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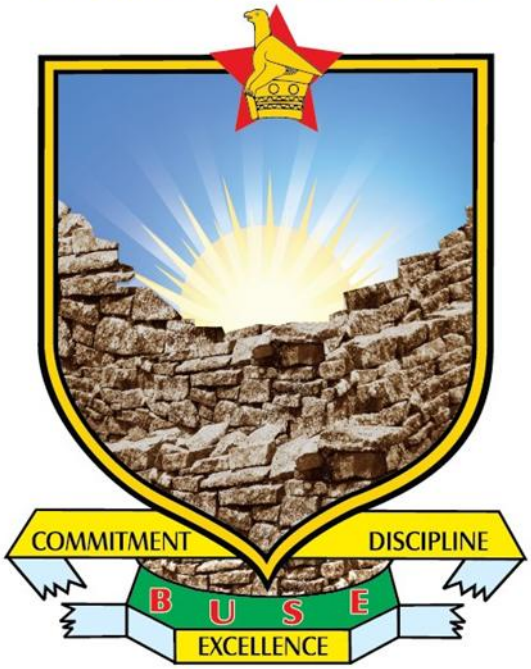

FACULTY OF SCIENCE

BIOLOGICAL SCIENCES DEPARTMENT

INFLUENCE OF PLASTIC CONTAMINANTS ON BIOGAS PRODUCTION FROM COW DUNG

BY

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B224161B

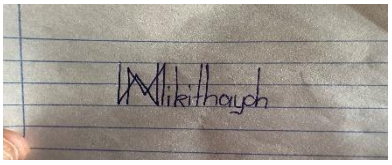
A RESEARCH PROJECT SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE BACHELOR OF SCIENCE HONORS DEGREE IN BIOTECHNOLOGY.

JUNE 2026

APPROVAL FORM

Title of research project: **"INFLUENCE OF PLASTIC CONTAMINANTS ON BIOGAS PRODUCTION FROM COW DUNG"**

The undersigned certify that they have read the research project and it is suitable for submission to the Faculty of Science, and checked for conformity with the faculty.

Signature of student  Date: 15/06/26

Signature of Supervisor:  Date: 15/06/26

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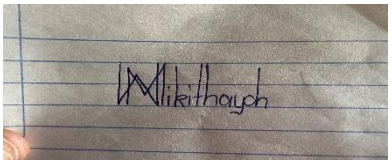
DECLARATION

I Whitney Mikithayo, confirm that the work I submitted for assessment is entirely original and written by me. It properly acknowledges other authors' works when they are included into it. There is a complete list of references used.

Signature of student  Date: 15/06/26

I, J. Ndava declare that I have supervised this thesis and I am satisfied that it can be submitted to the Biological Sciences Department, Faculty of Science and Engineering, at Bindura University of Science Education.

Signature:  Date: 15/06/26

DEDICATION

This project is dedicated to my husband and my dear mom Marjory, who have always supported, encouraged, and guided me through challenging times.

ACKNOWLEDGEMENTS

I want to start by expressing my appreciation to my academic mentor, Mr J. Ndava, for his perceptive observations, priceless suggestions, helpful information, practical guidance, and never-ending ideas, all of which were very helpful to me during my research. I also appreciate the help I received from the lecturers at the department of biological sciences.

I also want to thank my husband, my sister and my mom Marjory from the bottom of my heart. My strength came from their continuous support and encouragement. I'm grateful to each and every one of my friends and classmates for their assistance, encouragement, and counsel during my research. Finally, I'm grateful to God for loving me excessively.

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LIST OF ABBREVIATIONS

AD	Anaerobic Digestion
C/N ratio	Carbon: Nitrogen
HCL	Hydrochloric Acid
LDPE	Low Density Polyethylene
LPG	Liquefied Petroleum Gas
MPs	Micro-plastics
PP	Polypropylene
TS	Total Solids
VFA	Volatile Fatty Acids
VS	Volatile Solids

ABSTRACT

Anaerobic digestion is widely used for renewable energy production, with cow dung serving as a common and easily available substrate for biogas generation. However, increasing plastic pollution, especially from low-density polyethylene (LDPE), polypropylene (PP), and polyvinyl chloride (PVC), has raised concerns about its effects on ecosystems and biological processes. Previous studies have shown that contaminants in feedstock can negatively affect methane production during anaerobic digestion. Therefore, understanding the effect of plastic contamination on cow dung digestion is important for improving biogas production efficiency. This study investigated the influence of plastic contaminants on biogas production from cow dung by examining changes in pH, temperature sensitivity, digestion retention time, methane concentration, and cumulative gas production. A laboratory scale anaerobic digestion experiment was conducted to investigate the influence of plastic contaminants, specifically low-density polyethylene and polypropylene, on biogas production from cow dung. Fresh cow dung was collected and mixed with water to prepare a slurry in a 1:1 ratio. The slurry was divided into different treatment groups containing varying concentrations (5% and 10%), while the control sample contained cow dung with no plastic material. The digesters were sealed and maintained under anaerobic conditions at ambient temperature for a retention period of 30-46days. Parameters monitored during the experiment included pH, temperature, daily biogas volume, cumulative gas production, and methane concentration. Biogas production was measured using water displacement method, while methane concentration was determined using gas analysis methods. The collected data were compared to evaluate the effects of LDPE and PP contamination on the efficiency of AD and methane yield. The results showed that plastic contamination significantly reduced both the rate and total volume of biogas produced. Compared to the control sample, cumulative gas production decreased by 22% at 5% plastic contamination and 71% at 10% plastic contamination. Methane concentration also decreased from 62% in the control sample to 41% in contaminated samples, and digestion retention time increased from 30 days in the control to 46 days in slurries containing microplastics. The reduction in biogas yields may be due to the inhibition of methanogenic microorganisms by plastic particles, reduced mass transfer, and the release of plastic additives such as phthalates, which interfere with methane formation. These findings demonstrate that's microplastics negatively affect anaerobic digestion process. To maintain efficient biogas production, feedstocks should be screened for plastic contamination, and improved waste segregation and pre-treatment methods should be adopted. Future studies should investigate the long-term effects of aged microplastics and additive leeching on anaerobic digestion performance.

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CHAPTER 1

1.1 BACKGROUND OF THE STUDY

Anaerobic digestion is a biological process in which microorganisms decompose biodegradable material in the absence of oxygen (O_2). During this process organic waste such as cow dung is converted into biogas, a renewable energy source mainly composed of methane (CH_4), and digestate, a nutrient-rich by-product that can be used as fertilizer and soil conditioner. The anaerobic digestion involves a diverse community of microorganisms which include hydrolytic bacteria, acidogens, acetogens and methanogens (a group of *Archaea*). These microorganisms work together to break down complex organic matter in four main stages, namely, hydrolysis, acidogenesis, acetogenesis and methanogenesis. The stability and efficiency of these microbial communities are essential for achieving optimal methane production (Wang et al., 2021).

However, the increasing contamination of agricultural ecosystems with micro-plastics such as polypropylene (PP) and low-density polyethylene (LDPE), poses a potential environmental threat (Qin et al., 2020). These microplastics can enter livestock systems through contaminated feed and forage, for example, when small fragments of plastic silage wraps, bale nets or sheets break off and mix with animal feed. They may also be introduced through water contaminated by urban runoff, wastewater treatment, plant effluent and environmental deposition. In addition, soil acts as a major reservoir for microplastics, allowing them to accumulate in agricultural environments and eventually be excreted in animal manure (Wei et al.,2019).

Physicochemical analysis showed that MPs enhanced the hydrolysis of organic matter hydrolysis on day four of anaerobic digestion, resulting in the accumulation of volatile fatty acids in the initial stages of the process. Microplastics were also found to limit methane gas production by reducing acetate kinase activity while increasing the release of lactate dehydrogenase release from micro-organisms involved in the anaerobic digestation process (Rojas-Solorzano et al.,2022).

Recent studies have shown that microplastics can accumulate within digesters and affect anaerobic digestion. However, their specific effects on the methanogenesis stage and on the microbial community structure in cow dung digesters remain poorly understood and findings remain inconsistently reported (Wei et al., 2019). Therefore, this study aims to address this knowledge gap by systemically investigating the effects of commonly occurring microplastics on the microbial ecosystem responsible for methane production from cow dung.

1.2 PROBLEM STATEMENT

The increasing contamination of cow dung with microplastics from agricultural practices has become an emerging challenge for biogas production through anaerobic digestion. Different types of microplastics, possess varying chemical compositions, surface properties and potential for leeching, which may affect the microbial processes involved in governing methanogenesis is fragmented (Weithman et al.,2018). However, current knowledge on the effects of these microplastics on anaerobic digestion remains limited and fragmented.

Without a systematic investigation, it is difficult to determine whether microplastics inhibit biogas production, destabilize the digestion process or alter the microbial communities within ecology of digesters. These effects may result in reduced efficiency, economic losses and negative environmental impacts. Therefore, this study aims to address this knowledge gap by assessing the effects of different on methanogenesis of cow dung.

1.3 JUSTIFICATION

This study is important because it will help improve understanding of how plastic contaminants affect biogas production frow cow dung during anaerobic digestion. The increasing presence of plastics in agricultural and organic waste has become an environmental concern, and their effects on renewable energy systems need to be investigated. This study will contribute to environmental monitoring and management by providing information on the impact of plastic pollution on biogas production processes. The findings may support the development of better waste management practices and policies related to the use and disposal of agricultural plastics.

The research will also have economic and operational importance for the biogas industry as information obtained from the study can help biogas plant operators identify risks associated with MPs contaminated feedstocks and develop strategies to maintain stable methane production and efficient plant operation. Finally, the study will provide a foundation for future research by identifying the types of plastics that have the greatest effect on biogas production. The findings may encourage further studies on methods to reduce or prevent the negative effects of microplastics, including the development of suitable treatment and mitigation strategies.

1.4 AIM OF THE STUDY

To investigate the influence of polypropylene (PP) and low-density polyethylene (LDPE) plastic contaminants on anaerobic digestion of cow dung and their effects on methane production efficiency.

1.5.1 OBJECTIVES

1.To determine the influence of MPs on biogas yield under varying ambient temperature conditions.

2.To quantify volatile fatty acids (VFAs) accumulation during AD process

3.Assess the effect of plastic contamination on pH stability.

4.To compare cumulative biogas production in the control experiment and the contaminated jars with MPs.

1.5.2 RESEARCH QUESTIONS

1. Do MPs influence biogas yield under varying ambient temperature conditions?
2. To what extent do plastic contaminants cause accumulation of VFA and how the buildup correlate with methane production?

3. How does the presence of MPs affect pH stability during AD of cow dung?
4. How does cumulative production over a fixed retention period change across different contamination concentrations (control 0%,5%,10%)?

1.6 HYPOTHESIS

H_0 (Null Hypothesis): Diverse micro-plastics (PP, LDPE, PHA) at tested concentrations has no significant effect on the methane yield, process stability or microbial community in the anaerobic digestion of cow dung.

H_1 (Alternative Hypothesis): The addition of diverse micro-plastics will cause a significant, polymer-specific reduction in methane yield and process stability, including distinct structural shifts in the microbial community characterized by a decline in the abundance and diversity of methanogenic archaea.

1.7 RESEARCH DESIGN

The study will employ a completely randomized experimental design with a factorial arrangement of treatment.

- Statistical Analysis:
  *Data collection of biogas volume per 5days and calculation of the mean
  *Descriptive statistics to calculate mean SD of cumulative biogas
  *For visualization - graphs
  *Hypothesis testing use one way -ANOVA to compare cumulative biogas production or methane yield from digester against several test digesters with different concentrations.
  *Correlation/Regression analysis to see if there is linear relationship between plastic concentration and cumulative biogas yield
  *Presenting the means, standard deviations, ANOVA F-statistics and p value and the results in the appendix section

1.8 SIGNIFICANCE OF STUDY

Quantifies how microplastic contaminants disrupt critical anaerobic digestion parameters such as pH shifts, ambient temperature sensitivity, VFA accumulation and cumulative gas reduction thereby providing evidence-based thresholds for effects of plastic contaminants on biogas production from cow dung.

1.9.1 DELIMITATIONS

- The study will only focus on the anaerobic digestion of cow dung only, other organic wastes are not within the scope of this investigation.
- The investigation focuses on archaea and bacteria and does not include other microbial groups like fungi or protozoa.
- The experimental scope is limited to the effects of the physical presence and basic polymer chemistry of MPs, a detailed analysis of leachate chemistry.

1.9.2 LIMITATIONS

- The study uses batch assays, which may not fully capture the long-term continuous effects of MPs and potential microbial adaptation that could occur in a CSTR system.
- The results are limited to the tested MPS of a defined size and may not be directly be used to other polymer types, shapes.
- The molecular analysis identifies taxonomic changes but does not directly measure metabolic activity or gene expression.

1.9.3 ASSUMPTIONS

- Anaerobic cow dung used as inoculum contains a healthy diverse consortium of microbes capable of carrying out digestion.
- Plastics used are representative of common contaminants found in livestock and their chemical composition is known and consistent.

CHAPTER 2: LITERATURE REVIEW

2.1. HISTORY OF BIOGAS

Around 1600, the discovery of an unidentified combustible liquid in swampy areas led to the discovery of the decomposition of organic waste and the emergence of biogas (Klass, 1984). Scientist Thomas Shirley made the discovery in the UK in 1667. Italian physicis, a century after the composition was discovered determined it to be methane. Louis Pasteur makes use of this gas as fuel and for heisting and lighting systems. With anaerobic fermentation, he and his pupil Uyssey Gayon produced hundred litres of gas per cubic meter of materials which was manure and water at 350degrees celcius (Van Lier, 1997). Biogas was already being used for lighting during this time in India.

2.2. INTRODUCTION

Anaerobic Digestion (AD) is a crucial biological process whereby microorganisms break down biodegradable material in the absence of oxygen (O_2), converting the organic wastes like cow dung into biogas, a renewable energy source primarily composed of methane (CH_4), carbon dioxide (CO_2) and digestate. Cow dung is an ideal substrate for AD due to its rich microbial consortium and balanced carbon-to-nitrogen (C/N) ratio. The resultant digestate also serves as a nutrient-rich bio-fertilizer, promoting a circular economy (Surendra et al., 2014).

The process of anaerobic digestion simultaneously addresses waste management challenges and contributes to renewable energy portfolios.AD of cow dung can yield biogas with a methane content ranging 50%-75% making it a viable energy source. Under optimal conditions methane yield from cow dung can significantly be enhanced with studies reporting values as high as 192.53mL/gVS with 89% methane content.

However, the efficiency of this process is increasingly threatened by the emergence of plastic contaminants and their improper disposal into the environment particularly micro-plastics (MPs), defined as plastic particles smaller than 5mm.In agricultural practices, the pervasive presence of

MPs from sources like silage wraps, feedbags and general litter in organic wastes (livestock manure), has become a global environmental concern. Cattle manure has been found to contain significant levels of micro-plastics with one study detecting an average of 74particles per kilogram of wet matter. These contaminants enter anaerobic digesters alongside feedstock, introducing a complex set of biochemical challenges that can disrupt microbial processes, suppress methane yields and ultimately compromise the viability of biogas as a renewable energy source. These plastics, especially conventional non-biodegradable types, can introduce physical and chemical challenges to the AD process (Wei et al.,2019).

This literature review explores current research on the influence of plastic contaminants on the anaerobic digestion of cow dung, examining the mechanisms of impact, the differential effects of plastic types, and the implications for both biogas yield and digestate quality, as well as identifying gaps for further study.

2.3 THE ANAEROBIC DIGESTION (AD) PROCESS

AD is a complex biochemical process involving four key stages:

1. Hydrolysis: Complex organic polymers (carbohydrates, proteins, fats) are broken down into soluble monomers by hydrolytic enzymes.
2. Acidogenesis: Acidogenic bacteria convert these monomers into volatile fatty acids (VFAs), alcohols, ammonia, CO_2, and hydrogen sulfide.
3. Acetogenesis: The products of acidogenesis are further digested by acetogens to produce acetic acid, hydrogen, and CO_2.
4. Methanogenesis: Methanogenic archaea utilize acetic acid or CO_2 and H_2 to produce methane**.**

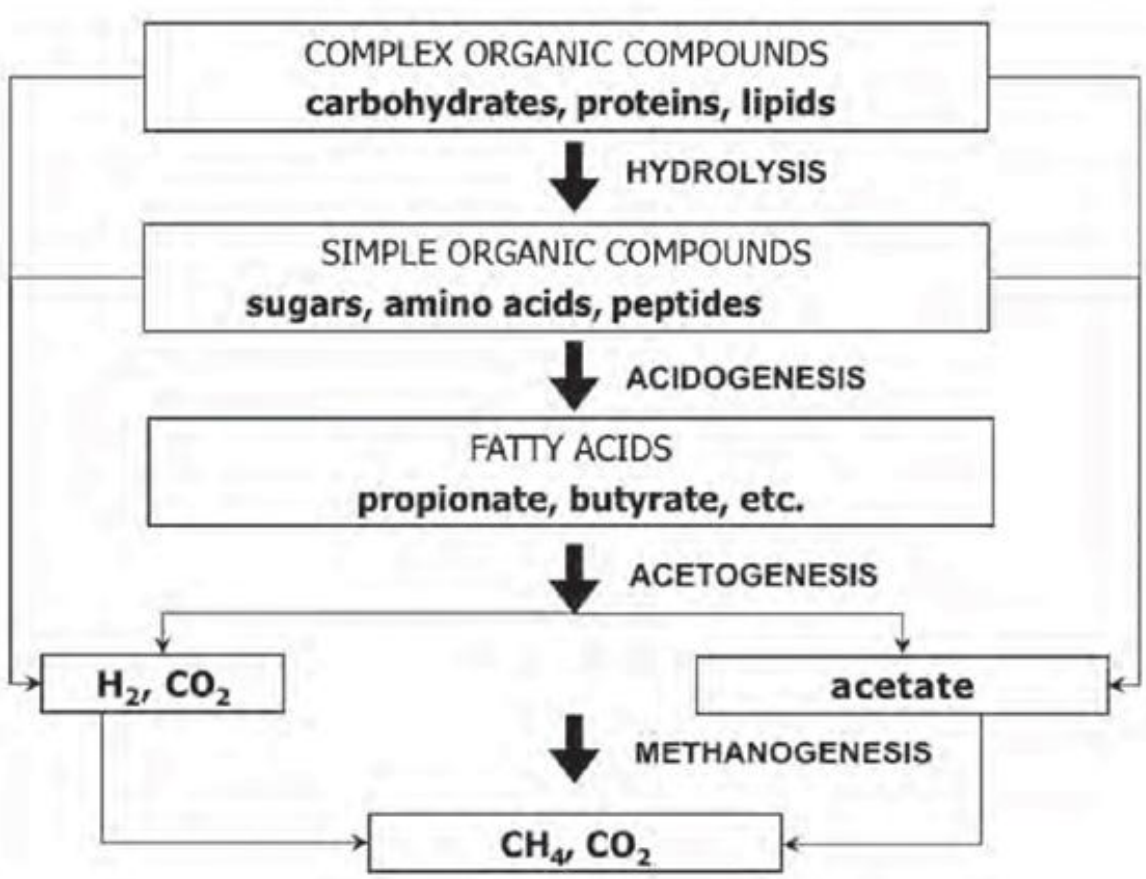

FIGURE1. Flow chart of anaerobic digestion process

The stability of this microbial consortium is crucial for efficient biogas production. Any disruption to these microbial populations or the enzymatic hydrolysis step can significantly hinder the entire process.

2.4 COW DUNG AS A SUBSTRATE FOR BIOGAS PRODUCTION

2.4.1 COMPOSITION AND CHARACTERISTICS OF COW DUNG

- Organic matter content: Cow dung contains 15-20% organic matter, cellulose (25-40%), hemicellulose (15-20%), lignin (10-15%), proteins, and lipids.
- Nutrient profile: Rich in carbon (C) and nitrogen (n) with a C:N ratio of 20:1 to 30:1 which is ideal for anaerobic digestion.

- pH range: Fresh cow dung has a pH range of 6,5-7,5, suitable for methanogen activity
- Moisture and solids: Has typically 80-85% moisture, 15-20% total solids (TS), with volatile solids (VS) compromising 80-85% of TS indicating high biodegradable potential.

2.4.2 MICROBIAL PROPERTIES OF COW DUNG

- Diverse anaerobic consortium: Naturally harbors hydrolytic, acidogenic, acetogenic and methanogenic microbes.
- Hydrolytic bacteria: for example, *Clostridium*, break down cellulose, hemicellulose and proteins into sugars, amino acids and fatty acids.
- Acidogens and Acetogens: Convert intermediates to acetate, H_2 and CO_2.
- Methanogens: Present in fresh dung due to the ruminant gut environment, reducing need for external inoculum.
- Enzyme activity: High cellulase, protease, and lipase activity accelerates initial hydrolysis steps.

2.4.3 SUITABILITY OF COW DUNG FOR ANAEROBIC DIGESTION

- Ready inoculum source: Contains methanogenic consortia, enabling self-starting digesters without external seeding.
- Balanced nutrients: Adequate trace elements (Fe, Ni, Mo) essential for enzyme cofactors in methanogenesis.
- Good buffering and low ammonia toxicity: Ammonia concentration typically below inhibitory levels preventing process failure.
- Wide operating range: Performs well at mesophilic (30-40^0 C) and thermophilic (50-60^0 C) temperatures with stable pH (6,8-7,5).

2.4.4 BIOGAS YIELD POTENTIAL OF COW DUNG

- Practical laboratory or field yield: Biogas yield 0.36m^3 /kg VS (50-65% methane)
- Factors enhancing yield: Pre-treatment (mechanical, thermal, or alkaline) can increase yield by 15-40%, co-digestion with energy crops or food wastes boosts methane yield.

- Impact of contaminants: Plastic contaminants physically block mixing, reduce substrate - microbe contact, and may leach toxic additives (phthalates) that inhibit methanogenesis (can lower biogas yield by 20-50%).

2.5. PATHWAYS OF PLASTIC CONTAMINATION IN COW DUNG

Recent surveys and analytical research methods report that livestock manure frequently contains micro-plastics and larger plastic debris. Plastics enter the AD feedstock primarily through:

- Silage Baling Films: These are widely used in modern dairy and beef farming to preserve fodder. Residual fragments often mix with the feed and appear in the manure.
- Feed Sacks and Packaging: Plastic bags used for concentrated feed supplements can be accidentally incorporated into feed or manure collection systems.
- Environmental Litter: Plastics from other agricultural activities or wind-blown debris can contaminate manure stockpiles.

  The most common plastics identified in these contexts include polyvinyl chloride (PVC), polyethylene (PE), polypropylene (PP), and PHA (Wei et al., 2019).

2.6. MECHANISMS OF PLASTIC INFLUENCE ON ANAEROBIC DIGESTION

The impact of plastics on AD is diverse, involving physical, chemical, and biological mechanisms.

2.6.1. PHYSICAL INHIBITION AND SYSTEM DISRUPTION

- Low-density plastics like LDPE films float and entrap other solids, forming a thick, impermeable scum layer on the surface of the digester. This crust hinders the release of biogas, causes pressure build-up, and creates stratification, preventing uniform mixing and heat transfer (Ariunbaatar et al., 2014).
- Long plastic strands or sheets can wrap around mixing impellers, clog pipes, valves, and pumps, leading to mechanical failures, increased maintenance costs, and operational downtime.

- The accumulation of non-degradable plastics physically reduces the working volume available for active digestion.

2.6.2. CHEMICAL INHIBITION AND TOXICITY

- Leaching of Additives: Many plastics contain chemical additives like plasticizers (e.g., phthalates), stabilizers, flame retardants, and colorants. Under the anaerobic conditions and varying pH of a digester, these compounds can leach out and exert toxic effects on hydrolytic and methanogenic microbes. Studies have shown that certain phthalates can inhibit methanogenesis, a critical step for methane formation (Li et al., 2015).
- MPs have a high surface area-to-volume ratio and can act as vectors for other environmental pollutants, such as heavy metals and persistent organic pollutants, introducing additional toxicants into the digester ecosystem (Wei et al., 2019).

2.6.3. BIOLOGICAL INTERFERENCE

- Inhibition of Hydrolysis: The hydrolysis of particulate organic matter is a rate-limiting step in AD. The presence of a physical plastic barrier can limit the contact between hydrolytic enzymes and their substrates (cow dung particles), thereby slowing down the initial breakdown process.
- Microbial Toxicity: Beyond additives, the plastic polymers themselves, especially as they degrade into smaller particles (nano-plastics), can be internalized by microbial cells, causing oxidative stress, membrane damage, and disruption of metabolic pathways (Seeley et al., 2020)

2.6.4. MICROBIAL COMMUNITY AND ENZYMATIC EFFECTS

MPs may physically interact with microbial cells, damage cell membranes, alter extracellular enzyme activity and shift community composition, thereby changing all the four processes of AD kinetics.

2.7. IMPACTS BASED ON PLASTIC TYPE

The influence of plastics is not uniform; it varies significantly with the polymer type and its properties.

- Conventional Plastics (PE, PP, PET): The literature consistently reports negative or neutral effects. A study by Wei et al. (2019) found that PE and PET MPs at high concentrations (over 100 items/g TS) could decrease methane production by up to 30% in batch digestion tests, primarily due to the induced oxidative stress on methanogens.
- Biodegradable Plastics (e.g., Polylactic Acid - PLA, Polyhydroxyalkanoates - PHA): The impact is more complex. In theory, they should degrade alongside organic waste. However, their hydrolysis rate in mesophilic AD (common for cow dung digesters) is often much slower than the retention time of the digester. This can lead to incomplete degradation, where the plastic fragments but does not fully mineralize, potentially exacerbating the microplastic problem in the digestate. Some studies report that the rapid acidogenesis of certain biodegradable plastics can lead to VFA accumulation and a drop in pH, inhibiting the sensitive methanogenic phase (Ariunbaatar et al., 2014).

2.7. REVIEW OF PREVIOUS STUDIES ON BIOGAS PRODUCTION FROM COW DUNG

2.7.1. CONTAMINATED FEEDSTOCKS IN ANAEROBIC DIGESTION

- Feedstock contamination is the introduction of non-biodegradable or inhibitory organic compounds into digesters.
- Types of contaminants studied: Microplastics like LDPE, PP, PVC, heavy metals for example Cu, Zn, Cd, antibiotics like tetracycline and sulfonamides and plastic additives like phthalates.
- Previous findings: (Wei et al., 2019) found that microplastics showed synergistic inhibition of anaerobic digestion of cow dung, (Mitchell et al.,2013) found that veterinary drug residues in cow dung lowered biogas yield by 25% and extended lag phase, (Alatriste-Mondragon et al.,2006) found that heavy metals in mature-based digesters caused methane reduction depending on concentration

2.7.2 PLASTIC WASTE EFFECTS ON MICROBIAL ACTIVITY

- Physical interference mechanisms: surface fouling in which plastic films coat microbial cells, blocking nutrient uptake and gas exchange, aggregate disruption in which plastic shreds break apart syntrophic bacterial-methanogen consortia, scum layer formation in which floating plastics trap biomass, causing washout and reducing active volume.

Chemical /toxicity mechanisms: leachate toxicity in which plastic additives like phthalates inhibit key enzymes, coenzyme M reductase and acetate kinase, oxidative stress in which microplastic leachates generate reactive oxygen species thereby damaging DNA and cell membranes of anaerobes.

- Key microbial groups affected: highly sensitive methanogens, moderately sensitive hydrolyzers, and tolerant but still impacted *Bacterioidetes*.

2.7.3 MICROPLASTICS IN BIODIGESTERS AND PREVIOUS STUDIES

- Sources of microplastics in cow dung-based systems: plastic silage wrap fragments, feed bag fibers eaten by cattle, grazing on pastures with plastic mulch residue or roadside litter, mixing with plastic waste during dung collection or storage.
- Types most studied: PE (low and high density, is the most common type), PP, PVC, PES, PS
- Previous studies findings;
    1. Li et al., (2024) did a real field survey on cow dung digesters in rural India which contained 2000-15000 microplastic particles/kg slurry, higher MPs correlated with 40-55% lower methane yields.
    2. Su et al., (2022) found out that PE microplastics inhibited hydrolysis phase, cellulase enzyme activity dropped by 45%.
    3. Wang et al., (2022) found out that PE in cow dung digesters lowered methanogen activity by 50% and increased VFAs accumulation, indicating acetogenesis inhibition.
    4. Luo et al., (2021) found out that PVC and LDPE microplastics reduced methane production in anaerobic sludge, decreased methanogenic bacteria by 80%.

2.8. MITIGATION STRATEGIES AND RESEARCH GAPS

Addressing this issue requires a multi-pronged approach:

- Pre-Digestion Separation: Implementing robust mechanical separation (e.g., sieves, shredders, and sorters) is the most effective first step to remove macro-plastics from cow dung feedstock.
- Policy and Awareness: Encouraging reduced plastic use on farms and promoting proper disposal of agricultural plastics.
- Development of Truly Anaerobic-Digestible Plastics: Research is needed to develop plastics whose degradation kinetics are synchronized with standard AD retention times.

  Key research gaps include:
- Long-term studies on the impact of real-world, mixed-plastic contamination in continuous-flow, farm-scale digesters, long term effects of mixed plastic types, biodegradation of plastics within digesters.
- The specific toxicological thresholds of different plastic leachates on key microbial populations.
- The fate and transport of nano-plastics generated during AD.

2.9. CONCLUSION

The pervasive contamination of cow dung with plastic waste poses a significant threat to the sustainability of biogas production. Evidence from the literature indicates that plastics, through physical disruption, chemical leaching, and biological inhibition, can reduce biogas and methane yields, cause operational failures, and compromise the quality of the digestate. While conventional plastics are largely inhibitory, so-called biodegradable plastics are not a panacea and may introduce new challenges. To safeguard the environmental and economic benefits of anaerobic digestion, it is imperative to implement stringent pre-processing measures to remove plastic contaminants and to foster innovations in both plastic material science and digester design. The success of biogas technology as a clean energy solution is intrinsically linked to effective waste management upstream.

CHAPTER 3

3.1 STUDY AREA

The research was conducted in Bindura. Fresh cow manure was collected at Chipindura High School. Laboratory work was done in the biology and chemistry lab at Astra campus, Bindura University of Science education. The study adopted a completely randomized design (CRD) to evaluate the impact of different types and concentrations of plastic contaminants on biogas yield, the experiment's control group with fresh cow dung only and the treatment groups with varying plastic type concentrations. All experimental biogas digester units are assigned to different treatments (plastic concentration), with treatment groups 0, 5 and 10% plastic contamination. One digester result does not influence the other.

3.2.1 SAMPLE COLLECTION

Fresh cow dung was collected from Chipindura High School in Bindura. Throughout the sample collection process random sampling technique was utilized to choose the sample participants. Fresh cow manure was collected in the morning from the paddocks where the cows rest overnight, samples were transported immediately to the laboratory in sterile, airtight, insulated and pre-chilled 20 litres containers with icepacks in zip-lock bags and stored at 4^0C to stop microbial activity before use. The total solids and volatile solids of the fresh dung were determined within twenty-four hours of collection, in which to find total solids one has to dry the cow dung in an oven at 105 degrees until constant weight is reached and cool in desiccator and for volatile solids burning the already dried sample at 550degrees and cool off.

3.2.2 PLASTIC CONTAMINANTS COLLECTION AND PREPARATION

Post-consumer plastic waste was collected from a local dumpsite, focussing mainly on two common polymer types, low density polyethylene (LDPE) from carrier bags and polypropelene (PP) from rigid packaging. The plastics were thoroughly washed with distilled water, air –dried and shredded to give small surface area.

3.3 SUBSTRATE AND INOCULUM PREPARATION

3.3.1 SUBSTRATE PREPARATION

The fresh cow dung was inter-grated and diluted with distilled water to achieve a target total of 8% concentration of total solids, a standard for wet anaerobic digestion. The pre-weighed and shredded plastic particles were mixed thoroughly with the dung water slurry. The control group received no plastic addition.

3.3.2 INOCULUM

To ensure a sturdy start to the anaerobic digestion process, a 10% inoculum of active anaerobic sludge was added to each bioreactor, the sludge was sourced from a functioning municipal wastewater treatment plant's anaerobic digester, providing a diverse and active microbial community.

3.4 ANAEROBIC DIGESTION

Bioreactors were assembled. A bioreactor tank, a gas collection device, and connection tubes that linked the tank and the gas collection unit were all included in the design. The bioreactors were 5 litres polypropylene containers. Five digestion tanks were used in the first experiment, with one serving as control. Cow dung slurry alone served as the control. The final four reactors were utilized with various substrate ratios. Cow dung and micro-plastics were combined in one reactor, one litre of each being added to another litre of the latter. The second mixture contained one part cow dung slurry and two parts micro-plastics, yielding a two-litre mixture. In the final reactor, two litres of cow dung slurry were made by combining one litre of micro-plastics with two litres of cow dung slurry. The volume was the same for all situations—two litres. The 1:2 and 2:1 ratio were calculated. There were labels on every reactor.

To prevent leakage, the digesting tanks were tightly shut. On the lids' tops, holes were bored through which tubes for gas recovery were attached. To stop any leaks, epoxy sealer was applied on top. In most cases, air leakage occurs from an object's interior to its exterior. To check for leaks, the bubble testing, an air leak test method was utilized. The researcher was keeping an eye out for bubbles brought by escaping air or gas while the tanks were submerged in water. Some containers had leaks; therefore, they were sealed again with sealant until no bubbles were visible. The gas recovery unit was connected to the digestion tanks.



FIGURE 1: Lab scale setups of the bio digestion tanks

The downward water displacement technique was used to collect gas. This technique involved inverting a one-litre measuring cylinder full of water in a plaster beaker to create the gas recovery unit. The measuring cylinder in the beaker was then turned upside down, and the connection tubing was placed inside. The measuring cylinder in the beaker was then turned upside down, and the connection tubing was placed inside. The water in the measuring cylinder was replaced by the gas as it was being created in the digestion tank and departed through the tubing. The amount of water that the gas displaced was used to calculate the volume of the gas. Thirty days were given for the tanks to ferment. For thirty days, the amount of gas produced was measured every twenty-four hours. On top of a wooden board is where the digestion tanks were stored. All of the above prepared conditions and controls were duplicated twice in different digesters for the second experiment. During the second experiment's pH and volatile fatty acid monitoring, each of the five conditions was created in a volume of 1 litre. Also, these were allowed to ferment for thirty days. The volatile fatty acids and pH levels were monitored daily throughout the duration of the fermentation process.

3.5 BIOCHEMICAL TESTS

3.5.1 pH MEASUREMENTS

For this analysis, a pH meter was used measuring after every 3 days. The researcher made sure the meter was on the pH measurement mode. To avoid carryover contamination of the tested solutions throughout the analysis, the pH electrode has to be carefully rinsed with distilled water between readings. To get rid of the additional rinse water, the electrode was carefully blotted on a cleaning tissue for the lab. Avoid rubbing the bulb as doing so may lead to the build-up of static charges.

Using a sieve to filter out any large substrate fragments, 100ml of each of the conditions under research was placed into a different beaker. In the testing solution, the pH electrode was dipped, and the pH meter's digital display displayed the results. When the pH reading is consistent, the pH is complete. For each circumstance, the temperature and pH values were noted.

3.5.2 TOTAL SOLIDS AND VOLATILE SOLIDS

Total Solids (TS%) is the percentage of solids left in the sample once the water has evaporated.

A sample of about 10 gm is obtained, poured into a foil plate, and dried in a furnace at a temperature of around 105 0C to a constant weight.

TS%= (Final weight/ initial weight) * 100

100 - Weighed and cooked in a crucible for two hours at 500 0C in a furnace is dried residue from a total solid analysis. Crucible residue was weighed after cooling.

VS %=[100-(V3-V1/V2-V1)] * 100

V1 = Crucible weight. V2 = Weight of crucible and dry residue. V3 = Ash and Crucible Weight (after cooling) (Suyog, 2011)

3.5.3 VOLATILE FATTY ACIDS (VFA) ANALYSIS

The 100 ml sample in the beaker was then filtered using filter paper after the samples' pH levels were measured. After filtration, 20 ml of the filtrate were added to a glass beaker with a capacity of 100 ml. The filtrate was treated with 0.1M HCl until a pH of four was obtained. It was then cooked on the hot plate for three minutes after adding the HCl. The beaker was taken off the hot

plate and allowed to cool for three minutes. The mixture was then titrated with 0.01M NaOH after cooling to raise the pH from 4 to 7. HCl and NaOH concentrations used for each sample are listed. The volatile fatty acid content was then determined using the following equation.

Total VFA content in mg/l acetic acid = (Volume of NaOH titrated) * 87.5

For preparation of solutions the following methods were used:

Concentrated HCl is about 37% HCl by mass, and is about 12 M, so 32% would be about 10 M. To make 1 M just make a 1/10 dilution: 1 mL 32 % HCl to 9 mL water. So to a make a 100ml solution, 10 mL 32 % HCl were added to 90 mL water. Always add acid to water.

1 molar NaOH Solution = 40 grams NaOH dissolved in 1 litre water and 0.01 molar NaOH Solution = 0.4 grams NaOH dissolved in 1 litre water. So, to get 100mls 0.01M NaOH, 0.04g NaOH granules were dissolved in 100ml of water.

3.5.4 NITROGN ANALYSIS

Kjeldahl is a procedure used to test for nitrogen content. A sample was weighed. The weighed sample was transformed into ammonium sulphate by digestion with sulphuric acid, alkalizing the solution, and determining the resulting ammonia by distilling it into a measured volume of standard acid. The excess was determined by titration.

3.5.5 CHEMICAL OXYGEN DEMAND

Chemical oxygen demand (COD) was measured using the closed reflux calorimetric method, the initial and final measurements are crucial.

3.5.6 MICROBIAL ANALYSIS

3.5.6.1 ISOLATION OF BACTERIA IN THE WASTE

One gram of waste was weighed and it was transferred into sterile test tubes. 10ml of sterile saline solution was transferred to the test tubes containing the waste samples. To obtain uniformity, the mixture was shaken. The mixture was allowed to set and the supernatant was served as inoculums. A sterile loop was used and the loop was full of the supernatant was collected and streaked on the nutrient agar plate. The plates were incubated for 48 hours at 37 degree celcious. The plates were carefully inspected for growth of bacteria, after the incubation period.

3.5.6.2 IDENTIFICATION OF PATHOGENIC BACTERIA

From the isolation above, some suspected colonies of pathogenic bacteria were identified with selection media. Gram negative rods were grown on MacConkey agar. Cocci shaped organisms were grown on mannitol agar. Pathogenic bacteria present in each of the wastes were identified in this media. They include B, subtills, Klebsiella, E. Coli, Staphylococcus ssp. and Salmonella spp.

3.5.7.3 ISOLATION AND IDENTIFICATION OF FUNGI

The same procedure used for isolation and identification of bacteria above was also used to identify and isolate fungi. Saboround dextrose agar (SDA) was used in place of nutrient agar. Using sterile loop, 1g of raw waste was collected and streaked on SDA plates. The plates were incubated at 25 to 28 degree celcious for 48 hours.

3.5.6.4 TOTAL VOLATILE COUNT (NUMBER OF LIVING MICRO ORGANISMS)

Surface viable count was used. The suspension obtained from the isolation of bacteria was diluted with sterile distilled water using sterile pipette. The aim was to obtain a dilution that contained approximately 30 cells per 0.015ml or 0.015 volumes per drop. Agar plates were divided into eight segments with an indelible marker. A drop of the suspension was inoculated on each segment. The plates were incubated for 24 hours at 37 degree celcious. Developed colonies were counted using the equation below.

Mean count = number of colonies in each segment/8

Total viable count = mean count x dilution factor/ vol. per drop

Dilution factor = 104

Volume per drop =0.015ml

3.6 ANALYSIS OF GAS PRODUCED IN THE BIOREACTOR

SYRINGE TECHNIQUE

The "Syringe Protocol" method (Singh, 2015), which involves the solution absorbing CO_2, was applied. For this, NaOH is used. The procedure only provides an approximation of the amount of methane present because other gases like NO and H_2S may also be present.

As NaOH absorbs CO_2 but not methane, a 50ml syringe with a flexible tube and diluted sodium hydroxide (NaOH) solution was used to estimate the proportion of carbon dioxide. By dissolving sodium hydroxide granules in roughly 100 ml of water, 100 ml of a diluted sodium hydroxide solution was created.

The syringe was originally filled with water to prevent air contamination before being used to draw a 20–30 ml sample of biogas produced during the experiment into the solution. A 10 ml sample of gas was obtained by pushing extra gas outside. 20 ml of the NaOH solution were absorbed. The syringe was shaken for 30 seconds while the tube's end was maintained submerged in the NaOH solution. The extra liquid was then forced out of the syringe, which was then directed downward, until the plunger level reached 10 ml. The liquid's volume was noted. Volume recorded for the two replicates was 3 ml and 4 ml, showing that 30–40% of the gas was absorbed, resulting in a balance of 65-60% methane produced. It is necessary to have nitrogen or another gas present if the flame does not burn correctly and you detect more than 50% methane (which is determined by a reading of less than 5 ml.

3.7 DATA COLLECTION

The information gathered for this study was quantitative. Information regarding amounts or information that can be quantified and recorded using numbers, is referred to as quantitative data.

Methods for collecting quantitative data are based on figures and mathematics. Random sampling and organized data gathering tools are the foundation of quantitative data collection techniques. The sample members in this study were chosen using a basic random selection method. The information gathered through the research's data gathering tools, which include the pH, VFA, and gas produced. With the conduct of experiments, data was collected.

3.8 DATA ANALYSIS AND INTERPRETATION

This part focuses on applying statistical techniques to test the study hypothesis and making conclusions. The information gathered was presented in chapter four using a variety of styles, including tables, graphs, and narratives and statistical calculations in the appendix. Analytical statistics were employed by the researcher to examine the quantitative data gathered. The variance and standard deviation are two statistical techniques that are employed. The findings were analyzed using a one-way ANOVA using a completely randomized design. Data analysis was done using R program. At 0.05, the level of significance was established. When interpreting the data, the researcher compared the findings to earlier study as well as the research questions.

CHAPTER 4

4.0 RESULTS

4.1 pH PROFILE OF BIOREACTOR DURING FERMENTATION

The pH profile of the anaerobic digesters served as a critical indicator of process stability throughout the fermentation period, initial pH in the bioreactors was noted to decrease with increasing amounts of cow dung in the mixture (Figure 3). On no cow dung set-up pH range maintained 7.2-7.4, which reflects cow dung's natural buffering capacity to maintain pH without adjustment. A significant severe drop at 5 and 10% PP contamination from 6.3-5.8 which shows severe obstruction, forming of scum layers and production of excess VFAs. LDPE reactor remained within ideal range throughout which shows that these plastics are less harmful to AD than PP. However, in all the bioreactor set-ups, the bioreactor pH dropped at the same level of gradient dropping with about 1.0 unit except for a substrate combination of 1:1 that gave 2.0 units. The profiles of pH change with time is shown in figure 3 below. The figure shows that there is decrease in pH as the PP and LDPE slurry fermentation process progressed. pH drops significantly from day 15 to day 20 in the LDPE slurry set, PP slurry reflected a drop of 2.0 units, the control which was cow dung slurry alone increased pH steadily and stabilizing between 7.2 and 7.4 for the remainder of the digestion period. There was no significant difference in the pH readings from day 0 to day 30 ($p<0.05$).

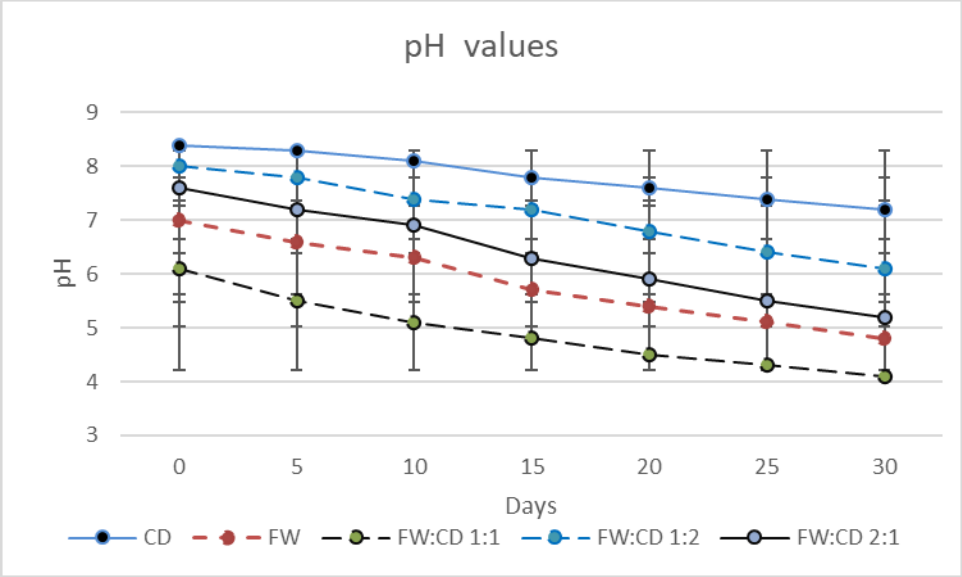

Figure 3: pH values recorded over the 30 days period.

4.2 EFFECT OF AMBIENT TEMPERATURE ON THE BIOGAS FERMENTATION PROCESS

Ambient temperature fluctuations significantly influenced biogas production efficiency across all treatment groups. Temperature was not being regulated or controlled. The mean temperature at 5 days intervals from the start of the fermentation process to the end of the process was being measured and is presented on figure 4 below. The temperature ranged from 26 °C to 32°C, with a mean of 29.1 ^{O} C. Temperature was increasing as the fermentation process progresses. Lowest temperatures were recorded from day 0 to day 5, can explain low rate in gas production on these days. From day 5 to day 15 temperatures were increasing in all digesters. From day 20to day 30 there were high temperatures as compared to other days. There was no a significant difference in temperature from day 0 to day 30 ($P<0.05$).

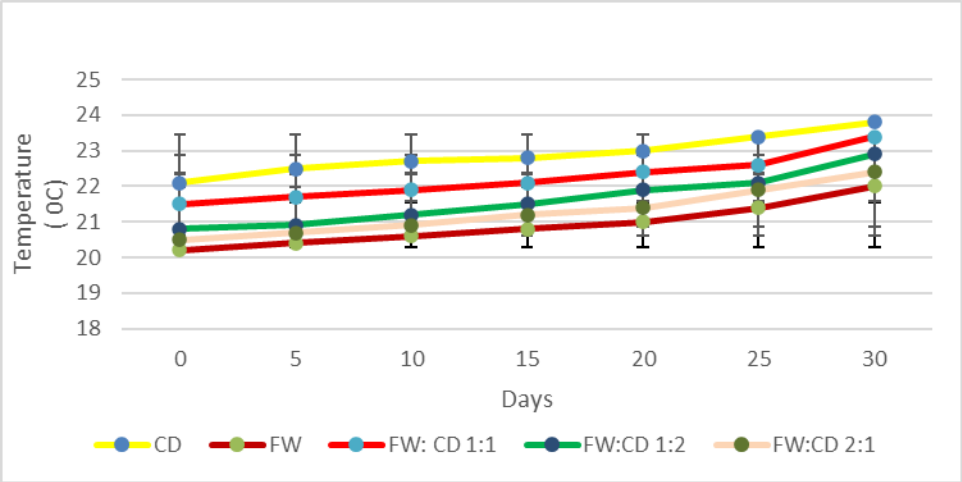

Fig 4: Temperature recorded for 30 days

4.3 VOLATILE FATTY ACIDS PRODUCTION

Cow dung slurry plus PP plastic contaminant set has the highest volatile fatty acid production which suggests persistent VFA accumulation which in turn suggests inhibition of methanogenic VFA consumption and inhibition of acetate kinase activity. Cow dung set alone has the least volatile fatty acid production. LDPE slurry has a slightly higher volatile acid production which suggests that MPs promoted organic matter hydrolysis and acidogenesis during the initial digestion stage. There was a significant increase in volatile fatty acids from day 20 to day 30 as shown in the figure 5 below. ($P>0.05$)

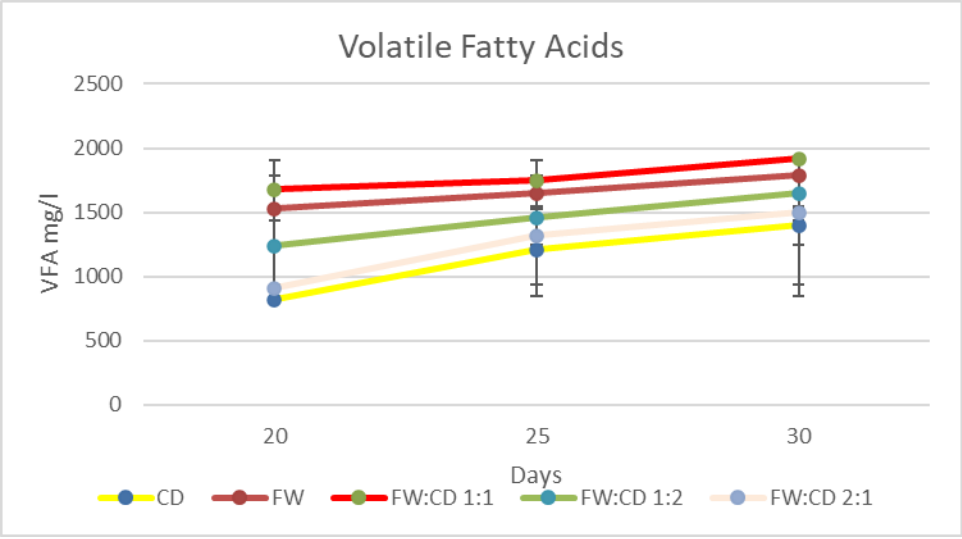

Fig 5: Volatile acids from day 20 to 30

4.5 BIOGAS VOLUME

Daily biogas production in the control group followed the pattern for AD of cow dung, a lag phase (1-5days) with low production, exponential phase(day6-18) with production at its peak, decline phase on days 19-30 whereby production gradually decrease day by day till the end of the experiment. The volume of gas produced was measured by water displacement method. Mean of cumulative gas volume at 5 days intervals from the start of the fermentation process to the end is being presented on figure 6 below. It is displayed that the amount of gas production increased as the process progresses. LDPE treated group demonstrated enhanced biogas production with a shorter lag phase and high peak production, whereas PP treated groups showed the severe inhibition with cumulative methane production decreasing compared to control. There is no significance difference in gas production between substrate ratios of treated groups. There was no significant difference in cumulative volume from day 0 to day 30 ($P<0.05$)

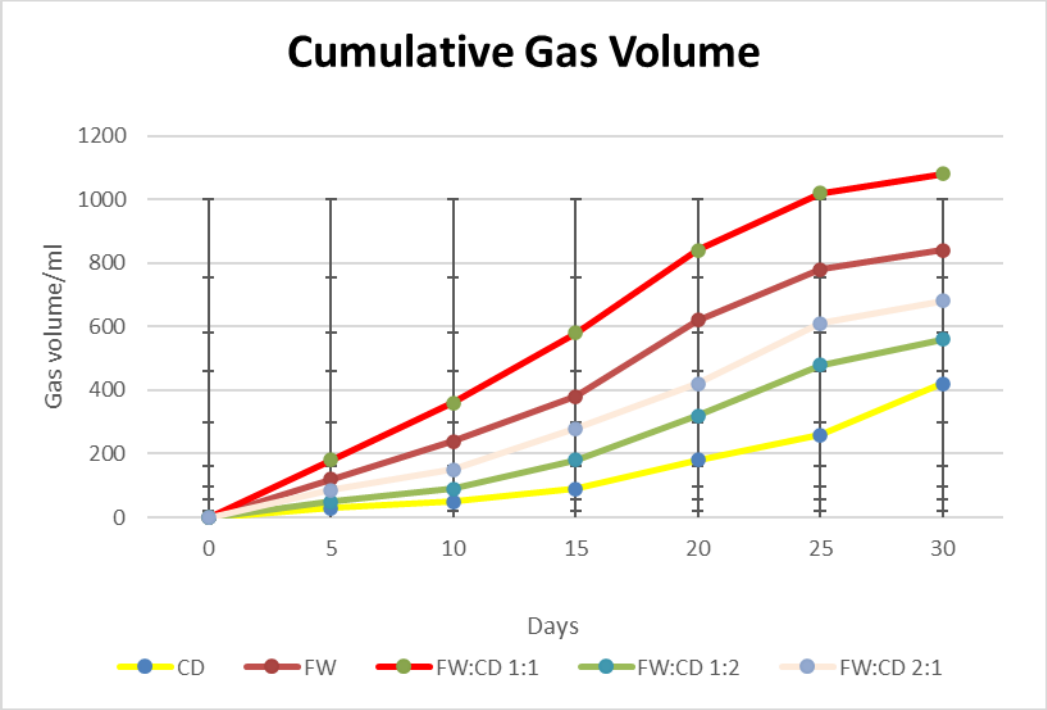

Fig 6: Cumulative biogas volume

4.6 BIOGAS BURNING

When we tested the gas produced from the control biogas bio-digester, a blue flame was produced, indicating high methane content and complete combustion. In the LDPE bioreactor tank there was bright yellow/orange flame, an indication of impurities because plastics inhibited microbes contributing to incomplete combustion and low methane levels. A weak yellow/orang-ish flame was observed while burning the gas in the PP bioreactor tank showing incomplete combustion.



4.6: BIOGAS BURNING FROM THE CONTROL BIODIGESTER

CHAPTER 5

5.0 DISCUSSION

5.1 pH PROFILE OF THE BIOREACTORS DURING FREMENTATION

The pH level is important for anaerobic digestion since it affects the action of the microorganisms that break down unprocessed materials to produce biogas. Methanogens work best in narrow range, with an ideal range of 6.0 to 6.5 for anaerobic digestion (Lantz, 2012), if lower microbes stop working and pH above 8 causes ammonia toxicity. The rate of biogas production increases as pH decreases due to the buildup of volatile fatty acids and amino acids (Mao et al.,2015). The pH values obtained in this investigation were still within the ideal range.

The pH graph shows that pH for all combinations decreased over the first ten days as a result of the rapid hydrolytic acidification process brought on by methanogens consuming accumulated VFA', due to the conversion of the acids into biogas, the pH drops from day 20 to day 30 was less noticeable. The pH decrease in all the combinations, is in agreement with the results of earlier researchers in biogas production in which it was generally agreed that at the early stages of the whole process of biogas production, acid forming microbes yield volatile fatty acids (VFA) following a drop in pH and deteriorating development of methanogenic bacteria (Cuzin, 1992).

The differential pH responses among plastic types can be explained by their distinct physicochemical properties, PP being hydrophobic and non-biodegradable, may provide surfaces that adsorb methanogenic Archaea while allowing free proliferation of acidogenic bacteria. PP-treated group showed the most severe pH depression, reaching 5,6 on day 6 a value below methanogenic tolerance threshold, these indicate that these plastics disrupted the syntrophic relationship between VFA producing bacteria and VFA consuming methanogens(Wang et al.,2021).These PP also cause pH depression in AD digesters due to preferential stimulation of acidogenic bacteria over methanogens .The pH reading of LDPE remained within the ideal range, demonstrating the stability of the digestive process.

Anaerobic digester's daily pH affects the mass transfer rate, which in turn affects digester performance. Methanogens consume VFAs more slowly because they are more sensitive to greater

ammonia concentrations. Slower VFA consumption causes them to accumulate and lowers the pH in the bioreactor. PH will decrease if acetogens outweigh methanogens, which can slow down methanogens and in the end result in digester malfunction. The pH in a digester can be kept in the ideal range by maintaining a balanced metabolism of acetogens and methanogens. Shah *et al* (2015) found that, a pH range of 6.8–7.5 has been recommended for strong inhabitants of methanogens. Based on the results from this study, it is advised to maintain a pH of 6,5–8,0 for digestion even though pH cannot be the sole factor determining digester stability. It is important to monitor pH in biogas generation because the microbes involved in anaerobic processes are responsive to pH and will slow when the substrate pH is outside the optimal range.

5.2 EFFECT OF TEMPERATURE ON BIOGAS PRODUCTION

The temperature regime used for this study was mesophilic, within the range of 26-40 ^{0}C and 35^{0}C being the optimum. Mesophilic digestion occurs at a lower temperature than thermophilic digestion and it is slower and produces less biogas, but mesophilic digesters are nevertheless popular since they consume less energy to heat their interiors than thermophilic digesters (Bail & Dhagat, 2001).

In this study the digesters didn't need to be heated because they were dependent on ambient air temperature. Temperatures were increasing from the start of the anaerobic process to the end. A low temperature from day 0 to day 5 in all combinations is because substrates were adapting to the environment. Cow dung slurry only 1:1 has the highest temperatures than all other combinations, LDPE then PP consecutively following. During the experimental period, temperatures were measured between 20^{0}C and 22^{0}C in all combinations. Since these temperatures are on the far left of the ideal pH scale, they were not conducive to make the highest volume of biogas. This is in agreement with Boe (2006) who studied the effect of temperature on the completion of biogas process from Duckweed and it was found that mesophilic temperatures at 35^{0}C were ideal for biogas production.

Due to the varied seasonal conditions, these reactors demonstrated seasonal variations in methane output. In these reactors, seasonal variations in methane production were observed in response to

the daily temperature variations from the first to the thirtieth day of digestion. During the digestion procedure, the bioreactors were placed on top of a wooden plank. The fact that wood does not conduct heat well makes it a good insulator (Suyog, 2011). This was done to keep digesters at a warm temperature because temperatures can drop very low, especially at night. As shown from the results, biogas production rises with temperature, as was the case on day twenty, when temperatures were a little higher than usual. Temperature monitoring is very important since it influences physical and biochemical processes during biogas production.

5.3 VOLATILE FATTY ACID PRODUCTION

According to this study, the pH decrease was brought on by the production of volatile fatty acids. The buildup of volatile fatty acids in the digesting tanks was not being controlled. Swati (2019), found that, keeping lower volatile fatty acid concentrations aids in increasing methane generation as well as improving waste conversion efficiency. In all the combinations from day 20 to day 30, volatile fatty acid production was increasing since they are an intermediate of anaerobic digestion which represents the metabolic activity of fermentative bacteria. This is in agreement with Bjornsson (2000) who studied the consequence of volatile fatty acids build up in biogas production. He established that volatile fatty acids are chemicals created in the middle stages of anaerobic digestion.

Due to constraint development of volatile fatty acids, it's very essential to regularly check the content of volatile fatty acids in the bioreactor. VFA levels that are consistently high indicate digester overload, which might eventually result in digester failure. Singh (2015) found a variety of methods, such as co-digestion, addition of certain metal ions, and agents that boost alkalinity, to lessen the harmful effects of VFAs. Volatile fatty acids monitoring is important for controlling steady reactor attainment and is used as a signal for appearing problems and are vital process observing parameters.

5.4 GAS VOLUME PRODUCED

The most crucial indicator in monitoring anaerobic digestion is biogas production. On daily basis measurements of biogas production make it easier to identify any stability problems that may arise. Methane and carbon dioxide make up the two primary components of biogas, together with small quantity of hydrogen sulphide (H_2S), ammonia, nitrogen, and hydrogen. Methane concentrations below 55% or CO_2 concentrations over 35–40% are signs of VFA build up and methanogen activity inhibition, respectively (Bedaso, 2019). The end product of anaerobic digestion, methane, which results from the breakdown of volatile materials, suggests that greater methane production indicates better digester efficiency in handling waste (Swati, 2019).

In this study, gas production increased with increase in retention time. Day 20 to day 30 had the highest volume of gas. The setup using 1:1 cow dung slurry only produced the topmost cumulative gas volume. The amount of biogas produced in this setup may have been caused by the presence of microorganisms with beneficial traits. LDPE contamination increased cumulative gas production by 5%, this enhancement while initially surprising has precedents in recent literature. LDPe plastics enhances direct interspecies electron transfer between species and methanogens. The least volume of gas was detected in the cow dung slurry plus PP setup this may be due to chronic stress caused through pH perturbation, VFA accumulation and possibly low-level leaching of additives. This is in agreement with Sehgal (2018) who found that fermentation of sole cow dung yield less volume of gas due to temperate anaerobic break down of about 45-50%. It is important to monitor gas volume at each stage of the process as these can act as an indicator of any potential problem.

5.5 BIOGAS BURNING

Biogas is mainly methane (CH_4) and carbon dioxide (CO_2) and may contain little quantity of hydrogen (H_2S), carbon monoxide (CO), moisture as well as siloxanes. In this study it was seen that the gas was burning with a yellow/orange flame in PP and LDPE bioreactors. It might be because there were more impurities and less gas was being produced which caused incomplete combustion. This is in agreement with Lantz (2012), who found that impurities like CO, H_2S in

raw biogas should be removed to ensure correct operation and burning efficiency of the gas. The control produced a blue flame which shows high methane content and complete combustion.

CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

This study investigated the influence of two common plastic contaminants, PP and LDPE at varying concentrations on anaerobic digestion of cow dung and subsequent biogas production over a 30-day retention period, over mesophilic conditions (35-37^{0}C) using batch type digesters, with biogas volume measured via downward water displacement and gas composition analyzed by gas chromatography.

The results of this research demonstrate that plastic contaminants exert effects on the anaerobic digestion process, with outcomes ranging from moderate stimulation to severe inhibition. PP contamination disrupts the syntrophic relationship between acidogenic bacteria and methanogenic archaea leading to process imbalance whilst LDPE contamination may provide additional surface area for biofilm formation and introduce accessible carbon sources that synergize with cow dung organic matter without overwhelming the methanogenic community.

6.2 RECOMMENDATIONS

Based on the experimental findings and the interpretation, the following recommendations can be proposed for different stakeholders involved in biogas technology, waste management and environmental policies, implementing feedstock screening protocols (for example manual sorting, density separation) , tolerate low to moderate LDPE contamination(concentrations below 5%), monitor pH and VFA levels regularly, maintain robust inoculum tanks, enforce labeling standards for plastic products, promote pre-treatment technologies, use biogas digestate as soil amendment with caution.

Recommendations for future studies being using co –contamination mixture effects as real world plastic contamination contain a variety of plastics, researchers to investigate whether the stimulatory effects observed with LDPE persist at nanoscale or transform into toxicity due to increased surface reactivity ,comprehensive life assessment comparing different plastics is needed to balance methane yield benefits against energy costs of plastics, research community should move toward reporting plastic contamination in terms of particle number, surface area, polymer

REFERENCES

Angelidaki, I., Ellegaard, L., & Ahring, B. K. (2011). Applications of the anaerobic digestion process. *Adv BiochemEngBiotechnol.* ,82: 1-33.

Bail, A., & Dhagat, N. N. (2001). Upflow anaerobic sludge blanket reactor- a review. *India.J. Environ. Health* , 43 : 1-82.

Bedaso, B. (2019). Volatile fatty acid production from fermentation of primary sludge without pH control.

Bjornsson, L. (2000). Intensification of biogas process by improved process monitoring and biomass retention. In *Department of Biotechnology, Lund University. Ph.D. Thesis.*

Boe, K. (2006). Online monitoring and control of the biogas process. In *Institute of Environment and Resources, Technical University of Denmark (DTU). Ph.D.Thesis.*

Bond, T., & Templeton, M. R. (n.d.). History and future of domestic biogas plants in the developing world. *Energy for Sustainable Development* , 15: 347-354.

Budiyono, I., Syaichurrozi, & Sumarduono, S. (2013). Biogas productio rate from cattle manure using rumen fluid as inoculums. *International Journal of Basic & Applied Sciences* , 10: 41-47.

Caviglia. (1999). Sustainable Agriculture in Brazil. *Economic Development and Deforestation. Edward Elgar* .

Cresson, R., Carrere, H., Delgenes, J. P., & Bernet, N. (2006). Biofilm formation during the start up period of anaerobic biofilm reactor - Impact of nutrient complementation. *Biochemical Engineering Journal* , 30: 55-62.

Cuzin, N. (1992). Methanogenic fermentation of cassava peel using a pilot plug flow digester. *Bioresource Technology.* , 41: 259-264.

Deublein, D., & Steinhauser, A. (2008). Biogas from waste and renewable resources. *Weinheim: Wiley-VCH* , 89-290.

Dioha, E., Dioha, J., & Nfor, B. (2012). Operating and financing biogas plant. *Journal of Energy Technologies and Policy 2* , 5: 25-37.

Duarte, A. C., & Anderson, G. K. (1982). Inhibition modeling in anaerobic digestion. *Water Science and Technology* , 14 (6/7), 749-763.

Garba, B., & Sambo, A. S. (1995). Effect of some operating parameters on biogas production rate. *Nigerian Journal of Renewable Energy* , 3: 1-2.

Horiuchi, J., Shimizu, T., Tada, K., Kanno, T., & Kobayashi, M. (2003). Selective production of organic acids in anaerobic acid reactor by pH control. *Bioresource Technology* , 82 (3), 209-213.

Karki, R., Chuenchart, W., Surendra, K. C., Shrestha, S., Raskin, L., Sung, S., et al. (2021). "Anaerobic co-digestion: current status and perspectives". *Bioresource Technology* .

Kayhanian, M., & Rich, D. (1995). Pilot- scale high solids thermophilic anaerobbic digestion of municipal solid waste with an emphasis on nutrient requirement. *Biomass and Bioenergy* , 8 (6), 433-444.

Kemausor, F., Adaramola, M., & Morken, J. (2018). A review of Commercial Biogas Systems anmd Lessons for Africa. *Energies* .

Klass, D. (1984). Methane from anaerobic fermentation. *Science, 223 (4640), 10211028* .

Kotsyurbenko, O. B. (2005). Trophic interactions in the methanogenic microbial community of low temperature terrestrial ecosystems. *FEMS Microbial Ecology* , 53 (1), 3-13.

Lantz, M. (2012). The Economic Performance of Combined Heat and Power from Biogas Produced from Manure in Sweden- A Comparison of Different CHP Technologies. *Applied Energy* , 98: 502-511.

Lepisto, R., & Rintala, J. (1999). Kinetics and characteristics of 70^0C, VFA- grown, UASB granular sludge. *Applied Microbiology and Biotechnology* , 52 (5), 730-736.

McKendry, P. (2002). Energy production from biomass (Part 2): Conversion technologies. *Bioresour Technol* , 83 (1) : 47-54.

Mshandete, A. M., & Parawira, W. (2009). Biogas technology research in selected sub-Saharan African countries- A review. *Afr. J. Biotechnol.* , 116-125.

Petersen, S. P., & Ahring, B. K. (1991). Acetate oxidation in thermophilic anaerobic sewage sludge digester: the importance of non aceticlastic methanogenesis of acetate. *FEMS Microbial Ecology* , 86, 149-158.

Pind, P., Angelidaki, I., & Ahring, B. K. (2003). A new VFA sensor technique for anaerobic reactor systems. *Biotechnology and B ioengineering* , 82 (1), 54-61.

Rahman. (2013). " Solid waste generation, characteristics and disposal at Chittagong university campus, Chittagong, Bangladesh". *Discov. Sci*, *vol. 4* , 25-30.

Russell, J. B., & Diez-Gonzalez, F. (1997). The effects of fermentation acids on bacterial growth. *Advances in Microbial Physiology* , 39, 205-234.

Schink, B. (1997). Energetics of syntrophic cooperation in methanogenic degradation. *Microbiology and Molecular Biology Reviews* , 61 (2) , 262-280.

Seghal, K. (2018). Current State and Future Prospects of Global Biogas Industry. *In Biogas; Biofuel and Biorefinery Technologies; Springer: Cham, Switzerland.* , 449-472.

Shah, A., Nawaz, A., Kanwal, L., Hasan, F., Khan, S., & Badshah, M. (2015). Degradation of poly (ecaprolactone) bya thermophillic bacterium RaIstonia sp. Strain MRL-TL isolated from hot spring. *Int. Biodeterior. Biodegrad* , 98, 35-4.

Singh, T. (2015). Production of biogas from kitchen waste using cow manure as co-substrate.

Surendra, K. C., Takara, D., Hashimoto, A. G., & Khanal, S. K. (2014). Biogas as a sustainable energy source for developing countries: Opportunities and challenges. *Renewable and Sustainable Energy Reviews* , 31, 846-859.

Suyog, V. I. (2011). *Biogas production from kitchen waste.* Rourketa, Crissa: B. Tech Thesis. National Institute of Technology.

Swati, H. (2019). *Anaerobic Digestion of Food Waste with Unconvectional Co-Substrates for Stable Biogas Production at High Organic Rates.*

Tiriwari, M., Guha, S., Harendranath, C. S., & Tripathi, S. (2006). Influence of extrinsic factors on granulation in UASB reactor. *Applied Microbiology and Biotechnology* , 71, 145-154.

Van Lier, J. (1997). High rate anaerobic waste wastertreatment under psychrophilic and thermophilic conditions. *Water Science and Technology* , 35 (10), 199-206.

Yadvika, Santosh, T., Sreekrishnan, R., Kohli, S., & Rana, V. (2004). Enhancement of biogas production from solid substrates using different techniques- a review. *Bioresource Technology* , 95, 1-10.

Zahariev, A., Dimo, P., & Anna, A. (2014). Biogas from Animal manure- perspectives and barriers in Bulgaria. *Annual Research and Review in Biology.* , 4 (5) 709-719.

APPENDIX

P<0.05, reject Ho as plastics affects biogas yield

In Pearsons correlation r is close to 1 which signifies a strong negative – more plastics equals to less biogas.

TABLE 1: pH

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
day 0	5	39.6	7.92	0.072
day 5	5	39.35	7.87	0.06575
day 10	5	39.25	7.85	0.03875
day 15	5	39.05	7.81	0.01175
day 20	5	39	7.8	0
day 25	5	37.7	7.54	0.013
day 30	5	37.2	7.44	0.013

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.997714	6	0.166286	5.432905	0.000787	2.445259
Within Groups	0.857	28	0.030607			
Total	1.854714	34				

TABLE 2: TEMPERATURE

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
day 0	5	103.9	20.78	0.022
day5	5	104.6	20.92	0.027
day 10	5	106	21.2	0.085
day15	5	108.6	21.72	0.037
day 20	5	108.2	21.64	0.073
day 25	5	108.8	21.76	0.038
day 30	5	109.6	21.92	0.012

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6.06	6	1.01	24.04762	7.75E-10	2.445259
Within Groups	1.176	28	0.042			
Total	7.236	34				

TABLE3: VFA

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
day 20	5	7700	1540	36750
day 25	5	7950	1590	36750
day 30	5	8200	1640	36750

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	25000	2	12500	0.340136	0.718317	3.885294
Within Groups	441000	12	36750			
Total	466000	14				

TABLE 4:CUMULATIVE GAS

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
day 0	5	0	0	0
day 5	5	465	93	1345
day 10	5	850	170	7200
day 15	5	1400	280	20950

day 20	5	2140	428	37470
day 25	5	3060	612	55670
day 30	5	3510	702	57720

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2102959	6	350493.1	13.60346	3.5E-07	2.445259
Within Groups	721420	28	25765			
Total	2824379	34				