ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 08, 2022

STUDIES ON MINIMIZATION OF MICROBIAL LOAD DURING FERMENTATION STAGE OF BEER

***Patil, N.S.,[1]Powar, V.V.,[2] Pawar, R. R.,[3] &Mote, G.V.[4]**

Department of Food Technology, D.Y.Patil Agriculture & Technical University, Talsande, Kolhapur(MH) India
* niranjanpatil127@gmail.com

Abstract

Microbiological quality control testing is indispensable in the brewing industry to uphold high standards of product quality. Microbial contamination, arising from various organisms such as bacteria, wild yeast, and mould, can significantly alter beer flavor and fermentation performance, resulting in undesirable taste, aroma defects, turbidity issues, and reduced yeast activity. This research work examines the critical role of microbial activity during fermentation stage &throughout the beer production process and highlights the need for active control measures to ensure the final product's integrity, to reduce the off flavor in the beer. This research aims to conduct an in-depth investigation into the effective strategies for minimizing microbial contamination during the fermentation process. However, the presence of undesired microbial contaminants during fermentation can adversely impact product quality. Consequently, this study focuses on comprehending the factors influencing microbial contamination during fermentation and devising methodologies to control and mitigate these contaminants, thereby enhancing process efficiency and product quality. The research aims to delve into the critical role that microbial activity plays throughout the brewing process, with a particular focus on the fermentation stage. Understanding how microorganisms interact and influence the brewing process is essential for producing consistent and high-quality beer. This thesis emphasizes the need for active control measures to maintain the integrity of the final beer product. Implementing robust quality control protocols and preventive measures can help prevent or mitigate the negative impacts of microbial contamination. This could encompass aspects such as equipment cleanliness, raw material quality, temperature control, and more. By addressing the issue of microbial contamination, the research seeks to enhance both the efficiency of the brewing process and the overall quality of the beer product. This improvement can lead to more consistent brewing outcomes and a higher level of customer satisfaction.

*(**Keywords**: Brewing, Fermentation, Beer)*

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 08, 2022

1. INTRODUCTION

Beer, a popular alcoholic beverage consumed worldwide, is typically crafted from malted cereal grains, hops to impart flavor, and a gradual fermentation process. It's a sophisticated alcoholic drink that boasts a wide array of flavor-active compounds found in various concentrations.

Brewing, the art of beer production, revolves around the conversion of grain starches into sugars, followed by sugar extraction with water. This sweet liquid is then fermented using yeast, resulting in the creation of the alcoholic and mildly carbonated beverage known as beer. Beer typically contains alcohol levels ranging from 2.5% to 13% (v/v) ethanol. Beers are commonly categorized based on their alcohol content, falling into the classifications of low-strength (approximately 2–3% alcohol), medium or average strength (around 5% alcohol), and high-strength or strong (>5–6% alcohol) beers.

After cooling and removing spent hops, the resulting liquid, known as 'hopped wort,' is pumped into fermentation vessels. Yeast is introduced with aeration to promote growth. In the anaerobic phase, yeast cells convert sugars into ethanol and carbon dioxide. Depending on fermentation temperature and yeast collection methods at the end of fermentation, beers are classified as 'bottom fermentation' or 'top fermentation.'

Fermentation typically spans about one week, yielding a 'green beer' or 'young beer,' which is not ready for consumption due to the presence of undesirable compounds that result from fermentation. During fermentation, yeast releases various molecules, including ethanol and CO2, which can influence the flavor. All brewing strains generate glycerol, vicinal diketone (VDKs), alcohols, esters, short-chain fatty acids, organic acids, and various sulfur-containing substances. The levels of these compounds in beer depend on factors such as yeast strain, precise fermentation conditions, including pitching rate, temperature, oxygen addition, fermentation and maturation duration. VDKs, particularly diacetyl, impart an undesirable buttery character to beer. These VDKs can be controlled by conducting a diacetyl rest, which involves raising the fermentation temperature slightly (15˚C-21˚C) when the gravity is a few degrees Plato from the terminal. Beers require a maturation or lagering period of several weeks at around 0°C to break down undesirable components before they can be considered ready for packaging. For extended preservation, beers may undergo pasteurization.

Microbiological quality control testing is indeed crucial in breweries to ensure high product quality. Microbial contamination, arising from bacteria, wild yeast, and mold, can significantly impact the flavor and fermentation performance of beer, leading to undesirable defects in taste, aroma, and turbidity, as well as reduced yeast performance. Throughout the beer production process, from raw materials to packaging, various microbial activities are involved. While some are desirable for traditional food fermentation, others can threaten the final product's quality and must be actively managed and controlled.

(Ref :- Vaughan, A., O'Sullivan, T., & Van Sinderen, D. (2005). Enhancing the microbiological stability of malt and beer a review. Journal of the Institute of Brewing.)

Beer is inherently a microbial product, and microbial activity plays a significant role in shaping its sensory characteristics, ultimately contributing to its overall quality. While the

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 08, 2022

fermentation of cereal extracts by Saccharomyces yeast is the most important microbial process in brewing, numerous other microbes can influence the entire brewing process. Despite beer's general inhospitable conditions for most bacteria due to its low pH, high CO2 and alcohol content, and presence of bittering agents, some beer spoilage bacteria, such as Lactobacillus spp., Pediococcus spp., Pectinatus spp., and Megasphaera spp., have adapted to grow undisturbed under these conditions. While these microorganisms usually do not pose health hazards to humans, they can cause off-flavors and lead to the loss of entire batches of beer. If contamination is suspected or detected, it is crucial to promptly investigate the entire brewing process chain to identify the source and take corrective actions to preserve the beer's quality.

In summary, maintaining strict microbiological quality control measures throughout the brewing process is vital for producing high-quality beer that meets consumer expectations. This involves monitoring and managing microbial activities to ensure the absence of spoilage microorganisms that can negatively impact the beer's flavor, aroma, and overall quality.

Microbiological quality control testing in breweries is essential for maintaining high product quality. The effects of microbial contamination, derived from organisms including bacteria, wild yeast and mould, can changes beer flavour and fermentation performance to gross flavour and aroma defects, turbidity problems and reduced yeast performance. Brewing beer involves microbial activity at every stage, from raw material to the packaging of beer. Most of these activities are desirable, as beer is the result of a traditional food fermentation, but others represent threats to the quality of the final product and must be controlled actively through careful management.

Beer, like any fermented food, is an immutably microbial product. Microbial activity is involved in every step of its process, defining the many sensory characteristics that contribute to final quality. While fermentation of cereal extracts by Saccharomyces is the most important microbial process involved in brewing, a vast array of other microbes affects the complete process. Microbial interdiction at every step of the barley-to-beer continuum greatly influences the quality of beer.
(Ref :-Faparusi, S. I., Olofinboba, M. O., &Ekundayo, J. A. (1973). The microbiology of burukutu beer.)
Therefore, in this study, our objectives were to reduce microbial contamination in fermentation stage & to find out the root cause analysis, 5 why for micro-organisms growth.

2. MATERIALSAND METHODS

Materials

Laminar air flow, Micropippete, Incubator, colony counter, compound microscope, Autoclave, Membrane filtration unit, Distillation unit, Petri plates, Parrafin wax, Distilled water, PPE, Bunsen burner, Inoculation loop, Pipette tips, Forceps, Aluminum foil, Spreader
Media : Raka-Ray (RR), YMCA, WLD, PCA, WLN, MAC-CONKEY, YMC

2.1Process flow sheet for plating of sample

Sample selection

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 08, 2022

Collection of sample with hygienic condition with proper PPE's

↓

Perform media plating

↓

Incubate plate

↓

Count CFU's

↓

Analyze Plating result

Fig:-**Flow sheet for plating of sample**

2.2Microbiologcal analysis

Total plate count

PCA was suspending 23.5 grams in 1000 ml distilled water. One milliliter of every dilution become pour plated on PCA in sterile petri plates and incubates for 72 hours at 30-32°C. Finally of the incubation period the petri plates were eliminated for counting the developed colonies in cfu/ml. CFU were counted usage a colony counter.

Yeast &mould count

The RBCA media is used. Plates were set in a hatchery at 25°C for 120 hours and perception was accounted for in CFU/ml of the article the article

Total coliform count

The Violet red bile agar (VRBA) medium is used for total coliform detection, the medium was moved sterile petri dishes, and after that an example was filled it. Plates were set in a hatchery at 38°C for 24 hours and perception was accounted for in CFU/ml of the article.

(Ref :- Pattison, T. L., Geornaras, I., & von Holy, A. (1998) International Journal of Food Microbiology)

2.3 Contamination Control Strategies

This study identified critical factors contributing to contamination, leading to the development and implementation of effective control strategies :

a) Water Treatment Optimization: By enhancing water treatment protocols, by maintaining hygiene during chemical dosing and regularly maintenance of machinery & UV lamp, water quality improved, resulting in a reduced initial microbial load and minimizing the risk of introducing contaminants during the brewing process.

b) Yeast Handling Improvements: Implementing dedicated yeast handling equipment and

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 08, 2022

rigorous cleaning practices proved effective in reducing contamination from yeast.

c) Process-Specific Cleaning Protocols: Tailoring cleaning-in-place (CIP) procedures to the characteristics of fermenting vessels ensured a more thorough removal of residues and contaminants.

3. RESULT & DISCUSSION

1. In Brewery the contamination found in the beer during fermentation that affects the flavor, pH, Haze, Yeast viability, Yeast consistency, off flavor, Fermentation velocity in the beer.

After study found that there are following chances from where the contamination is carriedMicro-organisms will thrive in the most inhospitable conditions. When they are deprived of their nutritional requirements, bacteria can often form spores which are more difficult to destroy than the active live bacteria. Consequently, they

2.Infect the beer from many different sources. The tables below identify the main sources of contamination.

Table 3.1.1: Source of contamination

Source of contamination	Comment
Water	The brewing water in the wort undergoes boiling as an essential step in the brewing process. Nevertheless, there remain potential risks of contamination, particularly during procedures such as Cleaning in Place (CIP) and the final rinse water stage.
Pitching yeast	Storing yeast may create conditions conducive to the growth of other microorganisms already present in the yeast slurry. This poses a significant challenge as contamination has the potential to spread throughout the brewery unless effectively managed through thorough plant cleaning and, in some cases, acid washing
Chilled Wort	Wort chillers and wort mains play a crucial role due to the nature of the wort. The heat exchangers, in particular, often encounter significant fouling, and inadequate cleaning can provide a protective environment for microorganisms. Wort, being rich in sugars, proteins, minerals, and oxygen, serves as a conducive medium for the growth of microorganisms.

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 08, 2022

WortAiration	Wort aeration system required free from contamination.
Yeast handling tanks	This can be a source of contamination because of nature of yeast.
Fermenting vessels/ Unit tank	Difficult to clean due to left over residue of yeast and hops. FV/UTs are the major sources of contamination because of the storage time of beer.

Samples were collected from multiple sources including water, cooled wort, Air used for aeration, yeast handling tanks, Pitching yeast and fermentation vessels. The microbial load was quantified using media plating techniques for both bacteria and fungi.

2. Sampling Results

In the Brewing Water, Cooled wort, Harvested yeast, CIP Sample the contamination is found

The following are the Root Cause & 5 Why Analysis For Microbial Contamination found in different sources:

A. Root Cause Analysis for water contamination:

Problem Statement: Microbial growth is occurring due to water contamination.
Immediate Cause: Presence of microorganisms in the water.
Underlying Cause: Inadequate water treatment and sanitation procedures.
Root Cause: Lack of proper water treatment protocols and monitoring.

5. Why Analysis:

i) Why is there microbial growth in water?

Because microorganisms are present in the water.

ii) Why are microorganisms present in the water?

Because the water treatment process is not effectively removing them.

iii) Why is the water treatment process not effectively removing microorganisms?

Because the treatment methods and equipment might be insufficient or improperly maintained.

iv) Why are the treatment methods and equipment insufficient or improperly maintained?

Because there might be a lack of regular maintenance and updates to the water treatment system.

v) Why is there a lack of regular maintenance and updates to the water treatment system?

There might be inadequate resources, or a lack of awareness about the importance of maintaining the system.

By addressing the root causes identified through the 5 Whys analysis, such as improving water treatment methods, ensuring proper maintenance, and raising awareness about the importance of water treatment, you can work towards mitigating microbial growth related to water contamination.

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 08, 2022

B) Root Cause Analysis for CIP sample:

Problem Statement: Contamination is occurring in the CIP sample.

Immediate Cause: Ineffective CIP process.

Root Cause: CIP process design and execution need improvement.

5Why Analysis:

1.Why is contamination occurring after CIP?

Because theCIP process is not effectively removing contaminants.

2.Why is the CIP process not effectively removing contaminants?

Because the cleaning solution might not be reaching all areas of equipment.

3.Why is the cleaning solution not reaching all areas?

Because there might be inadequate flow or pressure in certain parts of the equipment.

4.Why is there inadequate flow or pressure in certain parts of the equipment?

Because the equipment design might not be optimized for even distribution of cleaning solution.

5.Why is the equipment design not optimized for even distribution?

The equipment might not have been designed or updated with the latest insights into fluid dynamics and cleaning efficiency.

C) Root Cause Analysis for cooled wort contamination:

Problem Statement: Presence of microorganisms detected in cooled wort, impacting the quality of the brewing process.

Immediate Cause: Detection of microbial contamination in the cooled wort samples.

Underlying Cause: Ineffective cooling and aeration process allowing for microbial growth and contamination.

Root Causes:

a. Poor Aseptic Handling: Inadequate aseptic handling practices during the cooling phase, such as exposure to non-sterile surfaces, could introduce microorganisms.

b. Insufficient Aeration: Lack of proper aeration during the cooling process may create an environment conducive to microbial proliferation.

c. Contaminated Cooling Equipment: The cooling equipment, such as heat exchangers or pipes, may be contaminated, serving as a source for microbial introduction.

d. Temperature Fluctuations: Fluctuations in cooling temperatures may create pockets within the wort that are not adequately cooled, promoting microbial survival.

5 Why Analysis of Contamination found in Cooled Wort:

1. Why were microorganisms found in cooled wort?

Because the cooling process might not be effectively reducing the temperature to levels that inhibit microbial growth.

2. Why is the cooling process ineffective in reducing temperature?

Because the cooling equipment, such as heat exchangers, may not be functioning optimally, leading

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 08, 2022

to insufficient heat exchange.

3. Why is the cooling equipment not functioning optimally?

Because there is a lack of regular maintenance and cleaning, resulting in the buildup of contaminants on the heat exchange surfaces.

4. Why is there a lack of regular maintenance and cleaning of the cooling equipment?

Because a robust preventive maintenance schedule and cleaning protocols have not been established or consistently followed.

D) Root Cause Analysis for pitching yeast:

Problem statement: Microorganisms were found in the pitching yeast, compromising the fermentation process.

5 Why Analysis of Microorganism Contamination in Pitching Yeast:

1. Why were microorganisms found in pitching yeast?

Because the yeast propagation or storage conditions may not be adequately sterile.

2. Why are yeast propagation or storage conditions not adequately sterile?

Because the equipment used for yeast handling and storage might not be properly sanitized between uses.

3. Why is the equipment not properly sanitized between uses?

Because there may be gaps or lapses in the cleaning and sanitation procedures, allowing for the persistence of contaminants.

4. Why are there gaps or lapses in the cleaning and sanitation procedures?

Because there is a lack of rigorous training and supervision regarding yeast handling protocols, leading to inconsistencies in execution.

Based on the root cause analysis and 5 Whys, here are some potential strategies for improving the CIP process to reduce contamination:

Enhance Equipment Design: Collaborate with engineers to optimize equipment design for even distribution of cleaning solution, minimizing dead spots.

Monitor Flow and Pressure: Implement sensors to monitor flow rates and pressure throughout the CIP process, ensuring adequate coverage.

Review Cleaning Solutions: Evaluate the effectiveness of cleaning agents and adjust formulations if necessary to ensure thorough cleaning.

Update CIP Protocols: Develop detailed CIP procedures tailored to each piece of equipment, including flow rates, pressure settings, and cleaning durations.

Regular Maintenance: Establish a regular maintenance schedule to check and calibrate equipment, ensuring consistent performance.

Employee Training: Train staff to understand the importance of proper CIP execution and the impact on contamination prevention.

Validation and Verification: Implement validation procedures to confirm the effectiveness of the CIP process, including swab testing for cleanliness.

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 08, 2022

Continuous Improvement: Establish a feedback loop for process improvement, gathering insights from staff and analyzing post-CIP contamination level

Table 3.1.2:Results of colonies found in particular media. (Monthly avg. data)

SAMPLE NAME	MEDIA NAME	1st. Month (Avg.)	2nd Month (Avg.)	3rd Month (Avg.)	4th Month (Avg.)
BREWING WATER	**WLN**	**50**	**45**	**8**	**1**
	PCA	**10**	**9**	**5**	**0**
CIP PLATING	**WLN**	**TNTC**	**40**	**17**	**0**
COLD WORT	**WLN**	**17**	**17**	**8**	**2**
	MAC-CONKEY	**12**	**10**	**4**	**0**
HARVESTING YEAST FROM YST TANK	**WLD**	**25**	**12**	**8**	**1**
	YMC	**10**	**6**	**2**	**0**
FERMENTATION SAMPLE	**WLD**	**24**	**15**	**6**	**1**
	RR	**0**	**0**	**0**	**0**
	YMC	**12**	**8**	**2**	**0**

Fig 3.1.3:- Monthly Average data of analytical & Microbiological parameter for particular month.

Sr. no	**Parameter**	**For 1st month (Avg.)**	**For 2nd month (Avg.)**	**For 3rd month (Avg.)**	**For 4th month (Avg.)**
1	**pH**	**4.07**	**4.11**	**4.15**	**4.18**
2	**Haze**	**0.65**	**0.61**	**0.55**	**0.48**
3	**Yeast Viability**	**95%**	**96%**	**96.5%**	**97%**
4	**Yeast consistency**	**62%**	**64%**	**65%**	**66 %**
5	**Phenolic off flavor score out of 10**	**3**	**3**	**2**	**1**

Conclusion

The results of this study highlight the complex interplay between different stages of the fermentation process and their contributions to microbial contamination. While initial water quality and boiling effectively reduced microbial loads, challenges emerged during yeast handling and fermenting vessel cleaning. Residual microorganisms in yeast handling

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 08, 2022

tanks and the difficulty of cleaning fermenting vessels pose potential threats to product quality.

The success of contamination control strategies depended on addressing root causes. Optimizing water treatment processes improved water quality and minimized the introduction of contaminants. Enhancing yeast handling practices reduced the risk of contamination from yeast. Process-specific cleaning protocols for fermenting vessels underscored the significance of tailored approaches to effective decontamination.

This study underscores the importance of a comprehensive approach to minimize microbial load during the fermentation process. It emphasizes the necessity for collaboration between microbiologists, engineers, and brewers to continually refine processes and implement best practices. By adopting and adapting contamination control strategies, breweries can ensure the production of high-quality, uncontaminated fermented products that meet consumer expectations and industry standards.

"Fermenting vessels/Unit tanks are difficult to clean due to leftover residue of yeast and hops. FV/UTs are major sources of contamination because of the storage time of beer."

REFERENCES

Vaughan, A., O'Sullivan, T. and Van Sinderen, D., 2005. Enhancing the microbiological stability of malt and beer—a review. *Journal of the Institute of Brewing*, *111*(4), pp.355-371.

Hutzler, M., Müller-Auffermann, K., Koob, J., Riedl, R. and Jacob, F., 2013.Beer spoiling microorganisms–a current overview. *BrauweltInt, 31*, pp.23-25.

Hutzler, M., Riedl, R., Koob, J. and Jacob, F., 2012.Fermentation and spoilage yeasts and their relevance for the beverage industry-a review. *Brew Sci*, *65*, pp.33-52.

Sampaio, J.P., Pontes, A., Libkind, D. and Hutzler, M., 2017.Taxonomy, diversity and typing of brewing yeasts. *Brewing Microbiology*, p.85.

Back, W.: „Farbatlas und Handbuch der Getränkemikrobiologie“, vol. 1.,Fachverlag Hans Carl, Nuremberg, 1994.

Bohak, I.; Thelen, K.; Beimfohr, C.: „Description of Lactobacillus backi sp. nov., an obligate beer-spoiling bacterium“, MonatsschriftfürBrauwissenschaft (today: BrewingScience), 03/04, 2006, pp. 78-82.

Hutzler M.; Koob J.; Riedl R.; Jacob F.: “Classification, identification, and detection of beer spoiling microorganisms – A review”, World Brewing Congress, Portland, 28 July - 1 August 2012.

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 08, 2022

Juvonen R.; Suihko M. L.: "Mega sphaerapaucivorans sp. nov., Mega sphaerasueciensis sp. nov.andPectinatushaikarae sp. nov., isolated from brewery samples, and emended description of the genus Pectinatus", International Journal of Systematic and Evolutionary Microbiology, 2006, 56, 695-702, DOI10.1099/ijs.0.63699-0.

Qian F.: „Einfluss der Bierzusammensetzung auf die mikrobiologischeStabilität von Reisbier", 42. Technologisches Seminar 2009, FreisingWeihenstephan, 20-22 January 2009.

Suzuki K.; Funahashi W.; Koyanagi M.; Yamashita H.: "Lactobacillus paracollinoides sp. nov., isolated from brewery environments," International Journal of Systematic and Evolutionary Microbiology, 2004, 54, 115-117, DOI 10.1099/ijs.0.02722-0.

Marinangeli, P.; Angelozzi, D.; Ciani, M.; Clementi, F. and Mannazzu, I.: Minisatellites in Saccharomyces cerevisiae genes encoding cell wall proteins: a new way towards wine strain characterisation, FEMS Yeast Res., 4 (2004), no. 4-5, pp. 427-435

Abee, T., Krockel, L. and Hill, C., Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. Int. J. Food Microbiol., 1995, 28, 169–185.

Shekade, D. P., Patil, P. D., Mote, G. V., &Sahoo, A. K. (2018). Potential use of dragon fruit and Taro leaves as functional food: a review. *European Journal of Engineering Science and Technology*, *1*(1), 10-20.