



## Role of Microbial Hydrolysis in Anaerobic Digestion: Enhancing Biogas Production Efficiency

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**Abstract** – The first step of anaerobic digestion and the core of what makes anaerobic digestion efficient is microbial hydrolysis. Anaerobic digestion is often referred to as biogas or VOC generation; it is one of the most significant elements in solid waste management and renewable energy. This is the breakdown of organic waste materials into methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>). An understanding of these simpler substrates is critical to subsequent fermentation stages that produce biogas. However, microbial hydrolysis often acts as a rate-limiting step, particularly in complex substrates like lignocellulosic biomass. This paper provides an overview of the role of microbial hydrolysis in AD systems toward better yields of biogas and greater process efficiency.

This paper discusses some of the ways to optimize hydrolysis efficiency, including adding a hydrolysis phase separately to guarantee optimal conditions for the hydrolytic microorganisms and integrating techniques of pretreatment: biological, chemical, and physical, to speed up the degradation of substrates. Biological approaches of pretreatment using fungi, chemical procedures with alkaline or acidic solutions, and physical methods with mechanical shredding and thermal treatments were reportedly very promising for the increased hydrolysis rates.

Moreover, the paper depicts the optimization of steps for hydrolysis towards a better assimilation of biogas systems into material cycles. Enhanced microbial hydrolysis would eventually result in increased yields of biogas, better conversion of waste streams, and reduced emissions of greenhouse gases. This has been put forward along with the inhibitory effects of toxic compounds on microbial activity and the economic viability of pretreatment methods. Finally, the paper brings up to date potential solutions towards solving such challenges and increasing the economic and environmental sustainability of AD systems.

**Keywords:** Anaerobic digestion, microbial hydrolysis, biogas production, biomass pretreatment, renewable energy, circular economy.

### 1. INTRODUCTION

Anaerobic digestion is a benign biological process that has widely gained acceptance in the transformation of organic waste into renewable energy, as biogas, which is essentially rich in methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>). Therefore, anaerobic digestion production of biogas becomes an essential component of comprehensive waste management and circular economy strategies as the society remains on renewable energy provision. It ranges from agricultural residues through industrial effluents to



the solid wastes within municipalities. Methane produced in AD can be used for natural gas substitution and also contribute to the reduction of greenhouse gases and fossil fuel dependency.

However, microbial hydrolysis efficiency rather than the rate of hydrolysis and methane production happens to be a limiting factor for overall biogas production in substrates that contain lignocellulosic material or plant residues and industrial wastes, for example, because lignocellulose, which is made up of cellulose, hemicellulose, and a complex network of lignin, possesses very high resistance.

In contrast, appreciable progress has been observed in AD technology. Nevertheless, hydrolysis remains a bottleneck in the biogas process. In this regard, it is noted that organic matter breakdown in this process occurs slowly. This further implies that there would be limited supply of fermentable substrates in the subsequent stages of anaerobic digestion. These subsequent stages would further minimize the overall methane yields. Consequently, it is very important to enhance the efficiency of microbial hydrolysis to produce biogas, especially for those AD systems that utilize complex organic feedstocks.

One of the purposes of the paper is to demonstrate the importance of microbial hydrolysis during the course of anaerobic digestion and, through the consideration of such strategies as improving efficiency during the hydrolytic stage, some of the main approaches might be pointed out, of which the most significant one is represented by adding an extra hydrolysis stage. This approach offers a possibility for a set of conditions to be maximized for hydrolytic microorganisms exclusively, free from interference based on methanogenic activity. Pretreatment methods have been developed by researchers to accelerate hydrolysis, and depending on the type of treatments applied, this falls under mechanical, thermal, chemical, or biological treatments. Observations have been made whereby the pretreatments split up recalcitrant components, such as lignocellulose, thus allowing greater accessibility of the substrate for microbial degradation. Overall improved microbial hydrolysis tends to maximize the productivity of biogas and enhance the methane content and increase the scope for applications by AD based on a broader organic waste feedstock base.

The concurrent integration of pretreatment strategies and separate hydrolysis stages also follows circular economy principles, as it allows further valuable products to be produced, including volatile acids (VAs), which are a precursors for chemicals in biorefineries. This multi-product recovery approach contributes to sustainability within the AD system through maximization of material recovery in waste material, thus limiting associated environmental impacts. Ultimately, such maximalization of microbial hydrolysis in anaerobic digestion will be the key towards making biogas production more economically and environmentally sustainable, consistent with the world's progressive trend toward renewable energy.

## 2. MATERIALS AND METHODS

### 2.1 Microbial Hydrolysis's Role in Anaerobic Digestion

The large step in hydrolysis occurs at the microbial stage in anaerobic digestion where a complex organic molecule such as carbohydrates, proteins, and lipids is broken down into even simpler soluble compounds such as monosaccharides (simple sugars), amino acids, and fatty acids. The latter will provide substrates necessary for AD's subsequent fermentation and methanogenesis steps. Hydrolytic microbes secrete extracellular enzymes to degrade these complex molecules. For instance, cellulases hydrolyze cellulose into glucose, proteases hydrolyze proteins into amino acids, and lipases break down fats into glycerol and fatty acids.



The bacterial genera involved in hydrolysis primarily belong to Clostridium, Bacteroides, and Firmicutes. The microbes are generally resistant to anaerobic environments and have the enzymatic capacity required for hydrolyzing complex substrates. However, the recalcitrance of lignocellulosic biomass, which is extremely common in agricultural residues, forestry by-products, and selected wastes from industry, creates a bottleneck that prevents the growth activities of these microbes. The resistant and hard structure of lignocellulose, constituted by the arrangement of cellulose, hemicellulose, and lignin, confers resistance to enzymatic attack and slows down hydrolysis.

The hydrolysis rate can significantly influence the overall efficiency of biogas production: slow hydrolysis limits the availability of fermentable substrates like glucose to acidogenic bacteria, which in turn influences the subsequent methane production by methanogens. Hence, improving the hydrolysis rate and extent is one of the main objectives in optimizing anaerobic digestion systems. This paper discusses several approaches to the enhancement of microbial hydrolysis. A key focus throughout has been use of pretreatment strategies and separate stages of hydrolysis.

## 2.2 Pretreatment Strategies to Enhance Hydrolysis

Several pretreatment strategies have been developed to speed hydrolysis and augment biogas yields with enhanced biodegradability of organic substrates. However, some recalcitrant components, typically lignocellulose, cannot be degraded by microbes in standard anaerobic digestion conditions. Most of the pretreatment methods devised consist of chemical, thermal, mechanical, and biological processes, all of which aim at breaking through the physical or chemical structure of the substrate and then gaining access to hydrolytic microbes.

**Mechanical Pretreatment:** Using physical or mechanical methods like shredding, grinding, or milling tends to break down feedstock particles into smaller sizes. This maximizes the amount of surface available to microbial activities. Hydrolytic enzymes may now easily reach the substrate for the hydrolysis process and hence speed it up. Of course, physical or mechanical pretreatment efficiently enhances hydrolysis but may demand a high energy input. This then impacts the energy balance of the AD system.

**Thermal Pretreatment:** The thermal methods involve the heating up of the feedstock to a high temperature of between 100 and 200°C to break the lignocellulosic structure. It increases cellulose and hemicellulose exposure to microbial degradation. The process also uses thermal sterilization of the feedstock to eliminate pathogens and reduce the likelihood of inhibiting the process. High temperatures, on the other hand, can create inhibitory compounds, such as furans, which are harmful to microbes.

**Chemical Pretreatment:** Chemical treatments include alkaline (alkaline examples such as sodium hydroxide), and acid (acidic examples being sulfuric acid) treatments, whereby the lignin structure is disrupted and the hemicellulose is solubilized, thus leading to the enhancement of digestibility of the substrate. Alkaline treatment specifically works by breaking down the lignin structure strongly, liberating cellulose for further enzymatic hydrolysis. Acidic treatment, however, focuses on hemicelluloses and thus preferably is done concurrently with thermal pre-treatment to maximize the breakdown of lignocellulose.

**Biological Pretreatment:** Biological processes involve using fungi that are capable of breaking down lignin, such as Phanerochaete chrysosporium degradative microbes that degrade selective lignin and therefore provide for higher accessibility of cellulose for hydrolytic microbes. Biological pretreatment is considered eco-friendly and energy-friendly since the process is carried out in mild conditions with no hazardous by-



products. The only drawback with this process is the relatively slow reaction; several weeks must pass until lignin degradation becomes effective.

Various studies have proved that the inclusion of pretreatment strategies with anaerobic digestion significantly enhances the efficiency of microbial hydrolysis, and hence more yields are obtained from biogas. However this selection depends upon the type of feedstock, the feasibility of the processes involved, and the possible formation of by-products.

### 2.3 Separate Hydrolysis Stage in Anaerobic Digestion

The innovation lies in the introduction of an intermediate hydrolysis step before methanogenesis, an attempt to optimize the process for anaerobic digestion. Here, the reactor separates hydrolysis and acidogenesis so that specific conditions of hydrolytic and acidogenic microorganisms could be maintained and controlled. This strategy isolates the processes to optimize critical parameters: pH, temperature, and HRT which are quite different between the hydrolysis and the methanogenesis steps.

The hydrolytic microbes prefer a slightly acidic pH (5.5–6.5) and mesophilic temperature (30–40°C), whereas the methanogens require a nearly neutral pH (6.8–7.5) and can even work under both mesophilic and thermophilic conditions (50–60°C). Decoupling of both stages enables them to work at the best of their efficiencies, maximizing the efficiency of substrate conversion as well as the yield of methane.

Another stage, hydrolysis, makes it possible to recover valuable by-products, such as VFAs, which could be channeled into production in biorefineries as chemicals or further fermented into alternative sources of energy. This is beneficial not only for biogas but for the circular economy also, maximizing organic waste streams for utilization.

This means that introducing a separate hydrolysis stage, together with proper pretreatment strategies, seems quite promising to bypass the barriers surrounding microbial hydrolysis in anaerobic digestion. Optimizing the conditions for hydrolytic activity and improving substrate accessibility may significantly enhance the efficiency of biogas production, hence making AD systems more sustainable and economically viable.

## 3. RESULTS

### 3.1 Impact of Hydrolysis on Biogas Yields

The efficiency of microbial hydrolysis is essential in establishing biogas yield, particularly during anaerobic digestion of complex organic substrates, such as lignocellulosic biomass. Indeed, it has been increasingly demonstrated by researchers that a separate hydrolysis step, implemented into AD systems, greatly enhances methane production from challenging feedstocks. For instance, methane production in lignocellulose-containing systems has been shown to increase by 30–40% during optimized hydrolysis in a separate phase (Ziganshin et al., 2016). This process is mainly due to the degradation of organic polymers being more complete. For this purpose, strong microbial consortia like Clostridia and Bacteroides are specialized in the effective breakdown of cellulose, hemicellulose, and proteins into soluble intermediates like sugars, amino acids, and fatty acids.

The higher degradation rate of these complex molecules enhances the subsequent acidogenesis and methanogenesis phases with higher yields of biogas. The pilot and full-scale studies in several systems suggest that the efficiency of the hydrolysis stage relates directly to the overall methane production yields.



The methane yields are typically lower, and the process stability is negatively affected by low yields due to poor hydrolysis management of the systems.

### 3.2 Pretreatment Efficiency

The efficiency of pretreatment methods meant for the enhancement of hydrolysis has been variable, and some work with better biogas production processes dependent on the type of substrate and used method. Thermal pretreatment in combination with biological hydrolysis seems particularly efficient in further increasing the solubilization of organic matter. More or less similar increments of 20–50% in methane production were also reported in pilot-scale studies when thermal pretreatment, with temperatures between 70°C and 150°C, preceded biological hydrolysis (Hendriks & Zeeman, 2009). The heat would break the crystalline structure of cellulose and hemicellulose; this should make difficult-to-hydrolyze cellulose and hemicellulose components more susceptible to the action of enzymes produced by microbial consortia.

One promising approach to overcoming the lignocellulose barrier is biological pretreatment using lignin-degrading fungi. Fungi, such as *Phanerochaete chrysosporium*, secrete ligninases that degrade lignin, which, therefore, makes subsequent enzymatic hydrolysis of cellulose feasible. This is highly effective when used for agricultural residues and forestry waste because they contain relatively higher lignocellulose content. Reduction of 10–20% lignin content by lignin-degrading fungi enhances digestibility and increases methane production by as much as 30% from some substrates (Ziganshin et al., 2016).

## 4. DISCUSSION

### 4.1 Challenges in Microbial Hydrolysis

Although the general benefits of optimizing microbial hydrolysis in anaerobic digestion systems are well known, several key challenges also exist that limit the efficiency of this process. One of the primary challenges is overcoming the inhibition of hydrolytic enzymes caused by toxic substances present in particular feedstocks. For instance, feedstocks rich in nitrogenous compounds such as animal manure or food waste tend to create ammonia concentration that tends to be highly elevated during the digestion process. Ammonia inhibits the activity of many hydrolytic microorganisms, particularly those involved in protein and fat decomposition processes. This leads to lesser biogas yields (Chen et al., 2008). Similarly, phenolic compounds and heavy metals present in industrial wastewater streams can slow microbial activities thereby complicating the hydrolysis process further.

Another critical issue is slow digestion, which makes lignocellulosic materials difficult to degrade. Hence the applicability of anaerobic digestion should be extended beyond simpler substrates, which represent only a minor fraction of organic waste. Cellulose has a crystalline structure, and lignin is its major component; hence, lignocellulosic biomass is resistant to enzymatic attack, leading to incomplete hydrolysis and less methane yield. Pretreatments like thermal or biological treatments of the materials may help overcome this problem but would add extra costs and operational complexity.

One possible solutions to these issues are co-digestion, where the various streams of waste are mixed to mitigate nutrient imbalances as well as inhibitory compounds. Mixing nitrogen-rich manures from animals with carbon-rich agricultural residues helps dilute ammonia, a compound that inhibits microbial hydrolysis and elevates the hydrolysis of microorganisms. Co-digestion has been proven to enhance the rate and



extent of hydrolysis, hence making the AD system more stable, and elevating the methane yield (Murphy et al., 2016).

## 4.2 Integration of Hydrolysis into the Circular Economy

Hydrolysis is thus separated from methanogenesis in anaerobic digestion systems, which creates many advantages related to the circular economy as a whole. Isolating hydrolysis as a distinct stage enables better control over the conditions needed for better substrate degradation and higher yields of biogas and the potential recovery of valuable by-products, such as VFAs. These VFAs can be employed as chemical precursors in the production for biofuels, bioplastics, and other renewable chemicals. This would add to the increased list of products produced by AD systems and increase their economic viability.

Efficient organics to energy conversion, and associated valuable co-products recover valuable resources and reduce waste and the environmental footprint in line with circular economy principles. Optimized microbial hydrolysis can help increase the productivity of biogas output as part of a process contributing towards closed-loop systems where waste materials are continuously repurposed. It supports the realization of global sustainability goals, like reduced reliance on fossil resources, reduced emissions of greenhouse gases, and increased penetration of renewable energy technologies into regional material cycles (Ziganshin et al., 2016).

## 4.3 Environmental and Economic Benefits

The pretreatment strategies and separate hydrolysis steps offer environmental as well as economic advantages in achieving improved microbial hydrolysis. Improved hydrolysis leads to enhanced biogas yields, thus translating into higher amounts of energy recovered from organic waste as well as lower emissions of greenhouse gases. Optimizing the process of anaerobic digestion can allow for more diversion of organic waste away from landfills, where it otherwise generates emissions of methane without any energy recovery.

Economically, enhanced microbial hydrolysis can add more value to the economic efficiency of anaerobic digestion since methanogenesis will be more pronounced and hence less supplementation with other energy inputs. For instance, the recovery of VFA and other intermediate products during hydrolysis can introduce a new stream of revenue, further improving the economic sustainability of AD systems. For operators, this would also make improved biogas production a source of finance to invest in pretreatment technologies and optimized hydrolysis processes where renewable energy incentives or carbon credits are offered (Murphy et al., 2016).

## 5. CONCLUSION

An important step in the anaerobic digestion process, microbial hydrolysis directly impacts the efficiency of the biogas production process and, consequently, the stability of AD systems. Slow rates of degradation of complex organic substrates, especially for lignocellulosic material, represent one of the main bottlenecks to increasing biogas production. Pre-hydrolysis steps, integrated or separate, are nowadays considered breakthroughs to overcome this bottleneck.

These challenges can be overcome by improving conditions for hydrolytic microorganisms as well as including advanced pretreatment methods. Such pretreatments – thermal, biological, or chemical



treatments – have been applied in various studies on improving the solubilization of organic matter and hence increasing methane yields up to 50% in some cases. Inserting of a hydrolysis stage separately will allow, at least, control of a higher level compared to the actual operation for the process and recovery of valuable by-products such as volatile fatty acids intended for integration with circular economy models.

Considerable progress has been made, but a number of technical gaps remain in place. For instance, pretreatment methods typically lack sufficient economic feasibility, at least for small-scale operations. Pretreatment technologies are owing in many areas but also have to be efficient and cost-effective. Hydrolysis incorporation into a more general classification of AD systems is an important challenge in the case of complex or highly variable waste streams. Therefore, future research should be focused in developing adaptive AD systems with efficiency in the handling of diverse feedstocks and a maximum recovery of energy values with minimum adverse effects on the environment.

Optimization of microbial hydrolysis is the central thrust to improve the economies of operation and the environmental sustainability of anaerobic digestion systems. Closing technical gaps now existing will serve to promote improvements in hydrolysis efficiency, increase biogas production toward greater ranges of feedstocks, and speed the realization of renewable energy and a circular economy.

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