Maximizing biogas production from leftover injera: influence of yeast addition to anaerobic digestion system

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Maximizing Biogas Production from Leftover Injera: Influence of Yeast Addition to Anaerobic Digestion System

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Abstract

Injera is a staple food in Ethiopian dine. This study aimed to investigate on leftover Injera (LI) for producing biogas via anaerobic digestion (AD), while leftover injera is full of easily biodegradable components. Aiming to examine the impact of yeast addition on biogas production efficiency, it was found that the addition of 2% volatile solids (VS) of stimulated yeast, daily biogas output increased by 520 ml and 550 ml after 12 and 37 days of anaerobic digestion, respectively, with rather steady biogas production. The rate at which gas production increased was drastically cut in half when yeast was left out of the control group. Biogas production increased by only 60 ml despite the addition of two portions of substrate and yeast. Biogas output in the yeast group after fermentation was also up 33.2% compared to the control

group. The yeast group's anaerobic digesting system was more stable, as determined by the study of markers including volatile organic acids, alkalinity, and propionic acid. The findings can be used as a benchmark for future trials aiming to industrialise continuous anaerobic digestion, allowing for more flexible response to feed as waste LI as organic load.

Keywords:

Injera, anaerobic digestion, alkalinity, fatty acids, yeast, methane.

1. Introduction

Injera is a traditional Ethiopian flatbread made from fermented teff flour, which is naturally gluten-free and has a sourdough-like taste. Injera is a staple food in Ethiopian cuisine and is typically served with a variety of stews, curries and vegetable dishes. Teff based injera, a traditional fermented staple food in Ethiopia, that are predominantly found as waste after dining as leftovers. Moreover, still now, no studied have been investigated, hence, the current research aimed to investigated the viability of utilising leftover injera (LI) as substrate for biogas production, in addition, to examine for boosting the production level using addition yeast [1]. So for, different researchers [2] compare and contrast the effects of vacuum and nonvacuum packaging of injera, with and without of $C_7H_5NaO_2$ preservatives. Different microbes, such as Lactic Acid Bacteria and yeast strains can be used to accomplish sourdough fermentation, and that Injera is a popular fermented food item in Ethiopia was noted by different authors [3]. In reports elsewhere, researchers assessed the mineral, phytochemical, and microbial quality of injera made by replacing some of the teff flour with fenugreek seed flours [4]. The identification of the yeast responsible for Injera fermentation is crucial for achieving more consistency and expanding Injera production on a larger scale [5-7]. Open-fire cooking is a common practise in rural Ethiopian communities, especially for making sauce (called "Wot") and baking the staple food Injera.

Controlled cooking trials were conducted using electronic thermometers attached to the stovetop in real time [8]. The waste heat recovery system studied by Literatures [9] is meant to harness the energy contained in the waste gases produced by an institutional mirt stove, which may then be used to bake injera in a secondary mated. According to previous studies [10,11], barley grain was gathered from 4 different areas of Ethiopia and then utilised to manufacture kolo, porridge, bread, and injera. Literatures say [12,13] that the dialyzable (D) and total phenolic soluble nondialyzable (TPC) and total flavonoid contents (TFC) of tef injeras were determined by simulating their *in vitro* digestion for varying amounts of time [14].

In the present study, the *in vitro* dialyzability of leftover injera, a fermented gluten-free flat bread, was assessed using the modified inoculum [17]. It was found that its growth medium potential for treating food waste through anaerobic digestion (AD) with the aid of pH regulation matches with the optimal pH limit for the methanogenesis process, based on the emission of biogases *in vitro* from horses fed a concentrate diet [19,20].

Four rumen-cannulated animals, including two Rambouillet sheep, two Holstein steers, two Creole goats, and, provided the rumen liquor studied by different authors [21]. Researchers [22] employed lignite from the Konin area as a substrate for incubation studies to simulate the production of biogenic methane. Researchers [23] found that the wastes from yeast generation and biomass biorefinery operations for lipid and carotenoid extraction serve as substrates for anaerobic digestion. Methane (CH_4) and carbon dioxide (CO_2), both of which contribute to climate change and air pollution, are produced during ruminal fermentation [24]. Here, Anaerobic digestion (AD) was proposed to investigate [25], since the AD is a biological process that generates significant biomethane as an energy source from trash. Researchers found that the combination of inoculants had a substantial effect on the compost maturity of fermentation residue with a higher initial EC [26-28].

By creating favourable conditions (optimal pH, high ammonia content, and presence of urea) for the highly active ureolytic bacteria that authors [29] seek to isolate, the researchers were able to make their discovery. By increasing the number of bacteria in the fermentation medium, authors [30] found that methane output could be significantly increased [31]. AD is one of the most extensively utilised processes worldwide for turning solid organic waste into energy and other value-added products, and it was recently analysed. Anaerobic digestion of microbial biomass with animal dung at high solids was found to benefit from a three-stage process [32]. To maximise their biogas potential, authors [33] found that horse dung, chicken manure, and laboratory mouse excrement must be co-digested. There was a 32% dissimilarity in the bacterial biomass community and a 7% dissimilarity in the archaeal biomass community [36].

Clean energy and skilled cookstoves industry in Ethiopia needed in crucial contribution to the Injera bakery's design, assembly, and heat distribution investigation [37]. To help the vast majority of people who live in rural regions [38] and evaluated the various alternative energy sources that can be utilised to power baking stoves. Although subsidies make the units affordable for small farmers [39], the use of residential biogas energy has remained below

expectations. Hence, the households' decisions to embrace biogas technology in northern Ethiopia [40].

The purpose of this research was to examine how yeast interacts with the leftover injera (AD) system. During the anaerobic fermentation procedure, a yeast culture was added to the LI broth. However, a control group was also established, to which no yeast was added. The pH, alkalinity, volatile fatty acid (FFA) concentrations, and other parameters of these two systems, as well as their daily and cumulative gas output, were compared and analysed. The purpose of this study was to examine how yeast addition affected methane (CH_4) creation from LI in AD process. The findings can serve as a benchmark for future trials aiming to industrialize continuous anaerobic digestion using LI.

2. Material and methods

2.1. Materials

The LI was collected from the cuisine and dining lounge of Addis Ababa Science and Technology University in Addis Ababa, Ethiopia (Figure.1a) to utilise in this research. Initial LI dissections focused on removing the biggest bones and pericarps. The rest of the trash was tattered and frozen at -20 ºC. For acclimatization of fermentation, the infected sludge (Figure.1b) was taken from a remote biogas station located in Addis Ababa. The chemical and physical characteristics of LI and sludge are listed in Table 1. Active dry yeast for use in the anaerobic digestion process. Depending on the strain, yeast can have anywhere from 45-65% protein, 20-30% carbohydrates, 5% - 8% fat, and 7- 16% nucleic acid.

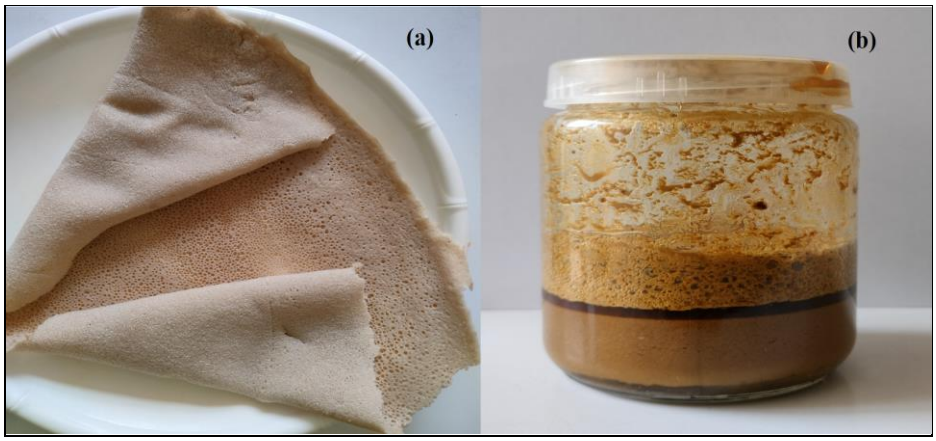

Figure 1. Leftover injera (a) and sludge sample (b) for inoculum

Table 1. Properties of leftover injera and inoculum sludge.

Factors	Leftover injera	Sludge for Inoculum
N*(%)	2.14 ± 0.15	2.61 ± 0.32
Volatile Solids (VS) (%)	25.2 ± 0.04	6.91 ± 0.05
Carbohydrate*(%)	46.4 ± 0.07	69.4 ± 0.05
Fat*(%)	32.4 ± 0.7	–
H*(%)	7.34 ± 0.16	5.73 ± 0.24
O*(%)	31.4 ± 0.18	26.3 ± 0.36
Total solids (%)	26.4 ± 0.05	14.6 ± 0.02
C*(%)	49.3 ± 0.05	28.3 ± 0.12
Protein*(%)	15.1 ± 0.09	15.3 ± 0.05
C/N	24.6 ± 1.7	12.4 ± 1.3
pH	4.82	8.96
	* - Dry basis	

2.2. Experimenting techniques

In this study, authors used batch mode to conduct two sets of experiments. For the AD reactions, 400 ml working volume and 500 ml of anaerobic bottles were used. Activated yeast and control (without yeast) AD studies were designed. In both experimental groups, the addition ratio of LI to sludge was 1:2.5 (based on VS). When fermentation gas production dropped dramatically, 2% (estimated as VS) activated *Saccharomyces cerevisiae* was added to the yeast group to see if it would increase stability, decrease VFA load which ultimately increase methane (CH_4) production (for day 12 of fermentation). Researchers have previously outlined the processes of adding and activating yeast. In order to mitigate glucose's negative impact on LI in AD system methane production, the same amount of glucose (2%, approximated as VS) was given to the control group was added to the yeast activation mixture. At the end of the anaerobic fermentation process, yeast and glucose were added again to evaluate if the full halt of gas production at this stage was due to the exhaustion of substrate or the inactivation of methanogens (no biogas generation, 37 days). Figure 2 depicts the experimental flowchart. The

extra air was removed from the two sets of reactors by filling them with nitrogen before being sealed and heated to 37 ˚C and rotated at 60 rpm for the experiments. There were 50 days of testing. At regular intervals, samples were withdrawn and parameters were calculated. Biogas output data was logged on a daily basis.

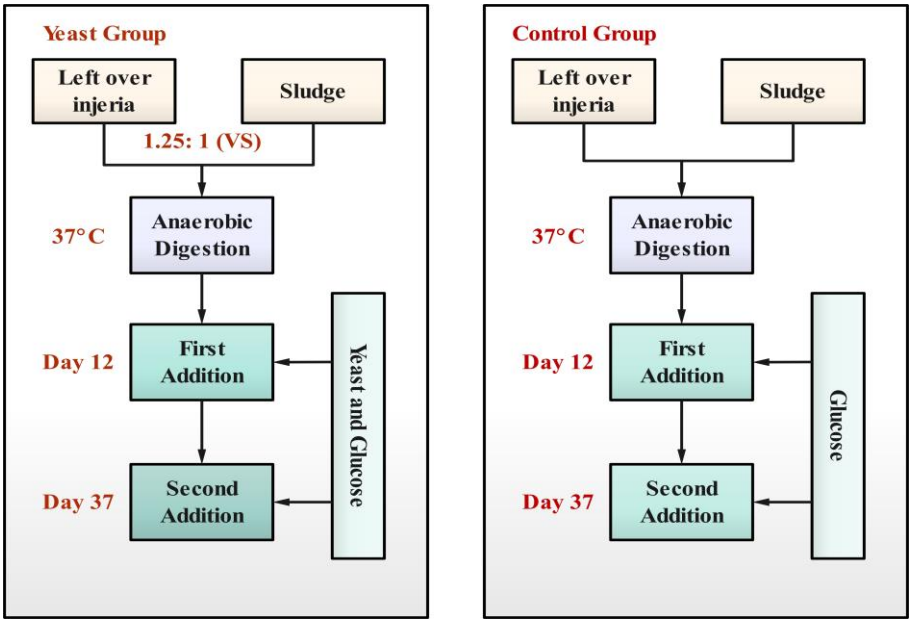

Figure 2. Conceptual flow for anaerobic digestion process.

2.3. Analysis techniques

Total solids (TS) and VS were calculated using a method approved by the State Environmental Protection Agency. The pH levels were measured with a digital acidity metre, model PHS-3C. The alkalinity was determined using a titration with bromocresol green and methyl red indicator. Measuring fat content with reference to GB 5009.6-2016 as per the national standard. The K9840 automatic nitrogen analyser was used to determine the protein concentration. It was determined by the following manner how the Volatile fatty acids (VFAs) were put together. The samples were initially centrifuged for 10 min at a rate of 12000g. A 0.24 m microporous membrane was used to dilute and filter the supernatant. The final measurements were obtained using a gas chromatography fitted with a DB-FFAP GC column (35 m × 0.55 mm x 0.5 m) and a spark ionisation detector. To gather the biogas, an alkaline drainage system was utilised.

3. Results and discussion

3.1. Anaerobic digestion biogas generation performance comparison between the two groups

Both the yeast and regulator groups were subjected to the experiments outlined in section 2.2. The only difference between them was that on days 12 and 37, 2% more yeast (in VS) was supplied to the yeast group compared to the control group. Two groups inside anaerobic fermentation had their daily biogas production was tracked, as well as their total biogas production over time (Figure 3 and 4). From the results it was observed that the biogas production dropped dramatically in both groups after 2-12 days. However, daily biogas output by both groups increased dramatically after day 12, when both groups were given the same quantity of glucose and when yeast was introduced to the yeast group. On day 13, biogas output was higher in the yeast group by 520 ml and higher in the control group by 330 ml compared to production before day 12 (Figure 3). From day 13 to day 37, the yeast group had an average gas output of 115.6 ml more than the control group. As seen in figure 4, the evolution of biogas output by the two experimental groups over time. Both groups' cumulative biogas production trends and gas output were similar when yeast was removed (1-12 days). Immediately after the initial addition of yeast, gas output increased by 26.5% compared to the control group. After the subsequent injection, the yeast group's cumulative biogas output reached 417 ml g^{-1} VS, which was 34.6% greater than the control group's production. The yeast group produced 451.3 ml g^{-1} VS of biogas over the course of fermentation (50 days), while the control group produced just 333.8 ml g^{-1} VS. In anaerobic fermentation, 1 g of protein (VS) can yield 496 millilitres of biogas under typical circumstances. Since yeast typically contains around 50% protein, producing 1 g of biogas from the yeast itself requires a total of 248 ml. The yeast group's biogas output was reduced by 4.6 ml g^{-1} VS, indicating that the yeast group's biogas production was 33.2% higher than the control group. The anaerobic digestion of methanogenesis in LI can be greatly enhanced by the addition of active yeast. Yeast was added to the substrate in another study to promote ethanol fermentation from the leftover injera. While the control group produced 35 ml g^{-1} VS of methane, the ethanol pre-fermentation group produced 254 ml g^{-1} VS. It fits quite well with an outcome of investigation.

This outcome could be explained by the fact that activated yeast inhibits the development of VFAs. After pitching yeast, mostly ethanol and acetic acid are produced because to S. cerevisiae's glycolytic metabolic process. Biogas generation is hampered by acidification in the AD system, which is seen as a major issue. When yeast was added to LI, instead of volatile

fatty acids (VFAs), ethanol was produced from the organic materials. Over time, the ethanol may be broken down into acetic acid, which the methanogens could then use to boost methane synthesis and keep anaerobic fermentation running smoothly. In anaerobic digesting systems, yeast can increase the concentration of CH_4-releasing bacteria.

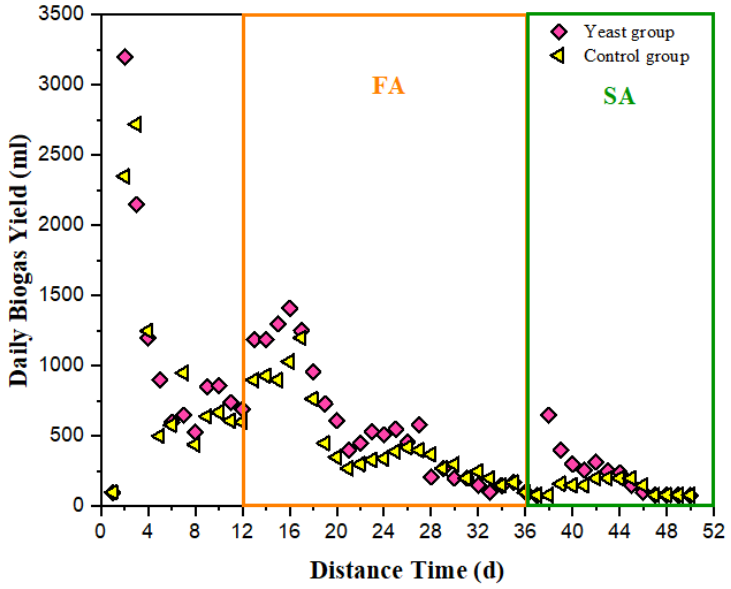

Figure 3. The yeast and control groups for daily biogas generation (FA-First addition period and SA-Second addition period)

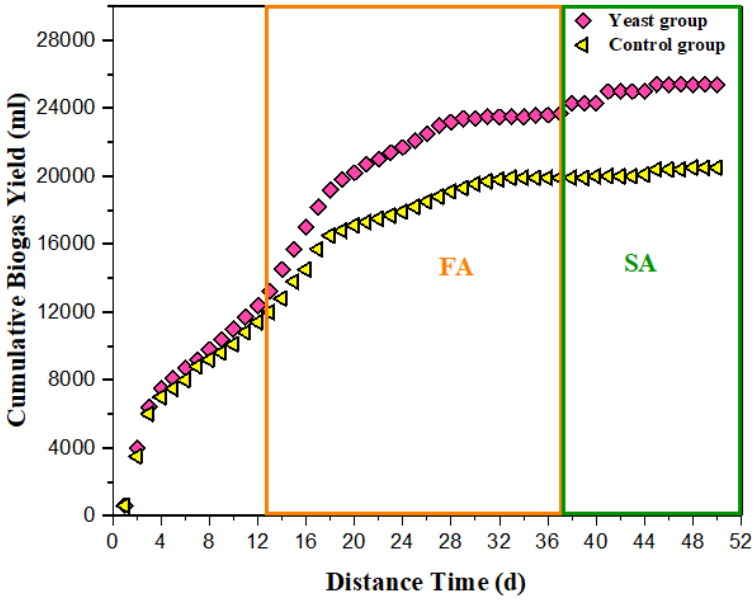

Figure 4. The yeast and control groups for total biogas production (FA-First addition period and SA-Second addition period)

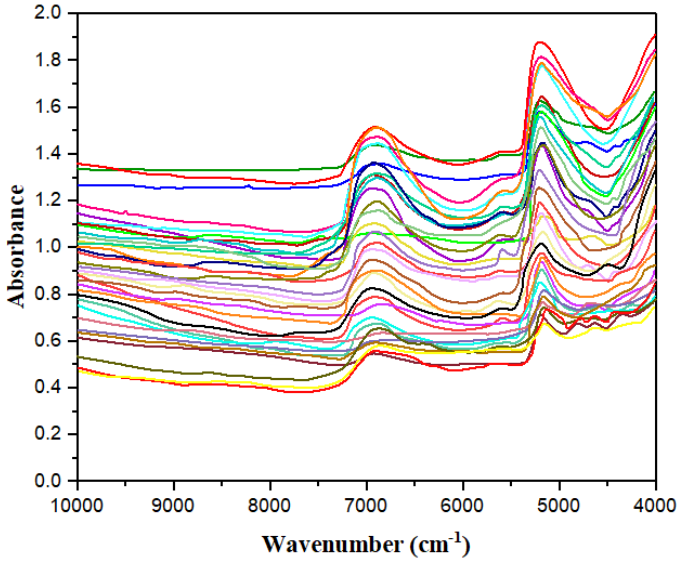

Figure 5. The NIR spectra of sbstrate sample

Figure 5 presents the NIR spectra of dried substrate sample. The two largest peaks in the sample's spectrum were primarily caused by water absorption and were seen at wave-numbers of roughly 6850 cm^{-1} and 5200 cm^{-1}. The first overtone with O–H stretching could be the source of the peak at 6850 cm^{-1}. The peak at 5200 cm^{-1} could be the result of an O-H deformation and stretching combo band. From the score plot of sample, the spectra of samples showed the strong influence of water at wavenumbers below 7000 cm^{-1}. Therefore, these particles reduce the path of water through which the light travels between the light source and the detector. According to the Lambert–Beer's law, this results in a lower absorbance at the water peaks. It is also true for the reflectance mode as showed in Figure 5. However, other molecules with the increase of sample concentration would absorb more light.

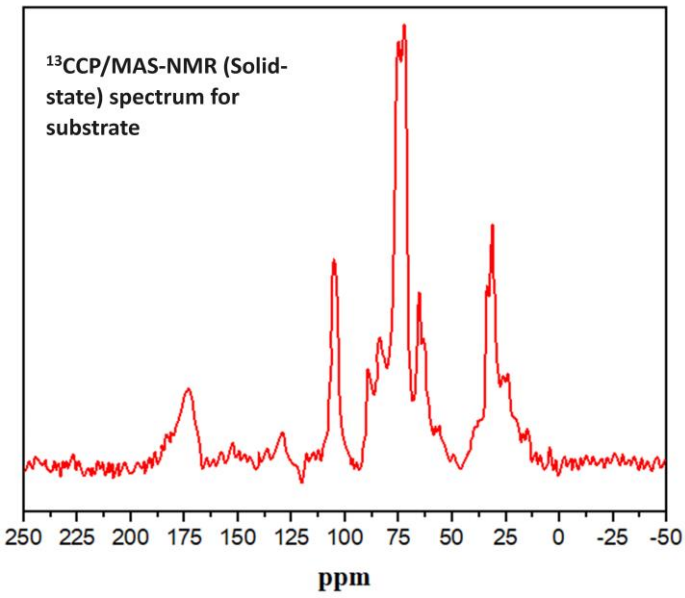

Figure 6. ^{13}CCP/MAS-NMR spectrum for substrate (second addition period).

Figure 6 show the solid-state ^{13}C NMR spectrum of substrate sample obtained from second addition period. The sample under the process presented a polysaccharide content of 57%. Nonetheless, both the amount of material left behind after fermentation and the ash content determined by proximate analysis were found to be greater. The sample that has stabilized was found to exhibit a modest increase in the aromatic and carboxyl/carbonyl regions, resulting in a low aromaticity index value. The inability of the digestion phase to facilitate the solubilization of the organic matter and subsequent wash out from the system with the percolation stream, which produced a stabilized product with a high polysaccharide content and low degree of aromaticity, might account for the advanced decomposition under anaerobic digestion of LI.

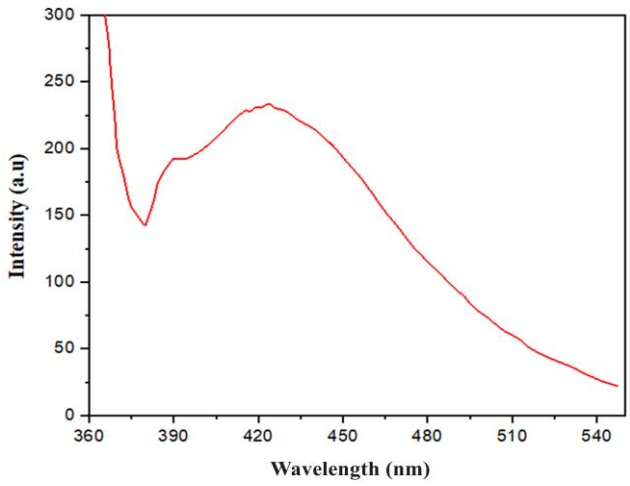

Figure 7. Emission spectra of substrate sample

Substance emission spectra are highly similar, typically exhibiting a broad peak devoid of any distinctive fluorescence. Here, greatest fluorescence intensity was measured between 410 and 450 nm in wavelength. The result showed that this material was humic-like substance, with a high hydroxyl and unsaturated carbon content contributing to its fluorescence features. The substrate's emission spectra in this work were characterized by a broad spectrum, and the maximum fluorescence intensity value's peak sites were both 425 nm (Figure. 7). The fluorescence intensity of the humic-like substance grew during the first stage of fermentation, showing that microorganisms were breaking down the organic matter. Meanwhile, under specific conditions, a novel chemical that resembled humic was created using breakdown products. Furthermore, the straightforward and easily degradable components underwent additional breakdown. As a result, the complex structures of humic-like substances may reduce the intensity of fluorescence.

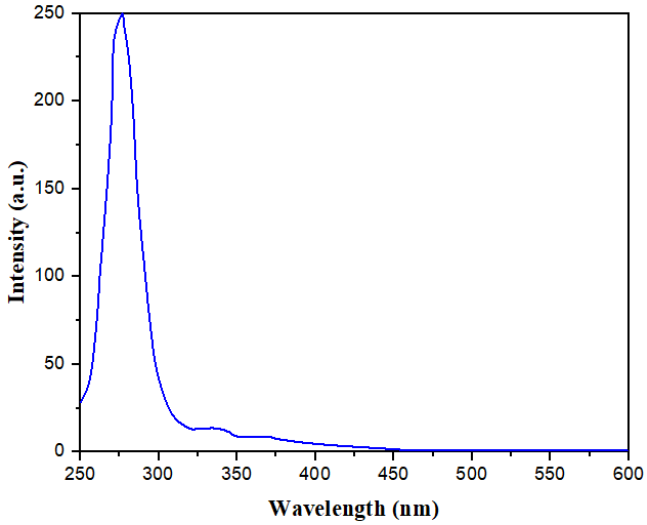

Figure 8. Synchronous Fluorescence spectra of the substrate

In order to evaluate the change in structure and component, Fluorescence spectroscopy was utilized to scan the excitation and emission monochromators simultaneously while maintaining a constant wavelength or energy offset between them. Figure 8 shows the synchronous fluorescence spectra that were acquired through the examination of a substrate sample generated by the second stage of the fermentation effluent. The fermentation effluent spectrum showed two primary peaks separated by varying relative fluorescence intensities: Peak I, the first peak, had an excitation wavelength of three main regions that could be identified: The protein-like region (255–310 nm), was linked to the existence of monoaromatic chemicals and

proteinaceous components. The presence of polycyclic aromatics with three to four fused benzene rings and two to three conjugated systems in unsaturated aliphatic structures is indicated by another region (310–360 nm), also known as the fulvic-like region, which was typically present in fulvic acids. The third peek, which ranges from 360 to 596 nm, was indicative of polycyclic aromatics, which have roughly 5-7 fused benzene rings. Humic substances were associated with this region, known as the humic-like region.

3.2. pH range, total alkalinity (TA), and TVFA compared between the groups of AD.

Stability was determined by studying markers such as volatile organic acids, alkalinity, and propionic acid. Adjusting the pH of anaerobic fermentation can speed up the hydrolysis and acidification process, while a too-high or too-low pH might slow the fermentation of organic molecules. Figure 9 shows that both groups' pH readings dropped with time, but neither group's pH dropped below 6.5. Since no oxygen was present during anaerobic fermentation, no acidification took place. This mechanism also explains why daily biogas output was not completely zero in the initial step of fermenting process, as seen in Figure 9. Both groups' pH increased rapidly next 8 days of AD fermentation, suggesting that the system itself acts as a buffer. pH levels did not significantly differ between the yeast and the controlling collection following the initial injection of yeast and glucose. Depending on the chemicals used, the pH of a CH_4 fermentation can change from 6.8 to 8.2, according to recent research. When it comes to managing microbial activities, the pH level is just as important. Acid-production bacteria thrive in a pH limit of 4.0 to 8.6, while methanogenic bacteria thrive in a pH range of 6.5 to 7.2.

As the TVFA concentration rises, the broth's alkalinity decreases considerably because HCO_3, CO_2, and other molecules that may accept proton H^+ can neutralise some of the acidic chemicals. Figure 10 demonstrates that both groups had similar shifts in alkalinity, while the yeast group ended up with somewhat more alkaline water. On day 37, for instance, the former's concentration was 1500.0 mg l^{-1}, whereas the latter was 1122.7 mg l^{-1}. The inclusion of yeast has the potential to increase hydrolysis of organics, leading to the production of neutral ethanol, while also decreasing the concentration of volatile fatty acids and the system's need for alkalinity.

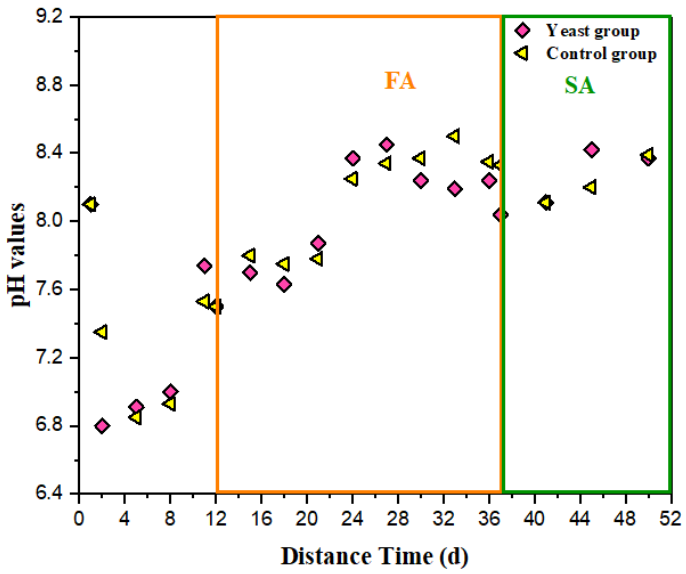

Figure 9. Evaluation of pH over time for yeast and the control group during AD

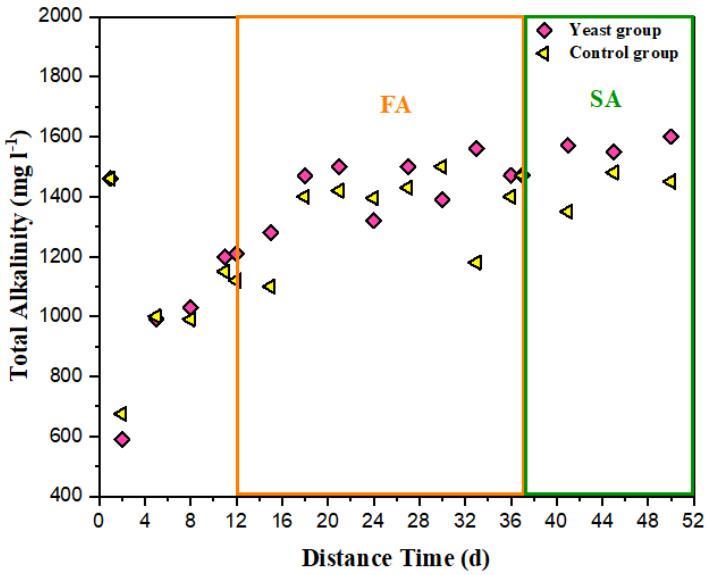

Figure 10. Evaluation of alkalinity over time for yeast and the control group

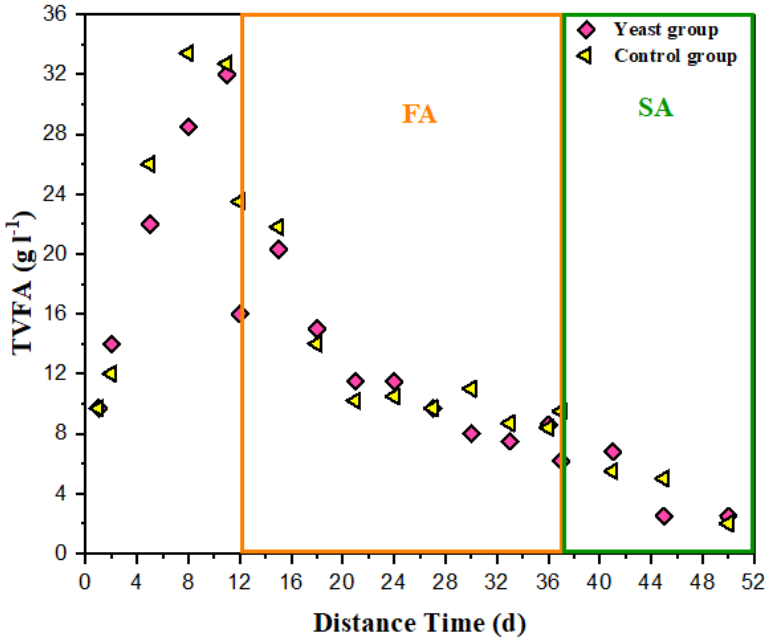

Figure 11. Evaluation of TVFA concentration over time for yeast and the control group during AD

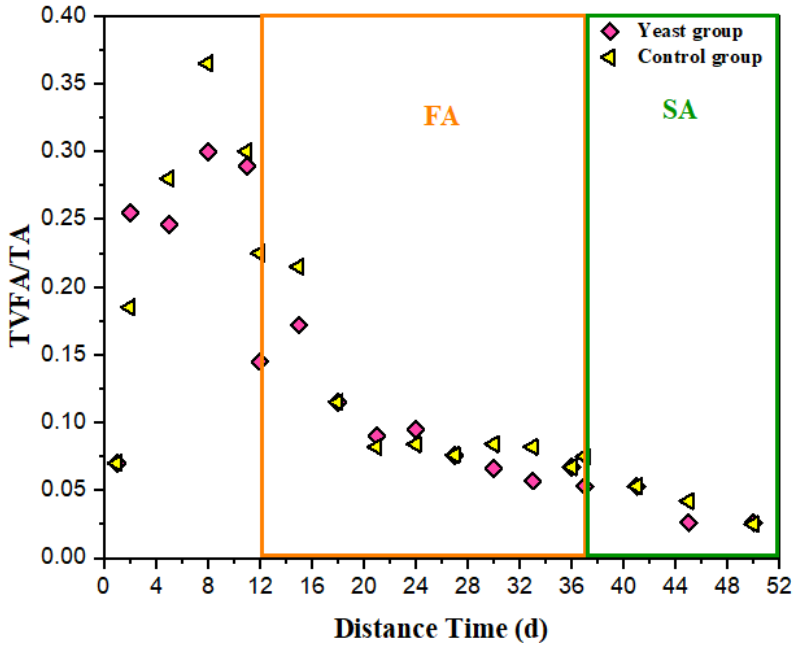

Figure 12. Evaluation of TVFA/TA concentration over time for yeast and the control group during AD

Volatile fatty acids (VFAs) are produced by methanogens during anaerobic fermentation of organic waste and serve as a substrate for the production of methane, both of which are

significant identifier of the metabolic activities of AD bacteria. If there are too many VFAs in the system, it will slow down. Figure 11 shows that early in the fermentation process, both groups had a sharp increase in TVFA concentration. According to the results as fermentation progressed, both groups' TVFA concentrations dropped, as expected as the pH and alkalinity improvement.

Figure 12 displays the difference between the two experimental groups as time was spent fermenting, as measured by the ratio of TVFA concentration to total alkalinity (TA) (total volatile fatty acids/total alkalinity). The system's alkalinity spiked quickly next to the adding of active yeast on 12 days fermentation (as seen in figure 12). As the yeast arrangement started to emit a lot of gas, the total volatile fatty acids/total alkalinity ratio kept going down. The yeast group had a lower TVFA/TA ratio than the control group, suggesting that yeast can be used to improve the stability of anaerobic digestion by adjusting alkalinity and decreasing TVFA concentration. In neither experimental group did the TVFA/TA ratio exceed 0.5. An outcome demonstrated that the arrangement had not become acidified; the drop-in gas generation prior to the adding of yeast was thus likely attributable to substrate depletion. Previous research has shown results that are consistent with this one. The TVFA/TA ratio can serve as an indicator of potential problems in the digestive tract. The TVFA/TA ratio demonstrates the balance between acid-forming and buffering substances in a system. The acid-resistance index is a sensitive indicator of the health of the anaerobic digestion system. When the TVFA/TA ratio is higher than 0.4, the acidity of the anaerobic system is on the verge of becoming unstable. The acidification of the anaerobic system is unbalanced, if the ratio of TVFA to TA is more than 0.6. Parameters like pH and alkalinity followed the same trend when comparing the two groups. An increase in alkalinity in the yeast set related to the control set, as well as a decrease in total volatile fatty acid concentration and total volatile fatty acid concentration relative to total acetic acid concentration, are indicators that the adding of stimulated yeast improves the stabilization of AD.

3.3. Analysis of the two groups' volatile organic acid composition during anaerobic digesting

Acetic acid (AC), propionic acid (PC), and butyric acid (BC) were the most common VFAs found in both groups (Fig 13 and 14). On day 8, the yeast group's acetic acid content peaked at 7.97 g/L, and then began to decline as butyric acid was consumed. Butyric acid levels dropped dramatically from day 11 to day 15. Increases in daily biogas output. As fermentation progressed, a higher concentration of propionic acid was produced, marking the beginning of

a propionic acid-type fermentation. VFAs with more than two carbon chains are easily collected during fermentation because methanogens cannot directly utilise them as a substrate. One frequent kind of short-chain fatty acid is propionic acid. From a metabolic standpoint, propionic acid is typically broken down into AC and H. The conversion of PC to AC is the more difficult of the two processes in the process since it is an endothermic reaction, which are notoriously hard to carry out in thermodynamics (butyric acid is the easier of the two).

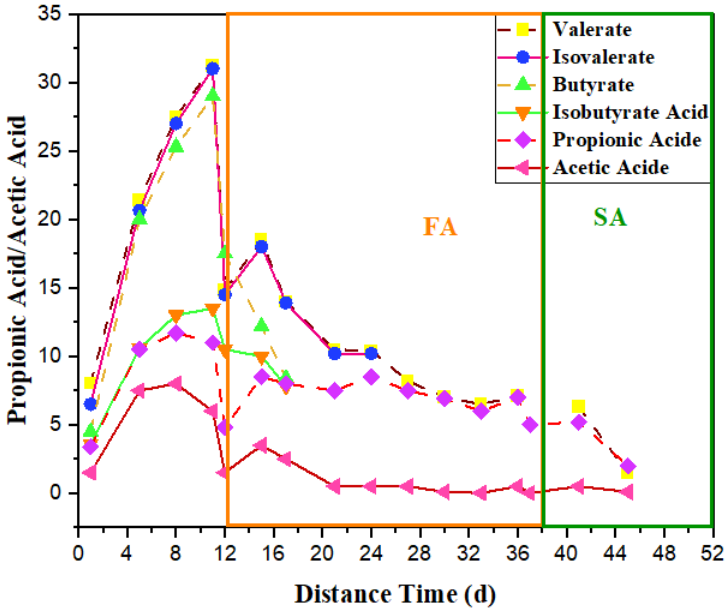

Figure 13. Yeast groups differ in their relative proportions of volatile fatty acids

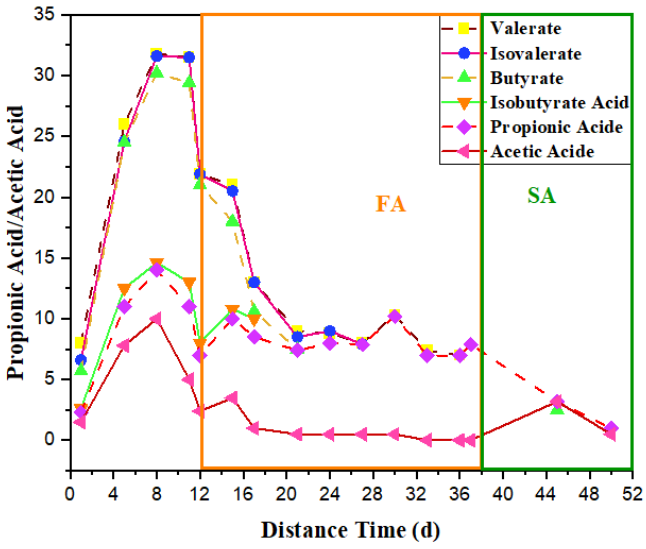

Figure 14. Control groups differ in their relative proportions of volatile fatty acids

Propionic acid has been argued to be an unfavourable substrate for microbes. As a result, methanogenesis is negatively impacted by fermentation of the propionic acid type because

organic acids are not used and instead accumulate. It was discovered that propionic acid can be produced most effectively in alkaline environments with high temperatures. On the other hand, it was proposed that a change in pH could increase the production of volatile fatty acids (FFAs), particularly propionic acid. When the system pH reached 8.0, the main enzyme activities linked with PA production was at its greatest. In the yeast group, the propionic acid concentration was lower after 12 days of fermentation compared to the control group, suggesting that yeast act as a preventative role once PC fermenting process has occurred in the AD fermenting system.

To confirm the presence of inhibitory propionic acidification in the digestive system, the PC/AC ratio can be measured. This ratio is highly predictive of the acidification of the system. Anaerobic fermentation is less stable when the PC/AC ratio is high. Figure 15 displays the propionic acid/acetic acid value shifts in the yeast collection in evaluation to the regulator collection. Since a lot of AC was employed in the first process of fermenting process, the PC/AC number kept going up as the anaerobic process went on. Additionally, propionic acid built up as a by-product because it has no immediate applications. As shown by the decreased PC/AC value in the yeast collection compared to the controller collection, yeast can be employed to inhibit propionic acid production. The addition of yeast increases ethanol synthesis but decreases lactic, propionic, and butyric acid production. To improve methane yield and lower acidification, produce C_2H_6O can be utilised as a minimum-release composites to release AC into the AD system.

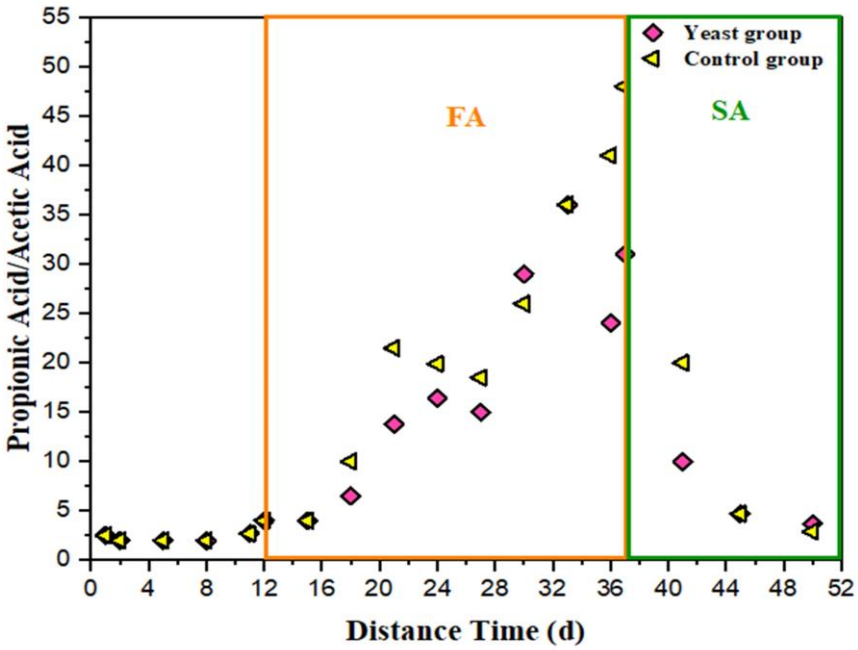

Figure 15. Yeast and control groups differ in their relative proportions of volatile fatty acids (PC/AC).

4. Conclusion

In this study, researchers looked into how adding yeast to LI would affect its rate of Anaerobic Digestion (AD). The results demonstrated that yeast addition can revitalise and boost biogas generation in AD system. Over-acidification was avoided to the yeast-enhanced AD's high volatile fatty acids consuming rate and less propionic acid content. Instead of fermenting LI into volatile fatty acids (VFAs), yeast was used. Methanogens have been shown to readily utilise acetic acid produced from ethanol, which would allow for more methane production and a more stable functioning. Therefore, the inclusion of yeast was proposed as a practical method to keep the AD system stable.

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