IJBPAS, May, 2025, 14(5): 2610-2620

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)
'A Bridge Between Laboratory and Reader'

www.ijbpas.com

LACTOBACILLUS DELBRUECKII SSP. INDIUS AG1: POTENTIAL CANDIDATE FOR DEGRADATION OF AGRICULTURE WASTES AND PRODUCTION OF BIOGAS BY ORSAT INSTRUMENT METHOD

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Received 15th Jan. 2024; Revised 19th Feb. 2024; Accepted 8th July 2024; Available online 1st May 2025

https://doi.org/10.31032/IJBPAS/2025/14.5.8980

ABSTRACT

The anaerobic digestion of organic materials, which is necessary to add chemicals to the continuous biochemical process, is largely mediated by microorganisms. Through a sequence of metabolic events, the microorganisms efficiently break down complex organic compounds to produce simple compounds like methane. In this investigation, inoculums used for biogas production was isolated from agriculture waste sources—*L. delbrueckii ssp. Indius AG1* at temperature (35ºC to 37ºC)—to assess their suitability for anaerobic digestion of sterile agriculture waste. The highest biogas production of 215 ml/2 kg sterile waste was obtained using a combination of inoculums about 10ml, and biogas characterization by orsat instrument method showed 47.7% of the methane content. Utilizing morphological, biochemical, and molecular methods, the microbial flora found in the high-yield reactor was identified. The abundance of *L. delbrueckii ssp. indius AG1*. The study suggests that biogas, which has the potential to displace fossil fuel, is not only a suitable method of managing waste but also a green energy source.

Keywords: Nan Sterile agriculture waste, Biogas, *L. delbrueckii ssp. indius AG1*, Molecular identification, Orsat instrument method

INTRODUCTION

India produces 108.34 million tons of fruit annually, and it produces 212.91 million tons of vegetables annually, the second-highest amount in the world. The lack of storage, transportation, and physical deformations results in the annual loss of almost thirty percent of this total crop (Indian Horticulture Annual Report, 2019). After processing, both local vendors and the industries produce wastes including peel, pomace, seeds, and so forth. Waste buildup at disposal locations causes a bad stench and a protracted natural decomposition, which raises health concerns **[1]**. This might potentially be used to produce biogas **[2]**. The breakdown of organic waste in the absence of oxygen produces biogas. Because it comes from biological material (biomass), it is also known as biogas. With the aid of enzymes, anaerobic bacteria break down organic waste into smaller molecules like methane, carbon dioxide, and hydrogen sulfide, which combine make up biogas. Since lignocelluloses makes up the majority of the biomass in agricultural waste composting, its breakdown is crucial to the process's success **[3, 4]**. The amount and type of material introduced to the system determines how much methane is produced during the anaerobic digestion of biologically degradable organic materials **[5]**. Consequently, there are numerous methods for producing energy through anaerobic digestion using leftover food, vegetable and fruit wastes, and cow dung. These days, dry fermentation, co-digestion, single-phase digestion, and two-phase digestion are the most popular ideas **[6]**. Features and Constituency of Biogas depends on the kind of substrate utilized to create biogas. It greatly influences its composition. Methane (50–70%), carbon dioxide (30–40%), hydrogen, nitrogen, and hydrogen sulfide are the main components of biogas. After it was determined that thermo-chemical pre-treatment and co-digestion were the most effective methods for optimizing biogas production from plant wastes, it was suggested that more research be done on the subject and that AD (Anaerobic digesters) digesters adopt this technology on a larger scale. Moisture content, C: N ratio, and TS were found to have an impact on the gas production **[7]**. Biogas is a gaseous fuel that is produced by fermenting waste and is useful for generating energy for heating, cooking, and powering cars **[8, 9]**. The temperature at which conventional anaerobic digestion occurs is mesophilic. However, compared to the mesophilic temperature range, the thermophilic temperature range is worth taking into account as an alternative since it permits faster reactor rates, more gas production, and higher rates of pathogen and weed seed destruction. But compared to the mesophilic process, the thermophilic process is more susceptible to environmental changes **[10, 11]**. The amount of waste being collected from homes and farms has grown significantly in recent years, gaining national importance. Biogas is produced spontaneously through the breakdown of organic waste. In an anaerobic environment, organic matter is formed by food and animal waste. Waste material that might otherwise damage the environment is used by biogas plants **[12, 13]**.

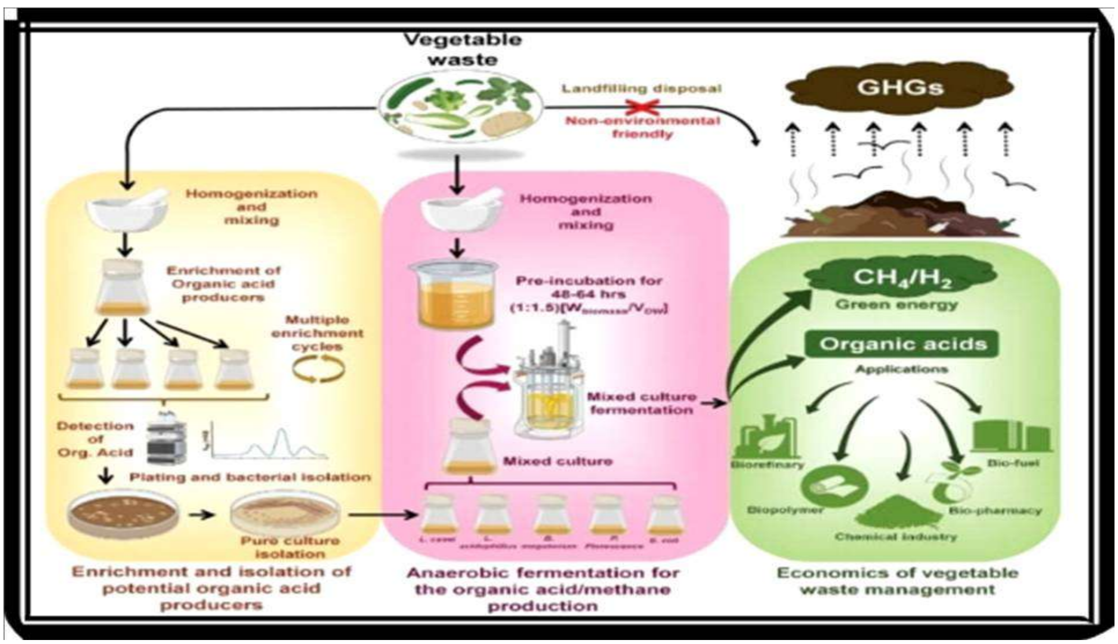

Figure 1: Schematic presentation of vegetable waste management proposed in this study to produce Biogas

2. MATERIAL AND METHODS

Isolation of lactic acid bacteria

Sample collection:

Fruit or vegetable waste was used to isolate lactic acid bacteria. To prevent contamination and spoiling, the samples were aseptically stored in a refrigerator at a low temperature (4°C) after collection.

Molecular Identification of lactic acid bacterial Species

After determining the genera of the isolated bacteria AG1 using a basic characterization procedure outlined in Bergey's Manual of Systemic Bacteriology, the isolates were identified using 16S rRNA analysis. The bacteria were then sent to "Genexplore Diagnostics & Research Centre Pvt. Ltd." Ahmedabad, Gujarat, India for 16S rRNA sequencing.

To produce biogas from waste by L. delbrueckii ssp. indius AG1

In this work, various types of agriculture wastes, from students' cafeteria of Noble University, Junagadh mixed *L. delbrueckii ssp. indius* AG1 were used as substrates for biogas production in 5 Litters sterile cylindrical plastic anaerobic digesters. In the above stated biogas digester one of the sterile cylindrical plastic anaerobic digester prepared for sterile agriculture wastes of students' cafeteria and lactic acid bacteria and the other digester contained sterile agriculture wastes without lactic acid bacteria as a control.

Biogas production:

Anaerobic waste digestion was applied to agricultural wastes employing *L. delbrueckii ssp. indius* AG1culture. After one day of incubation, biogas started to form, and the Orsat instrumentation method was used to measure the amount. Biogas didn't form until the first step. To create biogas, agricultural waste was utilized. The biogas formation reached its maximum after 20 days. The apparatus was maintained for the experimental observation for a few more days. There was no further biogas created after then. The following techniques are addressed regarding the building of the biodigester, feeding of the biodigester, and mode of biogas collection:

Contemplating Design

The specifications needed to build a bio digester include the digester's volume ($Vol_{digester}$), its gas storage capacity, the gas holder's volume (Vol_{Holder}), the retention duration, and the type and volume of agriculture waste that will be disposed of inside. Equation 1 in order to determine the unit size of a biogas unit:

The digester's volume (liters) = the retention time (day) × daily feed-in (liters/day)…. Equ.1

where the volume of digester is volume occupied by the sterile agriculture waste and the volume of gas storage. The digesters were fed at once but the calculation was based on daily feeding with the design criteria of retention period of 20 days, daily feeding of 0.24 kg and 0.24 kg of water for feeding i.e. 1:1 of sterile agriculture waste and sterile water

Computation of the Bio-digester

1 kg is equivalent to 1 liter; hence the total volume of digester's feed per day is given as:

Vol_{total} *of digester feed/day = 0.24 l of waste + 0.24 l of water = 0.48 l/day*

From equation 1,

$Vol_{digester}$= vol_{total} *of digester feed/day × retention period (day)*= 0.48 × 20 = 9.6L

Additionally, one-fifth of the digester's volume is indicated for the gas holder's volume:

Vol_{holder}=1/5× 9.6 =1.92L

Thus, the digester's entire volume is provided as follows:

Volume of digestion + volume of gas holder = total digester volume.

9.6 + 1.92 = 11.52 ~ 12L

Digester feeding

A halted feeding strategy was employed (batch feeding). This essentially entails filling the digester all at once and keeping the environment closed for the duration of the retention time. There were two digesters ready to be loaded. This single digester is used for the control (only sterile farm waste) and wastes (*L. delbrueckii ssp. Indius* AG1). The following are the steps taken when feeding the digester:

1. 2 kg of the wastes (sterile agriculture waste &*L. delbrueckii ssp. Indius AG1*) was weighed and 2L of sterile water was mixed thoroughly with of the waste in the ratio of 1:1 **(Table 1)**.

2. The mixture of the wastes were poured into sterile digester.

3. 2 kg of sterile agriculture waste without *L. delbrueckii ssp. Indius AG1* were weighed and mixed thoroughly with 2L of sterile water for the digestion **(Table 1)**.

Table 1: Ratio of Water Used to Waste

Utilized waste	Agriculture's weight waste	Inoculum	Quantity of sterile water utilized
Sterile agriculture waste	2 kg	-	2L
Digestion			
Sterile agriculture waste &*L. delbrueckii ssp. Indius AG1*	2 kg	10 ml	2L

Setup of an anaerobic digester

The experimental set up for the study using anaerobic digestion consists of plastic cane with a plastic cover and all the two anaerobic digesters were constructed at bench-scale experiments where the degradation of the sterile agriculture waste with lactic acid bacteria and one as control without lactic acid bacteria were accomplished in plastic cane with a capacity of 2 liters. Each plastic cane was sealed with its cover having two outlets. The first outlet was attached to an 8 mm internal diameter hose gas pipe and immersed up to a little above the bottom of the solution level in order to take samples without introducing air into the digester and indicate the quantity of gas produced inside the digester. Thus, a plastic cane was extended from the bottom of the substrate up to the plastic cane cover to prevent out flow of the substrate from the inside of the digester. The second outlet was above the top of the solution for gas collection.

Figure 1: Digester setup of the experiment

The whole cover and the hose gas pipe were sealed with gasket to protect air leakage from the environment. It was operated at ambient temperature in the hemophilic range (27°C-31°C), yet the temperature and moisture were monitored daily using thermo-hygrometer. A gas collector was provided for collection and determination of the amount of biogas. The content of methane concentration produced in the reactor was monitored daily. In the digesters' internal working temperature was maintained at the ambient temperature of the room using thick cover of sand and pH was regularly measured (every three days) throughout the digestion process.

Biogas yield and its quality

Gas chromatography analysis was used to determine the composition of biogas gas, while an indirect method was used to assess the volume and methane concentration of the gas produced in the anaerobic reactors. Both the volume of biogas produced and the gas's methane content were estimated using the indirect method. In order to determine how much biogas was created, each digester's downward displacement of water was used to calculate the volume of water displaced by the gas. The amount of CO_2 produced by the digesters was then used to estimate the methane content in the biogas. A 10% NaOH solution was used to filter the gas while the CO_2 dissolved and formed carbonate. As a result, the amount of NaOH ejected and the amount of methane in the gas are roughly identical. In the solution, other kinds of gasses were dissolved.

Figure 2: Orsat Setup

RESULTS AND DISCUSSION

Isolation and identification of lactic acid bacteria

Isolation and Characterization of potential lactic acid bacteria:

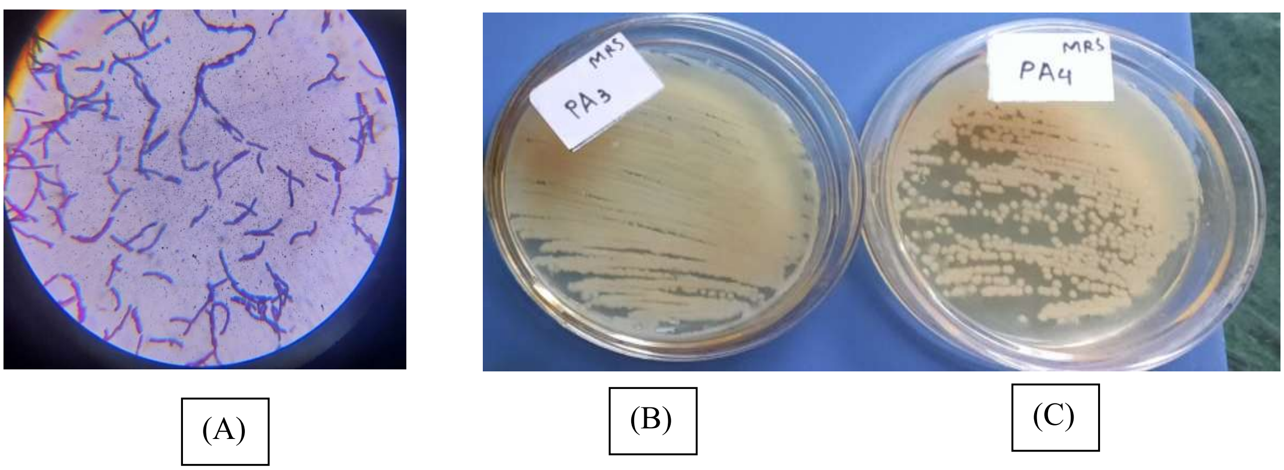

Figure 4: Cultural and Morphological characteristics of lactic acid bacteria
(A)Microscopic view showing gram positive long rods
(B and C) Growth on MRS agar plates

Utilizing 16s rRNA analysis, the isolate AG1 was identified.

Following the identification of the genera of the isolated bacteria AG1 using basic characterization as outlined in Bergey's Manual of Systemic Bacteriology, 16s rRNA sequencing was used to further identify the isolates to the species level. The bacteria were delivered to Ahmedabad, Gujarat, India's "Genexplore Diagnostics & Research Centre Pvt. Ltd." for this purpose.

Alignment report - 16S rRNA sequencing of

isolate *L. delbrueckii ssp. Indius AG1*
TTCCTTCGGGATGATTTGTTGGACGCTCG
CGGCGGATGGGTGAGTAACACGTGGG
CAATCTGCCCTAAAGACTGGGATACCACT
TGGAAACAGGTGCTAATACCGGATA
ACAACATGAATCGCATGATTCAAGTTTGA
AAGGCGGCGCAAGCTGTCACTTTAG
GATGAGCCCGCGGCGCATTAGCTAGTT
GGTGGGGTAAAGGCCTACCAAGGCAATG
ATGCGTAGCCGAGTTGAGAGACTGATCG
GCCACATTGGGACTGAGACACGGCCCAA
ACTCCTACGGGAGGCAGCAGTAGGGAAT
CTTCCACAATGGACGCAAGTCTGATGGAG
CAACGCCGCGTGAGTGAAAAAGGTTTTCG
GATCGTAAAGCTCTGTTGTTGGTGAAAAA
GGATAGAGGCAGTAACTGGTCTTTATTTG
ACGGTAATCAACCAGAAAGTCACGGCTA
ACTACGTGCCAGCACCCGCGGTAATACGT
AGGTGGCAAGCGTTGTCCGGATTTATTGG
GCGTAAAGCGAGCGCAGGCGGAATGATA
AGTCTGATGTGAAAGCCCACGGCTCAACC
GTGAAACTGCATCGGAAACTGTCATTCTT
GAGTGCAGAAGAGGAGAGTGGAACTCCA
TGTGTAGCGGTGGAATGCGTAGATATATG
GAAGAACACCAGTGGCGAAGGCGGCTCT
CTGGTCTGCAACTGACGCTGAGGCTCGAA
AGCATGGGTAGCGAACAGGATTAGATAC
CCTGGTAGTCCATGCCGTAAACGATGAGC
GCTAGGTGTTGGGGACTTTCCGGTCCTCA
GTGCCGCAGCAAACGCATTAAGCGCTCCG
CCTGGGGAGTACGACCGCAAGGTTGAAA
CTCAAAGGAATTGACGGGGGCCCGCACA
AGCGGTGGAGCATGTGGTTTAATTCGAAG
CAACGCGAAGAACCTTACCAGGTCTTGAC
ATCCTGCGCTACACCTAGAGATAGGTGGT
TCCCTTCGGGGACGCAGAGACAGGTGGT
GCATGGCTGTCGTCAGCTCGTGTCGTGAG
ATGTTGGGTTAAGTCCCGCAACGAGCGCA
ACCCTTGTCTTTAGTTGCCATCATTAAGTT
GGGCACTCTAGAGAGACTGCCGGTGACA
AACCGGAGGAAGGTGGGGATGACGTCAA
GTCATCATGCCCCTTATGACCTGGGCTAC
ACACGTGCTACAATGGGCAGTACACGAG
AAGCGAACCCGCGAGGGTAAGCGGATCT
CTTAAAGCTGTTCTC

VOLUME OF BIOGAS PRODUCED FOR EACH CO-DIGESTION WASTE

The volume of biogas produced over the 20-day retention period from sterile agriculture waste and *L. delbrueckii ssp. indius* AG1 control is displayed in **Figure 5**. On day 9 of the retention period, the production of gas from sterile farm waste and *L. delbrueckii ssp. indius* AG1 began with an average biogas production of 45 ml. On day 10, this increased to 97.5 ml, and on day 12, it decreased to 185 ml. The amount of biogas produced was 255 ml on day 13, then dropped to 185 ml the following day, and then increased until reaching a peak of 575 ml on day 16, at which point it started to decrease until the end of the retention period, resembling the work of (Aremu and Agarry, 2012).

Temperature of Slurry for digestion of sterile agriculture waste

The temperature during the digestion of sterile agricultural waste & *L. delbrueckii ssp. indius* AG1 and control is shown in **Figure 6**. For agriculture waste and *L. delbrueckii ssp. Indius AG1*, the temperature ranges from 25°C to 34°C to 29°C to 37°C, and it stays constant from day 15 to day 16. These temperatures, which range from 25 to 45°C, also represent a thermal stage of biogas production. Most biogas researchers have found that temperature plays a significant role in anaerobic digestion because lactic acid bacteria, specifically *L. delbrueckii ssp. Indius* AG1, are most productive in the 29°C–37°C temperature range. This experiment was conducted at a temperature below 30, which may have slowed the growth of methanogens and reduced the amount of methane produced as a result. This is similar to the report of (Ilori*et al.,* 2007) that the recovery time for biogas production as well as the quality and quantity of biogas produced from agricultural materials are a function of the nature, and composition of the digester feedstock.

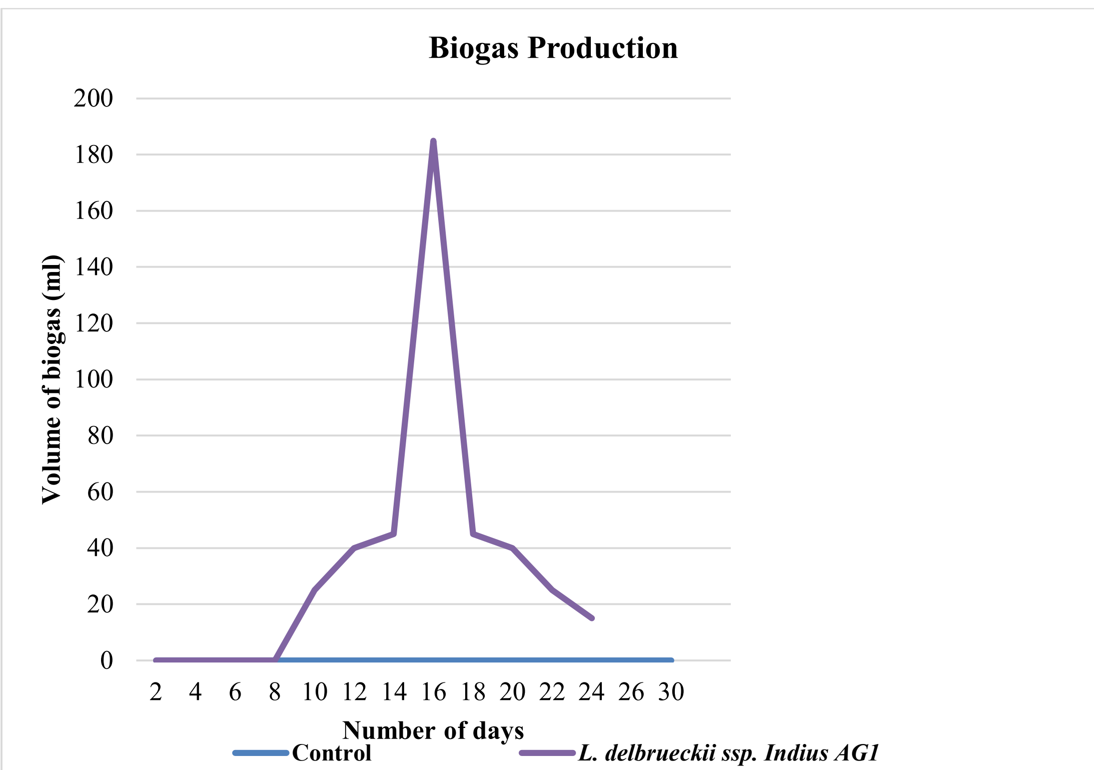

Figure 5: Volume of biogas of digestion of sterile agriculture waste against number of days

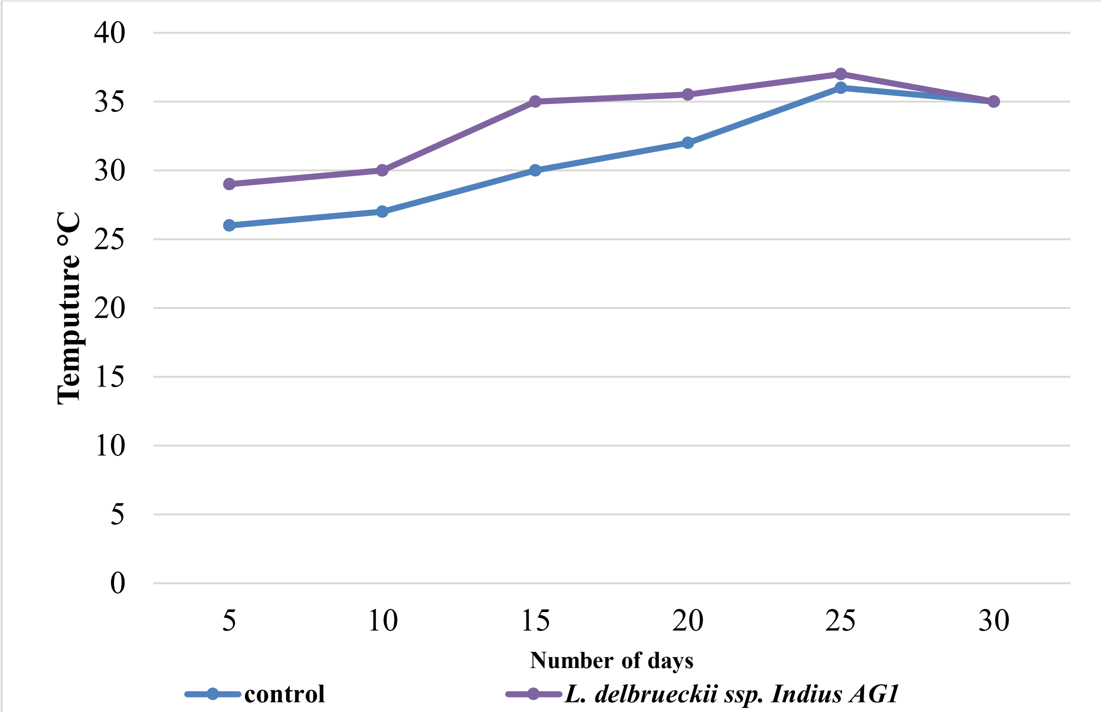

Figure 6: Temperature of slurry for waste digestion vs. Days

CONCLUSIONS

Anaerobic digestion of sterile agriculture waste (*L. delbrueckii ssp. Indius* AG1) can produce biogas, as demonstrated by the study on the production of biogas from this digestion process. These wastes are constantly present in our surroundings and, with the right management, can be a source of fuel. The investigation also showed that *L. delbrueckii ssp. Indius* AG1 has excellent potential for digestion of agricultural waste if digestion is to be employed, as well as for the creation of biogas if only one type of waste is to be used. The large volume of biogas yields should boost its use. Furthermore, it was discovered that temperature ranges that indicate a thermal stage of biogas production (25–40°C) also influence the volume yield of biogas production. For each agriculture waste (*L. delbrueckii ssp. Indius* AG1), the temperature at which biogas production peaked was reached on days 15 to 16, with respective temperatures of 32°C to 35°C. A significant first step toward utilizing one of the most abundant yet underutilized renewable energy sources in the world is the production of biogas from agriculture waste.

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