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Evaluation of lactic acid from selected vegetable wastes for Faecal sludge treatment

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ABSTRACT

Effective faecal sludge treatment is essential for pathogen reduction and compliance to World Health Organization standards for disposal or reuse. In developing countries, these treatments are frequently absent, ineffective, or immensely expensive. This study used an experimental research design to investigate the efficacy of lactic acid derived from selected vegetable wastes for the treatment of FS collected from an on-site sanitation system. Equal quantities of fresh cabbage, tomato, and carrot wastes were collected, pre-treated, and subjected to lacto-fermentation at 37 °C for six days. Daily monitoring of pH and lactic acid concentrations was performed using an electrode pH meter and a UV-Vis spectrophotometer, respectively.gas chromatography-mass spectrometry (GC-MS) was used to identify lactic acid in the fermented vegetable wastes through a derivatization reaction. FS treatment was performed in four reactors with varying lactic acid ratios (1:1, 1:0.5, 1:0.35), and a control, with *Escherichia coli* (*E. coli*) as the pathogen indicator for faecal contamination. *E. coli*, total solids, volatile solids, and odor levels were monitored over a 16-day treatment period in the four reactors. The fermentation process was deemed successful as indicated by the decrease in pH levels. Successful GC-MS detection of butyl lactate, the derivatized form of lactic acid was observed in the fermented vegetable wastes. Lactic acid concentrations post fermentation were 1.39 ± 0.09 mg/mL, 1.17 ± 0.13 mg/mL, and 1.61 ± 0.34 mg/mL for cabbage, tomato, and carrot wastes respectively. Statistical analysis revealed no significant differences ($p > 0.05$) in lactic acid concentrations among the vegetable wastes on day six of fermentation. Consequently, cabbage waste-derived lactic acid was selected for subsequent experiments in addition to the local abundance of cabbage waste. Total solids and volatile solids decreased across all the reactors over time. From day four, *E. coli* was undetectable in reactor 1:1; which also showed the highest reduction in odor levels. Therefore, reactor 1:1 treatment produced the optimal *E. coli* elimination and odor reduction conditions. This study demonstrates the potential of cabbage waste derived lactic acid for effective FS treatment.

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Introduction

Faecal sludge (FS) is the waste that arises from on-site sanitation systems and is not conveyed through a sewer. It exists as a partly digested or raw slurry or in a semi-solid form. It is generated through storing, collecting, or treating wastewater, including excreta and black water, either with or without grey water (*Shukla et al*., 2023). Globally, safe management of FS is crucial due to the significant health risks associated with exposure to unsanitised FS (*Chen et al*., 2023). The Sustainable Development Goal (SDG) 6 addressing sanitation requires that everyone should have a safely-managed sanitation facility in which excreta can be securely disposed of on-site or processed off-site and that open defecation be eliminated by 2030 (*Hyun et al*., 2019; Thevenon, 2020).

Global coverage of safely managed sanitation services stood at 58%, encompassing approximately 4.6 billion people in 2024. This marks a significant increase from 2015, when coverage was 48%, indicating a 10% rise over the nine years (United Nations Children's Fund & World Health Organization, 2024a). Despite this progress, 43% of the global population—about 3.5 billion people still lack access to safely managed sanitation services (United Nations Children's Fund & World Health Organization, 2024b). This includes 419 million individuals who practiced open defecation, which involves defecating in public places such as street troughs, bushes, or open water bodies (*Ubi et al*., 2021).

Faecal sludge is a significant source of pathogens, containing numerous bacteria, protozoa, viruses, and helminths (*Schlosser-Brandenburg et al*., 2023). Water-borne diseases are associated with lack of proper FS disposal systems (*Chan et al*., 2021). Safe handling and treatment of FS is therefore a key primary barriers in transmission pathways of such pathogens and a great public health concern (Tortajada & Biswas, 2018).

Current FS treatment methods, such as anaerobic digestion, constructed wetlands, composting, and pyrolysis, have limitations. Anaerobic digestion and constructed wetlands require significant infrastructure and land, while composting and pyrolysis are energy-intensive and relatively expensive (*Abdel-Rahman et al*., 2013; Hube & Wu, 2021; Zuberer & Zibilske, 2021). These challenges highlight the need for innovative, cost-effective, and scalable solutions.

Lactic acid offers a promising FS treatment alternative. Lactic acid is a versatile organic acid that can be produced through the fermentation of carbohydrate-rich substrates by lactic acid bacteria (LAB) (*Raman et al*., 2022). Recent studies have explored the use of organic waste such as food and agricultural waste as substrates for lactic acid production, combining waste valorisation with sustainable chemical production (*Odey et al*., 2018a). Vegetable waste, abundant and underutilized, is rich in carbohydrates, nutrients,and an ideal substrate for lactic acid production (*Wan-Mohtar et al*., 2023).

This study evaluated the feasibility of producing vegetable wate derived lactic acid through fermentation and its application as a treatment method for FS. The specific objectives were to identify and quantify the vegetable waste derived lactic acid, enumerate the levels of *E. coli*, total solids (TS), and volatile solids (VS) of FS treated with varying ratios of selected vegetable waste-derived lactic acid, and to evaluate the odour levels of FS treated with varying ratios of selected vegetable waste-derived lactic acid to assess its effectiveness in odour mitigation.

Materials and Methods

Study site

Samples were collected at the Mitunguu decentralized treatment facility in Mitunguu town Meru County, Kenya.

Faecal sludge sample collection

Faecal sludge was sourced from the decentralized treatment facility. Sample size and sampling method were acquired according to the method described by (*Velkushanova et al*., 2021). Five kilograms of FS were obtained via a composite grab sampling technique, which involved taking equal sample portions from an exhauster track at set intervals during emptying. The portions were then mixed to obtain a homogenous mixture and transported in an air-tight, closed high density polyethene container to Meru University of Science and Technology (MUST), Sanitation Research Institute's laboratory, where the initial FS characteristics, such as pH, total solids, volatile solids, and E.coli counts were recorded.

Vegetable wastes sample collection

Fresh cabbage, tomato, and carrot vegetable wastes serving as substrates for lactic acid production were sourced from Nkubu market, Meru County, Kenya,and transported to MUST Biological Sciences laboratory using a cooler box, where size reduction was carried out by use of a kitchen knife and a heavy-duty commercial blender (CK-777, China).

Experimental Setup

Anaerobic Fermentation of Vegetable Waste and Lactic Acid Recovery

One thousand grams of each substrate were thoroughly washed, blended using a heavy-duty commercial blender with 1000 mL of distilled water, and then homogenized to obtain a uniform mixture. The wastes were then poured into plastic containers and sealed to make them airtight and incubated at 37 °C for six days, as described by (*Omar et al.*, 2009). The lactic acid was recovered using the method described by (*Getaneh et al.*, 2021). The incubated vegetable wastes were frozen overnight, followed by thawing in a drying oven at 60 °C for 3 h, followed by filtration and centrifugation at 6000 rpm for 10 min using a centrifuge (Hermle Labnet Z216 MK, USA).

Qualitative Determination of Vegetable Wastes Derived Lactic Acid

The presence of lactic acid was determined using a GC-MS (Shimadzu GCMS-QP2010 SE Single Quadruple) after derivatization via acid-catalysed esterification. Lactic acid was subjected to a derivatization process via acid-catalyzed esterification. The analytes were prepared for GC–MS analysis through derivatization to enhance volatility and improve detection sensitivity. A volume of 10 µL of the sample was accurately measured into a clean glass vial. To this, 2 mL of n-butanol and two drops of 1 M hydrochloric acid were added. The mixture was gently vortexed and subsequently incubated in a water bath at 45 °C for 15 minutes to allow esterification to proceed. Following incubation, the mixture was cooled to room temperature. Thereafter, 500 µL of HPLC-grade water was added, followed by 1 mL of hexane. The resulting mixture was capped and shaken vigorously for 3 minutes, after which it was allowed to stand undisturbed until phase separation occurred. The upper organic layer was carefully separated, and a 100 µL aliquot of this layer was drawn using a micropipette.The aliquot was filtered through a nylon syringe with a 0.45 µm pore size filter. The filtrate was transferred into a clean GC–MS auto sampler vial for analysis. The GC-MS parameters were as follows: injection volume:1 µL, injection mode; split, with a split ratio of 10:1, injector temperature; 200 °C, the carrier gas; helium, supplied at a pressure of 11.3 psi, column flow rate; 1.1 mL/min, total flow; 15.2 mL/min, linear velocity; 38.2 cm/s, and purge flow; 3.0 mL/min. The oven temperature program was as follows, initial temperature; 50 °C (hold time 0.0 min), Ramp 1; 15 °C/min to 200 °C (hold 0.0 min), Ramp 2 ; 10 °C/min to 285 °C (hold 5.5 min). A BPX5, 30 m × 0.25 mm I.D., 0.25 µm film thickness column was used. The mass spectrometer was operated in electron impact ionization (EI) mode at 70 eV, scanning within the range of 40–550 m/z. Data acquisition and chromatogram processing were conducted using the instrument's dedicated software.

Spectrophotometric Quantification of Vegetable Wastes Derived Lactic Acid

Lactic acid was quantified using a spectrophotometric technique (*Borshchevskaya et al.*, 2016). A calibration curve was first prepared using a series of 2-fold dilutions of a lactic acid standard solution (1.2 g in 10 mL distilled water).The absorbance of the lactic acid-iron (III) chloride complex was measured at 390 nm using a spectrophotometer (Apel PD-UV-3000UV, Japan). Cabbage waste was selected as the source of lactic acid among the vegetable wastes due to its local abundance.

Assessment of Cabbage Waste Derived Lactic Acid in the Reduction of E. coli in FS

Four reactors containing 1:1, 1:0.5, 1:0.35, FS to lactic acid, and a control were set up as described by (Getaneh, 2021). Samples were collected from all reactor containers after every four days for the pH, VS, TS analysis, and *E. coli* enumeration for a period of 16 days. Faecal sludge treatment efficacy was assessed using *E. coli* as the indicator organism. The ability of cabbage waste derived lactic acid to eliminate *E. coli* was determined through a total viable plate count after plating on Violet Red Bile Agar (HIMEDIA), which is a selective media for *E. coli* (Basavaraju & Gunashree, 2022; Digo, 2015).

Determination of TS and VS Content of FS before and after Treatment

Total solids and volatile solids content were analysed using the 2540E standard method for wastewater examination.

Determination of pH

The pH of the samples was measured using a pH meter (Model Bante902, Zhejiang, China) standardized using buffer solutions at pH 7, 10, and 4.

Odour Evaluation of FS treated with Cabagge Waste Derived Lactic Acid

The odour level of the FS was determined according to method 2150B of water and wastewater analysis; Threshold Odour Test (T.O.N). The potency of the perceived odor was investigated by diluting the sample with odor-free water until the odor was just detectable. FS samples were added to labeled 500 mL conical flasks and topped up to 50 mL with odorless water. Odorless water was added to a separate flask for use as a standard. The flasks were covered with petri dishes and placed in a water bath (SY-21 6H, China) pre-heated to 60 °C for 10 minutes. The samples were then covered with a foil so that the contents within the flasks were not visible to the panelist. The flasks were randomized using codes and randomly placed on the bench top for the odor test panelists.

Data Analysis

The data obtained from each batch was first analysed using Excel by computing the averages of the three trials conducted per batch. The average values of the three batches were then calculated in order to generate the means, standard deviations, and standard errors.

Parameters	Unit	Initial faecal sludge characteristics Values
pH		5.63
Total solids	g/L	16.30
Volatile solids	g/L	4.3×10^4
E. coli	CFU/mL	1.02×10^4

Table 1: *Initial FS Characteristics*

Results and Discussion

pH and Lactic Acid Concentration of Fermeted Vegetable Wastes

The pH for cabbage, carrot, and tomato fermented vegetable wastes decreased after the 6-day fermentation period at 37 °C. A decrease in the pH levels from 5.90±0.05, 5.37±0.14, and 4.36±0.15 to 3.23±0.10, 3.20±0.16, 3.30±0.07 was observed for cabbage, carrot, and tomato wastes, respectively as shown in *Figure 1*. This result was an indication that fermentation had occurred. The drop in pH in all three vegetable wastes indicates the progress of fermentation. During this process, LAB convert sugars present in the vegetable wastes into lactic acid while causing a decline in pH (*Jabłońska-Ryś et al.*, 2022; *Kaur et al.*, 2019). The initial sharp decrease in pH observed after three days for the three fermented vegetable wastes suggests rapid fermentation activity, where LAB quickly utilizes the abundant fermentable sugars to produce energy while producing lactic acid as a metabolite, resulting in acidification. After which the pH levels stabilize, indicating that the fermentation process has stopped due to depletion of fermentable sugars (*Deshwal et al.*, 2021).

The steady final pH indicates a stable fermentation endpoint. These results suggest that most of the fermentation process occurred earlier in the process, followed by a stabilization phase of three days, which is the period taken to achieve a stable pH. These results agree with findings by (*Anderson et al.*, 2015; *Getaneh et al.*, 2021), which reported three days as the period during which the pH appears to stabilize.

A calibration curve ($y=0.382x - 0.0039$, $R2 = 0.9984$) was constructed from a standard lactic acid which was used to calculate the concentration of lactic acid in the fermented vegetable wastes. Lactic acid production was monitored over a six-day fermentation period, with statistical differences in mean concentrations across days evaluated using a one-way analysis of variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) post hoc test. As depicted in *Figure 1*, lactic acid concentrations exhibited a pronounced increase from Day 0 to Day 2, followed by a stabilization from Day 3 onward. The Tukey HSD test revealed that lactic acid production on Day 0 was significantly lower than on all subsequent days ($p < 0.001$), underscoring a rapid onset of fermentation and acid accumulation within the first 24 hours. This finding highlights the critical initiation phase of microbial activity immediately following the commencement of fermentation.

Similarly, the mean lactic acid concentration on Day 1 was significantly lower than those observed on Days 2 through 6 ($p < 0.001$), indicating a substantial increase in production between Day 1 and

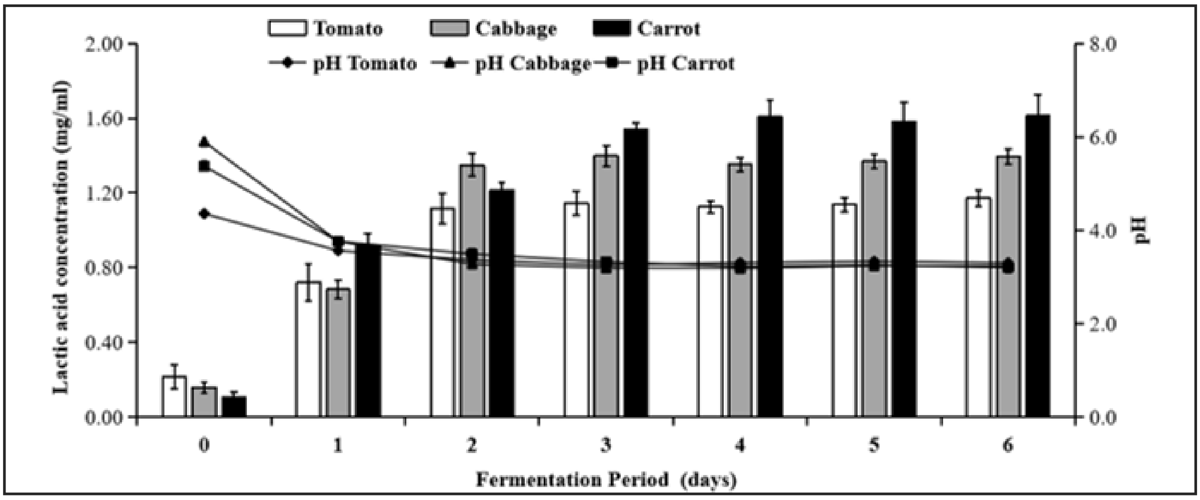

Figure 1: *pH changes and lactic acid concentrations of fermented vegetable wastes*

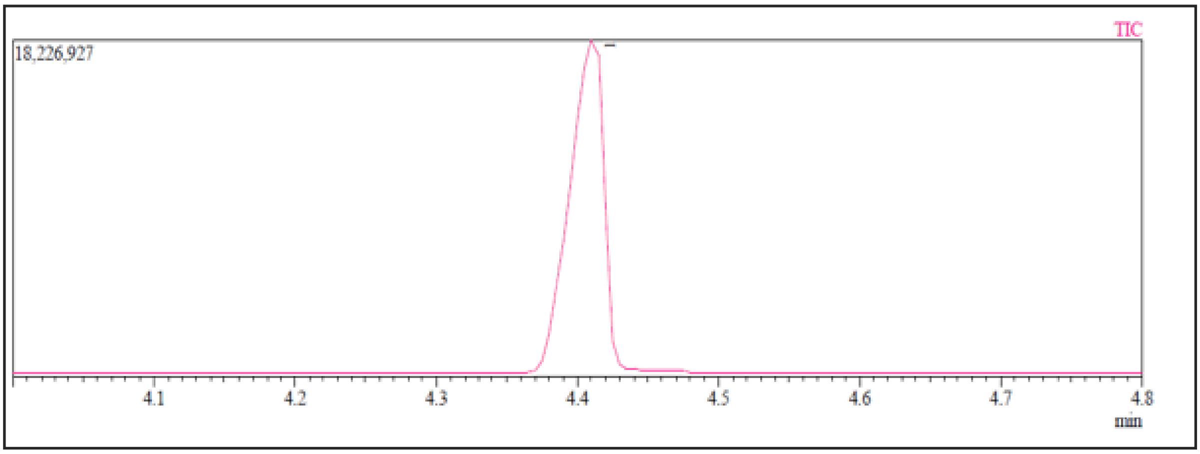

Figure 2: *Chromatogram of lactic acid standard derivative (butyl lactate)*

Day 2. The mean difference in lactic acid concentration between Day 1 and Day 2 was 0.395 mg/mL, with a statistically significant difference ($p = 0.001$). However, no significant differences were detected among Days 2, 3, 4, 5, and 6 ($p > 0.99$), suggesting that lactic acid production reached a plateau after Day 2. Statistical analysis revealed no significant differences ($p > 0.05$) in lactic acid concentrations among the vegetable wastes on day six of fermentation.Consequently, cabbage waste-derived lactic acid was selected for subsequent experiments, in addition to the local abundance of cabbage waste in the study area.

Qualitative Determination of Vegetable Waste Derived Lactic Acid using GC-MS

GC-MS was used to qualitatively determined the prescence of lactic acid in the fermented vegetable wastes. The results in *Figure 2* above show the detection of the standard analytical grade lactic acid in its esterified form butyl lactate derived from esterification of lactic acid and butanol.

GC–MS chromatograms of fermented cabbage, carrot and tomato wastes are shown in *Figure 3 (A),(B), & (C)*. These results confirm the production of lactic acid in the fermented vegetable wastes evidenced by the detection of butyl lactate in all the fermented samples. Additionally, low-abundance butane derivatives were detected, which are likely linked to derivatization reactions or from background matrix components.

pH of FS treated with varying ratios of cabbage waste derived lactic acid

Comparative analysis of pH in the reactors is shown in *Figure 4*. The control showed a steady increase in pH from slightly acidic 6.13 to moderately alkaline 8.20. This increase could be attributed to

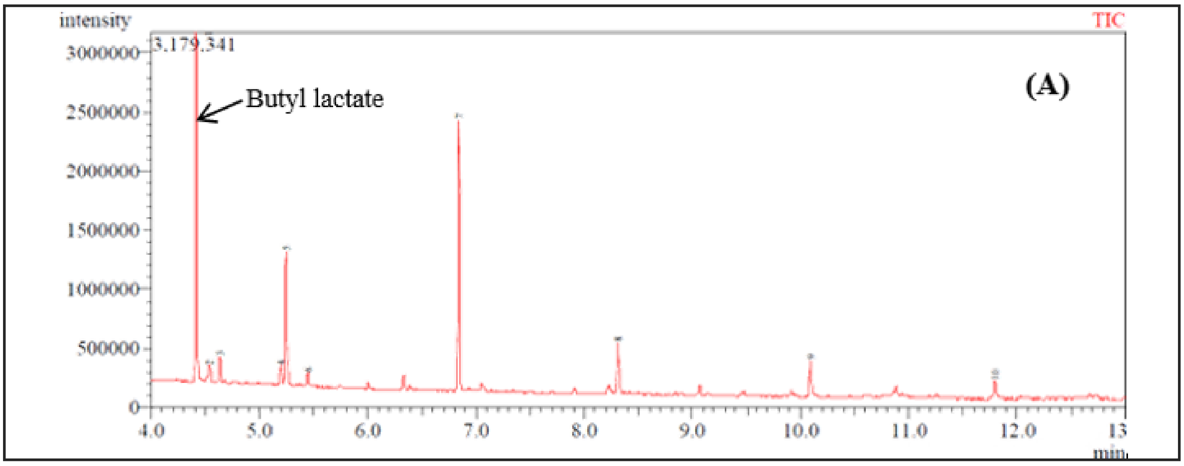

Figure 3 (A): *GC-MS chromatogram of cabbage fermented wastes. (1) Butyl lactate, (2) 1-Butoxy(methoxy)methoxy) butane, (3) Boronic acid, ethyl-,diethylester, (4) Butane, 1,1'-[ethylidenebis(oxy)]bis-, (5) Dibutoxy(dimethyl)silane, (6) Butane, 1,1'-[(1-methylethylidene)bis(oxy)bis, (7) Butane, 1,1-dibutoxy, (8) Tetradecane, (9) Hexadecane, (10) Heptadecane*

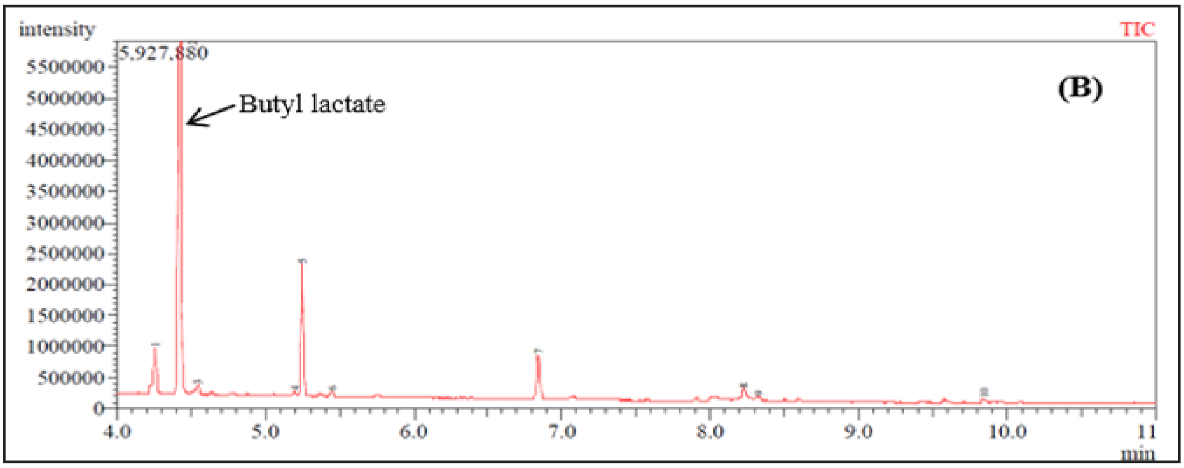

Figure 3 (B): *GC-MS chromatogram of carrot fermented wastes. (1) Butanoic acid, butyl ester (2) Butyl lactate (3) 1-Butoxy(methoxy)methoxy)butane, (4) Butane, 1,1'-[ethylidenebis(oxy)]bis-, (5) Dibutoxy(dimethyl)silane, (6) Butane, 1,1'-[(1-methylethylidene)bis(oxy)bis, (7) Butane, 1,1-dibutoxy, (8) Sulphuric acid dibutyl ester, (9) 1,1,3,3,5,5-Hexamethyltrisiloxane, (10) Butanedioic, dibutyl ester*

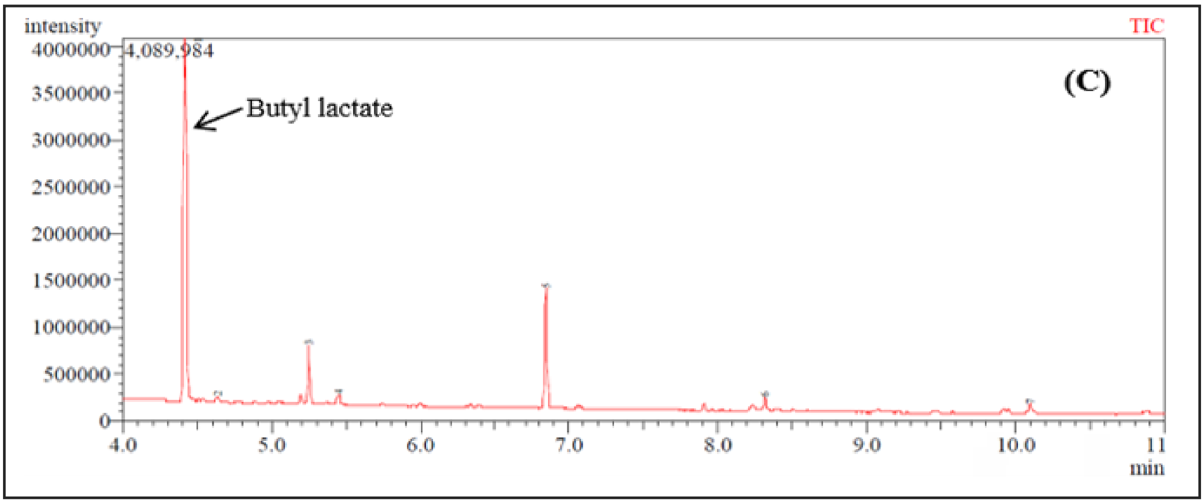

Figure 3 (C): *GC-MS chromatogram of tomato fermented wastes. (1) Butyl lactate, (2) Boronic acid, (3) Dibutoxy(dimethyl) silane, (4) Butane, 1,1'-[ethylidenebis(oxy)]bis- (5) Butane, 1,1-dibutoxy-, (6) Tetradecane, and (7) Hexadecane*

natural biochemical processes in the FS, such as ammonia production due to urea breakdown (*Chang et al.*, 2015). Reactor 1:1 had the highest pH decline by Day 4. The reactor exhibited a significant drop in pH, indicating a high concentration of lactic acid. The slight increase after Day 4 suggests some buffering capacity or metabolic changes in the sludge that partially counteract the acidity. These results are in agreement with (*Getaneh et al.*, 2021), findings where a decrease in pH to 4.05 by day five of treatment was observed.

In reactor 1:0.5, the initial pH (Day 0) was 6.13 while the final pH was 5.85. Reactor 1:0.5 exhibited a moderate decrease in pH compared to reactor 1:1. This suggests that the lower lactic acid concentration allowed for some neutralizing processes within the sludge. Reactor 1:0.35 showed the least change in pH, with a minor depression followed by a rise to slightly basic levels. These results suggest that lactic acid concentration significantly influenced the pH of FS.

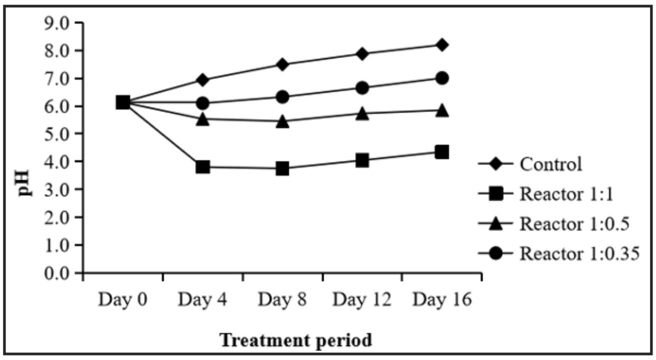

Figure 4: *pH of FS treated with varying ratios of cabbage waste derived lactic acid*

Levels of E. coli in FS treated with different ratios of cabbage waste derived lactic acid

Results of *E. coli* levels (cfu/mL) over a 16 day treatment period of FS with varying ratios of cabbage waste derived lactic acid. and a control are shown in Figure 5. There was a complete elimination of *E. coli* in reactor 1:1 by day 4, whereas, the levels of *E. coli* remained higher in reactors 1:0.5, 1:0.35 and the control. Reactor 1:1 was highly effective in eliminating *E. coli*. This is attributed to the low pH observed in reactor 1:1 (*Figure 4*) which inhibited the growth of *E. coli*, as acidic pH levels fall outside the optimal range for *E. coli*'s growth, likely leading to its elimination. Other research suggests that a pH range of 3.51 to 4.2 can effectively eliminate various pathogens (*Rodrigues et al.*, 2020). The antimicrobial property of lactic acid could have contributed to the limited growth of *E. coli* (*Anderson et al.*, 2015; *Getaneh et al.*, 2021).

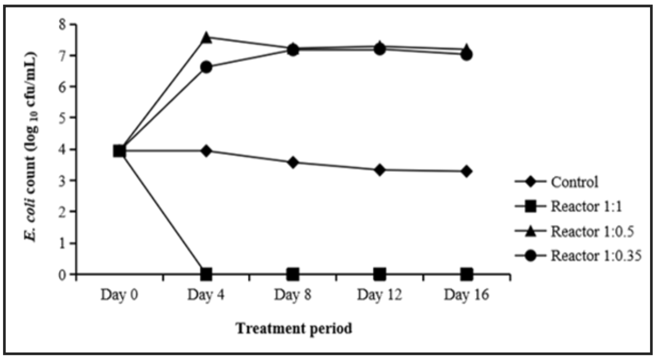

Figure 5: *Levels of E. coli in FS treated with varying ratios of cabbage waste derived lactic acid*

The results for reactors 1:0.5 and 1:0.35 agree with the findings by (Malambo, 2014), who reported an initial increase in *E. coli* count for the treatment reactors within the initial four days of treatment. The growth might have been supported by the utilization of different carbon sources such as glucose, lactose, and galactose that might have been available in the lactic acid extract (*Cutrim et al.*, 2016). These findings further suggest that reactors 1:0.5 and 1:0.35 conditions did not contain the threshold lactic acid amount or acidification levels required to eliminate or significantly lower the *E. coli* levels.

Trend of VS and TS of FS treated with varying ratios of cabbage waste derived lactic acid

Monitoring VS and TS provides insight into the efficiency of biodegradation processes. *Figure 6* shows the trend of VS and TS over the 16-day treatment period of FS with varying ratios of cabbage waste derived lactic acid. There was decrease in VS and TS in all the reactors. The gradual reduction of VS in the control is expected due to natural microbial activity and degradation processes occurring in the sludge without any additional treatments. Reactor 1:1, shows a similar pattern an indication of a slower but steady decomposition process compared to other reactors. On the other hand, reactors 1:0.5 and 1:0.35showed an initial rapid decrease in VS. This suggests a highly efficient microbial degradation process due to a suitable environment for microbial activity and organic matter breakdown.

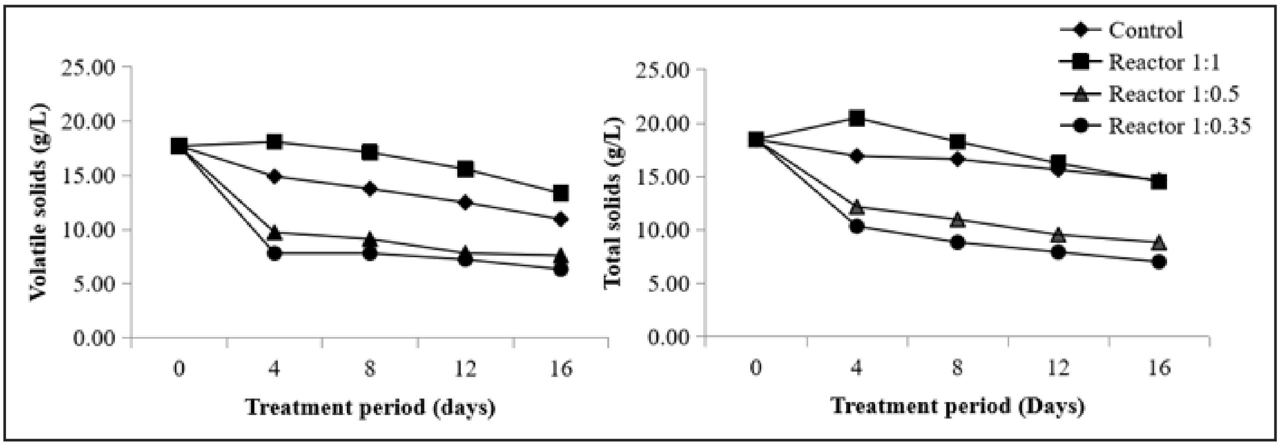

Figure 6: *VS and TS of FS treated with varying ratios of cabbage waste derived lactic acid*

The efficiency of VS reduction in FS treatment is critical for reducing the sludge's volume (*Zewde et al.*, 2021). Effective treatment results in lower organic load, making the remaining sludge more stable and less odorous (*Demirbas et al.*, 2017). High microbial activity and optimal environmental conditions facilitate the breakdown of organic matter in FS (*Cofie et al.*, 2016).

Similar pattern as that of VS was observed for TS in all four reactors. The reduction is consistent with typical sludge stabilization processes observed in untreated or minimally treated FS (Strande & Brdjanovic, 2014). This was also observed in study conducted by *Odey et al.* (2018) who reported a final TS of 33% in the 1:1 (FS: lactic acid) reactor, which was higher than the control (13%) and the 1:2 reactor (19.2%).

Odour Levels of FS treated with varying ratios of cabbage waste derived lactic acid

High level Threshold Odour Number (T.O.N)indicates a significant presence of odour, while a low T.O.N indicates low odour levels.**Table 2** presents the mean levels T.O.N of FS treated with varying ratios of cabbage waste derived lactic acid.

	Mean level T.O.N
Initial FS	37.33
Control	25
Reactor 1:1	7.33
Reactor 1:0.5	42.67
Reactor 1:0.35	26.67

Table 2: *TON levels of FS treated with varying ratios of cabbage waste derived lactic acid.*

The 1:1 ratio reactor showed the lowest T.O.N levels indicating the highest decrease in odour levels compared to the other reactors. The odour reduction was likely due to the low pH and the high concentration of lactic acid in the reactor, which inhibited the growth of bacteria that degrade proteins, such as sulphur-reducing bacteria. These bacteria typically produce odorous compounds, including dimethyl trisulfide, hydrogen sulphide (H_2S), dimethyl disulphide, methyl mercaptan, and dimethyl sulphide (*Choudhury et al.*, 2024; Newman, 2023). Low pH in the range 3-4.5 has been reported to reduce urea hydrolysis to ammonia (another odorous compound) by inactivating urease-producing bacteria (*Ray et al.*, 2018). T.O.N values for reactor 1:0.5 could have been due to the low lactic acid concentration and the relatively high pH that allowed growth of microbes throughout the treatment period (*Anderson et al.*, 2015; *Andreev et al.*, 2017). Continued microbial activity led to the breakdown of organic material, thereby producing large amounts of odour-causing compounds (*Zewde et al.*, 2021). Similar results were reported by *Anderson et al.*, (2015); *Getaneh et al.*, (2021); Odey et al., (2018), who reported decreased odour levels in higher lactic acid concentrations and higher odour levels in lower lactic acid concentrations treatments.

Conclusion

This study reveals a trade-off in using cabbage waste-derived lactic acid for FS treatment.The application of varying concentrations of lactic acid revealed distinct outcomes: high concentrations effectively eliminated *E. coli* and reduced odour, but were less effective in reducing VS and TS. Conversely, lower concentrations reduced VS and TS, but less effective in *E. coli* elimination and odour control. Optimizing lactic acid concentrations is crucial to balance sanitization and sludge stabilization. Further research should focus on refining these treatment ratios and exploring synergistic approaches to enhance both sanitization and solids reduction for sustainable FS management.

Conflict of Interest

The author declares no conflict of interest.

Ethics Statements

Informed consent of the participants was obtained, and the study protocol was approved by the National Commission for Science, Technology, and Innovation (NACOSTI) under research permit NACOSTI/P/25/415907. The approval was granted in accordance with national research regulations in Kenya.

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