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Review Article

Biochemical Dynamics and Sustainable Energy Production in Anaerobic Digestion: Microbial Insights and Innovations

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Abstract

Regarding sustainable wastewater treatment and renewable energy production, anaerobic digestion (AD) is an important technology. This review presents the microbial and biochemical processes involved in methane production in AD systems, focusing mainly on AD of domestic wastewater. The four stages, hydrolysis, acidogenesis, acetogenesis, and methanogenesis are described with relation to microbial consortia, enzymatic activities and coenzymes such as Coenzyme M and F_{420}. Recent advances in interspecies electron transfer (IET), in particular direct IET (DIET), also suggest that conductive materials such as biochar increase methane production and system robustness. The metabolism and substrate specificity of methanogenic archaea are discussed and the function of electron carriers in maintaining redox balance. Biogas upgrading technologies, namely membrane separation, pressure swing adsorption, biological scrubbing and hybrid systems are assessed in relation to the methane content and tolerance of microbes. The study highlights the utilization of microbial optimization and technological advancements to enhance the biomethane production in a circular and low carbon spectrum.

Anaerobik Sindirimde Biyokimyasal Dinamikler ve Sürdürülebilir Enerji Üretimi: Mikrobiyal Görüşler ve Yenilikler

Anahtar Kelimeler
Anaerobik Arıtma
Biyogaz artırımı
Koenzimler
Metan üretimi
Türler arası elektron transferi (IET)

Özet

Anaerobik sindirim (AS), sürdürülebilir atıksu arıtımı ve biyoyakıt üretimi için önemli bir teknolojidir. Bu derleme, evsel atıksu için AS sürecinin optimizasyonu da dahil olmak üzere, metan üretimi üzerine mikrobiyal ve enzimatik mekanizmaların etkilerini inceler. Hidroliz, asidojenez, asetojenez ve metanojenez kavramları mikrobiyal topluluklar, enzimler ve koenzimler (Koenzim M ve F_{420}) ile ilişkilidir. Özellikle elektrik transferi (IET) ile ilgili yeni bulgular, iletken malzemelerin örneğin biyokömüre benzer şekilde metan üretimini uyarabileceğini ve sistemin dayanıklılığını artırabileceğini önermiştir. Metanojenlerin metabolizması ve substrat özgüllüğü ile redoks dengesinin korunmasında rol oynayan elektron taşıyıcılarının işlevleri açıklanmıştır. Biyogaz iyileştirmesi için, membran ayırma, basınç salınım adsorpsiyonu, biyolojik yıkama ve çift sistem gibi çeşitli teknolojiler metan saflığı ve mikrobiyal tolerans açısından uygulanmış ve karşılaştırılmıştır. Bu çalışma, teknoloji geliştirmeyle uyumlu mikrobiyal optimizasyonun, döngüsel ve düşük karbonlu bir hızda biyometan katkısında ne kadar önemli olduğunu vurgulamaktadır.

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INTRODUCTION

Anaerobic digestion (AD) is a bio-physico-chemical process which takes place naturally in the environment and majorly involves the sequential degradation of biodegradable organic matter with a consortia of numerous microorganisms under anaerobic conditions with a result of producing methane rich biogas and nutrient rich digestate [1,2]. Anyway, this process is extensively applied in WWTPs, agricultural and industrial installations to manage and recover energy from organic residuals [1, 2]. AD consists of four main phases, including hydrolysis, acidogenesis, acetogenesis, and methanogenesis, which are dominated by different microbial guilds and controlled by factors like pH, temperature, organic loading rate (OLR), and toxicants [3].

AD, which was first developed for the stabilization of sludge, already plays an important role as a sustainable waste-to-energy technology, and is expected to contribute greatly to the mitigation of greenhouse gases [2,4]. AD-derived methane as a substitute for fossil fuels can provide low carbon energy and also reduce the release of man-made methane from organic waste, which is crucial as methane is recognised to have 25 times the global warming potential of CO_2 over a 100-year period [6,7]. The leftover digested slurry, which is rich in nitrogen (N), phosphorus (P) and potassium (K), has the possibility to be utilized as biofertilizer, even the performance of AD [8].

By means of the technology such as the AD, waste turns into renewable energy and soil amendments according to the circular economy principle [9]. Yet challenge remains in treating of domestic wastewater by such a process, since domestic wastewater is characterized by low organic strength, daily fluctuating of loadings, and high dilution. Such circumstances often stagnate the microbial activity and reduce methane quantity [7]. Thus, further understanding of how the microbial community behaves and the system biochemistry is necessary in order to optimize AD performance under these conditions.

Some recent developments have underscored the importance of microbial syntrophy, coenzymes, and electron transfer pathways, especially direct interspecies electron transfer (DIET), in stimulating methanogenesis [10,12,14]. Conductive supplementation (e.g., biochar) was found to enhance DIET, improve syntrophic stability, and enhance methane production, especially under perturbations (e.g., ammonia, fluctuating OLR) [7,8,10]. Although many works have investigated microbial performance, coenzyme pathways, or upgrading technology separately, a holistic and concerted review on the fundamentals of biochemical dynamics and optimal microbial performance for AD systems for domestic wastewater, is still lacking.

This mini-review seeks to fulfill that need by summarizing results from 24 recent studies (2008–2024) and especially on: (1) the macroscopic functions of the microbial communities and enzymes in the four biochemical phases of AD, (2) redox coenzymes and electron transferring Groups, participating in redox metabolism, (3) newly discovered electron transfer processes (DIET/MIET), (4) microbial enhancements with biochar assistance, and (5) microorganisms in technologies for biogas upgrading.

Integrating classical knowledge of microbial science and recent development achievements, this review is conducive to the rational re-engineered design of AD systems and provides new perspectives for biochemical optimization of methane production under domestic wastewater treatment.

METHOD

This mini-review was compiled by carrying out a systematic review of 24 articles from the scientific literature which span the years from 2008 to 2024 regarding anaerobic digestion (AD), microbial interactions, methanogenesis pathways and biogas upgrading technologies. The review articles were accessed through Scopus, Web of Science, and Google Scholar using

keywords like “anaerobic digestion”, “methane production”, “hydrolysis”, “acidogenesis”, “acetogenesis”, “methanogenesis”, “interspecies electron transfer (DIET/MIET)” and “biogas upgrading”. Only English documents were considered, including both theoretical and review studies providing biochemically, microbiologically, or technologically-related information useful for domestic and agricultural waste (co-)digestion. Preference was accorded to papers dealing with the microbial dynamics, enzymatic activity, co-enzyme/electron-carrying roles, syntrophic relationships and operational conditions such as OLR, HRT and ammonia values. Preference was also given to studies focusing on molecular techniques (e.g., high-throughput sequencing and meta-transcriptomics) and on reports of digester performance while subjected to stress. Results were categorized based on the four biochemical stages of AD to facilitate comparisons between systems. By merging the fundamental knowledge together with the new concepts, the present review is to consolidate what we know and how to make most of the knowledge to improve methane production and energy recovery from AD systems.

RESULTS

Biochemical Stages of Anaerobic Digestion

Hydrolysis: Breakdown of Complex Organics

Hydrolysis is a critical phase in AD and bases the decomposition of complex organic substances. Insoluble macromolecules such as polysaccharides, proteins, lipids, and nucleic acids are hydrolyzed down to smaller soluable monomeric building blocks, sugar units, amino acids, long-chain fatty acids, and nucleotides, respectively, by hydrolytic enzymes [1,11]. These components are further metabolized by fermentative bacteria in the subsequent AD phases. Cellulases, proteases, and lipases are the main extracellular enzymes that play a role. Cellulases degrade structural carbohydrates, e.g. cellulose and hemicellulose, to fermentable sugars, proteases cleave proteins into amino acids and lipases hydrolyze lipids to glycerol and fatty acids. These reactions are important for the liberation of organic carbon in the domestic wastewater where particulate biodegradables are prevalent. The microorganisms that catalyze this process are predominantly members of the Firmicutes, Bacteroidetes, Proteobacteria, and *Actinobacteria* phyla. *Clostridium* and *Bacteroides* are known to degrade carbohydrates and proteins whereas some of the Proteobacteria primarily metabolize lipids [1,6,11]. Hydrolysis frequently controls the AD rate overall, particularly for lignocellulose-rich substrates or low-biodegradability sludge [12, 13]. In order to circumvent this situation, pre-treatment techniques are used, thermal, mechanical or chemical, to enhance the solubilisation of the substrate and enzyme accessibility. In the end, the hydrolysis process governs the availability of substrates and affects the methane yield. The optimization of microbial structure, environmental conditions, and substrate properties is key to facilitating the best biogas production via digesting domestic wastewater.

Table **1**. Enzymatic Roles and Microbial Producers in the Hydrolysis Stage of AD

Enzyme	Substrate	Product	Microbial Producers	Reaction Description
Cellulase (EC 3.2.1.4)	Polysaccharides (e.g., cellulose)	Glucose (Monosaccharides)	*Clostridium*, *Bacteroides* (Firmicutes, Bacteroidetes)	Hydrolyses cellulose into glucose using water (hydrolysis)
Protease (EC 3.4.-.-)	Proteins	Amino Acids	*Clostridium*, *Bacillus* (Firmicutes)	Breaks peptide bonds in proteins to release amino acids
Lipase (EC 3.1.1.3)	Lipids (Triglycerides)	Glycerol + Fatty Acids	*Ralstonia*, *Pseudomonas* (Proteobacteria)	Degrades triglycerides into glycerol and fatty acids

Table 1 summarizes known extracellular hydrolytic enzymes common in the hydrolysis phase of anaerobic digestion, as well as some of the specific substrates and resulting products, and representative microbial genera. The hydrolytic bacteria such as *Clostridium*, *Bacteroides* and *Pseudomonas* are highly responsible for converting complex organic materials into simpler forms. This enzymatic action can aid low fermentation and methane content. These mechanisms have been well described in microbial ecology and anaerobic digestion systems [1, 6, 11]

Acidogenesis: Formation of Volatile Fatty Acids

In the second AD step, acidogenesis, hydrolysis derived monomers (glucose, amino acids, and fatty acids) are fermented to VFAs (acetate, propionate, butyrate), ethanol, lactate, CO_2, and H_2 by acidogenic bacteria [12]. A typical reaction is: $C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$. This reflects the dissimilation of glucose derived pyruvates into acids and gases as the prominent substrates for acetogenesis and methanogenesis [14]. Acetate is critical for acetoclastic methanogens, such as *Methanosaeta* and *Methanosarcina*, whereas hydrogen and carbon dioxide are requirements for hydrogenotrophic methanogens, such as *Methanobacterium* [3, 12, 14]. The balance and ultimate availability of these intermediates are critical for methane and biogas production. Over accumulation of VFAs or low-pH levels could inhibit the activity of methanogens and disrupt the system [15]. The main enzymes of the acidogenesis are pyruvate formate-lyase (forming Acetyl-CoA and formate), lactate dehydrogenase (forming lactate), hydrogenase (evolving H_2), acetate kinase (from acetyl phosphate to acetate), and alcohol dehydrogenase (forming ethanol). These enzymes are mainly synthesized by *Clostridium*, *Bacteroides*, *Peptostreptococcus*, *Zymomonas mobilis*, and *Lactobacillus*, depending on the substrate and conditions [12]. Acidogenesis, therefore, acts as crucial middle process between hydrolysis and methanogenesis, and it is the essential parameter for stable and higher rate performance of AD.

Table **2**. Key Enzymes and Microbial Producers Involved in Acidogenesis during AD

Enzyme	Substrate	Product	Microbial Producers	Reaction Description
Pyruvate Formate-Lyase (EC 3.2.1.4)	*Pyruvate*	*Acetyl-CoA and Formate*	*Clostridium, Bacteroides*	*Converts pyruvate into acetyl-CoA and formate, initiating downstream fermentation pathways.*
Lactate Dehydrogenase (EC 1.1.1.27)	*Pyruvate*	*Lactate and NAD^+*	*Lactobacillus*	*Converts pyruvate into lactate, regenerating NAD^+ for continued glycolysis under anaerobic conditions.*
Hydrogenase (EC 1.12.7.2)	*Reduced Ferredoxin*	*Hydrogen (H_2)*	*Clostridium, Peptostreptococcus*	*Facilitates the release of molecular hydrogen, a key electron sink and substrate for hydrogenotrophic methanogens.*
Acetate Kinase (EC 2.7.2.1)	*Acetyl Phosphate and ADP*	*Acetic Acid and ATP*	*Clostridium, Peptostreptococcus*	*Catalyzes substrate-level phosphorylation to produce acetate and ATP, contributing to energy conservation.*
Alcohol Dehydrogenase (EC 1.1.1.1)	*Acetaldehyde and NADH*	*Ethanol and NAD^+*	*Zymomonas mobilis, Clostridium*	*Reduces acetaldehyde to ethanol, regenerating NAD^+ and aiding redox balance in fermentative pathways.*

Table 2 summarizes main enzymes in the acidogenesis process of anaerobic digestion, with specific substrates and products, catalytic reactions, and microbial generaprivileged for this reaction. Fermentative bacteria specific including *Clostridium*, *Bacteroides*, *Peptostreptococcus*, *Zymomonas mobilis* and *Lactobacillus* are critical in directing the pyruvate conversion to volatile fatty acids (VFAs), hydrogen, and ethanol [14]. These are the key intermediates that mediate to acetogenic and methanogenic pathways, leading to effective production of methane in anaerobic systems [12].

Acetogenesis: Conversion of VFAs to Acetate and H_2

In the third step of AD, acetogenesis, the intermediate products like propionate, butyrate, and ethanol are transformed into acetate, hydrogen (H_2), and CO_2, the latter two being important substrates for methanogenic archaea [1]. This process is carried out through the action of syntrophic bacteria such as *Syntrophobacter wolinii*, *Syntrophomonas wolfei*, and Clostridium aceticum that are highly dependent on a close association with hydrogenotrophic methanogens. It is very important to control low hydrogen partial pressures, which make thermodynamically unfavorable acetogenic reactions possible [1,16].

The main pathways include:

- **Propionate oxidation**
  $CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2$
  (via *Syntrophobacter* species) [16]

- **Butyrate oxidation**
  $CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2$
  (via *Syntrophomonas* species) [16]

- **Ethanol oxidation**
  $CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + 2H_2$
  (via *Clostridium aceticum*) [1]

These processes are critical to the integrity of the microbial population in the digester. Without a functional hydrogenotrophic methanogenesis to remove the hydrogen that would otherwise

accumulate and inhibit acetogenesis, the system would be endergonic and unstable. Acetogenesis serves as an important metabolic linkage between acidogenesis and methanogenesis [1, 16].

Table **3**. Key Enzymes and Microbial Producers Involved in Acetogenesis during AD

Enzyme	**Substrate**	**Product**	**Microbial Producers**	**Reaction Description**
Propionyl-CoA Transferase (EC 2.8.3.1)	*Propionate*	*Acetate, H_2, CO_2*	*Syntrophobacter wolinii*	*Catalyzes propionate oxidation into acetate, CO_2, and hydrogen under syntrophic conditions to support methanogenesis.*
Butyrate Kinase (EC 2.7.2.7)	*Butyrate*	*Acetate, H_2*	*Syntrophomonas wolfei*	*Facilitates the conversion of butyrate to acetate and hydrogen, driven by low hydrogen partial pressure maintained by methanogens.*
Acetyl-CoA Synthase (EC 6.2.1.1)	*Ethanol*	*Acetate, H_2*	*Clostridium aceticum*	*Oxidizes ethanol to acetate and hydrogen, contributing essential substrates for hydrogenotrophic methanogens.*
Hydrogenase (EC 1.12.7.2)	*Reduced Ferredoxin*	*H_2*	*Pelotomaculum thermopropionicum*	*Enables the production of hydrogen from reduced ferredoxin during syntrophic metabolism to sustain interspecies hydrogen transfer.*

Methanogenesis: Microbial Pathways to Methane

In the last stage of anaerobic digestion, namely methanogenesis, methanogenic archaea metabolize the intermediates - mainly acetate, hydrogen and methanol - to methane (CH_4) and carbon dioxide (CO_2). This step is of critical importance for methane production and for the preservation of the upstream processes such as acetogenesis via hydrogen removal and thermodynamic feasibility [6, 12]. Phylogenetically diverse archaea, the methanogens consist of three groups based on the substrate utilized, acetoclastic, hydrogenotrophic, and methylotrophic.

Acetoclastic methanogens (e.g., *Methanosaeta concilii* and *Methanosarcina acetivorans*) disassemble acetate with the acetyl-CoA decarbonylase/synthase (ACDS) complex: $CH_3COOH \rightarrow CH_4 + CO_2$. This pathway is responsible for ~70% of the methane generated in domestic wastewater systems. *Methanosaeta* dominate at low acetate concentrations, but under fluctuating or higher concentrations, *Methanosarcina* is more competitive [11, 12]. Hydrogenotrophic methanogens, such as *Methanobacterium* and *Methanococcus*, utilize hydrogen to reduce CO_2: $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$. This pathway, which is catalyzed by enzymes such as formylmethanofuran dehydrogenase and various coenzyme F_{420}-linked proteins, serves the two purposes of methane production and elimination of hydrogen [6,11,12]. Methylotrophic methanogens, such as *Methanosarcina* and *Methanohalophilus*, catabolize methanol and methylamines: $4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$ $(CH_3)_3N + H_2O \rightarrow CH_4 + (CH_3)_2NH + CO_2$. These reactions that are catalyzed by methyltransferases with some application in systems treating industrial- or protein-rich effluents [11,12].

Table **4**. Key Methanogenic Pathways, Substrates, Products, Microbial Genera, and Enzymes in AD

Pathway	**Substrate**	**Product**	**Key Microbial Genera**	**Key Enzymes**
Acetoclastic Methanogenesis	Acetate (CH_3COOH)	Methane (CH_4) + Carbon Dioxide (CO_2)	*Methanosaeta concilii*, *Methanosarcina acetivorans*	Acetyl-CoA Decarbonylase/Synthase Complex (ACDS) (Part of EC 1.2.7.4)
Hydrogenotrophic Methanogenesis	Hydrogen (H_2) + Carbon Dioxide (CO_2)	Methane (CH_4) + Water (H_2O)	*Methanobacterium*, *Methanobrevibacter*, *Methanococcus*	Formylmethanofuran Dehydrogenase (EC 1.2.99.5), Methenyl-H_4MPT Cyclohydrolase (EC 3.5.4.27), Coenzyme F_{420}-dependent enzymes (EC 1.12.98.1)
Methylotrophic Methanogenesis	Methanol (CH_3OH), Methylamines	Methane (CH_4) + Carbon Dioxide (CO_2) + Water (H_2O)	*Methanosarcina*, *Methanohalophilus*	Methyltransferases (EC 2.1.1.-)

Table 4 summarizes the main methanogenic pathways in AD alongside with their specific substrates and products, related microbial genera and crucial enzymes involved in the methane production. Methanogenic archaea, including *Methanosaeta*, *Methanosarcina*, *Methanobacterium*, and *Methanohalophilus*, are key organisms for the degradation of intermediates such as acetate, hydrogen, and methylated compounds to methane. These different metabolic pathways are all involved in the final step for energy recovery in the anaerobic digestion process [6, 11, 12].

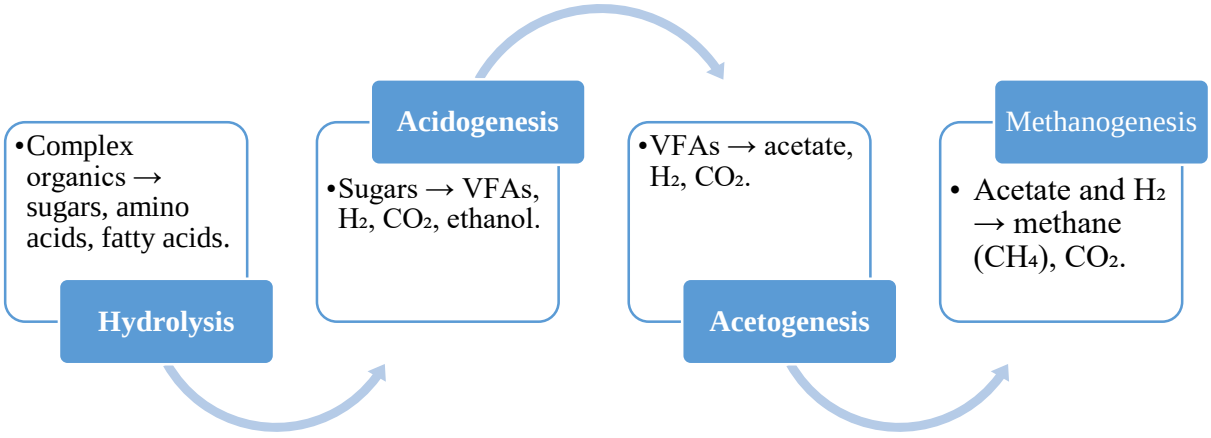

Figure **1**. Biochemical Stages of Anaerobic Digestion

Figure 1 represents the four fundamental chemical steps in AD, which are: hydrolysis, acidogenesis, acetogenesis and methanogenesis. In the hydrolysis process, complex organic material are disassembled to simple monomers as sugars, amino acids, and fatty acids. During acidogenesis, these monomers ar fermented into volatile fatty acid (VFA), hydrogen, carbon dioxide, and etanol. In acetogenesis VFAs are utilized to produce acetate, hydrogen, and carbon

dioxide. Last but not the least, the CH_4 and CO_2 are produced by acetate and hydrogen through methanogenic pathway which adds the final nail in the coffin to the biogas generation.

Role of Coenzymes and Electron Carriers in Methane Production

Methanogenesis relies on special coenzymes and electron carriers in which the redox reactions take place during the formation of methane. Key cofactors are coenzyme M (CoM, the carrier of the terminal methyl group); coenzyme F_{420}, a biologically indispensable low potential electron carrier in hydrogenotrophic methanogenesis; methanofuran (Key C_1-activator for CO_2-reduction); and tetrahydromethanopterin (H_4MPT, carrier of C_1 intermediates along the pathway) [17]. These molecules are supporting central core enzymes like formylmethanofuran dehydrogenase and methyl-CoM reductase in carbon reduction steps. Their activity is promoted by conductive materials (e.g. biochar) that upregulate coenzyme activity and enzyme expression in hydrogenotrophic and acetoclastic pathways [18, 19].

Table **5**. Major Coenzymes and Functions in Methanogenesis

Coenzyme	**Function**
Coenzyme M (CoM)	*Terminal methyl group carrier in methane formation, essential for the final step of methanogenesis.*
Coenzyme F420	Electron carrier involved in hydrogenotrophic methanogenesis; facilitates electron transfer via F420-dependent enzymes.
Methanofuran	*Initial CO_2 carrier in hydrogenotrophic methanogenesis, activating carbon for reduction.*
Tetrahydromethanopterin (H4MPT)	Transfers C1 intermediates along the methanogenesis pathway and participates in key redox steps.
Coenzyme B (CoB)	*Participates in the final reduction step along with Coenzyme M in the Methyl-CoM reductase complex.*
F420 Hydrogenase	Primary enzyme for electron transfer from H_2 to F420; its activity is strongly associated with enhanced methane production

Table 5 lists the important co-enzymes and electron carriers in methane biosynthesis in AD. These cofactors play essential roles in methyl group transfer, CO_2 activation, C1 intermediate shuttling and anaerobe redox balancing [17, 20]. The last reductive step is mediated by coenzyme M (CoM) and coenzyme B (CoB) through the methyl-CoM reductase complex. Coenzyme F_{420} acts as a central electron carrier in hydrogenotrophic methanogenesis, methanofuran and tetrahydromethanopterin (H_4MPT) are involved in C_1 transfer and early CO_2 reduction. Hydrogenase F_{420} transfers electrons from hydrogen to F_{420} to maintain methane production [17, 20].

Electron Transfer Mechanisms in AD

Interspecies electron transfer (IET) via mediated (MIET) as well as direct (DIET) pathways facilitates improvement in methanogenic efficacy in anaerobic digestion [12]. MIET relies on soluble shuttles including hydrogen, formate, riboflavin, or quinones to connect the two microbial partners, but it is constrained by kinetics and diffusion rates [21]. Conversely, in DIET, the direct electrical contact allows for rapid, thermodynamically favorable, electron transfer through conductive pili, multiheme cytochromes or a material (e.g., biochar, granular activated carbon, magnetite) [9,10]. DIET enhances reactor stability at high organic loading and ammonia stress [3,10], and facilitates the enrichment of electroactive bacteria e.g. *Geobacter* and *Methanosarcina* [3,19]. Studies by Azarmanesh et al. [22] and Zhang et al. [19] demonstrated the promotion of methane production and the enhancement of resistance of the

microbial community by biochar and magnetite. Infusible biochar is also an electron shuttle and biofilm scaffold promoting the DIET process [10, 22]. Its application is accompanied by the enhanced microbial activity, shock resistance and the overall recovery of the reactor under stress conditions [18,19].

Table **6**. Comparison of Direct Interspecies Electron Transfer and Mediated Interspecies Electron Transfer

Feature	**Direct Interspecies Electron Transfer**	**Mediated Interspecies Electron Transfer**
Mechanism	*Direct electron transfer through conductive pili, cytochromes, or external materials such as biochar or magnetite.*	*Electron transfer via soluble carriers like hydrogen, formate, riboflavin, or quinones.*
Electron Carrier	No external carriers required.	Requires diffusible electron carriers such as H_2, formate, or synthetic shuttles like riboflavin and phenazines.
Speed of Electron Transfer	*Faster due to direct physical contact and absence of diffusion limitations.*	*Slower due to diffusion of intermediates.*
Dependency	Requires close physical proximity or conductive connections between microbial species.	Can occur over longer distances; physical contact not required.
Key Advantages	*Improved methanogenesis, shortened lag phase, stability under high organic loading rates and ammonia stress.*	*Flexibility in spatial arrangements; suitable for diverse environments.*
Key Limitations	Spatial limitation; only functional between compatible microbes or conductive media.	Energy losses due to carrier synthesis; sensitive to hydrogen partial pressure
Examples of Applications	*Use of biochar, activated carbon, magnetite, or carbon cloth to enhance methanogenesis.*	*Used in systems with complex or spatially dispersed microbial communities*
Enhancement Strategies	Addition of conductive materials, promotion of electroactive microbial communities such as Geobacter and Methanosarcina	Addition of synthetic electron mediators like riboflavins or quinones

Table 6 compares DIET and MIET in anaerobic digestion. DIET advances electrons directly through conductive pili, cytochromes, or substances (e.g., biochar, magnetite) [9,14], making the reaction kinetics faster and increasing the stress resistance tolerance (to high OLR and ammonia) [3,14]. MIET (using carriers such as hydrogen, formate, or riboflavin) would have a more lenient spatial-arrangement requirement, but it would be less energy-efficient [14]. In DIET, biochar is usually added that enriched *Geobacter* and *Methanosarcina* population [9, 10].

Upgrading Biogas to Biomethane: Technologies and Microbial Integration

Anaerobically digested biogas comprises 50–70% CH_4, 30–50% CO_2, as well as impurities such as H_2S, moisture, and siloxanes. It needs to be upgraded to more than 95% of methane content to satisfy the grid or fuel quality requirements [23]. Water scrubbing extracts CO_2 and H_2S based on water, then 95–98% of natural gas (CH_4) is obtained with 3–5% methane loss and excessive water consumption [23]. Chemical absorption, with amines such as MEA, offers more than 99% purity, however it is an energy-consuming process, and has concerns regarding solvent quality [23] Pressure swing adsorption (PSA) with materials such as zeolites can achieve up to 96–98% CH_4 at low energy requirement (0.15–0.35 kWh/Nm^3) and 1.5–2.5% methane loss [23]. Membrane separation offers >97% purity in modular process but must undergo pre-treatment to prevent fouling [6, 23]. Cryogenic separation is capable of >99% CH_4 by CO_2 use cooling method, but requires high energy and complicated facilities [23].

Biological scrubbing transforms CO_2 to CH_4 by hydrogenotrophic methanogens that can offer gas of 95–98% purity with low environmental impact but high dependency on hydrogen, and microbial control is needed [17, 23, 24]. Hybrid technologies, which combine one or two methods (e.g. membranes and PSA), are more flexible but also can present a more complex operation [6, 23].

Table **7**. Biogas Upgrading Techniques: Performance Metrics, Advantages, and Operational Challenges

Technique	Methane Purity (%)	Energy Consumption (kWh/Nm^3)	Advantages	Challenges
Water Scrubbing	95–98	0.2–0.5	Simple, mature technology; widely used in WWTPs	High water consumption, CH_4 losses (3–5%)
Chemical Scrubbing	>99	0.4–0.8	High CH_4 purity, minimal CH_4 loss (0.1–0.2%)	Energy-intensive regeneration; amine degradation and environmental risks
Pressure Swing Adsorption (PSA)	96–98	0.15–0.35	Low energy use; modular design; widely implemented	CH_4 losses (1.5–2.5%); exhaust gas treatment required
Membrane Separation	>97	0.18–0.33	Compact, scalable, modular; high CH_4 recovery	Pre-treatment required; potential membrane fouling
Cryogenic Separation	>99	0.18–0.25 (up to 10% CH_4 energy)	Very high CH_4 and CO_2 purity; CO_2 valorization possible	High energy requirement; expensive and technically complex
Biological Scrubbing	95–98	Not specified	Environmentally friendly; uses renewable H_2; enables microbial integration	Microbial population control and consistent H_2 supply required
Hybrid Systems	Depends on combination	Depends on combination	Tailorable for specific feedstocks and upgrading goals	High installation and operational complexity and cost

Table 7 compares the main biogas treatment technologies- water and chemical scrubbing, PSA, membrane and cryogenic separation biological scrubbing and hybrid systems-according to methane purity, energy requirements, and operational aspects [6, 23]. Although the energy requirements for such systems of water scrubbing and PSA are modest, they provide limited methane recoveries [23]. Membrane and cryogenic processes can produce higher purities but, at the same time, are extremely expensive for the equipment and pre-treatment [6, 23] Biological scrubbing provides an environmentally friendly solution with methanogens, but requires a delicate control of hydrogen and microorganisms [17, 24]. Hybrid systems contribute with greater flexibility at the cost of complexity [6, 23].

CONCLUSION

Anaerobic digestion (AD) is a key technology for sustainable waste management, as well as for the recovery of renewable energy, by the conversion of organic matter into biomethane or methane enriched gas stream through well-defined microbial processes. This review emphasized the biochemistry of stages of the AD process (hydrolysis, acidogenesis, acetogenesis, and methanogenesis) and the contributions of archaeal methanogens, enzymes, and electron transfer mediators toward increased methane yield. DIET has been enhanced by conductive materials, and resulted in providing microbial syntrophy, better methane

production, and reactor stability. Coenzymes such as CoM and F_{420} also contribute to the orchestrated redox-chemistry required for the implementation of methanogenesis. Today biogas upgrading technologies (membrane separation, biological scrubbing, hybrid systems) allow to produce high purity biomethane for grid, fuel use or on site use. It is with prospective efforts geared toward:

- The construction of microbial communities may be rationally designed to amplify the effects on specific functions aimed to modulate in the AD.
- The variety of processes for the biological upgrading of bio-oils must increase to limit dependence on chemicals and ensure an environmentally-safe operation.
- It is necessary to develop advanced waste thermochemical treatment technologies for full resource recovery and high system efficiency.
- To promote DIET and stimulate the syntrophic relationship of microorganisms, more conductive materials should be added.
- An omics-based and on-line monitoring approach must be focused on to maximize the process performances and the system robustness.

Synchronizing microbial optimization with technical innovation is crucial for exploiting the full potential of AD in a low carbon circular bioeconomy.

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