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Isolation, Characterization And Identification Of Microorganisms From Domestic Waste Samples

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Abstract : Any substance or object which is unwanted or unusable is waste. Waste will remain waste until it has been fully recovered and no longer poses potential threat to the environment or to human health. Nature's own and oldest method of waste disposal is composting. On the basis of this background the present study aimed to study bioculture to minimise the duration required for preparation of compost from domestic waste.

As the nature of biodegradable waste is changing due to use of various pesticides, preservatives and chemicals, the nature of microorganisms present in waste is also changing, so our aim is isolation, identification, and characterization of different microorganisms from domestic waste with soil samples on suitable medium. Its application especially in composting is tested. Microorganisms isolated from domestic waste samples were *Pseudomonas fluorescens, Azotobacter spp., Lactobacillus casei, Cellulomonas flavigena, Kurthia spp., Erysipelothrix spp.. Planococcus citreus, Brochothrix spp., Actinomycetes pyogenes, and Caryophanon spp.*

Isolated organisms were grown in sugar cane molasses containing media to prepare bioculture. Spraying of this bioculture will convert domestic waste into compost in much less duration compared to conventional methods of composting, which is mainly due to natural flora Using this method we definitely reduce the area required for dumping grounds, as this method leads to proper utilisation of the space available for composting which is major problem for the growing cities. The limited revenues of municipal corporations make them ill-equipped, as high costs are required for all these disposal processes of MSW. As a result part of the MSW generated remains unattended and grows in the heaps at poorly maintained collection centres.

The choice of disposal site is more a matter of what is available than what is suitable. Therefore such techniques to reduce load on available land may prove to be very useful in future.

Keywords: Biodegradable,microorganisms,soil,environment,disposable,waste.

INTRODUCTION

Introduction:

1.1 Waste-

Waste is any substance or object which is unwanted or unusable. In living organisms, waste is the unwanted substances or toxins that are expelled from thein. More commonly, waste refers to the materials that are disposed of in a system of waste management. Once a substance or object has become waste, it will remain waste until it has been fully recovered and no longer poses a potential threat to the environment or to human health.

Waste generation encompasses activities in which materials are identified as no longer being of value and are either thrown away or gathered together for disposal. Waste handling and separation involves the activities associated with management of waste until they are placed in storage containers for collection. Handling also encompasses the movement of loaded containers to the point of collection. Separation of waste components is an important step in the handling and storage of solid waste at the source.

The functional element of collection includes not only the gathering of solid waste and recyclable materials, but also the transport of these materials, after collection, to the location where the collection vehicle is emptied. This location may be a materials processing facility, a transfer station or a landfill disposal site. The types of means and facilities that are now used for the recovery of waste materials that have been separated at the source include curbside collection, drop off and buy back centres. The separation and processing of wastes that have been separated at the source and the separation of commingled wastes usually occur at all materials recovery facilities, transfer stations, combustion facilities and disposal sites.

Waste (also referred to as rubbish, trash, refuse, garbage, or junk) is unwanted or unusable materials. In living organisms, waste is the unwanted substances or toxins that are expelled from them. More commonly, waste refers to the materials that are disposed of in a system of waste management.

Waste is directly linked to human development, both technologically and socially. The compositions of different wastes have varied over time and location, with industrial development and innovation being directly linked to waste materials. Examples of this include plastics and nuclear technology. Some components of waste have economical value and can be recycled once cecily recovered.
Waste is sometimes a subjective concept, because items that some people discard may have value to others. It is widely recognized that waste materials are a valuable resource, whilst there is debate as to how this value is best realized.

1.1. Types-
Municipal solid waste (MSW), also called urban solid waste, is a waste type that includes predominantly household waste (domestic waste) with sometimes the addition of commercial wastes collected by a municipality within a given area. They are in either solid or semisolid form and generally exclude industrial hazardous wastes. The term residual waste relates to waste left from household sources containing materials that have not been separated out or sent for processing

Biodegradable waste: food and kitchen waste, green waste, paper (can also be recycled). Recyclable material: paper, glass, bottles, cans, metals, certain plastics, etc. .

Inert waste: construction and demolition waste, dirt, rocks, debris.

Composite wastes: waste clothing. Tetra Paks, waste plastics such as toys. Domestic hazardous waste (also called "household hazardous waste") & toxic waste medication, e-waste, paints, chemicals, light bulbs, fluorescent tubes, spray cans, fertiliser and pesticide containers, batteries, shoe polish.

A compound that is biodegradable can be changed by the action of microorganisms to another compound. Biodegradable waste includes food and kitchen waste, green waste, paper tean also be recycled). Domestic hazardous waste (also called "household hazardous waste") & toxic waste also includes medication, paints, chemicals, light bulbs, fluorescent tubes, spray cs, fertiliser and pesticide containers, batteries, shoe polish.

1.2. Biodegradable waste-
Biodegradable waste is a type of waste, typically originating from plant or animal sources, which may be broken down by other living organisms. Waste that cannot be broken down by other living organisms may be called non-biodegradable.
A compound that is biodegradable can be changed by the action of microorganisms to another compound. Biodegradable waste includes food and kitchen waste, green waste, paper (can also be recycled). Domestic hazardous waste (also called "household hazardous waste") & toxic waste also includes medication, paints, chemicals, light bulbs, fluorescent tubes, spray cans, fertiliser and pesticide containers, batteries, shoe polish

Biodegradable waste can be commonly found in municipal solid waste (sometimes called biodegradable municipal waste, or BMW) as green waste, food waste, paper waste, and biodegradable plastics. Other biodegradable wastes include human waste, mamure, sewage. slaughterhouse waste.

Biodegradable matter is generally organic material such as plant and animal matter and other substances originating from living organisms, or artificial materials that are similar enough to plant and animal matter to be put to use by microorganisms. Some microorganisms have the astonishing, naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pharmaceutical substances, radionuclides and metals. Major methodological breakthroughs in microbial biodegradation have enabled detailed genomic, metagenomic, proteomic, bioinformatic and other high-throughput analyses of environmentally relevant microorganisms providing unprecedented insights into key biodegradative pathways and the ability of microorganisms to adapt to changing environmental conditions.

Biodegradation is the chemical breakdown of materials by a physiological environment. The term is often used in relation to ecology, waste management and environmental remediation (bioremediation). Organic material can be degraded aerobically with oxygen, or anaerobically. without oxygen. A term related to biodegradation is biomineralization, in which organic matter is converted into minerals. Biosurfactant, an extracellular surfactant secreted by microorganisms, enhances the biodegradation process. To make it more simple biodegradation means an object which disappears in its own time.

Biodegradable waste is an important substance due to its links with global warming. When it is disposed of in landfills, it breaks down under uncontrolled anaerobic conditions. This produces landfill gas which, if not harnessed, escapes into the atmosphere. Landfill gas contains methane, a more potent greenhouse gas than carbon dioxide.

1.3. Domestic waste-

Municipal solid waste (MSW), also called urban solid waste, is a waste type that includes predominantly household waste (domestic waste) with sometimes the addition of commercial wastes collected by a municipality within a given area. They are in either solid or semisolid form and generally exclude industrial hazardous wastes.

The problem of Domestic Waste is drawing increasing attention of the people as huge garbage is lying down uncollected beside the roads, streets, dustbins and on the ground which is causing threat to the environment as well as endangering public health.

This waste is generated as a consequence of household activities such as the cleaning, cooking, repairing empty containers, packaging, and the huge use of plastic carry bags. Many times this waste gets mixed with biomedical waste from hospitals and clinics. There is no system of segregation of organic, inorganic and recyclable wastes at the household level. Door-to-door collection is rarely practiced. Community collection bins are poorly managed and are usually no more than open dumps on the roadside.

The improper handling and management of Domestic Waste from households are causing adverse effects on the public at large and this deteriorates the environment.

The municipal workers are most affected by the occupational danger (hazard) of ste handling; they suffer from illnesses like eye problems, respiratory problems, gastro and skin problems. The persons who wander to collect the discarded things for selling purpose through wastes also suffer from various health problems like respiratory problems from inhaling particles, infection from direct contact with contaminated materials which lead to headache, diarrhea, fever and cough and cold.

The Municipal Solid Waste (Management & Handling) Rules, which was framed by the Central Government under the power coffered upon it by environment protection Act 1986, rules came into force from 2000. Even after passing 7 years of the rules there is a tremendous lack of literacy programmes on waste management and disposal techniques which keeps most of the people ignorant about waste management. Lack of awareness among the people increases the problems.

The apathetic Governmental attitude towards the disposal of waste is a problem that has led to difficulties in implementation of "The Municipal Solid Waste (Management & Handling) Rules, 2000" as it has led to ignorance towards uncollected domestic waste. The waste processing and disposal facility has to be set up by the Municipal Authority on their own or through the operator of the facility, as well as they have to follow the standards as specified under the Rules of 2000.

The improper and apathetic attitude towards management of Domestic Solid Waste has adverse effects on the society as well as environment causing various diseases like diarrhea, fever, cough and cold, headache, chicken-guinea etc; to the people living nearby areas of such local dustbins, to the municipal workers and also the animals like cows, birds who wander through these waste for food. Uncollected Domestic Waste is causing health hazards as well as polluting to the environment endangering the life of the people at large.

With the growing population the huge waste is being generated day by day. There is wide use of plastics, advanced technology and other materialistic things. This resulted in different characteristics of waste which became a complicated problem for management of Domestic Waste and disposal techniques.

This is such a burning problem concerned with the environment that needs to be carefully studied and researched as on every street waste is lying uncollected scattered around local bins and dumped around locally consequently there is occurrence of bad smell as well as hazard to the human health and to the passerby.

2 Waste disposal-

The disposal of garbage in the world is a problem that continues to grow with the development of industrialized nations and the growth of population. Since the beginning of time people have needed to find a way of disposing of their trash. In 18th century England and France, carters were paid by individuals to carry trash and discard it on the outskirts of town. Disposal in open pits became routine and Benjamin Franklin initiated the first municipal cleaning program in Philadelphia in 1757. Since then we have come a long way and have developed types of waste that cannot simply be dumped into a hole.

Waste disposal is a growing problem worldwide and is directly connected to industrial development and population growth. Since early modern times, disposing of waste has been an important concern for individuals and community officials. Although there have been recent advancements in waste disposal, it remains an overall public safety and environmental health issue that countries around the world continue to address.

Due to uncollected waste and improper disposal techniques drains also get clogged which lead to mosquitoes by which various diseases like malaria, chicken-guinea, viral fever, dengue etc. arise and affect the health of people adversely.

2.1.Methods-

There are many different methods of disposing of waste. Landfilling

A landfill, also known as a dump or rubbish dump (and historically as a midden), is a site for the disposal of waste materials by burial and is the oldest form of waste treatment. Historically, landfills have been the most common methods of organized waste disposal and remain so in many places around the world.

Landfills may include internal waste disposal sites (where a producer of waste carries out their own waste disposal at the place of production) as well as sites used by many producers. Many landfills are also used for other waste management purposes, such as the temporary storage, consolidation and transfer, or processing of waste material (sorting, treatment, or recycling).

A landfill also may refer to ground that has been filled in with soil and rocks instead of waste materials, so that it can be used for a specific purpose, such as for building houses. Unless they are stabilized, these areas may experience severe shaking or liquefaction of the ground in a large earthquake.

Incineration-

Incineration is the thermal destruction of waste. It is as old as throwing food wastes on a wood fire, and in many developing nations, garbage is still routinely burned in drums and boxes on city streets. Modern incineration systems use high temperatures, controlled air, and excellent mixing to change the chemical, physical, or biological character or composition of waste materials. The new systems are equipped with state-of-the-art air pollution control devices to capture particulate and gaseous emission contaminates. There are still many health concerns connected with incineration systems, especially for populations living near incinerators. However, the stringent regulations that have been enacted by federal and state regulators ensure that the design, operation, testing, and maintenance of these systems provide maximum safety and minimum risk to the surrounding area and inhabitants.

Incineration can be adapted to the destruction of a wide variety of wastes. This includes but is not limited to household wastes, often referred to as municipal wastes, industrial wastes, medical wastes, sewage, Superfund soils and liquids, and the hazardous wastes (liquids, tars, sludges, solids, and vent fumes) generated by industry. Unlike many other methods of waste disposal, incineration is a permanent solution. The major benefit of incineration is that the process actually destroys most of the waste rather than just disposing of or storing it.

Waste incineration involves the application of combustion processes under controlled

conditions to convert waste materials to inert mineral ash and glasses. The three Ts of combustion

(temperature, turbulence, and residence time) must be present along with sufficient oxygen for the reaction to occur:

The burning mixture (air, wastes, and fuel) must be raised to a sufficient temperature to destroy all organic components. The combustion airflow is reduced to the minimum level needed to provide the oxygen for the support fuel (gas, oil, or coal) and the combustible wastes without forming high levels of CO and unburned hydrocarbons. This will raise the temperature to the level needed for good combustion.

- Turbulence refers to the constant mixing of fuel, waste, and oxygen.

- Residence time is the time of exposure to combustion temperatures.

- Oxygen must be available in the combustion zone.

Open Dumping-

Open dumping is prohibited. An open dumping is defined as a land disposal site at which solid wastes are disposed of in a manner that does not protect the environment, are susceptible to open burning, and are exposed to the elements, vectors, and scavengers.

Open dumping can include solid waste disposal facilities or practices that pose a reasonable probability of adverse effects on health or the environment. An officially designated Municipal Solid Waste Landfill (MSWLF) is not an open dump. An officially designated Sanitary Waste Landfill is not an open dump. An officially designated Construction and Demolition Debris Landfill is not an open dump.

Ocean dumping-

Pollution of the open seas by human activities has become a serious problem. Ocean dumping is the dumping or placing of materials in designated places in the ocean, often on the continental shelf. A wide range of materials is involved, including garbage, construction and demolition debris, sewage sludge, dredge material, and waste chemicals. In some cases, ocean dumping is regulated and controlled, while some dumping occurs haphazardly by ships and tankers at sea or illegally within coastal waters. Incineration at sea of organic wastes, with subsequent dumping, has been allowed as a viable disposal process, both in the United States and in Europe.

An important, but little recognized source of ocean dumping is the elimination of bilge water from tankers carrying oil and other products. Bilge water can contain a number of toxic chemicals, as well as biological agents that can affect marine ecosystems and marine organisms, some of which are subsequently consumed by humans. Dumping of radioactive wastes and soil from contaminated nuclear defense sites has periodically been suggested as a viable disposal method, and canisters of nerve gas have been disposed of at sea. In addition to permitted ocean dumping, there is always the possibility of collisions, groundings, and accidents that result in de facto ocean dumping, often of materials not otherwise allowed.

3. Composting-

Comfort of the breakdown of organic material derived from and plants The "bushing done is sentricle in oxygen using process ef by the baterie, fun, insects and animate, which inhabit soil. to a compost heap these organisms generate heat as they deem negaric matter and break it into fine particles Composting insure tree and addeat method of waste disposal and soil fertilization.

Waste materials that are organic in nature, such as plant material, food scraps, and paper products, can be recycled using biological composting and digestion processes to decompose the organic matter. The remitting organic material is then recycled as mulch or compost for agricultural or landscaping purposes. In addition, waste gas from the process touch as methane) can be captured and used for generating electricity. The intention of biological processing in waste management is to control and accelerate the natural process of decomposition of organic matter.

Traditionally, gardeners have created their own compost using leaves, grass, shrub clippings and other useful organic materials found in the garden. Applying compost to soils provides an excellent conditioner and mulch, which fertilizes and provides soil structure, retains moisture and can restrict weed growth. Man-made compost is an alternative to the peat-based compost extracted from important natural wildlife sites.

In recent years there has been interest in the creation of garden compost from organic household waste, as a result of the growing awareness of the environmental problems created by the traditional disposal methods. In the UK around 30 million tonnes of domestic refuse is produced each year, which contains on average about 38% organic content, such as vegetable peelings, tea bags and food scraps

Composting is indeed a natural way of recycling, harnessing natural processes rather than machinery and man-made chemicals, but it takes people to do it.

Compost is not just decayed organic matter. Composting is applied microbiology at its most complex, involving the interactions of thousands upon thousands of different species of microorganisms (2 million individuals per gram) in a highly complex ecosystem. The composting process kills weed seeds and suppresses human and plant pathogens, which doesn' happen when leaves go down on their own. and other detritus rot down on their own.

Once applied, compost "balances" the soil flora: that is, for each of the scores or more of disease organisms that can affect each species of plant, at least 12 to 15 different species of bio control microorganisms need to be present, with the food and conditions they require, if the plant is to be healthy. Composting accomplishes that, among other things.

3.1. Natural process of composting

The natural process of compost is Leaves/other organic matter breakdown by organisms humus mixing of humus and soil with the aid of the organisms natural soil improvement. The composting process is carried out by three classes of microbes -
Psychrophiles-low temperature microbes
Mesophiles -medium temperature microbes
Thermophiles - high temperature microbes

Generally, composting begins at mesophilic temperatures and progresses into the thermophilic range. In later stages other organisms including Actinomycetes, Centipedes, Millipedes, Fungi, Sowbugs, Spiders and Earthworms assist in the process.

Isolation, characterization and identification of microorganisms from domestic waste samples

3.Isolation:-
Bacteria were isolated from each flask containing domestic waste samples at different stages of degradation using nutrient agar medium. The medium consisted of (g/L) peptone, 10: NaCl, 5: Yeast/meat extract, 3; and Agar, 30: pH 7.2.

Figure 3.1: Isolated colonies of microorganisms from degrading domestic waste on nutrient agar plate.

3.1Materials and methods

1.After 8 days a loopful of domestic waste samples from each flask were suspended in 1ml of distilled water.

2 Cellulosic waste suspension was then spread on the surface of two sterile nutrient agar containing plates. Similar procedure was followed for degrading vegetable and fruit waste.

3. Then one plate of each suspension was incubated at room temperature and the other at 37°C.

4. After two days of incubation 9 colonies with different morphologies were selected. Colony characteristics of each colony were recorded.

5. The flasks were further incubated.

6. After 15 days the similar procedure was repeated. At this time 8 colonies with different morphologies were selected and colony characteristics of each colony were recorded.

7. Single colonies with different morphologies were picked up and restreaked for a number of times on nutrient agar medium until single and uniform colonies were obtained.

Isolates were then preserved on nutrient agar at 4°C.

Isolate	Size(mm)	Shape	Colour	Margin	Elevation	Opacity	Consistency
G	1.5	Circular	Green	Irregular	Convex	Opaque	Sticky
Y	1.0	Circular	Yellow	Entire	Convex	Opaque	Mucoid
W	1.5	Circular	White	Irregular	Flat	Transparent	Sticky
R	1.5	Circular	White	Regular	Flat	Opaque	Mucoid
P	Pinpoint	Circular	White	Entire	Convex	Opaque	Mucoid
C	2.0	Circular	Cottony white	Regular	Flat	Opaque	Sticky
B	1.5	Circular	Yellow	Entire	Convex	Opaque	Mucoid
L	1.0	Circular	Shiny white	Entire	Convex	Opaque	Mucoid
O	0.5	Circular	Brown	Entire	Convex	Opaque	Mucoid
Rt	Pinpoint	Irregular	White	Irregular	Convex	Opaque	Sticky
Cn	1.02-2.0	Circular	White	Regular	Flat	Opaque	Sticky
Pi	Pinpoint	Circular	Pink	Entire	Convex	Opaque	Sticky
Br	Pinpoint	Circular	Brown	Entire	Convex	Translucent	Sticky
Dy	1.5	Circular	Dark yellow	Entire	Flat	Opaque	Sticky
Yo	1.0	Circular	Yellow	Entire	Convex	Translucent	Sticky
Ch	1.0	Circular	Chalky white	Entire	Convex	Opaque	Mucoid
Cr	1.5	Circular	Creamy white	Entire	Convex	Opaque	Mucoid

3.2. Result-

Seventeen isolates were obtained from degrading domestic waste samples. They were labelled as G. Y. W, R, P, C, B, L, O, Rt, Cn, Pi, Br. Dy, Yo, Ch, Cr.

Domestic Waste Sample	At Room Temperature	At 37^0C
Cellulose Waste	G,Y,W,Ch,R	R,P,Pi,Dy,Cn,Yo,G
Vegetable Waste	Y,P,W,Br,R	G,Y,W,Rt,O
Fruit Waste	B,W,R,L	G,C,Cr,W,L,P,O

3.3-Characterization and identification

3.3.1. Materials and methods

For identification and characterization of isolates following biochemical test were

performed referring to the Bergey's Manual of Determinative Bacteriology 9th edition.

For sugar fermentation Material

1) Sterile tube containing 1% peptone water + 0.5% sugar + phenol red and inverted

2) Suspensions of each isolate

IMViC Tests:-

The IMViC Tests were designed to differentiate gram negative organisms on the basis of their biochemical properties and enzymatic reaction in the presence of enzyme substrate.

Indole production test:

Tryptophan is an essential amino acid which is oxidized by the enzyme tryptophanase resulting in the formation of indole, Pyruvic acid and ammonia. Indole test is performed by inoculating the bacteria into tryptone broth, the indole produced during the reaction is detected by adding Kovac's reagent (Dimethylaminobenzaldehyde) which produces a cherry red reagent layer.

The following procedure used for indole production test:

● Preparation of tryptone broth 10gm of tryptone was dissolved in one liter of distilled water. The broth was sterilized in the autoclave at 15psi (121 C) for 15 minutes.

● All the tubes having tryptone broth were inoculated with microorganisms and one habe was un inoculated which was taken as comparative control.

● Inoculated and un-inoculated tubes were incubated at 35°C for 48hrs. After 48 hr of incubation, 1ml of Kovac's reagent was added to each tube including control.

● The tubes were gently shaken at intervals of 10-15 minutes.

● The tubes were allowed to stand to permit the reagent to come to the top. Methyl-red and Voges-Proskauer test (MP VP) The methyl red and Voges-Proskauer test are used to differentiate two major types of facultative anaerobic enteric bacteria that produce large amounts of acid and those that produce a neutral product acetone as end product. Both these are performed simultaneously because they are physiologically related and are performed on the same medium MR-VP broth. Opposite result are usually obtained for the methyl red and Voges-Proskauer tests, Le MR+, VP or MR, VP+ In these tests, if an organisms produces large amount of organic acids: like formic, acetic, lactic and succinic (and end product) from glucose, the medium remain red (a positive tests)after the addition of methyl red pH indicator (ie pH remaining below 4.4) in other organisms, methyl red will turn yellow (a negative test) due to elevation of pH above 6.0 because of the enzymatic conversion of the organic acids (produced during glucose fermentation) to known acetic end product such as ethanol and acetoin (acetylmethylcarbinol)

The following procedure used for MP/VP test, MP/VP broth (pH 6.9) tubes were prepared using the following composition

Requirements:-

● Peptone - 7.0gm

● Dextrose -5.0gm

● potassium phosphate -50gm

● Distilled water-1000ml

● 5 ml broth was poured into each test tube and sterilized by autoclaving at 151h pressure for 15 minutes.

● Two MP/VP tubes were inoculated with microorganisms and one tube was left uninoculated to be used as a comparative control.

● All tubes were incubated at 35°C for 48hrs.

● 5 drops of methyl red indicators are added to each tube. 12 drops of VP reagent 1 and 2-3 drops of VP reagent 2 were added to the other set of tubes and uninoculated control tubes.

● The tubes were shaken gently for 30 seconds with the caps off to expose the media to oxygen.

● The reaction was allowed to complete for 15-30 minutes.

Citrate utilization test:-

Citrate test is used to differentiate enteric bacteria on the basis of their ability to utilize or ferment citrate as the sole carbon sources. The utilization of citrate depends on the presence of an enzyme's citrates produced by the organism that breaks down the citrate into oxaloacetic acid. The citrate test is performed by inoculating by microorganisms into an organic synthetic medium, Simon's citrate agar, where sodium citrate is the only source of carbon and energy. Bromothymol blue is used as an indicator. When citric acid is metabolized CO_2 Sodium and water to form sodium alkaline product, which changes color of the indicator from green to blue and these constituents to test positive. Bromothymol blue is green when acidic (Ph 6.8 And below) and blue when alkaline (pH 76 And higher)

Requirements:-

- Simon's citrate agar medium (pH 6.9 was prepared using the following composition.
- Ammonium dihydrogen phosphate
- Dipotassium phosphate
- Sodium chloride
- Sodium citrate
- Magnesium sulfate
- Agar
- Bromothymol blue
- Distilled water 4

The following procedure used for citrate test

- All the constituents were dissolved except phosphate which were dissolved separately in 1000ml of water and volume was made up 1 liter, pH was adjusted to 6.9 and the medium was poured in the culture tubes and sterilized by autoclaving at 151b pressure for 15 minutes. The slants were subsequently prepared.

- The Simon's citrate agar slants were inoculated with microorganisms by means of stab and streak inoculation. One tube was left uninoculated to be used as comparative control. All the slants were incubated at 37°C for 48hrs Catalase test The catalase test facilitates the detection of enzyme catalase in bacteria. It is essential for differentiating catalase positive and catalase negative bacteria. The catalase test is also valuable in differentiating aerobic and obligate anaerobic bacteria.

Requirements:-

- Microscopic slide
- Dropper or Pasteur pipette
- 1% H2O2 The following procedure is used for catalase test,
- Collect a small amount of organism from a well isolated 18-24 hrs colony and place it on a microscopic slide.
- Using a dropper or Pasteur pipette place 1 drop of H2O2 onto the organism on the microscopic slide.
- Observing the formation of bubbles against a dark background enhances readability.

Oxidase test:-

The oxidase test is a biochemical reaction that assays for the presence of cytochrome oxidase, an enzyme sometimes called indophenol oxidase. In the presence of an organism that contains the cytochrome oxidase enzyme, the reduced colorless reagent becomes an oxidized coloured product.

Requirements:-

- Filter paper
- Bacterial culture
- Kovac's reagent
- Microscopic slide The following is the procedure for oxidase test
- Soak a small piece of filter paper in 1% Kovac's oxidase reagent and let dry
- Use a loop and pick a well-isolated colony from a fresh (18- to 24-hour culture) bacterial plate and rub onto a small piece of filter paper media and loops).
- Place 1 or 2 drops of 1% Kovac's oxidase reagent on the organism smear.
- Observe color changes.
- Microorganisms are oxidase positive when the color changes to dark purple within 5 to 10 seconds. Microorganisms are delayed oxidase positive when the color changes to purple within 60 to 90 seconds.
- Microorganisms are oxidase negative if the color does not change or it takes longer than 2 minutes.

3.4-Observation Table:-

	G	**Y**	**W**	**R**	**P**	**C**
Gram Character	Gram negative rod	Gram negative rod	Gram positive rod	Gram positive rod	Gram positive rod	Gram positive cocci
Motility	Motile	Non motile	Non motile	Non motile	Motile	Non motile
Sugars- Lactose	A/G	-	-	A	-	-
Fructose	A/G	A/G	A/G	A/G	A/G	-
Xylose	A/G	A/G	A/G	A/G	A/G	-
Maltose	-	A/G	A/G	-	A/G	-
Mannitol	A/G	-	-	A/G	A/G	-
Dextrose	A/G	A/G	A/G	A/G	A/G	A
Inositol	-	-	-	A	A	-
Sucrose	A/G	-	-	A/G	A/G	-
IMViC- Indol	-	+	+	-	+	-
Methyl red	-	-	-	+	-	-
Voges-Proskauer	+	+	+	+	+	+
Citrate	+	+	+	-	+	-

Enzymes- Oxidase	+	+	+	-	-	-

+= positive, - = Negative, A/G=Acid Gas production, ND=Not detectable

	B	**L**	**O**	**Rt**	**Cn**	**Pi**
Gram Character	Gram positive rod	Gram negative rod	Gram positive cocci	Gram positive rod	Gram positive rod	Gram positive cocci
Motility	Motile	Motile	Non motile	Motile	Motile	Non motile
Sugars- Lactose	-	-	A	-	-	-
Fructose	-	A/G	A/G	A/G	-	-
Xylose	-	A/G	A/G	A/G	-	-
Maltose	A/G	A/G	A/G	A/G	-	-
Mannitol	A/G	A/G	A/G	-	-	-
Dextrose	A	A/G	A/G	A/G	-	-
Inositol	A/G	-	A/G	A/G	-	-
Sucrose	A/G	A/G	A/G	A/G	-	-
IMViC- Indol	-	+	+	+	-	-
Methyl red	-	-	+	-	-	+
Voges-Proskauer	+	+	+	+	-	+
Citrate	-	+	+	+	-	-
Enzymes- Oxidase	-	+	+	+	+	-

+= positive, - = Negative, A/G=Acid Gas production, ND=Not detectable

	Br	**Dy**	**Yo**	**Ch**	**Cr**
Gram Character	Gram positive rod	Gram negative cocci	Gram positive rod	Gram positive cocci	Gram positive cocci
Motility	Non motile	Motile	Non motile	Non motile	Motile
Sugars- Lactose	A/G	-	-	-	-
Fructose	A/G	-	-	-	-
Xylose	A/G	-	-	-	-
Maltose	A/G	-	-	-	-
Mannitol	A/G	-	-	-	-
Dextrose	A/G	-	-	-	-
Inositol	-	-	-	-	-
Sucrose	-	-	-	-	-
IMViC- Indol	+	-	-	-	-
Methyl red	+	-	-	-	-
Voges-Proskauer	+	-	-	-	+
Citrate	+	-	-	+	-
Enzymes- Oxidase	+	-	-	+	-

+= positive, - = Negative, A/G=Acid Gas production, ND=Not detectable

Results:-

Eleven isolates were identified. Six were identified to their species level, while the others were identified to their genus level. Eleven isolates, G, L., O, P, C, W. R. Rt, Cn, Yo, Dy, were identified as *Pseudomonas rescence*, *Azotobacter spp.*, *Lactobacillus casei, Caryophanon spp. Actinomycetes pyrogens, Comebacterium cystitis. Brochothrix spp. Cellulomonas flavigena, Kurthia spp. calothrix spp.*, *Planococcus citreus*, respectively.

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