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Carrier Material and Microbial Selection for Enhanced Methane Production in *Ex-Situ* Biomethanation

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***Abstract* – Choosing the appropriate carrier material for ex-situ biomethanation is a critical factor to consider when developing biogas upgrading technologies. The chosen material for biomethanation in a biotrickling filter reactor functions as a substrate that immobilises microorganisms, which act as catalysts in the reaction for producing biomethane. This study conducted experiments on waste-derived materials, including glass foam and vulcanised wood ash material, in addition to polyurethane foam and expanded clay pellets. The manometric test measured the rate of CH_4 generation by quantifying pressure fluctuations. The results were confirmed by analysing final product gas samples using gas chromatography. To enhance the biomethane concentration in the end product, a strain of *Methanobacterium alcaliphilum* was evaluated alongside biogas digestate as the inoculum. This *Methanobacterium alcaliphilum* strain is a methanogen that utilises hydrogen and can thrive in a high pH environment. Thus, it has the potential to demonstrate improved biomethane production outcomes when a vulcanised wood ash material is used as the carrier material. Glass foam demonstrated excellent methane yields (up to 84 %), while vulcanized wood ash material was unsuitable due to its alkaline properties and inhibitory effects on microbial growth. Biogas digestate outperformed *Methanobacterium alcaliphilum* monoculture as inoculum, highlighting the need for further research on microbial community optimization, especially in larger reactor systems. These findings contribute to advancing sustainable biomethanation technologies and promoting circular economy principles.**

***Keywords* – Biomethanation; carbon farming; carrier material; gas chromatography; glass foam; *Methanobacterium alcaliphilum*; vulcanized wood ash material.**

Nomenclature	
VAM	Vulcanised wood ash material
EC	Expanded clay
GF	Glass foam
PUF	Polyurethane foam

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1. INTRODUCTION

Biogas production is a critical process for waste management and plays a significant role in agricultural sustainability. Modern farms face considerable challenges in maintaining operations without biogas plants, as their absence can lead to serious negative impacts on local communities, including unpleasant odours and disruptions to local ecosystems. Biogas production offers a technological solution for agriculture by efficiently processing waste while generating heat and electricity. Additionally, its by-product, digestate, serves as a nutrient-rich fertilizer, providing a more sustainable alternative to fossil-based fertilizers [1]. Biogas production is also a key component of the circular economy [2]. Streams once considered waste from industries, water management, and agricultural processes can be redirected to biogas facilities, where they are transformed into renewable energy, nutrient-dense organic fertilizers, and new material resources. Biomethanation, the process of converting CO_2 into methane using methanogenic microorganisms, plays a crucial role in carbon farming. This process can utilize CO_2 generated from various sources, including industrial emissions and agricultural waste, effectively recycling carbon within the ecosystem [3]. Beyond methane production, the process generates digestate, a nutrient rich by-product of anaerobic digestion, which further benefits agriculture. Applying digestate to agricultural soils boosts fertility and supports carbon sequestration. Packed with organic matter and essential nutrients, digestate enhances soil structure, stimulates microbial activity, and promotes carbon retention in the soil [4]. By recycling organic carbon back into the soil, farmers can achieve higher crop yields while advancing carbon sequestration initiatives, aligning their practices with climate sustainability objectives [5]. Biomethanation is one of the methods used in carbon farming, making it essential to conduct practical, application-focused research in this field [6]. Adopting biogas production and biomethanation as carbon farming strategies offers considerable economic advantages for farmers.

Traditionally, biogas upgrading has relied on energy-intensive and costly physicochemical methods. Biotechnology-based approaches, such as using biotrickling filter reactors for biomethanation, present a more efficient alternative, offering significant reductions in energy consumption and costs while being recognized as a promising solution for biogas upgrading [7]. The development of efficient biomethanation technologies is important for advancing biomethane production and optimizing biogas upgrading processes [8], [9]. An important aspect of this is the selection of effective carrier materials built within bioreactors, which play an important role in immobilizing methanogenic microorganisms that catalyse the conversion of CO_2 and H_2 into methane [10]–[12]. This process enhances the quality of biogas. While various materials of different compositions and generations have been studied to enhance biomethanation efficiency, more cost-effective and efficient solutions are still needed [10]. By utilizing sustainable materials for biomethanation technology, it is possible to improve methanogenesis efficiency while reducing environmental impact. With continued research and development, more filter material alternatives are expected to become available in the near future.

Previous studies have tested a variety of materials, such as natural zeolites, activated carbon, and ceramics, in biomethanation processes [10], [13]. However, these materials often pose challenges such as high costs, limited availability, or poor microbial adhesion. Glass foam and vulcanized ash, derived from industrial waste, present sustainable alternatives with the potential to address these limitations. This study explores the potential of using industrial waste-derived materials, such as glass foam [14], [15] and vulcanized wood ash material [16], as well as widely available alternatives like expanded clay [10] and polyurethane foam, as carrier substrates in biotrickling filter reactors [17]. These materials offer varying physical

and chemical properties that influence gas-liquid mass transfer and the efficiency of microbial biofilm formation [17].

Additionally, the composition and viability of microbial consortia are critical to the success of biomethanation [18]. The selection of microbial communities is a critical factor influencing the success of biomethanation processes. While mixed microbial consortia have traditionally been used in biomethanation studies due to their metabolic versatility and resilience, monocultures are increasingly being explored for their targeted and controlled performance in specific conditions. Previous studies have demonstrated that methanogenic monocultures, particularly hydrogenotrophic strains, can achieve high methane yields under optimized conditions [19]. In this study, biogas digestate, a by-product of anaerobic digestion, and *Methanobacterium alcaliphilum* [20], a monoculture strain adapted to alkaline conditions, were investigated as an inocula. The use of *Methanobacterium alcaliphilum* provides valuable insight into the performance of monocultures in biomethanation, particularly in conditions requiring adaptation to high pH. Comparing these inocula provides insights into optimizing microbial communities for methane production under varying conditions. This research contributes to identifying sustainable materials and microbial systems for enhanced biomethanation processes. Choosing these parameters for ex-situ biomethanation is a critical factor to consider when developing biogas upgrading technologies.

2. MATERIALS AND METHODOLOGY

2.1. Selecting Materials and Microorganisms

This study explores the potential of using industrial waste materials as filter substrates in biomethanation processes, focusing on woodchip ash and waste glass-based materials. These materials aim to serve as carriers for immobilizing methanogenic microorganism biofilms. Woodchip ash, often considered waste, can be valorised by converting it into aggregates with an increased surface area for gas-liquid transfer. Similarly, glass foam, made from recycled glass, offers high surface area, permeability, and chemical resistance, aligning with sustainable production trends. Additionally, expanded clay, commonly used in horticulture and construction [20], and polyurethane foam (PUF), a synthetic material with high porosity, were included for comparison. Both expanded clay and PUF provide well-documented performance data, enabling comprehensive evaluation [10], [15], [21]. Four filter materials were tested in the experiments, including polyurethane foam (PUF), expanded clay (EC), vulcanized ash material (VAM) as well as glass foam (GF).

Fig. 1. Filter materials used in the research: From left: PUF – polyurethane foam; EC – expanded clay, GF – vulcanised ash material; GF – glass foam.

Laboratory glass bottles with volumes of 250 mL were used as bioreactors in separate experimental setups. For each type of material, three replicate bioreactors were prepared to enable statistical analysis of the results. Additionally, control bioreactors without any filter

materials were included to assess whether the presence of filter materials enhances biomethanation efficiency compared to reactors without any materials.

The digestate serves as a stable microbial community well adapted to anaerobic environments, enabling efficient methanogenesis. Digestate is a by-product of existing biogas plants, making it an accessible and cost-effective source of inoculum for ex-situ biomethanation. Its use also reflects the actual conditions present in commercial biomethanation systems. For the experiments, the inoculum was sourced from the digestate of the biogas plant operated by "Agro Iecava" Ltd. After collection, the digestate was incubated at 37 °C for seven days, with excess gas being removed daily. Following degassing, the digestate was sieved to remove particles larger than 2 mm. The dry matter content was determined by drying the sample at 105 °C in an Ecocell oven for 24 hours and calculating the mass difference before and after drying.

To enhance the biomethane concentration in the final product, a strain of *Methanobacterium alcaliphilum* was tested alongside biogas digestate as the inoculum. These alkalophilic methanogens, originally isolated from lake sediments in the Wadi el Natrun region of Egypt, were obtained from the Leibniz Institute DSMZ-German Collection [23]. They are hydrogen-oxidizing and carbon dioxide-reducing methanogens, adapted to environments with low dissolved salt concentrations and pH levels ranging from 8 to 10. The *Methanobacterium alcaliphilum* strain used is a monoculture specifically chosen for its ability to thrive in alkaline conditions [20], [23]. This selection was based on the pH levels of the VAM material, which were measured to be around 10–11 [24]. Such high alkalinity was anticipated to align with the microorganism's optimal growth range.

Bacterial cell microscopy was used to monitor growing colonies of microorganisms (Fig. 2). Gram staining of bacteria was done to improve their visibility in the microscope and to facilitate identification and study.

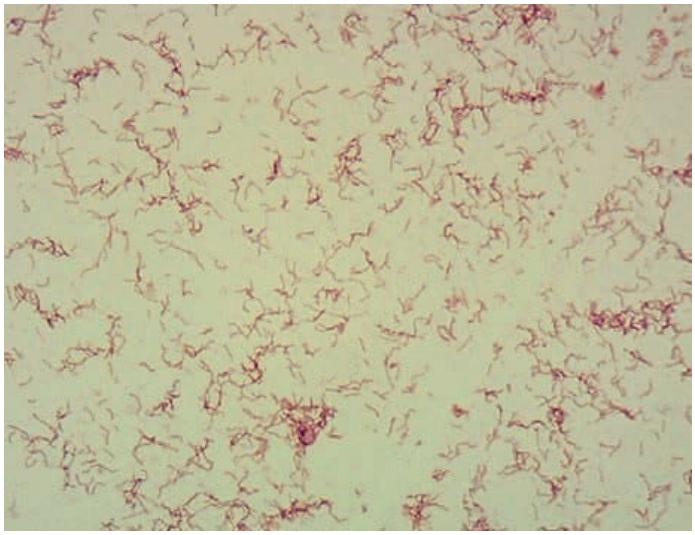

Fig. 2. Methanogenic bacteria: 200× magnification, stained bacterial cells.

2.2. Anaerobic Media

Methanogens utilizing H_2/CO_2 as substrates were cultured in an oxygen-free environment with a gas mixture of 80 % H_2 and 20 % CO_2, pressurized between 0.5 and 1 bar. To maintain optimal growth conditions, fresh gas mixtures were supplied periodically to prevent pressure drops and to remove CH_4 produced by the microorganisms. The medium for the methanogens required low redox potential (0.33 V) and minimal oxygen. To achieve this, oxygen was removed by boiling, maintaining an anaerobic atmosphere, and adding a reducing agent along with resazurin as an oxidation-reduction indicator [23]. For *Methanobacterium alcaliphilum*, the medium was prepared using pre-made solutions A, B, and C, adjusted to a pH of 8.3–8.4 [24]. After sterilization, the medium was reduced with H_2/CO_2 gas before inoculating the microorganisms at a pressure of 1.5 bar. In experiments involving biogas digestate, a basic anaerobic medium supplemented with macronutrients, trace elements, and vitamins was used, stabilized with cysteine hydrochloride and $NaHCO_3$ [25]. This medium was autoclaved, flushed with H_2/CO_2 gas and inoculated under similar anaerobic conditions.

2.3. Manometric Test

For the manometric test, each reactor was loaded with 200 mL wet filter material, 2 mL inoculum, and 30 mL anaerobic medium [26], [27]. A gas mixture of 80 % H_2 and 20 % CO_2 was introduced to sustain methane conversion. The serum bottles were tightly sealed with rubber stoppers, secured in place using aluminium caps to ensure an airtight environment. Three replicates were prepared for each filter material type, along with five control reactors that followed the same procedure but without inoculum. The control reactors accounted for pressure drops due to potential gas leakage during reactor punctures.

After sealing the reactors, needles were inserted through the rubber stoppers to fill them with a gas mixture, reaching an absolute pressure of approximately 1.5 bar. Pressure measurements were conducted using an Additel 672 pressure gauge, connected to the reactors via a needle inserted through the rubber stopper (Fig. 3).

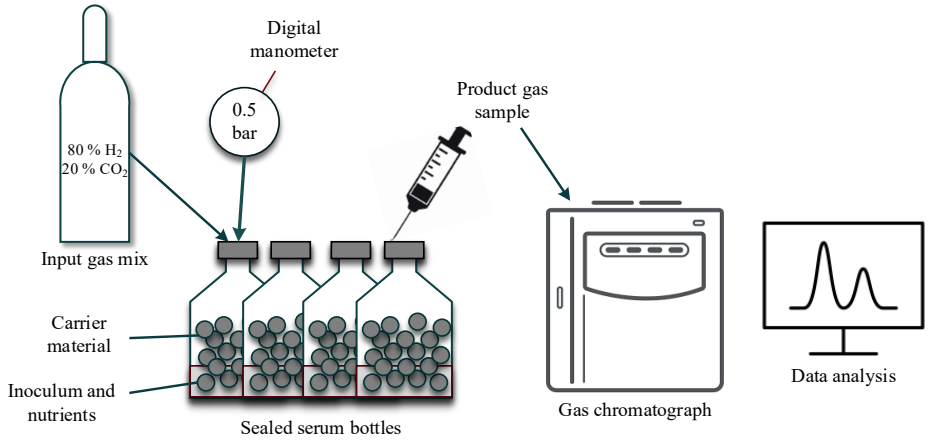

Fig. 3. Manometric test: filling serum bottles with a mixture of CO_2 and H_2 gas and analysing the samples. Needles attached to the measuring instruments with silicone tubing are inserted into the serum bottles through the rubber cap. The silicone tubing connects the needles of the measuring instruments in the serum bottle to the manometer and the mixture of CO_2 and H_2 gases. The CO_2 and H_2 gas mixture is injected to an absolute pressure of approximately 1.5 bar. After incubation at 37 °C, periodic pressure measurements are taken with a digital manometer. At the end of the test, samples of the gases are collected by syringe and then used for gas composition analyses with a gas chromatograph and the data are analysed.

The prepared serum bottles were then incubated at a constant temperature of 37 °C, positioned upside down. Daily measurements recorded pressure drops during the manometric test. The manometric test is divided into two phases: Phase I – enrichment and Phase II – testing. During both phases, pressure measurements are taken at regular intervals to track the rate of pressure decline. This trend reflects the rate of CH_4 production in the reactors. By analysing the pressure drop, which serves as an indicator of the hydrogenotrophic methanogenesis process, the CH_4 production potential of various filter materials can be effectively compared.

In the first stage, methanogenic microorganisms form a biofilm on the filter material. Pressure is measured daily using a digital gauge until it stabilizes for two consecutive days, marking the minimum pressure. The bottles are then refilled with H_2/CO_2 gas mixture to an absolute pressure of 1.5 bar and incubated. This process continues until the pressure drop within 24 hours reaches the minimum value, indicating the biofilm is fully developed. In the second stage, the bottles are refilled with H_2/CO_2 gas mixture and incubated again. Pressure measurements are taken more frequently at intervals such as 1, 2, 3, 5, 7, 10, and 24 hours to closely monitor and analyse the methanogenesis process and biomethane production dynamics.

2.4. Theoretical Methane Determination

The manometric method is a straightforward and cost-efficient approach for comparing various filter materials under laboratory conditions [26]. It utilizes materials similar to those used in biochemical methane potential test experiments (BMP), making it practical and compatible [28]. Its simplicity and affordability make it an excellent choice for evaluating filter material performance in ex-situ biomethanation setups. This method also allows for estimating the rate of hydrogenotrophic methanogenesis through stoichiometric calculations based on the established metabolic reaction. The methane production is directly influenced by the extent of hydrogenotrophic methanogen enrichment within bioreactors. Based on the Sabatier reaction equation [29], methanogenic microorganisms are known to generate 0.2445 moles of methane for every mole of hydrogen consumed (Eq. 1).

$$4.082H_2 + 1.031CO_2 + 0.008HCO_3^- + 0.008NH_4^+ \rightarrow$$
$$\rightarrow CH_4 + 0.039CH_{1,8}O_{0.5}N_{0.2(s)} + 2.066H_2O \quad (1)$$

The methane production can be calculated using the stoichiometric equation in combination with the ideal gas law. Assuming that the water generated remains in liquid form within the biofilm, the reaction leads to a reduction of 4.113 moles of total gas in the reactor (1.031 moles of CO_2 + 4.082 moles of H_2 – 1 mole of CH_4). This reduction is expected to correspond directly with a decrease in the total pressure inside the bioreactor. Therefore, the dynamics of hydrogenotrophic methanogenesis can be assessed by periodically monitoring the rate of pressure decrease in the reactor. These pressure readings are then used to estimate the theoretical methane yield. Once the experimental data were cleaned and organized, standardization was conducted to enable accurate comparison of results. This included adjusting measured gas pressures for temperature, gas volume, and water vapor pressure using Eq. (2) as outlined in the guidelines [30]:

$$V_{\text{std}} = V_{\text{meas}} \cdot \frac{(p_{\text{meas}} - p_{\text{H2O}})273.15\ \text{K}}{101.325\ \text{kPa}(T_{\text{meas}} + 273.15\ \text{K})}, \quad (2)$$

where
p_{meas} measured gas pressure, kPa;
T_{meas} gas temperature at the time of volumetric determination, °C;
P_{H2O} water vapour partial pressure, kPa;
V_{std} standardised gas volume, NmL;
273.15 K temperature;
101.325 kPa standard pressure.

Pressure measurements were initially taken as gauge pressure and later converted to absolute pressure by adding the ambient pressure at each measurement point to the recorded gauge pressure. To accurately account for potential gas losses caused by reactor punctures, the pressure drop in the control reactors was monitored, and this correction was applied to the calculated absolute pressure values. Methane production was determined using the ideal gas law and stoichiometric Eq. (1), which models the metabolism of hydrogenotrophic methanogens based on experimental data and adjusts methane yield according to the hydrogen mole fraction unit used. The total number of moles of gas produced was subsequently calculated using Eq. (3):

$$n_j = \frac{P_j V}{RT}, \tag{3}$$

where
n_j production of gas at a time point j, mol;
P_j pressure at a time point j, bar;
V reactor volume, L;
R ideal gas constant, L·bar·K^{-1} ·mol^{-1};
T temperature, K.

The calculated moles were standardised to normal conditions (1 atm pressure and 0 °C temperature), and the number of moles of methane produced (Eq. (4)) and the volume of methane produced (Eq. (5)) were determined:

$$n_{CH_4} = \frac{n_{j,std} - n_{j-1,std}}{4.113}, \tag{4}$$

$$V_{CH_4} = n_{CH_4} \cdot V_M, \tag{5}$$

where
n_{CH_4} methane gas content, mol;
n_{std} quantity of standardised gas, mol;
V_M ideal gas molar volume, L·mol^{-1};
V_{CH_4} methane mole volume, L·mol^{-1}.

2.5. Methane Measurements by Gas Chromatography

The gas samples were collected using syringes and then hermetically sealed with stoppers to prevent any leakage of gases before analysis. The collected gas samples were analysed using a Shimadzu Nexis GC-2030 gas chromatograph. It is equipped with two parallel analysis lines as well as a flame ionisation detector (FID) and a thermal conductivity detector

(TCD). A Restek Rt-Q-Bond column (30 m, 0.53 mm inner diameter, 20 µm film thickness) with an FID detector was used for the analysis of hydrocarbon compounds, which provides high sensitivity and accuracy in the detection of hydrocarbons. A TCD detector coupled to a three-column system was used to analyse H_2, N_2, CO, CH_4 and CO_2. This system consisted of a size exclusion pre-column (Restek Porapak Q 80/100), a size exclusion column (Restek Porapak Q 80/100) and a molecular sieve column (Restek Molsieve 5A 60/80). This sophisticated column system ensures efficient and accurate separation and quantification of the different gas components, allowing detailed data on the composition of the gases to be obtained.

3. Results and Discussion

3.1. Maximum Specific Volume of Methane Produced in Manometric Test

The experiment began with the development of a biofilm within the reactor, marking the first phase in manometric test. The biofilm was considered mature when the pressure drop or methane production peaked within a 24-hour period. Once the maximum pressure drop was achieved, the experiment transitioned to the second phase. During this stage, pressure measurements were taken at shorter intervals throughout the day, with the final measurement recorded 24 hours after the phase began. The specific methane production in the reactors was calculated using the Sabatier stoichiometric equation. The total methane production potential of each filter material can be evaluated by comparing the maximum methane yield or the lowest pressure recorded 24 hours after gas injection during the second stage.

The cumulative CH_4 production in the bioreactors revealed that the highest methane volumes were achieved with GF and EC filter materials. While their results were nearly identical, the EC material exhibited a slightly slower methanogenesis rate compared to GF during the initial hours (Fig. 4). Reactors without any filter material produced the next highest biomethane content, though it was three times lower than the levels observed in the EC and GF reactors.

The bioreactors containing VAM material produced the lowest methane levels, consistent with previous experiments [27]. This outcome is attributed to the high pH of the medium, which inhibits microbial growth. The cumulative methane production followed the order: GF > EC > Control > PUF > VAM.

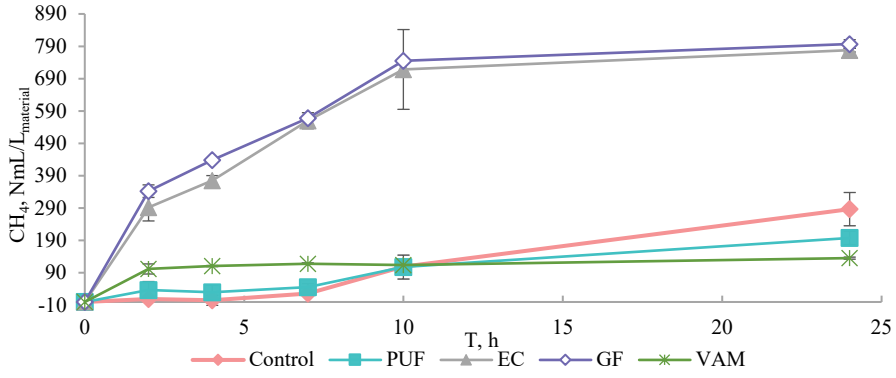

Fig. 4. Calculated specific volume of CH_4 produced in 250 mL reactors with digestate: Control – no material; PUF – polyurethane foam; EC – expanded clay; GF – glass foam; VAM – vulcanised ash material. Error bars – standard deviation. Maximum methane production decreased between GF > EC > Control > PUF > VAM filter materials.

In the next experiment, the strain of *Methanobacterium alcaliphilum* was tested to evaluate their adaptation to alkaline environments and their potential for biomethanation with VAM filter material. During the initial biofilm formation stage, it became evident that methane production would be lower, as the enrichment phase took twice as long as within reactors with biogas digestate. Despite the extended duration, the maximum pressure drop was still less than that observed with digestate. Consequently, the second stage of the manometric test was initiated to assess the impact of filter materials on methanogenesis using *Methanobacterium alcaliphilum*.

Significant differences between digestate and monocultures in the first phase highlighted that the two are not comparable, with digestate proving to be the more efficient solution.

During the second phase of the test, measurements taken within the first 24 hours revealed a similar, though relatively small, pressure drop across all reactors, including those without filter material. After performing stoichiometric calculations, the cumulative methane production was determined. The results showed that the highest average methane levels were observed in reactors containing VAM, EC, GF materials, as well as in the control reactors (Fig. 5).

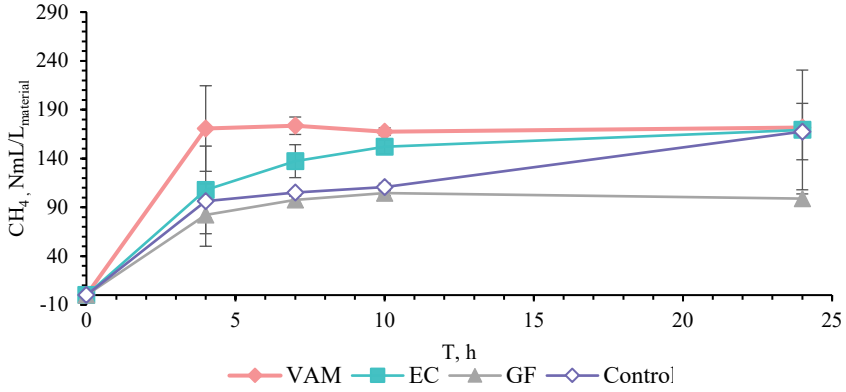

Fig. 5. Calculated specific volume of CH4 produced in 250 mL reactors with *Methanobacterium alcaliphilum* monoculture: Control – no material; EC – expanded clay; GF – glass foam; VAM – vulcanised ash material.

3.2. Gas Analysis

To confirm the results of the *ex-situ* biomethanation experiment conducted through the manometric test, the final product gas samples were analysed using gas chromatography to determine their chemical composition. This method allowed for the identification and quantification of H_2, N_2, CO, CH_4, and CO_2 in the samples. The analysis revealed that reactors with EC and GF filter materials produced the highest methane yields when biogas digestate was used as the inoculum.

Reactors containing glass foam produced the highest average CH_4 concentration at 79.7 %, followed by reactors with EC, which averaged 75.4 % (Fig. 6). The methane yield and content observed for glass foam and expanded clay are consistent with results from similar biomethanation studies. For example, studies using trickle-bed reactors with similar inoculum reported methane yields ranging from 70 % to 80 % [10]. However, the limited performance of VAM aligns with findings that alkaline substrates can inhibit microbial activity, particularly when heavy metal leaching occurs. Reactors with PUF material showed an

unusually low CH_4 yield of 5.6 %, comparable to the control reactors without filter material, which had 5.3 %.

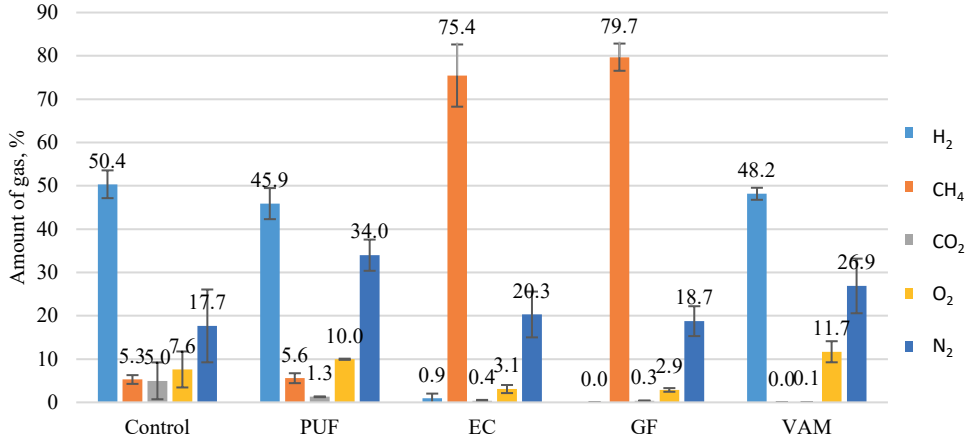

Fig. 6. Chemical composition of gas samples in which biogas digestate was used: PUF – polyurethane foam; EC – expanded clay; GF – glass foam; VAM – vulcanised ash material.

Reactors with the highest CH_4 levels had the lowest H_2 concentrations, indicating that microorganisms efficiently utilized hydrogen. Conversely, reactors with low CH_4 concentrations contained a significant amount of unconsumed H_2. While PUF performed well in previous experiments, this result may be an anomaly [24]. Microorganism viability could have been affected by growth conditions or nutrient availability. Since the enrichment phases of the manometric test varied among materials, microorganism inhibition may have occurred during the intervals between gas mixture additions. Additionally, a higher microbial population in the bioreactors would demand more nutrients, potentially influencing results.

Gas analysis of the *Methanobacterium alcaliphilum* monocultures revealed a minimal presence of methane in the tested materials. The highest amount of CH_4 was found in the control reactors without filter material at 18.56 % (Fig. 7).

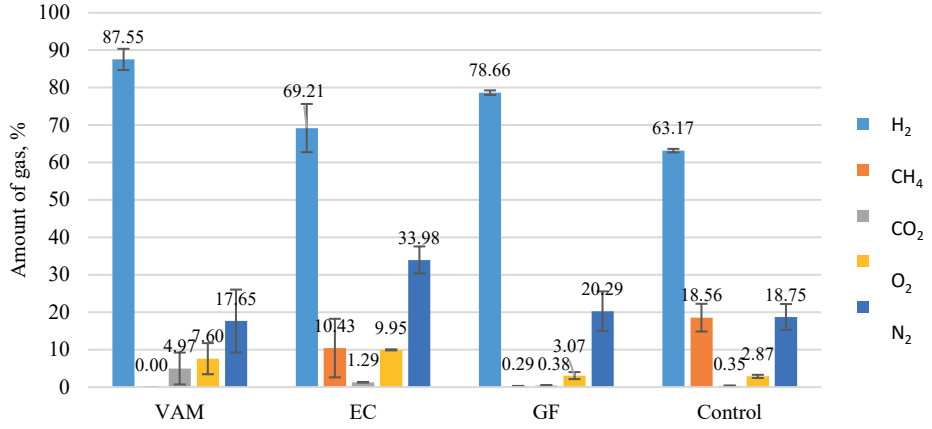

Fig. 7. Gas composition of the samples using *Methanobacterium alcaliphilum* monoculture. VAM – vulcanised ash material; EC – expanded clay; GF – glass foam.

This emphasizes the role of microbial inoculum over the carrier material in methane production. The highest result of methane in reactors filled with filter material was achieved in reactor with GF at 10.43 %.

A comparison of gas composition in this test indicates that reactors inoculated with *Methanobacterium alcaliphilum* monocultures exhibited more efficient methanogenesis compared to the control reactor without filter material. This suggests that inoculating *Methanobacterium alcaliphilum* monocultures along with filter materials enhances methane production efficiency. The lower methane yields observed with *Methanobacterium alcaliphilum* monoculture may be attributed to a combination of factors, including suboptimal growth conditions, and nutrient limitations. The extended enrichment phase and insufficient nutrient replenishment likely affected microbial viability and methanogenic activity. Although *Methanobacterium alcaliphilum* was specifically chosen for its ability to thrive in alkaline environments, it did not perform efficiently with VAM as the carrier material. The alkalinity of VAM, combined with potential heavy metal leaching, likely exceeded the microorganism's tolerance limits [31], inhibiting its metabolic activity and methane production despite its natural adaptation to moderately alkaline conditions.

Future studies will include PUF to explore its performance under similar conditions.

Regression analysis was performed to compare calculated and analysed CH_4 fractions in the digestate-inoculum experiment. The results showed a strong linear correlation (coefficient of determination 92) between estimated and measured CH_4 values, indicating that 92 % of the variation in methane abundance is explained by the calculations. This high coefficient of determination confirms the reliability of the model in predicting actual methane levels.

4. CONCLUSION

Biomethanation transforms CO_2 into methane while recycling organic matter into nutrient-rich digestate, simultaneously supporting renewable energy production, improving soil fertility, and promoting carbon sequestration. This combined advantage aligns agricultural practices with climate sustainability goals, offering farmers a sustainable approach to lowering emissions. Integrating biogas production and biomethanation into carbon farming can help establish resilient agricultural systems and advance eco-friendly waste management practices. This emphasizes the importance of advancing biomethanation technologies to support circular economy principles and carbon farming initiatives.

The study confirmed that glass foam - filter material derived from glass waste – has potential for use in biotrickling filter reactors for *ex-situ* biomethanation. However, filter material made from vulcanized wood ash does not show good results – no methane was produced in these bioereactors. While glass foam demonstrated excellent suitability, achieving up to 84 % CH_4 content and highlighting its innovative qualities, ash-based material did not perform well due to their alkaline nature and the potential release of heavy metals, which negatively affect microorganisms. Glass foam, with its high porosity, surface area, and low density, emerges as a promising material for biotrickling filter reactors, aligning with circular economy principles by repurposing waste into valuable resources. In contrast, vulcanized ash material (VAM) proved unsuitable for methanogenesis, regardless of the inoculum used, indicating the need for further research into waste ash recycling technologies.

These findings highlight the importance of selecting appropriate filter materials for biomethanation, as they directly impact microorganism immobilization and methanogenesis efficiency. Also, selecting microbial community for biomethanation plays important role in effectiveness of technology. In this case, biogas digestate inoculum outperformed

Methanobacterium alcaliphilum monoculture strain in methane production, emphasizing the need for further studies on microbial compositions, particularly in larger reactors. The manometric method, validated against gas chromatography, was effective for evaluating biomethane production in biotrickling filter reactors. While these experimental results may not directly translate to commercial reactors, they provide valuable insights into optimizing bioreactor configurations in early stages of technology development.

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