Lactic Acid Bacteria Isolation and Ethanol Production from Corn, Millet and Sorghum

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Abstract
The demand of bio ethanol as substitute of gasoline had rapidly increased. Today bio ethanol can be produced by dry grind or by wet mill process of food grains (Corn, Millet and Sorghum). Basically, the step involved in the production of bio ethanol includes grinding fermentation and distillation. This project shows bio ethanol production and extraction from the food grains. Also some of the lactic acid bacterial species that were involved in the fermentation process were isolated and identified, as Lactobacillus ferment, Lactobacillus lactics, Lactobacillus planterum, Lactobacillus bulgaricus and Weissella spp. This project work shows that with thehelp of these lactic acid bacteria and others like Saccharomyces cerevisiae, over 6.5% ethanol was obtained by distillation (78°C) 72hours of fermentation. The environmental factors and conditions that after favors the production of ethanol from the substrate sources includes: temperature, pH, anaerobic condition and the sterility of the materials used were put in place to avoid contamination by foreign microorganisms. Standard microbiological procedure was used to determine those lactic acid bacteria that involved in the fermentation of the food grains before the distillation was carried out.

Introduction and Literature review

The excessive presence of carbon dioxide in the atmosphere is a significant contributor to global warming, primarily driven by the widespread use of fossil fuels (Archer, 2005). In response, several regions, including India, Europe, Brazil, and the United States, have turned to biofuel production derived from plants as a strategy to mitigate carbon emissions (Roseiro et al., 2004).

Bio-ethanol, a renewable fuel produced from agricultural crops, has emerged as a cleaner alternative for road transport applications (Sanchez, 2007). By 2009, global ethanol fuel production had reached approximately 19.5 billion gallons (RFA, 2010). The demand for bioethanol as a road fuel is rising so rapidly that it is beginning to surpass supply levels (Kennedy et al., 2004). This has led to an increasing need for alternative plant species capable of generating large volumes of biomass for cost-effective ethanol production on an industrial scale. Common crops used in ethanol production include maize, sugar beet, sugarcane, corn, millet, sorghum, and cassava (Adelekan, 2010).

A critical concern in biofuel production is food security, particularly when food crops are utilized for energy generation. Therefore, crops selected for future biofuel production should ideally serve a dual purpose—providing both food and energy. One such promising crop is sweet sorghum (also known as Sorgo), a plant native to Africa

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that belongs to the Sorghum bicolor (L) Moench family (Bryan, 1990). Sweet sorghum is unique in that its grain can be harvested for human consumption while its stalk contains a sugary juice that can be extracted and converted into ethanol. The remaining fibrous stalk material, known as bagasse, can either be repurposed as animal feed or processed further for ethanol production (Dolciotti et al., 1998).

Sweet sorghum is widely regarded as a cost-effective source for ethanol production (Barbanti et al., 2006; Yun-long et al., 2006; Wang et al., 2009). The crop is grown in various parts of the world, with commercial cultivation in South Africa spanning approximately 120,000 hectares between 1995 and 2005, yielding an average of 2.9 tons per hectare (NDA, 2006). A fully mature sweet sorghum plant consists of about 75% cane, 10% leaves, 5% seeds, and 10% roots by weight (Grassi et al., 2002). Utilizing both the extracted juice and the bagasse for ethanol production can significantly enhance ethanol yields per hectare.

Fermenting sweet sorghum using yeast has the advantage of rapid ethanol conversion (Liu et al., 2008). However, yeast growth and ethanol fermentation require sufficient levels of carbon and nitrogen, which the naturally occurring inorganic salts in sweet sorghum juice may not provide in adequate amounts (Mei et al., 2009). Previous research has shown that the addition of yeast extract, ammonium, urea, calcium, and magnesium can improve both fermentation rates and ethanol yield (Bafrncova et al., 1999; Sipos et al., 2008; Jones et al., 2004).

A study conducted by Asli (2010) explored the impact of different nitrogen sources, pH levels, and dilution rates on sweet sorghum juice fermentation. The highest ethanol yield was achieved at a pH of 4.5 using ammonium sulfate as the nitrogen source, with an initial sugar concentration of 100 g/L. Similarly, Kundiyana et al. (2010) found that fermentation at a pH of 4.3 without urea supplementation or prior juice sterilization resulted in the highest ethanol production. Additionally, D'amore et al. (1989) optimized yeast concentrations for ethanol fermentation, concluding that yeast concentrations between 2% and 3.5% (w/v) produced the best ethanol yields.

Liu et al. (2008) further investigated the effect of different inorganic salts on ethanol production and found that ammonium sulfate produced the highest yields. This compound not only stabilizes the fermentation pH but also provides essential sulfur nutrients for yeast metabolism. During the fermentation process, sugar concentration must be carefully managed, as excessive sugar levels can create osmotic stress on yeast cells, leading to lower ethanol output (Roukas, 1996). Research by Zanette et al. (2007) demonstrated that lower dilution rates allow sufficient time for sugar-to-ethanol conversion, but prolonged retention can increase unwanted by-product formation.

This study examined the fermentation process of corn, millet, and sorghum harvested from different farms, with specific focus on pH levels, bacterial concentrations, temperature, and titratable acidity.

Lactic Acid Bacteria (LAB) and Their Industrial Relevance

Lactic acid bacteria (LAB), classified under the order *Lactobacillales*, are a group of Gram-positive,acid-tolerant microorganisms that do not form spores and lack respiratory mechanisms. They appear in both rod-shaped and spherical (coccus) forms and share metabolic and physiological characteristics. Commonly found in decomposing plant material and dairy

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products, LAB are known for their ability to produce lactic acid as the primary metabolic byproduct of carbohydrate fermentation. This acidification process has historically linked LAB to food fermentation, as the increased acidity helps inhibit spoilage organisms. Additionally, certain LAB strains produce protein-based bacteriocins, which provide further protection against spoilage and pathogenic microbes.

Beyond their fermentation role, LAB contribute to the sensory and textural properties of food products. Their widespread occurrence in food and their beneficial effects on the human microbiome have led to their classification as generally recognized as safe (GRAS). The primary genera of LAB include Lactobacillus, Leuconostoc, Pediococcus, Lactococcus, and Streptococcus, along with secondary members such as Oenococcus, Aerococcus, Carnobacterium, Enterococcus, Sporolactobacillus, Tetragenococcus, Vagococcus, and Weissella.

LAB thrives in acidic conditions, giving them a competitive edge in fermentation, as they can tolerate the low pH environment generated by their own lactic acid production. In laboratory settings, they are typically cultivated in media containing carbohydrates, as they rely on fermentation rather than respiration. They lack catalase enzyme activity and possess a structurally simple bacterial cell organization. Due to their significant role in food processing, LAB are among the most important microbial groups in the food industry (Scheff et al., 2002).

Fermentation Pathways in LAB

LAB species are classified based on their hexose fermentation pathways. Under glucose-rich and oxygen-limited conditions, homolactic LAB utilize the Embden-Meyerhof-Parnas pathway, breaking down one glucose molecule into two pyruvate molecules. To maintain intracellular redox balance, NADH is oxidized while pyruvate is reduced to lactic acid, yielding two ATP molecules per glucose molecule consumed. Homolactic LAB include genera such as *Lactococcus*, *Enterococcus*, *Streptococcus*, *Pediococcus*, and certain *Lactobacillus* species.

In contrast, heterofermentative LAB use the pentose phosphate pathway (also called the phosphoketolase pathway). In this process, glucose-6-phosphate is first converted into 6-phosphogluconate before undergoing decarboxylation to produce carbon dioxide. The resulting pentose-5-phosphate is then cleaved into glyceraldehyde phosphate (GAP) and acetyl phosphate. While GAP follows a similar metabolic route as in homofermentation, acetyl phosphate is reduced to ethanol through acetyl-CoA and acetaldehyde intermediates (Bolotin et al., 2001).

Overview of Corn (Zea mays)

Corn (*Zea mays*), a cereal crop from the grass family, originated in Central America and has since been cultivated worldwide. The introduction of corn to Western civilization dates back to 1492, when Columbus and his crew encountered the grain in Cuba. Unlike other major grains, which were introduced to the Americas from Europe, corn traveled in the opposite direction, spreading across European countries. Historians debate whether corn was first brought to Spain by Columbus himself or if its introduction occurred during his second voyage.

The term "corn" varies in meaning across different regions. In the United States, "corn" refers to maize, while in some countries, it designates the dominant grain of the region. In the United Kingdom, "corn" means wheat, whereas in Scotland and Ireland, it denotes oats. Biblical

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references to "corn" are often linked to wheat or barley (Li et al., 2008).

Corn was initially regarded as a botanical novelty in Europe but soon gained recognition as a valuable food crop. By the mid-1500s, it had spread across France, Italy, and southeastern Europe, reaching China and the Philippines by the late 16th century. While its exact point of origin remains debated, archaeological evidence suggests that maize was domesticated in the Tehuacán Valley of Mexico. Pollen grains retrieved from deep beneath Mexico City indicate the presence of maize approximately 80,000 years ago, while radiocarbon dating of corn cobs found in New Mexico's Bat Cave suggests cultivation as early as 5,600 years ago (Van den Berg et al., 2004).

Millet and Sorghum: Key Cereal Crops

Millets are a diverse group of small-seeded grasses cultivated globally for both human consumption and animal fodder. They are particularly significant in semi-arid regions of Asia and Africa, where they account for nearly 97% of production (Kaur, 2012). The resilience of millets under harsh climatic conditions, including drought and high temperatures, has made them a staple crop in countries such as India, Nigeria, Niger, and Mali.

Among millet varieties, pearl millet is the most widely grown, particularly in India and Africa. Other key millet species include finger millet, proso millet, and foxtail millet. Historical evidence suggests that millets have been cultivated for over 10,000 years, particularly in East Asia (Pradhan et al., 2010).

Sorghum, classified under the genus *Sorghum* in the *Poaceae* family, consists of approximately 25 species, most of which are native to Australia. However, sorghum has spread across Africa, Asia, and the Americas (Grassi et al., 2002). While some species are cultivated for grain production, others serve as fodder crops in pasture lands. Sorghum thrives in warm climates and has been naturalized in various regions worldwide (Geodestinies, 2002).

Sweet sorghum, a variety of *Sorghum bicolor (L) Moench*, is indigenous to Africa and holds significant potential for ethanol production (Bryan, 1990). It produces both edible grains and a sugary juice in its stalk, which can be processed into sugar or fermented into ethanol. After juice extraction, the remaining fibrous material, known as bagasse, can be utilized as animal feed or subjected to hydrolysis and fermentation for additional ethanol yield. Sweet sorghum is considered a promising, cost-effective crop for large-scale ethanol production (Barbanti et al., 2006; Yun-long et al., 2006; Wang et al., 2009).

Currently, there are over 4,000 sweet sorghum varieties cultivated globally (Grassi et al., 2002). In South Africa, commercial sorghum cultivation covered approximately 120,000 hectares between 1995 and 2005, with an average yield of 2.9 tons per hectare (NDA, 2006). The composition of ripe sweet sorghum typically includes 75% stalk, 10% leaves, 5% seeds, and 10% roots (Grassi et al., 2002). Utilizing both sweet juice and bagasse in ethanol production significantly enhances bioethanol output per hectare (Dolciotti et al., 1998).

Corn Stover as a Bio ethanol Source

Corn stover, the residual biomass left after corn harvest, is an abundant agricultural byproduct, with an estimated global production of 1.09 billion tons in 2018-2019 (Periyasamy et al., 2024). Rich in lignocellulose, corn stover contains approximately 38-40% cellulose, 28% hemicellulose, and 7-21% lignin (Li et al., 2010). Given its high biomass yield, it is

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increasingly being considered for large-scale bioethanol production (Amornraksa et al., 2020). Researchers such as Amornraksa et al. (2020) have also developed systematic separation processes to optimize bioethanol production from corn stover.

I.3 Uses of corn, millet and Sorghum

Importance of Corn, Millet, and Sorghum

Corn, millet, and sorghum play a significant role in food security and industrial applications across various regions of the world. These crops provide staple food sources, serve as livestock feed, and, to a lesser extent, act as raw materials for industrial processes. They are particularly valued for their starch content, which is used in adhesive production, textile and paper sizing, as well as in the confectionery and baking industries. In tropical regions, corn, millet, and sorghum are typically prepared through boiling, baking, roasting, or frying for direct human consumption (Devi et al., 2011).

Ethanol Production and Its Uses

Ethanol is a clear, liquid alcohol obtained through the fermentation of biological materials. Although ethanol has multiple applications, its role as a biofuel has gained increasing attention. The production process for ethanol is similar to beer brewing, where sugars are fermented to create alcohol. Once processed, ethanol is commonly blended with gasoline to enhance vehicle efficiency while reducing environmental pollution.

The term "alcohol" originates from the Arabic word *al-kuhul*, historically referring to a finely ground antimony powder used as an eye cosmetic. Ethanol is highly soluble in water and mixes well with most organic solvents, making it a valuable ingredient in various industries. It serves as a solvent in the production of perfumes, paints, lacquers, and explosives and is also used as a fuel source for engines and home heating systems. In pharmaceutical applications, solutions containing non-volatile substances dissolved in alcohol are called tinctures, while solutions of volatile solutes are referred to as spirits.

The Economic and Environmental Impact of Ethanol

The Renewable Fuels Association (RFA), an advocacy group for the ethanol industry, suggests that increased ethanol production has led to higher demand for corn, subsequently raising its market value. The association claims that this trend has benefited U.S. farmers by reducing the need for government farm subsidies. In a statement released in January 2007, the Chief Economist of the U.S. Department of Agriculture (USDA) projected that farm program payments could decline by approximately $6 billion due to the increased value of corn (Dominguez et al., 1998). However, the impact of rising corn prices on populations that rely on it as a staple food source remains a subject of debate.

From a sustainability perspective, ethanol derived from grains such as corn, millet, and sorghum contributes to a reduction in greenhouse gas (GHG) emissions compared to traditional gasoline. On a life-cycle basis, ethanol production results in approximately 20% lower GHG emissions than gasoline. With advancements in production efficiency and the adoption of renewable energy sources, this reduction could reach up to 52%. Ethanol also has the advantage of biodegrading naturally without causing environmental harm, making it a safer alternative to conventional fuel additives like methyl tertiary-butyl ether (MTBE).

2.0 Materials and methods

2.1 Sample source

Three different food grains (Corn, Millet and Sorghumn) bought at Osele market

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Ikare-Akoko were used separately for Bio-ethanol production.

Substrates and materials used

Food grains (Corn, Millet and Sorghum), conical flasks, spatula, Bunsen burner,stirring rod, beakers, measuring cylinder, test tubes, test tube rack, cotton wool, petridishes, aluminum foil, Nutrient Agar, Ethyl ethylene blue agar [EMB]. pippette, microscope, autoclave, incubator, Durham tubes, paper tape, hot plate, conical flask, sterile water, ethanol, Retort stand, pH metre, thermometer, glass slides, weighing balance, patula, beaker, fermenter, inoculating loop, slant bottles, IM of NaOH phenolphthalene.

2.2.1 Sterilization of materials used

Glass wares such as petri dishes, MeCartneybotles, test tubes, reagent bottles weresterilized in an autoclave at 121^0C for I5minutes. Inoculating loops was sterilized byflame before and after each use.

2.3 Preparation of culture media

Nutrient Agar (NA), which is a general purpose media, Ethyl ethylene blue agar(EMB) was used for the isolation and enuneration of bacteria. Potato Dextrose Agar(PDA) was used for isolation and cultivation of fungi. All the culture media were prepared according to manufacturer's action, brought to homogenous solution by allowing the soaking of the agar and was swirled and autoclaving was done at $12l^0$Cfor 15 minutes (Sumbo*et al.* , 1992).

Procedure for fermentation

Food grains (Corn, Millet and Sorghum) were obtained from Osele market Ikare Akoko, Ondo State Nigeria. They were washed in sterile distilled water. Three hundred grams of the grains were washed and placed in a clean bowl containing 2litres of distilled water separately and then grinded. The grinded mash was allowedto ferment for 3 days at room temperature 30 ± 20C (Ojokoh, 2007).

Determination of pH of fermented food grains [Corn, Millet and Sorghurm] mash

pH meter was used to measure the pH of fermented samples. This was done by first standardizing the pH meter (Hanna multi - parameter -H1-9828) fitted with glass electrodes with buffer solutions and then the pH reading of the sample at 0 hour, 24hours, 48 hours, and 72 hours (lland *et al*.. 2000).

Determination of the temperature of the fermented food grains (Corn, Millet and Sorghum) mash

The temperature of each mash was measured with thermometer at 0 hour, 24 hours,48 hours, and 72 hours (llan*det al*., 2000).

Isolation of lactic acid bacteria

Serial dilution was made for each mashed grains during fermentation. From the fermented mash, lml was taken aseptically with the aid of sterile calibrated pipette and dispensed into 9ml of distilled water in the first tube. The first test tube was shaken thoroughly and Iml from it was transferred with a sterile calibrated pipette in to a second test tube which was also shaken thoroughly. The dilutions were repeated up to one millimeter from this dilution was drawn aseptically using a sterilecalibrated pipette into some already labeled petri dishes. The petridishes were agitated gently in circular motion to ensure uniform on the distribution and uniform growth of the organisms on the different agar plates. The already prepared media were then poured on the sample in the petridish and was swirled gently and allowed to solidify. Plates containing the nutrient agar were incubated in an inverted position at 370C for 24 hours. While plates containing potato dextrose agar was incubated at27^0C for 72 hours. The analyses were done at 0 hours, 24 hours, 48 hours and 72hours the mashes. All the

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colonies found were counted manually and then multiplied by the corresponding dilution factor. The colonies formed on the PDA plates were counted as spore forming units per gram of sample (Cowan *et al*., 2014; Buchanan*et al*., 2007).

Sub culturing

Microorganisms grow in mixed population with so many species. To obtain a pureculture there is need for sub culturing. A pure culture is a population of cell thatarises from a single cell. A loop full of a colony that is farther from the other colonies is taken aseptically and spread over the surface of a prepared nutrient agar with asterile inoculating loop. This reduces the density of the microbial cells. The plates were incubated at 37°C for 24hours on inverted position.

Determination of titratable value of fermented food grains (Corn, Millet and Sorghum) mash

Twenty milliliter of the sample was dispensed into a conical flask and 2 drops of phenolphthalein indicator was added. The content of the flask was thoroughly mixed in the flask and titrated against 0.IM NAOH. The appearance of a pink colour marked the end point of the reaction (llande*t al*., 2000). The Titratable acidity (TTA)was then calculated using the formula below:

TTA= Average base titre (ml) x molarity of base (mol) x 100TTA

Volume of sample (ml)

Identification of lactic acid bacteria isolated

The identity of the bacteria isolates was confirmed by subjecting them to morphological characterization. The appearance of the colony of each organism of the agar was studied and characteristics such as shape, edge, colour, elevation and texture were observed to confirm their identities (Cullimore, 2000; Aneja, 2003:Roberts and Greenwood, 2003). After the periods of incubation the plates were observed for bacterial growth, colonies were then counted and isolates were morphologically characterised by observing the elevation, shape, margin and colour.

The sub cultured fungal isolates were identified through macroscopic observation oftheir colonies. Microscopic examination of the respective spores and hyphalappendages using wet mount method with lactophenol cotton blue and water as maintain respectively was also conducted for fungi (Sharma, 2009). Several physiological and biochemical attributes of the fungi isolates was evaluated according to methods described by Barnett*et al*., (2000).

Biochemical characterization generally carried out includes the Gram stain reaction, Motility test, Catalase test, Coagulate test and Sugar fermentation

Distillation

Distillation is the process of separating the ethanol from the solids and water in the mash. Alcohol vaporizes at 78°C and water at 100°C (at sea level). This difference allows water to be separated from ethanol by heating in a distillation column.

Results

The dilution factor, changes in pH, titratable acidity, temperature, bacteria coliform count of fermented Corn, Millet and Sorghum mash at 24,48 and 72 hours are shown in table 1. 2 and 3 below, table 4 shows the cultural characteristic of LAB isolates, table 5 shows the morphological, biochemical characteristics and identification ofisolates and were distillated after 72 hours of fermentation and Table 6 shows the result of the distillated mash after 72 hours of fermentation, which 1 litre of eachmash were distillated and 75cl of ethanol was obtained from 1litre of cornmash,6.8cl of

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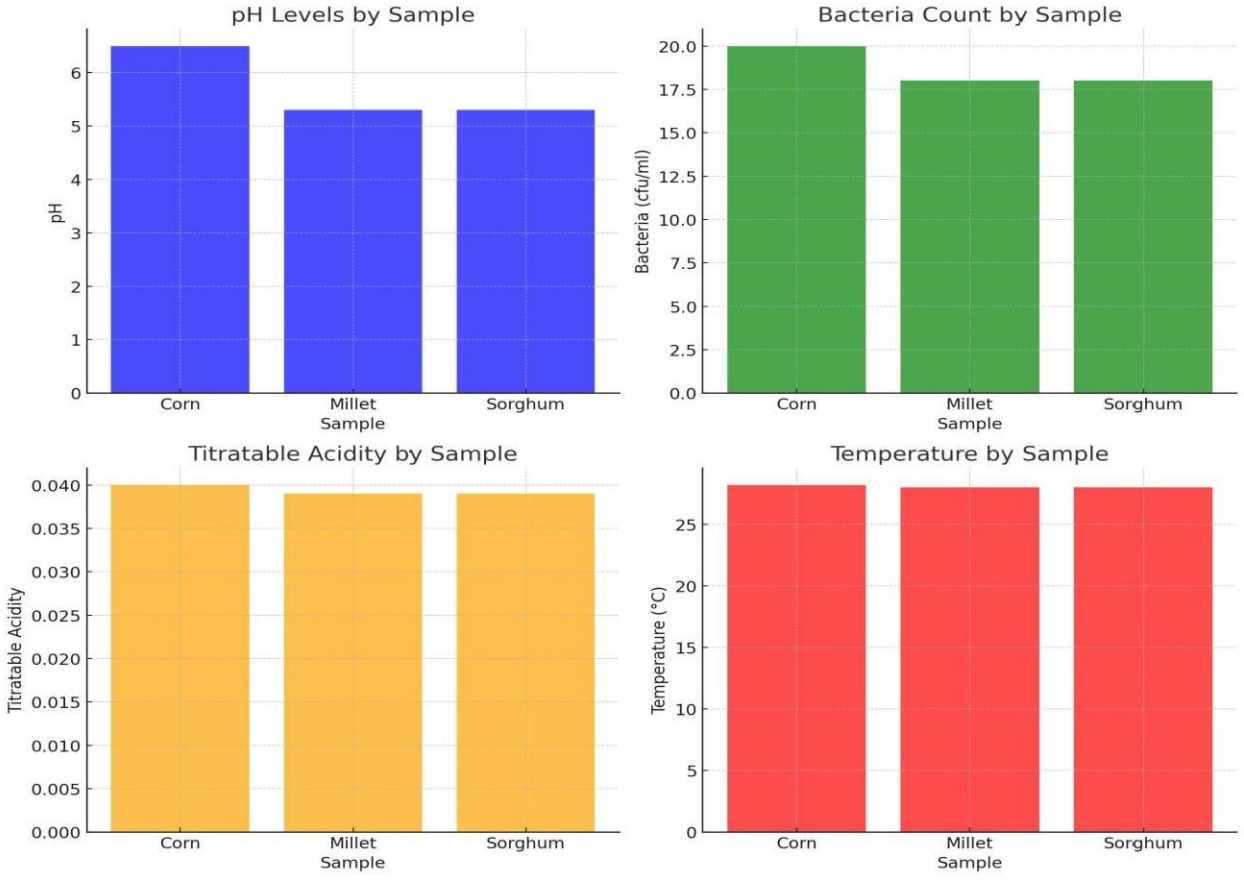
ethanol was obtained from 1litre mash of millet and 70cl of ethanol was gotten from llitre mash of sorghum.

The average relationship between the pH and time of fermented mash is represented graphically in Figure 1. Generally, there was decrease in pH of fermented Food grains (Corn, Millet and Sorghum) mash while increase was observed at 72hrs.

Figure 2 shows the average titrable acidity of the (Corn, Millet and Sorghum mash)samples as it increases with increase in time of fermentation.

Table 1: Some Physico-Chemical properties of the fermentation substrates and bacteria and fungi counts at 24hours during the process.

Time (Hours)	pH	Bacteria (cfu/ml)	Titratable acidity	Dilution factor	Temperature
Corn	6.50 ± 0.00	20	0.040	10^{-4}	28.2°C
Millet	5.30± 0.10	18	0.039	10^{-4}	28°C
Sorghum	5.30 ± 0.10	18	0.039	10^{-4}	28°C



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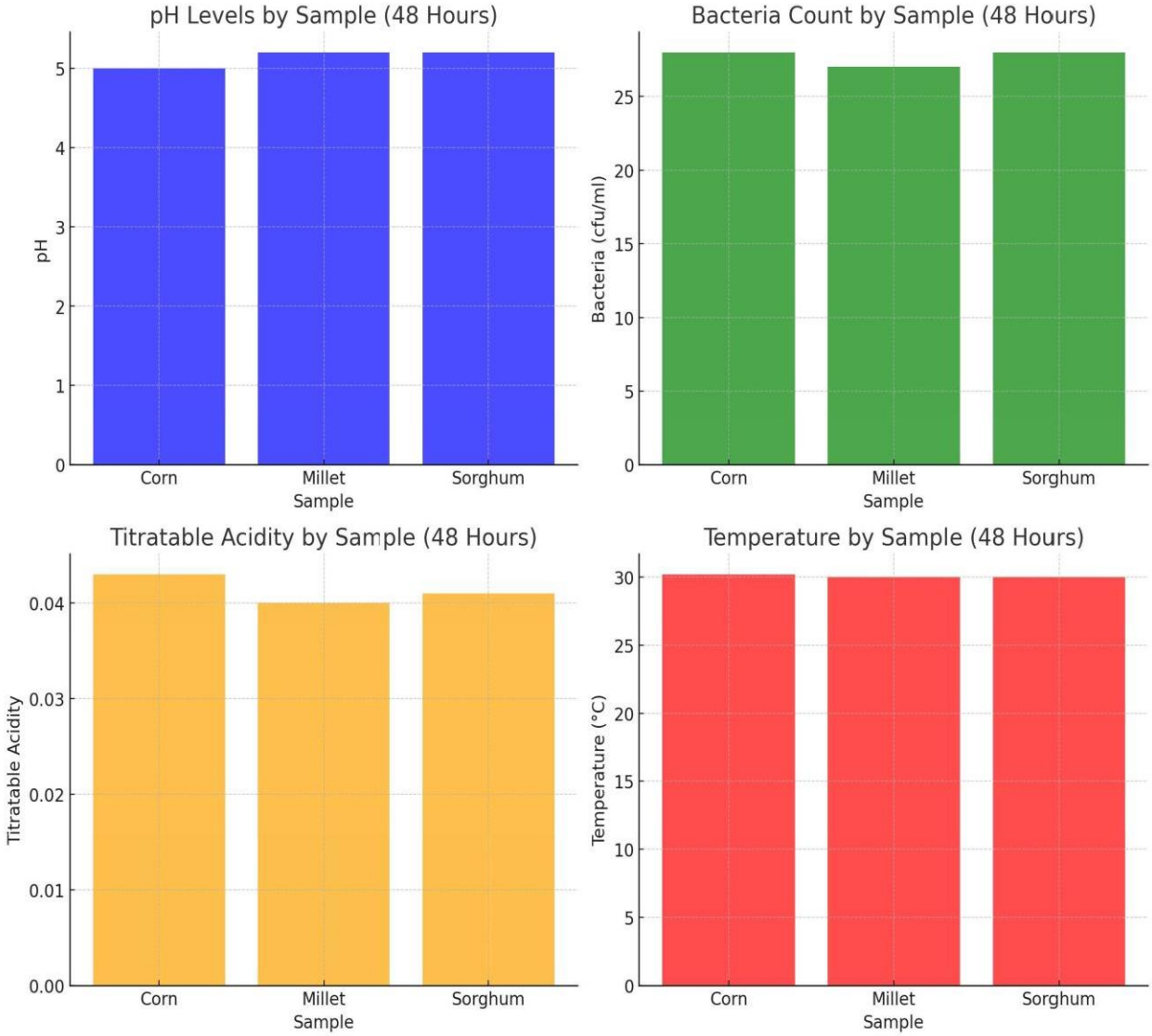
- **pH Levels by Sample** - Blue bars showing the pH values for Corn, Millet, and Sorghum.
- **Bacteria Count by Sample** - Green bars representing the bacterial counts (cfu/ml) for each sample.
- **Titratable Acidity by Sample** - Orange bars display ng the titratable acidity values.
- **Temperature by Sample** - Red bars indicating the temperature (°C) for each sample.

Table 2: Results of temperature, pH, titrable acidity, bacteria and fungi counts at 48hours of fermentation.

Time (Hours)	pH	Bacteria (cfu/ml)	Titratable acidity	Dilution factor	Temperature
Corn	5.00 ± 0.00	28	0.043	10^{-4}	30.2°C
Millet	5.20 ±0.10	27	0.040	10^{-4}	30°C
Sorghum	5.20 ± 0.10	28	0.041	10^{-4}	30°C



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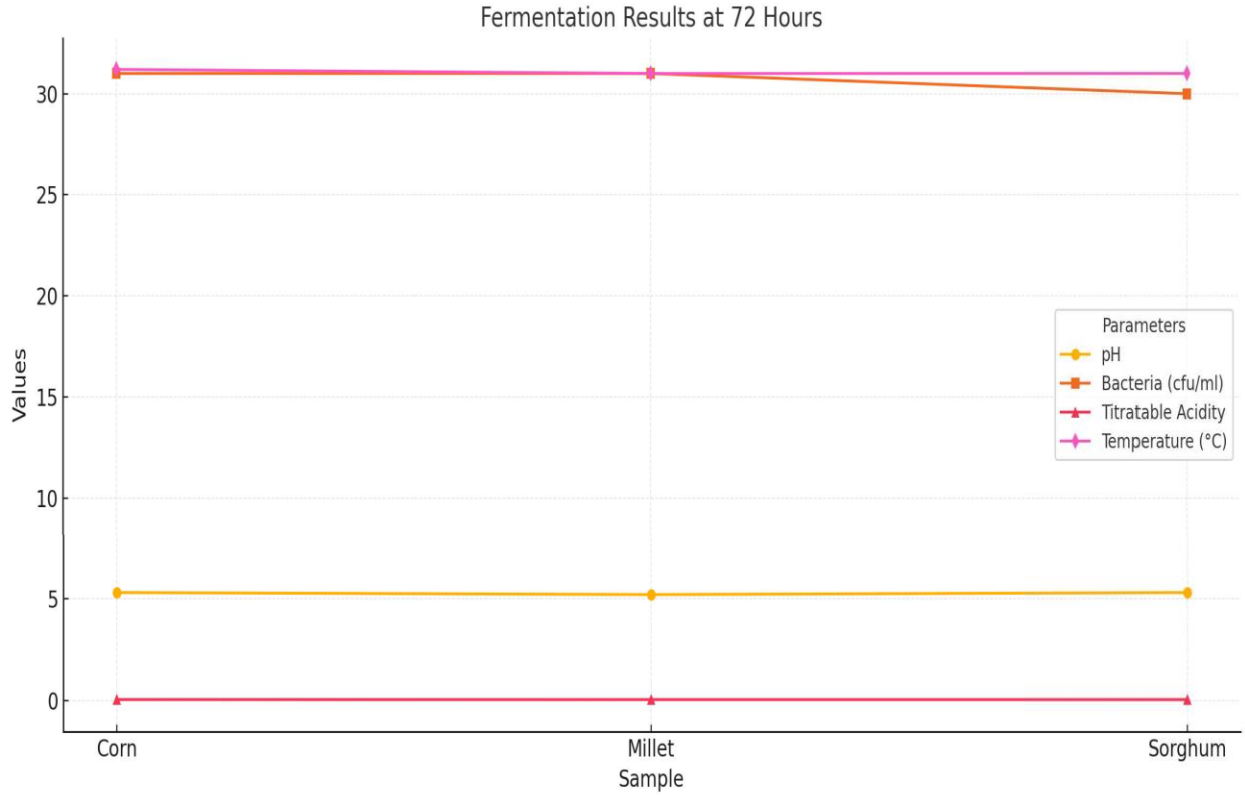
☐**pH Levels by Sample (48 Hours)** - Blue bars show the pH values for Corn, Millet, and Sorghum after 48 hours of fermentation.
☐**Bacteria Count by Sample (48 Hours)** - Green bars represent bacterial counts (cfu/ml) for each sample.
☐**Titratable Acidity by Sample (48 Hours)** - Orange bars display the titratable acidity values.
☐**Temperature by Sample (48 Hours)** - Red bars indicate the temperature (°C) for each sample.

Table 3: Results of temperature, pH, titrable acidity, bacteria and fungi countsat 72hours of fermentation.

Time (Hours)	pH	Bacteria (cfu/ml)	Titratable acidity	Dilution factor	Temperature
Corn	5.30 ± 0.00	31	0.044	10^{-4}	31.2°C
Millet	5.20 ±0.10	31	0.042	10^{-4}	31°C
Sorghum	5.30 ± 0.10	30	0.042	10^{-4}	31°C



The line graph combines all parameters for the fermentation results at 72 hours:

- **pH is shown with circular markers.**
- **Bacteria Count (cfu/ml) is represented with square markers.**
- **Titratable Acidity is illustrated with triangular markers.**
- **Temperature (°C) is marked with diamond shapes**

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Table 4: Cultural characteristic of LAB isolates

Codes of isolates	Shapes	Elevation	Colour	Surface	Margin
Lactococcus lactic	Circular	Raised	Creamy	Smooth	Entire
Lactobassillus ferment	Circular	Convex	Whitish	Smooth	Entire
Lactobassillus planterum	Circular	Flat	Creamy	Smooth	Entire
Lactobassillus bulgaricus	Circular	Flat	Whitish	Smooth	Entire

Table 5: Morphological, biochemical characteristics and identification of isolates

Cell morphology	Catalase	Gramstain reaction	Motility test	Colucose	Lactose	Sucrose	Probable organism
Cocci and clustered	-	+	-	+	+	+	Lactococcus lactis
Short rod , thin and clustered	-	+	-	+	+	+	Lactococcus plantarum
Long rod , thin and in chain	-	+	-	+	+	+	Lactococcus fermentum
Long rod , thin and clustered	-	+	-	+	+	+	Lactococcus bulgaricus
Long rod , thin clustered	-	+	-	+	+	+	Weissella Spp

- = negative reaction/absent

+ = Positive reaction / present

Table 6: Result of the distilled mash after 72 hours of fermentation

Mash distilled	The percentage of ethanol obtained
Corn	$7.5cl^3$
Millet	$68cl^3$
Sorghum	$70cl^3$

The results shows less than 8% of ethanol of the mash obtained as pure ethanol

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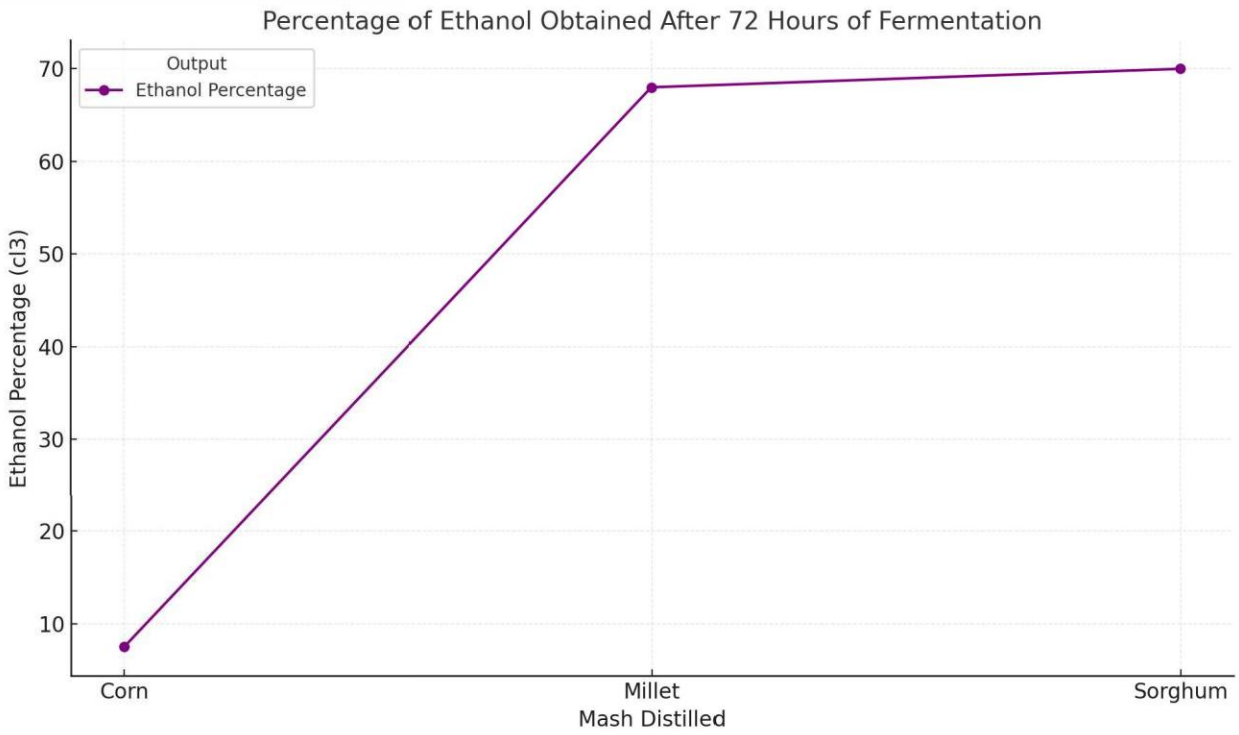

The line graph visualizes the ethanol percentages obtained from the distilled mash after 72 hours of fermentation:

- Corn shows the lowest ethanol percentage at 7.5cl^3.
- Millet and Sorghum are significantly higher at 68cl^3 and 70cl^3, respectively

Discussion and Conclusion

The isolated and identified lactic acid bacteria that involved in the fermentation of (Corn, Millet and Sorghum) for the bioethanol production include: *Lactobillusfermentium*, *Lactococcus lactic*, *Lactobacillus bulgaricus*, *Lactobillusplantarum andweissella* spp. The results of the analysis of the study were presented in the tables.Corn has the highest distilled ethanol while Sorghum has the least distilled bioethanol.This biothanol is produced when these organisms ferments 6-carbon sugars(mainlyglucose) via the glycolytic pathway where the glucan is converted to glucose by enzyme hydrolysis and fermentation of the glucose to ethanol by these bacteria. The mashed are fermented using these natural bacteria in a process that takes up to 72hours, the fermented mashed is separated into ethanol and residues (for animal feed production) via distillation. Fermentation involves the natural activities of these organisms (*Lactobillus fermentum*, *Lactococcus lactic*, *Lactobacillus bulgaricus*, *Lactobillus plantarum* and *weissella* spp.) to consume and metabolize sugars from the mashed (grinded Corn, Mil et and Sorghum). As sugars are consumed by bacteria, they are converted to ethanol and carbon dioxide. These bacteria are living organisms, so it is important to provide an environment that will support their growth and allow them to complete the conversion process.

The changes in pH of these fermented mash showed that there was decrease and increase in the pH throughout the fermentation. Raimbault and Tewe (20OT) indicated that the pH of a culture may change in response to metabolic activities. This may be due to the secretion of organic acids such as citric, acetic or lactic which causes pH to decrease.

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After fermentation the mash will contain solids, ethanol, and water. At this point the solution needs to be distilled to remove water and produce amore pure form of alcohol. Distillation is a process of separating various compounds based on differences in their boiling points. From an ethanol production standpoint, water and ethanol must be separated following fermentation. At sea level, ethanol as a boiling point of 78.3oC and water boils at 100^0C. If the water-ethanol mixture is heated to a temperature between 78.3^0C and 100^0C, the ethanol will turn into agas and the water will remain a liquid. The ethanol gas will evaporate, there by separating itself from the water. The ethanol vapors are then captured, cooled, and condensed back into a liquid. This process removes upwards of 90% of the water in the ethanol-water mixture; however, additional filtration is required to remove even more water if the ethanol will be used as a motor fuel. Generally, molecular sieves can be used to remove additional water, but this technology is too expensive and complex to implement at the low level, The ethanol can be useful as drinking, fuel, fluid in thermometers and in preventing biological specimens.

The study reveals that fermentation of food grains e.g. Corn, Millet and Sorghurm can be used for the bioethanol production by the means of fermentation and distillation. It is then recommended that farmers should grow more of these food grains of bioethanol production. This method of generating ethanol is highly satisfactory and hence recommended since almost 8% of the grains content can be gotten as ethanol; as it does not disturb the food chain of mankind and give for an environmental friendly uses.

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