Research Article:

EFFECTS OF TEMPERATURE, PARTICLE SIZE AND ENZYME ADDITION ON BIOGAS PRODUCTION IN-VITRO

Sanjaya Lamichhane* and Lars Erik Rudd
Blaestad Campus, Inland Norway University of Applied Sciences, Norway

*Corresponding author: sm79.lamichhane@gmail.com
Received date: 31 December 2021, Accepted date: July 30 2024
DOI: https://doi.org/10.3126/jafu.v6i1.78159

ABSTRACT

Biogas is an anaerobically produced ecofriendly renewable form of energy which can address the harmful effects associated with conventional source of fossil fuels. The objectives of present study were to examine effects of temperature, particle size and enzymes on biogas production. Moose (*Alces alces*) thrives in woody browse in semi arctic region and it was presumed that it hosts unique micro-organisms capable of producing fibrolytic enzymes. An experiment was conducted in the laboratory of Inland Norway University, Blæstad. Cow manure with 5% dry matter content and wheat straw with 3 different particle size were treated with 3 dose of Moose rumen bacteria (MRB) culture along with 3 temperature settings. A cubical model was selected according to DoE (Design of experiments) and 11 treatments with different combinations of treatments were tested. 100ml Erlenmeyer flasks were used as reactors, whereas gas was collected in 100 mL syringes. The data were then analyzed using MODDE pro and MS Excel. The study shows that increase in temperature and enzyme has positive main effects and interaction effects for achieving a high rate of biogas production. The study reveals the positive effect of using of MRB culture on the production of biogas since it contains novel microorganisms able to promote biomass digestion.

Key words: Biogas production, MRB, treatment combinations

INTRODUCTION

After the commencement of the industrial revolution human activities have resulted in a 40% increase in atmospheric carbon dioxide (CO_2) concentration with a profound increase from 280 ppm in 1750 to 406 ppm in 2017 (ESRL, 2018). Combustion of non-renewable energy sources emits greenhouse gases such as carbon dioxide, nitrogen oxides (NO_x), Sulphur dioxide (SO_2) and volatile organic compounds (WHO, 2018). Greenhouse gases in the atmosphere act like a blanket causing trapping of heat; the greenhouse effect. This effect causes a global increase of earth´s temperature and thus could lead to consequences like global warming, increased sea level, less ice and snow, drought and flooding, climate change and extreme weather incidents (Wellinger et al., 2013).

Biogas is a gaseous mixture of methane and carbon dioxide with small amounts of hydrogen sulphide (H_2S) and trace gases generated by the disintegration of organic matters in the absence of oxygen. Wide range of organic matters originating from household and industrial waste, manure, plants debris, sludge, sewage or any other biodegradable feedstocks have potential to produce biogas (Schnurer & Jarvis, 2010). Biogas is considered as a renewable source of energy because production-and-use cycle of biogas is continuous with no net carbon dioxide gain (McKendry, 2002). Biogas can be used directly for the purpose of combustion, heating and electricity generation or could be liquefied to bio-methane for use as a vehicle fuel. The process of biogas digestion also produces high value digestate, which is less odorful and have a higher agricultural value to use as a fertilizer (Ward et al., 2008). Millions of people from less developed regions of Asia and Africa are already using household digesters for their home purpose. Primarily, an effective digester can generate 200-400 m^3 of biogas with 50-75% methane content per ton of dry input (Jørgensen, 2009).

Moose (*Alces alces*), is a large ruminant in Cervidae (deer) family native to northern latitude territories such as northern United States, Canada, Russia and Scandinavia. Moose mostly browse deciduous or coniferous leaves, twigs, stems and also strips bark from trees. This feed contains large amount of lignin, tannins, polymers of cellulose and hemicellulose which is very harsh to digest since they largely act as barrier for absorption of nutrients (Ishaq et al., 2015). Rumen and reticulum of moose stomach fosters a large consortia of microbial population (bacteria, archaea, fungi and protozoa). Those microbiota plays a significant role to break down binds of nutrients and makes them readily available for absorption. The enzymes secreted by microbes like esterase, lignanse and cellulase helps them to carry out the digestion process at a higher rate (Bayané & Guiot, 2011). The bacterial isolates and culture from a moose was found to consist of 21 strains of *Streptococcus bovis*, 9 strains of *Butyrivibrio fibrisolvens*, 7 strains of *Lachnospira multiparus*, 2 strains of *Selenomonas ruminantium* and many more (Ishaq & Wright, 2012).

Therefore, we hypothesized that, ruminal fluid of moose might host novel microorganisms possibly of interest in biogas production. Those microbes would have a wide array of enzymatic action, capable of producing useful metabolites and probably have an unique potential to break down lignocellulose and hence, increase gas yield.

MATERIALS AND METHODS

Effects of temperature, particle size and enzyme concentration on biogas production were investigated in Blæsted campus, Norway on January 2018. Temperature was set to low, medium and high at 20˚C, 30˚C and 40˚C respectively. Particle size (PS) was set to low, medium and high at respective <1mm, 3mm and >5mm. Similarly, proportion of enzyme was set to low (0% v/v), medium (2.5% v/v) and high (5% v/v).

Design of the experiment

Experiment design was performed in accordance to MODDE Pro software. Three different factors were portrayed along x, y and z-axis, 8 different treatments were selected at the edges of cube. 3 of treatments were taken from the center points at the middle value. As a whole 11 treatments were obtained as shown in the figure.

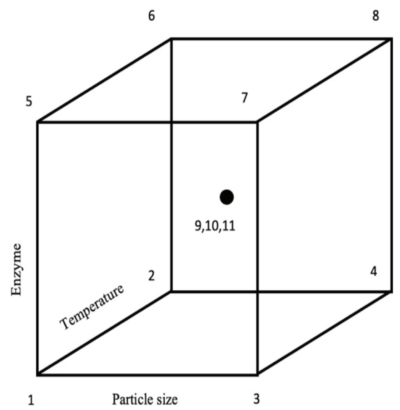

Fig. 1. Design of test

Table 1. Test condition of reactors

No. of Reactor	Temperature (˚C)	Particles size (mm)	Enzymes (%v/v)
1	20	1	0
2	40	1	0
3	20	5	0
4	40	5	0
5	20	1	5
6	40	1	5
7	20	5	5
8	40	5	5
9	30	3	2.5
10	30	3	2.5
11	30	3	2.5

Preparation of test

Wheat straw and cow dung manure were used as a substrate for our test to produce biogas in the laboratory. Dry wheat straw was collected from a local field around Blæstad campus. To find different particle size, straw was ground and then extracted from sieve with different mesh size. Here, cow dung provided the inoculum for the microbes to produce biogas. Percentage of dry matter presented in the manure was calculated by drying the samples to constant weight in a muffle furnace at 103˚C (standards EN 12880 and APHA 2540 B). Stable standard 5% dry matter was achieved in the manure solution by adding neutral water in the mixture. Different temperature settings were provided inside an incubator. To ensure equal volume of liquid input inside the digester 5% v/v (1.5ml) of distilled water was added in digester of no enzyme supplement. Likely, 2.5% v/v (0.7ml) of distilled water was added by the pipette to the digester having 2.5%v/v enzyme to replenish the amount of liquid. Since the main objective was to study effects of these 3 variables, the amount of manure and straw were kept constant in all experiments. Considering 100 mL capacity of our reactor, every flask was provided with 30 gm manure and 3 gm straw mixture (10:1 ratio) (standard VDI 4630).

11 different reactors were prepared for testing. After mixing of manure, straw and enzymes according to specified mixture, it was then fitted with rubber cork for insulation of gas. Syringe collector were fitted in all of the flasks with a bore in rubber corks. Piston of the syringe exerts some frictional force within its wall. To ensure comparable friction syringe collectors were tested with weights. Syringes were held upright and small weight (20g) was tied in the piston with a very fine thread. Small weights were continuously added until the piston just started to slide down i.e. friction of piston. Among many syringes, only those syringes with same frictional weight were selected for test (120g in our case).

Collection and culture of enzyme (MRB)

Bacterial isolate from moose rumen secreting cellulase activity, i.e. CMCase (endo beta 1,4 glucanase) and xylanase was used for the test. The isolate was prepared from rumen content of a road-killed Moose at the University of Bergen, but was proprietary to the company TransHerba AS, Elverum. Thus, the actual identity of the isolated organism was not disclosed but was given the name 'MRB 4' for practical reference. Master seed stock of the organism was stored at -80˚C. The protocol for growth and enzyme enrichment was done according to Thapa (2018). The MRB4 was pre-cultured in Anaerobic Basal Broth (ABB) from Oxoid. Pre-culture of MRB 4 was done in ABB and stirred at 30 ˚C for 20 hours. Optical density (OD) of the culture after 20h was 0.56 when measured at 600nm. 5 mL of the pre-culture was transferred to 100 mL ABB and incubated at 37°C for 17 h to OD_{600nm} 0.55. 20% sterile cellobiose was then added to the culture to a final concentration 0.4%. Addition of cellobiose was done to activate (trigger) the production of the CMCase in MRB 4. The MRB 4 was cultured further for 3 h to final OD 0.86. The enzyme activity at that stage was typically 0.4-0.5 CMCase units per mL (Thapa, 2018).

The activated culture (85ml) was transferred to a 10 cm dialysis tubing (Spectrapor 12-14kDa), sealed in both ends and concentrated by dialysis by immersion in solid PEG (polyethylene glycol). Dialysis in PEG was carried out in cold room (4°C) for 4.5 h and the concentrate was recovered. Collected volume was 30 mL, i.e. 2.9x concentration. The concentrated culture (containing bacteria and enzyme) was stored cold until used next day in biogas experiment. The concentrated enzyme preparation was dispensed at 0.75 or 1.5 mL to selected AD reactor mixtures according to the experimental plan (DoE).

Statistical analysis

The result of biogas yield in all of the reactors were statistically analyzed by using MODDE Pro software MKS UMETRICS, Umeåa Sweden) and MS Excel version 15.26.

RESULTS

Biogas collected in reactors

Gas collected in 11 different reactors fitted with designated treatments and mixture composition when analyzed after the completion of the test showed various volume of gas collection. Among them reactors no. 6 and 8, first achieved maximum limit of gas collection i.e. 100ml. Both of the reactors were provided with same amount of 5%v/v enzyme kept in 40°C but with different particle size.

Table 2. Amount and rate of gas production

Day	Time	Hrs.	Volume (ml) of gas collected in Reactor No.										
			1	2	3	4	5	6	7	8	9	10	11
1	12:00	0	0	0	0	0	0	0	0	0	0	0	0
1	21:45	10	0	5	0	3	0	0	0	15	10	10	5
2	8:15	20	0	5	0	9	0	20	5	35	10	15	5
2	2:15	26	3	5	0	13	5	35	5	45	13	18	15
3	8:15	44	5	5	0	15	5	80	5	90	40	45	40
3	12:15	48	5	5	0	20	5	100	7	100	45	50	45
Rate (ml/hr)			0.12	0.7	0	0.39	0.124	2.17	0.14	2.12	0.93	1.05	0.99

Interaction of treatment factors

When interaction between two different parameters, independent from the third variable was graphed from main table 2, following results appeared.

Table 3. Interaction between factors

Temperature*enzyme			Temperature*particle size			Enzyme*particle size		
	20°C	**40°C**		**20°C**	**40°C**		**0%**	**5%**
0%	5	5	**<1mm**	5	5	**<1mm**	5	5
0%	0	20	**<1mm**	5	100	**<1mm**	5	100
Avg.	2.5	12.5	**Avg.**	5	52.5	**Avg.**	5	52.5
S.D.	3.5	10.6	**S.D.**	0.0	67.2	**S.D.**	0.0	67.2
5%	5	100	**>5mm**	0	20	**>5mm**	0	7
5%	7	100	**>5mm**	7	100	**>5mm**	20	100
Avg.	6	100	**Avg.**	3.5	60	**Avg.**	10	53.5
S.D.	1.4	0.0	**S.D.**	4.9	56.6	**S.D.**	14.1	65.8

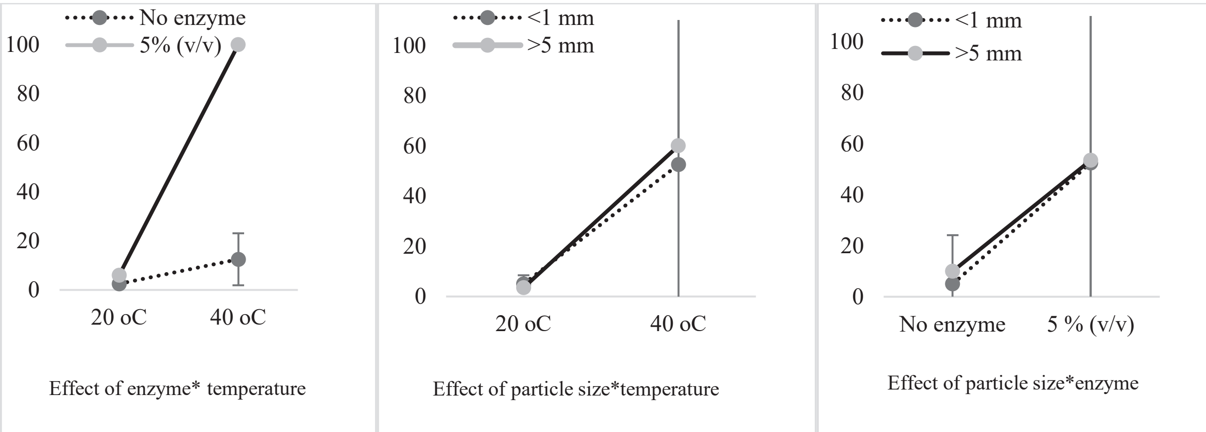

Fig. 2. Interaction plots

Temperature had a significant positive effect which was attenuated even more with the enzyme supplement. High temperature along with high dose of enzyme was found highest producing with 0 deviation from the mean (standard deviation). The contribution from particle size for gas production was not significant since the error at 40°C, SD is larger than the observed effect itself. Parallel lines signify that particle size doesn't have any contribution to biogas accumulation.

Main Effects

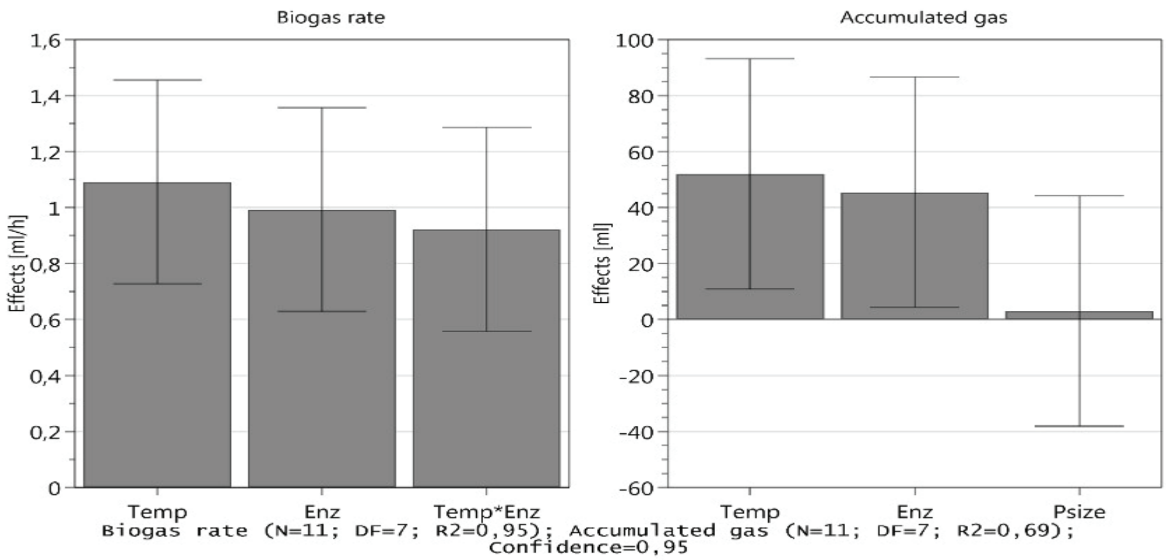

Fig. 3. Main effect plots

Bars in Fig. 3 are calculated main effects and their standard deviation (error bars). For biogas rate increased temperature and enzyme addition have significant positive effect. The Temperature*Enzyme factor also shows a positive interaction effect. Right panel also shows the same main effects of temperature and enzyme but having more error connected, although still significant. Particle size however, in this outcome, had no effect and the error is large.

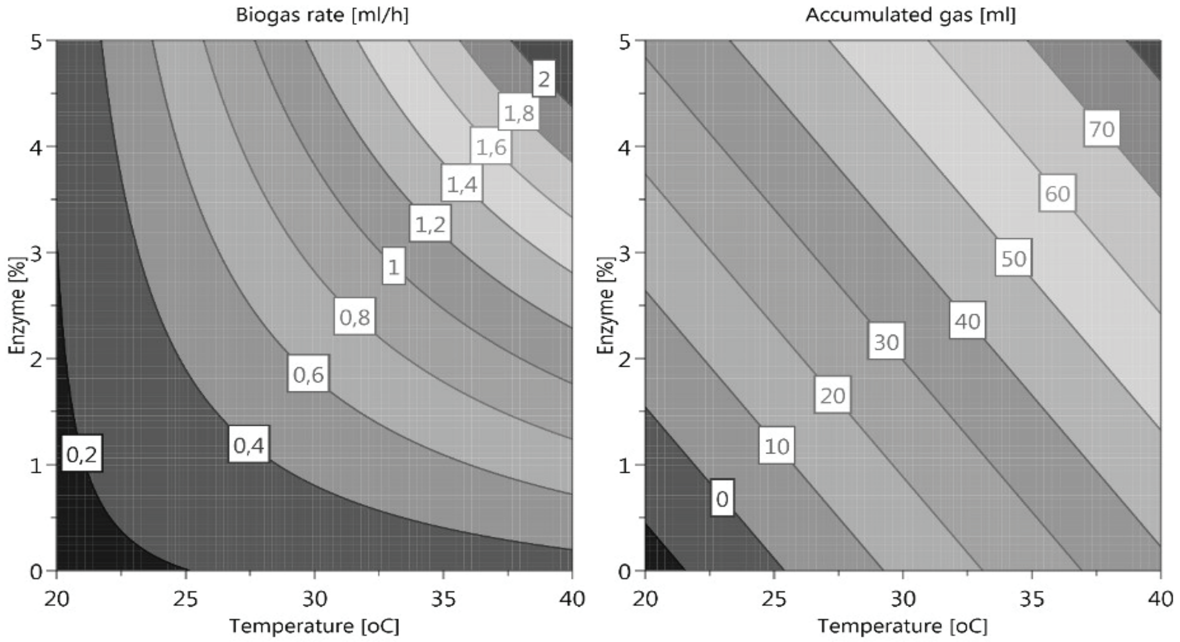

Fig. 4. Contour plot of temperature and enzyme

This is result diagram or contour plot (heat diagram). Based on the data a response surface was modelled by multilinear regression (MLR). Responses can be predicted for a given set of conditions (temperature and enzyme) shown as iso-lines or color bands. Both panels points to upper right corner as a 'hot spot' for best biogas conditions. Left panel is slightly curved, and reflects the interaction term of the two factors. Right panel have a linear form, i.e. interaction is not so pronounced. The contours surfaces are shown at particle size of 3 mm (no difference between the three size categories (contour plot, right panel)).

By the whole analysis we resulted that, for high rate of biogas production

1) Increase in temperature has positive effect
2) Increased enzyme has positive effect
3) Temperature and enzyme addition interaction has positive effect
4) Change in particle size from 1 to 5 mm has indifferent effect

DISCUSSION

Increase in temperature might cause increased microbial activity inside the digestor or could cause breaking of bonds between biomolecules of substrate . Rumen culture of moose might foster many microbiota which might have potential to breakdown complex substrate materials to release large amount of biogas the substrate. Various studies conducted by many researchers also supports our work in following ways.

Temperature effect

Increase in temperature is responsible for weaker hydrogen bonds between crystalline cellulose and the structural complexes in the biomass (Wellinger et al., 2013). Increase in temperature induce increased rate of biochemical reaction and thus increases the yield of biogas (Merlino et al. 2013). According to thumb rule, for every 10°C rise in temperature the rate of biochemical reaction will be doubled within certain limits which works in the case of anaerobic digestion process also (Jørgensen, 2009). As temperature increases, the substrate will be less viscous and have higher solubility which potentially increase the process of digestion.

Bergland et al. (2015) studied temperature effect on biochemical process of biogas production such as particle disintegration and substrate hydrolysis in a pilot experiment of 220-liter sludge bed reactor. Dairy manure when treated for 4 months in varying temperature 25°C, 30°C and 35°C resulted that temperature has 3.4 % per degree increase in rate at 25 – 30 ° C and 1.6 % per degree increase in rate at 30 – 35 °C. Similarly, Donoso et al. (2009) conducted batch test at a temperature range between 15-45°C with glucose, starch and acetic acid as substrate for acidogenesis, hydrolysis and methanogenesis respectively. The obtained result showed that temperature strongly influences all anaerobic processes with highest effect on the steps of acidogenesis. Assuming 5% decrease from operational temperature of the reactor, 50% slower kinetics of acidogenesis and 10% slower in hydrolysis rate occurred.

Enzyme effect

Same as temperature, enzyme was also found to have positive independent effect for increase biogas production. When the dose of enzyme increases it was found to increase biogas rate significantly. Same as ours, digestion of cattle manure under cellulolytic strains of mixed consortium and actinomycetes have been observed to improve biogas production in range of 8.4-44% when studied by Parawira (2012). Ishaq et al. (2015) performed fibrolytic test to examine biochemical potential of moose rumen microbiota to digest complex plant carbohydrates such as cellulose, cellobiose, xylan, starch and lignin. When 31 fibrolytic isolates were tested, 15 of them were found capable to digest all types of investigated plant components. They also suggested that those microbes have huge application to be used in agriculture and industrial sector.

Cellulose and xylan are the major constituents of lignocellulose (Rao & Li, 2017). The MRB culture have cellulases and xylanase activity (Thapa, 2018). Cellulases breaks large cellulose molecules into smaller mono or polysaccharides (Saini et al., 2015). Xylanase cleave the xylosidic linkages in xylan and reduce polymerization of biomass making it easy to degrade, thus reduced substrate yield more biogas (Saini et al., 2015).

Effect of particle size

In our work, PS does not found to have much effect on the rate and amount of gas collected. Though, many research suggested PS also have influence on gas production. Mshandete et al. (2006) from the substrate size of (2, 5, 10, 30, 50, 70 and 100mm) although found variable rate of gas production, the gas produced from 2 and 5 mm are almost equal. Large size of feedstock possesses less surface area for microbial activity and eventually could cause less methane yield and clogging of the digester (Sharma et al., 1988; Wellinger et al., 2013). Our study added that, despite a role of PS in biogas production, 1 mm to 5mm of straw substrate will yield relative same rate of biogas.

CONCLUSION

In our study, temperature and enzyme were found to have positive independent main effects in biogas production. The rate of production was even more supported when temperature and enzyme were applied in combination. It confirmed that the culture from rumen of Moose had some vital organisms which produce certain valuable substance that will eventually produce high biogas from the digester. The interaction effects showed that those organisms and/or the enzymes were reinforced with increase in temperature. Particle size did not show any significant relation to the rate of biogas production. Our study added that, despite of role of PS in biogas production, 1 mm to 5mm of straw substrate will yield relative same rate of biogas. 40°C temperature and 5%v/v enzyme application was found to have high rate of gas production among all combinations.

REFERENCES

Bayane, A., & Guiot, S. R. (2011). Animal digestive strategies versus anaerobic digestion bioprocesses for biogas production from lignocellulosic biomass. *Reviews in Environmental Science and Bio/Technology, 10*(1), 43–62. https://doi.org/10.1007/s11157-011-9242-6

Bergland, W. H., Dinamarca, C., & Bakke, R. (2015). Temperature effects in anaerobic digestion modeling. *Linköping Electronic Conference Proceedings*, 261–269.

Donoso-Bravo, A., Retamal, C., Carballa, M., Ruiz-Filippi, G., & Chamy, R. (2009). Influence of temperature on the hydrolysis, acidogenesis and methanogenesis in mesophilic anaerobic digestion: Parameter identification and modeling application. *Water Science and Technology, 60*(1), 9–17. https://doi.org/10.2166/wst.2009.332

Earth System Research Laboratory. (2018). Trends in atmospheric carbon dioxide. *Global Monitoring Division*. Retrieved from https://www.esrl.noaa.gov/gmd/ccgg/trends/global.html

Ishaq, S. L., Kim, C. J., Reis, D., & Wright, A. D. G. (2015). Fibrolytic bacteria isolated from the rumen of North American moose (*Alces alces*) and their use as a probiotic in neonatal lambs. *PLOS ONE, 10*(12), e0144804. https://doi.org/10.1371/journal.pone.0144804

Ishaq, S. L., & Wright, A. D. G. (2012). Insight into the bacterial gut microbiome of the North American moose (*Alces alces*). *BMC Microbiology, 12*, 212. https://doi.org/10.1186/1471-2180-12-212

Jørgensen, P. J. (2009). *Biogas—Green energy*. Faculty of Agricultural Sciences, Aarhus University. Retrieved from http://www.lemvigbiogas.com/BiogasPJJuk.pdf

McKendry, P. (2002). Energy production from biomass (part 2): Conversion technologies. *Bioresource Technology, 83*(1), 47–54. https://doi.org/10.1016/S0960-8524(01)00119-5

Merlino, G., Rizzi, A., Schievano, A., Tenca, A., Scaglia, B., Oberti, R., Adani, F., & Daffonchio, D. (2013). Microbial community structure and dynamics in two-stage vs single-stage thermophilic anaerobic digestion of mixed swine slurry and market bio-waste. *Water Research, 47*(6), 1983–1995. https://doi.org/10.1016/j.watres.2013.01.017

Mshandete, A., Björnsson, L., Kivaisi, A. K., Rubindamayugi, M. S., & Mattiasson, B. (2006). Effect of particle size on biogas yield from sisal fibre waste. *Renewable Energy, 31*(14), 2385–2392. https://doi.org/10.1016/j.renene.2005.10.013

Parawira, W. (2012). Enzyme research and applications in biotechnological intensification of biogas production. *Critical Reviews in Biotechnology, 32*(2), 172–186. https://doi.org/10.3109/07388551.2011.595384

Rao, M. P. N., & Li, W. J. (2017). Microbial cellulase and xylanase: Their sources and applications. In *Advances in Biochemistry & Applications in Medicine* (Chapter 4).

Saini, J. K., Saini, R., & Tewari, L. (2015). Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: Concepts and recent developments. *3 Biotech, 5*(4), 337–353. https://doi.org/10.1007/s13205-014-0246-5

Schnürer, A., & Jarvis, A. (2010). *Microbiological handbook for biogas plants*. Swedish Waste Management, Swedish Gas Centre Report 207.

Sharma, S. K., Mishra, I., Sharma, M. P., & Saini, J. (1988). Effect of particle size on biogas generation from biomass residues. *Biomass, 17*(4), 251–263. https://doi.org/10.1016/0144-4565(88)90018-1

Thapa, D. S. (2018). *Cultivation conditions for a moose (Alces alces) rumen bacterial isolate secreting cellulases* (Master's thesis). Inland Norway University of Applied Sciences, Hamar, p. 66.

Ward, A. J., Hobbs, P. J., Holliman, P. J., & Jones, D. L. (2008). Optimisation of the anaerobic digestion of agricultural resources. *Bioresource Technology, 99*(17), 7928–7940. https://doi.org/10.1016/j.biortech.2008.02.044

Wellinger, A., Murphy, J. D., & Baxter, D. (Eds.). (2013). *The biogas handbook: Science, production and applications*. Elsevier.

World Health Organization. (2018). *Household air pollution and health*. Retrieved from http://www.who.int/en/news-room/fact-sheets/detail/ambient-(outdoor)-air-quality-and-health