

PROFESSIONAL TRAINING - I

Submitted in partial fulfilment of the requirements for the award of Bachelor of Technology degree in Biomedical Engineering.

BY

M.V. SRI HARSHITHA (41240043)



DEPARTMENT OF BIOMEDICAL ENGINEERING

SCHOOL OF BIO AND CHEMICAL ENGINEERING

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade “A++” by NAAC

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI – 600119

OCTOBER-2023



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BONAFIDE CERTIFICATE

This is to certify that this Professional Training Report is the Bonafide work of
M.V. Sri Harshitha (41240043) Carried out the Professional training at, COL.Dr.
JEPPIAAR RESEARCH PARK entitled Exploring Bioanalytical Tools and Techniques
for Drug Discovery under my Supervision from 20th to 25th September.

YEAR COORDINATOR

Dr.A. Sabarivani,M.Tech.,PhD.

INTERNAL GUIDE

Ms.Sindu Divakaran,M.E.

HEAD OF THE DEPARTMENT

Dr.T.Sudhakar,M.Sc.,PhD.

Submitted for viva voce examination held on October 3rd 2023.

INTERNAL EXAMINER

Head of the Department
Department of Biomedical Engineering
Sathyabama Institute of Science & Technology
(Deemed to be University)
Category I University by UGC
Jeppiaar Nagar, Rajiv Gandhi Salai,
Chennai - 600 119

EXTERNAL EXAMINER

CERTIFICATE:



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Exploring Bioanalytical Tools and Techniques for Drug Discovery

CERTIFICATE

This is to certify that M.V Sri Harshitha has attended the Training session on “Exploring Bioanalytical Tools and Techniques for Drug Discovery” from 20th to 25th September 2023 jointly organized by the Department of Biomedical Engineering and Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology.

Dr. Krupakar Parthasarathy
Convener

Dr. T. Sudhakar
Convener

Dr. M. Radhakrishnan
Centre In-charge

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DECLARATION

I, M.V. Sri Harshitha; hereby declare that the Professional Training Report Entitled Exploring Bioanalytical Tools and Techniques for Drug Discovery done by me at COL.Dr. JEPPIAAR RESEARCH PARK is submitted in partial fulfilment of the requirements for the award of Bachelor of Technology Degree in Biomedical Engineering.

DATE:

PLACE: CHENNAI

SIGNATURE OF THE CANDIDATE

M.V.S Harshitha
M.V SRI HARSHITHA

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ABSTRACT:

This report provides a comprehensive overview of my internship at COL.Dr. JEPPIAAR RESEARCH PARK from 20th to 25th September. I had the privilege of getting to know more about Bioanalytical Tools and Techniques for Drug Discovery. This Internship encompassed a range of Activities including training in Instruments related to sterilization techniques and about techniques like PCR, RT PCR, Agarose gel electrophoresis etc. And I also got the knowledge about Bioprospecting of Actinobacteria. This report reflects on the what new knowledge I have gained and through my exposure.

In Conclusion, my internship gave me not only provided a platform for practical learning but also ignited a passion for the Bioprospecting and Research. It is my hope that this report serves as a testament to the valuable experiences gained during the internship and the contributions of various Research related activities in providing services to the society

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INTRODUCTION:

EXPLORING BIOANALYTICAL TOOLS AND TECHNIQUES FOR DRUG DELIVERY

Bioprospecting (also known as biodiversity prospecting) is the exploration of natural sources for small molecules, macromolecules and biochemical and genetic information that could be developed into commercially valuable products for the agricultural, aquaculture, bioremediation, cosmetics, nanotechnology, or pharmaceutical industries.

Instruments used in a bioprospecting lab and virology lab are mostly the instruments used in microbiology lab. They include Microscope, Laminar air flow, Incubator, Shaker, Ultraviolet chamber, Centrifuge, Lyophilizer, Hot air oven, Water bath, Auto clave, UV Spectrometry, Sonicator, Fume hood, Soxhelt apparatus etc ...

PCR is a method used to amplify DNA from a small amount of DNA template. RT-PCR uses reverse transcription to produce a DNA template from an RNA source that can then be amplified. Chromatography is a laboratory technique for the separation of a mixture into its components. Agarose gel electrophoresis is a method of gel electrophoresis used in biochemistry, molecular biology, genetics, and clinical chemistry to separate a mixed population of macromolecules such as DNA or proteins in a matrix of agarose, one of the two main components of agar.

A biofilm is a thick layer of prokaryotic organisms that have aggregated to form a colony. The colony attaches to a surface with a slime layer which aids in protecting the microorganisms. Biofouling or biological fouling is the accumulation of microorganisms, plants, algae, or small animals where it is not wanted on surfaces such as ship and submarine hulls, devices such as water inlets, pipework, grates, ponds, and rivers that cause degradation to the primary purpose of that item.

DIVERSITY AND BIOPROSPECTING OF ACTINOBACTERIA:

Actinobacteria are the ecologically and economically important prokaryotes with the unprecedented ability to produce novel metabolites. They are widely distributed in nature due to their physiological and metabolic versatility. Actinobacteria from marine ecosystem are recognized as a promising source for novel bioactive metabolites. Notably, mangroves are the most fertile and productive marine ecosystem due to their eco physiological conditions and vast microbial diversity. At the global level, there are many novel actinobacteria which have been reported from mangrove ecosystems and also explored for their bioprospecting potential.

However, in India, microbiologists have explored actinobacteria from selected mangrove ecosystems for the past five decades with special reference to culturable diversity and antimicrobial, antimycobacterial, anticancer, and enzymatic activities. So far, around 22 rare actinobacterial genera have been reported from the Indian mangrove ecosystems. However, several other novel actinobacteria with promising bioprospecting potential are yet to be explored.

Actinobacteria from Antarctica as a source for anticancer discovery:

Although many advances have been achieved to treat aggressive tumours, cancer remains a leading cause of death and a public health problem worldwide. Among the main approaches for the discovery of new bioactive agents, the prospect of microbial secondary metabolites represents an effective source for the development of drug leads. In this study, we investigated the actinobacterial diversity associated with an endemic Antarctic species, *Deschampsia antarctica*, by integrated culture-dependent and culture-independent methods and acknowledged this niche as a reservoir of bioactive strains for the production of antitumour compounds. The 16S Rrna-based analysis showed the predominance of the Actinomycetales order, a well-known group of bioactive metabolite producers belonging to the Actinobacteria phylum. Cultivation techniques were applied, and 72 psychrotolerant Actinobacteria strains belonging to the

genera *Actinoplanes*, *Arthrobacter*, *Kribbella*, *Mycobacterium*, *Nocardia*, *Pilimelia*, *Pseudarthrobacter*, *Rhodococcus*, *Streptacidiphilus*, *Streptomyces* and *Tsukamurella* were identified. The secondary metabolites were screened, and 17 isolates were identified as promising antitumour compound producers. However, the bio-guided assay showed a pronounced antiproliferative activity for the crude extracts of *Streptomyces* sp. CMAA 1527 and *Streptomyces* sp. CMAA 1653. The TGI and LC₅₀ values revealed the potential of these natural products to control the proliferation of breast (MCF-7), glioblastoma (U251), lung/non-small (NCI-H460) and kidney (786-0) human cancer cell lines. Cinerubin B and actinomycin V were the predominant compounds identified in *Streptomyces* sp. CMAA 1527 and *Streptomyces* sp. CMAA 1653, respectively. Our results suggest that the rhizosphere of *D. antarctica* represents a prominent reservoir of bioactive actinobacteria strains and reveals it as an important environment for potential antitumour agents.

Diversity and bioprospecting of actinobacteria from the Indian Himalayan region (IHR):

The Himalayas are referred to as the highest mountain ranges in the world. They are also one of the youngest to be formed. It is approximately 2400 km and consists of more than 40 mountains which exceed 7000 m in height. The entire Himalayan mountain range has its unique flora and fauna. The bacterial diversity of the Himalayan soil has been widely studied using 16S Rna gene cloning and sequencing. Clone library analysis revealed the dominance of actinobacteria especially genus *Bacillus*, *Rhodoplanes*, and *Streptomyces* in the soil. Moreover, *mycobacteria*, *12ermenters*¹², *cyanobacteria* were also found in the Himalayan soils.

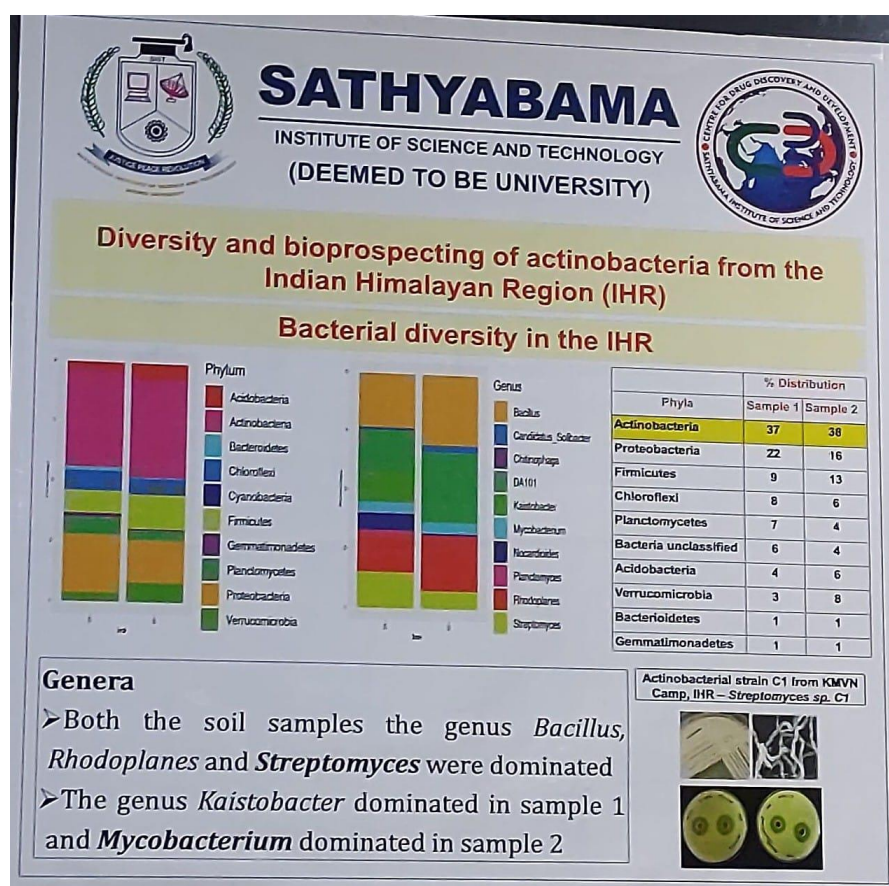


Figure 1: Diversity and Bioprospecting of actinobacteria from the Indian Himalayan Range

BIOPROSPECTING OF EARTHWORM CAST ASSOCIATED ACTINOBACTERIA:

The cast material from the agricultural soil around Kanchipuram city was collected and actinobacteria were isolated using Nutrient, Starch casein and Cellulose agar media. After incubation, 56 morphological different bacterial colonies were isolated. Cultural, morphological and physiological characteristics were indicated that 36 isolates belonged to the genus *Streptomyces*.

All the isolates showed good growth and some of them produced pigments on yeast extract malt extract agar medium. In antimicrobial activity test, out of 36, 12 isolates of *Streptomyces* were found to be actively antimicrobial activity against one or more tested human pathogens, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. It found the clear zone inhibition between isolates of *Streptomyces* and tested human pathogens.



Figure 2: Isolation of bacteria and actinobacteria from earthworm cast



Figure 3: Colony morphology of bacteria and actinobacteria

Antimicrobial activity of earthworm cast actinobacterial cultures were tested by adopting agar plug method. Actinobacterial cultures were grown on Yeast Extract Malt Extract (YEME agar) plates and were removed from the agar surface using sterile spatula. Tested pathogens (*Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) with 0.5 McFarland standards were transferred into nutrient agar plates using sterile cotton swab. All the plates were incubated at 37°C for 24 hours

STERILIZATION TECHNIQUES:

Sterilization refers to any process that removes, kills or deactivates all forms of life (particularly microorganisms such as fungi, bacteria, spores and unicellular eukaryotic organisms) and other biological agents such as prions present in or on a surface. Some sterilization techniques include: Laminar air flow, Auto clave, Hot air oven etc.

Laminar air flow is mere an indication of the direction of movement of air entering a closed compartment meant for aseptic processing of formulations or at working desk to avoid contamination. The air supplied is via “High-Efficiency Particulate Air” (HEPA) filters. These are filters with pore size of $0.45\ \mu$ and having an air velocity of $\sim 99\text{ km/s}$. Laminar air can be supplied in the desired area horizontally, vertically, or curvilinearly. It has a UV lamp used for sterilization of working desk.



Figure 4: Laminar air flow

Autoclave : The use of moist heat facilitates the killing of all microorganisms, including heat resistant endospores which is achieved by heating the materials inside the device at temperatures above the boiling point of water. According to the principle of gas laws, this can be achieved by raising the pressure inside the device. The usual procedure is to heat at 1.1 kilograms/square centimeter (kg/cm^2) [15 pounds/square inch (lb/in^2)] steam pressure, which yields a temperature of 121°C . At **121°C** , the time of autoclaving to achieve sterilization is generally considered to be **15-20 min**, depending on the volume of the load. If bulky objects are being sterilized, heat transfer to the interior will be slow, and the heating time must be sufficiently long so that the object is at 121°C for 15 min. Extended times are also required when large volumes of liquids are being autoclaved because large volumes take longer to reach sterilization temperature.



Figure 5: Autoclave

Hot air oven: A hot air oven is the most common method of sterilization in the laboratory working on dry heat. Sterilization is the process of removing or destroying all microorganisms including viruses, bacteria, and their spores from the article or surface without destroying their quality and quantity. It is a physical method of sterilization due to dry heat. Factors influencing sterilization by heat are nature of heat i.e dry or moist, temperature and time, number of microorganisms, nature of microorganisms, type of microorganism, and presence of organic material. Mode of action: protein denaturation, oxidative destruction of essential cell constituents, and toxic effects of elevated levels of electrolytes. It works on the principle of conduction where heat is absorbed by the exterior surface of an item and then passed onward to the next layer. This method was introduced by Louis Pasteur.



Fig 6: Hot air oven

FUME HOOD:

A fume hood (sometimes called a fume cupboard) is a type of local ventilation device designed to limit exposure to hazardous or toxic fumes & vapors in a lab. Fume hood is the single most essential component in a laboratory where chemical reactions are carried out. LabGuard is an expert leading fume hood manufacturer in India. We have a wide range of chemical fume hoods for our labs, be it an R&D lab, QC lab, educational lab or a third-party testing lab; you are all covered with LabGuard fume hoods. Fume hoods are designed to provide personnel protection from toxic or volatile chemicals by continuously delivering airflow away from the user to the work area. Fume hoods provide protection from more than just dangerous gases. The enclosed nature of the cabinet helps to protect experimenters from being hurt by debris if something explodes or splashes. Fume hoods are also usually made of flame-retardant materials, so unexpected fires tend not to be as damaging as they would be in an open area.



Figure 7: Fume hood

SHAKING INCUBATOR:

Shaker incubator A thermostatically controlled shaker incubator is another piece of apparatus used to cultivate microorganisms. Its advantage is that it provides a rapid and uniform transfer of heat to the culture vessel, and its agitation provides increased aeration, resulting in acceleration of growth. Shaking can be used to promote the growth and development of cells and microorganisms in a laboratory incubator for a variety of reasons. One reason is to increase the oxygen supply to the cells. In many cell culture applications, oxygen is an important factor that can affect the growth and metabolism of cells.



Figure 8: Shaking incubator

UV CHAMBER:

UV chambers have revolutionized the world of sterilization and disinfection, offering a safe and efficient way to eliminate harmful pathogens. In this comprehensive guide, we will explore the numerous benefits of UV chambers and shed light on their various applications. UV sterilization devices are game-changers in disinfection, offering a safe, efficient, and chemical-free solution against pathogens. Moreover, from healthcare facilities to the food industry and beyond, the applications of Ultraviolet Sterilization Device chambers are vast. By recognizing their benefits and practicing safety measures, businesses and individuals can effectively harness the power of Ultraviolet Sterilization Devices. Moreover, let's harness the power of Ultraviolet Sterilization Device light to promote a safer and more hygienic future. By utilizing these advanced technologies, we can effectively combat pathogens and create a cleaner environment for everyone.



Figure 9: UV Chamber

CENTRIFUGE:

Centrifugation is a term used to describe a method of separating mixtures using spinning and centrifugal force. Several characteristics can separate particles during centrifugation, including size, shape, density, and viscosity. The centrifuge utilizes the sedimentation principle due to gravitational force.

The centrifugation technique uses a centrifugal field to separate particles suspended in a liquid medium. These are put in the centrifuge's rotor either in bottles or tubes. Sedimentation is a process whereby gravity causes suspended particles to separate from fluids. The suspended substance may consist of powder or clay-like particles. Simple filtration filters particles larger than 5 micrometers from those less than 5 micrometers, which start Brownian motion and do not sediment under gravity. These particles can be separated with the help of the central force.



Figure 10: Centrifuge with 5810 rpm

RPM (revolutions per minute) means how fast the rotor in a centrifuge is spinning. When using RPMs, you should always look out for your rotor size (particularly radius). RPM means a rotor's revolutions (or rotations) per minute in the centrifuge. In other words, it describes how fast the rotor is spinning.

Even if two centrifuges with different motors rotate at the same speed (have the same RPM), the force acting on a centrifuged item depends on the radius of a particular rotor. Centrifuges will have a speed range that they are capable of achieving and will vary depending on the centrifuge. A low-speed centrifuge might spin at as low as 300 RPM, while a high-speed centrifuge could spin up to 15000 RPM.



Figure 11: Centrifuges with 12c and 8c

LYOPHILIZER:

Lyophilizer is also known as freeze dryer. A lyophilizer is the equipment execute dehydrate process that typically used to preserve perishable materials, to extend shelf life, or make the material more convenient for storage or transport. Lyophilizer works by freezing the material, then reducing the surround to allow the frozen water(ice) to sublimate.

That means, a lyophilizer will have systems of: refrigeration, vacuum, heating, drying chamber, vapor collect condenser and controller, as well as the temperature probes, vacuum sensors, software and so on. Lyophilization is based on a simple principle of physics called "SUBLIMATION". Sublimation is the process of transition of a substance from solid to the vapor state without passing through an intermediate liquid phase.

The process of lyophilization consists of:

- Freezing of the product to convert the water in the product to ice form,
- Sublimation of ice directly into water vapor under vacuum.
- Drawing off the water vapor
- Once the ice has been sublimated, the products are freeze-dried and can be removed from machine.

The principle advantages of lyophilization as a drying process are:

- Minimum damage and loss of activity in delicate heat-labile materials
- Speed and completeness of rehydration
- Possibility of accurate, clean dosing into final product containers
- Porous, friable structure

The principle disadvantages of lyophilization are:

- High capital cost of equipment (about three times more than other methods)
- High energy costs (2-3 times more than other methods)

A lyophilizer functions in three phases, with the first and most critical being the freezing phase. Proper lyophilization, otherwise known as freeze drying, can reduce drying times by 30%.

Freezing Phase: A lyophilizer uses various methods to freeze the product. Freezing can be done in a freezer, a chilled bath (shell freezer), or on a shelf in the lyophilizer. The lyophilizer cools the material below its triple point to ensure that sublimation, rather than melting, will occur. This preserves the material's physical form.

A lyophilizer most easily freeze dries large ice crystals, which can be produced by slow freezing or annealing. However, with biological materials, when crystals are too large they may break the cell walls, and that leads to less-than-ideal freeze drying results. To prevent this, the freezing is done rapidly. For materials that tend to precipitate, annealing can be used. This process involves fast freezing, then raising the product temperature to allow the crystals to grow.

Primary Drying (Sublimation) Phase: A lyophilizer's second phase is primary drying (sublimation), in which the pressure is lowered and heat is added to the material in order for the water to sublime. The lyophilizer's vacuum speeds sublimation. The lyophilizer's cold condenser provides a surface for the water vapor to adhere and solidify. The condenser also protects the vacuum pump from the water vapor. About 95% of the water in the material is removed in this phase. Primary drying can be a slow process. Too much heat can alter the structure of the material.

Secondary Drying (Adsorption) Phase: A lyophilizer's final phase is secondary drying (adsorption), during which the ionically-bound water molecules are removed. By raising the temperature higher than in the primary drying phase, the bonds are broken between the material and the water molecules. Freeze dried materials retain a porous structure. After the lyophilizer completes its process, the vacuum can be broken with an inert gas before the material is sealed. Most materials can be dried to 1-5% residual moisture.

Lyophilizers are sophisticated instruments that facilitate the process of lyophilization. Lyophilization or freeze-drying is a precise and costly technique used for the preservation of products, such as astronaut food, biopharmaceuticals, food products, and museum exhibition objects.

Lyophilization is common, but cost intensive and hence one of the key objectives during freeze-drying process development is to minimize the drying time (mainly primary drying time, which is the longest of the three steps in freeze-drying). However, increasing the shelf temperature into secondary drying before all of the ice is removed from the product will likely cause collapse or eutectic melt. Thus, from product quality as well as process economics standpoint, it is very critical to detect the end of primary drying.



Figure 12: Freeze dryer

SOXHLET APPARATUS:

Soxhlet extraction is a modern extraction technique in which we circulate the same solvent through the extractor several times. It is a type of continuous extraction technique but we can call it a series of short maceration. Soxhlet extractor needs the desired compound to be soluble in the solvent at a high temperature. One cycle of the Soxhlet extraction method involves extraction following the evaporation of the solvent. And theoretically, we can perform this cycle as many times as we want to get the maximum yield of the desired compound.

Soxhlet extractor makes the extraction process much more efficient than that of the traditional method. The answer lies in the principle of Soxhlet extraction. So, in this article, we will demonstrate how the Soxhlet extraction apparatus works with an example, and its benefits and drawbacks, as well as modifications made to eliminate those drawbacks. Soxhlet extractor extracts the components using the condensed vapors of the solvent. The condensed vapors come in contact with the sample powder and the soluble part in the powder gets mixed with the solvent.

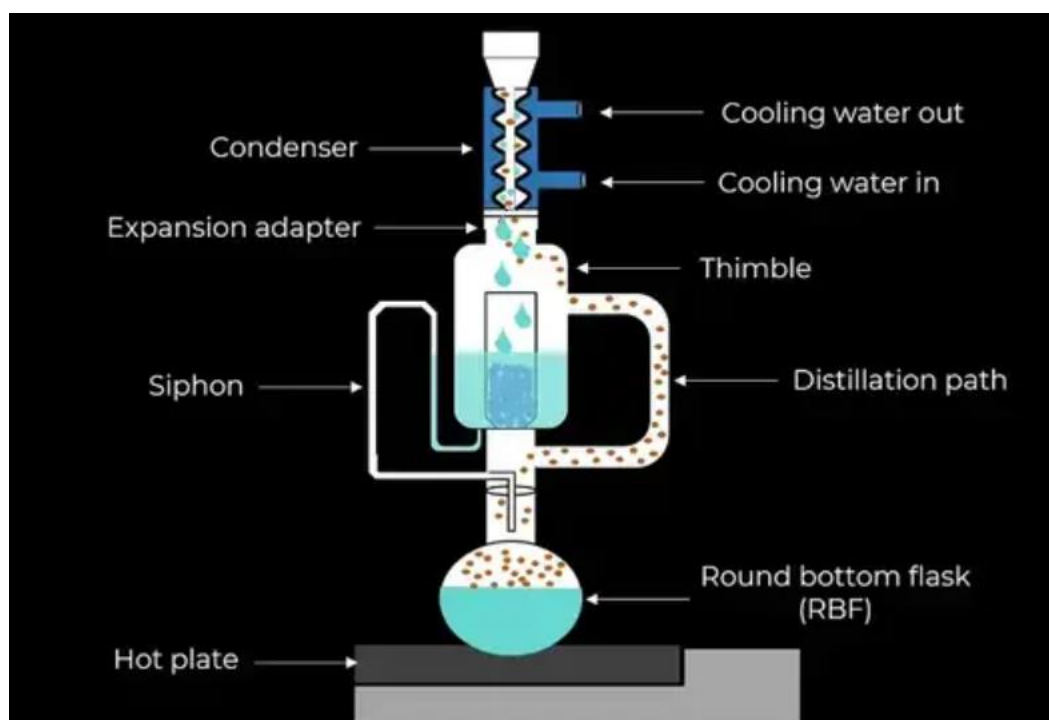


Figure 13: Soxhlet extraction assembly

Soxhlet extraction is one of several sample preparation methods that can be used to transfer targeted analytes from a sample matrix into a solvent, to permit subsequent analysis. With Soxhlet extraction, samples are repeatedly extracted with solvent regenerated by distillation through a periodic siphoning process. Because pure solvent is used for each extraction cycle, the removal of the analytes from the sample matrix is thermodynamically favored compared with simply soaking the sample with a single aliquot of solvent for the same period of time. This presentation will provide an introduction to the practice of 27erment extractions, and will demonstrate the technique for a typical sample.

The evolution of Soxhlet extractor is here critically discussed, and the conclusion from this overview is that the adoption of new technologies to improve its performance converts Soxhlet extraction in almost a “panacea” in this field.



Figure14: Ethnomedicine Soxhlet extraction

Solvent extraction of solid samples, which is commonly known as “solid–liquid extraction” but should rather be named as “leaching” or “lixiviation” to more strictly adhere to its physical–chemical foundation, is one of the oldest techniques of solid sample preparation. It serves, not only to remove and separate compounds of interest from insoluble high-molecular-weight fractions, but also from other compounds that could interfere with subsequent steps of the analytical process.

Classically, leaching has been widely carried out by maceration, based on the correct choice of solvents and the use of heat and/or agitation to increase the solubility of compounds and the rate of mass transfer. Despite the extensive use of maceration, particularly for isolation of natural products, this is characterized by long extraction protocols with low efficiency.

The Soxhlet extraction method uses a small amount of solvent and is very cost-effective. The Soxhlet extraction uses the solvent reflux and siphon principle to continuously extract the solid matter by pure solvent, which saves the solvent extraction efficiency and high efficiency. The solid sample is placed on a thimble-shaped filter paper, positioned into the Soxhlet extractor, and the device is assembled.

The solvent is added to the solvent reservoir flask and mounted onto a heating mantle. After heating, the condensed vapors of the solvent come in contact with the sample powder, and the soluble part of the powder gets mixed with the solvent for extraction. When the solvent surface exceeds the maximum height of the siphon, the solvent containing the extract is siphoned back. The flask is repeated, extracting a portion of the material each time so that the solid material is constantly used as a pure solvent and the extracted material is concentrated in the flask.

FERMENTOR:

A Fermentor is a device used to accomplish the fermentation process by using microorganisms, and for this reason, it is also called “**Biofermentor** or **Bioreactor**“. It is equipped with all the elements that are necessary to carry out the commercial production of substances like antibiotics, enzymes, beverages etc. in many industries. Fermentor is designed in such a way that a higher value of product could form by the microorganisms by utilizing a small amount of media.

It shows broad applicability in the food industry, wastewater treatment etc. Fermentation is the bioprocessing of the raw substrates through microbial biomass (mostly yeasts and bacteria).

The process is either carried out in a liquid substrate or anaerobic solids by utilizing which the microorganisms form a large quantity of desired metabolites. The fermented product can be collected from the bioreactor either at regular intervals (batch fermentation) or ongoing (continuous fermentation). In this context, we will discuss several contributions that led to the discovery of the fermentor along with the purpose, ideal properties, construction and different types of fermenters.

Ideal properties of a fermentor:

- A fermentor should be made of a good quality material that can withstand all the conditions inside the vessel.
- It should give high productivity.
- It should be able to handle the stream sterilization pressure.
- There should be all the control parameters to monitor the fermentation process like Ph electrode, temperature probe etc.
- A material used in the fermentor should be cheap that could give satisfactory results.

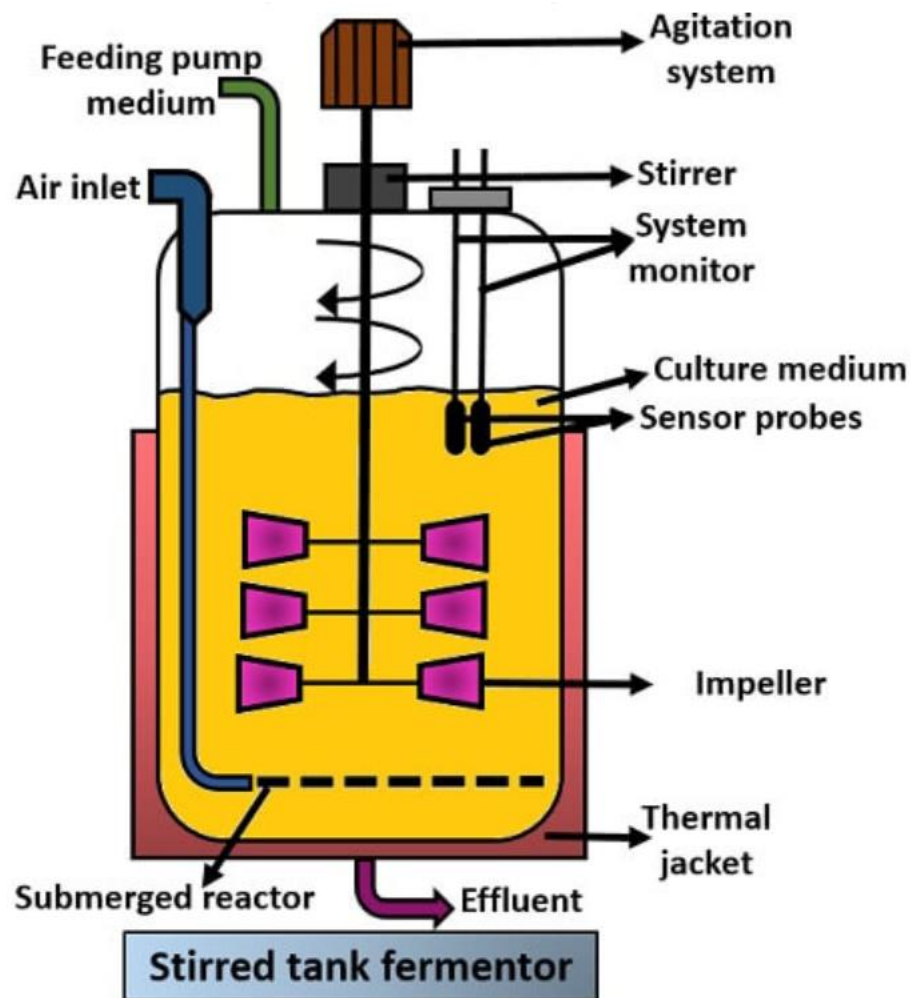


Figure 15: Stirred tank fermentor

A Fermentor can define as a closed cylindrical vessel which supports the biochemical and chemical activity of the microorganisms to carry the conversion of raw material into some useful product. It is also called a bioreactor, as it makes the use of microbial biomass to accomplish the fermentation process. Depending upon the use of stock culture, a fermentation process is subdivided into surface and submersion techniques. In surface fermentation, the biomass is cultivated on the surface of the raw substrate, while the microorganisms grow in the raw substrate during submersion fermentation.

PCR MACHINE:

[PCR](#) machine is also known as thermal cyclers or DNA amplifiers, or thermocyclers. PCR machines amplify small segments of DNA or RNA chosen from the genome with a primer. It is inexpensive and a very effective tool. It works under the concepts of complementary nucleic acid hybridization and [nucleic acid](#) replication to exponentially manufacture specific target DNA/RNA sequences by a factor of 10^7 in a couple of hours.

PCR machines are used in research labs and clinical diagnostics to replicate DNA, detect DNA sequences, perform DNA fingerprinting, forensic analysis, and molecular cloning, diagnose genetic diseases, and detect pathogens like Hep B and C viruses, HIV-1 causing AIDS, *Chlamydia trachomatis*, *Mycobacterium tuberculosis*, Human Papillomavirus, and Cytomegalovirus. This speed up other temperature-sensitive processes like restriction enzyme digestion or rapid diagnostics. These amplify segments of DNA using the polymerase chain reaction (PCR). PCR, sometimes known as “molecular photocopying”, is frequently hailed as one of the most significant scientific developments in molecular biology.

A single-stranded DNA template is used as a scaffold for the DNA polymerase enzyme, which guides the synthesis of DNA from deoxynucleotide substrates. When a specially created oligonucleotide is annealed to a longer template DNA. DNA polymerase adds nucleotides to the 3' end of the molecule. Thus, DNA polymerase can employ a synthetic oligonucleotide as a primer and extend its 3' end to create an extended stretch of double-stranded DNA when annealed to a single-stranded template with a region complementary to the oligonucleotide.

The polymerase chain reaction is a common technique practiced in genetic laboratories because it is a basic requirement for a genetic or molecular lab. Replication is a process of DNA synthesis, however, for us mimicking replication in a lab isn't possible.



Figure 16: PCR Machine

AGAROSE GEL ELECTROPHORESIS:

Agarose gel electrophoresis is a method of gel electrophoresis used in biochemistry, molecular biology, genetics, and clinical chemistry to separate a mixed population of macromolecules such as DNA or proteins in a matrix of agarose, one of the two main components of agar. The proteins may be separated by charge and/or size (isoelectric focusing agarose electrophoresis is essentially size independent).

Agarose gel electrophoresis is one of the most common electrophoresis techniques which is relatively simple and straightforward to perform but possesses great resolving power. The agarose gel consists of microscopic pores that act as a molecular sieve that separates molecules based on the charge, size, and shape.

Agarose gel electrophoresis is a powerful separation method frequently used to analyze DNA fragments generated by restriction enzymes, and it is a convenient analytical method for separating DNA fragments of varying sizes ranging from 100 bp to 25 kb.

DNA fragments smaller than 100 bp are more effectively separated using polyacrylamide gel electrophoresis whereas [pulse-field gel electrophoresis](#) is used to separate DNA fragments larger than 25 kb. Agarose gel electrophoresis can also be used to separate other charged biomolecules such as RNA and proteins.

The separation medium is a gel made from agarose. [Agarose is isolated from the seaweed genera *Gelidium* and *Gracilaria*](#) and consists of repeated agarobiose (L- and D-galactose) subunits. During gelation, agarose polymers associate non-covalently and form a network of bundles whose pore sizes determine a gel's molecular sieving properties. In general, the higher the concentration of agarose, the smaller the pore size.

To separate DNA using agarose gel electrophoresis, the DNA is loaded into pre-cast wells in the gel and a current is applied. The phosphate backbone of the DNA (and RNA) molecule is negatively charged, therefore when placed in an electric field, **DNA fragments will migrate to the positively charged anode**. Because DNA has a uniform mass/charge ratio, DNA molecules are separated by size.

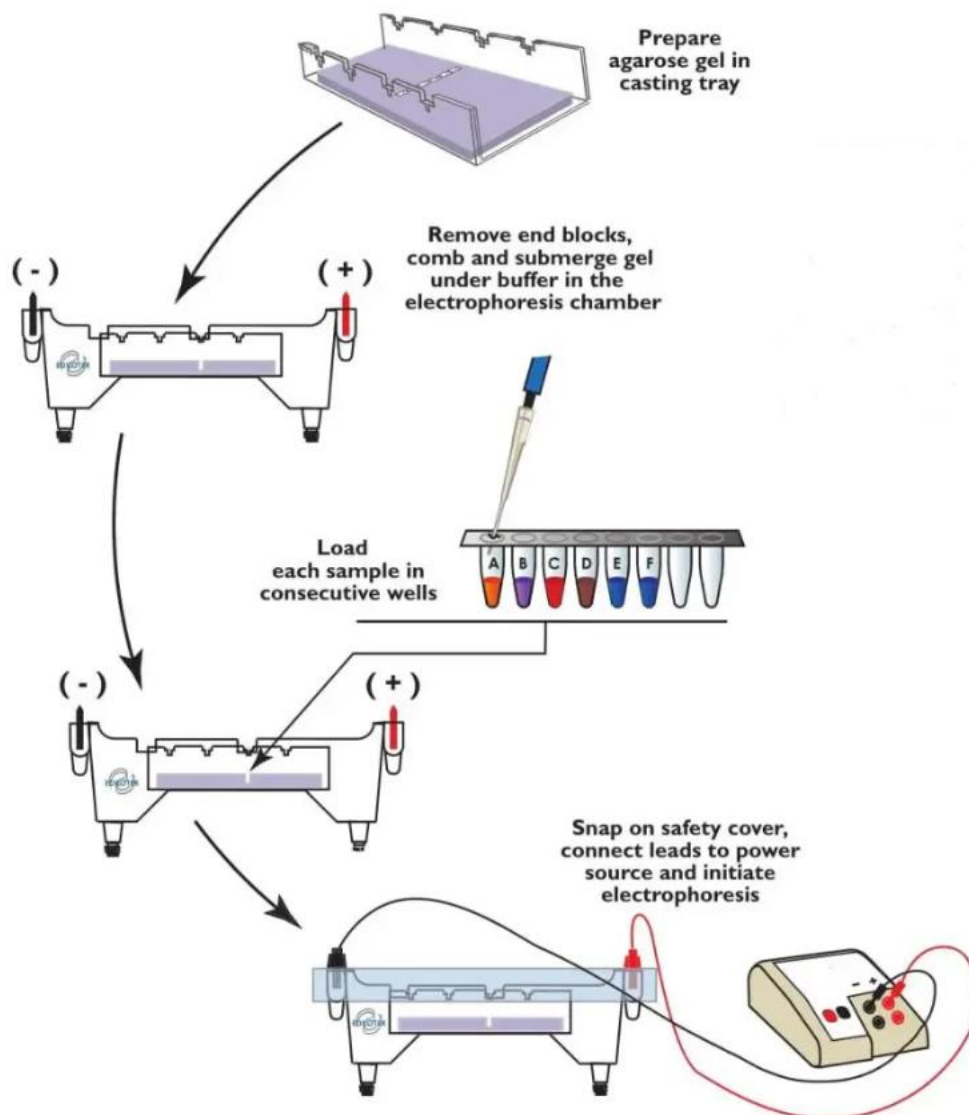


Figure 17: Agarose gel electrophoresis experiment overview

BIOFILM:

A biofilm comprises any syntrophic consortium of microorganisms in which cells stick to each other and often also to a surface. These adherent cells become embedded within a slimy extracellular matrix that is composed of extracellular polymeric substances (EPSs).

A biofilm is a thick layer of prokaryotic organisms that have aggregated to form a colony. The colony attaches to a surface with a slime layer which aids in protecting the microorganisms. There are a number of reasons why biofilms are formed, all of which promote growth and survival of the microorganisms.



Figure 18: Biofouling on ships

Biofouling, which is the build-up of microorganisms, plants, algae or small animals, is known to increase the roughness of the colonised surfaces. When those surfaces form the underwater parts of a ships' hull, the increased roughness will increase a ship's hydrodynamic drag. The immediate effect is a loss in ship speed at a constant power - or a power increase to maintain a constant speed.

Both of these have negative economic and environmental impacts through increased fuel consumption and atmospheric emissions, including GHG. For more severe biofouling conditions, such as a light layer of small calcareous growth (barnacles or tubeworms), an average length container ship can see an increase in GHG emissions of up to 55%, dependent on ship characteristics and speed.

Anti Fouling Paints are used to coat the underwater surfaces of ships to prevent sealife such as algae and molluscs attaching themselves to the hull, thereby slowing down the ship and increasing fuel consumption.

In the early days of sailing ships, lime and later arsenic were used to coat ships' hulls, until the modern chemicals industry developed effective anti-fouling paints using metallic compounds.

These compounds slowly "leach" into the sea water, preventing barnacles and other marine life from attaching to the ship. But studies have shown that these compounds persist in the water, killing sealife, harming the environment and entering the food chain.

One of the most effective biocides for use in anti-fouling paints, developed in the 1960s, was the organotin compound tributyltin (TBT), which has been proven to cause deformations in oysters and sex changes in whelks, as well as longer-term effects due to its persistence and bioaccumulation.

QUORUM SENSING:

Quorum sensing: cell-to-cell communication in bacteria Bacteria communicate with one another using chemical signal molecules. As in higher organisms, the information supplied by these molecules is critical for synchronizing the activities of large groups of cells.

Quorum sensing (QS) regulation allows bacteria to control their gene expression in response to their population density. The bacteria produce signaling molecules that accumulate during specific stages of growth, although their production level can also be influenced by the environment. At a threshold concentration, the signals activate a regulator that can induce or repress target genes

Bacterial cells within a population can act socially by coordinating their activities through cell-to-cell communication or 'quorum sensing'. This collective behavior depends on the production and extracellular release of small signal molecules. As the bacterial population density increases, so does the concentration of signal molecule such that the expression of quorum sensing-dependent target genes is activated (or repressed) once a critical threshold concentration of signal has been achieved. Quorum sensing permits a bacterial population to mount a cooperative response that improves access to nutrients, promotes defence against competitors, and survival in adverse environmental conditions.

Quorum sensing inhibitors target the communication system used by the biofilm community. Many of the virulence mechanisms produced by pathogenic microbes depend on communication between members of the biofilm. Gram- negative and positive bacteria as well as fungi utilize quorum sensing mechanisms.

Excessive and indiscriminate use of antibiotics to treat bacterial infections has led to the emergence of multiple drug resistant strains. Most infectious diseases are caused by bacteria which proliferate within quorum sensing (QS) mediated biofilms. Efforts to disrupt biofilms have enabled the identification of bioactive molecules produced by prokaryotes and eukaryotes.

These molecules act primarily by quenching the QS system. The phenomenon is also termed as quorum quenching (QQ). In addition, synthetic compounds have also been found to be effective in QQ. This review focuses primarily on natural and synthetic quorum sensing inhibitors (QSIs) with the potential for treating bacterial infections. It has been opined that the most versatile prokaryotes to produce QSI are likely to be those, which are generally regarded as safe.

Among the eukaryotes, certain legumes and traditional medicinal plants are likely to act as QSIs. Such findings are likely to lead to efficient treatments with much lower doses of drugs especially antibiotics than required at present. Most infectious diseases are caused by bacteria which proliferate within quorum sensing (QS) mediated biofilms. Efforts to disrupt biofilms have enabled the identification of bioactive molecules produced by prokaryotes and eukaryotes. These molecules act primarily by quenching the QS system. The phenomenon is also termed as quorum quenching (QQ). In addition, synthetic compounds have also been found to be effective in QQ. This review focuses primarily on natural and synthetic quorum sensing inhibitors (QSIs) with the potential for treating bacterial infections. It has been opined that the most versatile prokaryotes to produce QSI are likely to be those, which are generally regarded as safe.

SYNTHETIC AND NATURAL DYES:

Synthetic dyes are made from chemical compounds such as mercury, lead, chromium, copper, sodium chloride, toluene, or benzene. These are proven to be harmful to humans, especially those that work with them in production. They can also have a devastating impact on the environment – untreated dye that is dumped directly into waterways leads to water pollution. Plus, during the coloration process, a large percentage (10-15%) of dye used is released into the environment. When it gets into the water system of neighboring communities, it can pollute with toxic chemicals that can cause health problems, kill aquatic organisms and create concerns for surrounding farms.

Advantages of synthetic dyes:

- Consistent and reliable color results
- Achieve any color of your choice
- Cheaper to formulate than natural dyes
- Do not require the use of a mordant

Disadvantages of synthetic dyes:

- Contain harmful chemicals and carcinogenic components
- Can be absorbed into the skin and body and cause significant health issues – potentially leading to skin allergies and respiratory diseases
- Most are petroleum-based, making it difficult for wastewater from the dyeing process to be treated
- End up in oceans and water supplies through toxic runoff and residues from textile manufacturing and dyeing processes
- Cause harm to those that are involved in the production process and their surrounding communities

Natural dyes are colorants that are derived from plants, animals, fruits, insects, minerals, and other biological sources. They were initially popular in our history, but eventually synthetic dyes were favored due to their ease of use and color variability.

The natural coloration process can involve mordants and fixatives.

Some natural dyes require a mordant to adhere the dye to the fiber to prevent it from washing right out or fading away too soon. The use of a mordant will depend on the dye materials, fiber being dyed, and the length of time it takes for the dye to set.



Figure 19: Painting of art using natural dye

However, mordants are solutions that can contain dissolved metal oxides and since they are toxic, if not properly handled they can harm the dyer's health. The good news is that the use of copper, iron, tin, and chrome mordants have decreased over the years due to increased environmental awareness and health consciousness. Plus, certain mordants that are derived from plant sources (including oak galls and acorns) can work just as well on fibers. Alum, one of the most popular mordants used in the natural dyeing process, is one of the safest options.

Plant-based textiles (cotton, linens, etc.) may also require a fixative to help set the dye. Common fixatives are luckily natural materials, including salt, tannins, vinegar, and baking soda.

Advantages of natural dyes:

- Preserve the environment and are better for human health
- Lower human dependence on harmful products
- Biodegradable, compostable and degrade and decompose easily
- Non-toxic and hypoallergenic
- Contain antimicrobial properties
- Easy to extract natural color from plants, fruits, and flowers
- Higher UV absorption in fabrics they are used on – making them more protective for the skin from the sun's harmful rays
- Made from renewable resources

Disadvantages of natural dyes:

- More expensive than synthetic dyes – due to the source material and increased production time
- Color results can vary, depending on the fabrics and from crop season to crop season (but that's the beauty of nature!)
- Recipe standardization is difficult because color development depends on materials, colors and time

BIO SAFETY LAB:

The CDC [sets biosafety levels](#) to indicate what specific controls a laboratory must have in place for the containment of microbes and biological agents. Each biosafety level builds upon the previous level, thereby creating layer upon layer of constraints and barriers.

Each biosafety level — BSL-1 through BSL-4 — is defined based on the following:

- Risks related to containment
- Severity of infection
- Transmissibility
- Nature of the work conducted within the lab
- Origin of the microbe
- Agent in question
- Route of exposure

Biosafety levels Indictate the types of work practices allowed to take place in a lab setting; they also heavily influence the overall design of a facility and the type of specialized safety equipment in it.

Biosafety level 1 (BSL 1):

The lowest of the four biosafety levels, biosafety level 1 (BSL-1) applies to laboratory settings in which personnel work with low-risk microbes that pose little to no threat of infection in healthy adults — for example, a BSL-1 laboratory might work with a nonpathogenic strain of E.coli. BSL-1 labs typically conduct research on benches, do not use special contaminant equipment, and do not need to be isolated from surrounding facilities.

Biosafety level 2 (BSL 2):

Biosafety level 2 (BSL-2) covers all laboratories that work with agents associated with human diseases that is, pathogenic or infectious organisms that pose a moderate health hazard. Common examples of agents found in a BSL-2 lab include equine encephalitis viruses, HIV, and staphylococcus aureus (staph infections).

BSL-2 labs are required to maintain the same standard microbial practices as BSL-1 labs, as well as enhanced measures due to the potential risk the aforementioned microbes pose. Personnel working in biosafety level 2 laboratories are expected to take even greater care to prevent injuries, such as cuts and other breakage to the skin, as well as ingestion and mucous membrane exposures.

Biosafety Level 3 (BSL 3):

Once again building on the two prior biosafety levels, a biosafety level 3 (BSL-3) laboratory typically conducts research into or work on microbes that are either indigenous or exotic and can cause serious or potentially lethal disease through inhalation. Common examples of microbes found in BSL-3 labs include yellow fever, West Nile virus, and the bacteria that causes tuberculosis.

Microbes found within biosafety level 3 settings are so serious that work is often strictly controlled and registered through the appropriate government agencies. Laboratory personnel are also under medical surveillance and may require immunizations for the microbes they work with.

Biosafety level 4 (BSL-4):

Biosafety level 4 (BSL-4) labs are rare; however, a small number exist in the U.S. and around the world. As the highest level of biological safety, BSL-4 labs work with highly dangerous and exotic microbes, such as the Ebola and Marburg viruses. Infections caused by these types of microbes are often fatal and come without treatment or vaccines.

In addition to biosafety level 3 considerations, biosafety level 4 laboratories must follow these safety protocols:

- Personnel must change clothing before entering the facility and shower upon exiting
- All materials must be decontaminated before leaving the facility
- Personnel must wear the PPE from lower BSL levels, as well as a full-body, air-supplied.
- Access to a Class III biological safety cabinet.



Figure 20: Biosafety Laboratory

3D BIOPRINTING:

The field of tissue engineering has progressed tremendously over the past few decades in its ability to fabricate functional tissue substitutes for regenerative medicine and pharmaceutical research. Conventional scaffold-based approaches are limited in their capacity to produce constructs with the functionality and complexity of native tissue.

Three-dimensional (3D) bioprinting offers exciting prospects for scaffolds fabrication, as it allows precise placement of cells, biochemical factors, and biomaterials in a layer-by-layer process. Compared with traditional scaffold fabrication approaches, 3D bioprinting is better to mimic the complex microstructures of biological tissues and accurately control the distribution of cells. Here, we describe recent technological advances in bio-fabrication focusing on 3D bioprinting processes for tissue engineering from data processing to bioprinting, mainly inkjet, laser, and extrusion-based technique. We then review the associated bioink formulation for 3D bioprinting of human tissues, including biomaterials, cells, and growth factors selection. The key bioink properties for successful bioprinting of human tissue were summarized. After bioprinting, the cells are generally devoid of any exposure to fluid mechanical cues, such as fluid shear stress, tension, and compression, which are crucial for tissue development and function in health and disease.

The bioreactor can serve as a simulator to aid in the development of engineering human tissues from in vitro maturation of 3D cell-laden scaffolds. We then describe some of the most common bioreactors found in the engineering of several functional tissues, such as bone, cartilage, and cardiovascular applications. In the end, we conclude with a brief insight into present limitations and future developments on the application of 3D bioprinting and bioreactor systems for engineering human tissue.

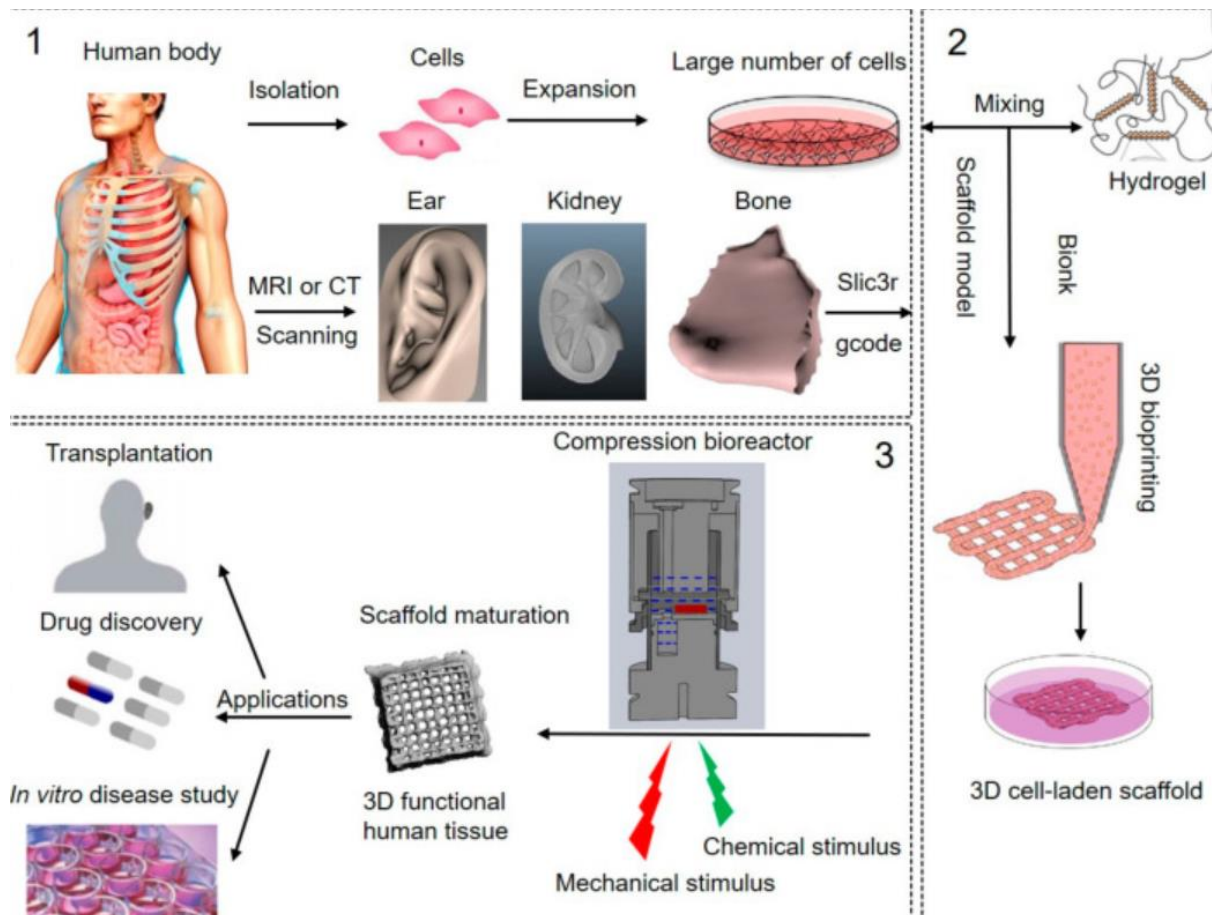
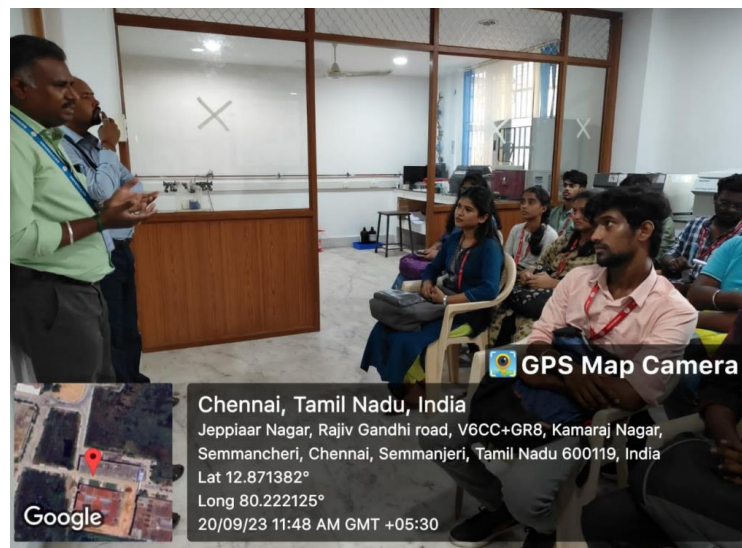


Figure 21: The process of 3D Bioprinting of Tissues

Bioprinting is a collection of additive manufacturing (AM) technologies, whose aim is to fabricate parts imitating real tissue and organ functionalities by combining both living and non-living materials in a specific three-dimensional (3D) spatial organization structure. As in traditional 3D printing or AM, the target is achieved through the use of computer-aided design (CAD) that represents the fundamental configuration of the target tissue or organ, in order to produce bioengineered structures that have various applications in regenerative medicine, tissue engineering, reconstructive surgery, drug discovery, pharmacokinetics, medical and basic cell-biology research

CONCLUSION:

During my internship experience at COL.Dr.Jeppiaar Research Park , I was able to develop my knowledge about different techniques and instruments related to bioprospecting and microbiology. I particularly found bioprospecting of actinobacteria and 3D Bioprinting to be useful in improving my knowledge. I found it to be valuable in developing my knowledge.



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