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Risk Based Evaluation and Study of Environmental Monitoring in a Food Industry

Shivani Agrawal* and Sujeet Mrityunjay

Department of Life Sciences, ITM University, Gwalior, India

Abstract

Environmental monitoring is the study of microbes which interact with surrounding ambience and monitor them by various method, in which time duration they multiply their progenies. We have monitored microbes in a food industry, as it is our responsibility to provide microbe free food. According to food and drug administration, environmental monitoring and their control is very crucial for every food industries. To evaluate the microorganism in a food industry as well as maintain the very low or negligible growth of microbes in our food by following the good laboratory practice and Cleaning In Place (CIP).

Keywords: Environmental monitoring • CIP • Zones • Listeria monocytogenes • Food laws and regulations

Introduction

Food is the basis to sustain a healthy life as evidenced from its consumption through centuries by human. We are plenty of foodstuffs available these days as part of new product development and flexible global participation of researchers and food technocrats. Their researches brings a lot reforms in traditional recipes through newest technological concepts (functional or nutraceutical) that address much of safety aspect for consumers health and is the prime focused concern [1]. On large scale production the government and public sector startup the food processing industries with some innovative techniques.

Food processing is a technique used by the food processing industry to turn raw agricultural products or livestock into consumable food products. Food processing is another term for the conversion of changing one food type into another. Primary food preparation entails transforming uncooked agricultural and livestock products into ones that can eventually be ingested by humans. Secondary food processing is the routine preparation of food for consumption. A few examples are creating bread, wine, sausages, etc., regardless of whether these are done at home or in vast factories. Tertiary food processing is the industrial stage of food preparation done for marketing objectives. Examples include meals served on aeroplanes, dishes that can be heated and served immediately, etc. Processing food has both benefits and drawbacks. To start a food processing business, one must adhere rigorously to labeling regulations to reassure consumers that the food is healthy and safe [2].

Strict labeling policies are the present hour need to stay fit and fine. Safe and wholesome supply of foods, high pace food demands, increase in processing aspect, minimization of fraudulent practices are the key points that somehow require laws and standards during transportation, storage, marketing and storage [3].

Laws and rules governed by food industry

Food law is a body of rules and regulations that controls how food is produced, distributed, sold, and consumed. Its goals are to safeguard consumer health and increase output. The provisions in the food regulations are specific to the various product categories that are outlined separately in each set of regulations. It comprises basic standards for quality to guarantee that the food is pure and prepared under sanitary circumstances. Depending on the needs of the food industry or whether it embraces international norms, each nation creates its own system of food regulation.

India has a broad geographic region and a diversity of seasons, which promote the growth of a wide range of flora and wildlife. Additionally, it has access to both fresh and saltwater sources, which offer coastal residents a variety of fresh meat products to enjoy and also help traders run their businesses. In addition to this, the availability of the most diverse spices and milk producing animals (cows, buffaloes, sheep, goats, camels, and yaks) is the driving force behind the need for a single agency to regulate these products. By creating a set of science based rules and norms relating to the manufacture and distribution of food items, the food safety and standard act, passed in 2006, makes it simpler. Despite being introduced in 2006, this law only became effective in 2011. In addition to ensuring the production of high-quality food, it safeguards

***Address for Correspondence:** Shivani agrawal, Department of Life Sciences, ITM University, Gwalior, India, Tel: 6232794021; E-mail: shivi151623@gmail.com*

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consumer health. Additionally, it governs issues pertaining to food imports, roles assigned to the government, sanctions, and surveillance, among other things [4].

The licenses required for starting a food processing industry in India

According to a variety of laws and regulations established by the Indian government, anybody who wants to start a business in food processing in India must obtain specific licenses. These licenses are noteworthy because they include the essential commodity act of 1955, the Food Safety and Standard Authority of India (FSSAI), the AGMARK, the vegetable oil product order of 1947, the meat food products order of 1973, the fruit products order of 1955, the milk and milk products order of 1992, and the prevention of food adulteration act of 1954.

ISO

Standardization plays an important role to make continuous improvement in every aspect of life. It helps to provide safe and good quality of products to the consumers and also bring economic benefits. Various international standards were established by International Organization for Standardization (ISO) related to food quality and environment protection. It is a Swiss based voluntary and independent international organization that was founded in 1947. It has 2281 international standards pertaining to safety and food quality, agriculture, and healthcare technology, and it is made up of 164 national standards bodies that work together to form a network of standards institutions. Additionally, it aids in facilitating business practices and has three membership levels:

Full members: Full members participate in technical and policy meetings of ISO and also have the right of voting. They can sell and adopt ISO standards nationally. ISO has 120 full members or member bodies including India, Italy and Australia.

Correspondent members: Correspondent members attend the technical and policy meetings of ISO as observers and they can adopt or sell the ISO international standards nationally. ISO has currently 39 correspondent members [5].

Subscriber members: Subscriber members cannot participate in the ISO work and do not adopt or sell ISO standards nationally. ISO has three subscriber members.

FSSAI

Any sort of food, whether processed or semi-processed, including genetically modified food, should adhere to the bare minimum of safety standards to give customers confidence that it won't hurt them. The standards established by the Food Safety and Standards Authority of India (FSSAI) must be followed by all owners and operators of food enterprises. It is an independent body that controls every step of the food manufacturing process to guarantee the creation of safe food. The administrative arm of the FSSAI is the ministry of health and family welfare (GOI). Its primary headquarters are in New Delhi, and it also has eight regional offices in Delhi, Mumbai, Chandigarh, Lucknow, Kolkata, Cochin, Chennai, and Guwahati. Additionally, it has 172 NABL accredited private labs, 88 State/UT laboratories, and 19 referral laboratories (FSSAI, 2011).

India has a substantial food processing sector. Another important issue is that the 130 million USD Indian food processing industry is likely to draw both domestic and foreign companies. Moreover, a few additional factors could increase the demand for the Indian food processing business. Despite the fact that our country is the world's top producer of milk, ghee, ginger, bananas, guavas, papayas, and mangoes, the local market in India offers 1.25 billion prospects for our growing population. Additionally, India is the world's second largest producer of wheat, rice, and a vast range of fruits and vegetables. Because of increased urbanization and the associated reduction of time, the demand for processed meals is rising. Our nation's food business is the fastest-growing behind China overall.

The Indian food processing business faces some difficulties. The food processing industry experiences some operational issues, much like any other industry. Moreover, they also experienced some other issues in a similar manner.

Access to raw materials: Because agricultural products are seasonal, the food industry has issues including production delays [6].

Storage space constraints: The inability of small-scale food processing businesses to keep their raw materials adequately is one of their issues.

Difficulties with transportation: This industry also depends heavily on transportation. The group or individual working in the food industry also encounters some issues.

If a problem arises with the same technical equipment or with problems such as spoiling, delayed delivery, product breakage and damage, etc., may happen: The type of machinery employed in a unit of production and industrial competitiveness both affect output rate. As the market for processed foods expands, so does industry competition. Despite these realities, the Indian food industry is developing and growing annually. The risk of food processing is also increased by the microbial contamination, which reduces industry productivity and expansion.

Food processing techniques are made to limit microbial development or inactivate microorganisms in food products in order to lower the danger of food borne pathogens or spoiling microorganisms. The management of microorganisms must take into account the machinery and surfaces of the processing plant. On the other hand, when adding antimicrobial compounds to product composition, it is necessary to consider the impact of other food components or food processing. The availability of techniques for isolation, identification, and molecular type when it comes to food-borne diseases can provide vital information on the spread of infections and the outbreaks that go along with them. Examining microbial interactions with food items and processes at each stage of the food production chains, from farm to fork, is important as a result [7]. So, here we do environmental monitoring for controlling the risk of microbes who affects the food in food industry.

The study of environmental issues and their effects is referred to as environmental monitoring. It is employed in the creation of environmental effective evaluation and, in some circumstances, human made decisions that could have an impact on the innate ambient [8]. The three main types of environmental monitoring are air, water, and soil. In a food Industry we monitor water and air. We

examine water borne microorganisms by taking sample of water from different plants and by pour plate technique identify the growth of microorganisms in sampled water. For air examining, we use air petri sampling device and with the same process, identifies the air borne microbes [9].

Microbiological environmental monitoring is a technique for proving adequate microbiological attribute in a critical ambient and rapidly determined substitute. It entails gathering information on microbial counts retrieved through specimen of ambience, surfaces, and humans in a clean environment. The information allows for the tracking of trends over time, as well as the detection of upward and downward changes in a given environment. Particle counts (that might indicate living creatures on transporters or inert material) are also analyzed as part of the programme, in addition to performing procedures for monitoring the amount and types of microorganisms. The goal of the microbiological environmental monitoring programme is to show that the controlled environment of a food processing facility's microbiological quality is within acceptable bounds and to pinpoint any shortcomings.

Monitoring the microbiological environment in a lab is essential for assessing the environment's quality for food processing. It is a specialized gadget created to monitor the effectiveness of cleaning and sanitation procedures. Microbial counts from samples of the air, surfaces, and people must be calculated [10].

Adverse effect of microorganisms in food industry

Microorganisms that are connected to food might be classified as "helpful," "pathogenic," or "spoilage." Spoilage microorganisms are microorganisms that can grow in food and change its flavor, consistency (body and texture), color, or appearance. Bacterial enzymes may also contribute to the progressive deterioration of frozen or dried foods when they are stored for an extended period of time. These changes lessen food's nutritional value and may eventually make it unfit for human consumption. For instance, perishable foods kept in the refrigerator, such as milk, fresh meat, poultry, fish, fruits, and vegetables, lose some of their quality traits during regular storage and eventually degrade, partially due to the activity of bacteria that might proliferate in the cold. Significant microbial growth (millions of organisms per g or cm^2) often occurs before quality losses are detectable. These modifications alert consumers to considerable microbial activity when they become apparent [11].

Foods contaminated with pathogenic microbes can affect people in a number of different ways. Salmonella and Shigella, for example, are two pathogenic germs that can enter the gastrointestinal tract through food. Before food is consumed, certain microbes, such as *Clostridium botulinum, Staphylococcus aureus,* and *Bacillus cereus*, can multiply and produce toxins. Foods may also harbour germs like clostridium perfringens and certain pathogenic *Escherichia coli* that produce poisons in living things.

In order to encourage the growth of beneficial microorganisms like lactic acid bacteria and yeasts, which are either naturally occurring or added on purpose, certain conditions are chosen for specific meals. Foods with a long shelf life and good organoleptic characteristics include cheeses, yoghurt, bread, pickles, and fermented sausages. We do environmental monitoring to limit the impact of microorganisms in the food business.

Microbiological environmental monitoring program

Monitoring the microbiological environment in a lab is essential for assessing the environment's quality for food processing. It is a clearly defined programme that tracks the effectiveness of routine cleaning and sanitation practises. The objective of the microbiological environmental monitoring programme is to demonstrate that the controlled environment of a food processing facility's microbiological quality is within acceptable limits and to identify any defects. It involves gathering microbial counts from samples of air, surfaces, and people [12]. FCSs are observed, including equipment components outside of processing equipment and those in contact with the product directly. MEMP might include air analysis. Microorganisms frequently use the food as a substrate to adapt to the conditions of food preparation. When creating a microbiological monitoring programme, which can be either a wide approach using indicator species or a focused approach on a specific organism, the food processing plant must consider which microorganisms are significant. As a result, one programme does not fit all of the facilities or products. The scope and goal of environmental monitoring programmes must be determined as a result. As a result, a microbiologist with adequate expertise of indicator species and environmental monitoring is necessary.

Requirement of a Microbiological Environmental Monitoring Program (MEMP)

Building solid precursor programmes in food processing facilities is essential to implementing an efficient HACCP based system and halting the spread of food borne illness. To create excellent and safe food items, a sanitary processing environment is required. Proper hygienic plant design and operations, as well as tight cleaning and sanitation regimes, are the best ways to achieve it. MEMP requires a systematic approach for a number of reasons, such as plant hygiene verification, the reduction of difficult to clean regions, the reduction of cross-contamination, and the improvement of cleaning protocol effectiveness [13]. The significance of MEMP deployment in the environment for processing food is summarized below.

Offers information to create a baseline for acceptable requirements for facility cleanliness. Inform the audience on the manufacturing environment's processing conditions. Locate potential contamination pathways. Provide details about the manufacturing environment's processing conditions. Identify potential contamination routes. Prevent microbiological contamination by identifying troubling trends and taking appropriate action. Validation and confirmation of cleanliness procedures in food plants. Knowledge of the environment's microbial ecology throughout processing. Validation and verification of a food plant's sanitation protocol. Being aware of the microbial ecology in the processing environment. Reduction of food recalls.

Microorganisms to monitor in the environment

Bacteria known as indicator organisms are employed to demonstrate the general food quality, the hygienic state of the

processing environment, and, to some extent, the presence or absence of pathogens. Regular environmental microbiological testing often concentrates on two things: first, determining the amounts of indicator organisms, and second, testing for particular pathogens. All sampling zones frequently test indicator organisms to determine whether contamination is present, absent, or within permissible limits. The following lists of bacteria are some of those targeted by microbiological EMP [14].

Total plate count: A common test that does not identify the species of bacteria present in a sample but rather acts as a gauge of the overall number of microorganisms on the surface is the total bacterial count, also known as an aerobic plate count. TPC is a subpar safety indicator because it has no connection to infections. Poor sanitation is indicated by ambient sample TPC concentrations above a specific threshold.

Coliforms: Because they are effective stand-ins for enteric pathogens, these bacteria have been used as hygiene markers of faecal contamination in water and ambient samples for a long time. Coliform analysis is a typical indicator of subpar sanitation and contamination after processing.

Enterobacteriaceae: This family has been used to indicate the quality and safety of food. Coliform counts may not be the best indicator of poor FCS cleanliness since Enterobacteriaceae are more resistant to the environment than coliforms. Although the coliform group and other pathogenic species are members of the Enterobacteriaceae family, this family cannot be used to monitor harmful bacteria. It is more reliable to test for specific pathogens to make sure the manufacturing environment is pathogen free.

Yeast and molds: Standard food processing and formulation controls have no effect on these fungi, which are eukaryotic in origin and result in the deterioration of food. Food processing areas are routinely examined for yeast and moulds since these bacteria can spread through the air *via* dust and aerosols.

Leisteria monocytogenes: The term "listeriosis" refers to a set of human illnesses caused by the food-borne bacterium Listeria monocytogenes. With a preferred temperature range of 30°C to 37°C, Listeria monocytogenes may grow at temperatures ranging from 1.5°C to 45°C.

Materials and Methods

Chemicals: Chromic acid solution, labolene solution, buffer solution, 70% I.PA. Solution

Preparation of chromic acid solution: Before starting to prepare the 2% chromic acid solution wear the gloves, safety goggles and face mask. Take the clean vessel and fill it with adequate amount of RO water, weighing potassium dichromate and mix in the RO water and stir it. Now take the concentrate sulphuric acid stirring it with RO water and potassium dichromate. Chromic acid solution is most corrosive. Checks the color of the solution, when the solution will show green color it discard with flow of water (Tables 1 and 2).

Preparation of labolene solution: For 0.5% solution in a bottle with 995 ml of pure RO water, measure 5 ml of the labolene solution and add it while thoroughly stirring. Glassware used in laboratories can be cleaned with it.

Media: Chloramphenicol yeast glucose agar, violet red bile agar, violet red bile glucose agar, eosin methylene blue agar (Table 1).

S.No.	Parameter / microorganism	Media used	Incubation temperature	Incubation time	Morphology
1	Yeast and mould	Chloramphenicol yeast glucose agar	25°C	3 days-5 days	Yeast has white and thread like structure, mould has fuzzy appearance
2	*E. coli*	Eosin methylene blue agar	37°C	24 hours	Green metallic sheath colonies
3	*Enterobacteriacease*	Violet red bile glucose agar	37°C	24 hours	Pink and pinpoint colonies
4	Coliform	Violet red blile agar	37°C	24 hours	Pink and pinpoint colonies
5	Total plate count	Plate count Agar	30°C	72 hours	White or cream color ellipsis

Table 1. Micro-organisam and its morphology.

Glassware: Petri plates, tips, pipette, loop, glass bottles, measuring cylinder, beaker.

Instruments: Autoclave, heating plate, laminar air flow, air petri sampling device, hot air oven, water bath.

Plate Count Agar (PCA)-30 gm/1000 ml: Final pH (at 25°C) 7.0 ± 0.1 (Table 2).

S.No.	Chemical	Quantity (gm per 1000 ml)
1	Tryptone	5

2	Yeast extract	2.5
3	Dextrose (Glucose)	1
4	Sodium chloride	6.5
5	Agar	15

Table 2. Chemicals and quantity.

For determining the plate counts of microorganisms in food, water, waste water, and clinical samples, it is indicated. 23.5 grams should be dissolved in 1000 ml of distilled or filtered water. Heat the medium until it boils in order to completely dissolve it. Sterilize by autoclaving for 15 minutes at 121°C and 15 pounds of pressure to 45°C-50°C. Mix thoroughly, and then transfer to sterile Petri dishes. Long chain amino acids, nitrogenous and carbonaceous substances, and other necessary nutrients are all present in tryptone. Vitamin B complex is supplied by yeast extract. The APHA advises using the pour plate method. In petri plates, the samples are diluted and the proper dilutions are added. These plates are filled with sterile molten agar, which is then gently rotated to achieve uniform mixing of the sample and agar. The surface inoculation method is preferred over the poured plate count approach because it yields superior results [15]. Plate count agar can also be used to count the number of bacteria in sterile rooms. Because it is a general-purpose medium, it might not be able to support the growth of finicky organisms. After 18 hours-48 hours of incubation at 35°C-37°C, cultural characteristics are observed. Between 10 degrees and 30 degrees celsius should be used for both the storage container and the prepared medium. Use before the stated expiration date on the label. Due to the product's hygroscopic nature, it is important to store the product dry after opening and carefully cover the bottle to avoid lump development. Lumps may occur if the product is not stored properly (Table 3). Store away from sources of ignition and severe temperatures in a dry, ventilated place. After usage, securely cap the container. Use before the labeled expiration date [16].

Violet Red Bile Agar (VRBA)- 41.53 gm/1000 ml: Final pH (at 25°C) 7.4 ± 0.1 (Table 3).

S.No.	Chemicals	Quantity (gm per 1000 ml)
1	Peptone	7
2	Yeast extract	3
3	Lactose	10
4	Bile Salts mixture	1.5
5	Sodium chloride	5
6	Neutral red	0.03
7	Crystal violet	0.002
8	Agar	15

Table 3. Chemical used for violet red agar.

Guidelines for the selective separation, recognition, and tally of the coli-aerogenes bacteria in clinical samples, milk, and other dairy products. Float 41.53 grammes in 1000 ml of pure or purified water. For the medium to thoroughly dissolve, heat while stirring until boiling. Remove autoclave. Once cooled to between 45°C and 50°C, add the inoculum into sterile Petri plates. The medium can, if necessary, be autoclaved for 15 minutes at 121°C and 15 lbs of pressure. Numerous genera of bacteria from the Enterobacteriaceae family make up the coliform group. The method of detection, lactose fermentation, has historically been employed to define this category. This group includes any gram negative, non-spore forming, aerobic, and facultatively anaerobic rod shaped bacteria that ferment lactose with the formation of gas and acid within 48 hours at 35°C. One of the usual tests is the inspection of foods, components, and raw materials for the presence of marker groups such coliforms. The coliaerogenes bacterial group is counted using violet red bile agar, a variation of MacConkey's original recipe. It depends on the utilization of the lactose and neutral red indicator systems, as well as the selective inhibitory substances crystal violet and bile salts. As a result, a lot of undesirable organisms are prevented from growing, and it is possible to identify some bacteria. Rapidly digesting organisms form purple colonies with purple haloes around them. Colonies with pale, greenish zones are produced by non-fermenters and late lactose fermenters [17].

By incubating VRBA in anaerobic conditions and/or at a higher temperature, *i.e.* at or above 42°C, selectivity can be enhanced. It is also not recommended by ISO. Yeast extract and peptone are sources of nitrogen, carbon, vitamins, and other essential growth components. The use of lactose, a fermentable carbohydrate, results in the formation of acids. The so created acidity is detected by neutral red indicator. The following gram-positive and unrelated bacteria are inhibited with the use of a mixture of crystal violet and bile salts. Sodium chloride keeps the osmotic equilibrium in check. Because other bacteria may also have the same effect, Violet red bile agar is not totally selective for enteric. For a positive identification, more biochemical testing is required. Individual organisms have different growth requirements, and they could have different growth patterns on the medium. The organisms listed on the COA have been examined in each lot of the medium. Users are advised to validate

the medium for any particular microorganism other than those listed in the COA depending on their particular requirements. Keep the prepared medium at 20°C-30°C and store it at 10°C-30°C in a tightly covered container. Use before the expiration date on the label. Since the product is hygroscopic, it must be properly stored dry after being opened, and the bottle must be tightly closed. The product may develop lumps if it is not stored properly. Store away from sources of ignition and excessive temperatures in a dry, well-ventilated location. After usage, securely cap the container. Use of the product before the indicated expiration date will maximize performance [18]. By autoclaving or incinerating used or useless preparations of this product, the user must ensure safe disposal. Any item that comes in contact with a clinical sample must be decontaminated and disposed of in accordance with current laboratory procedures. Dispose of infectious things in accordance with established laboratory procedures (Table 4).

Violet Red Bile Glucose Agar w/o lactose (VRBGA)-38.53 gm/ 1000 ml: Final pH (at 25°C) 7.4 ± 0.2.

S.No.	Chemicals	Quantity (gm per 1000 ml)
1	Peptone	7
2	Yeast exract	3
3	Sodium chloride	5
4	Bile Salts mixture	1.5
5	Dextrose	10
6	Neutral red	0.03
7	Crystal violet	0.002
8	Agar	12

Table 4. Chemical used for violet red glucose agar.

For the identification and counting of enterobacteriaceae, violet red bile glucose agar is advised. 40.62 grammes of the dehydrated medium should be dissolved in 1000 ml of distilled or filtered water. For the medium to thoroughly dissolve, heat it until it boils. Avoid autoclave. Put into sterile petri plates after thoroughly mixing. For the identification and counting of Enterobacteriaceae, a selective medium known as Violet Red Bile Glucose Agar (VRBGA) is advised. particularly the bile tolerant gram negative bacteria from non-sterile items and medicinal preparations. Nitrogenous substances and other nutrients necessary for bacterial metabolism are provided by pancreatic digest of yeast extract and gelatin. Because of the inhibitors, bile salts, and crystal violet, this medium is selective. Gram positive organisms, especially Staphylococci, are inhibited by crystal violet. Fermentation of lactose and glucose can be detected using neutral red indicator. In the presence of neutral red, glucose-fermenting bacteria create red colonies with pink-red haloes. Sodium chloride controls the osmotic balance of the medium. When the pH of the medium drops below 6.8, neutral red is absorbed and causes the dye to change color, giving the object its red color. Keep the prepared medium between 2 and 8°C and below 30°C in a firmly closed container [19]v. Use before the labelled expiration date (Table 5).

Chloramphenicol Yeast Glucose Agar (CYGA)-40 gm/1000 ml: Final pH (at 25°C) 6.6 ± 0.2.

S.No.	Chemicals	Quantity (gm per 1000 ml)
1	Yeast extract	5
2	Dextrose (Glucose)	20
3	Chloramphenicol	0.1
4	Agar	14.9

Table 5. Chemicals used for chloramphenicol yeast glucose agar.

Recommend for selective yeast and mould enumeration in milk and milk products. Float 40 grams in 1000 liters of distilled or filtered water. To completely dissolve the medium, heat it until it boils. Sterilize at 45°C-50°C using an autoclave that runs for 15 minutes at 121°C and 15 lbs of pressure. Transfer to sterilised Petri dishes after completely combining. For isolating and counting fungi, including yeasts and moulds, in milk and milk products, it is recommended to use a selective medium called chloramphenicol yeast glucose agar. The ISO committee recently recommended using this medium for yeast and mould counts. Yeast extract, which supplies vitamin B complex and nitrogenous elements, is included in the medium. The energy comes from dextrose. Associated bacterial flora is suppressed by the thermostable antibiotic chloramphenicol. This extends the prepared medium's usable lifespan to at least 4 months and enhances its shelf life. The prepared medium should be kept at 20°C-30°C and keep the container tightly closed between 15°C-25°C. Use before the labelled expiration date. Due to the product's hygroscopic nature, it is important to store the product dry after opening and carefully cover the bottle to avoid lump development. Lumps may occur if the product is not stored properly. Store away from sources of ignition and severe temperatures in a dry, ventilated place. After usage, securely cap the container. By autoclaving or incinerating used or useless preparations of this

product, the user must ensure safe disposal. Dispose of infected materials according to recognized laboratory procedures, and in accordance with current laboratory procedures, any material that comes into contact with the sample must be decontaminated and disposed away [20] (Table 6).

Eosin Methylene Blue Agar (EMB)- 35.96 gm/1000 ml: Final pH (at 25°C) 7.2 ± 0.2

S.No.	Chemicals	Quantity (gm per 1000 ml)
1	Peptone	10
2	Dipotasium hydrogen (phosphate)	2
3	Lactose	5
4	Saccharose (sugar)	5
5	Eoisin y	0.4
6	Methylene blue	0.065
7	Agar	13.5

Table 6. Chemicals used in eosin methylene blue agar.

For distinguishing between gram negative enteric bacilli in clinical and non-clinical specimens, it is indicated. Float 35.96 grams in 1000 ml of distilled or pure water. Blend the suspension until it is even. To completely dissolve the medium, heat it until it boils. Sterilize by autoclaving at 121°C for 15 minutes under 15 lbs of pressure, do not overheat. Cool to 45°C-50°C and shake the medium to oxidise the methylene blue (*i.e.,* restore its blue hue) and suspend the flocculent precipitate. Gram-positive bacteria are only partially inhibited by methylene blue and eosin-Y. In reaction to the fermentation of carbohydrates, these colors act as differentiating indications. Eosin and methylene blue are adjusted roughly to a 6:1 ratio. In order to provide gram negative bacteria's alternate source of carbohydrates that generally ferment lactose but occasionally do not or do so slowly, sucrose is added to the medium. When the pH drops, the coliforms develop purplish-black colonies by absorbing the methylene blue-eosin dye complex. The colony takes up the dye compound [21].

Non-fermenters most likely cause the pH of the surrounding media to rise by solubilizing the methylene blue-eosin complex, which results in colourless colonies. Eosin and methylene blue inhibit the growth of several salmonella and shigella bacteria. The isolates need to be confirmed by other tests. Peptone provides carbon, nitrogen, and other vital nutrients for growth. Due to the fact that lactose and sucrose are fermentable carbohydrates, they serve as energy sources. Methylene blue and eosin-Y are used as differential markers. The medium is buffed by phosphate. The medium plates can be immediately streaked with the test material. Plates with inoculations should be incubated and shielded from light. To obtain isolated colonies, normal methods should be followed. EMB agar should be infected alongside a non-selective medium. For the purpose of identifying isolated colonies, additional confirmation tests should be conducted. Keep the prepared medium at 20°C-30°C and store it at 10°C-30°C in a tightly covered container. Use before the expiration date on the label. The product must be properly stored dry after being opened because of its hygroscopic nature, and the bottle must be tightly closed. The product may develop lumps if it is not stored properly. Store away from sources of ignition and excessive temperatures in a dry, well-ventilated location. Following use, firmly close the container. Utilize the product before the mentioned expiration date for best results. By autoclaving or incinerating used or useless preparations of this product, the user must ensure safe disposal. Any item that comes in contact with a clinical sample must be decontaminated and disposed of in accordance with current laboratory procedures. Dispose of infectious things in accordance with established laboratory procedures [22].

Methods

Pour plate method: Take swab sample and mix properly with distilled water. After mixing, took 1 ml of sample and pour into petriplate. After pouring sample, pour the media and mix thoroughly, specific media for specific microrganisms. After solidifying the media, incubate the plate according to specified microorganism to grow. For total plate count we incubate the plate for 72 hours, for yeast and mould; incubate it for 7 days, for enterobacteriaceae, coliform and *E.coli* incubation period is 24 hours (Figure 1) [23].

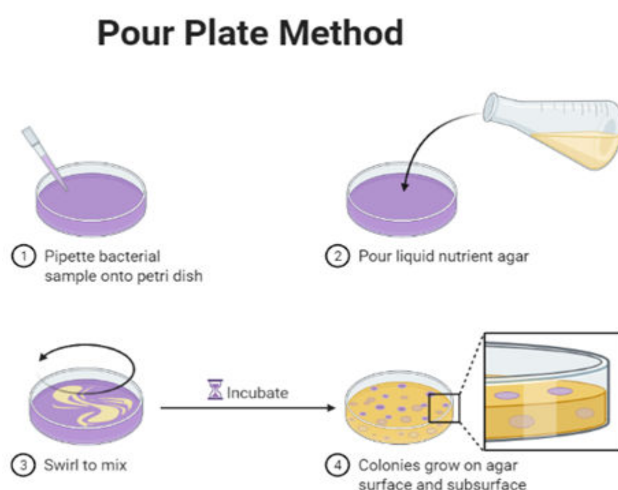

Figure 1. Microbe notes.

Streak plate method: A single species of microorganisms can be isolated into a pure strain using the streaking technique. Take swab sample and mix properly with distilled water. After mixing took 1 ml of sample and pour into petriplate. T-Streak refers to the three-phase streaking pattern. A sterile implement, such as a cotton swab or,

more frequently, an inoculation loop, is used to apply the streaks. A flame is used to disinfect the inoculation loop before use. After the loop has cooled, it is placed into an inoculum, such as a broth or patient specimen, which contains a variety of bacterial species. After that, the inoculation loop is moved in a zigzag pattern across the agar surface until around 30% of the plate has been covered. The plate is then rotated 90 degrees while the loop is re-sterilized. The zigzag pattern is continued by beginning in the previously streaked area and dragging the loop through it twice to three times. The zigzag pattern is continued by beginning in the previously streaked area and dragging the loop through it twice to three times. The loop collects fewer and fewer bacteria each time until it only collect a single bacterial cell that can develop into a colony. The first region of the plate ought to exhibit the plate's most vigorous growth. The final portion will have a lot of isolated colonies and the least amount of growth, whereas the second section will have fewer solitary colonies and more growth (Figure 2) [24].

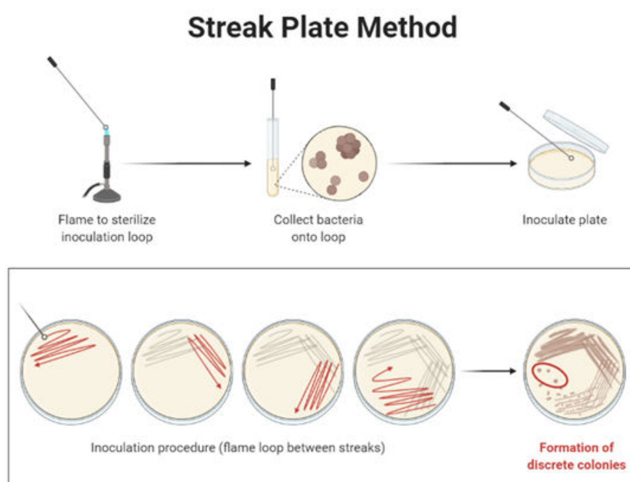

Figure 2. Microbe notes.

Swabbing

The swab method has been a tried-and-true technique for directly touching surfaces and sampling them for many years. The classic swabbing method uses an applicator stick with one end dipped in an extraction solution, typically 0.1 percent sterile peptone water, and the other end holding a sterile bud (such as cotton, rayon, calcium alginate, etc.). Utilizing the bud for sampling, the bacteria on a targeted area are removed by vigorously rubbing. Following sampling, the swab is sliced into the extraction solution to release the bacteria that were collected. The extraction solution is finally checked for microorganisms using the preferred method. Additionally, it is crucial that the swab solution be both compatible with detection techniques and safe for the retrieved bacteria. The remaining solution from the sampled surface is wiped or washed off when the swabbing is complete.

Water sampling

It is an indirect method of sampling used to examine objects or machinery that cannot be reached by direct sampling, such as tanks, pipelines, fillers, etc. Upstream of the procedure, sterile water is added, and samples are taken at various locations downstream. The microbiological load of the rinse water is assessed. The benefit of this method is that a huge surface area may be sampled, and no equipment disassembly is required. An alternative method involves soaking surfaces like plastic or wooden cutting boards in a rinse solution for a short period of time before testing the solution for bacteria. However, this rinse water sample is not appropriate for collecting biofilm because the flow-generated shear force could not be sufficient to remove it. In actuality, any residue or bacteria from the surface are transferred to an extraction or swabbing solution using either a particular or generic sample approach.

Air sampling

Another typical direct sample technique is duplicate organism direct remote contact, which involves employing microbiological media solidified in sterile dish (RODAC). The agar meniscus is briefly in touch with the surface being evaluated during sample. After being incubated at conditions that promote growth, the adhered cells are counted. This technique works better for gathering quantitative information on flat surfaces that have been cleaned and sterilized before sampling 1.

Environmental monitoring zones

Zone 1: It includes all surfaces with direct contact to food (e.g., slicers, mixers, conveyors, utensils, racks,work tables, etc.). Normally, during inspections to check for Salmonellae, food contact surfaces are not sampled. sampling of food contact surfaces, on the other hand, is crucial for inspections that aim to find Listeria monocytogenes.

Surfaces of products in contact: Employee hands, knives, racks, hoppers, screens, conveyor belts, air blowers, and work tables.

Zone 2: It consists of the territories that border zone 1 immediately. In research focusing on Salmonellae, this is the area where environmental contamination is most likely to directly affect a product's safety. Zone 2 in a small production area, including the exterior of equipment, the structure, food carts, equipment housing, gears, ventilation/air handling equipment, floors, etc., covers all non-food contact surfaces in the processing area. Zone 2 is the area surrounding the exposed product in a larger area where there may be a pathway for product contamination owing to mechanical or human action. If forklifts or foot traffic pass through a remote corner of the room, and similar traffic patterns also pass extremely close to a line where exposed food is transported or stored, that region may be classified zone 2.

Non-food contact surfaces that are near food and non-food contact surfaces include the outside framework of processing equipment, refrigerated units, equipment control panels, and switches.

Zone 3: It is the area next to zone 2 quickly. Through human or mechanical activities, a pathogen from zone 3 might infect zone 2 and spread to zone 3. Zone 3 might be made up of hallways and doors leading to areas utilised for food preparation or, in a large production room, places that are farther away from food handling equipment than conventional zone 2 areas.

Surfaces with less direct non-food contact that are situated in or close to the processing areas

Walls, floors, drains, hoses, air return covers, hand trucks, carts, and wheels.

Zone 4: It is the vicinity of zone 3 that, in the event of pathogen contamination, could cause contamination of zone 3 due to human or mechanical action. A warehouse for storing finished products, a cafeteria, a hallway, and a loading dock area are a few examples. Employee locker rooms are another example if they are not right next to the kitchens where the food is produced [25].

Surfaces not in contact with food that are not in the processing areas locker rooms, cafeterias, entryways, loading zones, storage places for finished goods, and work zones.

Zones 1 and 2, and to a lesser extent zone 3, should be where the majority of environmental samples are gathered. Zone 4 should be the location of very little, if any, environmental sample collection.

Incubation: We observe plates after an incubation period in an incubation room. To identify the shape and size of bacteria we do gram staining.

Verification and observation for the verification of bacteria use gram staining method.

This method helps with the categorization and distinction of microorganisms by dividing bacteria into gram positive and gram negative Bacteria. Crystal violet or methylene blue are used as the main colors in gramme staining. Gram positive organisms are those that, when viewed under a microscope still has their original color and are purple-brown in appearance. Organisms that is resistant to primary stain (Figure 3) [26].

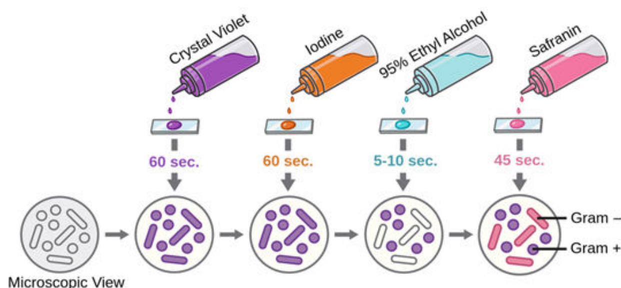

Figure 3. Labster theory.

Chemical analysis

For confirmation of microorganisms we follow chemical analysis by IMVIC Test.

One of these tests is represented by each letter in the acronym "IMVIC." In order to make it easier to pronounce, a lowercase I is placed after the letters "I" for indole, "M" for Methyl red, "V" for Voges-proskauer, and "C" for Citrate. The abbreviation IMVIC stands for four separate tests.

Indole test: The test is run in tryptophan broth, and the results are read following the addition of Kovac's reagent. After adding Kovacs reagent, the red coating at the tube's top indicates a positive outcome. Poor results are indicated by the lack of color shift at the top of the tube after the addition of the Kovacs reagent.

Methyl red test: A successful methyl red test will result in the development of red color after the addition of the methyl red reagent. The test is not positive if the color does not change after adding the methyl red reagent [27].

Voges-proskauer test: A negative VP test is indicated by the absence of color change after the addition of the Barritt's A and Barritt's B reagents. A positive Voges-proskauer test is indicated by the formation of a reddish-brown color after the addition of Barritt's A and Barritt's B reagents.

Citrate acid utilization test: The test is conducted with simmons citrate agar. A negative citrate utilization test is indicated by the absence of growth and any color changes in the test tube. A successful citrate reaction will show growth and a change in color to blue.

Observation

Observe the petri-plates after their incubation period and identify the hazardous microbes in examined samples (Table 7).

S.N.	Parameter /Microorganism	Morphology	Gram's Staining
1	*E.coli*	Green-metallic sheen colonies	Gram negative
2	Coliform	Pink pin point colonies	Gram positive
3	*Enterobacteriaceae*	Pink pin point colonies	Gram negative

Table 7. Observe the petri plates after their incubation period.

Discarding method

After an observation, we should discard the plates safely. For discarding the plates; first we wrap the petriplates in a discard poly bags then we put them in a discarding autoclave. After autoclaving we wash the plates with tap water and put them in a chromic acid solution for 24 hours. Before dipping the plates in a washtub with a 2 percent soap solution or a 0.5 percent labolene solution, rinse the plates with tap water. Apply a brush or scrubber to each plate. Each dish should be carefully cleaned with tap water to remove any soap residue. Rinse once more with distilled water.

Sterilization of glassware: Place the plates in an oven set to 60 degrees to 65 degrees celsius to dry. Once the petridishes have dried, bundle five to ten of them together and wrap them in cellophane or butter paper. Affix an indicator if necessary, and autoclave at 121°C and 15 pounds of pressure to verify the cycle time. Put labels on the containers with the use-before and sterilization dates (Tables 8-11).

Results

Results of microbial load in samples before sanitization

S.No.	Sample No.	Parameters	Ranges	Results					Observations	Conclusion
				Location 1	Location 2	Location 3	Location 4	Location 5		
1	Air Monitoring (Bakery Premixes Production Area)	Total Plate Count	NMT 100.0 cfu / m³	45	58	66	85	55	It is in Range but need to be avoided to more cuonts	Before any Product Preparation Cleaning and sanitization is required
		Yeast and molds	NMT 10 cfu / m³	8	9	10	14	8		
2		Parameters	Ranges	Location 1	Location 2	Location 3	Location 4	Location 5	Observations	Conclusion
	Air Monitoring (UHT Production Area)	Total Plate Count	NMT 100.0 cfu / m³	25	35	40	25	30	It is Air boarn organism should be avoided by cfrequent air monitoring and area sanitizations	Before any Product Preparation Cleaning and sanitization is required
		Yeast and molds	NMT 10 cfu / m³	4	7	12	5	11		
		Parameters	Ranges	Location 1	Location 2	Location 3	Location 4	Location 5	Observations	Cnclusion
3	Air Monitoring (Whipping Cream Production Area)	Total Plate Count	NMT 100.0 cfu / m³	55	35	40	20	35	It is Air boarn organism should be avoided by frequent air monitoring and area sanitization	Before any Product Preparation Cleaning and sanitization is required
		Yeast and molds	NMT 10 cfu / m³	16	10	13	5	11		

Table 8. Results of microbial load in samples before sanitization.

Results of microbial load in samples after sanitization

S.N.	Sample No.	Parameters	Ranges	Results					Observations	Conclusion
				Location 1	Location 2	Location 3	Location 4	Location 5		
1	Air Monitoring (Bakery Premixes Production Area)	Total Plate Count	NMT 100.0 cfu/m³	15	17	19	15	11	Sanitization is Required to control microorganisms	Very less amount of microbial growth is seen after sanitization
		Yeast and molds	NMT 10 cfu / m³	Absent	Absent	Absent	Absent	Absent		
2		Parameters	Ranges	Location 1	Location 2	Location 3	Location 4	Location 5	Observations	Cnclusion
	Air Monitoring (UHT Production Area)	Total Plate Count	NMT 100.0 cfu/m³	>10	>10	>10	>10	>10	Sanitization is Required to control microorganisms	Very less amount of microbial growth is seen after sanitization
		Yeast and molds	NMT 10 cfu/ m³	Absent	Absent	Absent	Absent	Absent		
3		Parameters	Ranges	Location 1	Location 2	Location 3	Location 4	Location 5	Observations	Cnclusion
	Air Monitoring (Whipping Cream Production Area)	Total Plate Count	NMT 100.0 cfu/m³	15	13	18	>10	12	Sanitization is Required to control microorganisms	Very less amount of microbial growth is seen after sanitization
		Yeast and molds	NMT 10 cfu/ m³	Absent	Absent	Absent	Absent	Absent		

Table 9. Results of microbial load in samples after sanitization.

Results of microbial load in samples before CIP (Cleaning in Place)

S.N.	Sample No.	Parameters	Ranges	Results					Observations	Conclusion
				Location 1	Location 2	Location 3	Location 4	Location 5		
1	Plant Hygiene Monitoring (Bakery Premixes Production Area)	Total Plate Count	NMT 100.0 cfu/m³	66	78	45	65	35	It is in Range but need to be avoided to more counts	Before any Product Preparation Cleaning and sanitization is require
		Enterobacter iaceae	NMT 10 cfu/ m³	<10	<10	<10	<10	<10		
2		Parameters	Ranges	Location 1	Location 2	Location 3	Location 4	Location 5	Observations	Conclusion

	Plant Hygiene Monitoring (UHT Production Area)	Total Plate Count	NMT 100.0 cfu/m³	55	35	15	25	20	It is Air born organism should be avoided by frequent air monitoring and area sanitizations	Before any Product Preparation Cleaning and sanitization is require
		Enterobacteriaceae	NMT 10 cfu/m³	<10	<10	<10	<10	<10		
3		Parameters	Ranges	Location 1	Location 2	Location 3	Location 4	Location 5	Observations	Conclusion
	Plant Hygiene Monitoring (Whipping Cream Production Area)	Total Plate Count	NMT 100.0 cfu/m³	55	35	40	20	35	It is Air born organism should be avoided by frequent air monitoring and area sanitizations	Before any Product Preparation Cleaning and sanitization is require
		Enterobacteriaceae	NMT 10 cfu/m³	<10	<10	<10	<10	<10		

Table 10. Results of microbial load in samples before CIP (Cleaning in Place).

Results of microbial load in samples After CIP (Cleaning in Place)										
S.N.	**Sample No.**	**Parameters**	**Ranges**	**Results**					**Observations**	**Conclusion**
				Location 1	**Location 2**	**Location 3**	**Location 4**	**Location 5**		
1	Plant Hygiene Monitoring (Bakery Premixes Production Area)	Total Plate Count	NMT 100.0 cfu/gm	<10	<10	<10	<10	<10	Cleaning is Required to control microorganisms	Very less amount of microbial growth is seen after CIP
		Enterobacteriaceae	NMT 10 cfu/gm	Absent	Absent	Absent	Absent	Absent		
2		**Parameters**	**Ranges**	**Location 1**	**Location 2**	**Location 3**	**Location 4**	**Location 5**	**Observations**	**Conclusion**
	Plant Hygiene Monitoring (UHT Production Area)	Total Plate Count	NMT 100.0 cfu/gm	Absent	Absent	Absent	Absent	Absent	Cleaning is Required to control microorganisms	Very less amount of microbial growth is seen after CIP
		Enterobacteriaceae	NMT 10 cfu/gm	Absent	Absent	Absent	Absent	Absent		
3		**Parameters**	**Ranges**	**Location 1**	**Location 2**	**Location 3**	**Location 4**	**Location 5**	**Observations**	**Conclusion**
	Plant Hygiene Monitoring (Whipping Cream Production Area)	Total Plate Count	NMT 100.0 cfu/gm	<10	12	10	8	Absent	Cleaning is Required to control microorganisms	Very less amount of microbial growth is seen after CIP
		Enterobacteriaceae	NMT 10 cfu/gm	Absent	Absent	Absent	Absent	Absent		

Table 11. Results of microbial load in samples after CIP (Cleaning in Place).

Discussion

Here we conclude that there is a huge microbial load before sanitization and clean in place. To provide safe and wholesome food for consumers worldwide, it is crucial to minimize the risk that food will act as a carrier for diseases. Given that the frequency of infectious diseases linked to food transmission is higher in low-income areas and that food-borne illnesses have a high rate of morbidity and mortality, they can impede socioeconomic growth. In order to help reach the sustainable development goals of 6.0 (clean water and sanitation), 2.0 (Zero hunger), 3.0 (good health and well-being), and 12.0, food safety is a crucial factor (responsible production and consumption).

Evaluation: The first step in selecting a sampling site is to map the manufacturing system and process steps (e.g. and equipment, development materials used (e.g. chrome steel, rubber, High Density Poly Ethylene (HDPE)), mapping and sampling sites. shall be located in zone 1 (product contact surface) and zone 2 (surface adjacent to the product contact surface). Verification of the indicators in these areas will provide the greatest value in terms of hygiene effectiveness. Zone sites human sampling of 1 provides additional quantifiable information, which can be used to indicate feasible loss of process controls or conditions that could lead to product infection from zone 1 sites of indicator organisms. Sampling in the usual manner can also be used to determine appropriate transit times for sketching distinctive tribes, providing clinical support over time. In addition, zone 1 and 2 sites Indicator tests provide a complementary method to monitor equipment condition and indicate the frequency of preventive protection or repair. For instance, trends closer to better numbers of indicator organisms in positive sites might also factor in the direction of the want for (extra frequent) alternative of gaskets or

other rubber and plastic elements. Incorporation of zone 3 websites into the indicator sampling plan can be treasured throughout investigations or root-motive evaluation, as these web sites are probably to have fluctuating levels of the unique goal micro organism, which may additionally result in erratic developments. Similar to choice of web sites for pathogen checking out indicator sampling sites have to be selected with the purpose of finding capacity issues rather than sites which can be clean to clean and sanitize or will constantly meet the perfect limits. For example, big, flat stainless steel surfaces are generally simpler to clean and sanitize (and therefore tend to not be the high quality sampling web sites, mainly if those are the only web sites used) at the same time as a fabric backed belt is greater difficult to clean and sanitize. The sampling plan must encompass a representative website online from each processing step in addition to websites that consist of each of the distinctive types of fabric used inside the production of gadget. Once you have chosen your location, you should also decide which sampling tool best suits your needs. If your online site has small areas of interest or hard-to-reach crevices, cotton swabs are a good choice. Sponges may be best for areas that are likely to be larger, as better mechanical motion allows for more collection. Clean to clean flat surfaces that require better sensitivity test techniques (due to expected low counts) allow media to be in direct contact with the surface. Effective environmental tracking applications are critical to measuring the overall effectiveness of microbiological controls in food processing centers and enabling organizations to be proactive.

Control

Cleaning in place: Modern food, dairy and beverage processors place top priority in the area of Clean In Place (CIP). CIP is defined as "cleaning the entire plant component or plumbing circuit with little or no operator intervention without disassembling or opening the equipment." At high turbulence and flow rates, the surface is scattered or sprayed and the liquid circulates in the system. The cleaning in place is done in following five steps:

Step 1 (Pre-rinse): A properly monitored and performed pre-rinse makes the rest of the cleaning cycle predictable and reproducible, making it a critical step in the CIP process. Moisten the surfaces of internal pipes and tanks. His 95% of the remaining residue is removed. The sugar will melt and the fat will begin to partially melt. Apply a non-chemical pressure test to the CIP flow channel.

Step 2 (Caustic wash): By softening fats, caustic washes make fat removal easier. The alkali used in caustic soda, sodium hydroxide, or caustic soda, also known as NaOH, has a very high pH in the concentration range of 0.5 perecnt to 2.0 percent. For heavily filthy surfaces, concentrations as high as 4 percent may be utilised. In the majority of CIP wash cycles, caustic is often utilised as the primary detergent. The efficiency of a pump can be improved by using a non-foaming formulation to assist reduce cavitation. The second wash can be recovered and reused, whereas the first wash is poured down the drain.

Step 3 (Intermediate rinse): Caustic wash leftover detergent is flushed away with fresh water. To guarantee proper cleaning, use the right equipment for each stage of the CIP cycle, including rinse. Tank levels in wash and rinse tanks are monitored by level transmitters and probes. To accurately manage the wash and rinse stages, flow transmitters make sure that spray devices have the best flow possible. Conductivity Chemical levels are checked by transmitters to make sure they are at the intended set point.

Step 4 (Final rinse): Also known as caustic soda, sodium hydroxide, or NaOH, the alkali used in caustic soda has a very high pH in the concentration range of 0.5 percent to 2.0 percent. Use city water, DI, or RO water to wash away any remaining detergent. The final rinse water of many systems can be collected and used as a pre-rinse for the next cleaning cycle. Subsequent pre-rinses are more efficient and cheaper due to the heat and chemicals still present from the last rinse.

Step 5 (Disinfectant rinse): A disinfectant rinse may be required before starting the next manufacturing process to kill bacteria. Various hypochlorite solutions (potassium, sodium, or calcium), commonly referred to as "hypo", have long been used as disinfectants in many CIP cycles. Chlorine is the main ingredient in disinfectant (bleach) rinses. More and more health care workers are now using Per Acetic Acid (PAA), a mixture of hydrogen peroxide and acetic acid, as an alternative to bleach based disinfectants.

Advantages of a cip system

- Errors are minimized by automating cleaning, which reduces the likelihood of human error, which could lead to a dangerous product.
- Employee safety is preserved since chemical exposure is reduced by keeping cleaning chemicals inside the system.
- Less time lost to cleaning during production means that the product is created in a longer period of time.
- Cleaning that is dependable and reproducible is a result of consistent and long-lasting product quality. Lower contamination leads to fewer product recalls and higher brand confidence.
- Repetitive cycle control reduces the quantity of water and energy used, saving on utilities.

Conclusion

Here, we conclude that we should do fumigation, sanitization and CIP on regular basis. Because when we do not follow these control measures, product has more chances of decaying and risky for human health. Also, we found that the growth of microbes is much more before doing CIP, sanitization and fumigation. But after sanitization, CIP and fumigation, product is 99% free from microbes. We suggest that before loading your food product, wait until your microbial results do not came. It is our responsibility to provide a qualitative food rather than quantitative food. Our company follows all the controlled measure to ensure their costumers that our food is good for their health, qualitative wise as well as texture wise.

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