaerobic Wastewater Treatment—Technological Basics, Development Directions, and Technological Innovations. *Energies* **2023**, *16*, 83. <https://doi.org/10.3390/en16010083>.
42. Gebreyessus, G.D.; Jenicek, P. Thermophilic versus Mesophilic Anaerobic Digestion of Sewage Sludge: A Comparative Review. *Bioengineering* **2016**, *3*, 15. <https://doi.org/10.3390/bioengineering3020015>.

43. Akinbomi, J.G.; Patinvoh, R.J.; Taherzadeh, M.J. Current challenges of high-solid anaerobic digestion and possible measures for its effective applications: A review. *Biotechnol. Biofuels Bioprod.* **2022**, *15*, 52. <https://doi.org/10.1186/s13068-022-02151-9>.
44. Gao, Q.; Li, L.; Wang, K.; Zhao, Q. Mass Transfer Enhancement in High-Solids Anaerobic Digestion of Organic Fraction of Municipal Solid Wastes: A Review. *Bioengineering* **2023**, *10*, 1084. <https://doi.org/10.3390/bioengineering10091084>.
45. Pilarski, K.; Pilarska, A.A.; Boniecki, P.; Niedbała, G.; Witaszek, K.; Piekutowska, M.; Idzior-Haufa, M.; Wawrzyniak, A. Degree of Biomass Conversion in the Integrated Production of Bioethanol and Biogas. *Energies* **2021**, *14*, 7763. <https://doi.org/10.3390/en14227763>.
46. Qin, Y.; Zhai, C. Global Stabilizing Control of a Continuous Ethanol Fermentation Process Starting from Batch Mode Production. *Processes* **2024**, *12*, 819. <https://doi.org/10.3390/pr12040819>.
47. Hayyat, U.; Khan, M.U.; Sultan, M.; Zahid, U.; Bhat, S.A.; Muzamil, M. A Review on Dry Anaerobic Digestion: Existing Technologies, Performance Factors, Challenges, and Recommendations. *Methane* **2024**, *3*, 33–52. <https://doi.org/10.3390/methane3010003>.
48. Hong, Y.; Bayly, R.A.; Salasso, D.; Cumin, J.R.; Sproule, D.E.; Chang, S. Method for Utilizing Internally Generated Biogas for Closed Membrane System Operation. U.S. Patent 8,580,113 B2, 12 November 2013. Available online: <https://patents.google.com/patent/US8580113B2/en> (accessed on 12 November 2013).
49. Andersen, L.J.N.; Jeppesen, M.D. Integration of Digester and Thermo-Chemical Digestate Treatment Equipment with Heat Recovery. International Patent WO 2023/208304 A1, 2 November 2023. Available online: <https://patents.google.com/patent/WO2023208304A1/en> (accessed on 2 November 2023).
50. Sidi Habib, S.; Torii, S.; S, K.M.; Charivuparampil Achuthan Nair, A. Optimization of the Factors Affecting Biogas Production Using the Taguchi Design of Experiment Method. *Biomass* **2024**, *4*, 687–703. <https://doi.org/10.3390/biomass4030038>.
51. Qian, S.; Chen, L.; Xu, S.; Zeng, C.; Lian, X.; Xia, Z.; Zou, J. Research on Methane-Rich Biogas Production Technology by Anaerobic Digestion Under Carbon Neutrality: A Review. *Sustainability* **2025**, *17*, 1425. <https://doi.org/10.3390/su17041425>.
52. Jaya Prasanna Kumar, D.; Mishra, R.K.; Chinnam, S.; Binnal, P.; Dwivedi, N. A comprehensive study on anaerobic digestion of organic solid waste: A review on configurations, operating parameters, techno-economic analysis and current trends. *Biotechnol. Notes* **2024**, *5*, 33–49. <https://doi.org/10.1016/j.biotno.2024.02.001>.
53. Cioabla, A.E.; Ionel, I.; Dumitrel, G.A.; Popescu, F. Comparative study on factors affecting anaerobic digestion of agricultural vegetal residues. *Biotechnol. Biofuels* **2012**, *5*, 39. <https://doi.org/10.1186/1754-6834-5-39>.
54. Edwards, T.M.; Puglis, H.J.; Kent, D.B.; López Durán, J.; Bradshaw, L.M.; Farag, A.M. Ammonia and aquatic ecosystems—A review of global sources, biogeochemical cycling, and effects on fish. *Sci. Total Environ.* **2024**, *907*, 167911. <https://doi.org/10.1016/j.scitotenv.2023.167911>.
55. Lee, D.-H.; Behera, S.K.; Kim, J.-W.; Park, H.-S. Methane production potential of leachate generated from Korean food waste recycling facilities: A lab-scale study. *Waste Manag.* **2009**, *29*, 876–882. <https://doi.org/10.1016/j.wasman.2008.06.033>.
56. Kim, J.; Park, C.; Kim, T.H.; Lee, M.; Kim, S.; Kim, S.W.; Lee, J. Effects of various pretreatments for enhanced anaerobic digestion with waste activated sludge. *J. Biosci. Bioeng.* **2003**, *95*, 271–275. <https://doi.org/10.1263/jbb.95.271>.
57. Wu, H.-M.; Li, X.; Chen, J.-N.; Yan, Y.-J.; Kobayashi, T.; Hu, Y.; Zhang, X. Food Waste Anaerobic Digestion Under High Organic Loading Rate: Inhibiting Factors, Mechanisms, and Mitigation Strategies. *Processes* **2025**, *13*, 2090. <https://doi.org/10.3390/pr13072090>.
58. Voidarou, C.; Antoniadou, M.; Rozos, G.; Tzora, A.; Skoufos, I.; Varzakas, T.; Lagiou, A.; Bezirtzoglou, E. Fermentative Foods: Microbiology, Biochemistry, Potential Human Health Benefits and Public Health Issues. *Foods* **2021**, *10*, 69. <https://doi.org/10.3390/foods10010069>.
59. Yan, X.; Deng, P.; Ding, T.; Zhang, Z.; Li, X.; Wu, Z. Effect of Temperature on Anaerobic Fermentation of Poplar Ethanol Wastewater: Performance and Microbial Communities. *ACS Omega* **2023**, *8*, 5486–5496. <https://doi.org/10.1021/acsomega.2c06822>.
60. Kabaivanova, L.; Petrova, P.; Hubenov, V.; Simeonov, I. Biogas Production Potential of Thermophilic Anaerobic Biodegradation of Organic Waste by a Microbial Consortium Identified with Metagenomics. *Life* **2022**, *12*, 702. <https://doi.org/10.3390/life12050702>.
61. Finore, I.; Feola, A.; Russo, L.; Cattaneo, A.; Di Donato, P.; Nicolaus, B.; Poli, A.; Romano, I. Thermophilic Bacteria and Their Thermozyms in Composting Processes: A Review. *Chem. Biol. Technol. Agric.* **2023**, *10*, 7. <https://doi.org/10.1186/s40538-023-00381-z>.
62. Menardo, S.; Gioelli, F.; Balsari, P. The methane yield of digestate: Effect of organic loading rate, hydraulic retention time, and plant feeding. *Bioresour. Technol.* **2011**, *102*, 2348–2351. <https://doi.org/10.1016/j.biortech.2010.10.094>.

63. Hmaissia, A.; Hernández, E.M.; Boivin, S.; Vaneckhaute, C. Start-Up Strategies for Thermophilic Semi-Continuous Anaerobic Digesters: Assessing the Impact of Inoculum Source and Feed Variability on Efficient Waste-to-Energy Conversion. *Sustainability* **2025**, *17*, 5020. <https://doi.org/10.3390/su17115020>.
64. Labatut, R.A.; Angenent, L.T.; Scott, N.R. Conventional Mesophilic vs. Thermophilic Anaerobic Digestion: A Trade-Off between Performance and Stability? *Water Res.* **2014**, *53*, 249–258. <https://doi.org/10.1016/j.watres.2014.01.035>.
65. Alcocer-García, H.; Sánchez-Ramírez, E.; García-García, E.; Ramírez-Márquez, C.; Ponce-Ortega, J.M. Unlocking the Potential of Biomass Resources: A Review on Sustainable Process Design and Intensification. *Resources* **2025**, *14*, 143. <https://doi.org/10.3390/resources14090143>.
66. Issahaku, M.; Agyemang Derkyi, N.S.; Kemausuor, F. A Systematic Review of the Design Considerations for the Operation and Maintenance of Small-Scale Biogas Digesters. *Heliyon* **2024**, *10*, e24019. <https://doi.org/10.1016/j.heliyon.2024.e24019>.
67. Makamure, F.; Mukumba, P.; Makaka, G. An analysis of bio-digester substrate heating methods: A review. *Renew. Sustain. Energy Rev.* **2021**, *137*, 110432. <https://doi.org/10.1016/j.rser.2020.110432>.
68. Aboudi, K.; Greses, S.; González-Fernández, C. Hydraulic Retention Time as an Operational Tool for the Production of Short-Chain Carboxylates via Anaerobic Fermentation of Carbohydrate-Rich Waste. *Molecules* **2023**, *28*, 6635. <https://doi.org/10.3390/molecules28186635>.
69. Harirchi, S.; Wainaina, S.; Sar, T.; Nojoumi, S.A.; Parchami, M.; Parchami, M.; Varjani, S.; Khanal, S.K.; Wong, J.; Awasthi, M.K.; et al. Microbiological Insights into Anaerobic Digestion for Biogas, Hydrogen or Volatile Fatty Acids (VFAs): A Review. *Bioengineered* **2022**, *13*, 6521–6557. <https://doi.org/10.1080/21655979.2022.2035986>.
70. Parajuli, A.; Khadka, A.; Sapkota, L.; Ghimire, A. Effect of Hydraulic Retention Time and Organic-Loading Rate on Two-Stage, Semi-Continuous Mesophilic Anaerobic Digestion of Food Waste during Start-Up. *Fermentation* **2022**, *8*, 620. <https://doi.org/10.3390/fermentation8110620>.
71. Srisowmeya, G.; Chakravarthy, M.; Nandhini Devi, G. Critical Considerations in Two-Stage Anaerobic Digestion of Food Waste—A Review. *Renew. Sustain. Energy Rev.* **2020**, *119*, 109587. <https://doi.org/10.1016/j.rser.2019.109587>.
72. Woźniak, A.; Kuligowski, K.; Świerczek, L.; Cenian, A. Review of Lignocellulosic Biomass Pretreatment Using Physical, Thermal and Chemical Methods for Higher Yields in Bioethanol Production. *Sustainability* **2025**, *17*, 287. <https://doi.org/10.3390/su17010287>.
73. Li, Y.; Zhao, J.; Krooneman, J.; Euverink, J.G.W. Strategies to Boost Anaerobic Digestion Performance of Cow Manure: Laboratory Achievements and Their Full-Scale Application Potential. *Sci. Total Environ.* **2021**, *755*, 142940. <https://doi.org/10.1016/j.scitotenv.2020.142940>.
74. Ivanovici, M.; Dumitrel, G.-A.; Gherman, V.D.; Todinca, T.; Pana, A.-M.; Ordodi, V.L. Anaerobic Digestion of Food Waste and Granular Inoculum: Study on Temperature Effect and Substrate-to-Inoculum Ratio on Biogas Production. *Fermentation* **2025**, *11*, 348. <https://doi.org/10.3390/fermentation11060348>.
75. Makamure, F.; Mukumba, P.; Makaka, G. Biogas Production from a Solar-Heated Temperature-Controlled Biogas Digester. *Sustainability* **2024**, *16*, 9894. <https://doi.org/10.3390/su16229894>.
76. Ugwu, S.N.; Enweremadu, C.C. Enhancement of Biogas Production Process from Biomass Wastes Using Iron-Based Additives: Types, Impacts, and Implications. *Energy Sources Part A Recovery Util. Environ. Eff.* **2022**, *44*, 4458–4480. <https://doi.org/10.1080/15567036.2020.1788675>.
77. Song, Y.; Qiao, W.; Westerholm, M.; Huang, G.; Taherzadeh, M.J.; Dong, R. Microbiological and Technological Insights on Anaerobic Digestion of Animal Manure: A Review. *Fermentation* **2023**, *9*, 436. <https://doi.org/10.3390/fermentation9050436>.
78. Pilarska, A.A.; Wolna-Maruwka, A.; Niewiadomska, A.; Grządziel, J.; Gałązka, A.; Paluch, E.; Borowiak, K.; Pilarski, K. Quantitative and Qualitative Changes in Genetic Diversity of Bacterial Communities in Anaerobic Bioreactors with Diatomaceous Earth/Peat Cell Carrier. *Cells* **2022**, *11*, 2571. <https://doi.org/10.3390/cells11162571>.
79. Sarker, S.; Lamb, J.J.; Hjelme, D.R.; Lien, K.M. A Review of the Role of Critical Parameters in the Design and Operation of Biogas Production Plants. *Appl. Sci.* **2019**, *9*, 1915. <https://doi.org/10.3390/app9091915>.
80. Serrano-Meza, A.; Moreno-Andrade, I.; Estrada-Arriaga, E.B.; Díaz-Barajas, S.A.; García-Sánchez, L.; Garzón-Zúñiga, M.A. Biomethane Production and Methanogenic Microbiota Restoration after a pH Failure in an Anaerobic Sequencing Batch Reactor (A-SBR) Treating Tequila Vinasse. *Fermentation* **2024**, *10*, 557. <https://doi.org/10.3390/fermentation10110557>.
81. Ibro, M.K.; Ancha, V.R.; Lemma, D.B. Impacts of Anaerobic Co-Digestion on Different Influencing Parameters: A Critical Review. *Sustainability* **2022**, *14*, 9387. <https://doi.org/10.3390/su14159387>.

82. Jarosz, Z.; Kaplan, M.; Klimek, K.; Dybek, B.; Herkowiak, M.; Wałowski, G. An Assessment of the Development of a Mobile Agricultural Biogas Plant in the Context of a Cogeneration System. *Appl. Sci.* **2023**, *13*, 12447. <https://doi.org/10.3390/app132212447>.
83. Venkiteshwaran, K.; Bocher, B.; Maki, J.; Zitomer, D. Relating Anaerobic Digestion Microbial Community and Process Function. *Microbiol. Insights* **2016**, *8* (Suppl. 2), 37–44. <https://doi.org/10.4137/MBI.S33593>.
84. Qian, W.; Peng, Y.; Li, X.; Zhang, Q.; Ma, B. The Inhibitory Effects of Free Ammonia on Ammonia Oxidizing Bacteria and Nitrite Oxidizing Bacteria under Anaerobic Condition. *Bioresour. Technol.* **2017**, *243*, 1247–1250. <https://doi.org/10.1016/j.biortech.2017.07.119>.
85. Lee, E.H.; Moon, K.E.; Kim, T.G.; Lee, S.D.; Cho, K.S. Inhibitory Effects of Sulfur Compounds on Methane Oxidation by a Methane-Oxidizing Consortium. *J. Biosci. Bioeng.* **2015**, *120*, 670–676. <https://doi.org/10.1016/j.jbiosc.2015.04.006>.
86. Beschkov, V.N.; Angelov, I.K. Volatile Fatty Acid Production vs. Methane and Hydrogen in Anaerobic Digestion. *Fermentation* **2025**, *11*, 172. <https://doi.org/10.3390/fermentation11040172>.
87. Szacherska, K.; Oleskiewicz-Popiel, P.; Ciesielski, S.; Możejko-Ciesielska, J. Volatile Fatty Acids as Carbon Sources for Polyhydroxyalkanoates Production. *Polymers* **2021**, *13*, 321. <https://doi.org/10.3390/polym13030321>.
88. Guo, Q.; Majeed, S.; Xu, R.; Zhang, K.; Kakade, A.; Khan, A.; Hafeez, F.Y.; Mao, C.; Liu, P.; Li, X. Heavy Metals Interact with the Microbial Community and Affect Biogas Production in Anaerobic Digestion: A Review. *J. Environ. Manag.* **2019**, *240*, 266–272. <https://doi.org/10.1016/j.jenvman.2019.03.104>.
89. Nsair, A.; Onen Cinar, S.; Alassali, A.; Abu Qdais, H.; Kuchta, K. Operational Parameters of Biogas Plants: A Review and Evaluation Study. *Energies* **2020**, *13*, 3761. <https://doi.org/10.3390/en13153761>.
90. Niya, B.; Yaakoubi, K.; Beraich, F.Z.; Arouch, M.; Kadmiri, I.M. Current Status and Future Developments of Assessing Microbiome Composition and Dynamics in Anaerobic Digestion Systems Using Metagenomic Approaches. *Heliyon* **2024**, *10*, e28221. <https://doi.org/10.1016/j.heliyon.2024.e28221>.
91. Singh, B.; Singh, N.; Čonka, Z.; Kolcun, M.; Siménfalvi, Z.; Péter, Z.; Szamosi, Z. Critical Analysis of Methods Adopted for Evaluation of Mixing Efficiency in an Anaerobic Digester. *Sustainability* **2021**, *13*, 6668. <https://doi.org/10.3390/su13126668>.
92. Singh, B.; Kovács, K.L.; Bagi, Z.; Nyári, J.; Szepesi, G.L.; Petrik, M.; Siménfalvi, Z.; Szamosi, Z. Enhancing Efficiency of Anaerobic Digestion by Optimization of Mixing Regimes Using Helical Ribbon Impeller. *Fermentation* **2021**, *7*, 251. <https://doi.org/10.3390/fermentation7040251>.
93. Farid, M.U.; Olbert, I.A.; Bück, A.; Ghafoor, A.; Wu, G. CFD Modelling and Simulation of Anaerobic Digestion Reactors for Energy Generation from Organic Wastes: A Comprehensive Review. *Heliyon* **2025**, *11*, e41911. <https://doi.org/10.1016/j.heliyon.2025.e41911>.
94. Neuner, T.; Meister, M.; Pillei, M.; Senfter, T.; Draxl-Weiskopf, S.; Ebner, C.; Winkler, J.; Rauch, W. Impact of Design and Mixing Strategies on Biogas Production in Anaerobic Digesters. *Water* **2024**, *16*, 2205. <https://doi.org/10.3390/w16152205>.
95. Pilarski, K.; Pilarska, A.A. Operation and Challenges of Biogas Technology: A Fundamental Overview. *Tech. Sci.* **2024**, *27*, 281–307. <https://doi.org/10.31648/ts.10701>.
96. Pilarski, K.; Pilarska, A.A.; Boniecki, P.; Niedbała, G.; Durczak, K.; Witaszek, K.; Mioduszewska, N.; Kowalik, I. The Efficiency of Industrial and Laboratory Anaerobic Digesters of Organic Substrates: The Use of the Biochemical Methane Potential Correction Coefficient. *Energies* **2020**, *13*, 1280. <https://doi.org/10.3390/en13051280>.
97. Meegoda, J.N.; Chande, C.; Bakshi, I. Biodigesters for Sustainable Food Waste Management. *Int. J. Environ. Res. Public Health* **2025**, *22*, 382. <https://doi.org/10.3390/ijerph22030382>.
98. Sihlangu, E.; Magama, P.; Chiyanzu, I.; Regnier, T.; Luseba, D.; Nephawe, K.A. Investigating the Influence of Organic Loading Rate, Temperature and Stirring Speed on Biogas Production Using Agricultural Waste in South Africa. *Agriculture* **2024**, *14*, 2091. <https://doi.org/10.3390/agriculture14112091>.
99. Alhraishawi, A.; Aslan, S. Effect of Salt Content on Biogas Production and Microbial Activity: Review Study. *Eur. Sci. J.* **2023**, *19*, 17. <https://doi.org/10.19044/esj.2023.v19n39p17>.
100. Kang, X.; Liu, Y.; Liu, W.; Wang, L.; Li, C. Fermentation and Biogas Production of Alkaline Wasted Sludge Enhanced in a Bioelectrolysis-Assisted Anaerobic Digestion Reactor under Increasing Organic Loads. *Sustainability* **2023**, *15*, 1443. <https://doi.org/10.3390/su15021443>.
101. Lu, Y.; Huang, G.; Zhang, J.; Han, T.; Tian, P.; Li, G.; Li, Y. Optimization of Anaerobic Co-Digestion Parameters for Vinegar Residue and Cattle Manure via Orthogonal Experimental Design. *Fermentation* **2025**, *11*, 493. <https://doi.org/10.3390/fermentation11090493>.

102. Nieścioruk, M.J.; Bandrow, P.; Szufa, S.; Woźniak, M.; Siczek, K. Biomass-Based Hydrogen Extraction and Accompanying Hazards—Review. *Molecules* **2025**, *30*, 565. <https://doi.org/10.3390/molecules30030565>.
103. Liu, J.; Olsson, G.; Mattiasson, B. Monitoring and Control of an Anaerobic Upflow Fixed-Bed Reactor for High-Loading-Rate Operation and Rejection of Disturbances. *Biotechnol. Bioeng.* **2004**, *87*, 43–53. <https://doi.org/10.1002/bit.20088>.
104. Ferguson, M.W.R.; Coulon, F.; Villa, R. Organic Loading Rate: A Promising Microbial Management Tool in Anaerobic Digestion. *Water Res.* **2016**, *100*, 348–356. <https://doi.org/10.1016/j.watres.2016.05.009>.
105. Kaneko, H.; Ozaki, Y.; Takezaki, J.; Daimon, H. Granular Activated Carbon and Organic Loading Interactions in Methane Fermentation: An Inverse Load-Dependent Relationship and Absolute Microbial Abundance Analysis. *Fuels* **2025**, *6*, 72. <https://doi.org/10.3390/fuels6030072>.
106. Maleki, E.; Bokhary, A.; Liao, B.Q. A Review of Anaerobic Digestion Bio-Kinetics. *Rev. Environ. Sci. Biotechnol.* **2018**, *17*, 691–705. <https://doi.org/10.1007/s11157-018-9484-z>.
107. Ripoll, M.; López, I.; Borzacconi, L. Hydrogenotrophic Activity: A Tool to Evaluate the Kinetics of Methanogens. *J. Environ. Manag.* **2020**, *270*, 110937. <https://doi.org/10.1016/j.jenvman.2020.110937>.
108. Ao, T.; Chen, L.; Zhou, P.; Liu, X.; Zhang, J.; Li, Y. The Role of Oxidation–Reduction Potential as an Early Warning Indicator and a Microbial Instability Mechanism in a Pilot-Scale Anaerobic Mesophilic Digestion of Chicken Manure. *Renew. Energy* **2021**, *179*, 223–232. <https://doi.org/10.1016/j.renene.2021.07.047>.
109. Shen, R.; Geng, T.; Yao, Z.; Yu, J.; Luo, J.; Wang, H.; Zhao, L. Characteristics of Instability and Suitable Early-Warning Indicators for Cornstalk-Fed Anaerobic Digestion Subjected to Various Sudden Changes. *Energy* **2023**, *278*, 127735. <https://doi.org/10.1016/j.energy.2023.127735>.
110. 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. <https://doi.org/10.1016/j.scitotenv.2020.142425>.
111. Rusanowska, P.; Zieliński, M.; Kisielewska, M.; Dudek, M.; Pauksztó, Ł.; Dębowski, M. Methane Production, Microbial Community, and Volatile Fatty Acids Profiling During Anaerobic Digestion under Different Organic Loading. *Energies* **2025**, *18*, 575. <https://doi.org/10.3390/en18030575>.
112. Manyi-Loh, C.E.; Lues, R. Anaerobic Digestion of Lignocellulosic Biomass: Substrate Characteristics (Challenge) and Innovation. *Fermentation* **2023**, *9*, 755. <https://doi.org/10.3390/fermentation9080755>.
113. Mikulski, D.; Kłosowski, G. Delignification Efficiency of Various Types of Biomass Using Microwave-Assisted Hydrothermal Pretreatment. *Sci. Rep.* **2022**, *12*, 4561. <https://doi.org/10.1038/s41598-022-08717-9>.
114. Arantes, V.; Saddler, J.N. Cellulose Accessibility Limits the Effectiveness of Minimum Cellulase Loading on the Efficient Hydrolysis of Pretreated Lignocellulosic Substrates. *Biotechnol. Biofuels* **2011**, *4*, 3. <https://doi.org/10.1186/1754-6834-4-3>.
115. Dipak, A.R.; Dutta, S.J.; Sherpa, K.C.; Jayaswal, K.; Saravanabhupathy, S.; Mohanty, K.T.; Banerjee, R.; Kumar, J.; Rajak, R.C. Co-Digestion Processes of Waste: Status and Perspective. In *Advanced Zero Waste Tools, Bio-Based Materials and Waste for Energy Generation and Resource Management*; Hussain, C.M., Kushwaha, A., Bharagava, R.N., Goswami, L., Eds.; Elsevier: Amsterdam, The Netherlands, 2023; Volume 5, pp. 207–241. <https://doi.org/10.1016/B978-0-323-91149-8.00010-7>.
116. Chen, J.; Ma, X.; Liang, M.; Guo, Z.; Cai, Y.; Zhu, C.; Wang, Z.; Wang, S.; Xu, J.; Ying, H. Physical–Chemical–Biological Pretreatment for Biomass Degradation and Industrial Applications: A Review. *Waste* **2024**, *2*, 451–473. <https://doi.org/10.3390/waste2040024>.
117. Brodeur, G.; Yau, E.; Badal, K.; Collier, J.; Ramachandran, K.B.; Ramakrishnan, S. Chemical and Physicochemical Pretreatment of Lignocellulosic Biomass: A Review. *Enzyme Res.* **2011**, *2011*, 787532. <https://doi.org/10.4061/2011/787532>.
118. de la Lama-Calvente, D.; Mancilla-Leytón, J.M.; Garrido-Murillo, I.; Rojas-Carrillo, J.; Borja, R.; Fernández-Rodríguez, M.J. Influence of Nitrogen Bioavailability on the Anaerobic Co-Digestion of the Aegagropiles of the Seagrass *Posidonia oceanica* with Different Nitrogen-Rich Substrates: Process Performance and Kinetic Analysis. *Appl. Sci.* **2025**, *15*, 2880. <https://doi.org/10.3390/app15062880>.
119. Atiqueuzzaman Khan, M.A.; Ngo, H.H.; Guo, W.; Liu, Y.; Nghiem, L.D.; Chang, S.W.; Nguyen, D.D.; Zhang, S.; Luo, G.; Jia, H. Optimization of Hydraulic Retention Time and Organic Loading Rate for Volatile Fatty Acid Production from Low-Strength Wastewater in an Anaerobic Membrane Bioreactor. *Bioresour. Technol.* **2019**, *271*, 100–108. <https://doi.org/10.1016/j.biortech.2018.09.075>.
120. Lee, I.S.; Parameswaran, P.; Rittmann, B.E. Effects of Solids Retention Time on Methanogenesis in Anaerobic Digestion of Thickened Mixed Sludge. *Bioresour. Technol.* **2011**, *102*, 10266–10272. <https://doi.org/10.1016/j.biortech.2011.08.079>.

121. Maldonado-Saeteros, S.; Baquerizo-Crespo, R.; Gómez-Salcedo, Y.; Pérez-Ones, O.; Pereda-Reyes, I. Influence of Temperature on Kinetics and Hydraulic Retention Time in Discontinuous and Continuous Anaerobic Systems. *Environ. Eng. Res.* **2023**, *28*, 210442. <https://doi.org/10.4491/eer.2021.442>.
122. Neto, A.d.S.; Taherzadeh, M.J. Guiding Microbial Crossroads: Syngas-Driven Valorisation of Anaerobic-Digestion Intermediates into Bio-Hydrogen and Volatile Fatty Acids. *Bioengineering* **2025**, *12*, 816. <https://doi.org/10.3390/bioengineering12080816>.
123. Schink, B. Energetics of Syntrophic Cooperation in Methanogenic Degradation. *Microbiol. Mol. Biol. Rev.* **1997**, *61*, 262–280. <https://doi.org/10.1128/membr.61.2.262-280.1997>.
124. Szpicer, A.; Bińkowska, W.; Stelmasiak, A.; Wojtasik-Kalinowska, I.; Czajkowska, A.; Mierzejewska, S.; Domiszewski, Z.; Rydzkowski, T.; Piepiórka-Stepuk, J.; Póltorak, A. Advances in Computational Fluid Dynamics of Mechanical Processes in Food Engineering: Mixing, Extrusion, Drying, and Process Optimization. *Appl. Sci.* **2025**, *15*, 8752. <https://doi.org/10.3390/app15158752>.
125. Nadeem, K.; Alliet, M.; Plana, Q.; Bernier, J.; Azimi, S.; Rocher, V.; Albasi, C. Modeling, Simulation and Control of Biological and Chemical P-Removal Processes for Membrane Bioreactors (MBRs) from Lab to Full-Scale Applications: State of the Art. *Sci. Total Environ.* **2022**, *809*, 151109. <https://doi.org/10.1016/j.scitotenv.2021.151109>.
126. Glass, J.B.; Orphan, V.J. Trace Metal Requirements for Microbial Enzymes Involved in the Production and Consumption of Methane and Nitrous Oxide. *Front. Microbiol.* **2012**, *3*, 61. <https://doi.org/10.3389/fmicb.2012.00061>.
127. Elazhar, M.; Bouchabchoub, A.; Elazhar, F.; Elmidaoui, A.; Takyi, M. Influence of Volatile Fatty Acids/Alkalinity Ratio on Methane Production during Mesophilic Anaerobic Digestion: Stability, Efficiency and Optimization. *Desalination Water Treat.* **2022**, *257*, 142–149. <https://doi.org/10.5004/dwt.2022.28580>.

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