REUTILIZATION OF MEDIA BY MICROBIOLOGY LABORATORY

A RESEARCH PROJECT

Submitted by

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UNDER THE GUIDANCE

OF

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"Dissemination of education for Knowledge, Science and culture"

- Shikshanmaharshi Dr. Bapuji Salunkhe

Shri Swami Vivekanand Shikshan Sanstha's

VIVEKANAND COLLEGE, KOLHAPUR
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PG DEPARTMENT OF MICROBIOLOGY

CERTIFICATE
OF
RESEARCH PROJECT COMPLETION

This is to certify that **Ms. Rajashri Prakash Patil** studying in M.Sc. part II Microbiology at Vivekanand College, Kolhapur (Empowered Autonomous) has sincerely completed research project work entitled **"REUTILIZATION OF NUTRIENT AGAR MEDIA UTILIZED IN MICROBIOGY LABORATORICS"** during academic year 2023-24.

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Introduction

Different culture media or nutrient media are used in cultivation and identification of microorganisms in microbiology laboratories as well as Biotechnology laboratory or pathological laboratories

common nutrient media used of cultivation of microorganism like Nutrient agar, Potato Dextrose agar, etc. also used some special media are also used for study of microorganism like macConkey's agar, manitol salt agar, milk agar, saburaud's agar, Ashby's agar, starch agar etc. Nutrient agar consists of peptone, beef extract and agar-agar. It is simple formulation but provides the nutrients necessary for the replication of a large number of microorganisms that are not to demanding. Beef extract contains water-soluble substances (Carbohydrates, vitamins, nitrogen Compounds and salts) Nutrient agar is used for cultivation of microbes Supporting growth of a wide range of non-fastidious organism Nutrient agar is popular because it can grow a variety of types of bacteria and fungi and contains many nutrients needed for the Bacterial growth. Potato dextrose agar is a versatile bacteria and fungi growing medium for yeast and molds.

MacConkey’s agar is used for a broad range of fungi but there are other agars that are more selective for specific types of fungi. These agar includes but are limited to malt agar and Sabouraud agar this agar are commonly used in the dairy industry for detecting the presence of yeasts and moulds in product Samples. For this purpose, the addition of tartaric acid is recommended. PDA Supplemented with chlortetracycline is recommended. for the microbial enumeration of yeast and mold from cosmetics.

MaeConkey's agar is a selective and differential culture medium for bacteria It is designed to selectively isolate found and enteric in the intestinal tract) bacteria and differentiate them based on lactose fermentation lactose fermenters turn red or pink on macconkey agar, and no fermenters do not change colour. The media inhibits growth of Gram positive organisms with crystal violet and bile salts allowing and isolation of bacteria. The media detects lactose fermentation by interior bacteria with the PH indicator neutral red; suspend 52gm of macconkey agar powder in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°c for 30 minutes, store the dehydrated Macconkey agar powder at 10-30°C and use before the expiry date on the label. Mannitol Salt agar is used as a selective media for the isolation of pathogenic *Staplococci*. It is recommended for the detection and emameration of coagulase positive *Staphylococci* in milk, food and other specimens. It is used to determine if the bacteria is halophilic (Salt loving) and if the bacteria can ferment mannitol. If the bacteria is able to grow then it is a halophilic bacteria, due to it's ability to grow in a high salt environments. The mannitol salt agar are preparing is by using Suspended 111 gm. of mannitol salt Agar in 100 ml of distilled water. Boil to: dissolve the medium completely. Sterilize by autoclaving at 121°c for 30 min. milk agar is also known as milk plate Count agar is a nutrient medium used for the enumeration of bacteria in milk and dairy products The milk agar contain Casein and glucose as carbon sources to promote

growth. A yeast extract is added to the medium as a source of vitamins. T microorganisms such as *Pseudomonas aeruginose* hydrolyse the casein. to form soluble nitrogen Compounds that form a clear zone surrounding. Colonies the milk agar preparing is so easy Suspend 51.5gm of in 1000 ml distilled water. Heat to boiling to dissolve the medium completely Sterilize by auto calving at 121°c for 30 min. Sabouraud dextrose agar is a non-Selective isolation medium used for the growth and maintenance of pathogenic and non-pathogenic fungi from clinical and non-clinical specimens. It is also used for recovery and total counting of yeasts and t moulds in environmental monitoring. This agar is naturally acidic which inhibits the growth of many bacteria. It is used to control the Sterility of pharmaceutical and cosmetic products. Composition of this agar is (glucose) and pH. Suspend peptone, dextrose 65 gms of Sabouraud dextrose agar in 1000 ml of distilled water. Heat to boiling dissolve the medium to completely. Sterilize by autoclaving at." Is lbs. pressure:

Ashby's mannitol agar is used for cultivation of *Azotobacter species* that can use mannitol and atmospheric nitrogen as source of carbon and nitrogen respectively Ashby media are formulated as described by subba Rao, 1977 It is used for isolation Azotobactor a non-symbiotic nitrogen fixing bacteria which user mannitol as a carbon source and atmospheric nitrogen as nitrogen sourse suspend 40.7 gms in 1000ml of distilled water heat to boiling to dissolve the medium completely.

Starch agar consists of heat stable digestive Products of protein, as would be found in nutrient agar these provide minerals, and wide variety In ever provide amino acids the acids, nutrients used by a of bacteria for growth able Starch agar is a differential medium that tests the ability of an organism to produce, certain exoenzymes, including a amylase and oligo -1, 6-glucosidase, that hydrolyze starch In industries like food. Industry, dairy industry, pharmaceutical industry etc. Various media used for identification of microorganisms in quality control laboratories, for example, Baird Parker agar etc.

Baird parker agar is a type of agar used for the selective isolation of gram-positive Staphylococci species. It contain lithium chloride and telturite to inhibit the growth of alternative microbial 'flora, while the included puruvate and glycine, promote the growth of Staphylococci.

Soybean casein digest agar is a general purpose medium for isolation and cultivation of a wide variety of fastidious and non-fastidious microorganism. Soybean casein digest agar is used for total aerobic microbial count and antimicrobial preservative effective test, it is also used for testing bacterial contaminants is cosmetics and for a multitude or purpose including maintainace of stock culture plate count phage typing colicin typing and as a base for media containing blood. These medium is recommended for sterility testing of mold and lower bacteria. It is also recommended as sterility testing medium and for sensitivity testing by the tube dilution method for antimicrobial agent. soybean casein digest agar contain pancreatic digest of casein and papaic digest of soybean which provides amino acids long chain peptides and essential nutrients required for the growth of microorganisms. Sodium chloride maintains the osmotic balance.

Violet red bile agar is modification of MacConkeys original formulation is used for the enumeration of Coli aerogenes bacterial group. It realize on the use of the selective inhibitory components crystal violet and bile salt and the indicator system lactose and neutral red. Thus the growth of many unwanted organisms is suppressed while tentative identification of bacteria can be made. Organism which rapidly attack lactose produced purple colonies surrounded by purple halos. This medium is also used for the determination of coli aerogens contain of water, milk, and other dairy products.

Cetrimide agar base is a culture medium used to selectively isolate and identify *Pseudomonas aeruginosa*. Cetrimide is a quaternary ammonium that inhibits a large number of bacteria, including those of the genus Pseudomonas, other than *Pseudomonas aeruginosa*. Cetrimide Agar is used to isolate, and detect pigment production by *Pseudomonas aeruginosa. Pseudomonas aeruginosa* produces a variety of water-soluble pigments. Pigment production is determined by visual examination of the plates for coloration of the colonies.

Along with this different agar media broth are also used for cultivation or identification of microorganisms for e.g. Nutrient broth, peptone nitrate broth, glucose phosphate broth, decarboxylation test broth

Nutrient broth is a general purpose medium used for the cultivation of a wide variety of fastidious and non-fastidious microorganisms with non-existent nutritional requirements. Peptone and yeast extract provide nitrogen compounds, vitamin B complex, amino acids and other nutrients essential for growth. Sodium chloride is used to maintain osmotic balance and glucose is the source of fermentable carbohydrates in the medium

The nutrient broth is the same formulation as the nutrient agar, only the agar has been omitted. Nutrient broth is used as a pre-enrichment medium when testing certain foods and dairy products for *Salmonella* spp. In dried or processed foods, *Salmonella* can be sub-lethal and in low numbers. The presence of other bacteria and components of food samples can interfere with the growth and recovery of *Salmonella* spp. Pre-enrichment in a non-selective medium such as nutrient broth helps repair cell damage, dilutes toxic or inhibitory substances, and provides a nutritional advantage to *Salmonella* over other bacteria. Nutrient broth is included in many standard method procedures for testing foods, dairy products and other materials. Glucose broth is used for cultivation and fermentation studies of microorganisms.

Glucose broth was developed to exclude the ingredients like beef extract that would contain small amount of carbohydrates.

All these media contain different components like wide Peptone, meat extract, yeast extract, agar powder, vitamins, different sugars like, Glucose, sucrose, lactose, maltose, fructose, xylose, manitol etc.

These components are costly in nature.

In college laboratories, industries & pathological laboratories huge expenses are done on these media after usage, these media are discarded as a waste.

After one use, media are thrown so their disport is one problem in front of lab, because throwing as it is in environment can create pollution problems, due to their nutritious nature.

These media are used in huge quantity in college laboratories. Due to their costly ingredients the utilization of these media is expensive for them.

Hence a study was undertaken to reuse these media. The study indicates that the nutrient agar medium can be used for nearly 14 times after Sterilization.

Review of Literature

Louis Pasteur was the first person to use culture medium for cultivating bacteria in the laboratory in year 1860.

This medium consisted of yeast ash, sugar & ammonium Salts,

In 1881, W. Hesse used his wife's agar as a solidifying agent for bacterial growth.

Agar was discovered in 1658 by minora Tarazaemon in Japan.

In 1882, koch was the first to use agar in microbiology - Agar is phylocolloid, water soluble polysaccharide, extracted from a group of red-purple marine algae including Gelidium & Gracilaria.

Agar production in the United States was started just before the beginning of World War II as a strategic material.

In 1940's, bacteriological-grade agar manufactured by the American Agar company. of San Diego, California, served as reference agar for the evaluation of the characteristics OR other culture media components, such as peptones.

After this major development industry of culture media got a stand-up & microbiology become best emerging field of biology. Cultivation of bacteria is easy in laboratory

Agar is classically used in Final concentration of 1-28 for hardening of culture media. Lesser amounts (0.0570 0.5%) are added to media to study motility (0.5% W/V) & for anaerobes growth (0.1%) & microphiles.

There are various types of agar media that are used in microbiological culture development. for e.g. Nutrient agar, Mac-monkeys agar, Blood agar,

1905: Alfred macconkey, based at the listed institute of preventative medicine in elstree, used bile salts to select for lactose fermenting bacteria in faucal samples

the level of conjugation in the bille salts determines its selectivity profile: conjugated bile salts are less inhibitory and allow the growth of staphylococci and enterococci; while more disassociated salts such as desoxycholate are much more selective, only allowing growth of enterobacteriacae.

1912: churchman showed that derivatives of triphenylmethane such as gentian violet and brilliant green dyes were inhibitory to bacteria, particularly gram positives; and crystal violet causes some inhibition of fungi

1923: Muller described a medium using iodine and sodium thiosulphate which react together to form tetrathionate. The selectivity of tetrathionate depends on whether or not an organism possesses the enzyme tetrathionase. Salmonellae and proteus species possess the enzyme, so can grow in the presence of tetrathionate

By the 1930s, bacteriologists understood more fully the importance of growth factors upon bacterial nutrition and by the 1950s the mechanisms of coenzymes and biochemical pathways became better understood

Another important development was the addition of antibiotics to media. In the 1960s that antibiotics were used in culture media, for the first time, as selective agents for example, Thayer-martin's publication in 1964 detailing a formulation for the isolation of Neisseria gonorrhoeae and n. meningitides, using a mixture of vancomycin, colistin and trimethoprim, was one of the most widely documented early examples of antibiotics being used in a selective medium. in parallel with the development of selective media, diagnostic media was produced in the early 20 (th) century. The main driver for this was the diagnostic importance of hemolytic reactions. In 1919 James brown used a blood agar to study the hemolysis reactions of the genus streptococcus and from this was able to differentiate alpha, beta and gamma hemolysis, an important step. For differentiating different species of staphylococcus, where the correct identification is important for medical

The discovery of new microorganisms presents new challenges and this requires new isolation methods. for example, in 1977 joseph mcdade and Charles c. Shepard identified *legione pneumophilia* as the pathogen which caused legionnaires disease which required the development of new media both for clinical and water testing applications.

Other notable developments include a special medium for recovering bacteria from water systems (reasoned and geldrich's R2A agar). in addition, in 1983 barry marshall demonstrated that isolates from gastric and duodenal ulcers all contained a campylobacter-like organism later called helicobacter pylori more recently, in 2002 the first vancomycin resistant *staphylococcus aureus* were found in michigan and Pennsylvania, in the USA, which led to the development of differential media. With another more recently identified bacterium a special chromogenic media was developed to specifically detect the emerging pathogen enterobacter sakazakii from infant formula milk.

Specialist media like chromogenic media represents another relatively recent development. Chromogens are molecules designed to mimic metabolic substrates which are colourless until they are cleaved by the target enzyme. Once cleaved the molecule becomes both insoluble and coloured, so builds up within the cell. This means that colonies of an organism which possess the enzyme can be easily differentiated from those that do not. by designing a selective base medium and adding chromogenic substrates media can be designed that allow differentiation and identification of groups of organisms. a large number of chromogenic media are now available for organisms as wide ranging as e. coli and coliforms, salmonella listeria monocytogenes, urinary tract pathogens, *clostridium perfringens*, and *candida species.*

Media manufacture in the twenty-first century displays continuities and similarities with the pioneering manufacturers of the past. The main 'active' ingredient of culture media remains pertones. Today peptones are still largely manufactured from meat (such as liver, heart and veal); but also from milk and vegetable sources (of which pea sources are the most common). Pertones are hydrolyzed using either acid or enzymes. In producing pertones the quality needs to be tightly controlled with limits on pertone manufacture and quality control specifications for parameters such as the residual moisture, ash, amino and total nitrogen, phosphates, salt, ph., metal ion content, as well as microbiological tests.

2.1 What is culture medium?

The media is a source of nutrients to support the growth of organisms, the media help in the growth, counting of microbial cells, and of microorganism. The Culture medium can be liquid or gel.

2-2 Common ingredients of culture media-

1)Peptone

It acts as and nitrogen. Source of carbon

2) Beet extract -

It acts as source of amino acids, vitamins, minerals

3) Agar-Agar -

It is used as solidifying agent.

4) Distilled water-

It is also used.

2.3. How to prepare culture medium-

1) Weigh the exact amount of each ingredient on weighing machine.

2) Dissolve these ingredient's in distilled water.

3) Adjust pH of the medium if needed.

4) Add agar powder in 1%. Quantity.

5) Autoclaved the media at 121°C for 30min.

6) After sterilization, the media were poured in sterile petri plates

2.4. The Culture media are classified by different ways -

1) Based on the physical state-

1. liquid media

2. Solid media

3. semisolid media

2) Based on the presence or absence of Oxygen-

1. anaerobic media

2. aerobic media

3) Based on nutritional factor-

1. Simple media

2. Synthetic media

3. Complex media

4. special media

1) Based on the physical State-

i) liquid media

These media show the growth Of a large number of bacteria. It is called broth. That allows bacteria to grow uniformly with turbidity.

Example of liquid media- "Nutrient agar, Tryptic Soy broth, phenol red carbohydrate broth.

ii) Solid media

It is used for the isolation Of bacteria Solid media as a pure culture. allow the growth of bacteria in the form of Colonics.

Example of Solid media- Nutrient agar, MacMonkey's agar, Blood agar, starch agar.

iii) Semi Solid media –

These media show the motility of bacteria. These media are useful for microaerophilic bacteria. These media have agar Concentration of 0.5% or less.

It has jelly consistency.

Example of semi-solid media-

Hugh and Leitson's Oxidation fermentation medium, mannital motility media.

2)Based on the presence / absence of oxygen

Microorganism have different requirements. for growth depending on oxygen requirements Based on the presence or absence of Oxygen there are 2 types of media-

Aerobic media -

In these media, it is easy to Cultivate microbes, on solid media. The growth occurs by keeping the culture in the incubators.

Example of Aerobic media- medium. Nutrient agar, cooked meat

Anaerobic media -

This media is for anaerobic bacteria which require low oxygen level.

Example of Anaerobic media- Robertson cooked meat isolation for clostridium species.

3) Based on Nutritional factor -

1) Simple media-

It's a general purpose media that supports growth of non- the growth fastidious microbes, and it is primarily used for the isolation of microorganism. Example of simple media-

nutrients broth, peptone water, nutrient agar.

2) synthetic media -

A synthetic medium is a defined medium. A defined medium is a medium in which there is no yeast, plant or animal tissue present, and all the chemicals employed are known. these wase made from only the was pure ingredient's Example of Synthetic media- Beet extract and peptone in nutrient broth.

3) complex media-

Complex media contain additional Components for bringing out specific qualities or providing the unique nutrients needed for the bacterium growth.

Example of Complex media-

MacConkey's agar media, chocolate agar media.

4) special media-

special media are of seven types

i) Enriched agar media-

The media, which comprises all the nutrients needed for the of different kinds of microorganism is enriched media

Example of Enriched agar media-

1) Blood agar

2) Chocolate agar

3) Macconkey agar

ii) selective media-

This media shows the growth of selective, microbes or desired microorganisms and invited the growth of unwanted microbes. This inhibition occurs by adding bile salts antibiotics, dues, PH adjustment media is agar based.

Example – Mannitol agar - selective for Staphylococcus- aurous.

iii) Differential media -

This media shows visible changes to the presence of an indicator. It differentiates bacteria based on colony colour growing on the same plate; biochemical characteristics show organisms growth with chemical indicator like neutral red, phenol red, methylene blue.

Example - Mannitol salt agar, Blood agar.

iv) Enrichment media –

It is a liquid medium, which also permits the at a low growth of desired bacteria density. The media provides an environment and additions as selective media and inhibits unwanted bacteria from growing. It is for the isolation. of the soil and fecal microorganisms-

Example - *Salmonella Typhi* - Selenite F broth used For Isolation of organisms.

v) Transport media-

The media transport Specimen after collection to control the overgo Cultivation of organisms. for the with media act as temporary Storage It is also maintains the viability of pathogens in the specimen and prevents.

Example- Them from drying. Pikes medium transport helps to Streptococci from throat.

vi) Indicator media-

When bacteria multiply in these media containing an indicator, they tend to change their colour. Maccokey's media are is also an example of an indicator media.

vii) Sugar media-

It contain 1% sugar, which can be any fermentable substance like glucose mannitol, sucrose and lactose. The generation of acid following the fermentation of sugar transforms the medium into pink due to the presence of an indicator Also, to show that gas is produced, Durham's tube is kept inverted inside the sugar tube and gas bubbles are observed.

Types of special purpose culture media- <u>3 types</u>

1) Assay media

The media assay vitamins, amino acids and antibiotics.

Examples-

Antibiotic sensitivity test the media used is muller- Hinton agar has 1.7% agar for better diffusion of antibiotics. It also contain Starch, which absorbes toxins released by bacteria. In this media plates zone of inhibition is seen around antibiotics

2) Minimal media-

Minimal media is a defined medium with different compositions depending on microorganisms cultured. It contains a Carbon Source like sugar Isuccinate and inorganic salt like magnesium, nitrogen, sulfon phosphorus. Carbon is a source of energy magnesium and ammonium Salts are the source of ions for metabolism Stimulation phosphate is a buffering agents

This media uses the selection of recombinants for the growth of wild type microorganisms.

3) Fermentation media-

This media for the optimum microorganisms Fermentation media produced high yields of the products media provides energy and nutrients for growth, Substrate and medium gives the for the synthesis of in the fermentations product Fermentation media contain major and minor components-

Major components- Carbon & nitrogen for energy.

Minor components-

This contains inorganic Salts, growth factors, Vitamins, buffer, antifoaming agents, dissolved oxygen, gases, growth inhibitors, enzymes, and nutrient in fermentation media depend on the organism and type of fermentation process.

Application of culture media-

1) To identity the cause of infections.
2) To identify Characteristics of microorganism.
3) To isolate pure culture.
4) To store the culture stock.
5) To observe biochemical reactions
6) To test microbial contamination in any sample.
7) To check antimicrobial agents and preservatives effect.
8) To observe microbes Colony types, it colour shape, cause.
9) To differentiate between different Colonies.
10) To create antigens for laboratory use.
11) To estimate viable count.
12) To test antibiotic sensitivity.

3.0 Material and Method-

3.1. Reuse of nutrient agar media- 1st cycle-

3.1.1 - Collection of used various agar media -

1. Different types of agar media was discarded after using in laboratory.

2. These agar media were collected and plates were counted.

Different methods used in recycling were-

a) Use of UV light.

b) Filtration.

3.1.2 Use of UV light-

All used media plates were exposed to UV rays for 10 minutes. Then agar was cut into pieces and Sterilized by auto calving

3.1.3. Filtration-

All used agar media was cut into pieces and sterilized by autoclaving. Then the molten medium was filtered through muslin cloth and again media Was Sterilized by autoclaving

3.1.4. - Growth removal and sterilization-

3.1.4.1 - Removal of growth -

1. After collecting used agar plates, plates was opened and growth of organisms was removed using nicrome wire loop or spatula.

2. These plates was thoroughly washed with water to remove maximum growth.

3.1.4.2 -Cutting agar media -

1. After removing growth of organism agar was cut in small pieces.

2. The empty flask was weighed down.

3. All pieces of agar were placed into the flasks was noted down.

4. The weight of agar was calculated as = B-A.

3.1.4.3. - Sterilization of agar media -

1. The flask containing agar pieces was sterilized at 121 c for 30 min.

3.1.4.4. - Poring of agar media -

1. After solidification of agar plates, the fresh culture of bacteria was streaked on agar plates by using nicrom wire loop under aseptic condition.

3.1.4.5. - Solidification of agar plates

The media plates were were allowed to temperature.

3.1.4.6. - checking efficiency of reduced agar media-

After solidification of agar plates, the fresh culture of bacteria was streaked on agar plates, by using nichrom wire loop under aseptic condition. Different bacterial cultures were used in the experiment like *E:col Salmonella Klebsilla, Staph. aureous, P. mirabilis,*

3.1.4.7. - Incubation and observation of plates-

After completion of streaking the plates were incubated at 37°C for 24 hours. After incubation, plates were observed for growth of respective organism.

3.2 - Reutilization of agar media used in 1st cycle – 2nd cycle –

After incubation, the plates showing growth of bacteria were counted and again the growth of bacteria was scrapped and same procedure 3.1.2 to 3.1.8 was repeated.

3.3 - Reutilization of agar media used in 3nd cycle.

Same procedure was used further.

In this way procedure was repeated till the plates showing totally no growth of organism.

3.4 Reuse of liquid nutrient media 1st cycle -

1. Collection of used nutrient broth -

100 ml nutrient broth used during practical was collected.

2. The broth was centrifused at 5000 rpm for 20 mins to remove bacterial growth.

3. The pellet was discarded and supernatant was used further.

4. The supernatant collected and measuring cylinder and collected in flask.

5. It was then sterilized at 121°c for 20 minutes.

6. After sterilization a loopful was streaked on sterile nutrient agar plates, plates we was incubated after incubation plates ware observed.

3.5. - Reuse of PDA medium -

Used PDA plates from, laboratory were collected and recycled in the same way as mentioned in above procedure.

3.6 - Reuse of MA medium -

Used MA Were plates from laboratory collected and recycled in the same way as mentioned in above procedure.

3.7. - Reuse Of KB medium -

The KB agar plates used during practicals were collected and growth was scrapped and agar was cut into pieces and agar was Weighted and sterilized by autoclaving.

4.0 Results and Discussion -

4.1. Use of UV light -

The plates after UV exposure and sterilization showed turbidity hence this method was not continued.

4.2 Filtration -

The plates which were proceeded by filtration method, showed turbidity and there was difficulty in observation of growth. Similarly method was tedious hence this method was not continued in the experiment.

4.3 . Growth removal and Sterilization -

The plates were proceeded by growth removal and sterilization method all plates of first recycle agar media Showied good growth after 24 hours of incubation. Good result upto 14 recycled. In these method agar media are easily recycled.

The results show that no addition of any components the agar media was recycled and good results are observed.

In these method used different cultures like *S. aureous salmonella proteous mirabilis Pseudomonas* and *E.coli.* All showed heavy growth the nutrient agar after 24 hrs incubation in the 1st recycling procedure. This indicates that all nutrients are not used by bacteria as well as resteriliza-tion of nutrient agar doesn't affect nutrient value of media. more the amount of medium was showed remained same. There wasn't any loss of medium as the Same number of plates were got in 1^{st} recycles.

The same results as above were Obtained for all the organism upto 9 recycles. After 9^{th} recycling i.e. form 10^{th} recycle, growth of all orgarlism was seen to decrease.

The amount of nutrient agar was Seen decreased at 11th recycle. At this point, the plates number was seen to decreases number. by only one as the original

In the 1^{st} recycle we used 6 nutrient agar plates. After removing growth of organisms, the agar was washed, cut, collected in out the flask and weighted down. The agar medium, left in each recycle is represented in table -1.

Table 1 – Nutrient agar recycles -

Sr. no	Cycle no	No of plate recycled	Weight of agar	No of plate formed	Turbidity	Growth
1.	1	6	132.2 gm	6	NO	Growth
2.	2	6	130.40gm	6	NO	More
3.	3	6	128 gm	6	NO	More
4.	4	6	126.3 gm	6	NO	More
5.	5	6	123 gm	6	NO	More
6.	6	6	120.42 gm	6	NO	More
7.	7	6	115.5 gm	6	NO	More
8.	8	5	111.14 gm	5	NO	
9.	9	5	109 gm	5	NO	
10.	10	5	103.31 gm	5	NO	
11.	11	5	98.22 gm	5	NO	
12.	12	5	97.21 gm	5	NO	
13.	13	5	95.54 gm	5	NO	Less
14.	14	5	92.22 gm	5	NO	Less

4.4. Recycling Of NB -

In these method *S. aureous* culture are used they showed bacterial growth at the Nutrient broth after 24hrs incubation in the 1st recycle. The broth was centrifuged upto 9 recycled the bacterial growth Nutrient broth in are 9 heavy in recycled. This indicates that all nutrients are not used by the organism as well as recentrifugatinon of Nutrient broth doesn't affeet nutrient Value of media. The amount of NB as the of same.

In the 1 recycled we used 50ml Nutrient broth. Broth was centrifuged centrifugation, process remove bacterial growth.

The pellet was discarded and supernatant was used after a recycled. NB was left in each recycle is represent in table 2.

Table – 2.

Sr.no	Cycle no	No of tube recycled	Weight of Nutrient	No of plate formed	Turbidity
1.	1	4	50 ml	4	NO
2.	2	4	50 ml	4	NO
3.	3	4	49 ml	4	NO
4.	4	4	48 ml	4	NO
5.	5	4	46.33 ml	4	NO
6.	6	4	45 ml	4	NO
7.	7	4	44 ml	4	NO
8.	8	4	42 ml	4	NO
9.	9	4	40 ml	4	NO

4.5. Recycling of PDA-

In these method *candida albicans* culture are used. They showed heavy growth of the potato dextrose agar after 48 hours in the 1^{s} recycling procedure. This indicates that all as nutrients are not used by the organism as well resterilization of PDA doesn't affect nutrient Value of media. The amount of medium was showed remained same. There wasn't any loss of medium as the same number of plates were got in 1st recycle. The same results as above were obtained for organism upto 4 recycles.

In the I recycle we used 4 PDA plates. After removing growth of organisms, the PDA was washed, cut, collected the flask and weighted down. The PDA medium, left in each recycle is represented in table 3.

Table 3 –Potato dextrose agar recycles.

Sr. no	Cycle no	No of plate recycled	Weight of PDA	No of plate formed	Turbidity	Growth
1.	1	4	86.23 gm	4	NO	More
2.	2	4	85 gm	4	NO	More
3.	3	4	83.80 gm	4	NO	More
4.	4	4	82 gm	4	NO	More

4.6. Recycling of MCA -

In these methods *E.coli* Culture are used. They showed heavy growth at the macConkey's agar after 24 hrs. incubation in the 1st recycling procedure. This indicates that all nutrients are not used by the organism, as well as resterilization of PDA doesn't affect the nutrient value of media. The amount of medium was showed remained same, there wasn't any loss of medium as the same number of plates were got in ist recycle. The same results as above were obtained for organisms upto 3 recycles.

In the recycle after removing We growth MCA was washed; cut used 2 MCA plates, of organises, the Collected the flask, and weighted down. The MCA medium left in each recycle is represented in table 4.

Table 4 - MacConkey's agar recycled. –

Sr. no	Cycle no	No of plate recycled	Weight of MCA	No of plate formed	Turbidity	Growth
1.	1	2	43.22 gm	2	NO	More
2.	2	2	41.20 gm	2	NO	More
3.	3	2	40 gm	2	NO	Less
4.	4	2	38 gm	2	NO	Less

4.7. Recycling of KB agar -

KB agar when resterilized, it was found difficult to solidify. Hence agar powder was added (1%) and resterilized. But no solidification was observed further exptiment was continued by adding of 2% agar powder However, after this Procedure also no solidification was seen thus it can be concluded that KB agar can not be recycled.

Table – 5. - B.Sc. – I -

Sr.no	Name of practical	Nutrient medium used	No. of students	Agar medium used (ml)	Agar Agar
1.	Preparation of cuture media	Nutrient Agar	60	60×20 =1200 ml	12 gm
2.	Detection of Amylase activity	Nutrient Agar	60	60×20 =1200 ml	12 gm
3.	Detection of caseinase activity	Nutrient Agar	60	60×20 =1200 ml	12 gm
4.	catalase activity	Nutrient Agar, Slant	60	60×5 = 300ml	3 gm
5.	Standard plate count method (SPC) of milk.	Nutrient Agar	60	60×100=6000 ml	60 gm
6.	Isolation, colony characters, Gram staining, motility of Staph, aureous.	Nutrient Agar	60	60×20=1200 ml	12 gm
7.	Isolation, colony characters. Gram staining, motility of *Bacillus species*	Nutrient Agar	60	60×20 =1200 ml	12 gm
8.	Isolation, colony characters, Gram staining motility of *E-coli.*	Nutrient Conkey agar	60	60×20 =1200 ml	12 gm

Table – 6. - B.Sc – II -

Sr.no	Name of practical	Nutrient medium used	No. of students	Agar medium used (ml)	Agar Agar
1.	Isolation of *salmonell species*	MCA	50	50×20 =1000 ml	10 gm
2.	Isolation *of proteous species*	MCA	50	50×20 =1000 ml	10 gm
3.	UV survival carve	NA	50	50×100 =5000 ml	50 gm
4.	Isolation of *s.aureous*	NA	50	50×20 =1000 ml	10 gm

Table – 7. - B.Sc. – III

Sr.no	Name of practical	Nutrient medium used	No. of students	Agar medium used (ml)	Agar Agar
1.	To isolate phosphate Solubilizing 'bacteria from soil-	KB agar	54	54×20 =1080 ml	11 gm
2.	To isolate *xanthomonas citri* from diseased plant materia like infected lemon.	PDA	54	54×20 =1080 ml	11 gm
3.	Isolation and identification Of the same by morphological, Cultural and biochemical Characteristics - *Pseudomonas aeruginosa*.	NA	54	54×20 =1080 ml	11 gm
4.	Isolation and identification of *klebsilla pneumoniae*	MCA	54	54×20 =1080 ml	11 gm
5.	Isolation and identification Of *candida albicans*	PDA	54	54×20 =1080 ml	11 gm
6.	6. Determination of MIC of Streptomycin against *E-coli* by broth methed.	NB	54	54×20 =1080 ml	11 gm
7.	Determination of sensitivity of common pathogens. to antibiotics by paper disc method.	NA	54	54×20 =1080 ml	11 gm
8.	Bio-assay of *penicillin*	NA	54	54×40 =2160 ml	21.5 gm
9.	Spc. of tomato sauce	NA	54	54×100 =5400 ml	54 gm
10.	Effect of UV Light on bacteria and graphical presentation of results	NA	54	54×100 =5400 ml	54 gm

Table – 8. - M.Sc. – I

Sr.no	Name of practical	Nutrient medium used	No. of students	Agar medium used (ml)	Agar Agar
1.	Isolation and morphological study of *Aspergillum, Penicillium, Rhizopus.*	PDA	40	40×20 =800 ml	8 gm
2.	Isolation and morphological Studies of *Actinomycetes* by coverslips techniques.	PDA	40	40×20 =800 ml	8 gm
3.	Isolation and characterization of thermophile bacteria.	NA	40	40×20 =800 ml	8 gm
4.	Isolation and characterization of Acidophilic bacteria.	NA	40	40×20 =800 ml	8 gm
5.	Isolation and characterization of Halophile bacteria and halatolerant bacteria	NA	40	40×20 =800 ml	8 gm

Table – 9. - M.Sc. – II

Sr.no	Name of practical	Nutrient medium used	No. of students	Agar medium used (ml)	Agar Agar
1.	Detection of air micro flora	NA	20	20×100 =2000 ml	20 gm
2.	Detection of surface micro flora of working place	NA	20	20×100 =2000 ml	20 gm
3.	Finger disinfection microbial testing	NA	20	20×20 =400 ml	4 gm
4.	Microbiological analysis of butter	NA	20	20×100 =2000 ml	20 gm
5.	Microbiological analysis of cheese	NA	20	20×100 =2000 ml	20 gm

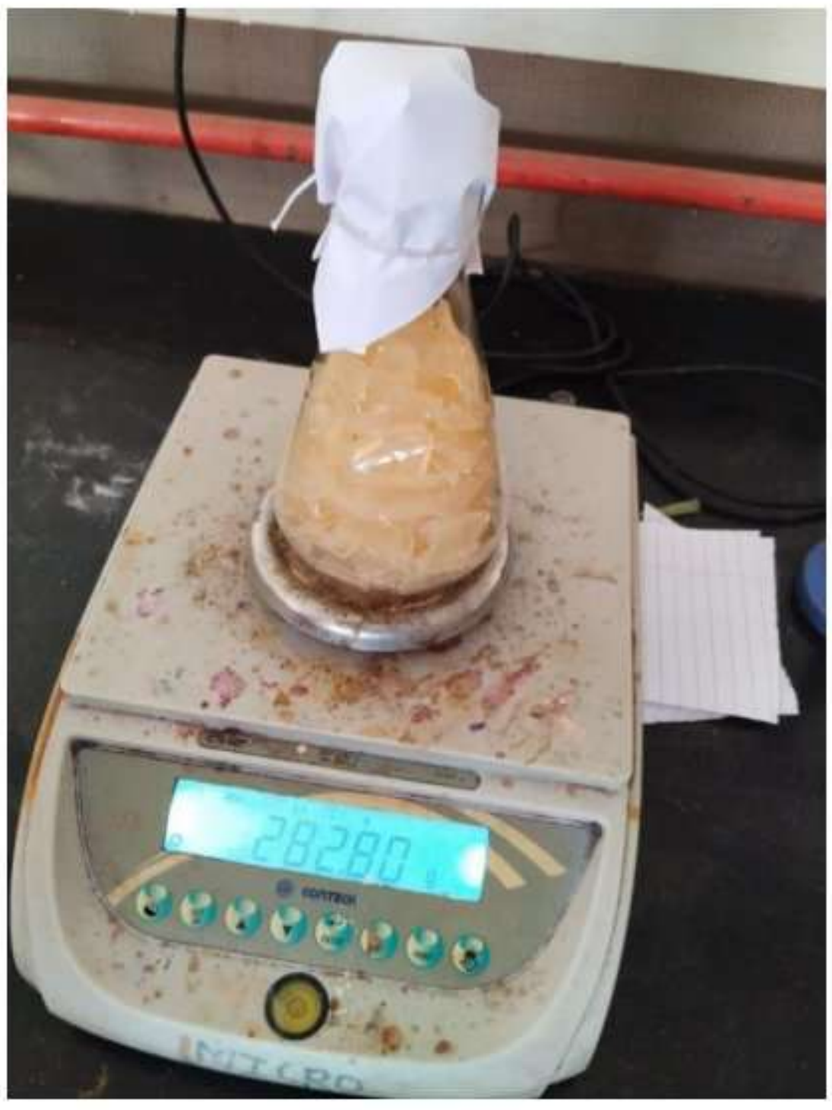
Table - 10 - Total amount of different media used by B.Sc. - I, B.Sc. - II, B.Sc. - III, M.Sc. - I, M.Sc. - I, student during an academic year - 2023 – 2024.

Sr.no	Class (ml)	NA (ml)	MCA (ml)	PDA (ml)	NB (ml)	Agar –agar (gm)
1.	**B.Sc. - I**	12300	1200	-	-	135
2.	**B.Sc. - II**	6000	2000	-	-	80
3.	**B.Sc. - III**	15120	1080	2160	1350	186
4.	**M.Sc. - I**	2400	-	1600	-	40
5.	**M.Sc. - II**	8400	-	-	-	84
	Total	44,220 ml	4280 ml	3760 ml	1350 ml	524gm

Nutrients Agar Pieces

1) After Resterilization

2) Before Resterilization

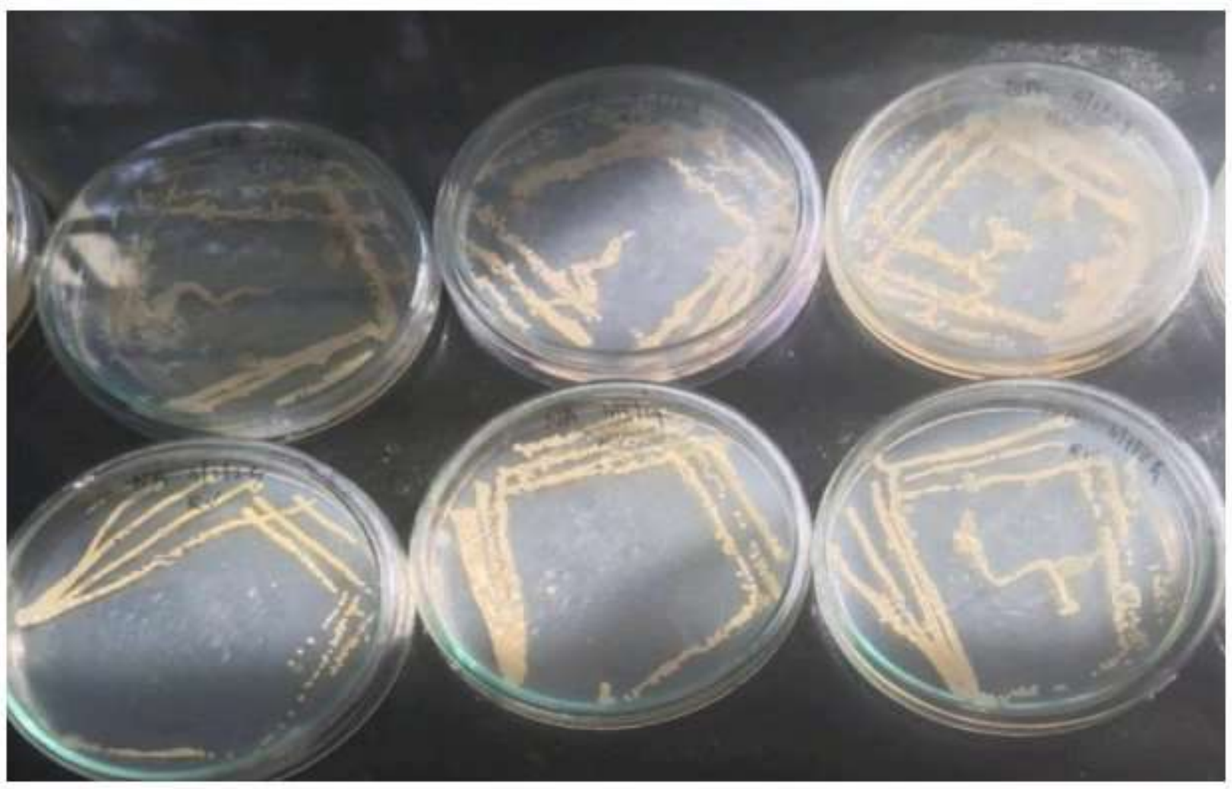


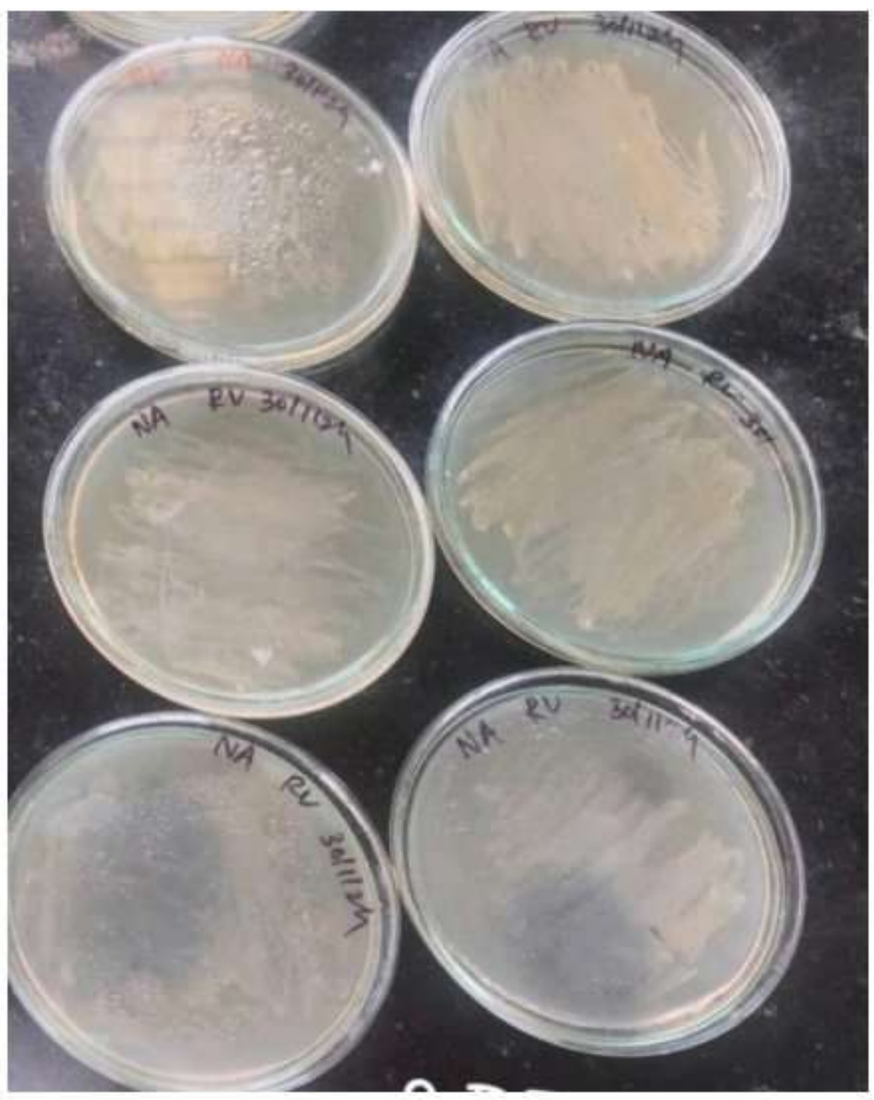
Weight of Nutrient agar

Nutrient agar plates recycles

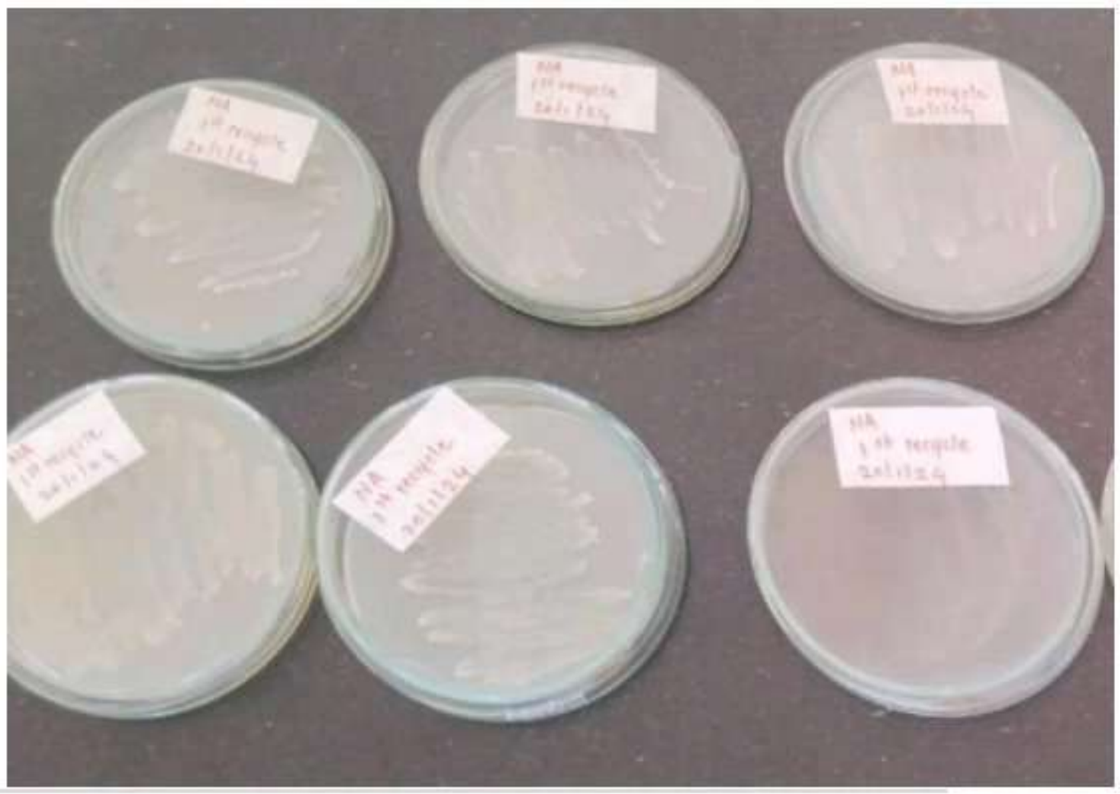
1st Recycle

2nd Recycle



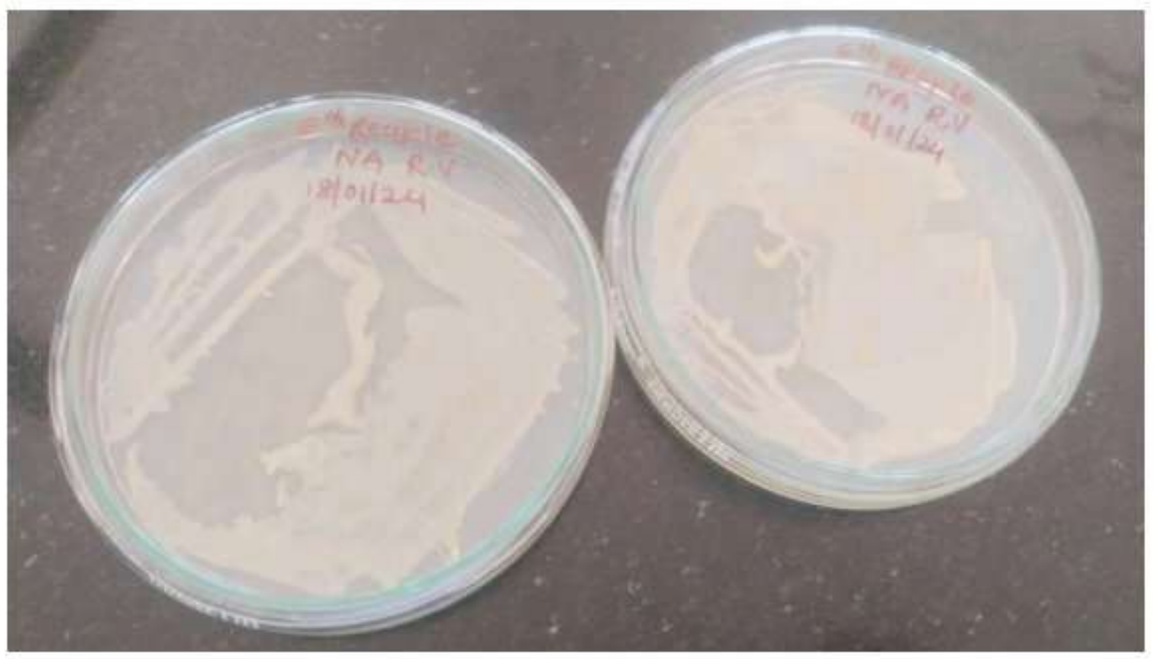


3rd Recycle

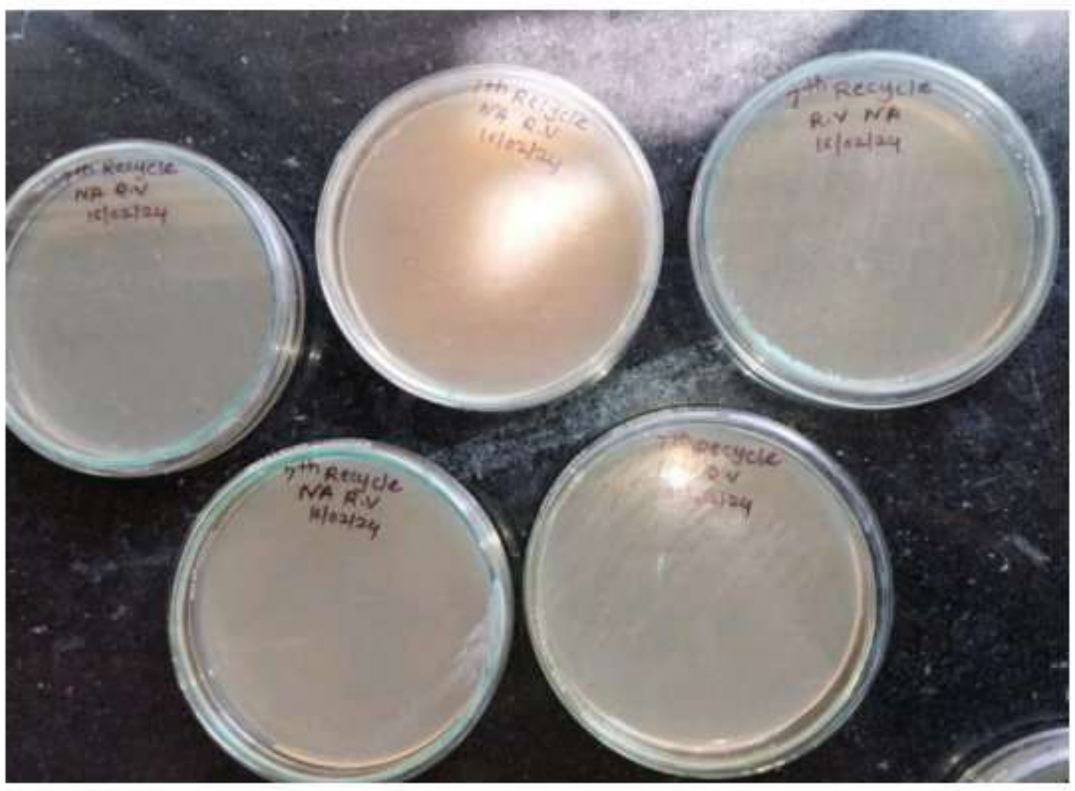


4th Recycle

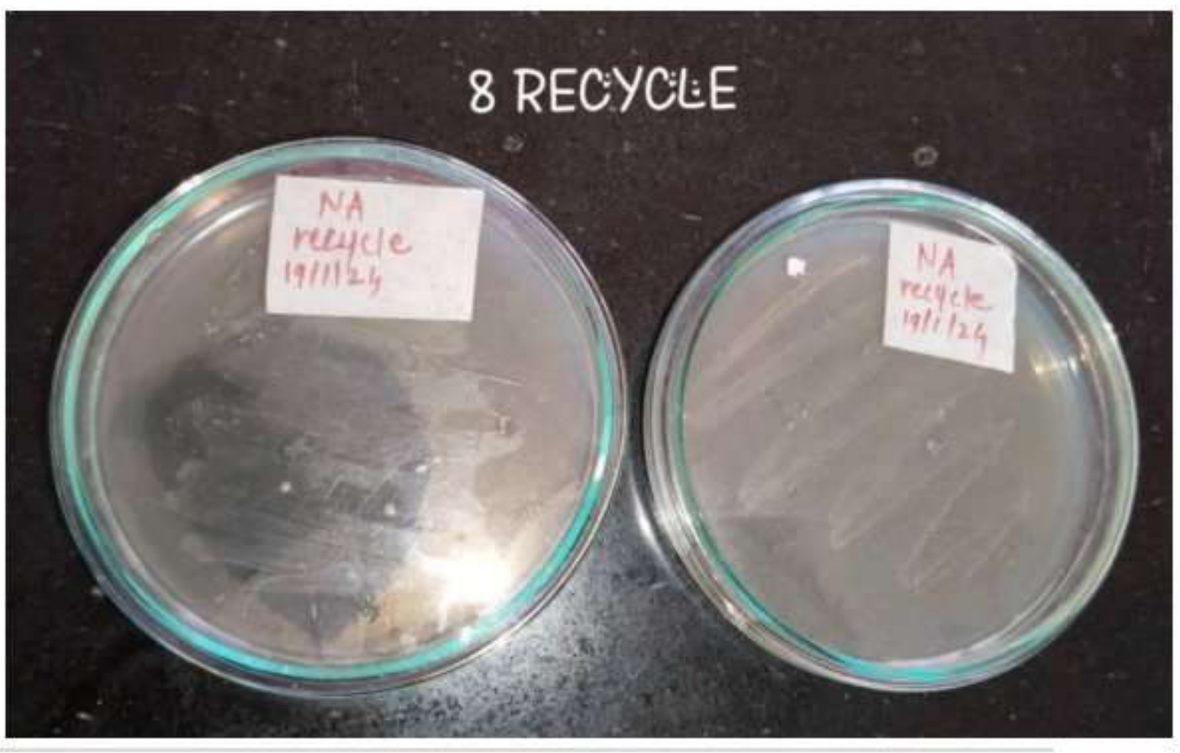
5th Recycle



6th Recycle



7th Recycle



8th Recycle

9th Recycle

10th Recycle

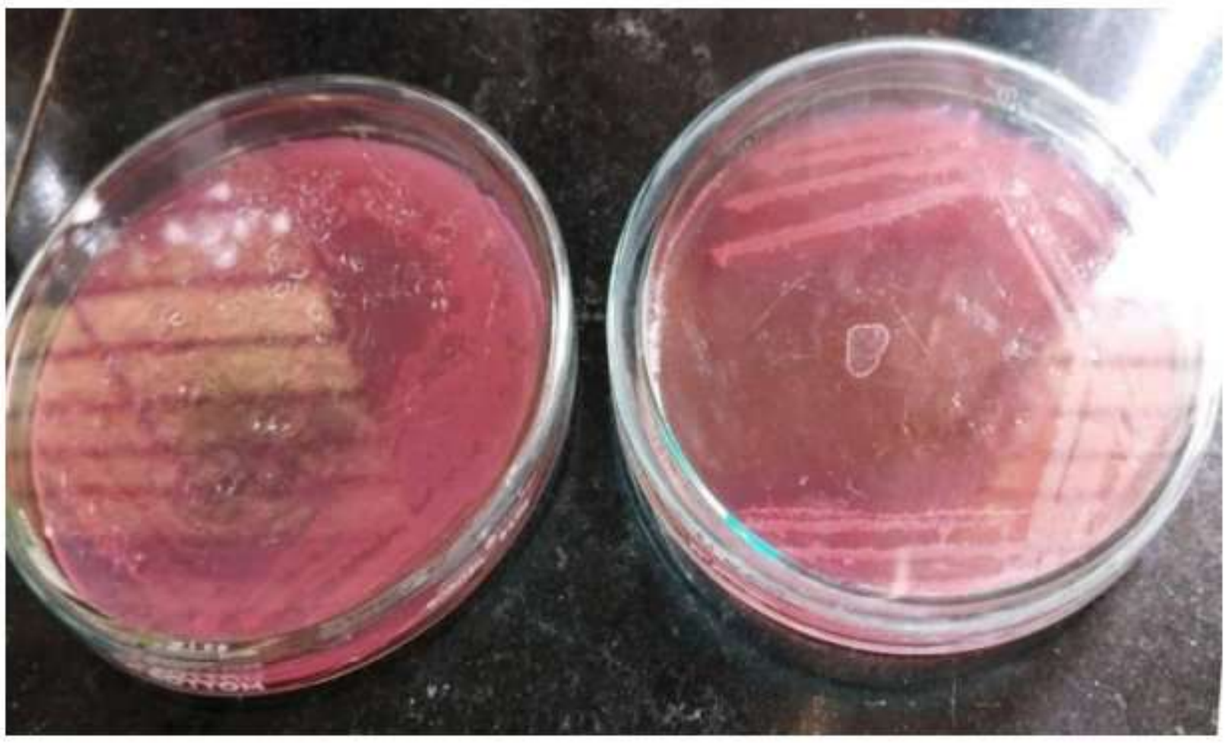
11th Recycle

12th Recycle

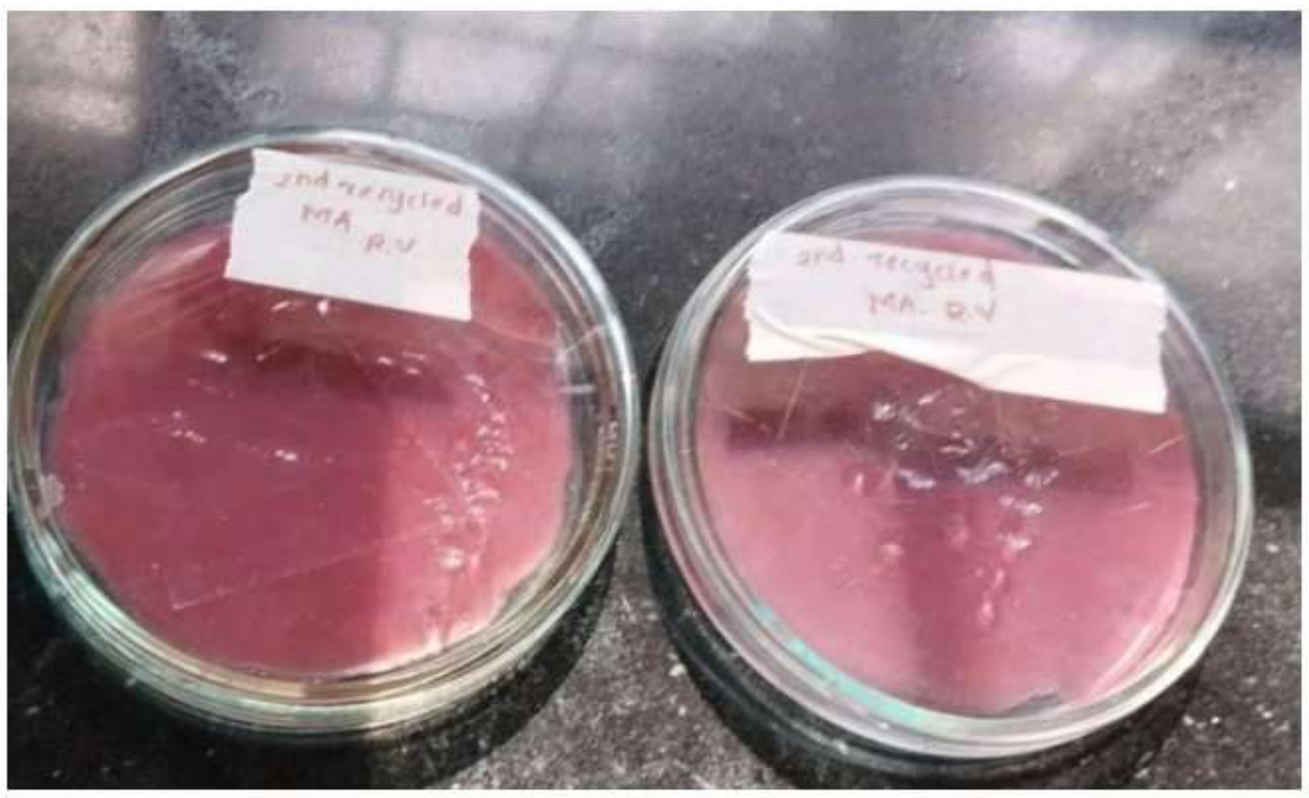
13th Recycle

14th Recycle

MacConkeys Agar Plates Recycle –



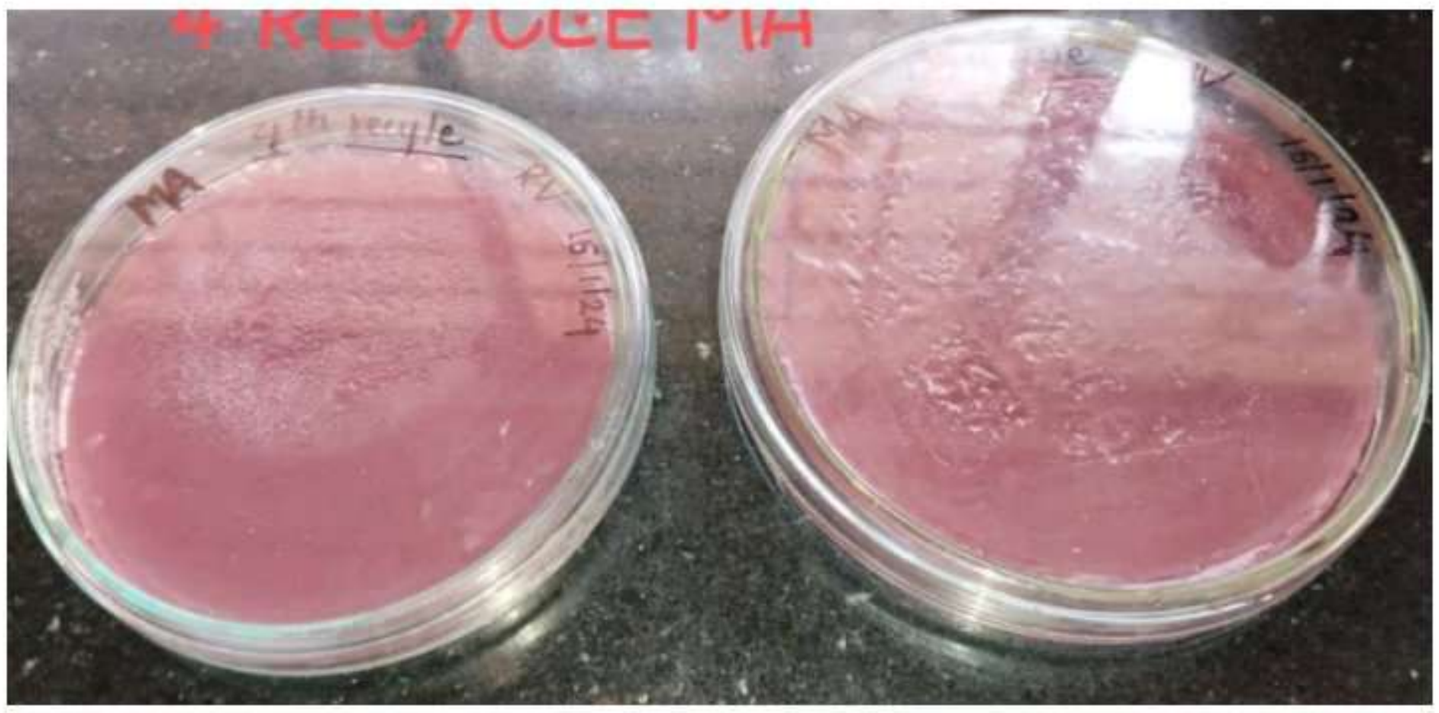
1st Recycle

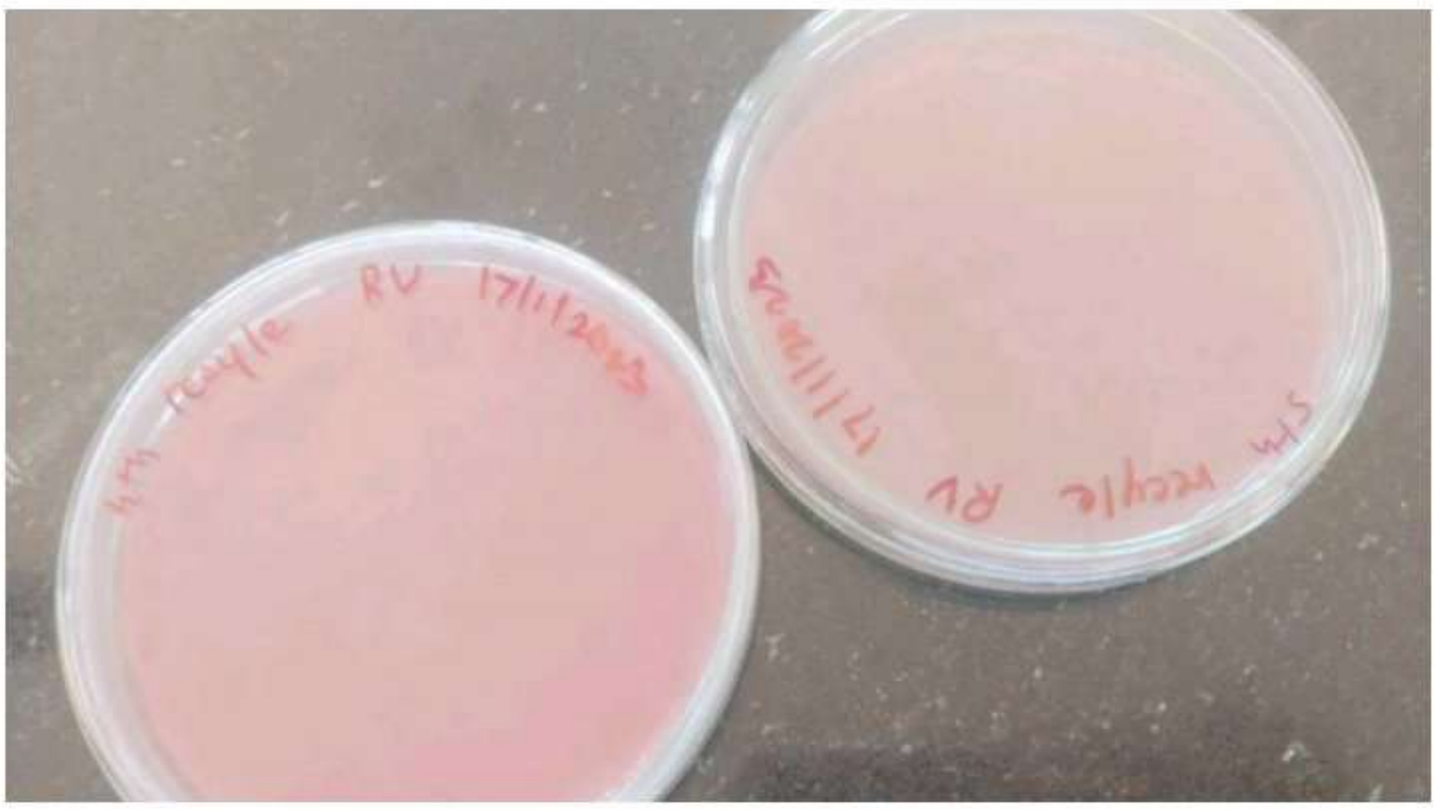


2nd Recycle

3rd Recycle

4th Recycle





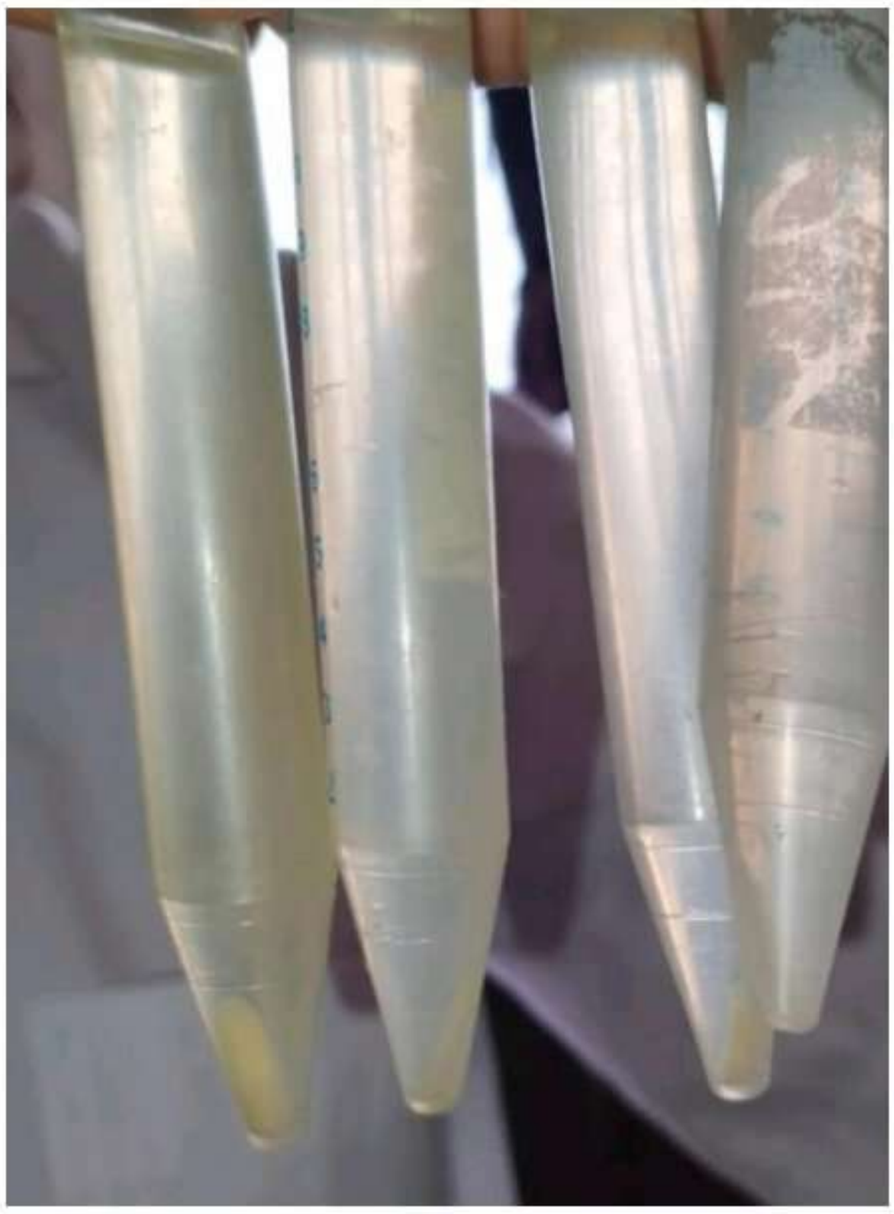
5th Recycle

Potat Dextrose agar plates Recycle -

1st Recycle

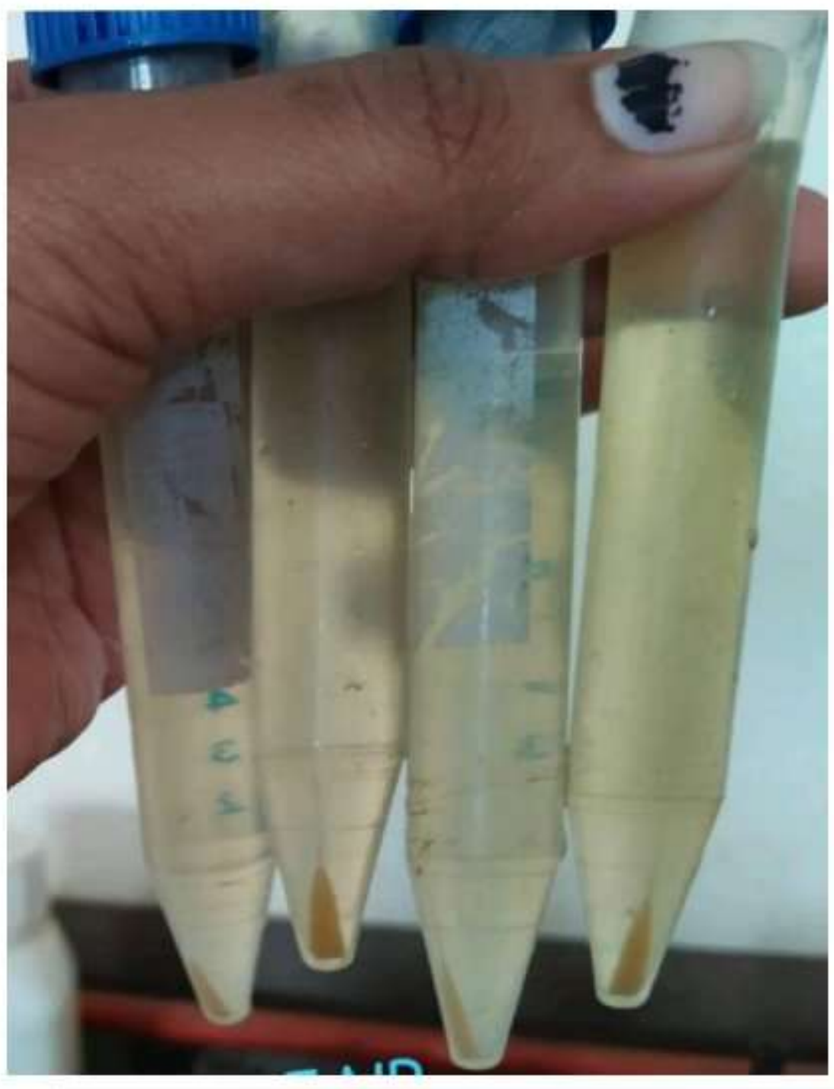
2nd Recycle

Nutrient Broth Recycle –



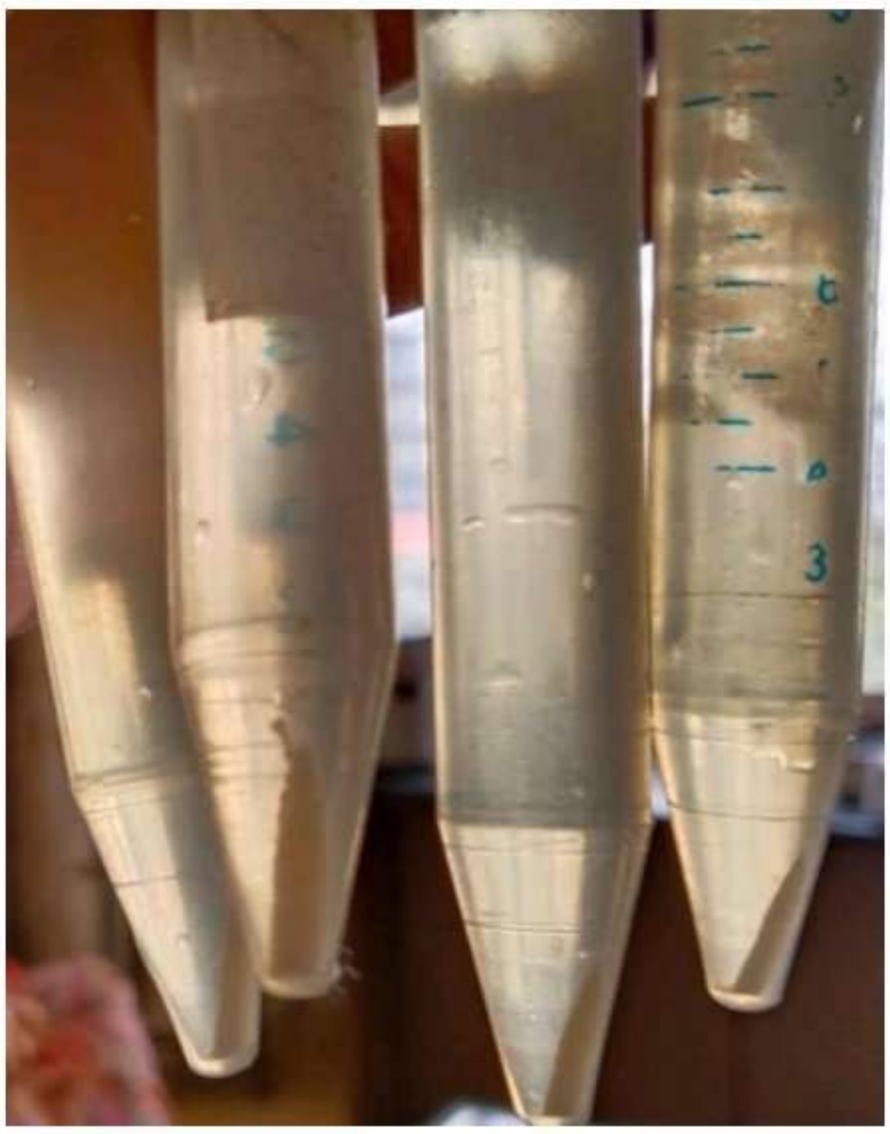
1ST Recycle

2nd Recycle

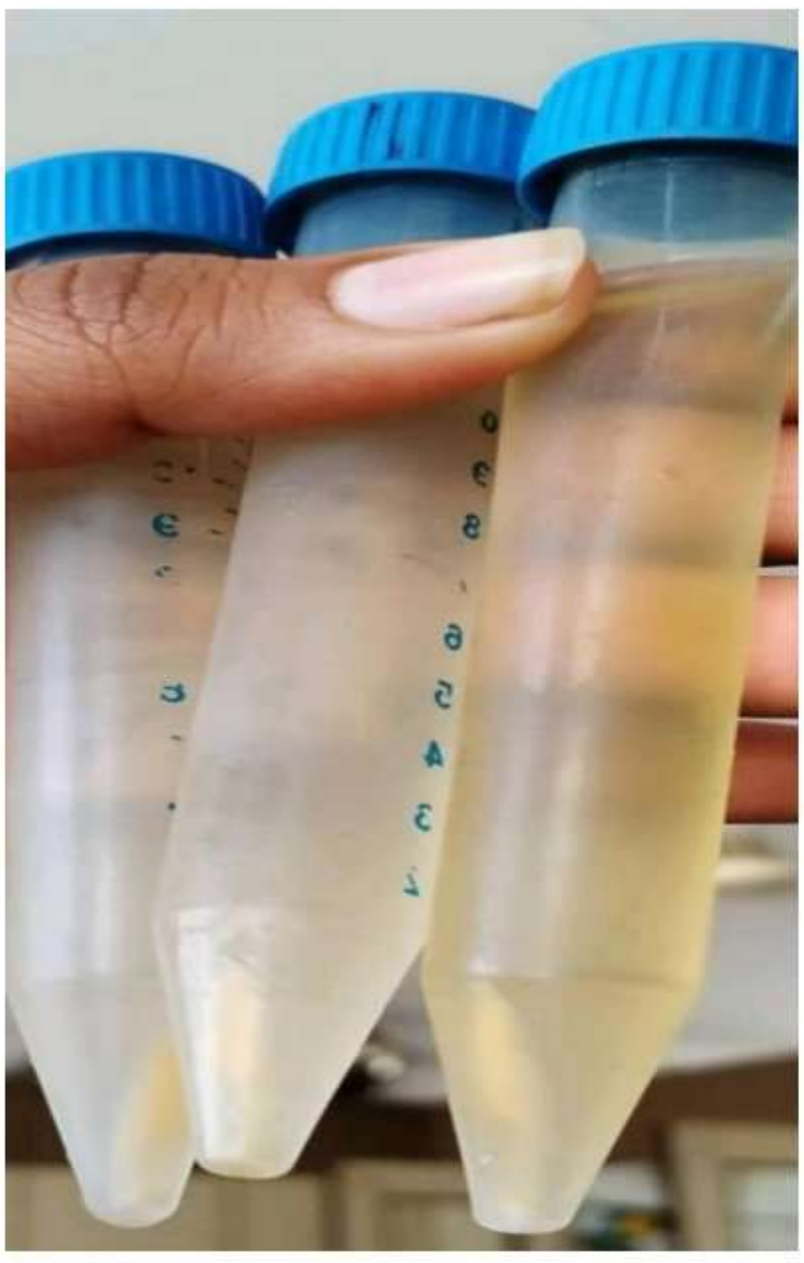


3rd Recycle

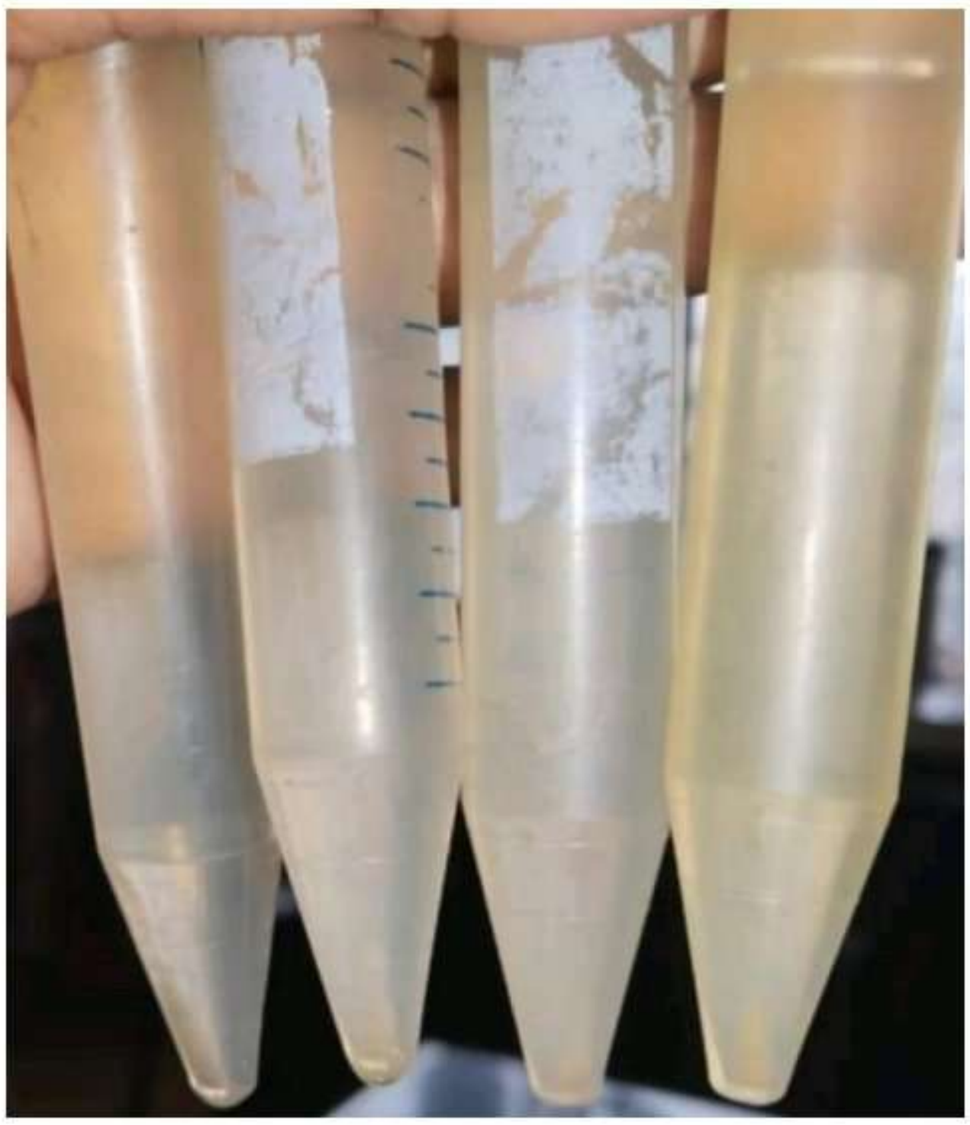
4th recycle



5th recycle



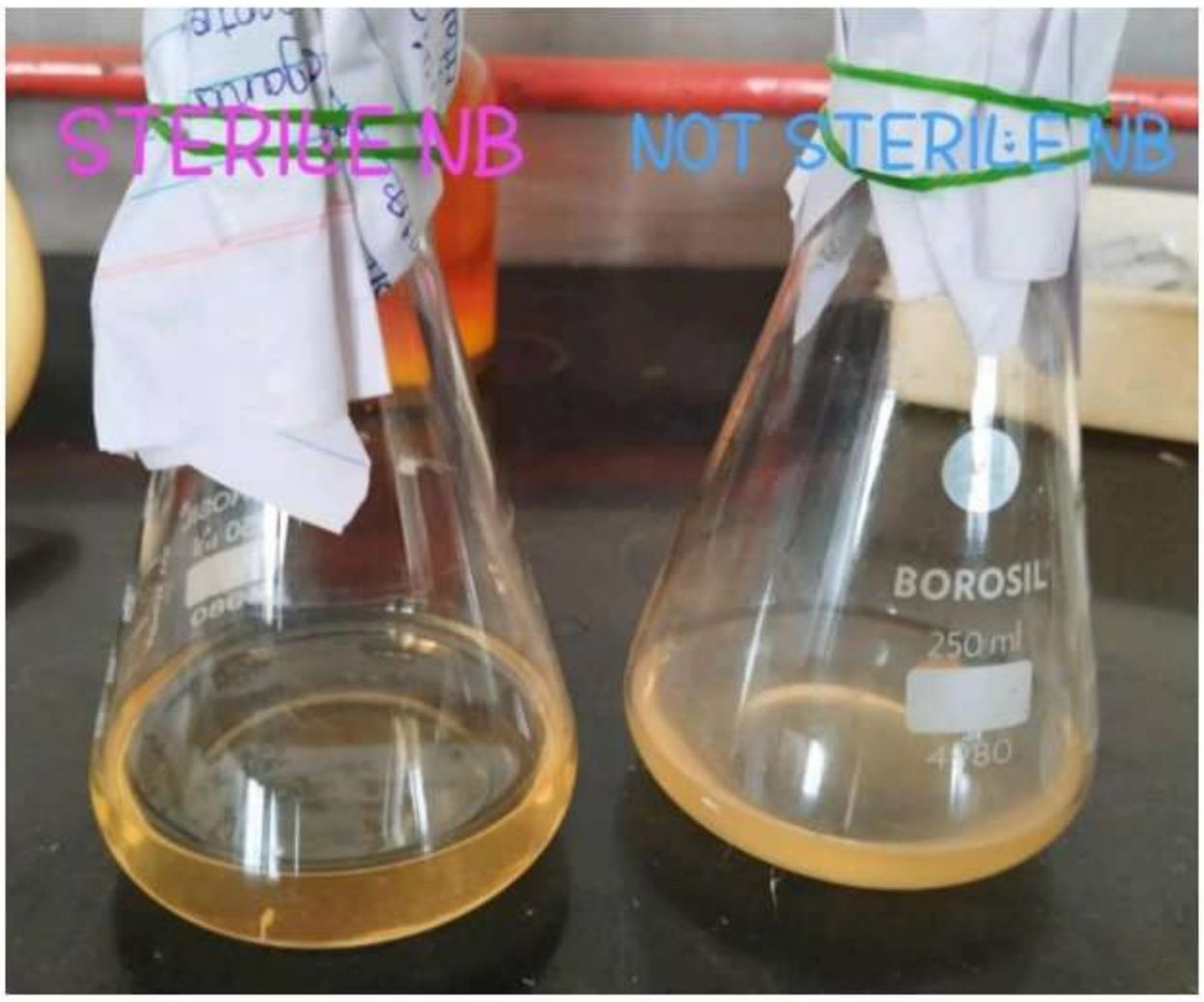
6th recycle



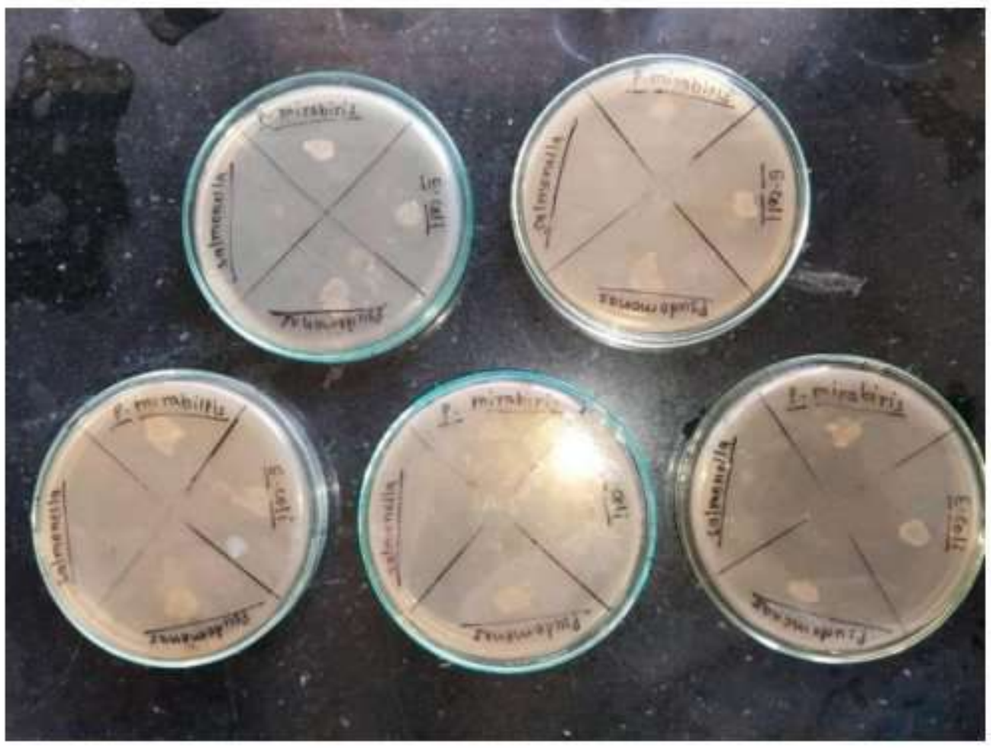
7th Recycle

8th Recycle

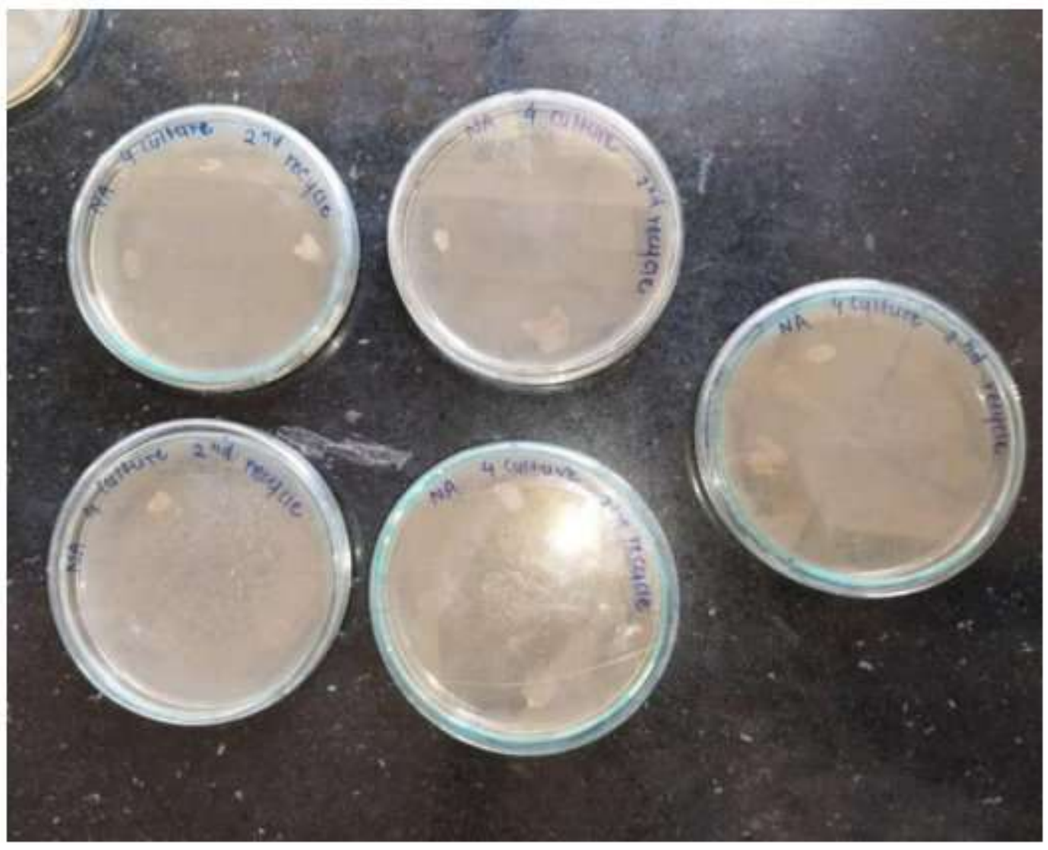
9th Recycle



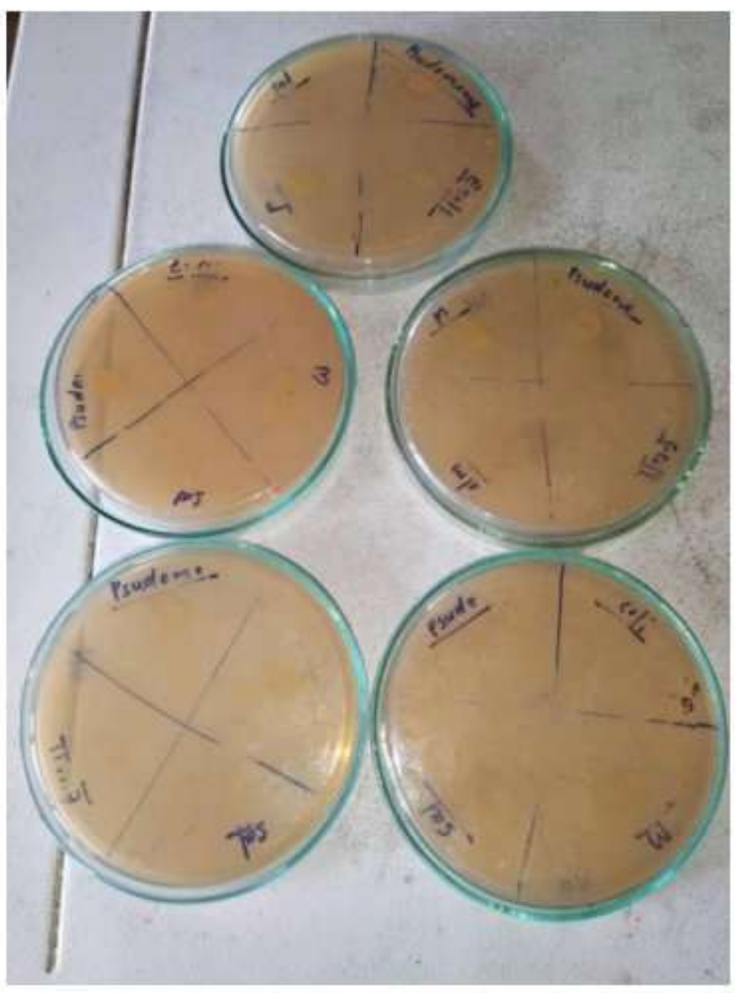
4 Culture Recycle –



1st Recycle



2nd Recycle

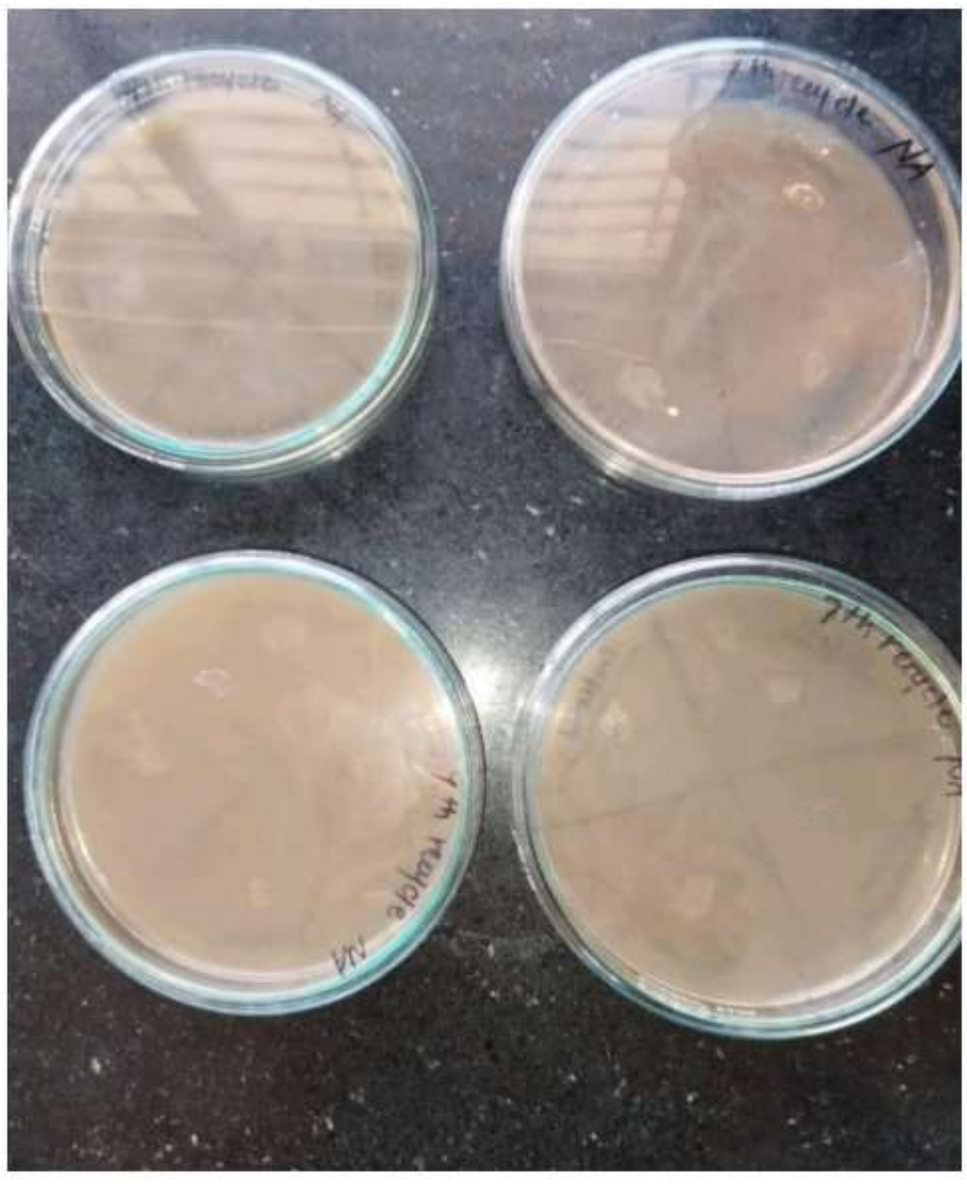


3rd Recycle

4th Recycle

5th Recycle

6th Recycle



7th Recycle

5.0 - Summary and conclusion:

In the study of recycling of nutrient media various methods were used. UV exposure method didn't give good results. Second method is the filtration, in filtration method the contaminated media was then filtered through mesh cloth. But the media gets Solidify in mesh cloth. Observed Similarity turbidity was in the media. Another method are Used in recycle agar media is growth removal, sterilization method gave good results, and hence method was continued. No any addition of component the result was good.

There is significant result of recycling of media, metabolic products or microorganism are not hindering the growth cycle. Results of second day shows that Nutrition of culture media is not completely utilized in first cycle. It remains present after 24 hours of incubation. Essential trace elements. Need to be analysed, trace elements has major role in the growth of microorganisms and agar which is major and costly component of culture media is Showed no changes in colour and other Properties till 7^{th} recycle, Media is recycled it save money, labour time environmental hazards.

Recycled agar media gets prevents the environmental pollution.

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