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Biogas Upgradation by CO_2 Sequestration and Simultaneous Production of Acetic Acid by Novel Isolated Bacteria

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Abstract: Anaerobic digestion produces biogas, which is a proven bioprocess for generating energy, recovering nutrients, and reusing waste materials. Generally, the biogas generated contains methane (CH_4) and carbon dioxide (CO_2) in a 3:2 ratio, which limits the usage of the biogas to only cooking gas. To further enhance the application of biogas to vehicular fuel and natural gas grids, CO_2 must be removed for an enhanced calorific value. This study seeks to lower greenhouse gas emissions by sequestering carbon dioxide from biogas. CO_2 sequestration by microorganisms to upgrade the biogas and simultaneously convert the CO_2 into acetic acid is a less explored area of research. Therefore, this research focuses mainly on the analysis of CO_2 consumption % and acetic acid yield by novel isolated bacteria from fruit waste and mixed consortia obtained from cow dung and digested samples. The research finding states that there was a 32% increase in methane yield shown by isolated strain A1, i.e., CH_4% was increased from 60% to 90%, whereas only an 11% increase was shown by consortia, which was an increase from 60% to 80%. The highest biogas upgradation was shown by the A1 strain at 30 °C incubation temperature and pH 8. The A1 strain demonstrated the highest recorded yield of acetic acid, reaching a concentration of 2215 mg/L at pH 8. A pH range of 7–8 was found to be the best-suited pH, and a mesophilic temperature was optimum for CO_2 consumption and acetic acid production. The major objective is to create an effective method for improving biogas so that it is acceptable for different energy applications by lowering the carbon dioxide content and raising the methane content. This development signifies a significant advancement in the enhancement of biogas upgradation, as well as the concurrent generation of value-added goods, thereby establishing a sustainable platform technology.

Keywords: biomethane; acetogens; mix consortia; pure isolates; GHG emissions

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1. Introduction

The substantial global energy demand substantially exacerbates the ongoing environmental crisis. According to the findings of the Intergovernmental Panel on Climate Change (IPCC) Working Group III (WG3), the primary sources of global greenhouse gas (GHG) emissions can be broken down into a wide variety of sectors, such as the energy sector, industry, buildings, transportation, agriculture, forestry, and other land uses. The assessment conducted by the United Nations on the Sustainable Development Goals (SDGs) emphasizes the critical need to accelerate endeavors pertaining to modern renewable energy (SDG 7) and the shift in economies toward carbon neutrality (SDG 13) (United Nations. Department of Economic and Social Affairs. The Sustainable Development Goals: Report 2022. UN, 2022). After petroleum,

biomass emerges as the pre-eminent repository of carbonaceous materials that are readily available on the earth. Utilizing this resource for the purposes of industrial bio-manufacturing signifies a significant advancement in the endeavor to establish a circular economy on a worldwide level [1–3]. Through the process of anaerobic digestion, it is possible to convert various types of biomass feedstocks into biogas [4,5]. The estimation of potential biogas generation from waste sources indicates a substantial energy resource, amounting to approximately 36 Kilojoules per year. This value corresponds to approximately 8% of the current total energy demand, which is estimated to be around 12 watt/h out of a total energy demand of approximately 165 watt/h [6]. The embrace of biogas generation is witnessing a significant upswing in Europe, while in the United States, the utilization of various resources remains comparatively untapped. This variation can be ascribed to the differing rates of energy sector transitions in the two areas [7,8].

The incorporation of various bio- and/or chemical procedures helps to alleviate the possible constraints that can emerge when depending exclusively on an independent process [9]. The aim of biogas enhancement is to address the challenges linked with biogas generation by efficiently removing or reusing the contaminants found in the biogas. The existence of CO_2 within biogas has been identified as having a significant influence on its heat content. Moreover, the substantial existence of CO_2 has been noted to reduce the energy potential of biogas. Moreover, the presence of minute quantities of NH_3, hydrogen sulfide H_2S, and siloxanes in biogas has been linked to potential harm to infrastructure [4].

Presently available technologies for enhancing biogas encompass a range of approaches, including absorption, adsorption, membrane-based filtration, and cryogenic separation. Nevertheless, it is essential to acknowledge that these methods encounter specific difficulties concerning expenses, energy usage, productivity, and robustness [4]. Promising novel biogas enhancement methods offer substantial potential in providing timely economic and ecological benefits for biogas generation, thereby promoting its extensive adoption and aligning with the principles of a circular economy [7].

Approximately 280 biogas facilities worldwide are actively engaged in the biogas enhancement process, utilizing a range of different technologies [10]. The dominant methods that have gained extensive acceptance include adsorption approaches like pressure shift adsorption, absorption techniques like pressurized water cleansing, physical or chemical absorption, membrane procedures utilizing elevated or reduced pressure, and cryogenic separation [11,12]. The expenses linked with these methods are relatively high, owing to the need for either functioning in high-pressure settings, incorporating chemical enhancements, or using specialized membranes [11,13]. Furthermore, it is crucial to emphasize that the extraction of CO_2 from biogas using these methods poses a significant constraint regarding the loss of CH_4. Therefore, an opportunity exists for the development of innovative methods geared toward improving the overall effectiveness of biogas enhancement procedures. At the same time, these techniques should also aim to reduce the economic load linked to investment operations. Gas purification with the biological method is an exceptionally promising technology due to its remarkable efficiency and cost-effectiveness in both investment and operations [14–16].

Various biological processes have been used in the field of biogas upgrading. For instance, one approach involves utilizing microalgae to effectively capture CO_2 from biogas [11]. Another method involves the utilization of hydrogenotrophic and methanogenic microbes, which can convert CO_2 and H_2 into CH_4 either in situ or ex situ. However, the exploration of selecting suitable microorganisms for the purpose of enhancing biogas upgrading in an efficient and cost-effective manner remains an unexplored area of research.

Lately, there has been a notable increase in the focus directed toward acetogenic microorganisms in the field of bioenergy technology.

This intensified fascination arises due to their impressive ability to efficiently transform C1 compounds, like CO_2, via the acetyl-CoA metabolic pathway. Due to this metabolic mechanism, these bacteria can produce valuable substances, including organic acids, that carry substantial promise for application within the chemical sector [17]. The bacteria

currently under study, including a striking variety of 23 unique genera and over 100 recognized species, have been definitively categorized as obligate anaerobes. Their metabolic processes lead to the impressive generation of billions of metric tons of acetate each year, establishing it as their primary fermentation byproduct worldwide [18]. Acetogenic microorganisms have been identified in a broad spectrum of environments, displaying an impressive capacity to adjust to diverse ecological circumstances. These settings include various soil varieties and the digestive tracts of termites. Remarkably, acetogens have showcased their aptitude for flourishing in settings marked by substantial variations in temperature, pH levels, and salinity [19]. Diverse pure acetogenic cultures have found application in the realm of biochemical manufacturing. With the examination of pure cultures, it has been determined that various microorganisms, including *Acetobacterium woodii* and *Clostridium* spp., exhibit a remarkable ability to convert CO_2 and H_2 into liquid products [20].

Lately, there has been an increasing focus on the application of both blended community and uncontaminated culture fermentation. This is primarily ascribed to the myriad advantages linked to these methodologies, particularly process resilience, especially within the framework of continuous operations. Considering the combined discoveries, it is reasonable to contemplate transforming CO_2 obtained from biogas into more valuable foundational components, particularly acetic acid, instead of generating CH_4. This transformation can be accomplished using either mixed culture acetogenic groups or pure isolates.

The primary objective of the current investigation was to establish an innovative bioprocess that incorporates the utilization of acetogenic bacterial consortia and pure isolates for the purpose of effectively enhancing the yield of CH_4 through CO_2 fixation, ultimately resulting in the production of acetic acid. Consequently, a comprehensive set of experiments was conducted to ascertain the optimal experimental parameters by manipulating the pH and incubation temperature of the reaction medium.

With the development of a novel methodology aimed at effectively sequestering carbon dioxide derived from biogas, this study endeavors to mitigate the adverse effects of greenhouse gas emissions, thereby contributing to global efforts in combating climate change. The present study presents an opportunity to explore the feasibility of establishing a sustainable and economically viable means of producing acetic acid. In addition, the production of biogas with the utilization of organic waste, coupled with the concurrent conversion of CO_2 present in biogas into acetic acid and cleaner energy, serves to enhance the overall worth of waste streams while also contributing to the effective management of waste. The implementation of biogas upgrading processes has been found to have a significant impact on the overall efficiency and cost-effectiveness of biogas utilization. By upgrading biogas, the requirement for supplementary purification measures can be minimized, thereby offering the potential for substantial cost savings. In short, this study possesses the potential to yield extensive contributions with its comprehensive approach toward addressing prevalent environmental challenges. Furthermore, it has the capacity to generate promising economic opportunities while simultaneously advancing scientific knowledge in the field. Moreover, the investigation aims to foster the sustainable utilization of biogas and other renewable energy sources, thereby promoting a greener and more sustainable future. The phenomenon under consideration possesses the capacity to exert influence across various sectors, thereby bolstering endeavors toward global sustainability.

2. Materials and Methods

2.1. Bacterial Isolation and Culture Maintenance

The bacteria were isolated from fruit waste and cow dung samples. Fruit waste consisting of a high sugar content and cow dung having a high amount of acetogens were selected for isolating acetic acid-producing bacteria. All the isolations were performed using the serial dilution method, and the selection of the isolate was performed based on the halazones formed over the glucose yeast extract and calcium carbonate (GYC) media. Selected isolates were evaluated for acetic acid production using the acid–base titration

method. The highest-yielding isolates were selected for biogas upgradation in a previous study [21]. All the isolates were maintained on GYC agar plates at 30 °C. For the enrichment and maintenance of mixed consortia, basal anaerobic media were used.

2.2. Batch Experimental Conditions

A basic anaerobic media was used for the gas fermentation process. This media has a complex composition, including all kinds of essential nutrients, micro-macronutrients, vitamins, etc., for the proper metabolism of anaerobic bacteria. The detailed components of the basal anaerobic media are presented in Table 1 [22]. Further, the pH of the media was altered by using 1 M NaOH and 1 M HCl solutions. The batch experiment was carried out in 100 mL serum bottles. Each bottle contained 34 mL of media and 41 mL of biogas in a fixed ratio of 3:2 (CH_4:CO_2). The biogas used in this study was collected from an anaerobic digester running on the cow dung. The upgrade process was started using 10% inoculum. The bottles were sealed and autoclaved to maintain the sterile conditions. After the sterilization, inoculum, vitamin solution, and biogas were added. The bottles were incubated at 30 °C at 150 rpm in a horizontal position for better gas–liquid exchange for 7 days. All the batch bottles were analyzed for gas content using GC and acetic acid yield using HPLC.

Table 1. Detailed components for the preparation of basal anaerobic media.

Solution A (g/L)	Solution B (g/L)	Solution C (g/L)	Solution D (g/L)	Solution E (mg/L)
NH_4Cl-100	$K_2HPO_4 \cdot 3H_2O$-200	Resazurin-0.5	$FeCl_2 \cdot 4H_2O$-2	Biotin-2
NaCl-10			H_3BO_3-0.05	Folic acid-2
$MgCl_2 \cdot 6H_2O$-10			$ZnCl_2$-0.05	Pyridoxine acid-10
$CaCl_2 \cdot 2H_2O$-5			$CuCl_2 \cdot 2H_2O$-0.038	Riboflavin-5
			$MnCl_2 \cdot 4H_2O$-0.05	Thiamine hydrochloride-5
			$(NH_4)6MO_7O_{24} \cdot 4H_2O$-0.05	Cynocobalamine-0.1
			$AlCl_3$-0.05	Nicotinic acid-5
			$CoCl_2 \cdot 6H_2O$-0.05	P-aminobenzoic acid-5
			$NiCl_2 \cdot 6H_2O$-0.092	Lipoic acid-5
			EDTA-0.5	DL-pantothenic acid-5
			Conc. HCl-1 mL	
			$Na_2SeO_3 \cdot 5H_2O$-0.1	

Add solution A-10 mL, B-2 mL, C-1 mL, D-1 mL, E-1 mL to 975 mL of distilled water. Add cysteine hydrochloride-0.5 g and $NaHCO_3$-2.6 g into 10 mL distilled water and mix this solution to the media. Sparge N_2: CO_2 (4:1) gas mixture into the media to maintain a neutral pH. Fill the media into the serum bottles as per the requirement and autoclave the media.

2.3. Experimental Analysis

Experiments were conducted for 7 days and compared the CO_2 consumption between the pure isolates and mixed consortia. The experiments were conducted at varying pH values and incubation temperatures, which are depicted in Table 2. Gas chromatography (TRACE 1110, ThermoFisher Scientific, Nashik, India) was conducted at MNIT Jaipur, Rajasthan, for all the experiment sets, which were conducted in triplicates for gas content and CO_2 consumption. The CH_4 and CO_2 percentage was analyzed using the gas chromatography protocol reported in Hniman, Prasertsan, and O-Thong, (2011) [23], and the column used was a carbon-packed column of 60/80 mesh, 1 m × 3.20 mm. The amount of acetic acid produced was analyzed on the 7th day using HPLC. Before the HPLC analysis for the detection of acetic acid, the liquid was centrifuged to remove any solid particles and filtered through a syringe filter, and each sample was diluted 10 times with distilled water. The column used for the detection of acetic acid was an Aminex HPX-87H Column 300 × 7.8 mm, 0.5 m. H_2SO_4 was used as a mobile phase with a 0.6 mL/min flow rate. Equation (1) was used for the calculation of CO_2 consumption.

$$CO_2 \text{ consumption}\% = \frac{\text{Volume of } CO_2 \text{ utilized(mL)}}{\text{Volume of } CO_2 \text{ inserted(mL)}} \times 100 \quad (1)$$

Table 2. Operating conditions for biogas upgradation analysis.

Experiments	Parameter	Operating Condition		
1	Effect of different pH values			
	pH	6	7	8
	Biogas ratio		3:2 (CH_4:CO_2)	
2	Effect of different temperatures (°C)			
	Temperature (°C)	30	35	40
	Biogas ratio		3:2 (CH_4:CO_2)	

3. Result and Discussion

3.1. Comparision of Biogas Upgradation Using Mixed Microbial Consortia and Pure Isolates

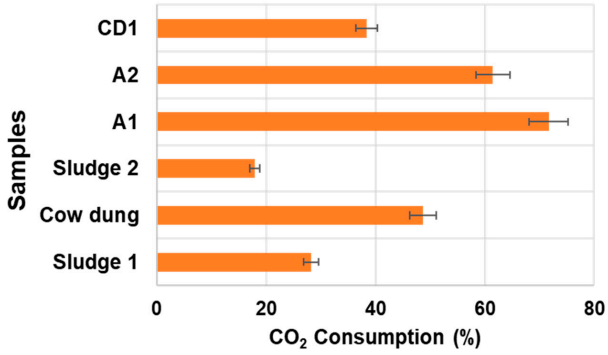
For the comparison of the CO_2 consumption of the mixed consortia and pure isolates, batch anaerobic digestion was performed in serum bottles. The experiments were conducted in triplicates, where the CO_2 consumption was analyzed daily for seven days with the help of gas chromatography. The analysis showed that the %CO_2 consumption was less in the initial 1–2 days of incubation, as some of the CO_2 may have been solubilized and metabolized by the bacteria.

The addition of resazurin to the basal anaerobic media converts the color of the media from blue to pink, which indicates the cell viability in the media during the total reaction time. Figure 1 depicts the experimental setup using a serum bottle. The mixed consortia were obtained from cow dung and two different kinds of digestate, one from the anaerobic digester working on cow dung (Sludge 1) and another working on the sewage sludge (sludge 2), were stabilized and maintained on the nasal anaerobic media. The isolates (A1, A2, CD1) were isolated from fruit waste [21]. The isolates were maintained on the GYC media at 37 °C. The CO_2 consumption in the mixed microbial consortia of S1, S2, and cow dung was 28.2%, 48.2%, and 17.94%, respectively. Figure 2 depicts a comparison between the pure isolates and mixed consortia in the production of acetic acid, CO_2 consumption, and biogas upgradation. Compared with this, CO_2 consumption was higher in the individual isolate, i.e., A1 showed 60.76%, A2 showed 75.32%, and CD1 showed 71.66%. Similarly, the acetic acid production of S1, S2, and cow dung was much lower than the individual isolates. There was an approximate 60% rise in acetic acid production in individual isolates compared with microbial consortia. The acetyl-co-A pathway is followed by acetogens, which consume the CO_2 and H_2 produced during metabolism and produce acetate [24]. Some of the acetoclastic and acetogenic bacterial species follow the Wood–Ljungdahl Pathway (WLP), in which bacteria fix the CO_2 and H_2 to acetic acid and butyric acid. The metabolic pathways demonstrated by acetoclastic microorganisms display variability, a phenomenon that can be ascribed to both the particular microbial species and the surrounding environmental conditions. Acetoclastic microorganisms of a specific nature have been observed to display a notable preference for anaerobic behavior, thriving in environments that are distinguished by low to negligible levels of oxygen. In contrast, it is worth noting that certain acetoclastic microorganisms exhibit a notable level of adaptability, which enables them to accommodate diverse metabolic pathways and adapt to varying environmental conditions. Approx. 2 moles of CO_2 is converted to 1 mol of acetyl co-A, and further, it is reduced to acetate [25]. Equation (2) shows the reaction taking place during the WLP, forming acetate from the reduction of CO_2 and a negative Gibbs free energy showing a thermodynamically spontaneous reaction [26].

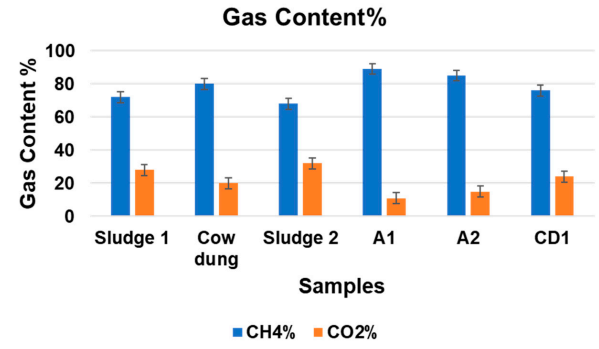
$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \ (\Delta G^\circ = -75.2 \text{ kJ/mol}) \quad (2)$$

(**a**) (**b**)

Figure 1. Serum bottles (**a**) before inoculation and (**b**) after inoculation.



(**a**)



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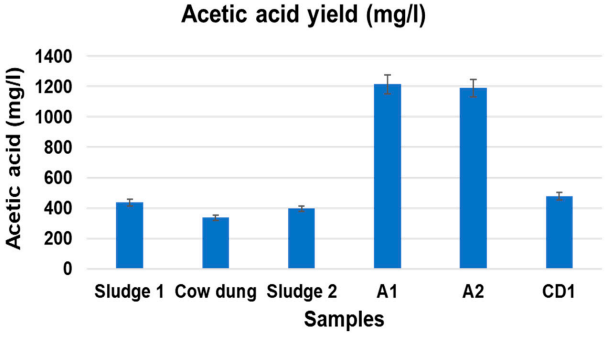


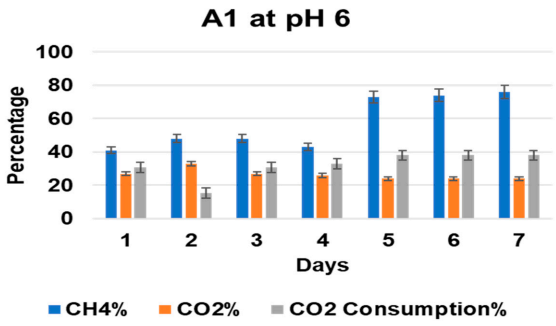
(**c**)

Figure 2. Graphs depicting (**a**) percent CO_2 consumption, (**b**) percent gas content, and (**c**) acetic acid yield. Incubated at 30 °C at 150 rpm for 7 days.

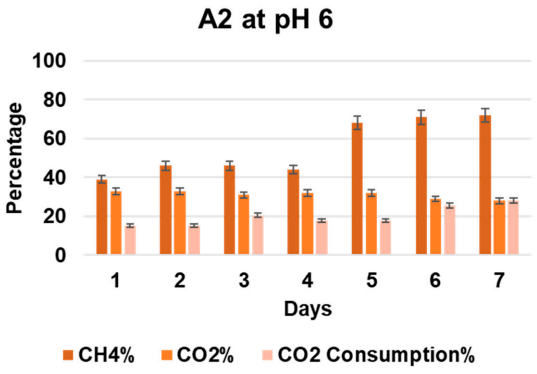
This study was performed in batch mode because the study of the metabolic pathway of acetogenic bacteria has shifted toward batch experiments so that it can be better commercialized [27]. Commonly used pure isolates of acetogenic bacteria used for acetic acid synthesis using CO_2 are *Murella*, *Clostridium Acetobacterium*, etc., species [27–30]. Most of the studies are on mixed cultures of acetogenic bacteria as they utilize a varied substrate, making it easier for environment biotechnologists to deal with waste [31]. Gas fermentation using microbial consortia poses a potential challenge in terms of reduced productivity, primarily attributed to the acetogenesis and hydrogenotrophic competitive methanogenesis. This competition arises due to both processes vying for the same substrate. Additionally, the acetate produced during the fermentation process can be diverted toward the production of methane through acetoclastic methanogenesis [32].

3.2. *Effect of pH on Biogas Upgradation and Acetic Acid Production*

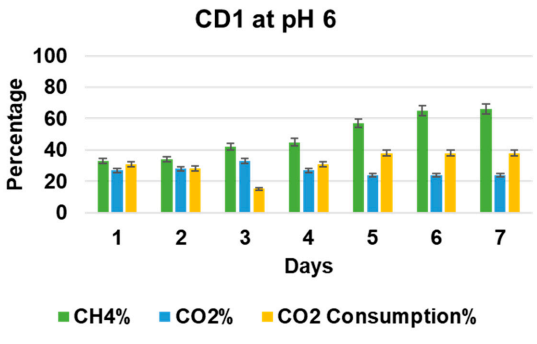
Pure isolates give a higher percentage of biomethane and higher CO_2 consumption; hence, further experiments with varying temperatures and pH values were conducted. The pH of the media was changed to three levels: 6, 7, and 8, keeping the temperature constant at 30 °C. The ratio of biogas was constant throughout the experiment, i.e., 3:2. The percent change in the gas content and the CO_2 consumption is depicted in Figure 3.



(**a**)

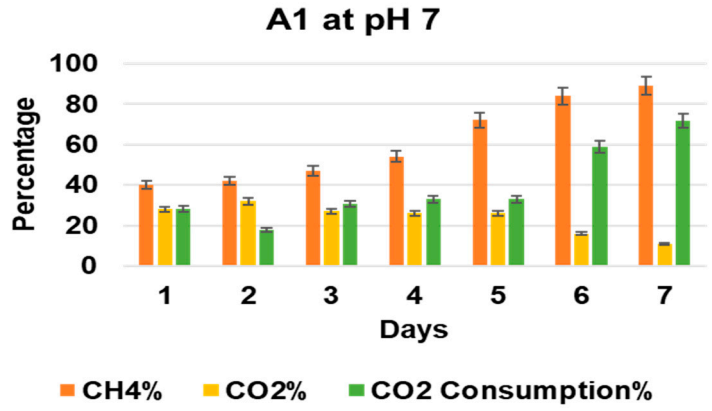


(**b**)



(**c**)

Figure 3. *Cont.*



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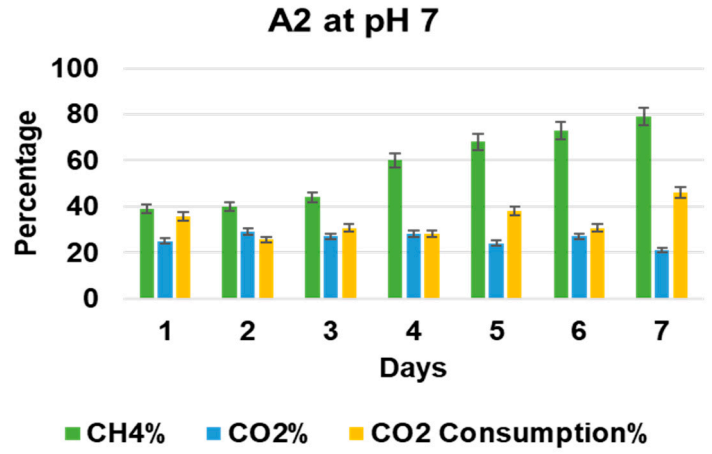

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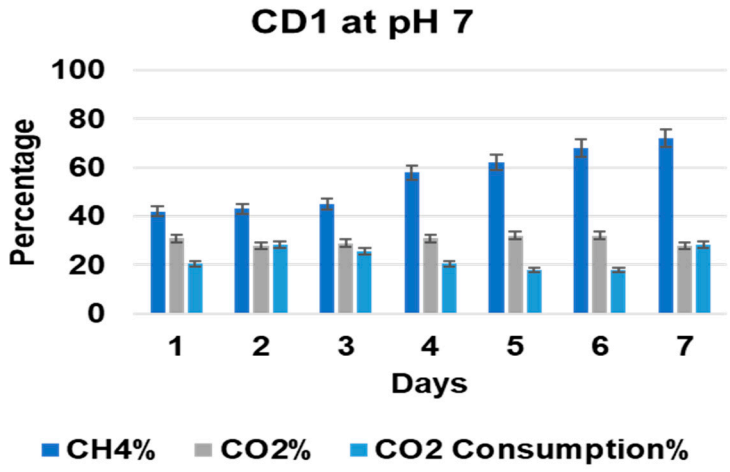
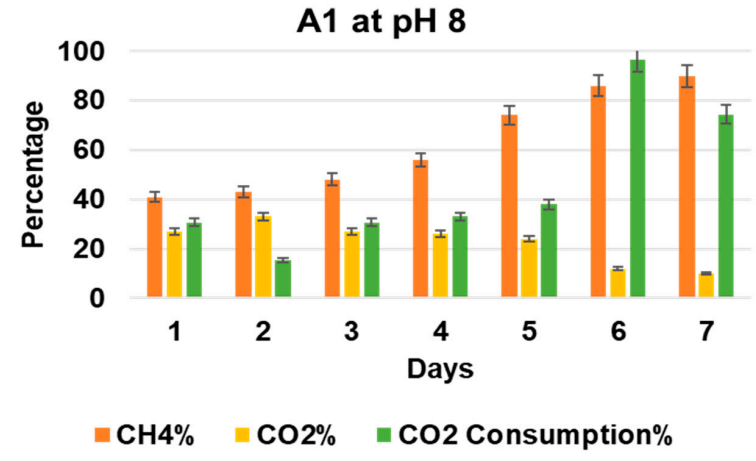


(**f**)

Figure 3. *Cont.*



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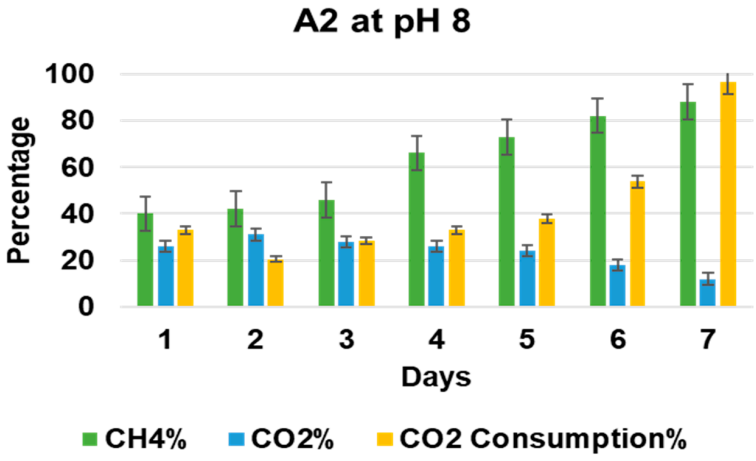
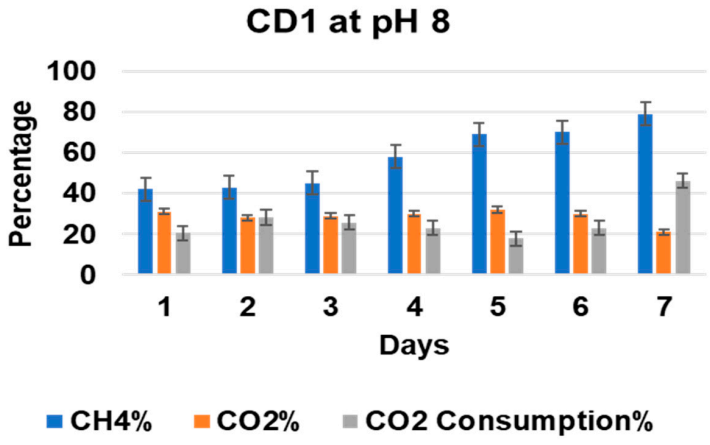


(**h**)

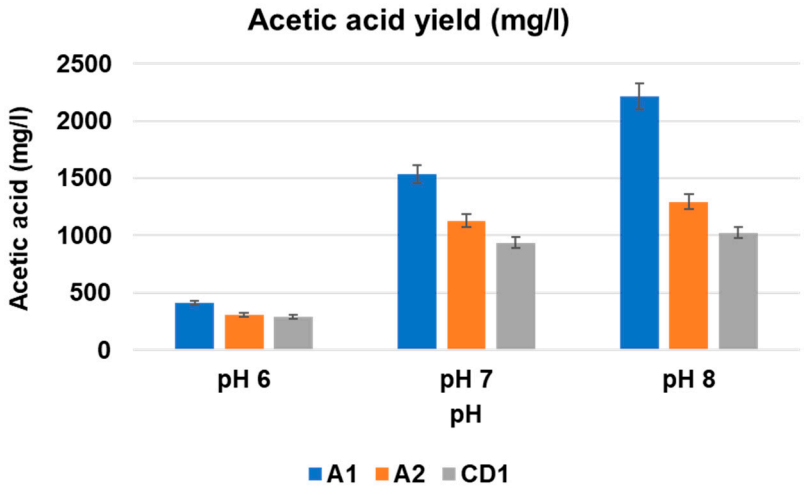


(**i**)

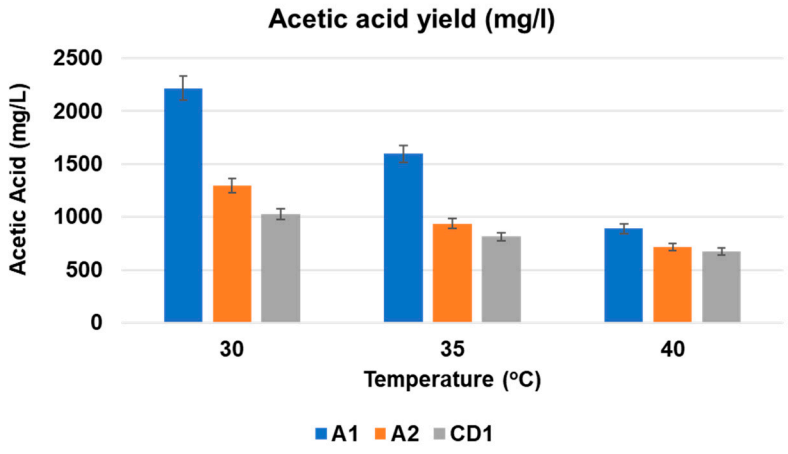
Figure 3. Graphs depicting CH_4%, CO_2%, and CO_2 consumption% by (**a**) A1 at pH 6, (**b**) A2 at pH 6, (**c**) CD1 at pH 6, (**d**) A1 at pH 7, (**e**) A2 at pH 7, (**f**) CD1 at pH 7, (**g**) A1 at pH 8, (**h**) A2 at pH 8, and (**i**) CD1 at pH 8.

At an alkaline pH, i.e., 8, maximum CO_2 was consumed and converted into acetate. At an acidic pH of 6, CO_2 consumption was much lower, and at pH 7, the CO_2 consumption was comparable to that at pH 8. The methane content was enhanced from 61% to 90% by the A1 isolate. All three isolates, i.e., A1, A2, and CD1, were higher compared with the results

at pH 6 and 7. The acetic yield (Figure 4a) was 2215 mg/L, which was the highest of all the sets of experiments. In all three pure isolates, A1 showed the maximum CO_2 consumption and highest acetic acid production, followed by the A2 isolate and the CD1 isolate. It was found that there was a 47% increase in CH_4 percentage by the A1 strain at pH 8, which was the highest, followed by A1, which showed a 44% increase, and CD1, which showed a 29% increase in methane percentage. At pH 6 and 7, the CH_4 percentage was much lower, showing a 46%, 30%, and 18% increase in CH_4 percentage by A1, A2, and CD1, respectively. Similarly, 26%, 18%, and 8% were the % increases in CH_4 shown by A1, A2, and CD1, respectively, at an acidic pH, i.e., pH 6. This trend can be observed in Figure 3a–c. The acetic acid yield was found to be 200 times more at pH 7 than at pH 6 by all three strains. At pH 8, A1 produced 44% more acetic acid than at pH 7, followed by A2, which produced 14% more, and CD1, which produced 10% more than at pH 7 (Figure 4a). Similar results were reported in a study by Atasoy et al., 2020 [33], in which it was evident that the maximum yield of acetic acid was seen at an alkaline pH. These pH conditions enhance the CO_2 consumption-ability of bacterial cells, and cell growth also increases. A pH beyond 8 and lower than 7 may cause cells to die and decrease the metabolism rate; hence, the pH range of 7–8 was found to be the best-suited pH for CO_2 consumption and acetic acid production.



(**a**)



(**b**)

Figure 4. Graphs depicting the acetic acid yield (**a**) at varying pH values and (**b**) at varying temperatures.

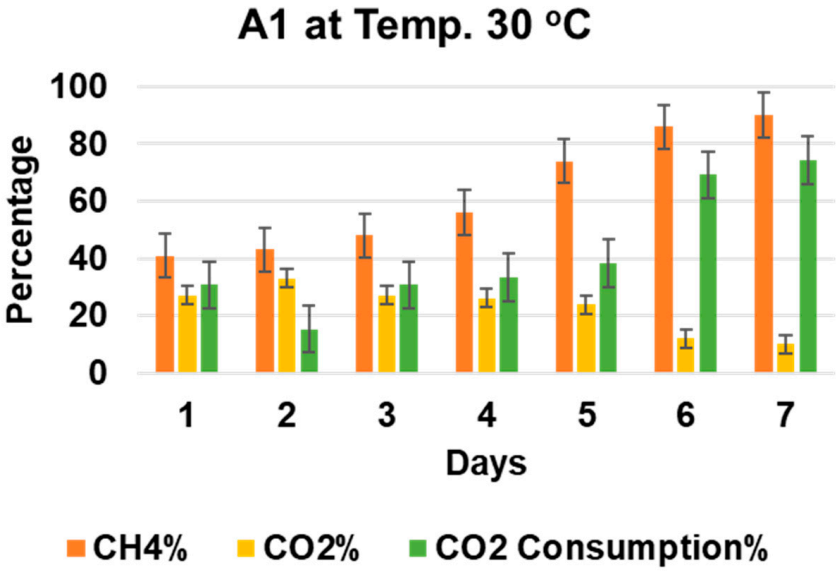
A crucial factor that significantly influences the final metabolites generated during anaerobic gas fermentation is the pH of the medium [34]. Studies have reported that a high amount of acetic acid production by progressively growing bacterial colonies is found at an alkaline pH or a pH closer to neutral. When the pH of the fermentation process is reduced, the acetic acid that is generated undergoes protonation and is subsequently transported across the cellular membrane into the cytoplasm. Once inside the cytoplasm, the acetic acid is deprotonated, leading to a disturbance in the stability of the cell [35]. This cytoplasmic stress in bacterial cells is overcome by the production of alcohols, hence reducing the overall yield at an acidic pH. In this study, A1, A2, and CD1 produced 412.5, 307, and 290 mg/L at pH 6, which is very low. This yield was enhanced as the pH increases, as at higher pH or alkaline pH, acetic acid converts into acetate form and remains stable. The rate of hydrogen generation was observed to be higher at lower pH values compared with higher pH values. However, it is important to note that when the pH dropped below a value of 7, it was observed to have a negative impact on bacterial growth. Due to all of the above, it was determined that a pH value of 7 would serve as the initial starting point for all experimental procedures. The natural rise in the acid in the medium of fermentation was observed due to an overproduction of metabolites, specifically acetic acid. A potential reduction in pH may manifest itself as a consequence of the dissolution of carbon dioxide, which subsequently results in the liberation of hydrogen ions (H^+). Alternatively, it may also arise due to the generation of certain products, such as acetic acid, by the bacteria.

The effect of ambient humidity was not analyzed in this study; however, many studies have shown that an increased presence of elevated ambient humidity was observed to exert a favorable influence on methane production. This phenomenon can be ascribed to the creation of an optimal environment for methanogenic bacteria, which hold a pivotal role in the conversion of organic material into methane. The efficacy of biological biogas upgradation systems can be significantly heightened when exposed to elevated ambient humidity levels [36]. This can be attributed to the accelerated decomposition of substrates and a reduced occurrence of obstructions. Furthermore, the energy consumption of biological biogas upgradation systems can be substantially diminished with the augmentation of ambient humidity. This reduction is primarily a result of obviating the need to heat the biogas, leading to noteworthy energy conservation [37]. However, elevated ambient humidity fosters the proliferation of mold and various other microorganisms, thereby heightening the risk of contamination within biological biogas upgradation systems. The augmentation of ambient humidity leads to an amplified presence of water vapor, consequently diminishing the quality of biogas. Moreover, the heightened humidity levels can also potentially induce corrosion in the metallic components of biological biogas upgradation systems [38]. While high ambient humidity increases the solubility of hydrogen sulfide in water, which improves the effectiveness of bioscrubbing, it also increases the amount of water vapor from biogas, which improves biofiltration [39].

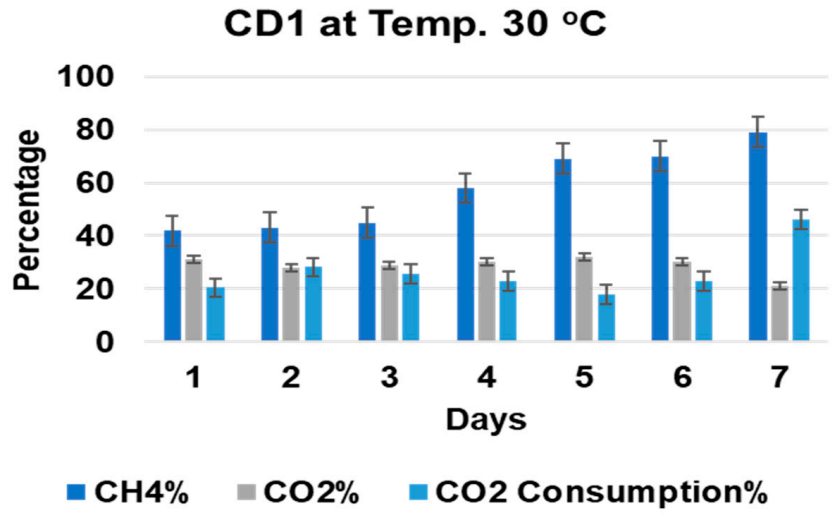
3.3. Effect of Incubation Temperature on Biogas Upgradation and Acetic Acid Production

Three different levels of the incubation period, i.e., 30 °C, 35 °C, and 40 °C, were tested for CO_2 consumption and acetic acid production. It was found that the maximum CO_2 consumption and methane yield was at 30 °C and 35 °C. At 40 °C, the CO_2 consumption was the least and the CH_4 yield was decreased by 10–15%. There was a 47% increase in the CH_4 yield by the A1 isolate, a 44% increase in CH_4 yield was shown by the A2 strain, and a 29% increase in the CH_4 yield by CD1 at a temperature of 30 °C. Similarly, there was a 34%, 26%, and 23% increase in CH_4 by A1, A2, and CD1, respectively, at a temperature of 35 °C. The acetic acid yield also varied as per the change in the temperature. As the incubation temperature increased, the yield of acetic acid decreased. At 35 °C, there was an increase of 79% of acetic acid by isolating A1 as compared with the yield at temperature 40 °C. Similarly, the A2 isolate produced 31% more acetic acid than that of the A2 isolate at 40 °C, and 20% higher acetic acid was produced by CD1 at 35 °C than at 40 °C. At 30 °C, the acetic acid yield was highest as the CO_2 consumption was also very high (Figure 4b). There was

a 39%, 28%, and 26.2% rise in the acetic yield by A1, A2, and CD1, respectively, at 30 °C as compared with the acetic yield at 35 °C (Figure 5). From the results, it is evident that the decline in acetic acid formation at higher temperatures is due to the decreased metabolism rate of the bacterial cells. The optimum temperature for the maximum biogas upgradation for all three isolates (A1, A2, CD1) was 30–35 °C. More of the acetogenic species of bacteria are found in mesophilic conditions than in thermophilic conditions [40]. Singla et al. [41] reported similar work in which, at a mesophilic temperature, the CO_2 consumption was found to be maximum.

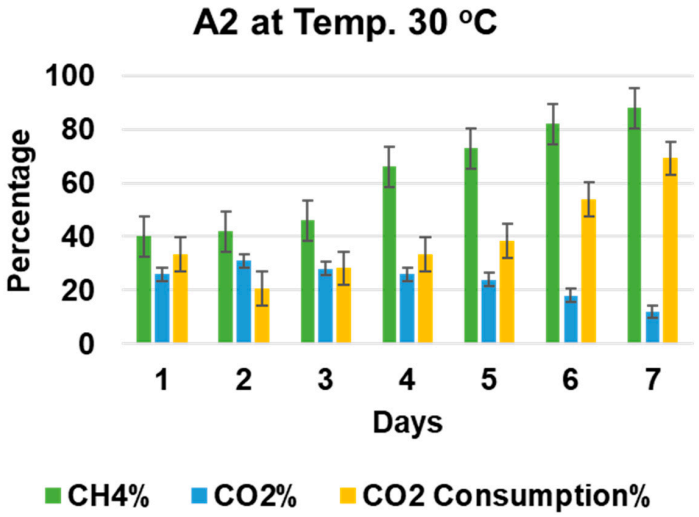


(**a**)

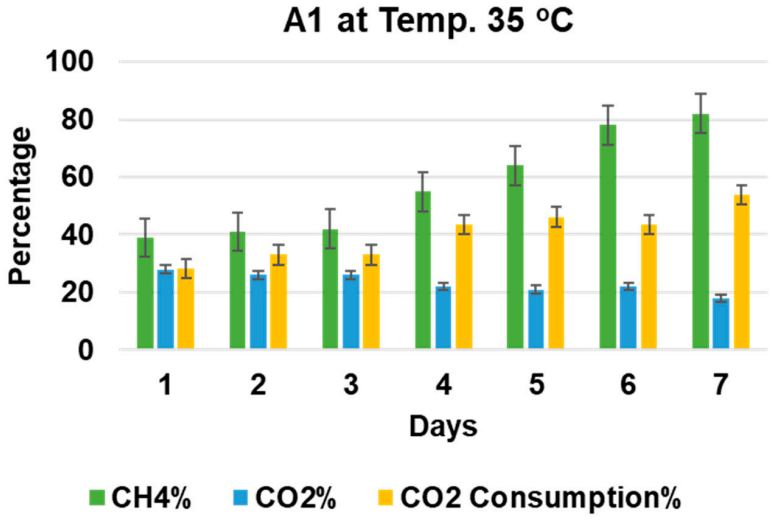


(**b**)

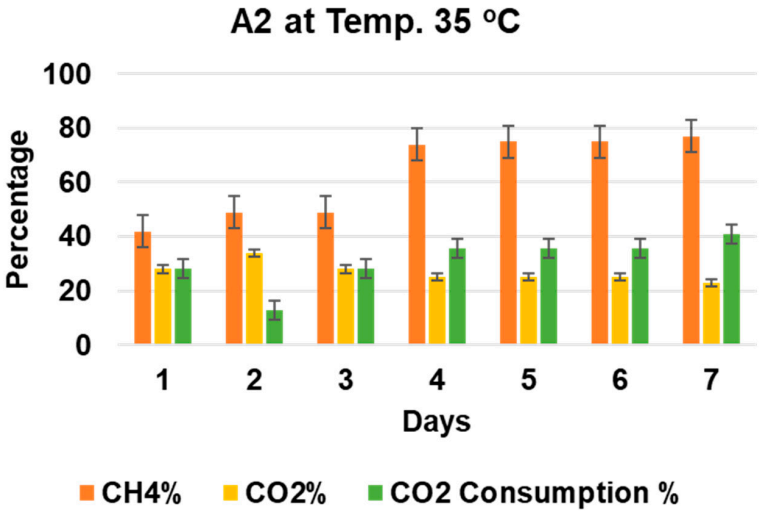
Figure 5. *Cont.*



(**c**)

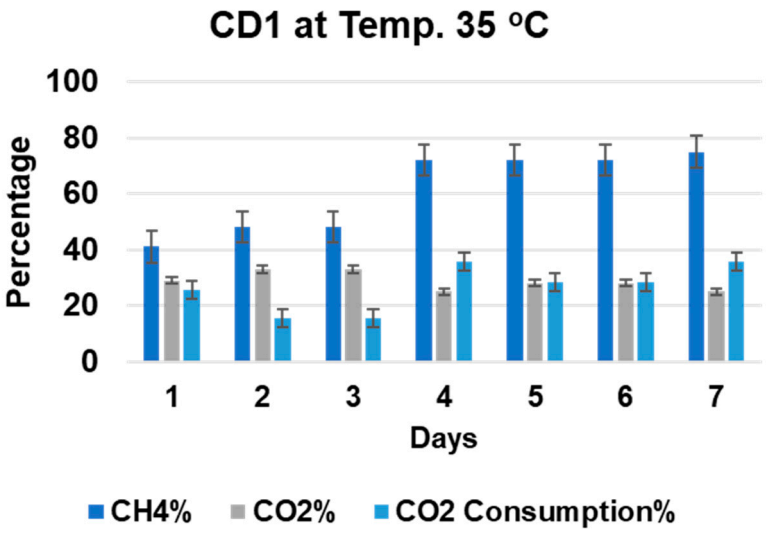


(**d**)

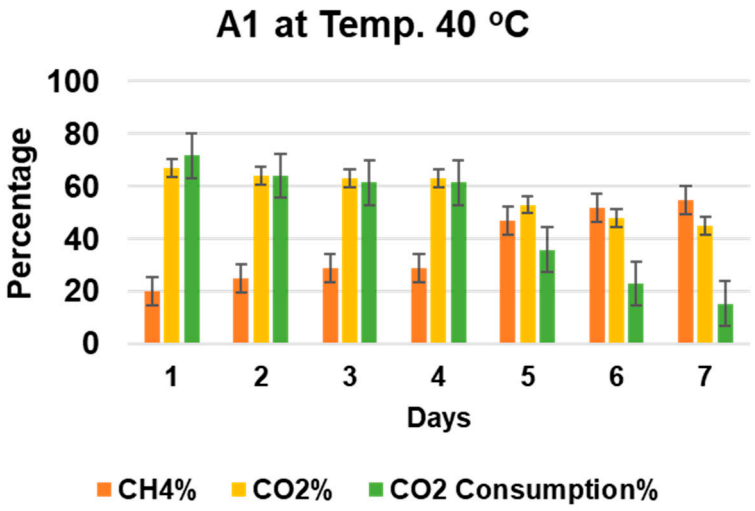


(**e**)

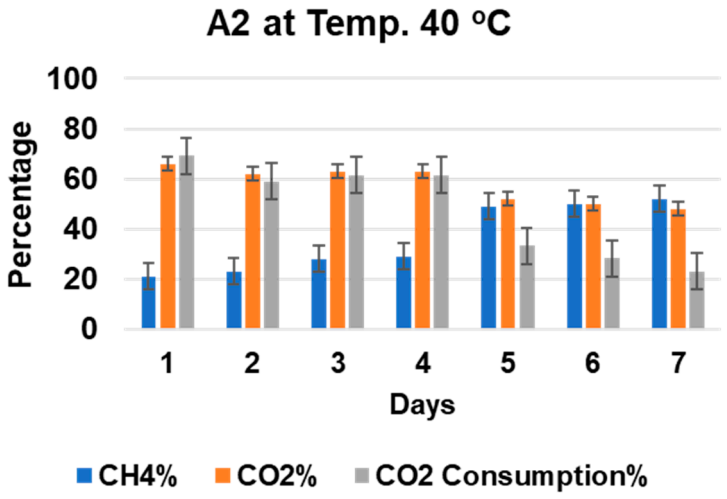
Figure 5. *Cont.*



(**f**)

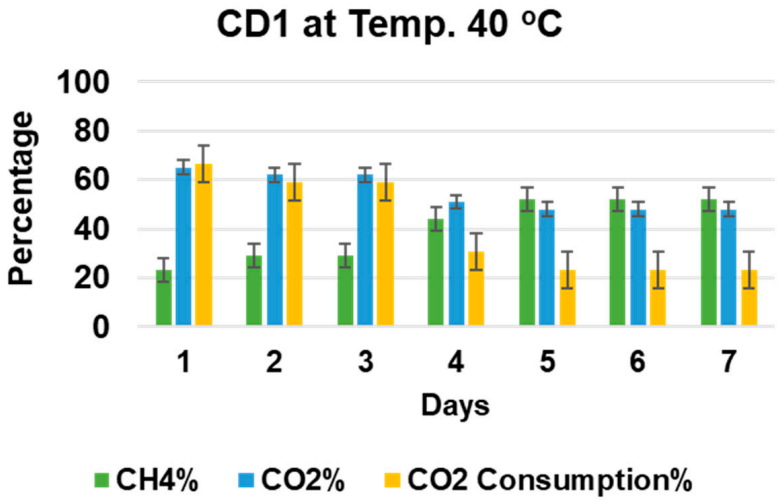


(**g**)



(**h**)

Figure 5. *Cont.*



(**i**)

Figure 5. Graphs depicting CH_4%, CO_2%, and CO_2 consumption% by (**a**) A1 at temperature 30 °C, (**b**) A2 at temperature 30 °C, (**c**) CD1 at temperature 30 °C, (**d**) A1 at temperature 35 °C, (**e**) A2 at temperature 35 °C, (**f**) CD1 at temperature 35 °C, (**g**) A1 at temperature 40 °C, (**h**) A2 at temperature 40 °C, and (**i**) CD1 temperature 40 °C.

3.4. Effect of pH and Temperature on CO_2 Consumption

By comparing the CO_2 consumption of mixed consortia and the pure isolates (A1, A2, CD1), it was found that the pure isolates are better at consuming CO_2 than the mixed consortia. Out of the three isolates, A1 showed the highest CO_2 consumption, followed by A2 and then CD1. From the analysis, it was found that 28.2%, 48.7%, and 17.9% of CO_2 was consumed from the biogas by sludge 1, sludge 2, and cow dung, respectively. In the case of pure isolates, it was found that 71.7%, 61.5%, and 38.4% of CO_2 was consumed by A1, A2, and CD1, respectively. The pure isolates were tested at varied temperatures and pH values. At pH 6, the CO_2 consumption by A1 varied from 30.7% to 38% from day 1 to day 7 of the incubation period. In the case of A2 and CD1, this value was less and varied from 15.3% to 28.2% and from 15.3% to 38%, respectively. The variation in CO_2 consumption and CO_2% in biogas was discrete based on incubation day. A higher amount of CO_2 was consumed when H_2 gas was in a higher amount. Ref. [42] reported that an optimum ratio of H_2:CO_2:CH_4 (2:1.5:1) gives the maximum amount of biomethane and the highest amount of CO_2 consumption. This analysis was performed at varied pH values (6, 7, and 8), and it was found that the optimum pH for maximum yield was 7 and 8. The percent increase in CO2 consumption at pH 7 in the A1 strain was from 28.2% to 71.7%, and at pH 8, it was from 30.7% to 74.3%. In a similar study, it was reported that the optimum pH for the maximum consumption of CO_2 from raw biogas by mix consortia is an alkaline pH [43]. Table 3 represents the comparison between the results of the present study and the reported data for the biogas upgradation. Comparing the results from this study, it was found that the mixed consortia can upgrade biogas at a higher purity than the pure isolates. Also, it was found that pure isolates can produce a higher amount of acetic acid as compared with the mixed consortia. The reported results also depict that mixed consortia work better in an acidic pH, and the pure isolates work better in an alkaline pH.

Table 3. Reported data for the biogas upgradation and acetic acid yield.

Substrate	Temperature (°C)	pH	CH_4%	Acetic Acid (mg/L)	References
Sludge from AD	~54	<6	77	50	[44]
Sludge from AD	~35	<6	96	40	[44]
Digestate from AD	~35	<7	96	290	[42]
Peatland soil	~29	>7	98	540	[43]
Pure isolates	30	8	90	2215	This study

4. Conclusions

The objective of this investigation was to assess and compare the capacity of mixed consortia and pure isolates in upgrading biogas. The experimental setup involved the utilization of three distinct isolates, namely, A1, A2, and CD1, as well as three samples, specifically, cow dung, sludge 1, and sludge 2. The results of this study indicate that the isolated strain A1 exhibited a significant improvement in the methane yield of produced biogas, with a notable increase of 32%. In contrast, the consortia demonstrated a comparatively lower enhancement, showing only an 11% increase. Strain A1 exhibited the most significant biogas upgradation when subjected to an incubation temperature of 30 °C and a pH of 8. The A1 strain demonstrated the highest recorded yield of acetic acid, reaching a concentration of 2215 mg/L at pH 8. The present approach presents a pioneering technique for enhancing the quality of biogas using biological processes. This method offers a promising avenue for advancing biogas upgradation methodologies while concurrently generating acetic acid.

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