An Internship Report

On

Microbiology and Industrial Irradiation Divion Research Activities

AT

Institute of Food and Radiation Biology
(Atomic Energy Research Establishment,Savar)

Submitted To :

Prof.Dr.Md Bellal Hossain
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Submitted By :

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LETTER OF APPROVAL

Date: 22nd December 2018

To

Prof.Dr.Md Bellal Hossain
Prof.& Head
Department of Nutrition & Food Engineering
Daffodil International University.

Subject: Submission of Internship Report.

Dear Sir,
I am here by submitting my Internship Report, which is a part of the NFE Program curriculum. It is great achievement to work under your active supervision. This report is based on Microbiology and Industrial Irradiation Research Activities at IFRB(AERE,Savar).I have got the opportunity to work in Microbiology and Industrial Irradiation Division at Institute of Food and Radiation Biology of Atomic Energy Research Establishment,Savar for thirty days, under the direct supervision of Dr.Tabassum Mumtaz, Principle Scientific Officer.

This project gave me both academic and practical exposures. First of all I have gained knowledge about the Microbiology and Irradiation uses on various aspects. Secondly, the project gave me the opportunity to develop a network with the corporate environment and top level personnels.

I shall be highly obliged if you are kind enough to receive this report and provide your valuable judgment. It would be my immense pleasure if you find this report useful and informative to have an apparent perspective on the issue.

Sincerly Yours,
Rasal Ahmed Bhuiyan
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ACKNOWLEDGEMENT

First of all, I wish to express my gratitude to the Almighty God for giving me the strength to perform my responsibilities as an intern and complete the report within the stipulated time. I am deeply indebted to my supervisor **Dr.Md.Bellal Hossain Prof. & Head, Department, of Nutrition & Food Engineering, Faculty of Allied Health Sciences,** Daffodil International University for his whole-hearted supervision during my organizational attachment period. I am very grateful to **Dr. Md. Khorshed Alam,** Director of IFRB, AERE,Savar, for giving me permission to carry out this research work at this Institute. I am also express my heartfelt appreciation to **Dr.Tabassum Mumtaz**, Principal Scientific Officer as my organizational supervisor to conduct this research very enthusiastic. It would have been very difficult to prepare this report up to this mark without their guidance.I sincerely like to thanks my Co-Supervisor **Ms.Nasima Akter Mukta, Lecturer,** Department of Nutrition and Food Engineering, Daffodil International University, for his valuable guidance, inspiration to conduct this research very successful.

I also would like to thanks **Mr.A.K.M. Sarwar Inam,Associate Professor,and Moonmoon Haque, Associate Professor** for their countless inspiration and encouragement during my student life in this department. My gratitude goes to entire NFE Department of Daffodil international University for arranging Internship Program that facilitates integration of theoretical knowledge with real life situation. I must mention the wonderful working environment and group commitment of this organization that has enabled me to deal with a lot of things.

I would like to express my warmest thanks to **Md.Reaz mahmud** ,Assistant Technical Officer,& **Md Emran Hossain,** Co-ordination Officer, Department of Nutrition & Food Engineering. I express my deep gratitude to the office/labs stuff of the Department of Nutrition & Food Engineering under faculty of Allied Health Sciences, Daffodil International University as well as MIID,IFRB(AERE,Savar).

ABSTRACT

Microbiology department is the learning of the microorganisms which inhibit, create, and contaminate food, also including the research of microorganisms causing food spoilage, pathogen that may cause disease especially if food is inappropriately cooked or stored, those used to produce fermented foods such as cheese, yogurt, bread, beer, and wine, and those with other useful roles such as produce probiotics.In this short period of time I worked on microorganism specially bacteria detection in water and home prepared foods.There are some parameters that needs to check in microbiology department like total viable bacterial count,total coliform count,E-coli identification etc.I did experiments on total viable bacteria count and coliform bacteria detection by using different bacterial media.

EXECUTIVE SUMMARY

This report is prepared on the basis of my One-month practical experience at the microbiology and Industrial Irradiation Division of Institute of Food and Radiation Biology(IFRB) of Atomic Energy Research Establishment (AERE),Savar,Dhaka. This internship program helps me to learn about the practical knowledge of microbiology department activities Institute of Food and Radiation Biology IFRB is one of the largest National Research and development (R&D) Organization which conducts Research and development activities in the field of Food Science and Technology .This Institute plays an active role in transferring technologies developed by our Scientist to the commercial entrepreneurs of our country .

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List of Abbreviation

AERE : Atomic Energy Research Establishment

MIID : Microbiology and Industrial Irradiation Department

NFE : Nutrition and Food Engineering

DIU : Daffodil International University

IRFB : Institute of Food and Radiation Biology

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CHAPTER-1

INTRODUCTION

Water is an essential part of our life.From dining to bathroom everywhere there is need of water.For drinking water needs to maintain its quality that meets with the requirements,mostly use of water in washing purpose in kitchen and other places.Before useing water make sure it does not contain any harmful pathogen like e-coli that can make you ill.I have worked on microbiology based experiments which is related to water and food.The main focus of experiments is to obtain physical,chemical and microbiological status of different samples of water and foods.Another aspect of this tests to certify the water or food as safe to consume. As sample i used pond water and tap water. The experiment consist of checking physical parameters,chemical parameters and microbiological parameters of samples.

1.1 Origin of the report

Internship Program of daffodil International University is a requirement for completion of degree for the NFE students.The main purpose of the internship is to meet the students with the job world. As an intern the main target was to match up the theoretical concepts into real life experience.

The internship program have following purposes:
- To have an idea of activities of the MIID,IFRB,AERE,SAVAR
- To obtain practical knowledge.
- To match up the practical field with the lessons learned in DIU
- To fulfill the requirement of NFE Program.

This report is the result of two months long internship program conducted in Institute of Food and Radiation biology and is prepared as a requirement for the completion of the NFE program of Daffodil International University.

1.2 **Objectives of the report**

The objective of the report can be viewed in two types:
- General Objective
- Specific Objective

General Objective:
This internship report is done primarily to complete the Bachelor of Nutrition and Food Engineering (NFE) degree requirement under the Faculty of Allied Health Science in daffodil
International University.

Specific Objective:
More specifically :
- To give an overview of Institute of Food and Radiation Biology(IFRB),AERE
- To focus on the microbiological activity of water sources
- To obtain real-time practical work experiences

CHAPTER-2

OVERVIEW OF THE INSTITUTE

2.1 History of Institute

Atomic Energy Research Establishment (AERE) is a leading research set-up of Bangladesh Atomic Energy Commission (BAEC) for peaceable application of nuclear energy in various fields of physical, biological and engineering sciences. AERE planned in 1974 and came into existence in 1975 by the acquisition of 259 acres of land at Ganakbari, Savar which is about 40 km away from Dhaka City and about 4 km north of National Martyrs' Memorial at Savar. The AERE started its journey as a development project and the development process is going on.

2.2 Objectives of the institute

Institute of Food and Radiation Biology(IFRB)

- Develop effective and environment friendly control measures of insect pests for protection and preservation of stored and field crops
- Monitor pesticide residues in food and environment for safeguarding human health
- Sterilize medical products, pharmaceuticals and food products by gamma radiation
- Process and product development for food preservation by radiation & combination treatment
- Conserve agro-wastes into food, feed and chemicals through the combination of nuclear and microbial biotechnology

CHAPTER-3

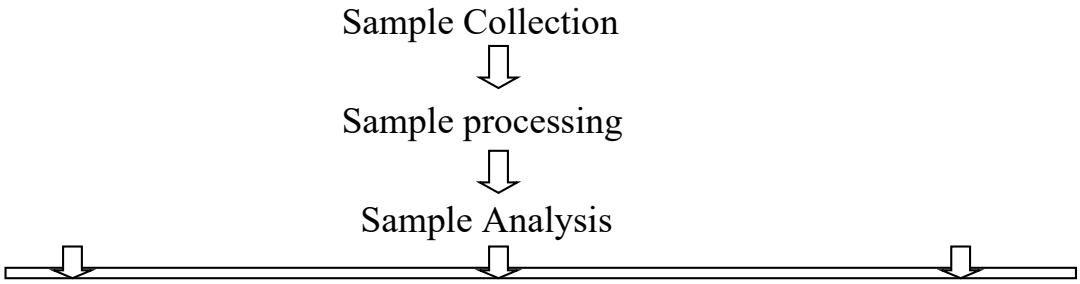
Design of the Study

3.1 Short description on the study

The experiment was done in Microbiology and Industrial Irradiation Division of Institute of Food and Radiation Biology at Atomic Energy Research Establishment(Savar,Dhaka).The study was to measure water quality according to physical,chemical and microbiogical status of the sample(pond water and tap water).

3.2 Methods to follow

Flow diagram :



Physical Parameters	Chemical Parameters	Microbiological
*Odour *Colour *pH *Conductivity	*Hardness *BOD/COD	*TVBC *TCC/FCC >Presumptive test >Confirmatory test >Completed test

3.3 Equipments used in the study

1. Digital balance
2. Digital pH meter
3. Laminar air flow
4. Incubator
5. Drying oven

6. Autoclave
7. Suction pump
8. Filtration unit
9. Colony counter
10. Microscope

3.4 Apparatus used in the study

1. Beaker
2. conical flask
3. Measuring cylinder
4. Spachula
5. Petri dish
6. Test tube
7. Durham tube
8. Forcep
9. Pipette
10. Pipette filler
11. Dropper
12. Glass slice

3.5 Bacterial media used in the study

1. Lactose Broth
2. Nutrient Agar(Plate count agar)
3. MacConkey Agar
4. Eosin Methylene Blue(EMB)

3.6 Sample Collection(Pond water)

Two Samples were collected from pond.One sample was from open area where sunlight reaches and another sample from shade area where sunlight can not

reach.Samples were collected in the sterile sampling bottle which was autoclaved.The water was collected from pond in quick time.After collection of water immediately closed the cork so that any other particle can not move into it.

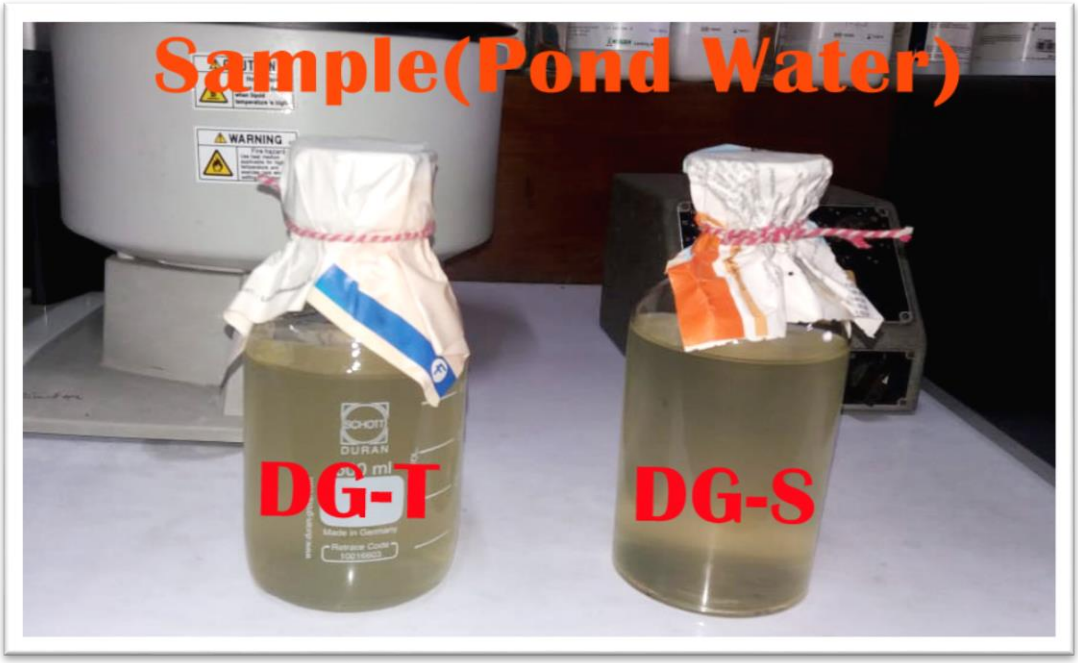


Fig 1 : Sample Collection(Pond water)

3.7 Sample Processing(Pond water)

Two sample were named as DG-S(Sunlight) and DG-T(Tree).After collection of water from pond kept it in laminar air flow for further analysis.Serial dilution was done to $10^{-1} – 10^{-5}$.

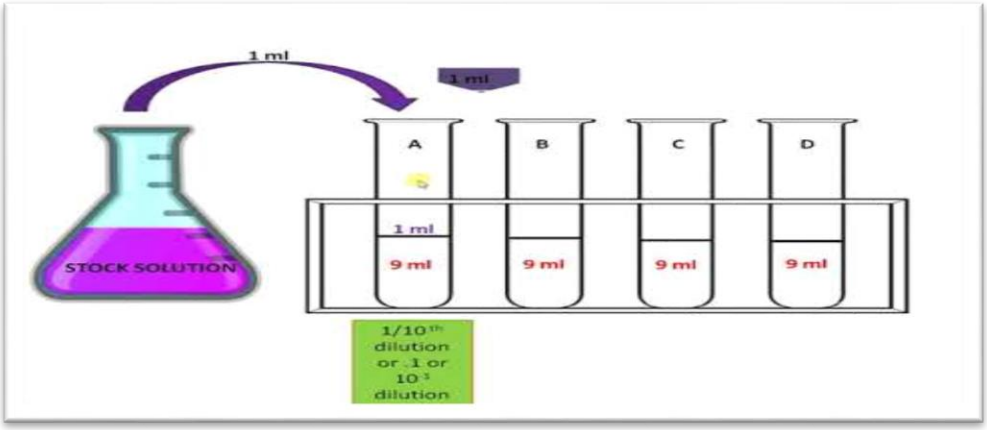


Fig 2 : Serial dilution

3.8 Sample Analysis(Pond water)

3.8.1 Physical Parameters

I. **Odour**

Odour was checked by 3 members

II. **Color**

Color was checked by 3 members

III. **pH**

pH was checked by Electronic pH meter.Placed the pH electrode in sample and shows the pH value in the display screen

IV. Conductivity

Not done

3.8.2 Chemical Parameters

I. Hardness
Not done

II. BOD/COD
Not done

3.8.3 **Microbiological Parameters**

I. Total Viable Bacterial Count

For this test Nutrient Agar media prepared and autoclaved with other requirements.After autoclaving moved them to laminar air flow.Sample diluted to 10^{-1} – 10^{-5} and D3 and D4 used in plates for test.Nutrient Agar media poured

into plates and waited for setting.Then spreaded 100ul sample into plates with the spreader.Then incubated for 24 hours at 37C.

II. Total Coliform Count

Total Coliform test consist of three stages presumptive,confirmatory and completed test

Presumptive Test :

For presumptive test lactose broth media used to identify evidence of coliform.After preparing lactose broth media placed it into test tubes with durham tube and then autoclaved.Then transferred to laminar air flow for next process.20ml Direct sample of .DG-S and DG-T added to test tube containing lactose broth and kept in incubator at 37C for 24 hours.

Confirmatory Test :

E-Coli(Gram Stain test) :

Used MacConkey Agar media to identify the possible coliform as gram positive or negative.MacConkey agar inhibits the growth of gram positive bacteria.
After autoclaving the prepared media moved to laminar air flow for inoculation. Poured media into plates and waited for set up the gel.Then added 100ul of diluted sample of 10-4 and 10-5 into plates and spreaded finely.Then kept it in incubator at 37C for 24 hours.

Gram staining :

Preparation of fixed smear

⇩

Cover with crystal violet(1 min)

⇩

Cover with Alcohol(6-7 Sec)

⇩

Cover with Safranin(1 min)

⇩

Air Dry

⇩

Microscope Reading

Completed test : Confirmation test shows no sign of Coliform then no need this test.

3.9 **Sample Collection(Tap water)**

Sample collected from household tap using sterile bottle which was autoclaved.

3.10 **Sample processing**

After collecting the sample kept in laminar air flow and done the serial dilution for 10^{-1},10^{-2},10^{-3}.

3.11 **Sample Analysis(Tap water)**

3.11.1 Physical Parameters

I. Odour

Odour was checked by 3 members

II. Color

Color was checked by 3 members

III. pH

pH was checked by Electronic pH meter.Placed the pH electrode in sample and shows the pH value in the display screen

IV. Conductivity

Not done

3.11.2 Chemical Parameters

I. Hardness

Not done

II. BOD/COD

Not done

3.11.3 **Microbiological Parameters**

I. Total Viable Bacterial Count

For this test Nutrient Agar media prepared and autoclaved with other requirements.After autoclaving moved them to laminar air flow.Sample diluted to 10^{-1} – 10^{-3} and D2 and D3 used in plates for test.Nutrient Agar media poured into plates and waited for dry.Then spreaded 100ul sample into plates with the spreader.Then incubated for 24 hours at 37C.

II. Total Coliform Count

Total Coliform test consist of three stages presumptive,confirmatory and completed test

Presumptive Test :

For presumptive test lactose broth media used to identify evidence of coliform.After preparing lactose broth media placed it into test tubes with durham tube and then autoclaved.Then transferred to laminar air flow for next process.20ml Direct sample of .tap water added to test tube containing lactose broth and kept in incubator at 37C for 24 hours.

Confirmatory test :

E-Coli test(Gram negative identify):

Used MacConkey Agar media to identify the possible coliform as gram positive or negative.MacConkey agar inhibits the growth of gram positive bacteria.

After autoclaving the prepared media moved to laminar air flow for inoculation. Poured media into plate and waited for set up the gel.Using filtration unit 100ml sample passed through membrane filter then placed the membrane into MacConkey plate. Then kept it in incubator at 37C for 24 hours.

E-Coli Test for Home made cheese

Eosin Methylene Blue Agar

Eosin Methylene Blue Agar(EMB) used to identify bacteria characteristics.It inhibits the growth of gram positive bacteria.If the sample contain gram negative bacteria only they can survive.After preparing the EMB placed it into autoclave for sterilization.Then moved to laminar air flow for next process.
Poured EMB media into plates and added 100ul of prepared cheese sample into it .Then spreaded finely with the glass spreader.Then kept it into incubator at 37C for 24 hours.

CHAPTER-4
Results and Disscussion

4.1 Physical Parameter(Pond water)

4.1.1 Odour : odour was soily and unpleasant.

4.1.2 Color : Brownish

4.1.3 pH : 6.21

4.2 Microbiological Parameters(Pond water)

4.2.1 Total viable bacterial count

Sample	Dilution 10^{-3}	Dilution 10^{-4}
DG-S	64	55
DG-T	70	43

TVBC(DG-S) = Coloni count/sample taken * Dilution size

$= 55/0.1*10^{-4}$

$= 5500000$

$= 55*10^{5}$ cfu

TVBC(DG-T) = Coloni count/sample taken*Dilution size

$= 43/0.1*10^{-4}$

$= 4300000$

$= 43*10^{5}$ cfu

4.2.2 Total Coliform Count

Presumptive test

This test was done for both the sample DG-S and DG-T.Lactose broth used as media for detecting the evidence of gas formation.
Both the sample produced gas in the tube of lactose broth.The durham tubes floated up with containing gas.This test shows that sample may contain coliform bacteria.

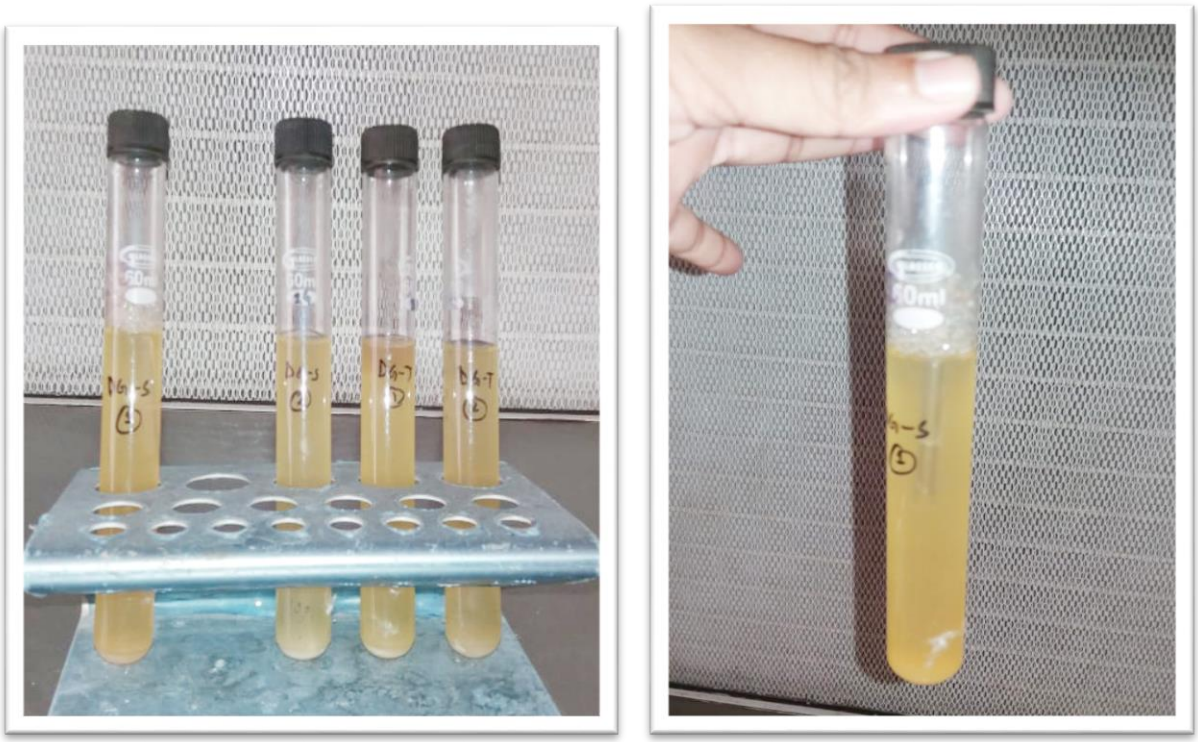


Fig 3 : Lactose broth gas formation

Confrimatory test

MacConkey Agar(Gram stain): There are some growth of bacteria on MacConkey agar media.To differentiate the bacteria we did gram staining to check it is gram positive or negative.Gram staining shows that it is rod shaped but contains violet color which means it is gram positive bacteria.So we found that this bacteria is not from coliform group(gram negative).

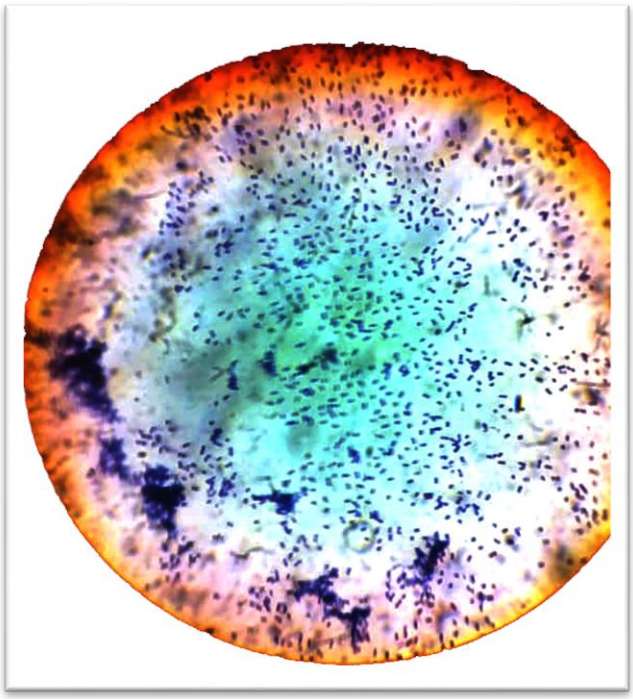


Fig 4 : Gram staining

4.3 Physical parameters (Tap water)

4.3.1 Odour : No smell

4.3.2 Color : Colorless

4.3.3 pH : 6.48

4.4 Microbiological Parameters(Tap water)

4.4.1 Total Viable Bacteria Count

Sample	Dilution 10^{-2}	Dilution 10^{-3}
Tap water	53	4
Tap water	39	5
Average	46	4

TVBC = Coloni count/sample taken * Dilution size

= $46/0.1*10^{-2}$

= 46000

= $4.6*10^{4}$ cfu

TVBC = Coloni count/sample taken * Dilution size

= $4/0.1*10^{-3}$

= 40000

= $4.0*10^{4}$ cfu

Fig 5 : Colony count(tap water)

4.4.2 Total Coli form Count

Presumptive test

Tap water sample was placed in Lactose broth media into test tubes.
Sample produced gas in the tube of lactose broth.The durham tubes floated up with containing gas.This test shows that sample may contain coliform bacteria.

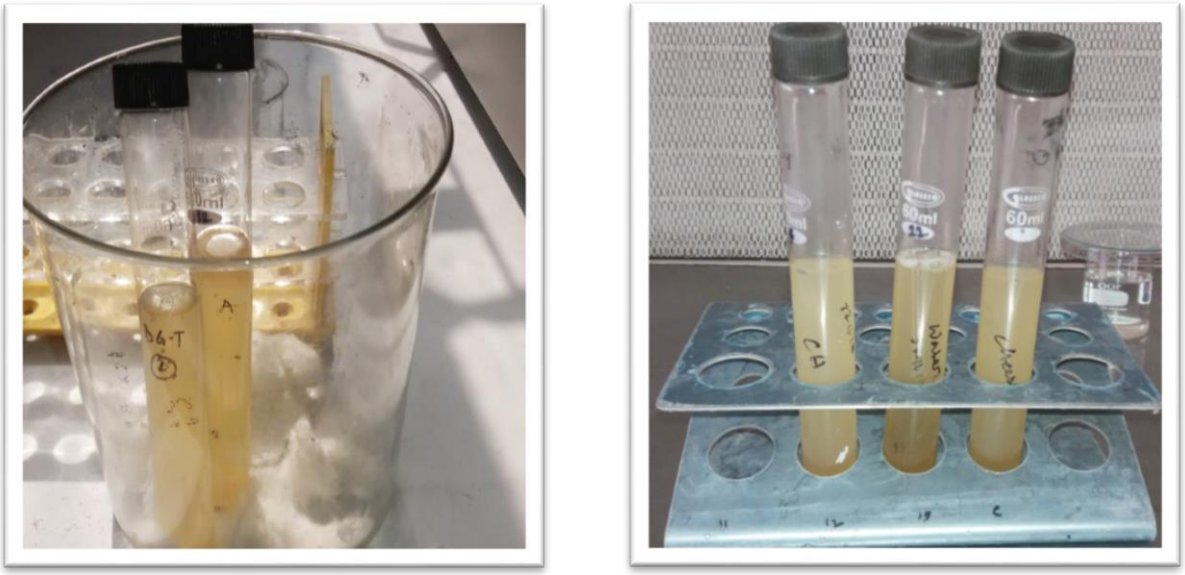


Fig 6 : Lactose broth gas formation(tap water)

Confrimatory test
MacConkey(Gram stain): There were no sign of bacteria growth on macConkey Agar media plates.This test confirmed that sample does not contain any coliform bacteria(gram negative).

Fig 7: MacConkey Agar plate (Tap water)

4.4.3 E-coli test for homemade cheese

EMB test : This test confirms the presence of coli form bacteria.It will show different characteristics to identify which group bacteria it is.Usually EMB inhibits the growth of gram positive bacteria.coli aerogenes organisms produce characteristic colonies
Escherichia : small coloniews,dark,almost black greenish,metallic sheen.
Enterobacter : Large,Pinkish mucoid colonies,dark centers,rare metallic sheen

The result shows no sign of bacteria growth on EMB media plates.That means sample does not contain E-Coli.

Fig 8 : EMB media plates(Cheese)

CHAPTER-5

INSTRUMENT DESCRIPTION

5.1 Laminar Air flow :

Laminar Air Flow provides a work area with aseptic/sterile conditions for the tissue culture. Laminar Air Flow has continuous displacement of air (it provides streamline flow of air) that passes through HEPA (High Efficiency Particulate Air) filter that removes the particulates from the air.Laminar Air Flow are equipped with a UV lamp that should be turned on about 10-20 minutes before being used to sterilize the shell or cabinet or the surface of the Laminar Air Flow to avoid any kind of contaminations. Wipe down the surface with ethanol before and after each use.Laminar Air Flow can be vertical and horizontal. In the Vertical Laminar Air Flow the air blows down from the top of the cabinet.(Acmas,2014)

5.2 Incubator :

The microbiological incubator is deployed in research and industry in a wide variety of applications with living organisms. Cell cultures and micro-organisms must be incubated in a controlled atmosphere. In the standard incubator and the cooled incubator, the temperature is controlled, and in addition in the CO2 incubator the carbon dioxide content, humidity, and in some cases the oxygen and nitrogen content are also controlled. (Atmosafe ,n.d).(Labcompar ,n.d)

5.3 Autoclave :

An autoclave is generally used to sterilize medical equipments, laboratory gadgets, medicinal items, and other things. It can sterilize solids, liquids, hollows, and instruments of various shapes and sizes. Autoclaves vary in size, shape and functionality. A very basic autoclave is similar to a pressure cooker; both use the power of steam to kill bacteria, spores and germs resistant to boiling water and powerful detergents. In microbiology autoclave use mostly for removing pathogens from media, apparatus and other required materials that used in sterile condition (Tuttnauer Team ,2015).

5.4 Microscope :

Microscopes are instruments designed to produce magnified visual or photographic images of small objects. The microscope must accomplish three tasks: produce a magnified image of the specimen, separate the details in the image, and render the details visible to the human eye or camera. (Abramowitz and R.Spring,n.d)

5.5 Colony Counter :

It is an instrument that is used for counting the colony in the petri dishe.
It consist of light,magnifying glass.

5.6 Moisture Analyzer :

It is a digital automatic moisture analyzer.Sample will placed into it and the device provide the moisture content of the sample as percentage

5.7 Drying Oven :

The function of a Drying Oven is to remove moisture from a product. Depending upon the process and production requirement, a Batch or Conveyor configuration is available. To meet both process and safety requirements, the exhaust system is engineered to accommodate specific moisture release rates. Multiple heat zone configurations can be provided to maximize drying efficiency. (Craig Application engineer,n.d).

CHAPTER-6

CONCLUSION

In this short period of study I learned a lot.I was a bit weak in microbiology and its related section.But after this study I know many things regarding microbiology.though it was short period but I tried to acquire as much as possible.In this study I learned about water analysis regarding microbiology.To declare the water as safe to drink needs to maintain some requirements.One of the important is coliform bacteria.Make sure it is not present in water.I studied to identify coliform bacteria like E-Coli and total bacterial count in water.End of the day it was enjoyable lesson

CHAPTER-7

References

Acmas(may 17,2014) Laminar Air Flow

Retrived from http://www.acmasindia.com/blog/tag/laminar-air-flow/

Tuttnauer Team (Nov 2,2015) .Autoclave sterilization process guide .
Retrieved from https://tuttnauer.com/blog/autoclave

Atmosafe (nd). Incubator

Retrived from https://www.atmosafe.net/en/glossary/incubator.html

Labcompare (n.d) Laboratory incubator.
Retrived from https://www.labcompare.com/General-Laboratory-Equipment/74-Laboratory-Incubators/

Abramowitz and R.Spring(n.d).Basic concepts of optical microscopy
Retrived from https://www.olympus-lifescience.com/en/microscope-resource/primer/anatomy/anatomy/

Craig Application engineer (n.d) drying oven
Retrived from https://www.internationalthermalsystems.com/industrial-ovens/drying-oven/