OPTIMISATION OF BIOGAS YIELD THROUGH ENZYMATIC PRETREATMENT OF WASTEWATER

MD SAZIDUR RAHMAN[1], ABDULLAH AL MAMUN[1*], MD ZAHANGIR ALAM[2], NUR KHAIRIYAH BINTI BASRI[1] AND MD MUNIR HAYET KHAN[3]

[1]*Cataclysmic Management and Sustainable Development Research Group (CAMSDE), Department of Civil Engineering, International Islamic University Malaysia (IIUM), Gombak, 53100 Kuala Lumpur, Malaysia.*

[2]*Department of Chemical Engineering & Sustainability, International Islamic University Malaysia (IIUM), Gombak, 53100 Kuala Lumpur, Malaysia.*

[3]*Faculty of Engineering and Quantity Surveying (FEQS), INTI International University, Persiaran Perdana BBN, Nilai 71800, Negeri Sembilan, Malaysia.*

**Corresponding author: mamun@iium.edu.my*

ABSTRACT: Anaerobic digestion is preferred to produce biogas from highly polluted organic wastes, which also has the potential for renewable energy. However, the slow degradation of complex organic compounds available in organic wastes poses difficulties in this process. Lower biogas yields and longer hydraulic retention times (HRT) are caused mainly due to the complex nature of the substrates. This work reports the progress and potential of applying enzymatic pretreatment to improve biogas production using anaerobic digestion systems. Enzymatic pretreatment is the process of breaking down complex organic molecules into simpler ones, which are more readily biodegradable compounds in hydrolysis steps utilising enzymes, such as cellulase, protease, and lipase. The efficiency of the enzymatic pretreatment is measured by the increase in biogas production. Although this field is relatively new, according to the literature from previous studies, enzymatic pretreatment significantly increases the biodegradability of organic wastes compared to untreated substrates, producing higher biogas yields in shorter HRTs. However, the improvement varies depending on the type of waste and the process parameters used. The enzymatic pretreatment of municipal sludge, pulp & paper sludge, and POME has been reported to improve biogas yield by 23.1, 26.0, and 52.17%, respectively. The HRTs of the above-mentioned anaerobic processes are recorded as 11 days, 62 days, and 38 days, respectively. These results highlight the potential of enzymatic pretreatment to enhance biogas production in anaerobic digestion. By improving efficiency, this approach offers a more sustainable means for waste management and energy generation. There is huge potential for further research to explore the use of mixed enzyme combinations and optimal dosages to evaluate the scalability of enzymatic pretreatment for industrial applications.

KEY WORDS: *Anaerobic Digestion, Enzymatic Pretreatment, Biogas Production, Multi-enzyme Cocktail*

1. INTRODUCTION

Anaerobic digestion is a biological method used to treat wastewater in the absence of oxygen, with biogas production as a by-product. However, the process is often hindered by the slow degradation of complex organic matter, particularly in industrial wastewater, resulting in a lower biogas yield and an extended hydraulic retention time (HRT) [1]. Biogas generation, a form of renewable energy, has gained global attention for its dual benefit in energy security and waste management [2]. Wastewater, rich in organic compounds, offers a viable substrate for biogas production. However, the efficiency of anaerobic digestion is contingent on the effective breakdown of these complex compounds to enhance methane yield [3]. The efficiency and speed of anaerobic digestion largely depend on the pretreatment process.

Biogas generation has received a lot of interest as a renewable energy source because of its potential to improve both energy security and environmental sustainability [4]. Wastewater, which contains a significant amount of organic matter, provides an ideal feedstock for biogas production [5]. However, the efficiency of anaerobic digestion, and thus biogas yield, is strongly dependent on the breakdown of complex organic molecules in wastewater into simpler forms that are accessible to methanogenic microbial populations [6].

One of the most difficult issues in anaerobic digestion is the partial degradation of complex substrates such as cellulose, hemicellulose, and lignin, which make up a large portion of the organic matter [6]. To improve the biodegradability of such substances, pretreatment methods such as chemical, thermal, and mechanical treatments are used [7]. Enzymatic pretreatment has emerged as a viable method for increasing biogas yield by promoting the hydrolysis of complex organic [8]. Enzymes such as cellulases, lipases, and proteases catalyse the breakdown of larger molecules into smaller substrates, increasing their accessibility to anaerobic microbes [9].

1.1 Enzymatic Pretreatment for Biogas Production

Biogas, composed primarily of methane (CH_4) and carbon dioxide (CO_2), is produced through the anaerobic digestion of organic matter by microorganisms in the absence of oxygen [4]. The process involves four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, where complex organic compounds are broken down into simpler molecules and ultimately converted into biogas [10]. The efficiency of biogas production is influenced by the biodegradability of the substrate and operational conditions [11].

Enzymatic pretreatment can enhance the biodegradability of organic waste by breaking down complex compounds and thereby improving the efficiency of the anaerobic digestion process [12]. By facilitating the breakdown of lignocellulosic materials and other recalcitrant compounds, enzymatic pretreatment helps to increase biogas yields and reduce the hydraulic retention time (HRT) required for effective digestion.

1.2 Challenges in Biogas Production from Wastewater

Industrial wastewater, such as palm oil mill effluent (POME), municipal wastewater, and pulp and paper sludge, contains a substantial proportion of lignocellulosic materials, lipids, and proteins that pose challenges for biodegradation. Lignin, which is a critical component in lignocellulosic biomass, acts as a physical barrier preventing microbial access to cellulose and hemicellulose [13]. The crystalline structure of cellulose further complicates the hydrolysis process. Similarly, lipid-rich wastes form a complex matrix that inhibits microbial activity, which requires long HRTs for efficient breakdown [14]. Without adequate pretreatment, the anaerobic digestion of such wastewater results in suboptimal biogas yields and lengthy digestion periods, which significantly affect process viability [15].

Enzymatic pretreatment offers a solution by improving the hydrolysis phase of anaerobic digestion, which is often the rate-limiting step in biogas production [16]. Studies show that enzymes such as cellulases, proteases, and lipases can significantly reduce the time required for hydrolysis by breaking down complex molecules into simpler, more biodegradable substrates [17]. This increases the availability of soluble sugars, peptides, and fatty acids, which are more readily utilised by methanogens, thus improving biogas yield and reducing HRTs [18]. Without adequate pretreatment, wastewater digestion can result in long retention times, inefficient biogas production, and high organic load in the effluent, making the process economically and environmentally less feasible.

In the Malaysian context, where palm oil production generates vast amounts of Palm Oil Mill Effluent (POME), the challenge is especially evident. Despite government support through Feed-in Tariff (FiT) schemes, tax incentives, and the Green Technology Financing Scheme (GTFS), biogas adoption has been limited - only 92 out of 450 palm oil mills had biogas facilities by 2016, largely due to logistical issues in remote areas and the absence of mandatory biogas capture regulations [19].

1.3 Benefits of Enzymatic Pretreatment

Enzymatic pretreatment has been proven effective across various types of wastewaters. For instance, the enzymatic treatment of municipal sludge has been reported to improve biogas yield by 23.1%, while pulp and paper sludge saw an improvement of 26%, and POME yielded a remarkable increase of 52.17% [20]. These improvements are accompanied by reduced HRTs - 11 days for municipal sludge, 62 days for pulp and paper sludge, and 38 days for POME - compared to untreated controls [21]. These results highlight the importance of optimising the enzymatic process by selecting the right enzyme cocktail and adjusting parameters such as pH, temperature, and enzyme dosage to maximise efficiency and economic feasibility [22].

Moreover, the environmental benefits of enhancing biogas production through enzymatic pretreatment are significant. It supports the goal of reducing greenhouse gas emissions by providing a more sustainable and efficient method for managing organic waste while promoting the use of renewable energy [23]. Future research should focus on optimising enzyme combinations, scaling up applications for industrial systems, and reducing the cost of enzymes to make this approach more economically viable [24].

2. METHODS

This paper is based on secondary data and peer-reviewed publications. No primary experiments or tests were conducted for the data collection. Rather, secondary data and journal sources were used to assess the potential of pretreatment to enhance biogas production. A structured literature review approach was adopted to ensure the reliability and relevance of the findings. Academic databases, including Scopus, Web of Science, and Google Scholar, were systematically searched using targeted keywords such as “enzymatic pretreatment,” “biogas production,” “anaerobic digestion,” and “organic wastewater.” Inclusion criteria focused on studies that provided quantitative data on biogas or methane yield improvement following enzymatic pretreatment. As shown in Fig. 1, the methodology flowchart illustrates the steps involved in writing this paper.

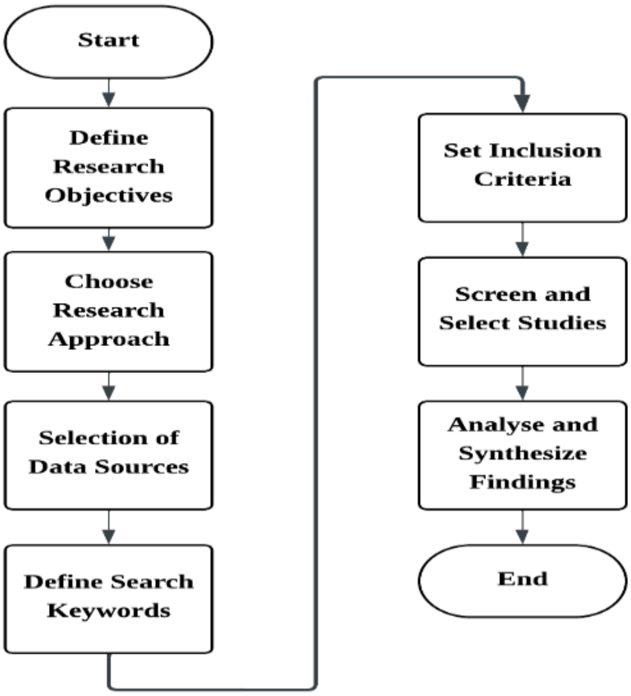

Fig. 1: Methodology flowchart

2.1 Pretreatment Method

Pretreatment of organic substrates is required to overcome the challenges provided by complex substances such as lignin, cellulose, and lipids, thereby making the substrates more digestible to microorganisms [25]. Several pretreatment methods have been developed to improve biogas production, including physical, chemical, thermal, and biological methods. Physical pretreatment, such as grinding or milling, lowers the particle size and increases the surface area available for microbial attack [26]. Chemical procedures involve the use of acids, alkalis, or oxidising agents to degrade lignin and dissolve hemicellulose and cellulose [27]. Thermal pretreatment is the process of heating the substrate to a high temperature, causing cell walls to break down and making organic content more accessible [28]. Biological pretreatment, especially enzymatic pretreatment, has gained a lot of attention because it is more eco-friendly, precise, and requires less energy compared to chemical or heat treatments [29]. Enzymatic pretreatment uses enzymes to selectively break down complex polymers, making the organic matter more easily degradable during anaerobic digestion and increasing the biogas generation and process efficiency [30].

2.2 Advantages and Challenges of Enzymatic Pretreatment

Enzymatic pretreatment offers several advantages over other pretreatment methods, such as chemical or thermal processes. Enzymes are highly specific, meaning they target bonds within organic compounds, resulting in efficient hydrolysis without producing harmful by-products. This makes enzymatic pretreatment environmentally friendly, as it does not require harsh chemicals or high energy inputs [31]. Moreover, enzymes work under mild conditions, such as moderate temperatures and neutral pH, which aligns well with the conditions within anaerobic digesters [9, 32]. However, enzymatic pretreatment also presents challenges, particularly in terms of cost and scalability [33]. The production of enzymes can be expensive, and large amounts of enzymes may be required for industrial-scale operations [34]. Additionally, enzymes are often substrate-specific, meaning that different enzymes or enzyme blends are required for different types of waste [35]. This specificity can make the pretreatment process more complex to implement in a full-scale biogas plant. Overcoming these challenges, such as through the development of more cost-effective enzyme production techniques or the use of enzyme recovery systems, is crucial for the widespread adoption of enzymatic pretreatment in the biogas industry [36].

Hydraulic retention time (HRT) is the average time the substrate spends in the digester for microbial breakdown and biogas production [37]. Shorter HRTs can result in incomplete substrate degradation, reducing biogas outputs, whereas longer HRTs allow microorganisms to digest complex organic matter completely [38]. Optimising HRT during enzymatic pretreatment is important because it improves substrate digestibility, allowing for a possibly shorter HRT without losing biogas output [39]. However, balancing HRT is critical, since excessively lengthy retention times can lower process efficiency and increase operating costs [40].

The presence of inhibitors can significantly reduce the effectiveness of anaerobic digestion by decreasing microbial activity required for methane production [41]. Ammonia, sulphides, volatile fatty acids (VFAs), heavy metals, and long-chain fatty acids (LCFAs) are some common inhibitors that can accumulate during digestion or be found in the substrate [42]. For example, high ammonia concentrations suppress methanogenic archaea, whereas sulphides, which are frequently formed from sulphur-containing substances, might disturb microbial activities [43]. Phenolic compounds can also act as strong inhibitors during enzymatic pretreatment [44].

3. RESULTS AND DISCUSSION

Not much information is available in the published materials on the use of enzymes to improve the biogas yield in anaerobic digestion of highly polluted wastewater. This section critically analyses and summarises the results obtained in the past research works published in the journals. Enzymatic pretreatment improves biogas production over a wide range of substrates, as shown in Table 1.

The enzymes' capacity to break down complex organic substances such as lignin, cellulose, hemicellulose, and lipids into more digestible forms for anaerobic microorganisms, leading to an increase in methane yield. The different types of improvement among substrates show the importance of substrate composition, enzyme selectivity, and the type of enzymatic action [30]. Pretreatment of POME with xylanase resulted in a 160% increase in methane (CH_4) production compared to untreated controls [45]. This substantial increase can be due to xylanase's ability to convert hemicellulose, a major component of the lignocellulosic biomass contained in POME, into xylose. The xylose generated during enzymatic hydrolysis is more easily metabolised by the microbial community responsible for biogas production in anaerobic digestion. In contrast, fleshing, a by-product of the leather industry, showed a modest 15% increase [46]. Although rich in lipids, fleshing is more recalcitrant and may contain inhibitory substances such as salts and heavy metals. Lipase helps by converting triglycerides into fatty acids and glycerol, but the limited accessibility and possible inhibition may reduce the overall methane enhancement.

Table 1: Enzymatic pretreatment results on different substrates

Substrate	Enzyme	Results	Ref.
POME	Xylanase	CH_4 production increased by 160% compared to the control	[45]
Fleshing	Lipase	Biogas production increased by 15%	[46]
Willow	Laccase	33% biogas yield improvement	[47]
Manure	Laccase	Methane yield improvement by 19.8 m^3	[49]
Corn stover	Blend of cellulase	Methane production increased by 111%	[48]
Sugar beet pulp and spent hops	Celustar XL and Agropect pomace	Biogas yield increased by 19% and 13%	[50]
Pulp and paper bio-sludge	Protease	Biogas yield increased by 26%	[51]
Waste-activated sludge	Mix of amylase, protease, cellulase, lipase	Biogas yield increased by 34%	[52]
Sewage sludge	Isolated protease and bacteria	Biogas volume increased by 3.65 and 5.77 times, respectively	[53]

Substrates rich in lignocellulosic biomass, such as willow and corn stover, also benefited from pretreatment. Willow showed a 33% increase with laccase [47] likely due to lignin removal that exposed cellulose and hemicellulose for digestion. Corn stover, on the other hand, responded with a 111% increase [48] using a cellulase blend, which highlights its high cellulose content and the effectiveness of enzymatic hydrolysis in unlocking fermentable sugars. These results underscore the importance of substrate-enzyme compatibility.

Manure, although relatively less fibrous, showed a measurable increase (19.8 ± 0.4 m^3 methane) with laccase [49]. The improvement suggests the presence of partial lignin, which inhibits degradation; laccase helps to alleviate this effect. Similarly, sugar beet pulp and spent hops, both agro-industrial residues, showed moderate improvements (19% and 13%) with enzymes targeting pectin and cellulose [50]. These modest gains suggest partial accessibility of biodegradable components even before pretreatment.

Protease treatment of pulp and paper bio-sludge resulted in a 26% increase in biogas yield [51]. The protease likely helps in breaking down proteins into amino acids, making them more accessible for anaerobic microorganisms. The complex protein structure of pulp and paper bio-sludge may have initially hindered microbial activity, but the enzymatic treatment improved biodegradability. Similarly, waste-activated sludge, treated with a combination of amylase, protease, cellulase, and lipase, saw a 34% increase in biogas yield [52]. The use of multiple enzymes enhances the breakdown of various organic components, improving overall substrate digestibility.

Fig. 2 shows the increase in biogas production after enzymatic pretreatment on different substrates. Jose tall wheatgrass, treated with cellulase and hemicellulase, saw a 31% increase in biogas production, highlighting the effectiveness of these enzymes. Algae: Spirulina platensis, with cellulase and cellobiose, showed a 24.8% increase. While effective, the increase was lower than that of wheatgrass. Switchgrass had the highest increase at 39%, suggesting that its high cellulose content makes it highly responsive to enzymes. Microalgae treated with cellulase showed only an 8% increase, indicating limited biogas yield from this substrate. However, when microalgae were treated with a mix of cellulase, xylanase, and glucohydrolase, the increase rose to 15%, showing the benefit of using a combination of enzymes.

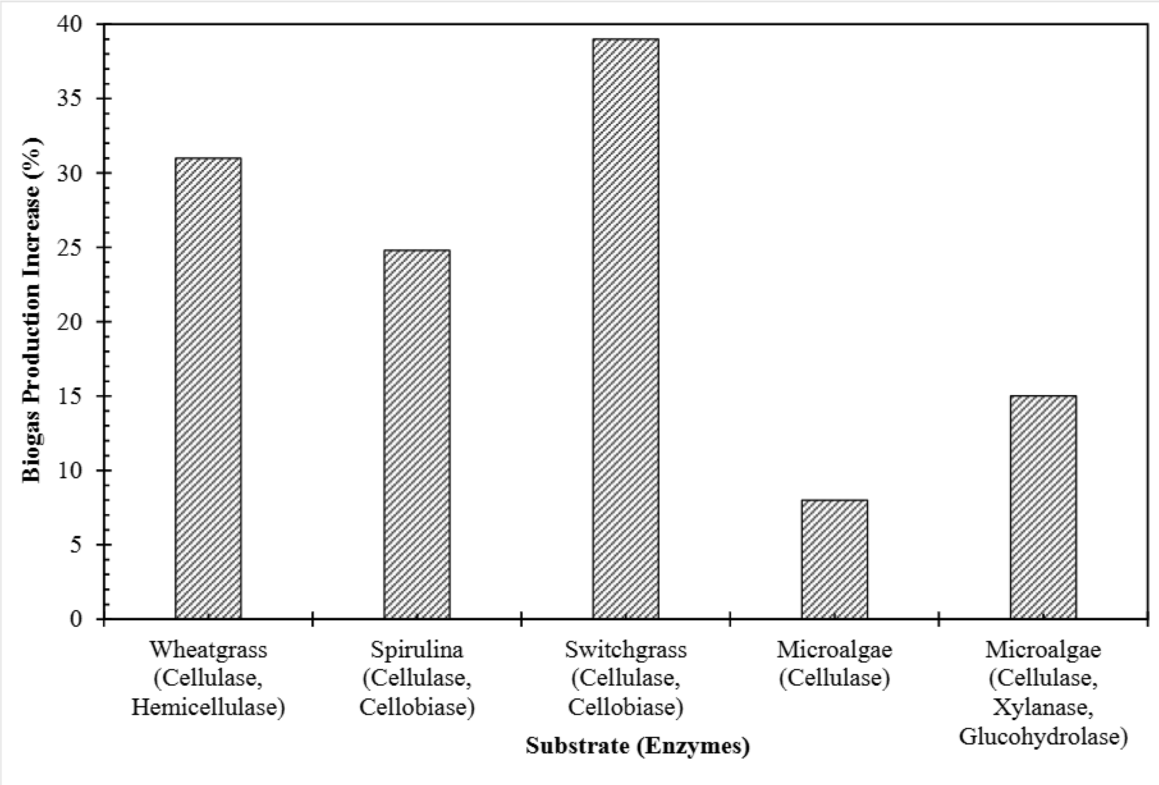

Fig. 2: Increase in Biogas Production by Enzymatic Pretreatment on Various Substrates [58, 59, 60]

To further enhance biogas yield, the use of enzyme blends, such as combining cellulase and xylanase, has proven effective, especially for substrates with mixed polymeric structures. Optimising parameters such as enzyme dosage, contact time, temperature, and pH is critical to ensure efficient pretreatment without incurring excessive costs.

However, while enzymatic pretreatment offers significant advantages, scalability and cost remain major challenges. Industrial enzymes can be expensive, and their application on a commercial scale often requires large volumes and precise conditions [54]. Strategies such as the development of recombinant microorganisms for on-site enzyme production, enzyme immobilisation and recovery, and the use of crude enzyme extracts from low-cost sources are being explored to reduce operational costs [55, 56].

From an environmental perspective, enzymatic pretreatment supports sustainable waste management. Enhanced methane yields reduce the volume of residual waste and increase energy recovery, which can substitute fossil fuels and help lower greenhouse gas emissions. Moreover, improved conversion reduces the organic load in digestate, making it safer for land application and contributing to circular economy goals in wastewater and agricultural waste management [57].

4. CONCLUSIONS

Enzymatic pretreatment is a promising approach to enhance biogas yields from various organic waste substrates by improving the breakdown of complex compounds such as lignin, cellulose, and lipids. Process specificity, low energy requirements, and compatibility with anaerobic digestion make enzymatic pretreatment an attractive option for sustainable energy recovery and wastewater treatment. However, its widespread implementation remains limited due to the high cost of enzymes and challenges related to scalability. To address these limitations, future research should focus on developing cost-effective enzyme production methods, including recombinant enzyme expression, on-site enzyme generation using microbial cultures, and the use of crude or waste-derived enzyme sources. Additionally, pilot-scale studies are essential to validate laboratory findings under real-world conditions and assess operational feasibility. Addressing these research gaps will be critical to advancing enzymatic pretreatment as a practical and economically viable strategy in biogas production and environmental management.

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REFERENCES

[1] Ferrer, I., Garfi, M., Uggetti, E., Ferrer-Martí, L., Calderon, A., & Velo, E. (2010). Biogas production in low-cost household digesters at the Peruvian Andes. Biomass and Bioenergy, 34(12), 1709-1716.

[2] Holm-Nielsen, J. B., Al Seadi, T., & Oleskowicz-Popiel, P. (2009). The future of anaerobic digestion and biogas utilisation. Bioresource Technology, 100(22), 5478-5484.

[3] Li, C., Champagne, P., & Anderson, B. C. (2018). Enhanced biogas production from anaerobic digestion of solid waste: Current status and prospects. Bioresource Technology, 291, 121883.

[4] Jameel, M. K., Mustafa, M. A., Ahmed, H. S., Mohammed, A. jassim, Ghazy, H., Shakir, M. N., Lawas, A. M., Mohammed, S. khudhur, Idan, A. H., Mahmoud, Z. H., Sayadi, H., & Kianfar, E. (2024). Biogas: Production, properties, applications, economic and challenges: A review. Results in Chemistry, 7(2211-7156), 101549. https://doi.org/10.1016/j.rechem.2024.101549

[5] Un, C. (2025). Assessing Biogas from Wastewater Treatment Plants for Sustainable Transportation Fuel: A Detailed Analysis of Energy Potential and Emission Reductions. Gases, 5(1), 6. https://doi.org/10.3390/gases5010006

[6] Nayeri, D., Mohammadi, P., Parnia Bashardoust, & Eshtiaghi, N. (2024). A comprehensive review on the recent development of anaerobic sludge digestions: performance, mechanism, operational factors, and future challenges. Results in Engineering, 22, 102292–102292. https://doi.org/10.1016/j.rineng.2024.102292

[7] Amin, F. R., Khalid, H., Zhang, H., Rahman, S. u, Zhang, R., Liu, G., & Chen, C. (2017). Pretreatment methods of lignocellulosic biomass for anaerobic digestion. AMB Express, 7(1). https://doi.org/10.1186/s13568-017-0375-4

[8] Pranesh Kannappan Karthikeyan, Himiyage Chaminda Hemaka Bandulasena, & Radu, T. (2024). A comparative analysis of pre-treatment technologies for enhanced biogas production from anaerobic digestion of lignocellulosic waste. Industrial Crops and Products, 215, 118591–118591. https://doi.org/10.1016/j.indcrop.2024.118591

[9] Liu, Z., & Smith, S. R. (2020). Enzyme Recovery from Biological Wastewater Treatment. Waste and Biomass Valorization. https://doi.org/10.1007/s12649-020-01251-7

[10] Goswami, R., Chattopadhyay, P., Shome, A., Banerjee, S. N., Chakraborty, A. K., Mathew, A. K., & Chaudhury, S. (2016). An overview of physico-chemical mechanisms of biogas production by microbial communities: a step towards sustainable waste management. 3 Biotech, 6(1). https://doi.org/10.1007/s13205-016-0395-9

[11] Meegoda, J., Li, B., Patel, K., & Wang, L. (2018). A Review of the Processes, Parameters, and Optimization of Anaerobic Digestion. International Journal of Environmental Research and Public Health, 15(10), 2224. https://doi.org/10.3390/ijerph15102224

[12] Olatunji, K. O., Ahmed, N. A., & Ogunkunle, O. (2021). Optimization of biogas yield from lignocellulosic materials with different pretreatment methods: a review. Biotechnology for Biofuels, 14(1). https://doi.org/10.1186/s13068-021-02012-x

[13] Zhao, X., Zhang, L., & Liu, D. (2012). Biomass recalcitrance. Part II: Fundamentals of different pretreatments to increase the enzymatic digestibility of lignocellulose. Biofuels, Bioproducts, and Biorefining, 6(5), 561–579.

[14] Browne, J. D., & Murphy, J. D. (2013). Assessment of the resource associated with biomethane from food waste. Applied Energy, 104, 170-177.

[15] Appels, L., Baeyens, J., Degrève, J., & Dewil, R. (2008). Principles and potential of the anaerobic digestion of waste-activated sludge. Progress in Energy and Combustion Science, 34(6), 755–781.

[16] Taherzadeh, M. J., & Karimi, K. (2008). Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review. International Journal of Molecular Sciences, 9(9), 1621–1651.

[17] Zheng, M., Yang, L., Luo, S., & Wang, Y. (2014). Pretreatment of lignocellulosic biomass for enhancing biogas production. Energy Procedia, 61, 1748–1751.

[18] Kim, M., Ahn, Y. H., & Speece, R. E. (2015). Comparative process stability and efficiency of anaerobic digestion; mesophilic vs. thermophilic. Water Research, 36(17), 4369–4385.

[19] Hoo, R., Leow, F., Lee, M., & Low. (2020). Producing Biogas from Palm Oil Mill Effluent in Southeast Asia -the Green Elephant in the Room? https://esi.nus.edu.sg/docs/default-source/esi-policy-briefs/producing-biogas-from-palm-oil-mill-effluent-in-southeast-asia.pdf

[20] Wang, Q., Noguchi, K., & Yamasaki, S. (2020). Improvement of biogas production from sewage sludge in anaerobic digestion by microbial pretreatment with mixed cultures of hyperthermophilic bacteria. Renewable Energy, 151, 795-802.

[21] Sawatdeenarunat, C., Surendra, K. C., Takara, D., Oechsner, H., & Khanal, S. K. (2016). Anaerobic digestion of lignocellulosic biomass: Challenges and opportunities. Bioresource Technology, 215, 304-313.

[22] López-González, J. A., Vargas-García, M. C., López, M. J., Suárez-Estrella, F., & Moreno, J. (2014). Different enzymatic activities during lignocellulosic waste composting. Bioresource Technology, 157, 182-189.

[23] Angelidaki, I., Ahring, B. K., Ellegaard, L., & Johansen, K. (2003). Applications of the anaerobic digestion process. Biomethanation II, 1–33.

[24] Pang, Y., Liu, Y., Ren, J., Wang, X., & Chen, Z. (2021). The application of enzymes in anaerobic digestion: Current status and future perspectives. Bioresource Technology Reports, 15, 100748.

[25] Hamraoui, K., Gil, A., El Bari, H., Siles, J. A., Chica, A. F., & Martín, M. A. (2020). Evaluation of hydrothermal pretreatment for biological treatment of lignocellulosic feedstock (pepper plant and eggplant). Waste Management, 102, 76–84. https://doi.org/10.1016/j.wasman.2019.10.020

[26] Maurya, D. P., Singla, A., & Negi, S. (2015). An overview of key pretreatment processe for biological conversion of lignocellulosic biomass to bioethanol. 3 Biotech, 5(5), 597–609. https://doi.org/10.1007/s13205-015-0279-4

[27] Wang, L., Li, G., Chen, X., Yang, Y., Liew, R. K., Abo-Dief, H. M., Lam, S. S., Sellami, R., Peng, W., & Li, H. (2024). Extraction strategies for lignin, cellulose, and hemicellulose to

obtain valuable products from biomass. Advanced Composites and Hybrid Materials, 7(6). https://doi.org/10.1007/s42114-024-01009-y

[28] Saha, B., Khwairakpam, M., & Kalamdhad, A. S. (2021). Thermal pre-treatment – A prerequisite for the reduction of hydrolysis stage during anaerobic digestion of Ageratum conyzoides. Materials Science for Energy Technologies, 4, 34–45. https://doi.org/10.1016/j.mset.2020.12.002

[29] Chen, S., Zhang, X., Singh, D., Yu, H., & Yang, X. (2010). Biological pretreatment of lignocellulosics: potential, progress and challenges. Biofuels, 1(1), 177–199. https://doi.org/10.4155/bfs.09.13

[30] Yuh Xiu Liew, Yi Jing Chan, Manickam, S., Mei Fong Chong, Chong, S., T. Joyce Tiong, Jun Wei Lim, & Pan, G.-T. (2020). Enzymatic pretreatment to enhance anaerobic bioconversion of high strength wastewater to biogas: A review. Science of the Total Environment, 713, 136373–136373. https://doi.org/10.1016/j.scitotenv.2019.136373

[31] Abolore, R. S., Jaiswal, S., & Jaiswal, A. K. (2023). Green and Sustainable Pretreatment Methods for Cellulose Extraction from Lignocellulosic Biomass and its Applications: A Review. Carbohydrate Polymer Technologies and Applications, 7, 100396–100396. https://doi.org/10.1016/j.carpta.2023.100396

[32] Hasan, M. M., M. Mofijur, Uddin, M. N., Kabir, Z., Irfan Anjum Badruddin, & Yunus, M. (2024). Insights into anaerobic digestion of microalgal biomass for enhanced energy recovery. Frontiers in Energy Research, 12. https://doi.org/10.3389/fenrg.2024.1355686

[33] Jain, S., & Kumar, S. (2024). Advances and challenges in pretreatment technologies for bioethanol production: A comprehensive review. Sustainable Chemistry for Climate Action, 5, 100053. https://doi.org/10.1016/j.scca.2024.100053

[34] Saravanan, A., Kumar, P. S., Vo, D.-V. N., Jeevanantham, S., Karishma, S., & Yaashikaa, P. R. (2021). A review on catalytic-enzyme degradation of toxic environmental pollutants: Microbial enzymes. Journal of Hazardous Materials, 419, 126451. https://doi.org/10.1016/j.jhazmat.2021.126451

[35] Lewis, T., & Stone, W. L. (2023, April 24). Biochemistry, proteins enzymes. National Library of Medicine; StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK554481/

[36] Mustafa, A. H., Rashid, S. S., Rahim, M. H. A., Roslan, R., Musa, W. a. M., Sikder, B. H., & Sasi, A. A. (2022). Enzymatic Pretreatment of Lignocellulosic Biomass: An Overview. Journal of Chemical Engineering and Industrial Biotechnology, 8(1), 1–7. https://doi.org/10.15282/jceib.v8i1.7030

[37] Savand-Roumi, E., Salehiyoun, A. R., & Mohtasebi, S. S. (2023). Use of a biogas-specific e-nose with machine learning to identify biogas pattern changes linked to hydraulic retention times in an anaerobic digester: A case study. Fuel, 357, 130013. https://doi.org/10.1016/j.fuel.2023.130013

[38] Quevedo, H. (2024, April 29). In the biogas production process, what is HRT? Portal Energia E Biogás® - a Maior Plataforma de Conteúdo Sobre Digestão Anaeróbia E Biogás Do Brasil. https://www.biogaseenergia.com.br/in-the-biogas-production-process-what-is-hrt

[39] Yang, Z., Yang, D., Hua, Y., Chen, X., Wang, X., Gong, H., Dong, B., Li, X., & Dai, X. (2024). Dual optimization in anaerobic digestion of rice straw: Effects HRT and OLR coupling on methane production in one-stage and two-stage systems. Journal of Environmental Management, 370, 123041. https://doi.org/10.1016/j.jenvman.2024.123041

[40] Cruddas, P., Nikolaos Asproulis, Antoniadis, A., D David Best, Collins, G., Estefanía Porca, Jefferson, B., Cartmell, E., & McAdam, E. J. (2021). The impact of hydraulic retention time on the performance of two configurations of anaerobic pond for municipal sewage treatment. Environmental Technology, 43(25), 3905–3918. https://doi.org/10.1080/09593330.2021.1937331

[41] Chen, Y., Cheng, J. J., & Creamer, K. S. (2008). Inhibition of anaerobic digestion process: A review. Bioresource Technology, 99(10), 4044–4064. https://doi.org/10.1016/j.biortech.2007.01.057

[42] Tian, H., Karachalios, P., Angelidaki, I., & Fotidis, I. A. (2018). A proposed mechanism for the ammonia-LCFA synergetic co-inhibition effect on anaerobic digestion process. Chemical Engineering Journal, 349, 574–580. https://doi.org/10.1016/j.cej.2018.05.083

[43] Harirchi, S., Wainaina, S., Sar, T., Nojoumi, S. A., Parchami, M., Parchami, M., Varjani, S., Khanal, S. K., Wong, J., Awasthi, M. K., & Taherzadeh, M. J. (2022). Microbiological Insights into Anaerobic Digestion for biogas, Hydrogen or Volatile Fatty Acids (VFAs): a Review. Bioengineered, 13(3), 6521–6557. https://doi.org/10.1080/21655979.2022.2035986

[44] Gufe, C., Jambwa, P., Marumure, J., Makuvara, Z., Khunrae, P., & Kayoka-Kabongo, P. N. (2024). Are phenolic compounds produced during the enzymatic production of prebiotic xylooligosaccharides (XOS) beneficial: a review. Journal of Asian Natural Products Research, 26(8), 867–882. https://doi.org/10.1080/10286020.2024.2328723

[45] Prasertsan, P., Khangkhachit, W., Duangsuwan, W., Mamimin, C., & Thong, S. (2017). Direct hydrolysis of palm oil mill effluent by xylanase enzyme to enhance biogas production using two-step thermophilic fermentation under nonsterile conditions. Special Issue on the Asia Biohydrogen and Biorefinery Symposium (ABBS 2016), 58 October 2016, Jeju Island, South Korea, 42(45), 27759–27766.

[46] Sri Bala Kameswari, K., Kalyanaraman, C., Porselvam, S., & Thanasekaran, K. (2011). Enhancement of Biogas Generation by Addition of Lipase in the Co-Digestion of Tannery Solid Wastes. CLEAN - Soil, Air, Water, 39(8), 781–786.

[47] Schroyen, M., Vervaeren, H., Vandepitte, H., Van Hulle, S. W. H., & Raes, K. (2015). Effect of enzymatic pretreatment of various lignocellulosic substrates on production of phenolic compounds and biomethane potential. Bioresource Technology, 192, 696–702.

[48] Brémond, U., de Buyer, R., Steyer, J.-P., Bernet, N., & Carrere, H. (2018). Biological pretreatments of biomass for improving biogas production: an overview from lab scale to full-scale. Renewable and Sustainable Energy Reviews, 90, 583–604.

[49] Kudanga, T., & Le Roes-Hill, M. (2014). Laccase applications in biofuels production: current status and future prospects. Applied Microbiology and Biotechnology, 98(15), 6525–6542.

[50] Ziemiński, K., Romanowska, I., & Kowalska, M. (2012). Enzymatic pretreatment of lignocellulosic wastes to improve biogas production. Waste Management, 32(6), 1131–1137.

[51] Bonilla, S., Choolaei, Z., Meyer, T., Edwards, E. A., Yakunin, A. F., & Allen, D. G. (2018). Evaluating the effect of enzymatic pretreatment on the anaerobic digestibility of pulp and paper biosludge. Biotechnology Reports, 17, 77–85. https://doi.org/10.1016/j.btre.2017.12.009

[52] Kim, T.-H., Song, D., Lee, J.-S., & Yun, Y.-M. (2023). Enhanced Methane Production from Pretreatment of Waste Activated Sludge by Economically Feasible Biocatalysts. Energies, 16(1), 552–552. https://doi.org/10.3390/en16010552

[53] Agabo-García, C., Pérez, M., Rodríguez-Morgado, B., Parrado, J., & Solera, R. (2019). Biomethane production improvement by enzymatic pre-treatments and enhancers of sewage sludge anaerobic digestion. Fuel, 255, 115713. https://doi.org/10.1016/j.fuel.2019.115713

[54] Hugendra Rishay Moodley, Laurah Gutu, Ayinde, W. B., Ikumi, D., & Basitere, M. (2024). Enhancing anaerobic digestion efficiency in dairy waste water treatment: a comprehensive review of enzyme-based pre-treatment by microorganisms in South Africa. Water Practice and Technology. https://doi.org/10.2166/wpt.2024.095

[55] Dos Santos Ferreira, J., de Oliveira, D., Maldonado, R. R., Kamimura, E. S., & Furigo, A. (2020). Enzymatic pretreatment and anaerobic co-digestion as a new technology to high-methane production. Applied Microbiology and Biotechnology, 104(10), 4235–4246. https://doi.org/10.1007/s00253-020-10526-x

[56] Mohidem, N. A., Mohamad, M., Rashid, M. U., Norizan, M. N., Hamzah, F., & Mat, H. bin. (2023). Recent Advances in Enzyme Immobilisation Strategies: An Overview of Techniques and Composite Carriers. Journal of Composites Science, 7(12), 488. https://doi.org/10.3390/jcs7120488

[57] Pan, S.-Y., Tsai, C.-Y., Liu, C.-W., Wang, S.-W., Kim, H., & Fan, C. (2021). Anaerobic co-digestion of agricultural wastes toward circular bioeconomy. IScience, 24(7), 102704. https://doi.org/10.1016/j.isci.2021.102704

[58] Romano, R. T., Zhang, R., Teter, S., & McGarvey, J. A. (2009). The effect of enzyme addition on anaerobic digestion of Jose Tall Wheat Grass. Bioresource Technology, 100(20), 4564–4571. https://doi.org/10.1016/j.biortech.2008.12.065

[59] El-Mashad, H. M. (2015). Biomethane and ethanol production potential of Spirulina platensis algae and enzymatically saccharified switchgrass. Biochemical Engineering Journal, 93, 119–127. https://doi.org/10.1016/j.bej.2014.09.009

[60] Passos, F., Hom-Diaz, A., Blanquez, P., Vicent, T., & Ferrer, I. (2016). Improving biogas production from microalgae by enzymatic pretreatment. Bioresource Technology, 199, 347–351. https://doi.org/10.1016/j.biortech.2015.08.084