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# INNOVATIVE BIOENERGY SOLUTIONS FOR DEVELOPING NATIONS: DARK FERMENTATION AND ANAEROBIC DIGESTION OF ABATTOIR WASTE

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**ABSTRACT**

This study investigates the energy recovery potential of abattoir waste by subsequent Dark Fermentation (DF) and Anaerobic Digestion (AD) processes. The 2-reactor system assesses the effect of recycling and parametric optimisation. A simulation model based on the Anaerobic Digestion model 1 (ADM1) was developed and applied for biogas production, which defines carbohydrates, lipids and proteins (CLP) as the main components of anaerobic digestion. The motivation for this work emanates from that a major cause for failure of anaerobic plants is inconsistent feedstock and process design, thus, process modelling provides a cheaper and more reliable evaluation of process components and parameters. Furthermore, a great amount of abattoir waste is generated, coupled with difficulty in disposal thus raising the waste management cost. The study investigated the effect of CLP on biogas yield and assessed the outcome from a co-digestion of manure, blood, and tissue as major abattoir waste streams. Impact on hydrogen yield was in the order of lipids>carbohydrates>proteins whereas for methane it was carbohydrates>lipids>proteins. Manure had the highest impact on methane yield rate, followed by blood, and tissue, whereas hydrogen production was in the order of blood, tissue, and lastly manure, which performed poorly. Recycling improved methane yield by 32%. The study provides optimisation data and linear correlation models for estimating yield based on the three substrates. The study furthermore presents hydrogen and methane potential of various abattoir waste stream. Based on the South African waste stream, there is potential to generate 0,068-156,26 GW of energy from abattoir waste.

## 1. INTRODUCTION

Biological processes such as anaerobic digestion are receiving global attention due to their positive socioeconomic impact and contributions to environmental remediation. Likewise, many developing countries such as South Africa are tapping into technologies such as anaerobic digestion as a cost-effective and environmentally benign method that can be used in the valorisation of abattoir waste. South Africa is experiencing a steady growth in meat consumption leading to a rise in slaughterhouse (abattoir) facilities and an increased throughput. This has resulted in increased waste generation from the sector. The waste generated in these facilities includes manure, wastewater, offals, blood, rumen content, confiscates (lungs, livers, kidneys), feathers, hooves, animal tissue (Neethling, 2014; Roberts et al., 2009). The South African Red Meat Abattoir Association (2021) reported over 9,544,704 slaughters of cattle, sheep and pigs in 2021. This accounts for around 1,078,182 tons of waste produced, assuming a waste production rate of 60%, 55%, and 60% for a 500kg cow, 50kg sheep, and a 100kg pig respectively. It was reported that about 46-50% of a cow, 38-40% pig, 28-32% chicken waste, 22% turkey, and 48% sheep/goat ends up as waste after slaughter (Tolera & Alemu, 2020). There was a reported 2 586 334 cattle, 3 547 843 sheep and 3 410 527 pigs slaughtered from the 436 abattoirs in 2021 by the Red Meat Association. There is also around 2 033 000 slaughters of goats in the country (Qekwana, 2012). About 2,3 million chickens were curled in 2018 (DTI, 2019). In 2016, 1500 tonnes of broiler meat was produced from 265 formal abattoirs (CSIR, 2018). It was furthermore stated that about 12,8 litres per bird was produced as wastewater from the poultry abattoirs (CSIR, 2018). Jayathilakan et al. (2012) provided a waste stream analysis for the cattle, pig, sheep and poultry by-products.

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Whole carcass constituted 77,5%, 63% and 62,5% for pig, cattle and sheep body weight respectively. Other wastes include Liquid Blood with 3%, 18%, and 2,4% respectively, and fats with 3%, 4% and 3% respectively. For hides and skin, the order is 6%, 6%, 15% and organs there is 7%, 16%, and 10% of the body weight. Chest and abdomen are 10% for pigs and sheep, and 11% for cattle. Feet constitute 2% for each animal stated. According to the paper, poultry feathers are 7-8% of live weight, blood is 3,2-3,7%, gizzards and proventriculus equal 3,5-4,2%, feet with 3,5-4% and intestines and glands constituting 8,5-9%.

According to the Water Research Commission, over 7.2 million $m^3$ of wastewater is discharged from the South African abattoir industry with a Chemical Oxygen Demand (COD) of 730-9930 mg/l, pH of 5.7-8.4, suspended solids of 189-3330 mg/l, and a Kjeldahl Nitrogen of 0.71-24 mg/l (GDARD, 2009; Müller, 2017; Water Research Commission, 2017). Much of the wastewater is discharged via municipal lines after pretreatment, due to the high cost of treatment for reuse/recycling. The organic load for slaughterhouse waste other than wastewater is reported as 470-960 mg/l O2 (BOD5) and 960-1280 mg/l O2 (COD) (Borowski & Kubacki, 2015; Boughou et al., 2018; Matheri et al., 2017; Palatsi et al., 2011; Singh et al., 2018; Staroń et al., 2017).

The high organic load of the waste has made it difficult for abattoirs to effectively manage it, and it was found out that there is a continuous yearly increase in deviations from adherence to regulations by abattoirs. This is especially worse for small scale abattoirs (Roberts et al., 2009). As a consequence, outbreaks such as listeriosis in 2017, and pathogenic avian influenza in 2023, which resulted in human death and culling of over 7,5 million egg laying chickens was observed (DTI, 2019; Tchatchouang et al., 2020; Thomas et al., 2020). The common disposal methods in South Africa include waste disposal facilities (WDF), burial on farms or private land, and less common methods such as incineration, composting, rendering, and anaerobic digestion (Western Cape Government, 2016). There is a need to assess opportunities for valorisation of the waste stream to maximise the value of abattoir waste, which may effectively improve the economics of the facilities.

The recent developments in waste management as countries heed the need for environmental protection and climate change have led to new regulations regarding waste handling and disposal. In South Africa, non-infectious abattoir waste falls under the classification of class B landfills (low risk hazardous waste landfills), of which many municipalities have only a few or none, thus raising the difficulty in disposal logistics (Gogela et al., 2017). This has had dire implications for the abattoir industry, which has begun experiencing some of the highest increases in the cost of disposal due to stringent disposal regulations and long-distance transportation costs. As a trade-off to the waste management costs, a study by Gogela et al. (2017) drew a financial feasibility for a medium to large scale biogas plant producing facility (>50 kilowatt-electric (kWe)) at an abattoir site in South Africa, whereby an abattoir can benefit directly from the energy produced. South Africa is furthermore aiming to cut organics to landfill and is leading toward a landfill ban, and municipalities such as Western Cape are already implementing such restrictions since 2019. As such, it will be beneficial for waste producers to find alternative waste treatment technologies to cut the rising costs of handling and disposal.

This study assesses the energy recovery potential of abattoir waste via dark fermentation and anaerobic digestion. There is little information about the valorisation of abattoir waste for hydrogen production, and especially optimisation information and potential for upscaling in South Africa. This is due to the high protein and fat content of the waste stream. Nevertheless, whereas it makes for a good substrate for AD, the slow hydrolysis rates and process inhibition makes them a less desirable feedstock (Palatsi et al., 2011). Thus, the aim was to investigate the availability and energy potential of the South African abattoir waste stream. The study presents methane and hydrogen yield potential of the typical abattoir waste stream components, and their corresponding energy based on their abundance. The composition of the waste stream is obtained from literature, whereas energy yield was achieved using the Aspen Plus simulation model. There is limited literature providing detailed biochemical waste stream characterisation data, especially on carbohydrates, proteins and lipids. However, articles such as Palatsi et al (2011), and Hejnfelt and Angelidaki (2009) provide more comprehensive characterisation data which was useful for simulating the anaerobic process. The 2-stage process has hydrogen production via dark fermentation, followed by methane production in the second stage. As per our understanding, dark fermentation has the highest potential for hydrogen production and feasibility for upscaling among biological pathways, and a technological integration of hydrogen and methane via AD provides the highest feasibility towards commercialisation due to the advancement in the application of the conventional anaerobic digestion (Ahmed et al., 2021; Dell'Orto & Trois, 2022). Combination with other technologies such as photofermentation, gasification, microbial electrolysis cells and microbial fuel cells has been studied in various literature (Osman et al., 2020; Sekoai et al., 2018; Sittijunda et al., 2022). The underlying motivation for integration, however, is that less than 20% of biodegradability is achieved during dark fermentation (Jain et al., 2024), and furthermore, multistage processes were seen to improve the yield, reduce the overall fermentation period, improve the energy balance of the process and provide a better handling of the substrates. Dawei (2008) achieved a 21% improvement to hydrogen yield in a 2-stage arrangement, and an 88% increase by recycling and sparging methane from the second reactor. The author also found out that a multistage system is more tolerable to inconsistent feedstock or varying feed composition. Other authors also found considerable gains conversely by sparging hydrogen gas in the methane reactor and gaining 42% in energy yield while enhancing biogas quality by 20% (Ghorbanian, 2014). The findings of this work are an important knowledge base for anaerobic digestion of abattoir waste, basing on the fact that a major reason for failure of such plants is the feedstock and substrate compositional inconsistencies. Therefore, understanding how the composition affects yield can be useful information for upscaling and maintaining a digester, par-

ticularly in the case of a double stage process. Developing countries can benefit from this innovative solution to ease the landfilling capacity and exploring opportunities for energy recovery from costly waste streams such as abattoir wastes. The simulation developed is not limited to abattoir waste only but can be applied to any biodegradable waste resource.

## 2. MATERIALS AND METHODS

### 2.1 Model building and development

The anaerobic process was developed and modeled using Aspen Plus software, as a modification to previous models by (Rajendran et al., 2014; Serrano & Knud, 2011). The model and reaction inputs were based on the Anaerobic Digestion Model 1 (ADM1 model) as described in (I. Angelidaki et al., 1993, 1999). The basic model development, including the set of reactions and reaction kinetics was obtained from the mentioned literature, and applied to a 2-stage process for the production of hydrogen and biogas in separate reactors. Greater details of the methodology are therefore found in the literature stated (Rajendran et al., 2014; Serrano & Knud, 2011). Based on the ADM1 model, the input components were defined as water, lipids (triolein, tripalmitin), proteins (soluble and insoluble), carbohydrates (cellulose, hemicellulose) and inert compounds. These form the basis for anaerobically degrading materials. The Non-Random Two Liquids (NRTL) method was also maintained as in previous studies due to appraisal for accurate calculation of mole fractions, and activity coefficients and suitability for gas and liquid phases and since it was already validated against experimental and plant data for anaerobic digestion modelling. Due to the limiting nature of the hydrolysis phase, the set of reactions were modelled separately in a stoichiometric reactor (Rstoic), which required the extent of reaction and reaction stoichiometry as inputs. Modelling hydrolysis separately allows for testing the effects of pretreatment on conversion efficiencies. In the current model, eleven hydrolysis reactions were added, gathered from ( I Angelidaki et al., 1993; I. Angelidaki et al., 1999; Rajendran et al., 2014; Serrano & Knud, 2011) (Table A1). The justification and validation for the reactions is provided in the stated literature. The remaining major metabolic pathways, which are acetogenesis, acidogenesis, methanogenesis, amino acid degradation reactions and hydrogen pathways (Dark Fermentation) were simulated using Continuously Stirred Tank Reactors (CSTR). CSTR units are kinetic reactors and as such reaction kinetic information was provided, based on the first order reaction kinetics (I. Angelidaki et al., 1993, 1999; Rajendran et al., 2014). The first-order kinetic framework allowed for simplicity and compatibility with Aspen Plus. Furthermore, due to the limited substrate-specific data, a major gap in the current literature, identical or similar rate constants were used in some cases as described in the cited literature. Amino acid degradation reactions were added separately to allow for clearer tracking of nitrogen transformations, but microbial biomass was not explicitly included in the reactions to avoid over-parameterisation, in line with the mentioned literature. The hydrolysis reaction set stated in this work may not be exhaustive, and more reactions can be added to enhance the model's capability to specific substrates. Hydrolysis reactions and subsequent pathways depend on the specific substrate composition, captured in the form stated above. Therefore, the hydrolysis reactions stated are not simultaneously active and depend on the particular composition of the input substrate and adding or removing hydrolysis reactions may not impact the model's accuracy. Two CSTRs were used to simulate hydrogen pathways in the first reactor and methanogenesis in the second reactor. 42 reactions were used to represent the major metabolic pathways (Table A2). A mixer was used for the initial mixing of the input substrates (to create homogeneity) prior to the hydrolysis reactor, and another for mixing the recirculate (which was simulated at 30%) with the liquid product from the dark fermentation reactor. Recirculation was only applied to the methanogenesis reactor. A heat exchanger (pre-heater) was added before each unit to ensure that the input material was at the stipulated temperature. A phase separator separated the produced gas from the aqueous phases. All units were modeled at atmospheric pressure. The sequential flow of material started with the initial mixing of input materials in a mixer, and the homogenous mixture was sent to a stoichiometric reactor (hydrolysis), followed by reaction rate calculations, before passing to kinetic reactors CSTR(1) (dark fermentation) and toward CSTR(2) (methane production). In the case of recirculation, the digestate from CSTR(2) was sent to a splitter, and the recirculate mixed with the liquid product from CSTR(1). Aspen performed the mass and energy transfer convergence calculations.

To capture the non-linearity of the process, a more dynamic approach was followed to model the kinetics rather than fixed-rate constants. The power law kinetics incorporated process inhibition and environmental dependency terms. Inhibition terms included substrate concentration, acetates, butyrate, Long Chain Fatty Acids (LCFA), pH, ammonia, hydrogen partial pressure, and temperature (modelled using Arrhenius equation). At each simulation step, the power law kinetic reactions were calculated, depending on the input species and conditions, which introduced non-linearity into the system, as the reaction rate changed in response to the varying concentrations and conditions. These factors were applied to the kinetic parameters for acidogenic, acetogenic, amino acid degradation, methanogenic and dark fermentation reactions. The inhibition factors were calculated and applied to the reaction rate (K) before running the CSTRs, where the major metabolic pathways were computed.

Complex organic matter (input components) underwent hydrolysis according to the extent of reaction in Table A1, and the products in the liquid phase were directed to the CSTRs where reactions in Table A2 were implemented. Materials from hydrolysis are sent to specific Calculator blocks (Aspen Flowsheeting tool), which performed reaction rate calculations and accounted for inhibition. These reaction rates were then exported to the CSTRs. Reaction rates were calculated for acidogenesis, acetogenesis, methanogenesis, dark fermentation, glycerol, valeric acid, butyric acid, propionic acid, linoleic acid, amino acid, palmitic acid, and oleic acid according to the products from

hydrolysis reactions. The calculator blocks imported substrate flow rates, VFAs, ammonia flow, and temperature, from the hydrolysis product stream. The Arrhenius equation was used to calculate the effect of temperature, and pH was calculated based on the chemical equilibrium constants for the VFAs according to the work of (I. Angelidaki et al., 1999).

Despite the efforts to simulate the non-linear behavior of the anaerobic digestion process, by incorporating dynamic reaction rate modelling, several limitations persist, which must be noted when expanding the results beyond the current scope. The simulation work was done based on the oversimplification of the ADM1 model, to make it suitable for the ASPEN platform. Whereas, it is suitable for the Aspen framework, using fixed reaction kinetic data introduces a limitation to the modeling of complex microbial community interactions. A more linear output may be observed, as a result, particularly when operating at low concentrations, and isothermal conditions, where the effects of inhibition are not pronounced. Furthermore, the temperature effects were incorporated using the Arrhenius equation to influence the reaction rate constant, which is an oversimplification of the enzymatic, microbial shifts that may occur from temperature changes. Lastly, thermodynamic models such as Henry's law used for gas-liquid interactions in Aspen are not designed for biological systems. As such, to address the solubility limitations, a conservative approach was used for carbon dioxide accounting, and all carbon dioxide in both the gaseous and liquid streams was counted for to overestimate the gas in biogas.

### 2.2 Substrate characterisation and model validation

The characterisation information used as input for the simulation is presented in Table A3. The information was gathered from literature. The data was used for both the simulation runs and validation against the experimental data. Carbohydrates, lipids and proteins were validated against the experimental results of Gu et al, 2024, which studied the effect of the aforementioned on hydrogen and methane production. Hydrogen and methane yields were compared with the experimental results based on the starting composition and operating conditions. Hejnfelt and Angelidaki, (2009) and Budiyono et al (2011), and Al Rubaye (2019) provided sufficient compositional data for their slaughterhouse and manure substrates to be mimicked under simulation conditions, with clear results for comparison.

### 2.3 Energy potential for biogas

Energy recovery potential (E) for biogas was calculated as follows:

$$Theoretical\ Energy\ Potential=[Vmethane\ (m^3/day)*HHVmethane]+[Hydrogen\ (m^3/day)*HHVhydrogen] \quad (1)$$

$$Practical/usable\ Energy\ Output=[Vmethane\ (m^3/day)*LHVmethane]+[Hydrogen\ (m^3/day)*LHVhydrogen] \quad (2)$$

$HHVmethane=39,8\ MJ/m^3$
$LHVmethane=35,8\ MJ/m^3$
$HHVhydrogen=12,7\ MJ/m^3$
$LHVhydrogen=10,8\ MJ/m^3$

## 3. RESULTS AND DISCUSSION

### 3.1 Model Validation

A model validation was conducted from various experimental results. In a study assessing methane potential from pig slaughterhouse waste, Hejnfelt and Angelidaki (2009) conducted thermophilic tests on substrates with a composition of 22% carbohydrates, 11% proteins and 55% lipids at a pH of 7,5 in a 26-day retention time. A methane yield of 369 l/kg feed was obtained. The same composition in the model yielded 374 l/kg $CH_4$ which was a 1,35% difference. In an experimental result presented by Al-Rubaye et al, (2019) for a cattle manure feed of 0,33 l/day with a retention time of 15 days, a biogas composition of 49,89% methane was reported, and this compared well with the simulation which obtained 48,76% methane and 51,10% carbon dioxide. The feed composition was 0,083 glycine, 0,204 cellulose, 0,086 hemicellulose, 0,257 glucose, 0,015 triolein, and 0,355 inerts. An experimental study by Budiyono et al. (2011) on the potential for cattle manure with a characterisation of 70% carbohydrates, 8% proteins, 2% fat and 20% ash, achieved a biogas composition of 48,89% carbon dioxide and 47,87% methane. A slight difference was found with the simulation which read 52,50% carbon dioxide and 47,30% of methane. Hydrogen simulation was tested against Gu et al. (2024) who conducted batch experimental work under mesophilic conditions with a feedstock of carbohydrates, proteins, lipids, and cellulose rich substrates. In the study, dark fermentation was conducted for 52 hrs and the digestate anaerobically digested to a further 40 days. The results showed that carbohydrates rich substrates yielded the highest cumulative hydrogen amount, owing to the faster degradation and vulnerability to microbial attack compared to proteins and lipids. Gu et al. (2024) further associated this with the rather straightforward structure of carbohydrates, a high moisture content, and high levels of cellulose and hemicellulose exposed to pretreatment. Carbohydrates hydrogen yield was 47,90% and 38,70% higher than that of the protein and lipids, respectively. For methane production, the study obtained about the same yield with only a slight advantage for lipid rich (60% lipids), followed by carbohydrates rich (60% carbohydrates) and protein rich (60% protein) substrates. In the simulation, hydrogen production was highest in protein rich, followed by carbohydrates and lipids. Methane production was in the order of carbohydrates rich, protein rich and lipids rich, respectively. An observation of the results from the publication points to the possibility of process inhibition of proteins and lipids during the dark fermentation test which caused such low yields. Furthermore, these substrates are known to degrade over an extended period, with long lag phases. In fact, Hejnfelt and Angelidaki (2009) realised a lag phase of 20 days when anaerobically digesting pork fat.

A test was done to observe the model's response to temperature. To this cause, cattle manure substrate, characterised above (Al-Rubaye et al., 2019), was used under mesophilic (35°C), themophilic (55°C) and hyperthemophilic (75°C) conditions at a feed rate of 1 l/ day and total solids of 6,62 g/l with a 5- and 20-day retention time for DF and AD respectively. With both DF and AD operated

under same conditions, the highest yield was obtained for hyperthermophilic, followed by thermophilic and mesophilic. Hyperthemophilic yield for hydrogen, methane and carbon dioxide, respectively was found to be 0,918 $m^3$/day, 0,170 $m^3$/day, and 0,243 $m^3$/day for DF, and <0,001 $m^3$/day, 0,759 $m^3$/day and 0,844 $m^3$/day for AD. Thermophilic yield were 7,52%, 7,74% and 5,79% lower for hydrogen, methane and carbon dioxide, whereas for mesophilic runs the yield dropped by 11,50%, 12,20% and 11,80% respectively. The average composition for DF was 69.00 +/- 0.14% hydrogen, and 12.00 +/- 0.037% methane, whereas for AD the average was 47,45 +/- 0.085% methane and no hydrogen. The results were typical with increased kinetic performance at higher temperature. Whereas the results present a more direct increase in kinetic performance, and thus higher bacterial growth; microbial activity and COD removal optimality are complex and rely on many conditions combined, which is why AD results are inconsistent and difficult to standardise. Therefore, studies such as Manogram et al. (2023) observed an 8-fold increase in yield from psychrophilic to mesophilic co-digestion of chicken manure and fruit bunch, but a 3-fold reduction when the operating conditions were set to thermophilic. Hejnfelt and Angelidaki (2009) achieved methane yields at 8 times lower for thermophilic as compared to mesophilic runs during the digestion of slaughterhouse waste, at equal feeding rates in a CSTR. In fact, an attempt to increase the load resulted in reactor failure for the thermophilic test. The composition for methane was 72,50 +/- 1,50%. In the same study, feedstock with 20% and 5% mixed pork waste yielded the same methane yield and composition under thermophilic tests, whereas under mesophilic tests, at 5% pork waste, the yield increased by 138 l/kg.

High protein and lipid substrates are more prone to inhibition due to ammonia concentration which increases with temperature and organic load (Ghimire et al., 2015). Angelidaki and Ahring (1999) thus recommended operating high nitrogen bearing substrates at a lower pH and high dilution. This improves the C/N ratio and maintains ammonia in its liquid phase as ammonium instead of the gaseous form, which is toxic to methanogens. In Dawei, (2008), it was highlighted that at extreme thermophilic conditions, hydrogen yield can reach the maximum theoretical yield of 4 mol of hydrogen per mole of glucose, and the bacteria shows better tolerance for high hydrogen partial pressures. In the study, better handling was achieved through enriching bacteria culture. The same result was reached by Okonkwo (2020) where mixed cultures improved resilience of the process to short- and long-term temperature fluctuations, improving resilience to thermal failures. As mentioned before, pH inhibition was accounted for in the model, however, as a limitation, the correlation used yielded the standard highest yield at pH of 6,5. Increasing and decreasing the pH produced a similar effect in terms of pH inhibition figures to the reaction kinetics. As such, using the same manure feed, the same results were observed for pH values of 6,0, and 7,0 as well as 5,5 and 7,5. The results showed a reduction of 3,4% methane yield when pH is changed by 0,5 and 15,4% at a pH change of 10 units. Thus, in this study, all reactions were performed at pH 6,5 to remove the effect.

### 3.2 Carbohydrates, Proteins and Lipids

As mentioned before, carbohydrates, lipids, and proteins are core components for anaerobic digestion, which can be used for accurate modeling of the process. Hence this section is dedicated to understanding the combined effect of these components on hydrogen and methane yields. Optimising these substrates can provide reliable information on the effect of co-digestion substrates on process outcome. To allow for proper running of the model, all three components were included in all the runs, and the main component accounted for 80% total solids, whereas the other two constituted 10% each. DF was run at a thermophilic temperature, and the second stage was mesophilic. High temperatures favour hydrogen producing bacteria and heat shock is often applied to restrict methanogens (Dell' Orto, 2017).

A sensitivity test was conducted on Aspen Plus at a feed rate of 0,1-1 l/d for each of the substrates. Figures 1-3 show process outcome as total volume produced from both stages. The plots show the effect of codigesting carbohydrates and lipids at a constant protein rate of 0,5 l/day. Increasing or decreasing the protein content, shifts the graphs upwards or downwards uniformly with the least maximum achievable yield of hydrogen at 936,374 l/d and the highest maximum at 1087,63 l/day corresponding to 0 l/d and 1 l/d of proteins respectively. Keeping lipids constant showed a much higher effect on the achievable hydrogen yield with only 398,992 l/day produced at 0 l/day lipids. The interaction of proteins and lipids yielded a maximum of 832,524 l/day at 0 l/day of carbohydrates. This shows that proteins had the least effect of the three. For methane production, codigestion of carbohydrates and lipids only, resulted in the maximum methane yield of 433,165 l/day, whereas carbohydrates and proteins only, achieved a yield of 451,034 l/day. In the absence of carbohydrates, the highest methane yield was 675,529 l/day.

The discussion above is emphasised in the perturbation plots which presents the effect of a point change of one component using a line graph, while keeping the other two constant. There is a linear relationship between the components load and yield of hydrogen, methane and carbon dioxide. Lipids had the highest effect on hydrogen yield followed by carbohydrates and proteins respectively. As mentioned, this is unlike the findings of Gu et al, (2024), where carbohydrates achieved the highest yield, and lipids appeared to be inhibited. However, similar results were obtained for methane yield, where proteins and lipids showed a high and comparable response, and carbohydrates had the least effect. A reason for the disparity in hydrogen production could be that in each run Gu et al (2024) was feeding 40% of carbohydrates for protein and lipids rich substrates, and 60% carbohydrates when studying its effect as the main component, compared to 20% when studying the other two. Whereas in this study, the main constituent was 80% and the others 10%. The faster biodegradability of carbohydrates allowed it to be utilised in the first stage, for a retention time of 2 days compared to the slower degrading lipids and proteins. Whereas, the lipids and proteins have a higher yield potential, the biodegradability of carbohydrates is the highest

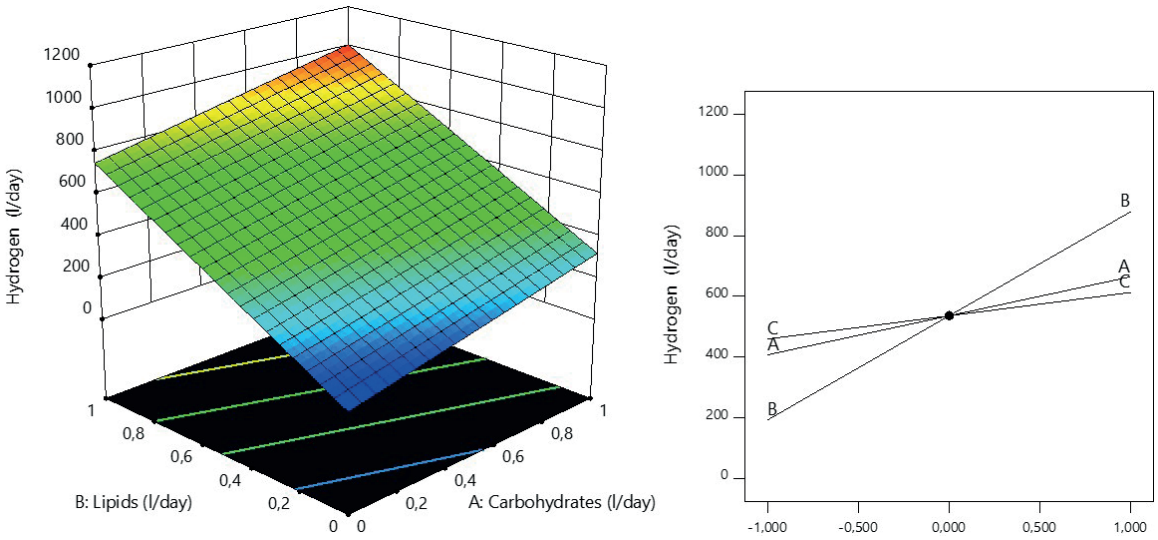


**FIGURE 1:** The effect of carbohydrates, lipids and proteins on hydrogen yield. A = Carbohydrates; B = Lipids, C = Proteins.

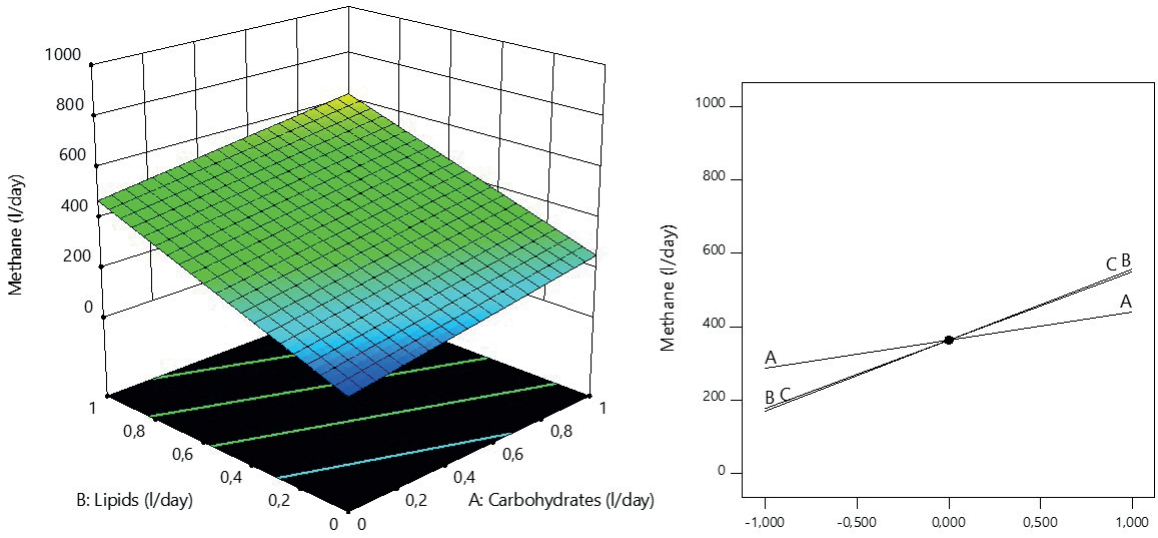


**FIGURE 2:** The effect of carbohydrates, lipids and proteins on methane yield. A = Carbohydrates; B = Lipids, C = Proteins.

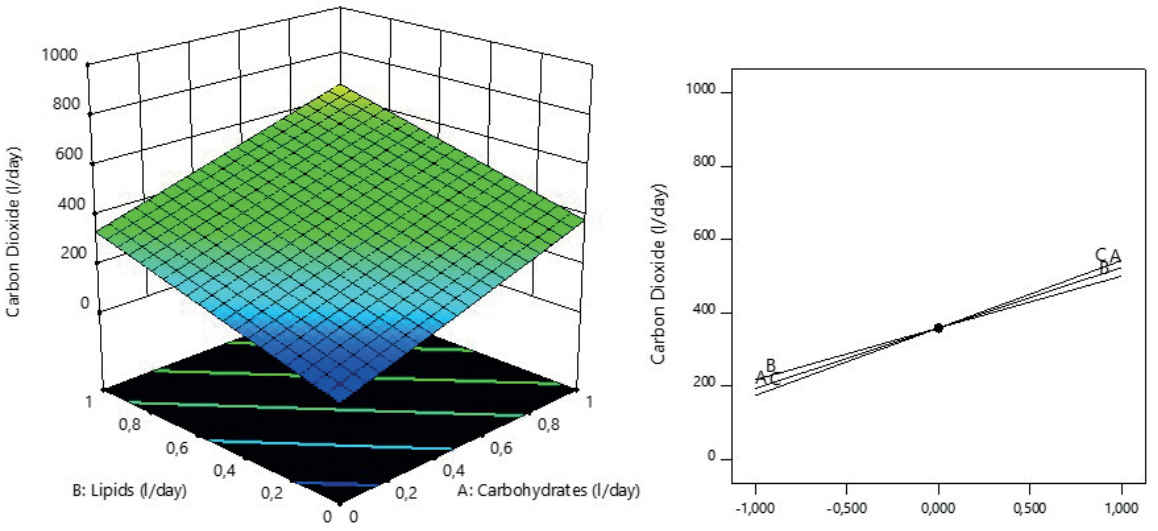


**FIGURE 3:** The effect of carbohydrates, lipids and proteins on carbon dioxide yield. A = Carbohydrates; B = Lipids, C = Proteins.

and this is significant in experimental tests (Alhraishawi & Aslan, 2022). The biogas potential of the three substrates is 990-1452 ml/g for lipids, 480-630 mg/l for proteins, and 373-921 for carbohydrates (Alhraishawi & Aslan, 2022). The theoretical yield of methane is in the order 0,99 $m^3$ $CH_4$/kg, 0,63 $m^3$ $CH_4$/kg, and 0,42 $m^3$ $CH_4$/kg for lipids, proteins and carbohydrates, respectively (Alves et al., 2009).

To further this, process inhibition due to ammonia and volatile fatty acids accumulation, biomass blockages, foaming and floatation from nitrogen rich substrates such as proteins and lipids is very common in lipid and protein digestion (Alhraishawi & Aslan, 2022; Breure et al., 1986; Ortner et al., 2014). Hejnfelt and Angelidaki (2009) observed foaming on digestion of lipids rich substrates especially in CSTRs, and this was attributed to the degradation of Low Chain Fatty Acids (LCFA), which decreased the bio-accessibility of the LCFA and other particles. In the studied range, for this work, ammonia production was at 3,1 +/- 0,1% of the biogas for proteins and lipids. Ammonia production was in the order of protein>lipids>carbohydrates. Hydrogen sulphide production was also in the same order (Table 1). Codigestion at 1 l/day for each substrate reduced the amount of ammonia by 23%, and hydrogen sulphide by 5,8%. The digestate observed an increase in acetate by 55%, whereas ethanol, propionate and butyrate were reduced in volume (Table 2).

**TABLE 1:** Biogas and energy recovery potential for various abattoir substrates Feed = 1 l/day.

| | Biogas (l/day) | | | | | Theoretical Energy | Practical Energy |
|---|---|---|---|---|---|---|---|
| **Substrate** | **$CH_4$** | **$H_2$** | **$CO_2$** | **Ammonia** | **Hydrogen sulphide** | **kWh/day** | **kWh/day** |
| Protein (P) (no recycle) | 1523,34 | 2243,96 | 2986,98 | 215,15 | 71,92 | 24,76 | 21,89 |
| Lipids (L) (no recycle) | 1522,95 | 2249,87 | 2989,05 | 215,12 | 71,92 | 24,78 | 21,9 |
| Carbohydrates (C) (no recycle) | 1548,07 | 2253,26 | 3001,51 | 214,23 | 71,92 | 25,07 | 22,16 |
| P: L: C<br>At 1 l/day each | 1804,03 | 1777,46 | 2948,07 | 166,14 | 67,68 | 26,22 | 23,28 |
| Cattle rumen | 933,13 | 882,43 | 975,25 | 192,70 | 58,11 | 13,44 | 11,93 |
| Goat rumen | 1161,86 | 1405,95 | 1519,04 | 180,77 | 52,41 | 17,81 | 15,78 |
| Cattle Blood | 955,19 | 836,81 | 964,74 | 207,97 | 63,36 | 13,52 | 12,02 |
| Cattle muscle | 975,85 | 829,24 | 982,98 | 216,40 | 66,59 | 13,72 | 12,2 |
| Cattle Manure | 826,57 | 838,39 | 964,10 | 165,73 | 30,57 | 12,1 | 10,74 |
| Confiscates (liver, lung, kidneys) | 860,10 | 864,29 | 859,94 | 148,30 | 44,66 | 12,56 | 11,15 |
| Pig tissue + fat | 984,77 | 1188,76 | 971,52 | 111,16 | 33,74 | 15,09 | 13,37 |
| Pig stomach | 832,75 | 934,08 | 840,13 | 123,32 | 36,65 | 12,51 | 11,09 |
| Chicken Feathers | 1106,38 | 817,29 | 1119,39 | 286,76 | 88,32 | 15,12 | 13,46 |
| Poultry wastewater | 845,26 | 920,44 | 847,89 | 128,43 | 37,78 | 12,60 | 11,17 |

**TABLE 2:** Digestate stream for various abattoir substrates Feed = 1 l/day.

| **Substrate** | **Acetate** | **Propionate** | **Butyrate** | **Ethanol** | **Keratin** | **Protein** | **Cyano-acetate** |
|---|---|---|---|---|---|---|---|
| **l/day** | | | | | | | |
| Protein (P) (no recycle) | 0,4018 | 0,1425 | 1,099 | 1,314 | 0,1098 | 0,1093 | 0,1117 |
| Lipids (L) (no recycle) | 0,4020 | 0,1425 | 1,098 | 1,314 | 0,1098 | 0,1093 | 0,1118 |
| Carbohydrates (C) (no recycle) | 0,4182 | 0,1425 | 1,052 | 1,314 | 0,1098 | 0,1093 | 0,1157 |
| P:L:C<br>At 1 l/day each | 0,6205 | 0,0847 | 0,2682 | 1,243 | - | 0,1053 | 0,1592 |
| Cattle rumen | 0,2287 | 0,0824 | 0,0537 | 0,0649 | 0,1358 | 0,0872 | 0,0556 |
| Cattle Blood | 0,2197 | 0,0800 | 0,0242 | 0,0389 | 0,1321 | 0,0955 | 0,0541 |
| Cattle muscle | 0,2199 | 0,0788 | 0,0156 | 0,0389 | 0,1268 | 0,1006 | 0,0542 |
| Confiscates (liver, lung, kidneys) | 0,2279 | 0,0819 | 3,360E-05 | 0,0389 | 0,1205 | 0,0667 | 0,0578 |
| Pig tissue + fat | 0,2930 | 0,1158 | 0,0097 | 0,0389 | 0,1218 | 0,0500 | 0,1218 |
| Pig stomach | 0,2236 | 0,0893 | 0,03424 | 0,0389 | 0,1180 | 0,0543 | 0,1180 |
| Goat rumen | 0,3166 | 0,1200 | 0,2784 | 0,3509 | 0,1867 | 0,0772 | 0,0837 |
| Chicken feathers | 0,2276 | 0,078947 | 0,0254 | 0,03892 | 0,1476 | 0,1340 | 0,0527 |
| Poultry wastewater | 0,2240 | 0,088596 | 0,0185 | 0,0389 | 0,1261 | 0,0558 | 0,0601 |
| Cattle manure | 0,2143 | 0,076712 | 0,0473 | 0,1725 | 0,1098 | 0,0450 | 0,0541 |

Past research has proven that such inhibition can be overturned and adsorption by materials such as biochar is being studied. Biochar can also be instrumental in reducing the carbon content of the process. Whereas Angelidaki and Ahring (1999) suggested co-digesting protein and lipids rich substrates at high dilution and mesophilic temperatures, Alves et al, (2009) noted that appropriate equipment and feeding scheme can improve the feasibility of working with this feedstock. The study proposed a specialised reactor system with a primary biomass retention through floatation and a secondary biomass retention through settling. Neves et al, (2009) suggested progressive addition of lipids rich substrates while staying below the threshold values for Low Chain Fatty Acids (LCFA). Breure et al (1986), recommended spatial separation during the hydrolysis of high protein concentrations after discovering that glucose adapted mixed bacteria turned to struggle degrading proteins. Separation of hydrolysis and fermentation was applied in this work. Lastly, carbon dioxide generation was found to follow a more linear trend to a point change in substrates load and there is minimum difference in cause and effect of the three components.

Following the study of the interaction of the components to the yield, three optimisation criteria were set. First with the aim to maximise hydrogen yield, second one maximising methane, and the third one minimising carbon dioxide. It was found out that at an equal feed rate of 1 l/day, the highest hydrogen yield of 1084 l/day was produced, with 875 l/day of methane and 937 l/day of carbon dioxide. This also served as the highest achievable methane production in the studied range. Carbohydrates were found to generate a very high amount of carbon dioxide particularly in the dark fermentation stage, with lipids generating the least. As such minimising carbohydrates and increasing lipids was the best way to obtain low yields of carbon dioxide while maintaining high yields of hydrogen and methane. The least carbon dioxide was measured at 0 l/day carbohydrates, 1l/day lipids and 0,520 l/day proteins where 318,74 l/day carbon dioxide was produced with 753,73 l/day hydrogen, 464,371 l/day methane. Based on the simulation, a linear model was developed with an adjusted and predicted R2 > 0,99 and sequential and lack of fit p-values <0,0001. Equations 3-5 shows the correlation for estimating the biogas yield at varying feed rates of the three components.

$$Hydrogen\ l/day = 257{,}349*A + 686{,}277*B + 153{,}356*C - 12{,}303 \quad (3)$$

$$Methane\ l/day = 153{,}120*A + 372{,}375*B + 386{,}567*C - 92{,}699 \quad (4)$$

$$Carbon\ dioxide\ l/day = 330{,}038*A + 282{,}587*B + 367{,}772*C - 130{,}901 \quad (5)$$

where:
A = Carbohydrates (l/day)
B = Lipids (l/day)
C = Proteins (l/day)

## 3.3 Co-digestion of manure, animal tissue and blood

Manure, animal tissue and blood forms some of the highest wastage at abattoir facilities. As such, these offers the potential for viability in conversion to biogas and a great benefit for energy production. Such a waste stream is available in the long term and can be useful in sizing an anaerobic plant, with a guarantee for a consistent feed composition, a gap that exist in the biogas industry and a major cause of failure for biodigesters. Animal blood and tissue are rich in proteins and low in lipids and carbohydrates (Table A3), whereas manure has a slightly higher carbohydrate. In an experimental setup, manure offers a microbial community and a buffering capacity which can be useful for maintaining pH. The following section details the results from the co-digestion of manure, animal tissue and blood.

### *3.3.1 Substrate impact on biogas production*

The impact of substrate composition was investigated over the range of 0-1 l/day of each of animal manure, blood, and tissue. The study compares the effect of introducing recycling in the second reactor with a 30% recycling rate. As such recycling affected methane and carbon dioxide yields only. This was because the first reactor which in a typical 2-stage arrangement acts as an acidification reactor, is expected to be smaller and take much lesser loads. The first reactor was simulated at thermophilic temperature, whereas the second reactor was operated under mesophilic conditions. The reason for this is because there is favorable hydrogen production kinetics, and the first reactor may be incorporated as a pretreatment stage due to the hazardous nature of the abattoir waste stream, which is often required to be pasteurised prior to use. Both were operated at 1 atm. Figures 4-6 show the effect of Manure and blood at constant tissue of 0,5 l/day without recycling (upper image) and with recycling (lower image). Increasing or decreasing the amount of tissue shifted the plot up or down respectively. The highest possible output for hydrogen at 0 l/day of tissue was 85 l/day. The highest yield dropped down to 70 L/day when animal blood was set to zero and up to 153 L/day when excluding animal manure. The results shows that animal manure had a negative effect on hydrogen yield, and blood has the highest impact. The perturbation plot reflects this observation showing the linear slope of blood having the highest response to a unit change, followed by tissue and animal manure.

At a feed of 1 l/day for each substrate, the maximum achieved methane was 643,089 l/day. The highest possible output of methane at 0 l/day tissue was 550 l/day, and the highest possible at 0/day of blood was 500 l/day. Manure showed the highest impact on methane production with only 260 l/day achievable at 0 l/day manure. This is shown in clearer detail in the perturbation plots where A has a much steeper slope compared to B and C. Animal tissue had the least influence on methane yield. Following the three criteria for optimisation, the highest yield of methane was observed at an equal feed of 1 l/day of each substrate, producing 649,04 l/day methane, 148,74 l/day hydrogen, and 898,53 l/day of carbon dioxide. The effort to maximise hydrogen while maintaining a high methane production was observed at the same feed rate. An attempt at minimising carbon dioxide, resulted in the reduction in

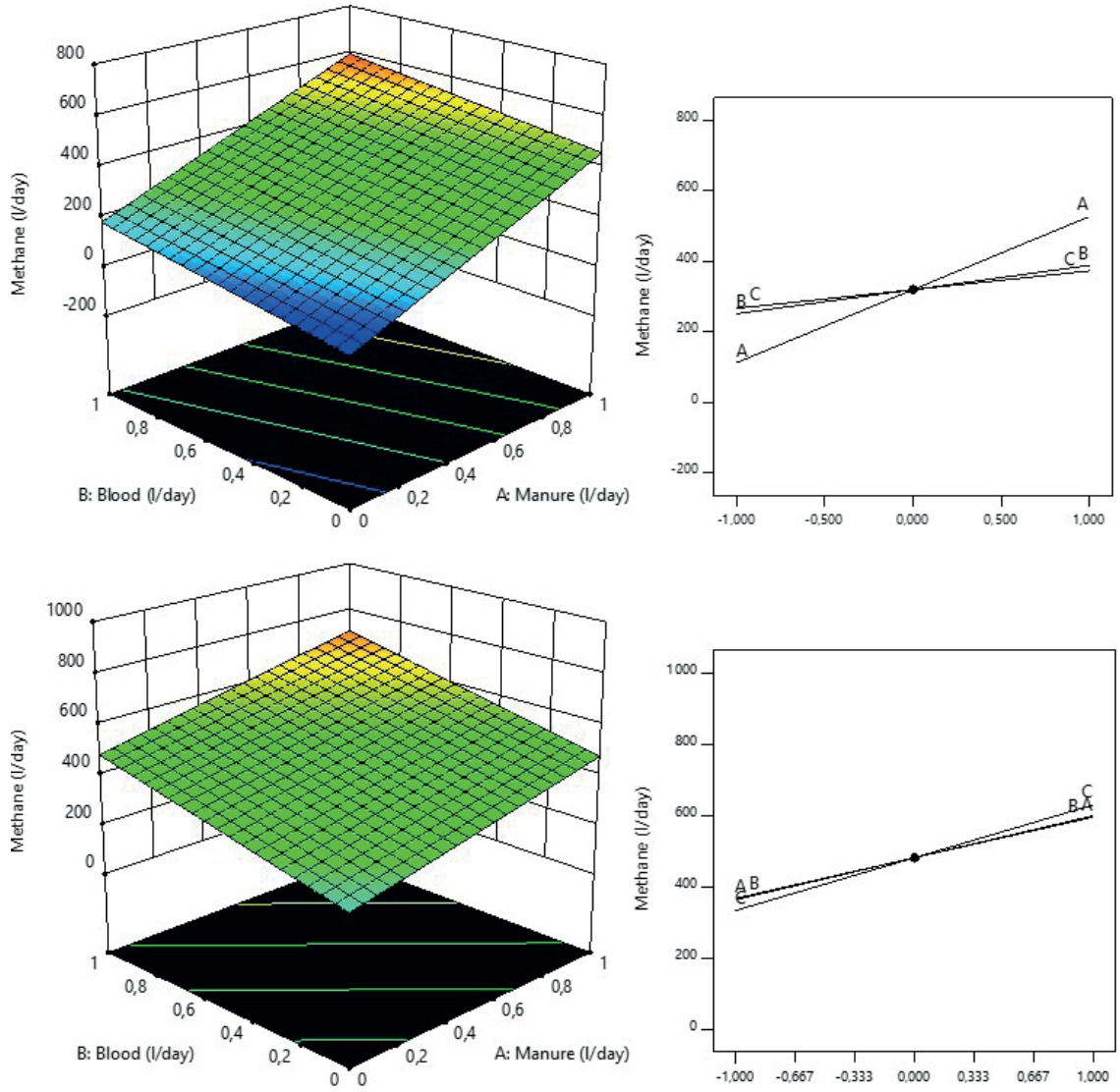


**FIGURE 4:** The effect of manure, blood and tissue on methane yield. upper image = without recycling, lower image = with 30% recycling. A = Manure, B = Blood, C = Tissue.

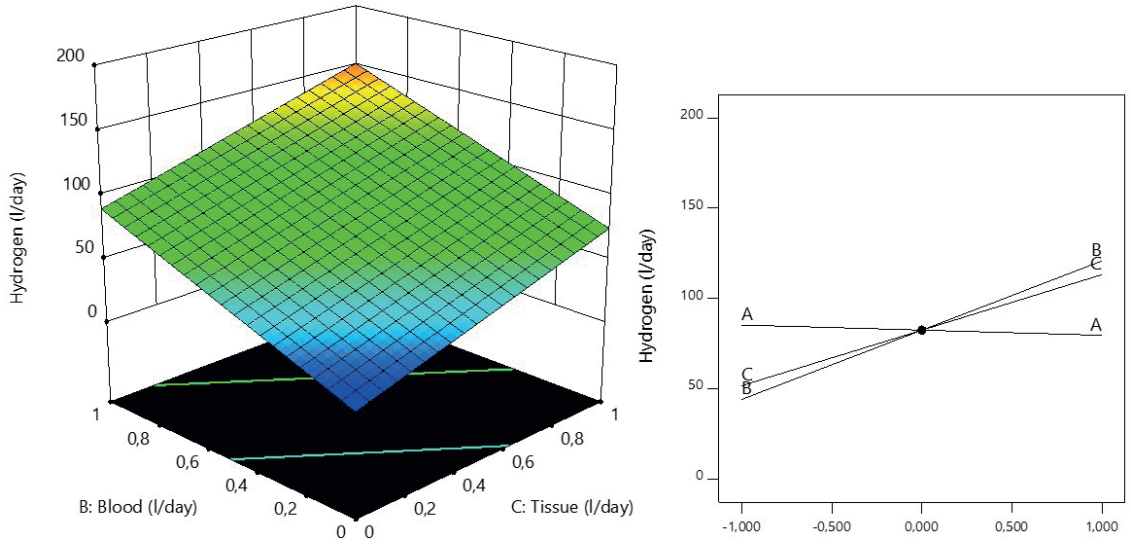


**FIGURE 5:** The effect of manure, blood and tissue on hydrogen yield. A = Manure, B = Blood, C = Tissue.

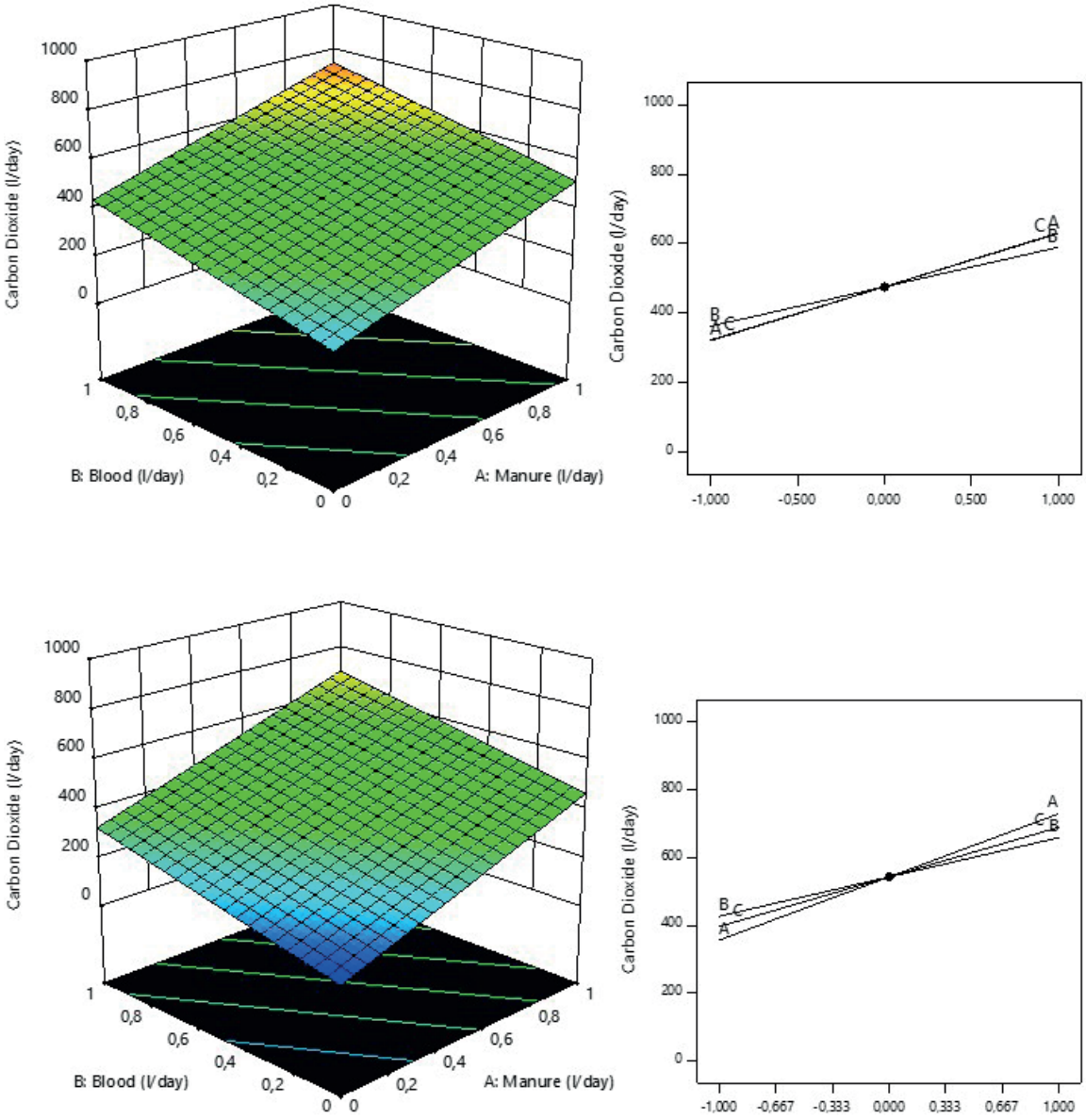


**FIGURE 6:** The effect of manure, blood and tissue on carbon dioxide yield. upper image = without recycling, lower image = with 30% recycling. A = Manure, B = Blood, C = Tissue.

the yield of methane and hydrogen. The optimum feed for the criteria was 0,73 l/day manure, 1 l/day blood, and 0,088 l/day tissue, which yielded 438,48 l/day methane, 94,25 l/day hydrogen, and 531,55 l/day carbon dioxide. Based on the results presented, it is clearly visible that manure may not be the best co-digestion substrate for hydrogen production, but it is good for methane production as reflected in equation 6-8 below and various experiments (Gaogane, 2021). As such in a 2-stage process, it is best to introduce the substrate in the second stage.

$$Hydrogen\ l/day = 76{,}756*B + 61{,}496*C - 5{,}701*A + 16{,}190 \quad (6)$$

$$Methane\ l/day = 414{,}221*A + 137{,}294*B + 106{,}092*C - 8{,}568 \quad (7)$$

$$Carbon\ dioxide\ l/day = 306{,}433*A + 227{,}074*B + 310{,}181*C + 54{,}842 \quad (8)$$

where:
A = Manure (l/day)
B = Blood (l/day)
C = Tissue (l/day)

There were observable changes in substrate impact on yield when recirculation was introduced with recycling a small portion of the digestate to the second reactor. The results for recycling showed the maximum yield of methane at 1 l/day of each substrate as 860,83 l/day. At 0 l/day tissue, the maximum yield was 564 l/day, which went up to 625 l/day in the absence of animal blood. The effect of manure was much closer to that of animal blood, as the highest methane yield was predicted at 632 L/day at 0 l/day of manure. This is presented in the perturbation plot with the highest effect on yield observed for animal tissue. The carbon dioxide production rate was mostly influenced

by the feed rate of animal manure, followed by animal blood and tissue. At zero tissue, carbon dioxide produced was 699 l/day, and at no blood addition, the maximum produced was 760 l/day, whereas in the removal of animal manure the yield was only 619 l/day. Under optimisation with basis for methane yield, the maximum amount of methane was found at a feed rate of 0,993 manure, 0,995 blood, and 0,987 tissue, with a production of 854,25 l/day methane, and 984,48 l/day carbon dioxide. This was a 32% increment in the maximum achievable methane value at 30% recycling compared to non-recycling conditions. The best effort to minimise carbon dioxide while maintaining the highest possible methane production was defined at 0 manure, 0,999 blood, and 0,480 tissue. Biogas yield had a significantly lowered methane yield of 478,46 L/day and 466,27 L/day carbon dioxide. The corresponding coefficients for estimating biogas yield in the case of recycling are presented below:

$$Methane\ l/day = 228{,}187*A + 235{,}487*B + 296{,}114*C + 101{,}045 \quad (9)$$

$$Carbon\ dioxide\ l/day = 372{,}965*A + 232{,}095*B + 293{,}313*C + 93{,}596 \quad (10)$$

where:
A = Manure (l/day)
B = Blood (l/day)
C = Tissue (l/day)

### 3.4 Biogas composition of abattoir waste and potential for energy recovery

Table 1 shows that for a 1 l/day feed of cattle rumen, 933 l/day of methane and 882 l/day of hydrogen can be produced. Goat rumen can produce a higher amount with methane and hydrogen production of 1161 l/day and 1405 l/day respectively. A study on the mono-substrate feed of cattle rumen fluid achieved 3,28 ml/ml rumen fluid at STP using a rumen fluid extracted hydrogen enhancing bacteria culture namely Staphylococcus (Maman et al., 2024). The study achieved the highest yield at a pH of 6,5. An advantage of rumen fluid is that it is internal predigested and does not require any pretreatment, and it is also abundant since a single cow generates 90-100 L of rumen per cow, which is often disposed (Maman et al., 2024). Because of the predigested form, the rumen fluid can be used as both an inoculum and substrate.

Cattle blood at the same feed rate can produce 955 l/day of methane and 836 l/day of hydrogen. Hejnfelt and Angelidaki, (2009) achieved 562 l/kg waste from a batch digestion of blood at 5% concentration. Increasing the concentration, reduced the yield significantly on the study. In the study mentioned, batch experiments on mixed pork waste achieved theoretical yields, reaching over 600 l/kg VS methane yield. There was no difference in the yield at mesophilic and thermophilic, showcasing reliability as feedstock. Increasing the feedstock to 50%, the theoretical yield was also reached, but only at mesophilic temperatures. Reaching the theoretical yield means pretreatment is not required to enhance the performance, making pork waste a favourable feedstock. Under CSTR conditions, methane yield was 45% of the theoretical yield at both 5% and 20% mixed pork concentrations, but mesophilic temperatures elevated the yield to 74% of the theoretical yield at 5% concentration. Pork fat achieved a methane yield of 562 l/kg waste and meat and bones at 10% concentrations achieved a methane yield of 580 l/kg VS. In the current study, pig tissue + fat observed a methane yield of 984,77 l/day, and cattle muscle was lower with 975,85 l/day. Hejnfelt and Angelidaki, (2009) achieved a yield of 550 l/kg VS meat pieces at 20% concentration. A higher methane yield was obtained for cattle muscle compared to internal organs (liver, kidney) but the converse was observed for hydrogen production. The result was in agreement with (Cieciura-Włoch & Borowski, 2019). The difference was that Weronika and Borowski (2019) achieved significantly lower hydrogen yields in both batch experiments. Ammonia concentration was significantly higher in the biogas of cattle muscle, blood and chicken feathers of which according to Hejnfelt and Angelidaki, (2009), digesting them under mesophilic temperatures is a better choice to reduce the total ammonia-N in the free form.

Sawyer et al. (2019) estimated cattle manure production of 136 161 tons per year in South Africa, and potential for recovery of methane and hydrogen gas was found to be 826 l/day and 838 l/day, respectively. Chicken feathers produced a high methane yield of 1106 l/day and 817 l/day of hydrogen. Due to the high organic load of wastewater, a high amount of biogas can be recovered with yields of up to 845 l/day methane, and 920 l/day hydrogen. These figures show a great potential in energy gains from anaerobic digestion of abattoir waste stream in a 2-stage process. The main composition of digestate was acetate, propionate, ethanol, butyrate, cyano-acetate and unreacted proteins. Similar results were observed for digested manure, in comparison to the experiment by Hussien et al. (2024) where the digestate was composed mainly of acetate, followed by propionate and butyrate. A review by Ghimire et al. (2015) and Xiong et al, (2024) showed that these are major digestate constituents regardless of substrates, temperature and pH in various experiments. Using the percentage weight of waste per animal, the total slaughter per year, and energy yield per litre per day, the total energy production in GWh was calculated and presented in Table 3. Cattle rumen and blood showed great potential for energy production with over 2075 GWh/d and 3750 GWh/d energy, respectively, generated from double stage anaerobic digestion. Poultry wastewater based on the figure stated above is over 19,2 million litres and has potential to produce 240 GWh/d.

## 4. CONCLUSIONS

A simulation model was developed to estimate the outcome of a 2-stage digestion of abattoir waste. The simulation was based on the ADM1 model which uses carbohydrates, lipids and proteins as the main components of anaerobic digestion. The effect of codigestion of cattle manure, blood and tissue was studied on hydrogen and methane production. The study found out that in a codigestion of blood, manure, and tissue, blood had the highest impact on hydrogen yield, whereas manure was the

**TABLE 3:** Energy potential from abattoir waste stream in South Africa.

| Substrate | $CH_4$ l/day | $H_2$ l/day | Total Energy (MJ/day) | Total Energy (GWh/day) |
|---|---|---|---|---|
| Cattle rumen | 933,13 | 882,43 | 7,46E+09 | 2071,25 |
| Goat rumen | 1161,86 | 1405,95 | 3,63E+08 | 100,80 |
| Cattle Blood | 955,19 | 836,81 | 1,35E+10 | 3750,64 |
| Cattle muscle | 975,85 | 829,24 | 1,90E+09 | 528,66 |
| Cattle Manure | 826,57 | 838,39 | 5,89E+06 | 1,64 |
| Confiscates (liver, lung, kidneys) | 860,10 | 864,29 | 1,12E+10 | 3099,43 |
| Pig tissue + fat | 984,77 | 1188,76 | 6,63E+08 | 184,14 |
| Pig stomach | 832,75 | 934,08 | 3,66E+08 | 101,75 |
| Chicken Feathers | 1106,38 | 817,29 | 1,52E+07 | 4,22 |
| Poultry wastewater | 845,26 | 920,44 | 3,40E+12 | 944116,58 |

most producer of methane. It was found out that there is so much potential in energy production from the abattoir waste stream for developing countries such as South Africa, which has seen growth in meat demand over the years. A key observation is that the waste stream has potential to generate between 0,068-156,26 GW of energy. It was pointed out that substrates such as animal manure and rumen tissue can play an important role as both inoculum and substrates for methane and hydrogen production respectfully, and they can be digested effectively without pretreatment. As such results like these can form a great benchmark for the abattoir industry for the uptake of 2-stage anaerobic digestion for waste management, energy production, and utilisation.

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