

Analytical and Microbiological Quality Evaluation of Beer Production

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Abstract: Brewery science involves the production of beverages using fermentation processes with microorganisms, and this is among its key attributes. Beer is a widely consumed and popular beverage. Its nutritional, medical, and pleasant sensory properties are essentially maintained in breweries. Quality control (QC) has an important perspective mainly with production, as a repressive function. Brewery science leads to quality evaluation and analysis to avoid mistakes in producing and distributing the final product. In this study, the application of advanced, traditional, and regularly implemented methods for the detection of microorganisms in beer, yeast analysis, and yeast propagation is discussed. Advanced instrumental techniques for the reliable, rapid, selective, and relatively sensitive analysis of beer are widely used for quality evaluation and research. The dissertation work involves a general quality and microbiological evaluation of beer using morphological and physiological methods to determine microorganisms. Analysis methods were performed using different culture media by microbiological plating techniques: spread plate and membrane filtration for the identification of surface microorganisms in beer yeast analysis and yeast propagation, and by Hybriscan[®]D for beer analysis, and by the alcolyzer method for alcohol content. The results showed that beer microbiological purity varied across various fermentation processes and other treatments applied in the brewery. Yeast analysis and propagation are well characterized by multiplication rate, fermentation speed, and aroma for good-quality beer. Hybriscan[®]D sandwich hybridization is a rapid test for beer spoilage microorganisms and is more effective and faster than classical methods. Measured alcohol content by alcolyzerantompaar in the final product or beer is always based on standard specifications

Keywords: Quality Evaluation, Quality Control, Beer Production, Microbial Analysis, Yeast Propagation, Hybriscan[®]D Beer, AlcolyzerAntomPaar

I. INTRODUCTION

Brewing beer is a complex biochemical and industrial process in which several critical stages, including brewing, cellaring, fermentation, and packaging, must be continuously monitored to prevent microbial contamination. Contamination of raw materials, brewing equipment, yeast cultures, or the brewery environment can significantly affect beer quality and prevent the final product from meeting the brewer's expectations [1]. Although beer possesses inherent antimicrobial properties such as low nutrient availability, acidic pH, hop-derived antiseptic compounds, enzymatic activity, ethanol content, and processing steps like filtration and pasteurization, these factors only limit the growth of microorganisms rather than ensuring complete sterility [2]. Consequently, modern brewery science emphasizes the production of beer that is safe, stable, and of consistent quality.

Microbial contamination in beer is commonly associated with quality defects, including off-flavors, haze formation, turbidity, and reduced shelf life [3]. Effective control of unwanted microorganisms is therefore one of the most critical aspects of brewery operations [4]. Comprehensive analysis of raw materials, intermediate products, and finished beer is essential and is achieved using a combination of classical microbiological techniques and advanced analytical methods. In breweries where sterile filtration or pasteurization is not employed, strict quality assurance procedures are



particularly important to maintain high levels of cleanliness [5]. Despite significant improvements in brewery hygiene, secondary contamination during processing and packaging remains a major cause of microbiologically spoiled beer [6]. Routine cleanliness monitoring, hygienic sampling, and increased awareness of sanitation practices contribute to extended shelf life, improved production efficiency, and reduced rejection rates. Therefore, breweries must safeguard beer quality and safety by thoroughly analyzing raw materials, equipment, and final products for contaminants introduced during or after fermentation.

Several microbiological quality evaluation assays are recommended in brewery quality control, including growth promotion testing and other advanced analytical approaches. Quality control assays assess the nutritional suitability of culture media to ensure adequate microbial growth, which is essential since culture media are widely used in routine microbiological analysis [7]. Such evaluations form the foundation of reliable microbial detection and monitoring in brewery environments.

Quality evaluation and analytical monitoring play a crucial role in minimizing errors during the production and distribution of beer. The present study focuses on the application of both conventional and advanced methods routinely used for the detection of microorganisms, beer quality analysis, yeast evaluation, and yeast propagation. Product quality is maintained through systematic control measures implemented at every stage of the brewing process. This work involves a comprehensive microbiological and quality evaluation of beer using morphological and physiological methods for microorganism identification. Microbial analysis was performed using various culture media through the spread plate and membrane filtration techniques. In addition, yeast analysis and propagation studies were conducted, and beer quality was further assessed by determining alcohol content using the AlcoLyzer method. Overall, quality control is a vital tool for ensuring the production of microbiologically pure, high-quality beer while preventing errors during manufacturing and distribution.

II. BREWERY PROCESS AND QUALITY CONTROL

The major stages involved in beer production include milling, mashing, lautering, boiling, whirlpooling, cooling, fermentation, maturation, filtration, packaging, and distribution. Each step represents a critical control point where microbial contamination may occur if hygiene is compromised [1].

III. MATERIALS AND METHODOLOGY

3.1 Microorganisms Encountered in Brewery

Beer production is vulnerable to microbial contamination at various stages, such as wort production, fermentation, maturation, and packaging. Several bacteria and yeasts are frequently encountered in brewery environments, some of which cause beer spoilage, while others are non-spoilage contaminants [1,2]. The common microorganisms encountered in brewery operations and their characteristic features are presented in **Table 1**.

Table 1. Microorganisms Encountered in Brewery

Microorganism	Characteristics
Acetic Acid Bacteria	Gram-negative, catalase-positive, short rods, non-spore forming, acid producers, motile; produce acetic acid/vinegar odor
Enterobacteria	Gram-negative, catalase-positive, short rods, non-spore forming; facultative anaerobes; produce diacetyl, DMS, phenolic odors
Lactobacillus	Gram-positive, catalase-negative rods; heterofermentative; produce lactic acid, acetic acid, ethanol, and CO ₂
Pediococcus	Gram-positive, catalase-negative cocci; homofermentative; lactic acid as major end product
Bacillus	Gram-positive, catalase-positive, spore-forming rods; common in water and wort but rare in beer
Micrococcus	Gram-positive, catalase-positive cocci; aerobic; generally non-spoilage organisms
Pectinatus	Gram-negative, obligate anaerobic curved rods; produce acetic acid, propionic acid, and H ₂ S



Megasphaera	Gram-negative, obligate anaerobic cocci; produce H ₂ S, butyric, valeric, and caproic acids
Zymomonas	Gram-negative, catalase-positive rods; highly motile; produce ethanol and CO ₂
Clostridium	Gram-positive, spore-forming anaerobic rods; produce butyric acid, causing off-flavors

3.2 Culture Media and Inhibitors Used in Brewery

Various selective and differential culture media were used to isolate, enumerate, and identify microorganisms commonly encountered in brewery environments. These media support the growth of target microorganisms while suppressing non-relevant flora, thereby ensuring reliable microbiological quality control of beer and brewing materials [1,2,4,8].

3.2.1 Culture Media

Modified Wallerstein Laboratory Nutrient (MWLN) Agar:

MWLN agar was prepared by dissolving 37 g of MWLN agar powder in 1 L of distilled water with continuous stirring and boiling. The pH was adjusted to 6.5 ± 0.2 using 1.0 N HCl or 1.0 N NaOH as required. The medium was autoclaved at 121°C for 15 minutes. MWLN agar was used to cultivate the microorganisms commonly encountered in brewery samples [8].

Modified Wallerstein Laboratory Differential (MWLD) Agar:

MWLD agar was prepared by dissolving 37 g of MWLD agar in 1 L of distilled water. The pH was adjusted to 6.5 ± 0.2 , and acridine, a selective agent, was added. The medium was autoclaved at 121°C for 15 minutes and used for the detection and differentiation of brewery-associated microorganisms [8].

Raka Ray Agar:

Raka Ray agar was prepared using a medium supplemented with 10 mL of Tween 80 and 5 mL of 0.1% (w/v) cycloheximide, yielding a final concentration of 74.9 g/L. The medium was autoclaved at 121°C for 15 minutes. It was used for the selective isolation of anaerobic lactic acid bacteria, particularly *Lactobacillus* and *Pediococcus* species [9].

Lysine Agar:

Lysine agar was prepared by dissolving 66 g of medium in 1000 mL of distilled water with the addition of 10 mL of potassium lactate. The medium was boiled to dissolve completely, cooled to 50°C, and supplemented with 1 mL of 10% (w/v) lactic acid. The medium was not autoclaved and was poured aseptically into sterile Petri plates. Lysine agar was used for the identification of non-*Saccharomyces* wild yeasts [10].

Yeast Extract–Dextrose Chloramphenicol Agar:

This medium was used for yeast and mold analysis. Detailed preparation procedures were followed as per standard analytical methods for yeast and mold examination [2].

Yeast Malt Copper (YMC) Agar:

YMC agar was prepared by dissolving 41 g of medium in 1000 mL of distilled water and autoclaving at 121°C for 15 minutes. Copper sulfate solution was added to the medium at 45–50°C before pouring plates, resulting in a final concentration of 250 ppm CuSO₄. This medium permits the growth of wild yeasts while inhibiting standard brewing yeasts [5].

MacConkey Agar:

MacConkey agar was prepared by dissolving 55.07 g of medium in 1000 mL of distilled water and autoclaving at 121°C for 15 minutes. It was used to detect enteric bacteria [11].



Yeast Extract Agar:

Yeast extract agar was prepared by adding 23 g of medium to 1000 mL of distilled water and used for the enumeration of total water-borne microorganisms [1].

Plate Count Agar:

Plate count agar was prepared by dissolving 23.5 g of medium in 1000 mL of distilled water and autoclaving at 121°C for 15 minutes. This medium was used for total microbial enumeration [4].

Nutrient Agar:

Nutrient agar was prepared by adding 28 g of medium to 1000 mL of distilled water and autoclaving at 121°C for 15 minutes. It served as a general-purpose medium for total microbial count [4].

Wort Agar:

Wort agar was prepared by dissolving 48.3 g of medium in 1000 mL of distilled water containing 2.35 g of glycerol, and autoclaving at 121°C for 15 minutes. It was used for the cultivation and enumeration of yeast [1].

Lauryl Tryptose Broth / Endo Agar:

Lauryl tryptose broth and Endo agar were prepared by dissolving 35.6 g of broth and 15 g of agar in 1000 mL of distilled water, then autoclaving at 121°C for 15 minutes. These media were used to identify and cultivate coliforms in water samples [11].

3.2.2 Inhibitors Used

Selective inhibitors were incorporated into culture media to suppress specific microbial groups:

Cycloheximide (0.1% w/v): Inhibits yeast growth while permitting bacterial growth [12].

Tetracycline (10 mg/L): Inhibits bacterial growth and allows yeast proliferation [2].

Copper salts (300 ppm): Inhibit brewing yeasts while permitting wild yeast growth [5].

Phenyl ethanol (0.3% v/v): Inhibits Gram-negative bacteria [2].

Vancomycin (30 mg/L): Inhibits non-beer spoilage Gram-positive bacteria [4].

3.3 Microbiological Plating Techniques

3.3.1 Spread Plate Technique

Microorganisms in beer samples were enumerated using the spread plate method. Agar medium (15-20 mL per plate) was equilibrated at 46-50°C and supplemented with cycloheximide where required. Samples (0.2 mL) were aseptically spread on the medium surface using a sterile glass spreader, allowed to dry for 15 min, and incubated at 37°C in an inverted position for 48 h. Colonies formed were counted and recorded. Prepared plates were stored at 5-10°C and used within one week [13].

3.3.2 Membrane Filtration Technique

For liquid samples with low microbial counts (<1 CFU/mL), membrane filtration was employed. Sterile 0.45 µm pore size membranes were placed on support screens using flamed forceps, and 100 mL of beer samples were filtered under vacuum. The membranes were then transferred to agar medium and incubated at 25-37°C for 48 h. Colonies on the membrane surface were enumerated. Care was taken to avoid foam formation during filtration [13].



3.4 Yeast Analysis

3.4.1 Yeast Cell Count:

Yeast cell numbers were determined using a Neubauerhemocytometer. Samples were diluted tenfold with 0.2% saline, and cells in five medium squares were counted under a compound microscope. Cells equal to or larger than half the mother cell were considered individual cells; smaller budding cells were excluded [14–16]. Yeast concentration (cells/mL) was calculated using the formula:

$$\text{Yeast count} = \text{No. of bacteria} \times 25 \text{ squares} \times 102 \times 10^4$$

3.4.2 Yeast Viability:

Yeast viability was assessed using 0.01% methylene blue staining. Equal volumes of sample and stain were mixed, and 500 cells were counted across 9-10 squares of the hemocytometer. Cells that reduced the dye were considered viable. Viability (%) was calculated as:

$$\% \text{ Viable cells} = \frac{\text{Total no. of cells counted} - \text{No. of dead cells} \times 100}{\text{Total no. of cells Counted}}$$

3.4.3 Yeast Solids Determination:

By Weight:

$$\% \text{ Yeast solids} = \frac{\text{Weight of yeast (sediment)} \times 100}{\text{Weight of slurry}}$$

Centrifugation was performed at 3000 rpm for 10 min to obtain the sediment.

3.4.4 Yeast Propagation

Yeast propagation was carried out under sterile conditions with continuous aeration and zinc supplementation (1 ppm) to ensure optimal growth and viability (Fig. 1). Wort served as the growth medium, sterilized at 121°C for 15 min and cooled to the appropriate pitching temperature for each stage [15]. Inoculum to medium ratios did not exceed 1:10.

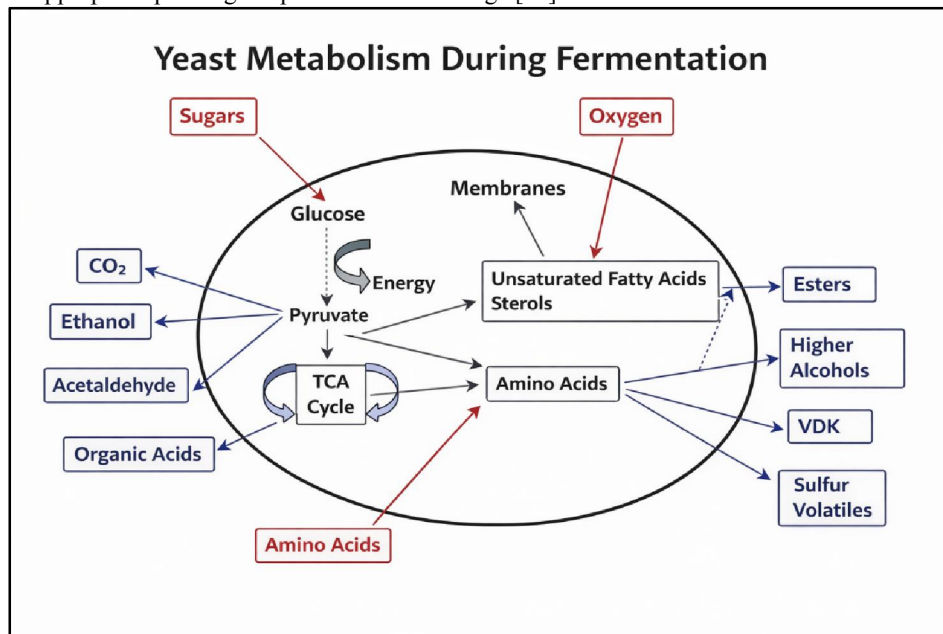


Fig. 1: Schematic overview of the complete yeast propagation workflow



Laboratory Stages:

Stage 1: Sterile saline or water was used to suspend yeast from the slants. Two flasks (500-750 mL each) were inoculated and incubated at 25-27°C for 48 h with constant oxygenation.

Stage 2: Cultures were transferred to 5-7.5 L wort in Carlsberg flasks at 20-22°C for 48 h. Cell count and viability were monitored.

Yeast Room Stages:

Stage 3-4: Wort was boiled for 30 min and cooled to 10-18°C. Cultures were inoculated at $\leq 1:10$ ratio and incubated at 13-18°C for 24-36 h with constant aeration. Cultures were periodically checked for cell count, viability, and contamination.

Plant Stages:

Stage 5: Sterile wort was transferred through plate heat exchangers, and fermentation was performed at 10-12°C for 24-36 h.

Stage 6: Fermentation continued until the end gravity was reached. The yeast was cropped, chilled to 4°C, and used for pitching in subsequent brews.

The yeast propagation process at the laboratory and plant stages, highlighting the progressive growth and maintenance of culture purity, is illustrated in Fig. 2 (a & b).



(a)



(b)

Fig.2.(a),(b). Detailed depiction of laboratory and plant stages, showing culture growth and purity maintenance.

3.5 Beer Analysis:

3.5.1 Beer Microbial Analysis Using Hybriscan®D

The microbial quality of beer was assessed using the Hybriscan®D Beer rapid test system (Hybriscan®D, Germany), which detects RNA of beer-spoiling microorganisms via sandwich hybridization [17].

Sample preparation: Beer samples (100-1000 mL) were filtered through 0.45 μm membranes and incubated in 5 mL enrichment medium at 28°C for 24-30 h.

Cell lysis and hybridization: Enriched samples were centrifuged, lysed with buffers A, B, and C, and incubated with Test Solution D in microtiter wells. Binding plates were used for hybridization, followed by enzyme incubation and substrate reaction.



Detection and quantification: Optical density was measured at 450 nm. Results were compared with standard plate counts. The system detects common beer-contaminating microorganisms, including *Lactobacillus brevis*, *Lactobacillus plantarum*, and *Lactobacillus buchneri*.

3.5.2 Alcohol Content Determination

Alcohol content was measured using the AlcoLyzer Anton Paar system (Anton Paar, Austria) [18–20].

Sample preparation: 20 mL of beer (Kingfisher Mild, 330 mL) was placed in sterilized vials.

Measurement: Alcohol (% v/v), apparent extract, real extract, specific gravity, and density were recorded. Daily calibration was performed using water and ethanol solutions to ensure accuracy.

IV. RESULTS AND DISCUSSION

4.1 Microbiological Plating Techniques

Microbiological plating techniques, including the spread plate and membrane filtration methods, were used to assess the purity of the beer sample. No colonies were observed on either the spread plate or the membrane filter after incubation, indicating that the beer was pure and free of contamination (Fig. 3a, 3b). Control plates were monitored throughout the experiment, and any presence of colonies in the controls would have invalidated the test. The absence of colonies demonstrates effective microbiological control, confirming compliance with quality standards for brewery products [21]. These results align with standard practices where the detection of spoilage organisms is critical to maintain product quality [8,15].



Fig 3. (a) Membrane filtration under LAF

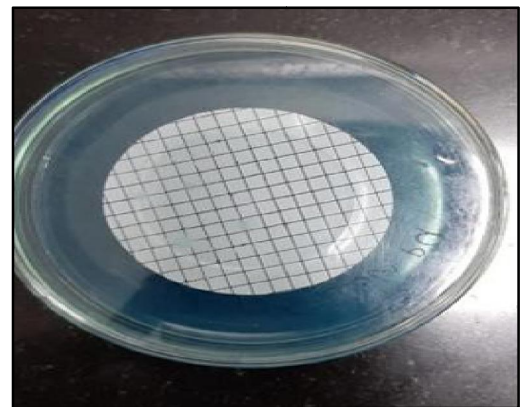


Fig 3. (b) Absence of colonies on membrane (m WLN)

4.2 Yeast Analysis

Yeast cell count in the sample was determined using a hemocytometer, yielding a total of 125×10^6 cells/mL. Viability assessment showed that 96.15% of cells were viable, and yeast solids by weight were calculated as 67.70% (Table 1, Fig. 4, Fig. 5). The high viability and consistent yeast solids confirm that the yeast culture was healthy and suitable for fermentation. Proper yeast cell health directly affects fermentation rate, beer flavor, and overall quality [22].



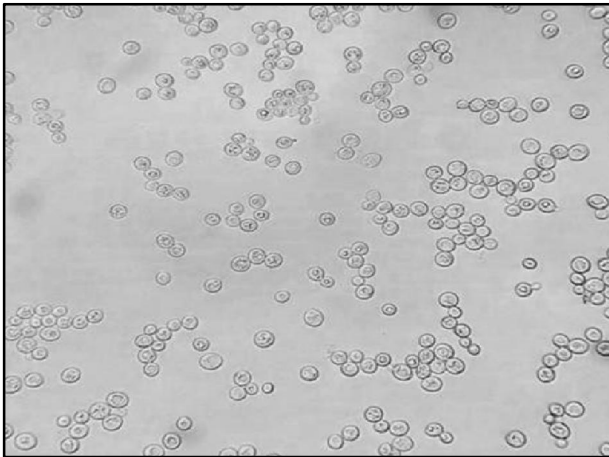


Fig 4. Yeast cell image by microscopy (Yeast biotechnology)



Fig 5. Yeast solid after centrifugation

The microbiological analysis of propagated yeast revealed minimal contamination across stages, with freshly propagated yeast showing the lowest microflora counts (Fig. 8). Selective media such as M.W.L.N, Raka Ray, MRS, W.L.D, Y.M.C.A., Lysine, MacConkey, and Plate Count Agar were used to differentiate bacteria, wild yeast, and molds. Propagated yeast maintained purity, which contributed to stable fermentation characteristics and consistent product quality. The total microflora for propagated yeast and different yeast generations showed that proper propagation methods effectively limit contamination and enhance fermentation performance (Table 2, Fig. 6).

Table 2 - Total Microflora of Beer Fermented with Propagated and Different Yeast Generations

Yeast Type / Generation	Total Microflora (log N)
Fresh propagated yeast	0
Propagated yeast – 4 generations	0.4
Propagated yeast – 7 generations	0.6
Dried yeast in fermentation	1.2

Quality evaluation of fermentation using propagated yeast demonstrated stable physicochemical parameters. pH decreased gradually from 5.1 at 24 hours to 4.2 during the maturation phase, while diacetyl levels were minimal, and total microflora decreased from 20 to 3 CFU/mL, indicating successful microbial control (Table 3). These results highlight the importance of using pure, propagated yeast to maintain beer quality, prevent off-flavors, and extend shelf life.



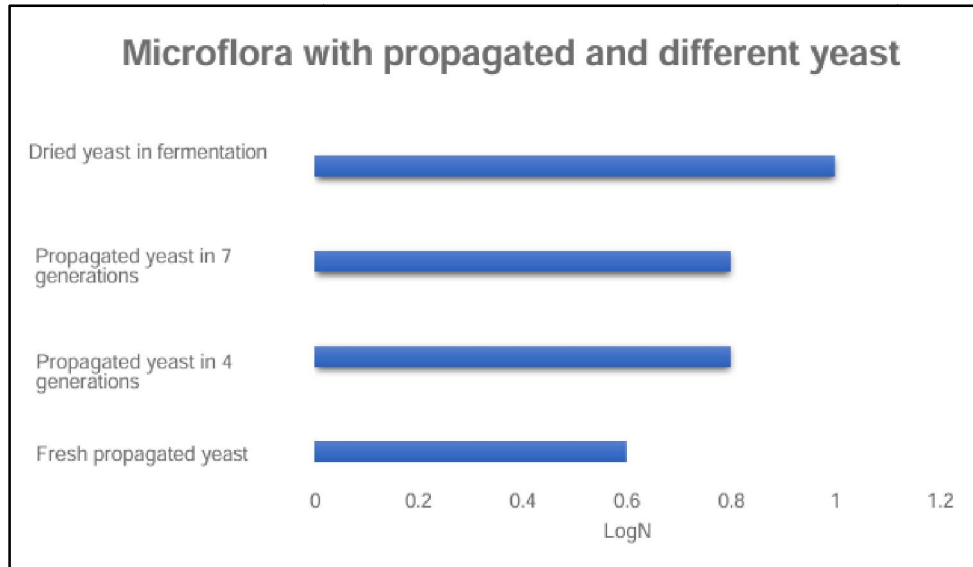


Fig 6. Total microflora of beer fermented with propagated yeast and also with different yeast

Table 3. Fermentation is carried out with propagated yeast in a brewery

Fermentation is carried out with propagated yeast in a brewery					
Physicochemical and Microbiological analysis	24 hours	3 Days	7 Days	Incooling	Maturation phase
pH	5.1	4.7	4.2	4.2	4.2
Temperature(°C)	9	10	7	1	0
Diacetyl(mg/l)			0.075	0.052	0.048
Acidity(glactic acid/100ml beer)				1.8	1.8
Total microflora	20	15	10	7	3

4.4 Hybriscan®D Rapid Test for Microorganisms

The Hybriscan®D rapid test system was employed to detect beer-spoiling microorganisms using RNA-targeted probes. The test efficiently detected all relevant microorganisms, including Lactobacillus, Megasphaera, Pediococcus, and Pectinatus. Comparison with traditional plate count methods revealed that Hybriscan®D detected more microorganisms and provided faster, more reliable results (Fig. 7). This demonstrates that ribosomal RNA-based detection is more sensitive and effective for monitoring microbial quality in beer production.



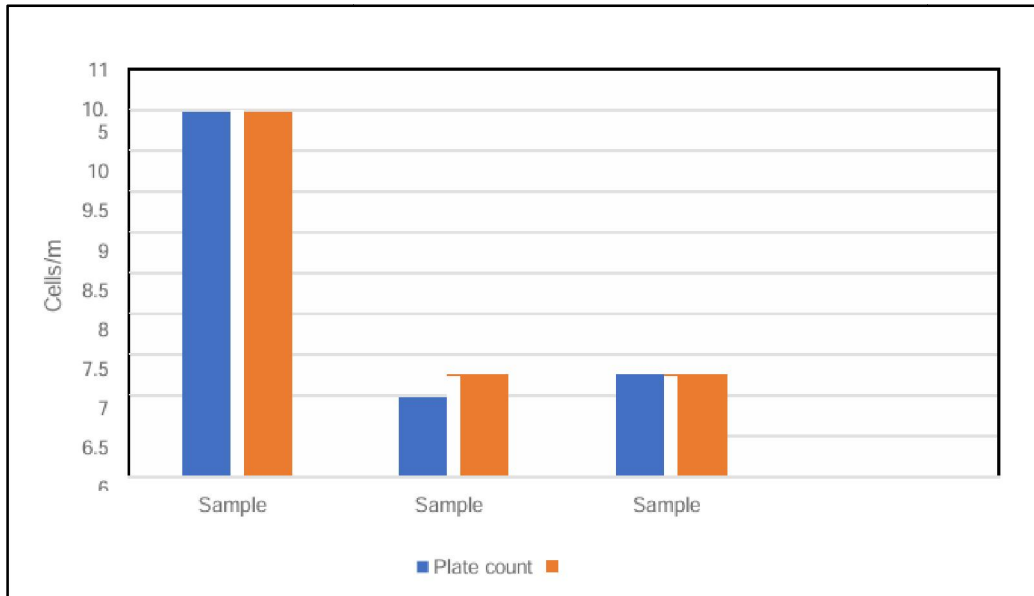


Fig 7. Comparison of cell numbers of the Hybriscan® method versus the plate count method in three different samples

4.5 Alcohol Content Analysis

The alcohol content of Kingfisher Mild (330 mL can) was measured using the AlcoLyzer Anton Paar system. The sample contained 4.87% v/v alcohol, with apparent extract (Ea) of 2.07% v/v, real extract (Er) of 3.84% v/v, specific gravity of 1.00807, and density of 1.00626 g/cm³ (Fig. 8). These measurements confirm the accuracy and precision of instrumental analysis in quality control, ensuring compliance with standard specifications.



Fig.8. Displayed alcohol content for Kingfisher mid (330 ml can) is 4.87 %



V. CONCLUSION

This study assessed the microbial quality of beer at multiple stages of brewery production to evaluate the impact of microbial populations on the final product. The results emphasize that microbial contamination can compromise beer quality, highlighting the importance of stringent quality control and assurance practices. Active microbial detection throughout production, coupled with proper media and identification techniques, enables accurate monitoring and proactive management of contamination risks. Maintaining hygiene and continuous surveillance ensures the production of high-quality, contamination-free beer, safeguarding both product integrity and consumer satisfaction. Effective microbial control is therefore critical not only for beer quality but also for operational efficiency and the economic success of breweries.

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Credit Authorship Contributions Statement

Ms. Aparna A. Dhumal: Analytical experiments, data validation, manuscript preparation.

Ms. Kajal R. Gaikwad: Microbiological analyses, result interpretation, writing-original draft preparation.

Dr. Vishal Naik: Supervision, methodology guidance, writing- review and editing.

Declaration of Competing Interest

The author declares no conflict of interest, financial or personal, that could have influenced the work reported in this paper.

Consent to Publish Declaration

Not applicable.

Ethics and Consent to Participate Declarations

Not applicable.

Data Availability

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

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