# Study The Effect of Adding Proteins to Cellulose and Lipids on Biogas Production

Fareed A.Radhi<sup>1</sup>, Fawziea M. Hussien<sup>2</sup>, Johain J. Faraj<sup>3</sup>

- <sup>1</sup> Middle Technical University Engineering Technical College-Baghdad, Baghdad Iraq
- <sup>2</sup> Middle Technical University Engineering Technical College-Baghdad, Baghdad Iraq
- <sup>3</sup> Middle Technical University Engineering Technical College-Baghdad, Baghdad Iraq
- <sup>1</sup>abc0029@mtu.edu.iq, <sup>2</sup>Fawizea\_material@mtu.edu.iq, <sup>3</sup>Johaintech@mtu.edu.iq

### Abstract

This research aims to know the strength of the effect of adding proteins represented by meat residues to the vegetable wastes (VW) mixture on the one hand, and the lipids waste (LW) mixture on the other hand, on the production of biogas, knowing that cow dung is an essential component in each of the above mixtures. Two digesters (bioreactors) were used, the first contains meat waste, vegetable waste and cow dung, and the second contains meat waste, lipids waste and cow dung. The main factors were measured: the concentrations of methane, carbon dioxide, hydrogen, Ammonia, and pH. In addition, the daily biogas production rate and the cumulative volume of biogas were calculated for both bioreactors. The highest methane production for digester No.1 was 95 (g/kg.VS), while the highest methane production for methane of digester No.2 was 139 (g/kg.VS). The daily biogas production for digester No.1 was 4.4 (L/day) while for digester No.2 was 5.6 (L/day), in addition to cumulative biogas for digesters No.1 and No.2 were 282.45 (L) & 353.82 (L). The anaerobic digestion occurred at the ambient temperature range (19 to 30 °C), and the hydraulic retention time was 64 days.

# Keywords: Biogas, Protein, Lipids, Co-digestion, Bioreactor.

## 1. Introduction

Many countries addressed sustainability as one of the critical concerns. Reduced consumption of fossil fuels is needed to achieve sustainability. Renewable energy production through wind, biomass, solar, tidal wave and numerous other excellent practices will contribute to environmental sustainability in future. Cost of taxes and fuels is another factor driving the quest for more cost-effective and environmentally sustainable energy sources for households and even whole countries. Anaerobic digestion of organic waste (cattle manure and food wastes) to produce biogas is another method for generating electricity in addition to decreased food waste. Furthermore, the anaerobic digestion method aids to make less odour (decontaminate) waste. It can serve as a superior feedstock for the composting operation,

resulting in fertilizer with a high level of nutrient content to help crop growth. Co-digestion is an anaerobic digestion technique that results in a more effective digestion process, increasing methane yields from some organic materials due to the beneficial synergistic effects of mixed materials with complementary properties and the availability of lost nutrients by the cosubstrate[1]. The digestion process has four phases. These stages must be controlled early on when beginning a digester, so if the process is properly maintained, methane production should require minimal chemical testing and treatment. Before methanogenic bacteria may begin to produce methane, bacteria must first consume the oxygen in the substrate and then break it down into volatile fatty acids and fermented alcohols. The steps are as follows: Hydrolysis stage: Enzymes degrade and liquefy smaller molecules in the material, as well as large polymers. Acidogenesis stage: Fermentation of the hydrolysis products (soluble monomers) produces volatile fatty acids (VFAs) and alcohols. Acetogenesis stage: Acetogenic bacteria degrade alcohols and volatile fatty acids (VFAs), generating acetic acid, hydrogen, carbon dioxide and in this process. Moreover, finally, the Methanogenesis stage: Acetic acid and hydrogen, are converted to CO<sub>2</sub> and methane by methanogenic bacteria[2]. The addition of protein (chicken wastes) to food waste will increase gas production by up to 30%[3]. Co-digestion of paper sludge, food waste, and pulp at various waste mixture ratios was performed. In comparison to the digestion of a single substrate, the authors found that codigestion produced more methane, performed better at removing organics, and had a higher buffering ability[4]. The results revealed that the co-digestion mechanism had a substantial impact on biogas and methane yields. The maximum biogas yield obtained for the Cafeteria Waste mixing ratio: Waste Vegetables: Food wastes (0.5:1.0:1.5, 1.0:1.5:0.5, 1.5:0.5:1.0, and 1.0:1.0:1.0) were 33.92, 35.52, 36.55, and 43.87 L/d on the 25th, 24th, 24th, and 21st days, respectively[5]. Biogas methane yield increased from 352 (mL/g.VS) to 447 (mL/g.VS) then eventually to 679 (mL/g, VS) when food waste mixture (FW) with a C/N ratio of 17 was blended with vegetables, vegetable wastes (VW), and meat to increase its C/N ratio to 26 and 30 before the process of anaerobic digestion.[6]. Batch assays determined the methane potentials of starch, meat-and-bone, lipids, skin, hair, meat, raw waste, and ribs to be 497, 225, 487, 561, 582, 575, 619, and 359 dm<sup>3</sup>/kg, respectively. At 37 ° C, co-digestion of 5% pork by-products with pig manure produced 40 per cent more than manure digestion alone.[7]. When 5% fish lipids were applied to a cattle deposits anaerobic reactor, the methane yield increased from 25-50 m<sup>3</sup> biogas/m<sup>3</sup>[8]. The "pasteurization" process prior to co-digestion of (food waste, slaughterhouse waste, animal blood, cow dung, potato residue, card ) has risen in the methane yields of blood about (+15%) and potato about waste (+12%) only[9]. A research was conducted to evaluate the efficiency of co-digestion of fish and vegetable waste; the results indicated that co-digestion produced 463 L/kg.VS of methane. [10]. The waste of biotreated fish's perch was co-digested with vegetable waste in anaerobic conditions [11]. Co-digestion of fish and strawberry waste in anaerobic conditions optimized by[12]. Discontinuous digester with a capacity of 2 litres operating at 38 degrees Celsius and containing (blood and wastewater) as the first mixture and slaughterhouse residues as the second mixture to produce combustible methane[13]. Another research of co-digestion of discarded adult nappies with expired food materials, including meat wastes, resulted in the recovery of 0.427 MJ/L of content with pasteurization of meat pretreatment[14]. Specific Biogas Production reached 704 m<sup>3</sup> /kg.VS for the chicken litter-waste oil feedstock mixture[15]. Three scenarios were compared to system of reference of easy compositing and rendering: (1) the anaerobic digestion of the slaughter house trash in combination with the OFMSW, (2) the anaerobic digestion of the slaughter house wastes, and (3) the combination of anaerobic digestion of sewage sludge and slaughterhouse trash. The study's results indicated that the overall energy input from start to finish consumption and emissions of GHG associated with treating the the slaughter house wastes with the anaerobic digestion and the anaerobic co- digestion were 567 MJ/ton of the slaughter house wastes and 400834 kg-

CO2/ton of the slaughter house wastes, respectively, while that the overall energy input from start to finish consumption and the emissions of GHG associated with making control scenarios were 1900 MJ/ton of the slaughterhouse wastes and 96.4 kg-CO2 /ton of the slaughterhouse wastes The results show that anaerobic digestion of the slaughterhouse wastes has the potential to significantly reduce the US meat industry's the emissions of GHG while also producing bioenergy to help ensure the country's energy security[16]. Anaerobic codigestion of kitchen waste is being investigated for acid inhibition. The findings indicate that the total gas yields for the four biodegradable from maximum substrates to a minimum substrate are as follows: celery cane, soybean, lipid meat, and rice[17]. Meat bone meal (MBM) co-digestion with crude glycerol (CG) and dairy manure (DM) under anaerobic conditions was investigated. Anaerobic digestion (mono type) findings indicated, the CG provided the highest yield of methane 480 (mL/g.VS), followed by 410 (mL/g.VS) for MBM then 170 (mL/g.VS) for DM. Methane yield increased as MBM content increased in anaerobic co-digestions, but decreased as CG content increased[18].

#### 2. Methods

# 2.1. The experimental design

The bioreactors (digester) technology has been designed and built to generate biogas. It consists of two 25-liter plastic anaerobic bioreactor as shown in figure (1). Each bioreactor comprises three terminals (supply feedstock, outlet biogas, and outlet sludge), each of which is controlled by a manual ball valve of varying size, as well as a floating piston-cylinder storage tank for biogas, as seen in figure (2). Additionally, these digesters have several sensors for measuring and obtaining crucial variables gathered by an Arduino processor, such (Hydrogen, methane, Carbon dioxide, pH, & Ammonia). These sensors are regulated based on the current manufacturer's standards documentation.



Figure 1. The bioreactor



Figure 2. The floating storage

#### 2.2 The Substrate

The first bioreactor's mixture is meat residues, and vegetable wastes got from kitchen residue and cow dung to create a microbial culture within the bioreactor. The second bioreactor's mixture is cooked lipids mixed with meat residues at a proportion ratio, as shown in table (1). Meat residues are minced about 1- 2 cm. The addition of meat leftovers, vegetable wastes, and cow dung improves mixing efficiency and increases the area available for cell proteins to break down biomass, resulting in quicker breakdown and a shorter anaerobic period. The blender (automatic mixers) is used to combine substrates and water until a homogeneous mixture is obtained, which is then placed in the digesters, while the functional digestive capacity is 20 L. After the mixture is fed to each bioeactor, the air is removed using a suction that meets the manufacturer's requirements (litres per minute). This procedure is repeated for a minimum of 6 minutes to guarantee that the reactor is oxygen-free or oxygen-reducing. Under (19 to 30 °C), the operation lasted a total of 64 days. Throughout this time period, readings for CH<sub>4</sub>, CO<sub>2</sub>, H<sub>2</sub>, NH<sub>3</sub>, pH, daily biogas output, and total biogas output per bioreactor have been taken.

 $\overline{\mathbf{V}\mathbf{W}}$ Water Bioreactor No. MR Lipid Ratio CD **Total** 0/0 % 0/0 % % Vol. (L) 1 20 10 0 75:25:0 10 60 20 2 0 15 15 0:50:50 10 60 20

Table 1. The concentrations of substrates are used in the digestion process.

## 3. The Results and Discussion

Many graphs (figures) will be used to illustrate the findings for two bioreactors, including the impact of protein on biogas output. Graph (figure) No.3 illustrated the daily generation of methane by bioreactor No.1 (vegetables, meat, CD, and water); it started in methane production on day nine about of 9 (g/ kg.VS), then it rises gradually until reach at first peak about of 50 (g/ kg.VS) on day 16. The production of bioreactor No.1 decreased sharply at 10 (g/ kg.VS) on day 20, to increase sharply until it reached 55 (g/ kg.VS) on day 23 then rose gradually until it reaches the maximum peak at 91 (g/ kg.VS) on day 39, after that the production of this bioreactor decreased gently with some fluctuations until it reaches at 60 (g/ kg.VS) on day 64. Bioreactor No.2 which is contained on (meat residues, lipids waste, CD, and water) started the methane production on day ten at about 10 (g/ kg.VS). It rises steadily until day 21 at about 30 (g/ kg.VS), its jump to 71 (g/ kg.VS) on day 23. The production has some fluctuations whereas it reachs a maximum peak at about 139 (g/ kg.VS) on day 30, the production of methane falls to rise again and remains as a pattern until day 63 at about 110 (g/ kg.VS).

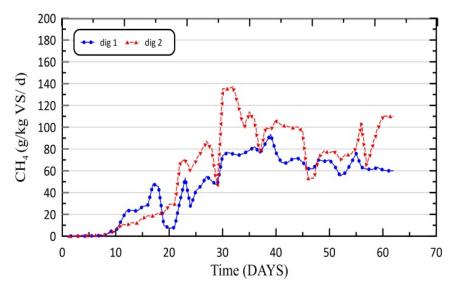


Figure 3. Methane daily production for bioreactors (dig) 1 and 2

The daily carbon dioxide production for bioreactor No.1 is observed starting on day four at about 2.5 (g/ kg.VS), then rise to 12 (g/ kg.VS) on day six until it reaches the first peak of 20 (g/ kg.VS) on day 7 to fall and rise again at the same value on day 12. The emission of CO<sub>2</sub> decrease and increase again at the maximum peak of 33 (g/ kg.VS) on day 16 to decrease gradually then rise slightly. It has semi-stable emission until day 55; the value of CO<sub>2</sub> emission is 25 (g/ kg.VS) then decrease gradually at the final day of about 12 (g/ kg.VS). The bioreactor No.2 started carbon dioxide emission on day three at value 3 (g/ kg.VS). This emission rises gradually until it reaches 20 (g/ kg.VS) on day 13. The emission of CO<sub>2</sub> increases again to value 40 on day 22 to fall and rise at value 44 (g/ kg.VS) on day 28, then it increases sharply at the maximum value, which is 62 (g/ kg.VS). The emission of carbon dioxide decreases gradually with several fluctuations until it reaches the final day. The value is 12.6 (g/ kg.VS), as shown in figure (4) as below. It is noted that the emission of carbon dioxide for the AD of protein and lipids higher than the AD of protein and cellulose due to lipids have hydrocarbons contents than cellulose and the chemical bonds of cellulose and protein stronger than lipids[19].

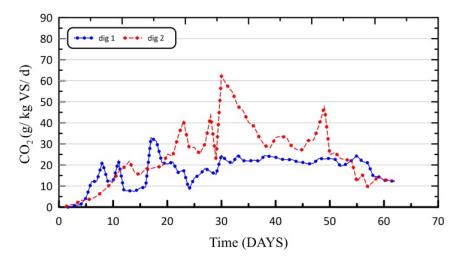


Figure 4. CO<sub>2</sub> daily production for bioreactor (dig) 1 and 2

The pH value has recorded for two bioreactors for this study. Bioreactor No.1, which is contained (VW, MR, and CD), is noted that started pH value at 7.1 on the first day then decreases slightly to 6.8 and keep on at this value for few days, then fall once at the value of 6.6 on day 15. The pH value rises until it reaches 7.6 on day 46; after that, it fluctuated between ranges (7.2 to 7.4) until it reached 7.3 on the final day. Bioreactor No.2, which is contained (LW, MR, and CD). The initial magnitude of the pH was about 6.6 on the first day, then pH decreases slightly and rises once again at the same value (6.6). After that, the pH decreases to below 6.2 as slowly on day 21, then increases gradually with an increase in ambient temperature until it reaches about 7.8, then decreases to below 7.6 on the final day. The reason for the decrease in the pH value at the beginning of the AD is an accumulation of volatile fatty acids "VFT" in the bioreactor No.2 [20], as shown in figure (5) below.

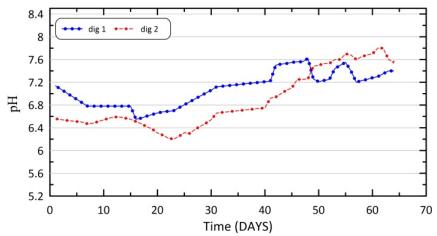


Figure 5. The relation of pH value and days for bioreactor (dig) 1 and 2

The ammonia concentration for two bioreactors (digesters) which shown in figure (6) as below. The production of Ammonia for bioreactor No.1 started on day 15 at below 50 ppm/day, then increased to 100 ppm/day on day 16. In this bioreactor (digester) had six peaks, which are 250, 320, 350, 350, 380, and 450 ppm/day for days 20, 29, 30, 49, 54, and 60 respectivily. In constrant, bioreactor (digester) No.2, which is started in the production of Ammonia on day 14 at 50 ppm/day, then increased sharply to 150 ppm/day on day 15. This bioreactor had maximum peak at 499 ppm/day on day 30, then decreased sharply on day 34 at 250, after that it had fluctuated for some days, then it increased gradually until it reached to 500 ppm/day on the final day. The reason of the production of Ammonia for bioreactor No.2 higher than bioreactor No.1 is the concentration of protein in bioreactor No.2 higher than bioreactor No.1 which consider Ammonia's source, in addition, to increasing in ambient temperatures during the fermentation period [21].

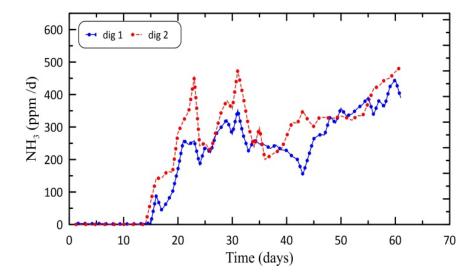


Figure 6. The concentrations of Ammonia for bioreactors (dig) 1 and 2

The hydrogen concentrations for bioreactors No.1 and No.2 illustrated in figure (7) below. The hydrogen production for bioreactor (digester) No.1 started on day two at 60 ppm/day, then increased gradually to the first peak and considered the maximum peak at 550 ppm/day on day 16. The production of H<sub>2</sub> had many fluctuations until it reached 400 ppm/day on day 39, then decreased gradually to 250 ppm/day on the final day. In the constraint, bioreactor (digester) No.2 had hydrogen production lower than bioreactor No.1 initially, but it increased gradually then sharply to reach 600 ppm/day on day 25. After that, this production had fluctuated for several days, and it decreased slightly until it reached the final day at 350 ppm/day. The reason hydrogen production for bioreactor No.2 is higher than bioreactor No.1 results in the accumulation of hydrogen production for mixing (protein and lipid) higher than mixing (protein and cellulose) in the acetogenic oxidation phase[22].

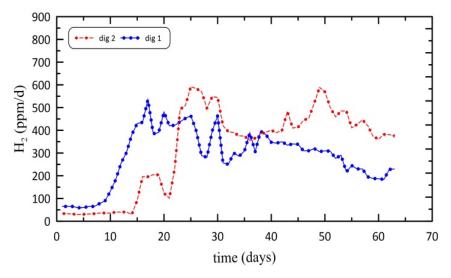


Figure 7. Hydrogen concentration for bioreactors (dig) 1 and 2

ISSN: 1007-6735

Biogas production for the two bioreactors (digesters) influent with ambient temperature during the digestion period, as illustrated in figure (8), which shows the effect of the ambient temperature on the daily biogas production for the two bioreactors mentioned above. Bioreactor No.1 is later than bioreactor No.2 in biogas production, which is started on day four, while bioreactor No.2 started on day two. The reason for this action is that cellulose needs more time for decomposition, through the figure above, when the ambient temperature increases or decreases, biogas production increases or decreases. Bioreactor No.2 is higher than bioreactor No.1 in the daily biogas production, and the accumulation of biogas for bioreactor No.2 and No.1 was 353.82 and 282.45 L, respectively as shown in figure (9).

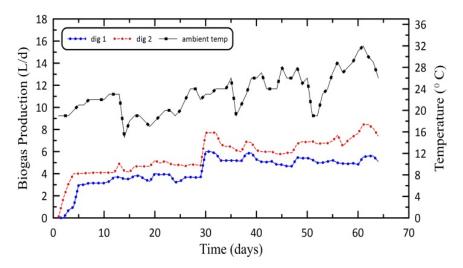


Figure 8. The daily biogas production for bioreactors (dig) 1 and 2

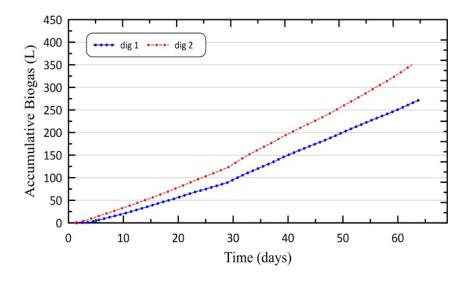


Figure 9. The accumulation of the biogas production for bioreactors (dig) 1 and 2

## 4. Conclusions

The aim of this study is to know the effect of adding proteins to each of cellulose and fat separately on the productivity of biogas, which lasted for 63 days. Through this

experiment, it was found that the bioreactor that contains proteins and fats has a higher productivity in biogas than the reactor that contains proteins and cellulose, as a result that the proteins generate amino acids, which are a good source of volatile fatty acids, from which hydrogen and acetic acid are generated[23]. Thus, methane is generated, in addition to the high carbon content in fats, which makes it produce methane gas in a large amount, unlike cellulose, which needs time to decompose[24].

# Acknowledgements

I would like to express my heartfelt gratitude and appreciation to my superiors, Prof.Dr. Fawziea M. Hussien and Dr. Johain J. Faraj, for their unwavering assistance and encouragement during my M.Sc. thesis and related research; their advice, inspiration, vast expertise, and recommendations were extremely beneficial in the planning and execution of this dissertation.

## **Nomenclature**

AD	Anaerobic Digestion
FW	Food Wastes
FOG	Fat, Oil, and Grease
VW	Vegetable Wastes
Dig	Digester
VS	Volatile Solid
TS	Total solid
CD	Cow Dung
No.	Number

#### References

- [1] D. T. Sponza and O. N. Ağdağ, "Effects of shredding of wastes on the treatment of municipal solid wastes (MSWs) in simulated anaerobic recycled reactors," *Enzyme Microb. Technol.*, vol. 36, no. 1, pp. 25–33, 2005.
- [2] R. Dana, Micro-Scale Biogas Production: A Beginners Guide. Citeseer, 2010.
- [3] M. A. Hasan *et al.*, "Biogas production from chicken food waste and cow manure via multistages anaerobic digestion," in *AIP Conference Proceedings*, 2018, vol. 2016, no. 1, p. 20011.
- [4] Y. Lin, D. Wang, J. Liang, and G. Li, "Mesophilic anaerobic co-digestion of pulp and paper sludge and food waste for methane production in a fed-batch basis," *Environ. Technol.*, vol. 33, no. 23, pp. 2627–2633, 2012.
- [5] M. R. Al Mamun and S. Torii, "Anaerobic co-digestion of cafeteria, vegetable and fruit wastes for biogas production," in 2014 International Conference on Renewable Energy Research and Application (ICRERA), 2014, pp. 369–374.

- [6] M. I. Tanimu, T. I. M. Ghazi, R. M. Harun, and A. Idris, "Effect of carbon to nitrogen ratio of food waste on biogas methane production in a batch mesophilic anaerobic digester," *Int. J. Innov. Manag. Technol.*, vol. 5, no. 2, p. 116, 2014.
- [7] A. Hejnfelt and I. Angelidaki, "Anaerobic digestion of slaughterhouse by-products," *Biomass and bioenergy*, vol. 33, no. 8, pp. 1046–1054, 2009.
- [8] P. Weiland, "Biogas production: current state and perspectives," Appl. Microbiol. Biotechnol., vol. 85, no. 4, pp. 849–860, 2010.
- [9] Y. Zhang, S. Kusch-Brandt, S. Heaven, and C. J. Banks, "Effect of Pasteurisation on Methane Yield from Food Waste and Other Substrates in Anaerobic Digestion," *Processes*, vol. 8, no. 11, p. 1351, 2020.
- [10] N. B. Akshaya and S. Jacob, "Unification of Waste Management from Fish and Vegetable Markets Through Anaerobic Co-digestion," Waste and Biomass Valorization, vol. 11, no. 5, pp. 1941–1951, 2020.
- [11] A. Mshandete, A. Kivaisi, M. Rubindamayugi, and B. O. Mattiasson, "Anaerobic batch codigestion of sisal pulp and fish wastes," *Bioresour. Technol.*, vol. 95, no. 1, pp. 19–24, 2004.
- [12] A. Serrano, J. A. Siles, M. C. Gutiérrez, and M. Á. Martín, "Optimization of anaerobic codigestion of strawberry and fish waste," *Appl. Biochem. Biotechnol.*, vol. 173, no. 6, pp. 1391–1404, 2014.
- [13] A. Marcos, A. Al-Kassir, A. A. Mohamad, F. Cuadros, and F. López-Rodríguez, "Combustible gas production (methane) and biodegradation of solid and liquid mixtures of meat industry wastes," *Appl. Energy*, vol. 87, no. 5, pp. 1729–1735, 2010.
- [14] K. Tsigkou *et al.*, "Expired food products and used disposable adult nappies mesophilic anaerobic co-digestion: Biochemical methane potential, feedstock pretreatment and two-stage system performance," *Renew. Energy*, vol. 168, pp. 309–318, 2021.
- [15] J. J. Chávez Fuentes, SMART-FARMS: Transforming chicken litter into a reliable source of energy through AcoD. 2017.
- [16] S. Wang, K. Sahoo, U. Jena, H. Dong, R. Bergman, and T. Runge, "Life-cycle assessment of treating slaughterhouse waste using anaerobic digestion systems," J. Clean. Prod., vol. 292, p. 126038, 2021.
- [17] B. Dai et al., "Acid inhibition during anaerobic digestion of biodegradable kitchen waste," J. Renew. Sustain. Energy, vol. 7, no. 2, p. 23118, 2015.
- [18] F. J. Andriamanohiarisoamanana *et al.*, "Anaerobic co-digestion of dairy manure, meat and bone meal, and crude glycerol under mesophilic conditions: Synergistic effect and kinetic studies," *Energy Sustain. Dev.*, vol. 40, pp. 11–18, 2017.
- [19] T. Menzel, P. Neubauer, and S. Junne, "Role of Microbial Hydrolysis in Anaerobic Digestion," *Energies*, vol. 13, no. 21, p. 5555, 2020.
- [20] J.-G. Park, B. Lee, S.-Y. Jo, J.-S. Lee, and H.-B. Jun, "Control of accumulated volatile fatty acids by recycling nitrified effluent," J. Environ. Heal. Sci. Eng., vol. 16, no. 1, pp. 19–25, 2018.
- [21] M. Vandermies and P. Fickers, "Bioreactor-scale strategies for the production of recombinant protein in the yeast Yarrowia lipolytica," *Microorganisms*, vol. 7, no. 2, p. 40, 2019.
- [22] A. Rabii, S. Aldin, Y. Dahman, and E. Elbeshbishy, "A review on anaerobic co-digestion with a focus on the microbial populations and the effect of multi-stage digester configuration," *Energies*, vol. 12, no. 6, p. 1106, 2019.

- [23] T. Karuppiah and V. E. Azariah, "Biomass pretreatment for enhancement of biogas production," *Anaerob. Dig.*, 2019.
- [24] R. Gumisiriza, J. F. Hawumba, M. Okure, and O. Hensel, "Biomass waste-to-energy valorisation technologies: a review case for banana processing in Uganda," *Biotechnol. Biofuels*, vol. 10, no. 1, pp. 1–29, 2017.