

## Review on Biochemistry of Biogas Generation

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### Abstract

Anaerobic digestion or anaerobic biodegradation is regarded as the complex biochemical and biological activities accomplished through the collaboration of various bacterial species. Its outcome is “biogas”, mostly comprised of methane and carbon dioxide that can be applied as fuel, electricity and cooking gas to reduce greenhouse gas emission introducing cost-efficient renewable technology for social economics. Through an anaerobic digestion process, several biological steps and various kinds of bacteria are introduced simultaneously. Therefore, Effective control systems and monitoring are necessary to optimize the process and achieve the desired biogas yields. This literature review introduced the basic

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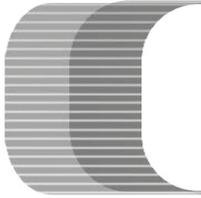
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background of the biochemical process of anaerobic digestion to catch desired biogas yields. Moreover, Life cycle assessments of biogas in order to attain decreased CO<sub>2</sub> foot print will be mentioned. Based on the existing knowledge, the composition of complex biopolymers in various feedstock impacts the biogas yields especially the highest fat and protein embrace longer lag time than high carbohydrate in substrates.

**Keywords:** Anaerobic, Biogas, Biochemical Process, Digestion



## บทบทวนเรื่องชีวเคมีของการสร้างแก๊สชีวภาพ

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### บทคัดย่อ

การย่อยสลายแบบไม่ใช้ออกซิเจนหรือการย่อยสลายทางชีวภาพแบบไม่ใช้ออกซิเจนเป็นกระบวนการทางชีวเคมีและชีวภาพที่ซับซ้อนผ่านความร่วมมือกันของสิ่งมีชีวิตหลายสายพันธุ์ ผลลัพธ์ของกระบวนการคือ “แก๊สชีวภาพ” ที่ส่วนใหญ่ประกอบไปด้วยแก๊สมีเทนและแก๊สคาร์บอนไดออกไซด์ ซึ่งสามารถนำไปเป็นเชื้อเพลิงไฟฟ้าและแก๊สหุงต้ม ทั้งนี้ เพื่อลดการปล่อยแก๊สเรือนกระจกและเสนอเทคโนโลยีหมุนเวียนที่มีราคาถูกลงสำหรับเศรษฐกิจเชิงสังคมได้ โดยที่กระบวนการย่อยสลายแบบไม่ใช้ออกซิเจน ประกอบด้วย ขั้นตอนทางชีววิทยาที่หลากหลาย ที่มีแบคทีเรียหลายชนิดเกี่ยวข้อง ดังนั้น จึงจำเป็นต้องมีระบบควบคุมและติดตามที่มีประสิทธิภาพในการทำให้กระบวนการดังกล่าวเกิดประโยชน์สูงสุด และได้มาซึ่งแก๊สชีวภาพที่ต้องการการทบทวนวรรณกรรมขึ้นนี้ นำเสนอข้อมูลพื้นฐานของกระบวนการ

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ชีวเคมีของการย่อยสลายแบบไม่ใช้ออกซิเจนเพื่อสร้างแก๊สชีวภาพที่ต้องการ นอกจากนี้ยังมีการพิจารณาเรื่องการวัดวัฏจักรชีวิตของแก๊สชีวภาพเพื่อลดปริมาณแก๊สคาร์บอนไดออกไซด์ จากข้อมูลที่มีอยู่ องค์ประกอบของพอลิเมอร์ชีวภาพที่ซับซ้อน จากวัตถุดิบส่งผลต่อเกิดแก๊สชีวภาพ โดยเฉพาะอย่างยิ่งไขมันและโปรตีนระดับสูงสุดทำให้มีช่วงหน่วงเวลา นานกว่าการมีคาร์โบไฮเดรตระดับสูงในสารตั้งต้น

**คำสำคัญ :** ไม่ใช้ออกซิเจน แก๊สชีวภาพ กระบวนการเคมีชีวภาพ การย่อยสลาย

## 1. Introduction

The production of energy in the form of biogas, which is regarded as biochemical conversion process of anaerobic digestion, can decrease the organic content of wastes, promising technique and the most environmentally friendly. Anaerobic digestion, decomposition substrates, abundant in micro and macro nutrients answering digestate which valuable to fertilizer for plants. Biogas is primarily composed of methane, carbon dioxide, and trace gases [1]. Two stage anaerobic digestion process, Optimal pH between 5.5 and 6.5, hydrolysis and acidogenesis describe in the first step whereas in the pH range of 7.8-8.2, methanogens act with the highest activity in the following stage. To avoid methanogen toxicity, AD systems must maintain low unionized  $H_2S$  levels, as  $HS^-$  is less harmful. In sulfate-rich wastewater, neutral or slightly acidic pH favors toxic  $H_2S$  formation. In protein-rich substrates, pH should be adjusted above 8.0 to shift toxic unionized ammonia ( $NH_3$ ) into its safer form, ammonium ( $NH_4^+$ ) [2]. Depending on temperature, biochemical reactions happened that growth of neither bacterium and the activity takes part in acetogenesis or methanogenesis between  $40^\circ C$  and  $50^\circ C$ . The composition of methane depends on organic loading rates, ( $> 9$  g VS/L digester volume per day) of OLR is increased in thermophilic while ( $< 6$  g VS/L digester volume per day) is higher in mesophilic range. However, total biogas yields are lower in mesophilic than thermophilic range which its feasibility relies on the efficiency of thermal energy production (Table 1) [3].

**Table 1** : Temperature in AD Process [4]

Optimal Parameters Temperature	Advantages	Disadvantages
Psychrophilic (below $25^\circ C$ )	lack of heat exchange units	larger digester volume for efficient degradation
Mesophilic ( $20 - 40^\circ C$ ) (usually $35^\circ C$ )	higher stability	requires lower energy cost
Thermophilic ( $50 - 65^\circ C$ ) (typically $45^\circ C$ )	higher growth of methanogenic bacteria, reduced retention time, increased digestibility of solid substrates, adjusting liquid and solid portions	greater amount of disproportion and higher energy requirement

### ***1.1 Microbial Communities in Anaerobic Digestion***

Anaerobic digestion is orchestrated by a succession of microbial guilds, each specializing in a distinct biochemical transformation. The process starts with hydrolytic bacteria involving *Clostridium* spp., *Bacteroides* spp., and *Acetivibrio* spp., which secrete extracellular enzymes such as cellulases, proteases, and lipases to degrade complex macromolecules as carbohydrates, proteins, and lipids into soluble monomers especially sugars, amino acids, and fatty acids [5]. These monomers are then fermented by acidogenic (fermentative) bacteria, including *Streptococcus lactis*, *Lactobacillus plantarum*, and *Leuconostoc mesenteroides*, which produce volatile fatty acids such as acetic, propionic, and butyric acids, along with ethanol, hydrogen, and carbon dioxide. The volatile fatty acids are further metabolized by acetogenic bacteria, notably *Syntrophomonas* spp., *Syntrophobacter* spp., and *Pelotomaculum* spp., which operate in syntrophic association with methanogens. These bacteria convert volatile fatty acids into acetate, hydrogen, and carbon dioxide, relying on methanogens to consume hydrogen and maintain thermodynamically favorable conditions. Acetoclastic refers to microorganisms that produce methane by splitting acetate (a simple organic acid). These microbes are vital in anaerobic digestion, especially in the final stage, where acetate is the main intermediate. Their activity helps convert organic waste into biogas efficiently. Hydrogenophilic methanogens are microbes that produce methane by using hydrogen ( $H_2$ ) and carbon dioxide ( $CO_2$ ) as their main energy sources. They thrive in anaerobic environments and are especially active in the early stages of anaerobic digestion, when hydrogen is abundant. The final stage is governed by methanogenic archaea, which include hydrogenotrophic species such as *Methanobacterium* spp. and *Methanococcus* spp., and acetoclastic species like *Methanosarcina* spp. and *Methanosaeta* spp. and. These archaea convert acetate and hydrogen with carbon dioxide into methane, completing the anaerobic digestion cycle and generating biogas [6]. The metabolic interdependence among these microbial groups ensures system stability and efficient energy recovery, with each group's activity tightly regulated by environmental parameters such as pH, temperature, and substrate composition.

### 1.2 Role of Enzymes in Biochemical Conversion

During digestion, enzymes show a central role in the biochemical conversion of organic matter into biogas. This process unfolds in four main stages—hydrolysis, acidogenesis, acetogenesis, and methanogenesis—each driven by specific microbial communities that secrete enzymes to catalyze distinct reactions. In the initial hydrolysis stage, complex macromolecules including cellulose, proteins, and lipids are broken down into simpler monomers like sugars, amino acids, and fatty acids. This transformation is enabled by extracellular enzymes including cellulases, proteases, and lipases, which are secreted by hydrolytic bacteria such as *Clostridium*, *Bacteroides*, and *Acetivibrio*. These enzymes cleave the chemical bonds within polymers, making the substrates accessible for further microbial metabolism. Once hydrolysis produces soluble monomers, acidogenic bacteria such as *Streptococcus lactis* and *Lactobacillus plantarum* ferment these compounds into volatile fatty acids, alcohols, hydrogen, and carbon dioxide. Enzymes involved in this stage include dehydrogenases and decarboxylases, which facilitate redox reactions and carbon rearrangements [7]. The resulting volatile fatty acids are then processed by acetogenic bacteria like *Syntrophomonas* and *Pelotomaculum*, which rely on enzymes such as acetyl-CoA synthetase and hydrogenase to convert these intermediates into acetate, hydrogen, and carbon dioxide. Importantly, these reactions are thermodynamically unfavorable unless hydrogen is continuously removed by methanogens, highlighting the syntrophic relationship between acetogens and methanogens. In the final methanogenesis stage, methanogenic archaea such as *Methanobacterium* and *Methanosarcina* utilize specialized enzymes to convert acetate and hydrogen with carbon dioxide into methane. Key enzymes involve methyl-CoM reductase, which catalyzes the final step of methane formation, and formylmethanofuran transferase, which initiates the reduction of carbon dioxide [8]. These enzymes are highly specific and operate under strict environmental conditions, such as optimal pH and temperature, which must be maintained to ensure system stability and high methane yield.

To enhance biogas production, researchers have explored strategies such as bioaugmentation with enzyme-producing microbes, pretreatment of substrates with commercial enzyme blends, and the use of nanoparticles to deliver trace elements that stimulate enzymatic activity. These approaches improve substrate accessibility, accelerate reaction rates, and increase methane output [9]. Overall, enzymes are not just passive facilitators but active drivers of the entire anaerobic digestion process, orchestrating a complex network of biochemical transformations that convert waste into renewable energy.

### ***1.3 Inhibitory Factors Affecting Digestion***

Anaerobic digestion, the biological process used in biogas production, can be impaired by several inhibitory factors that disrupt microbial activity and biochemical efficiency. One major inhibitor is ammonia, especially in its free form, which can accumulate from protein-rich substrates and become toxic to methanogenic archaea—particularly under elevated pH and temperature conditions. Similarly, excessive accumulation of volatile fatty acids such as propionate and butyrate leads to acidification, undermining the stability of microbial consortia and inhibiting methane formation. Hydrogen sulfide, a byproduct of sulfur-containing substrates, is another problematic compound, as it is toxic to microbes and corrosive to equipment. The presence of heavy metals, often introduced via industrial waste, can interfere with enzymatic functions by binding to critical cellular components. Long-chain fatty acids derived from lipid-rich waste can also inhibit digestion by adsorbing onto microbial membranes and hindering nutrient exchange. Environmental stressors like high salinity disrupt microbial water balance and enzyme activity, while the emerging presence of microplastics and nanomaterials in feedstocks may interfere with microbial structure and metabolism. Additionally, imbalances in pH—either acidic or alkaline—can destabilize microbial communities, and temperature fluctuations outside the mesophilic or thermophilic ranges may cause microbial stress or loss of activity [10]. Operational factors, such as overloading with organic material, can lead to metabolic bottlenecks, acid accumulation, and even reactor failure. Mitigation strategies include controlled feeding, co-digestion for nutrient balance, use of buffering agents, and pretreatment methods to reduce the impact of inhibitory compounds, all of which contribute to maintaining a stable and efficient biogas production system.

### *1.4 Biogas Upgrading and Utilization Techniques*

Biogas upgrading and utilization are critical processes for transforming raw biogas into high-quality energy products such as biomethane, which can be used for electricity generation, heating, transportation fuel, or grid injection. Upgrading involves removing impurities like carbon dioxide, hydrogen sulfide, and water vapor to enrich the methane content and increase its energy value. Techniques include pressure swing adsorption, water and chemical scrubbing, membrane separation, and cryogenic distillation, each offering varying degrees of methane purity and scalability depending on operational needs. Innovative biological approaches, such as microbial conversion of carbon dioxide and hydrogen into methane using hydrogenotrophic methanogens or algae-based phototrophic systems, are also gaining attention for their sustainability. Once upgraded, biomethane can be used in combined heat and power units for onsite energy efficiency, as compressed or liquefied fuel for vehicles, or injected into existing natural gas grids after meeting quality standards [11]. Additionally, biomethane serves as a feedstock for producing chemicals like bio-methanol, bio-DME, or hydrogen through reforming processes. Optimization strategies such as co-digestion, smart monitoring systems, and carbon reuse applications are vital to enhancing energy yield and system integration. Together, these techniques elevate biogas from a waste-derived byproduct to a versatile and renewable energy resource.

## **2. Biochemical Process of Anaerobic Digestion**

Organic substrates are degraded through biochemical reactions via anaerobic micro-organisms under oxygen free environments, regarded as an anaerobic digestion process. During AD process, the biochemical decomposition phases of organic materials can be separated into four layers such as hydrolysis, acidogenesis, acetogenesis and methanogenesis which are directly connected in this way that byproduct of one stage is the substrate of the next stage (Fig. 1) [12].

### *2.1 Hydrolysis*

Hydrolysis refers to the breakdown of chemical bonds through the addition of water, where anions and cations interact with H<sub>2</sub>O molecules, changing pH in the process to design breaking H-O bonds noted as the first step of the AD process (Table 2). Using hydro-lases from microorganisms, polysaccharides, proteins and lipids of polymeric organic materials convert to simple monomers such as sugars, amino acids and fatty acids. Based on the nature

of the substrate, the rate of decomposition changed. During the hydrolysis step, unattractive VFA or the creation of toxic byproducts including complex heterocyclic compounds, causes the rate limiting step especially organic feedstock while easy biodegradable feedstock is the rate limiting step for methanogenesis. In hydrolysis phase, substrate contains lipids and protein takes a few days but carbohydrates in substrate lasts a few days in addition lignocellulose and lignin can happen few days, and uncompleted anaerobic digestion process. The first step of hydrolysis of anaerobic digestion is crucial because large molecules cannot be easily adorable finally the final stage of simple monomer is introduced [13]. To accomplish anaerobic digestion, various microorganisms introduce extracellular enzymes for fats, proteins and sugars etc., especially degradation of protein is known as proteolytic and then different sugars are demonstrated saccharolytic [14]. In the hydrolysis,  $\text{CH}_3\text{COO}^-$  and  $\text{H}_2$  can be directly applied by methanogens via fermentative microorganisms where VFA (higher chain organic compounds) are caused, whereas relatively large molecules are turned into smaller molecules, (ethanoic acid  $\text{CH}_3\text{COOH}$  acetic acid) [15].

## ***2.2 Acidogenesis***

In the next phase of the fermentation stage, hydrolysis of microbes still take part, such as Acetobacterium, Eubacterium and Enterobacterium counted. Sugars and amino acids of hydrolysis products are converted into short chain organic acids such as propionic acid, acetic acid and butyric acid of VFAs, low alcohols,  $\text{H}_2\text{S}$ ,  $\text{H}_2$ ,  $\text{CO}_2$  and  $\text{NH}_3$ . In this phase, as the intermediate products, the concentration of hydrogen controls the descriptions of ends products produced truly the number of reduced compounds decreased, and the partial pressure of hydrogen was increased [16].

## ***2.3 Acetogenesis***

The performance of acidogenic stage anaerobic oxidation is mentioned as the third phase, which is converting volatile fatty acid especially butyric acids and acetic acids into carbon dioxide, hydrogen and butyric acids. Based on the interaction between volatile fatty acid degradation and hydrogen partial pressure, Propionic acid degradation is still thermodynamically less advantageous than butyric acid, on the other hands 65-95% of  $\text{CH}_4$  from acetic acid directly. Acetogenic bacteria can active low hydrogen concentration surrounding requiring 10.4 and 10.6 of low hydrogen partial pressure. Acetogenesis in syntrophy, Syntrophus, Clostridium and Syntrophomonas which are numerous genera leading to yield hydrogen gas and mainly carried out oxidation and methanogens for next step [17] [18].

Table 2 : Hydrolysis Reaction [18]

Reactant	Products	Enzymes	Time Elapsed
Carbohydrate	Short chain sugars	Cellulase	Hours
Lignin	Aromatic compounds	Hemicellulase	Uncompleted and degradable slowly
Lignocellulose	Short chain sugars	Amylase	
Protein	Amino acid	Proteinase	Days
Fat	Glycerin and fatty acid	lipase	Days
$C_6H_{10}O_4 + 2H_2O \rightarrow C_6H_{12}O_6 + 2H_2$			

#### 2.4 Methanogenesis

In anaerobic digestion, approximately 70% of  $CH_4$  is produced via methanogenesis, which describes the state of the digestion and the extent of biological activities. More-over, the highest methane production led to well performance and stability of anaerobic digestion. In this phase, acetoclastic methanogens apply acetate to release  $CH_4$  and hydrogen oxidizing and  $CO_2$  reducing transform carbon dioxide and hydro-gen to receive  $CH_4$ . Temperature, feeding rate, pH and substrate type of operation conditions influence methanogenic microorganisms. Principally, enormous oxygen and temperature fluctuations, especially more than  $3^\circ C$ , involve methanogenic bacteria actively, which ceases AD functions. Methylo-trophic methanogenesis, hydrogenophilic or hydrogenotrophic methanogenesis e.g. methanobacterium arpho-philicum and acetoclastic or acetotrophic methanogenesis such as methanosarcina bakei, methanosaeta are noticed as the main three pathways for methane production. Using acetoclastic methanogenesis pathway, domestic sewage 70%  $CH_4$  has been received. Based on the nutrient requirements and pH optima, acidogenic/hydrolytic bacteria and methanogenic Archea are different [19]. Generally, the microorganisms contained in hydrolysis and acetogenesis expand speedily than methanogenesis of various organisms, therefore, methanogenesis display as the rate limiting step. However, based on substrate types, hydrolysis or methanogenesis can be the rate limiting process especially if substrate degrade easily, methanogenesis can be the rate limiting steps whereas if hydrolysis is the limiting steps [15] [20].

### 3. Biochemical Properties of Various Feedstocks

A large fraction of vegetable substrates contains carbohydrates in the form of soluble polysaccharides and sugars with insoluble polysaccharides; breaking carbohydrates means the first energy releasing reaction. Rapid acidification decreases the methanogenic activity in the bioreactor because of accumulation of VFA and lower pH of vegetable wastes. The nitrogen in protein is needed for growth of bacteria and the carbon in carbohydrate supports the energy, in addition, the nitrogen is slower than carbon of bacteria activity in AD [20]. The highest carbohydrate contents of vegetable waste demonstrated the highest biogas and methane yields in comparison with coffee husk or oil palm empty fruit bunches (OPEFB) and five Fruit-Based Agro-Industrial Wastes (FBAIW) (banana, jackfruit straw, apple, orange, and pineapple peel waste) showed higher methane production than five agricultural crop residues (ACRs) (vegetable waste, rice straw, coffee husk, maize straw, and oil palm empty fruit bunches (OPEFB)). Because of the fibrous nature of ACRs, cause negative effect on the rate and overall biogas production, increasing lignocellulose composition. In lignin, cellulose is enclosed go to biodegradation rate can be prohibited [21].

During the digestion of this waste must seek and solve the heat and energy sufficiently to hit the desired energy demand of the anaerobic digestion plant, suitable pretreatment methods and better crop residues of biodegradation [22]. The lag phase of carbohydrate enriched materials described the highest biogas yields with shorter digestion mainly the feed to microorganism ratio F/M ratio (0.6). However, over 0.6 becomes longer initiation with the highest organic loading rate due to the difference in growth rates of lactose and glucose via carbohydrate metabolism. The management of municipal solid waste could be used by using organic fraction of municipal solid waste (OFMSW), environmentally friendly feedstock [23]. Fruit-vegetable waste (FVW) and food waste (FW) are noted as one of the majorities of organic fraction of municipal solid waste (OFMSW), including soluble simple sugar and high protein [24]. Anaerobic digestion can overload easily due to the highest biodegradable organics FVW and FW, leading to acidogenesis rapidly. Consequently, in equity between production and degradation of VFAs happens uncontrolled to motivate a pH drop and the volatile acid accumulation [25]. Preventing excessive acidification immediately can add buffers (e.g. bicarbonates, etc.), but it cannot solve the pH recovery and severe acidification mainly influences microorganisms in anaerobic digestion and makes it expensive [26]. In anaerobic co-digestion of food waste and fruit-vegetable waste, methanogenesis and methane output by adding powdered activated carbon (PAC) and granular activated carbon (GAC). However, PAC reduced acidification from accelerating of VFAS consumption more than GAC [27].

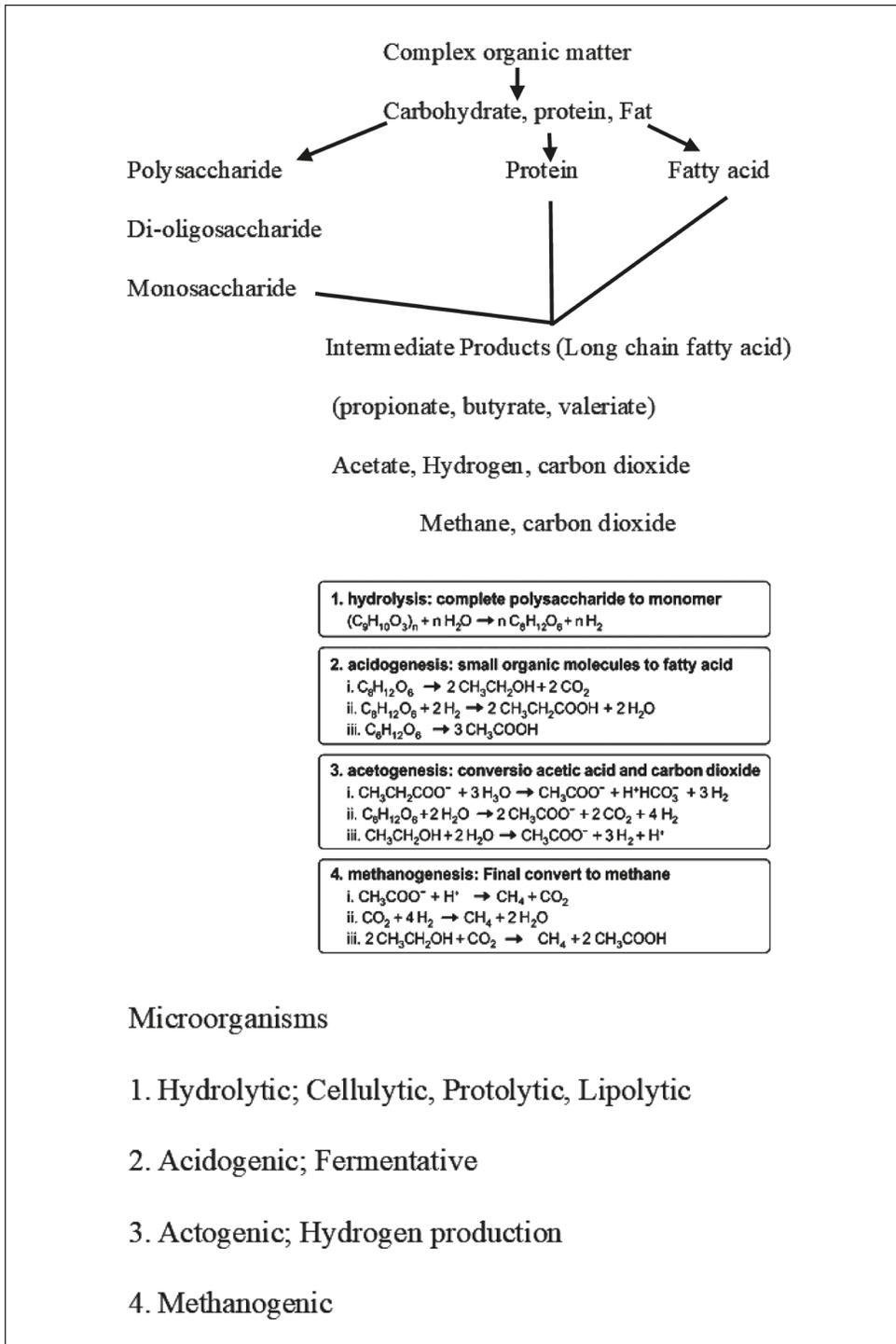


Figure 1 : Biochemistry of Biogas Production [12][15].

For biogas production, cellulose-based waste as an abundant availability such as waste textiles and lignocellulose would be applied as a substrate because of its high carbohydrate content but their structure resists microbial hydrolysis. Nevertheless, in biogas application, lignocellulose materials are resisted and waste textiles are unreal. In a biogas process, during hydrolysis of polysaccharides, microorganisms including Acetibitio, Clostridium and Bacteriodes are capable and some of these microbes secrete various enzymes closed together into cellulosomes. Cellulosomes located on the cell wall of the organisms, in addition, stable, large, multi-enzyme complexes specialized in the bonding to and breaking cellulose on the cell surface as reside with projection noticeable. In lignocellulose substrates, polysaccharides are hemicellulose and cellulose which can be converted into simple sugar by hydrolyzing but lignin, opposes hydrolyzing during microbial close halting hemicellulose and cellulose. Therefore, pretreatment can handle breaking by lignin structure then hemicellulose and cellulose easily degradable, moreover, the porosity of substrate is enhanced and the crystallinity of cellulose is reduced. The cocoa residues with hydrothermal pretreatment can increase biogas production to enhance hydrolysis of the hemicellulose from cocoa residues [22]. Theoretically, the microalgae biomass of all organic matter, such as carbohydrate (0.851), Protein (1.014) and lipids (0.415 L/g of VS), would be moved into biogas yields. However, Microalgae biomass of anaerobic digestion can receive low biogas yield because of a compact cell wall of microalgae cells, but hydrothermal pretreatment can increase biogas performance and halt the microalgae cell wall of its compact structure [27]. The algal biomass (Enteromorpha) and metal nanoparticles (NPs) treated by microwave (MW) pretreatment of total biogas yields were observed 53.60ml/gTS (Co NPs + MW pretreatment). A algae cell wall comprises hemicellulose and cellulose locate in the internal layer while carbohydrates and glycoprotein exist in the external layer. However, by using MW pretreatment, hydrolyzing carbohydrates and polysaccharides of glycosidic bond to simple sugar, attacking metal NPs, dissolution of the internal layer, NPs hydrolyze cellulose to oligosaccharides cyclodextrins and cellobioses, the positive energy balance occurred [26]. Feather and horn contain keratin, insoluble protein which prevents proteolytic enzyme mainly polypeptides with cross-linked disulfide, hydrophobic interaction, hydrogen bond as strong structure. Approaching alkali or strong acid or harsh physical pretreatment can digest keratin simply. After aerobic wastewater treatment, activated sludge contain high organic component and substrate can be applied for biogas yields, exocellular polymeric substance can resist activated sludge digestibility [17].

Under several acidification systems, methanogenic digestion efficiency and anaerobic digestion stability can be described as low efficiency of methanogens. Different forms of municipal non-herbaceous as well as herbaceous phytomass, low concentration of lignocellulose with non woody parts of non herabaceous phtomass possessed abundant crystalline cellulose and high degree of lignification [28]. The low lignocellulose, lignin and low cellulose crystallinity of lawn cutting increased biogas production. When anaerobic digestion of landfill waste, MSW intact with 10 mg Ag/kg solids of silver nanoparticles AgNPs, the accumulation volatile fatty acid as well as acetic and concomitant decrease pH that happened halts methanogenesis. However, AgNPs can help acidogenic bacteria activity but can cease methanogens [29].

Feedstocks affluent in semi cellulose and cellulose with adequate proteinaceous material can enrich biogas yields. Proteinaceous feedstock decrease biogas, whereas complex polysaccharides materials are desirable for methane yields. Ruminant animal manure holds the required micronutrients and methanogens, therefore, 90% of water and solid waste and cow dung and are 1:1 (w/w) to get 10% of solid content because adding undigested organic material to the digester, the greater will catch biogas yields [30]. Co-digesting poultry and cattle manure decreased methane production and fatty acids accumulation slightly. The highest RMP values correlated with degradation of volatile fatty acid, cellulose and hemicellulose improved in the digestate. When handling the digestate and storage, Manure mentions enriched risks for methane emissions and low degradation.

Chicken food waste might be co-feed with cow manure, 75% CM 25% CC and 100% CC, the larger molecular size of chicken meat leads to the production hydrogen instead of methane when pH goes down compared to 100% CM, the smallest molecular size easily to degrade and digest. The presence larger protein size can decrease the hydrolysis process, maintaining the pH when pH is low whereas dairy manure (DM) the highest fiber content of containing lignin, hemicellulose and cellulose and also no VFA due to high pH. Dairy manure, such as recalcitrant feedstock can vary the rate of methane yields depending on the degree of hydrolysis. Mixture of wine- making waste with liquid cattle manure resulted in a slow methane production and initial methane formation later due to hemicellulose and cellulose degraded slowly and then lignin strength acid [31].

A lower C:N ratio shows increased ammonia level and increased pH, which are harmful methanogens while a high C:N ratio receives lower gas level. Mixing animal manure or solid waste with sewage is a perfect blending carbon and as mixing of solid waste with sewage or animal manure [29]. In the bio digester, the composition of water is lower; various acid accumulation happens that can block the fermentation and thick scum on the surface, especially water must be between 60 and 90% total weight of waste however substrate: feed: waste should be accounted appropriately [30]. The digestion activities of cow manure, pretreatment, codigestion, additives (carbon based material, composites and Fe based nanomaterials) are noteworthy more attention and also bioelectrochemical fields are introduced as the future reactor of AD [32].

During anaerobic digestion, some factors including cellulose relevant factors (polymerization degree and cellulose crystallinity and indirect factors such as chemical contents (acetyl group, cellulose and hemicelluloses), display the crucial sector in inhibiting the decomposition of substrate. The cross-linked polysaccharide networks, have synergetic effect closely impact each other on bioconversion but are not concerned with influencing factors. As a protection barrier, direct factor affecting biomass hydrolysis construct a spatial network enhance partial cell wall composition, leading to higher surface accessibility moreover decreasing starting enzymatic hydrolysis. Nevertheless, there still have to overcome the decomposition of AD, pretreatments methods and cost and high energy input [33].

The desired biogas production was achieved by supplementation ultra-fine bubble water (UFBW) into waste activated sludge (WAS) via AD because of its mass transfer of nutrients to the microbial cells enhancing the methane yields (14-21%). Adding various gas-NBW without adding chemical can enhance the hydrolysis of organic solids to be more environmentally friendly at low energy consumption. During anaerobic digestion of waste activated sludge would be mentioned by adding  $N_2$ -NBW that increases the enzyme activities of extracellular hydrolases leading to increase hydrolysis of WAS; in addition, it can rise 29% of methane production. At the hydrolysis-acidification stage of  $CO_2$ -NBW and air-NBW addition of AD of refractory cellulose (a high loading of 3.5 (VS cellulose/VS inoculum)) could be received the highest volatile fatty acid 11-30%. Mainly air-NBW addition of AD was described 20% enhancement in cellulose crystallinity reduction (81%) and then 18% increase in  $CH_4$  production (263.6 mL/g-VS reduced) compared to  $CO_2$ -NBW supplementation. Adding oxygen nanobubble water ( $O_2$  NBW) to anaerobic digestion of cellulose can improve the process. It enhances cellulose breakdown by 8% to 14% and boosts methane production by 8% to 30%. This takes because it creates a small oxygen-rich environment that supports produce more volatile fatty

acids during the early stages of digestion, especially when there is a lot of cellulose. This method works better than using air NBW, nitrogen NBW, or artificial nitrogen and oxygen mixtures. Adding nitrogen nanobubble water ( $N_2$  NBW) and oxygen nanobubble water ( $O_2$  NBW) during anaerobic digestion of sludge using corn straw can improve methane production. The methane yields reached 127 milliliters per gram of volatile solids with  $N_2$  NBW and 142 milliliters per gram with  $O_2$  NBW. These increases occurred even though the reduction in cellulose crystallinity was not significantly different between treatments. However, corn straw with more crystalline cellulose showed the greatest reduction in crystallinity. In a separate study, a two-stage anaerobic digestion of food waste with air nanobubble water addition increased the activity of four extracellular hydrolases. This enhancement led to a 24 percent higher methane yield compared to using  $N_2$  NBW [34-41].

Biogas yield in anaerobic digestion (AD) is highly impacted by the composition of the feedstock, as various organic materials possess distinct properties affecting microbial activity and methane production. Energy crops like maize silage and sugar beet offer the highest biogas yield (50 - 220  $m^3$ /tonne wet weight) and methane content (55 - 70%), due to their easily degradable carbohydrates. In contrast, animal manure generates lower yields (12 - 48  $m^3$ /tonne) with moderate methane levels (50 - 65%), primarily because of its low carbon-to-nitrogen (C/N) ratio, which may lead to ammonia inhibition unless co-digested. Food waste provides a favorable balance, with methane contents reaching up to 75%, although its composition varies. Lignocellulosic feedstocks such as crop residues and straw require pretreatment due to their high lignin content, which hinders microbial breakdown. Moisture and volatile solids (VS) are crucial — higher VS implies greater biogas potential, while dry materials may demand dilution [42]. Additionally, certain industrial wastes and fatty feedstocks can inhibit microbial pathways if not managed properly. Co-digestion strategies are often employed to optimize microbial efficiency, balance C/N ratios, and mitigate toxicity, resulting in improved process stability and biogas output.

**Table 3 :**

Feedstock Type	Biogas Yield ( $m^3$ /t WW)	Methane (%)	References
Energy crops	50 - 220	55 - 70	[43]
Animal manure	12 - 48	50 - 65	[44]
Food waste	20 - 140	60 - 75	[45]
Sewage sludge	9 - 16	55 - 65	[46]
Crop residues	20 - 100	50 - 60	[47]

Substrates rich in fats and proteins generally lead to longer digestion lag times in biogas systems due to several interconnected biochemical and microbial factors. Carbohydrates are rapidly broken down by enzymes like amylases, making them more accessible to microbes and allowing for quicker biogas production. In contrast, fats require lipases and proteins require proteases, both of which act more slowly and demand greater energy for hydrolysis. Furthermore, the microbial communities involved in degrading fats and proteins are highly specialized. Fat degradation relies on slow-growing syntrophic bacteria to break down long-chain fatty acids, while protein digestion involves proteolytic microbes that release ammonia through deamination. These byproducts, basically long-chain fatty acids and ammonia, can inhibit key methanogenic bacteria, disrupting the balance of the digestion process [48]. As a result, acetogenesis and methanogenesis proceed more slowly when digesting fats and proteins. Although these substrates offer high methane yields, their structural complexity, inhibition risks, and reliance on slower microbial pathways contribute to a delayed onset of gas production compared to simpler carbohydrate-based materials.

#### **4. Academic Majors Related to Biogas Biochemistry**

Academic involvement in the biochemistry of biogas generation spans a wide range of university majors and research disciplines. Biochemistry mentions a central role by examining enzyme activity and the metabolic pathways of microbes involved in anaerobic digestion. Microbiology complements this by studying the behavior of methanogens and acidogenic bacteria crucial to methane production. Environmental science integrates sustainability frameworks and evaluates the environmental impacts of biogas systems through tools like Life Cycle Assessment. Chemical engineering focuses on optimizing reactor design and upgrading technologies, while biotechnology contributes through microbial enhancement and genetic modifications to boost gas yields. Agricultural science explores feedstock diversity and nutrient recovery, often aligning biogas initiatives with farming practices. Renewable energy and environmental engineering emphasize system integration, smart monitoring, and waste treatment infrastructure. Materials science supports the development of innovative components such as nanoparticle additives and thermal insulators, enhancing digester performance [49]. Finally, sustainability studies address policy, equity, and alignment with the United Nations Sustainable Development Goals, creating biogas research a truly interdisciplinary endeavor.

## 5. Life Cycle Assessment and Anaerobic Digestion Challenges

Life Cycle Assessment (LCA) is a crucial role for evaluating the environmental sustainability of biogas systems. It supports a cradle to grave analysis by examining each stage of the biogas lifecycle—from sourcing feedstock and transporting materials to anaerobic digestion, biogas utilization, and digestate management. At each step, LCA quantifies the inputs, such as energy, water, and raw materials, and the outputs, including emissions, waste, and other environmental pollutants. This comprehensive assessment shows identify critical environmental hotspots and trade-offs, such as methane leakage during storage that may offset the climate benefits of replacing fossil fuels, or digestate application that could contribute to eutrophication. LCA also supports meaningful comparisons between biogas and conventional energy sources, often showing that biogas can reduce greenhouse gas emissions by up to 80 to 90 percent when effectively managed [50]. Beyond impact evaluation, LCA informs regulatory compliance and sustainability certification, like the European Union's Renewable Energy Directive and ISCC. It demonstrates design improvements, including optimal feedstock blending and technology upgrades to minimize resource consumption and environmental harm. Integrated with digital modeling and scenario analysis, LCA equips stakeholders to make data-driven decisions aligned with circular economy principles and the United Nations Sustainable Development Goals.

The low life cycle  $\text{CO}_2$  footprint sustainably used wastes, sugarcane, short rotation coppice, miscanthus and sugarcane would be helpful to the elevation of state-of-the art biogas option in addition biomass- to -biogas and biogas end- use phases  $\text{CO}_2$  foot print must be decreased by serving various innovative techniques. Pressurized anaerobic digestion (PAD) based biogas plants release pretty low direct  $\text{CO}_2$  footprint ( $13\text{kgCO}_2/\text{MW hf}$ ) which is one of arising biogas productions [51]. Aerobic digestion of microalgae biomass to produce biogas with a solar driven hydrothermal pretreatment mentioned levelized cost of energy ( $0.17 \text{ \$/m}^3$ ), greenhouse gas emission ( $-166.13\text{g CO}_2\text{-eq / (kWh biogas)}$ ) and net energy ratio (input/output) (0.69) distributive. The highest biogas yield was a good advantage in reducing net energy and decreasing price over hydrothermal pretreatment, the enhancement of nitrogen recovery can also delay greenhouse gas emission [52]. Based on a biogas plant of municipal sewage

sludge of life-cycle assessment (LCA) represented as negative greenhouse gas emission ( $-0.2385 \text{ kg CO}_2 \text{ eq/m}^3$ ) and then digested can apply in chemical fertilizer, finally, has positive impact on the environments [53]. The global warming ( $6.76 \times 10^{-15}$  to  $2.04 \times 10^{-5} \text{ PDF}\cdot\text{m}^2\cdot\text{yr}\cdot\text{m}^{-3}$  GWP, land use change ( $4.92 \times 10^{-8}$  to  $4.78 \times 10^{-6} \text{ PDF}\cdot\text{m}^2\cdot\text{yr}\cdot\text{m}^{-2}$  LUC), water consumption ( $1.19 \times 10^{-12}$  to  $4.28 \times 10^{-6} \text{ PDF}\cdot\text{m}^2\cdot\text{yr}\cdot\text{kg}^{-1}\text{WCP}$ ) of waste-derived biogas could be applied for a green energy based on Life cycle assessment [32]. Using Life Cycle Assessment (LCA) and Cost-Benefit Analysis (CBA) for development of AD systems, must be introduced as appropriate assessment tools [54].

In New Zealand, Direct interspecies electron transfer (DIET) enhancing additives particularly phenazine, biochar and active carbon but neutral red (phenazine) in landfill site can elevate biogas generation according to a case study [55]. In biogas technology adoption in Ethiopia especially in rural area, must seek appropriate substrate potential. To be applied for internal combustion engines and power generator sets, calorific values of biogas must be increased [56]. The advantages of biogas lead to share local energy sector, reduce environmental impact, control methane emission, receive organic fertilizer and create income for farmers [57]. Nevertheless, it is still limiting the adaptation of potential feedstocks as a result of process inhibitions, decrease biodegradability and digester disruption, in consequence multidisciplinary research communities to carry on its business scale implementation and adoption [58][59].

Life Cycle Assessment (LCA) demonstrates an essential role in evaluating the environmental, economic, and social dimensions of anaerobic digestion (AD) systems. Key impact categories involve climate change potential from greenhouse gas emissions, eutrophication due to nutrient-rich digestate runoff, acidification from ammonia and sulfur compounds, and ozone formation from volatile organic compounds—all of which influence ecosystems and human health. These are complemented by indicators of human and ecotoxicity, resource depletion, and land and water usage. Beyond environmental metrics, AD systems intersect with socio-economic and policy dimensions: Social LCA and Life Cycle Costing (LCC) frameworks assess labor conditions, community wellbeing, and the economic viability of biogas solutions, while policies including Thailand's

Bio-Circular-Green (BCG) model or the EU's Renewable Energy Directive offer strategic support but require integrated governance to address fragmentation and ensure equitable development [60]. Regional variability and data uncertainty remain critical challenges, as feedstock types, climate conditions, and technological infrastructure differ widely between locations—impacting emissions, performance, and outcomes. Addressing these uncertainties calls for robust methodological tools, including Monte Carlo simulations, pedigree matrices, and sensitivity analyses to improve data quality and transparency. This comprehensive view enables more inclusive and adaptable LCA applications in the evolving landscape of AD technologies [61].

## 6. Challenges and Future Direction

Biogas production represents promising opportunities for renewable energy and circular resource systems, yet it struggles a range of complex challenges that span environmental, technical, economic, and societal dimensions. Feedstock variability, whether from agricultural residues, manure, or food waste, can significantly influence microbial activity and methane yield in order to fluctuating moisture content and nutrient composition. The anaerobic digestion process itself is highly sensitive to changes in temperature, pH, hydraulic retention time, and the accumulation of volatile fatty acids, often heading to instability or inhibition, basically from ammonia or protein-rich substrates. Inhibitory compounds including salts, heavy metals, or antibiotic may disrupt microbial communities, requiring careful substrate management. Methane leakage from poorly sealed digesters and storage systems undermines both climate goals and energy recovery, while digestate quality must be handled to prevent contamination of water or soil through pathogen or excess nutrients. High infrastructure costs and operating expenses, particularly for small scale applications, pose barriers to widespread adoption, and resisted technical skills in certain regions slow implementation [61]. Furthermore, fragmented policies, unclear subsidies, and inconsistent regulations hinder harmonized development, while public resistance shown by concerns over hygiene, odor, and safety can stall community level deployment. Overcoming these hurdles calls for targeted research, capacity building, robust regulatory frameworks, and inclusive design strategies tailored to local contexts.

Biogas production is involving as a cornerstone of sustainable energy through anaerobic digestion processes, which are enchantingly being refined for higher efficiency and combination into broader circular systems. Future direction demonstrates upgrading biogas to biomethane for grid injection or applied as vehicle fuel, combining anaerobic digestion with wastewater treatment, hydroponics, and nutrient recovery and producing green hydrogen by steam methane reforming [62]. The deployment of smart monitoring tools including Internet of Things relied sensors and advancements in microbial engineering for tailored microbial communities promise enhanced resilience and methane yields.

However, several challenges persist. Feedstock variability influences gas composition and yield due to differences in moisture and nutrient content. Process instability can result from changes in pH, ammonia accumulation, and temperature fluctuations. Infrastructure costs are a major barrier in many contexts, especially for small scale applications. Methane leakage resulting from inadequate sealing or technical flaws compromises both environmental objectives and economic feasibility, while fragmented policies and inconsistent standards limit regional collaboration and consistent implementation. To address these obstacles, current research focuses on pretreatment techniques including thermal, chemical, and enzymatic methods to enhance hydrolysis efficiency, co digestion strategies that balance carbon to nitrogen ratios using diverse feedstocks, nanoparticle additives to stimulate microbial activity, and mathematical modeling for predictive control and performance optimization. Life Cycle Assessment is a valuable tool for evaluating environmental impacts and tradeoffs across biogas systems. Applying design thinking offers innovative approaches including user centered digesters tailored to local conditions, modular systems for flexible deployment, dual feeding mechanisms for operational continuity, insulation to support optimal temperatures, and effective digestate management for nutrient recovery [63]. These strategies closely support the United Nations Sustainable Development Goals, particularly Goal 7 for affordable and clean energy, Goal 13 for climate action, Goal 12 for responsible consumption and production, and Goals 2 and 6 which promote food security and clean water access. Altogether, biogas systems contribute significantly to climate resilience, resource efficiency, and inclusive development.

## 7. Conclusion

Different kinds of waste into efficient bioenergy are a promising approach for biogas production. An aerobic digestion treatment of biogas production which is crucial to understand the basic biochemistry of AD. In the biogas digester, sugars and soluble polysaccharide go through fermentation, complex polysaccharides lead fermentation and hydrolysis, fat and protein go deamination, hydrolysis and fermentation to biogas yields. The high lignin content of feedstock can resist the hydrolysis and prevent the cell disruption by microorganisms that is one the main challenge of production an enhanced biogas yield. Moreover, free ammonia content of amino acid of fermentation can impact on acetate-utilizing methanogens. In various fields, two stage operation has showed the highest methane production and biogas purities in compared to single stage operation. Depending on the anaerobic digestion technology of its operation conditions and the nature of raw substrates, basic research must approach biogas combustion process deeply. An increased use of biogas would contribute to the sustainable development by reducing the greenhouse-gas emissions and the use of fossil fuels.

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### *Author Contribution*

KT: reviewing, formal analysis, writing original draft and preparing final draft and for-matting. WT: visualization, investigation, and validation. NA: preparing writing draft and project administration. AS: reviewing, and editing and preparing final. AK: conceptualization, supervision, reviewing, and editing and preparing final and original draft and formatting.

### *Conflicts of Interest*

We have no conflicts of interest to disclose.

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