Maharani Lakshmi Ammanni College for Women **Autonomous**

Affiliated to Bengaluru City University
Re-accredited by NAAC with "A" grade, Recognised by UGC
under Section 2(f) and 12(b) of the UGC Act 1956
Conferred the Status of 'College with Potential for Excellence' by UGC

MAHARANI LAKSHMI AMMANNI COLLEGE FOR WOMEN AUTONOMOUS

ENTREPRUENERSHIP TRAINING PROGRAMME- MODULE- 1

IN

Production, extraction and partial purification of Industrially important enzymes from bacterial and fungal source and formulation of enzyme-based soaps and detergents

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Course Details	
Name of the course	**Entrepreneurship Training Program**
Course module :I	Production, extraction and partial purification of Industrially important enzymes from bacterial and fungal source and formulation of enzyme based soaps and detergents
Course Co-ordinator	1. Dr. Jolitha A.B, PG Biotech Coordinator, Dept. of Biotechnology, mLAC. 2. Dr. Nagalxmi B.N, HOD Dept. of chemistry, mLAC
Collaborating Faculty and Departments	1. Dr. K.M Harini Kumar, Prof, Dept.of Plant Biotechnology, University of agricultural Sciences, GKVK campus 2. Dr. Nagalaxmi B.N, HOD Dept. of chemistry, mLAC 3. Dr. Manjula K.R, Asst. prof. Dept. of Biotechnology, Reva University 4. Mr. Suraj Madhavan, Co-founder and Director Innvocept Solutions. Business Development and Project Management 5. Mrs. Vandana Parashar, BiSEP faculty, mLAC 6. Mrs. Chaya M.R, Associate Prof, Dept. of chemistry, KLE's Nijlingappa College, Bangalore 7. E- cell, mLAC
Stream **Subject**	Science Biotechnology/Biosciences
Duration	3 months
Eligibility	Any Life Science Undergraduate, Post Graduate students, UG/PG diploma students with minimum 55% in their qualified examination
Course Intake	20 students
Total hrs	72
Criteria for evaluation	1. Submission of business plan for the project

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	work undertaken 2. Viva- voce

Course Description

- **Note: on approval of the proposal, the progamme will tie-up with MSME approved industries for internship and product development**
- **The course is designed in consultation with the MSME Development institute, Ministry of MSME, Govt of India, Bangalore**

The objective of this program is to impart knowledge & skills in biotechnology or biosciences enabling them with tools and capabilities to own a Start-ups in this sector.

- The programme intends to give a conventional training in the entrepreneurship skills in enzyme/dye production and soap and detergent manufacturing
- It also aims at imparting innovative technology to bring novelty in their products like more eco-compatible, less carbon footprint etc.
- Emphasizes on students to think out of the box to integrate technology to make the product unique and consumer friendly

The course comprises of two modules THEORY and PRACTICAL hands-on skill training. Students will be introduced to the entire process of starting and nurturing a biotechnology company. Technical & financial skills to forecast growth & health of their company.

THE PRACTICAL COMPONENT HANDS-ON TRAINING ON

- Production, Extraction and Purification of commercially important enzymes from bacteria and fungus
- Production of soaps and detergents utilizing the extracted enzymes.
- Formulation of enzyme-based detergents/ cleaning solution (also other applications of the enzymes produced which will be given as a project to the students)
- Labelling, Packing and Marketing

Learning Objectives- Theory

- To familiarize students with various aspects of of entrepreunership skills
- Road map for bioscience students who are interested in owning a startup/ company
- Scope of Entrepreneurial ventures, forecasting, business problems faced during early stages of a start up
- Self-sustainability

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- Financial Management
- Insights into funding protocol and procedures
- IP, Legal and other issues related to bioentrepruenership

Learning outcome

- Analyze the business environment in order to identify business opportunities,
- Consider the legal and financial conditions for starting a business venture,
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- Critical analysis of the multifaceted application of the product manufactured
- Evaluate the effectiveness of different entrepreneurial strategies,
- Interpret their own business plan, construct a business model and team

Learning Objectives- Hands-on skill training

- Insight into the significance of R& D in bioentrepreunership programme
- Primary requirement to set up a production facility
- Importance of GLPand GMP and generation of SOP'S
- Selection, maintenance and costing of the raw materials used for production
- Importance of consistency in results for product quality, troubleshooting etc
- Costing analysis Scale up process and yield calculation

Learning Outcome- Hands-on skill training

- Stimulate innovative methods of production, reduce costs and improve product quality
- Planning and execution of and setting up an experiment from the start to finish
- Handling and maintenance of biological products such as enzymes, Instruments etc

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Details of Syllabus: Theory-24hrs

Unit	**Topics**	No. of lecture hours
1.	Introduction to bioentrepreneurship – biotechnology in a global scale; Importance of entrepreneurship; advantages of being entrepreneur	**02**
2.	Introduction to Biotechnology Industry freedom to operate; types of bio-industries – bioservices- bioindustrial, Agribio and biopharma and business incubators Entrepreneurship/intrepreneurship Translational biotechnology industry, insight into the commercialization pathways for drug, medical device, diagnostic companies	**4**
3.	Starting a Company/ Startup Commercialization Knowledge Survey (CKS) Corporate structure (LLC, LLP, C-Corp, S-Corp, etc.) ownership/vesting Regulations for transfer of profitable technologies; quality control; technology transfer agencies; Understanding of regulatory compliances and procedures	**4**
4.	Business basics The business model canvas Biotechnology business models accounting basics (financial statements) valuation (What's that company worth?) Business Strategy; Entry and exit strategy; pricing strategy; negotiations with financiers, bankers, government and law enforcement authorities; dispute resolution skills; global thinking; mergers & acquisitions.	**5**
5	Funding process Business plan preparation; business feasibility analysis by SWOT, socioeconomic costs benefit analysis; funds/support from Government agencies like MSME/banks and private agencies like venture capitalists, business plan proposal for virtual startup, statutory and legal requirements for starting a company/venture	**5**

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6.	**Intellectual Property and regulatory Strategies** **Basic concept of intellectual properties (patents, Trademarks, Copyrights)** **FDA, Drug control body, ISO etc** **Understanding of regulatory compliances and procedures (CDSCO, NBA, GCP, GLA, GMP).**	**4**

Course Plan			
	Hours	**Total duration**	**Total no. of hrs**
No theory/week	2hrs x 2 days =4hrs week	2 months	24 hrs
No. of Practical sessions/week	3hrs x 2days= 6 hrs/ week	2 months	48hrs
Project duration		1 months	1 months
Industry internship		10 days	10 days
Semester evaluation		15 days	15 days

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Skill development training – MODULE I

Production, extraction and partial purification of Industrially important enzymes from bacterial and fungal source and formulation of enzyme based soaps and detergents

- **Note: Along with the systematic approach towards formulating enzyme based detergents and other surface cleaning agents, the module will also train the other industrial applications of enzymes**
- **The module will aslo train the students in conceptualization of product improvement in terms of manufacturing**
- **surfactants and detergents that are eco-friendlier and less polluting the land and water bodies Incorporation of more organic based components in developing the product**
- **products that use less water consumption and less frothing**
- **Students will be asked to plan their R& D as a part of their dissertation and bring innovation in the product development**

Production, extraction and purification of enzymes from bacterial source				
Sl. no	**Organism**	**Source of organism**	**Enzymes**	**Type of fermentation**
1	***Bacillus subtilis*** will be used for demonstration (Subject to change as per the availability)	Soil, air, water (Different sources), sewage water, municipal waste, domestic waste and any other low cost resources	Alpha amylase Alkaline proteas Lipases	Submerged and solid state fermentation
2	***Aspergillus Sp.***	Soil, air, water (Different sources), sewage water, municipal waste, domestic waste and any other low cost resources	Alpha amylase Alkaline proteas Lipases	Solid state fermentation

Learning outcome of the module

- Techniques to isolate commercially important organisms from different sources (Air, Water,
- Soil, Sewage, waste, etc) and their characterization
- Lab scale and pilot scale up production of
- Different fermentation techniques- Solid state and submerged fermentation
- Downstream processing techniques
- Characterization of the enzymes
- Purification techniques for the enzymes

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enzymes and their optimization
- Enzyme characterization and kinetics
- Different purification techniques (Downstream Proccessing)
- Formulation of detergents and determination of their efficiency
- Production, extraction and purification of industrially important enzymes – amylase, proteases, lipases
- Inoculum characterization and preparation
- Screening techniques for microbes- Bacteria and fungi
- Media preparation, standardization for optimal production
- Setting up and fermentation process, trouble shooting and monitoring
- Immolization techniques
- Production of detergents- powder and liquid
- Compatability test for the enzymes with the detergents
- Determination of the efficiency of the enzyme based detergents
- Pilot scale up production of the enzyme- Batch fermentation
- Maintenance of records
- Quality check and consistency of the product
- Labelling and packing techniques of the product
- Costing, calculation of production rate with respect to the raw materials
- Profit analysis, market analysis, marketing and improvement strategies for their product

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Details of phase wise execution of the hands-on skill training

Phase-I- Isolation, screening and culturing of micro-organisms for enzyme production		
Objectives/action plan	**Details**	**Observation/comments**
a) Isolation, screening and growth of the amylase/alkaline protease and amylase producing organisms- fungi and bacteria	i) Media for screening amylase/ alkaline protease and lipase producing organisms – starch agar media ii) Media preparation and standardization of media for optimum growth of pure colonies iii) characterization and confirmation of the colonies	organisms in the starch – agar media that produce clear zone around the colonies when iodine solution, was added were selected as amylase producing organisms Casien hydrolysis will be taken as an indicator for alkaline protease producing organisms Fatty acid hydrolysis in the media will be used for sceening lipase enzyme producing organisms
b) production of enzymes from the isolated colonies	i) Inoculum and primary culture preparation for – Alpha Amylase Alkaline Protease Lipases ii) standardization and optimization of physical parameters like, Temp, pH, nutrient requirement, optimum production time/day. For enzyme production, organisms will be cultured in 250ml Erlenmeyer flasks containing 50 ml of the liquid medium (the medium for optimum amylase/alkaline protease and lipase production). 1% (v/v) inoculum will be added and incubated at 37ºC for 24h on a rotary shaker (200 rpm). Production media was supplemented with different carbon and nitrogen source such as sucrose, glucose, lactose, peptone, yeast extract and ammonium sulphate at different concentration (1– 5%) to determine the highest yield of enzymes	Spore suspension will be used for fungal cultures After inoculating the media with the culture, the media will be kept at 37∘ C for 48 hr and the O.D will be checked. Inoculum having OD equivalent to O.D600 = 0.450 will be considered for the primary culture The nutritional and the physical parameters would be recorded and used for the scale up process
c)extraction and	The culture supernatants after centrifugation (5000xg,	Enzyme assay will be done for

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determination of activity of the enzymes	for 10 min) will be used for the enzyme assays	different time interval like 2 days 5 days 7 days etc and the optimal production time will be recorded
d) Partial purification of the extracted crude enzymes by ammonium sulphate precipitation method	Culture medium for each enzyme production will be taken, cells were separated by centrifugation at 5000 rpm for 30 min. The supernatant containing the enzyme will be concentrated using addition of 10–100% ammonium sulphate precipitation. Fractionated enzyme samples were then subjected to dialysis process for further purification Protein estimation will be performed by lowry's method to check the purity	Different percentatages of ammonium sulphate will be testes Organic solvent precipitation will also be perfomed and the methods will be standardized
Pilot scale up production of the enzyme	Production enzymes by pilot scale fermentor high lipase / protease amd amylase producing protocol will be incorporated for batch fermentation in 5litre pilot scale fermentor. Optimized protocol, 2.5 L of enzyme producing medium will be sterilized at 121 C for 20 minutes and then sterilized media was poured into the fermentor containment system under aseptic condition. bacterial inoculum as optimized will be added and Standard operation conditions were: agitation rate 150–400 rpm, temperature 37 C, air-flow rate is 1 vvm and fermentation time 48 h, with pH 6 ± 0.5 will be monitored. Samples (20 ml) were taken at regular intervals, cells were removed by centrifugation at 5000 rpm for 30 min, and the culture supernatants will be evaluated for enzyme activity. Biomass concentration will be measured by turbidimetry at 610 nm in a colorimeter	Standard operating conditions will be maintained as optimized for different enzymes in the scale up fermentation process.

Phase II- Determination and optimization of the microbial enzyme activity	
Objective/ action plan	**Details**

a) Determination of Alpha amylase activity	Alpha amylase activity of the extract was measured by DNS method [30]. In brief the reaction mixture containing 1% soluble starch, 20 mM phosphate buffer (pH = 7), and fermented extract was taken and incubates at 37∘ C for 20 minutes followed by the addition of 3,5-dinitrosalicylic acid (DNS). The amount of the reducing sugar liberated during assay was estimated by measuring color development at 540 nm by UV-VIS spectrophotometer. 1U of amylase activity is defined as the amount of enzyme that liberated micromole of maltose per minute under standard assay condition
b) Determination of alkaline protease activity	**isoelectric precipitation for isolation and determination crude alkaline protease** alkaline proteases are active only above pH 8. 0.5 N NaOH will be used to raise the pH of the filtrate. 0.5 N NaOH will be added dropwise with continuous stirring of the filtrate till precipitates began (isoelectric point). The pH of the solution will be noted. Isoelectric point of alkaline protease from bacterial source is around 10 and from fungi is 9. The filtrates containing the precipitates were allowed to stand for 30 min, after which the protein free solution in the upper region of the beaker will be decanted.The will be transferred into Eppendorf tubes and subjected to centrifugation at 3000 rpm for 10 min. the precipitate will be dissolved in suitable buffer Ninhydrin test will be performed to detect hydrolysis of casein by enzyme alkaline protease
c) Determination of Lipase activity	Lipase activity will be deter-mined spectrophotometrically using p-nitrophenol palmitate (pNPP) as substrate. The absorbance will be measured at 410 nm for the first 2 min of reaction. One unit (1U) was defined as that amount of enzyme that liberated 1µmol of *p*NPP per minute
Note: The isolation and enzyme activity will be determined at different pH, substrate concentration and temperature. **The thermal stability, shelf life (Activity when stored at different Temp and other physical conditions also will be studied and optimized)**	

Phase- III production of soaps and detergents	
Objectives	**details**
a) Preparation of Detergents (synthetic)	Place 5 ml of dodecanol into a 100-mL beaker, 2 mL of concentrated sulfuric acid, H2SO4will be

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	added stirring allow the mixture to stand for 10 minutes. Into a 250-mL beaker fill one-third full of ice, add about 10 g of sodium chloride and mix thoroughly. Make up the volume to 75 ml. Mix 5 ml of 6 M sodium hydroxide with 10 mL of water in a small beaker, add 4 drops of phenolphthalein indicator as the pink color of the phenolphthalein begin to fade, pour the sodium hydroxide solution into the dodecanol-sulfuric acid mixture and stir until the pink colour disappears. After the 10 minutes pour the detergent mixture into the ice-salt bath the powder detergents will be precipitated, Stir to break up large lumps of detergent. Filter the precipitated detergent mixture through 2-3 layers of cheesecloth .Wash the collected detergent twice with 10 ml portions of ice-cold water squeeze excess water from the solid detergent.
b) Preparation of soaps	**saponification of fatty materials,** oils, greases, or tallows are "saponified" - by soda (to obtain hard soap) or potash (to obtain soft or liquid soap). Apart from the soap, a by-product - *glycerine* - forms during the chemical reaction. This may be separated off or may be left, depending on the nature of the manufacturing process. 10 g of the oil was measured will be warmed, and added to 0.2N NaOH solution. The caustic will be added and stirred well using a stirring rod until it blends with the fat. Small amount of sodium carbonate, sodium sulphate and sodium silicate will be added into the soap mixture and stirred properly until it blends. The mixture will be heated in a heating tub to 110^0C. molten Sodium sulphate is added during the soap is clarified.Sodium silicate is added when the soap is being cooled down. Sodium silicate hardens the soap. Ribbon test' to check the completion of the saponification process. physical test - the taste test – was also done to

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	determine the level of caustic. After this the soap is mould into a proper shape and kept in a filter paper. This soap is taken in air oven to dry up the moisture. Before keeping in the oven the weight of soap is taken. Soap is kept for 12 hrs in the oven maintained at 300-3500C. The weight of the soap is again taken. Yield is calculated by dividing the weight of the soap by the weight of the oil taken multiplied by 100.
The preparation of soap can be done in coconut oil, palm kernel oil, animal fats, oil waste, factory grease for student assignments	
c) Addition of the additives to the detergent	1. Surfuctants-alkyl benzene sulphonate(8-18%) **2.** Builders (20-45%)- Zeolite, sodium dipolyphosphates **3.** Bleaches (15-30%)- sodium perborate **4.** Fluorescers (0.1%)- fluorescent dyes **5.** Fillers (5-45%)- sodium sulphate, sodium silicate **6.** Suspension agents- carboxy methyl cellulose **7.** Foam control agents- silicones
d) Test for the properties of soaps and detergents	1. Emulsifying properties 2. Behavior in hard water 3. Test for alkalinity 4. Test for reaction with mineral acids
Phase-IV Immobilization of the enzymes and compatibility test with detergents	
Objectives	**Details**
a) Standardization of the dilution of enzymes with suitable buffer	i) Enzymes will be diluted and their activity will be determined after dilution before subjecting to immobilization ii) Different buffers, pH will be used to determine the optimum activity and dilution
b) Immobilization of the microbial enzymes by sodium alginate-calcium chloride entrapment method	Enzyme diluted with respective buffer will be pre-incubated for 20 min at 37 ^{o}C. To this, equal volume of 2% sodium alginate will be added and mixed. Using a micropipette, this solution will be

	transferred in drops into ice cold 0.1 M CaCl2 solution. The beads so formed were collected and stored for further assays. The activity of the immobilized enzymes will be determined as mentioned earlier.
c) Study Effect of pH, Temperature and substrate on the immobilized enzymes	i. For temperature studies, the incubation time of immobilized enzyme- substrate mixture will be incubated for 20 min at different temperatures from 15º C, 20 º C 30 º C, 35 º C, 40 º C, 45 º C, 50 º C, 55 º C 60 º C, 65 º C and 70 º C followed by the assay of enzyme activities ii. Different time intervals ranging from 20 – 60 min for the incubation at different temperature will also be assessed iii. Effect of pH will be studied studied with potassium phosphate and sodium acetate buffers of pH 7, pH 8, pH 9, pH 10 and pH 11
d) To study the effect of organic solvents and metal salts on the immobilized enzymes	i. To check the organic solvent stability of enzyme, check the organic solvent stability of enzymes, isopropanol, methanol, hexane, ethyl acetate and petroleum ether will be incubated with the immobilized enzyme beads followed by the enzyme activity determination. 20, 50, 70 and 100% solvents will be used ii. The effects of various metal salts such as CaCl , MgSO , KCl, NaCl and CuSO at 1 and 3 mM concentrations will be studied. The results will be obtained at different temp, PH and time duration for solvent and metal salts compatability
e) Study of detergent- immobilized enzyme compatibility	i. Compatibility of immobilized enzyme with lab based detergents like Tween 20, SDS and Triton X-100

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	ii. Compatibility test of the immobilized enzymes with commercial detergents like Surf Excel, Wheel, Ariel and Patanjali and other detergents as described above iii. Compatibility of immobilized enzymes for non commercial detergents (indegeneously manufactured in the skill development program.) To 5% solutions of detergents, Immobilized enzyme beads will be added and incubated for different time intervals, temperature, pH, followed by the determination of enzyme activity as mentioned earlier.
f) Wash perform analysis of the enzyme-detergent mixture Efficiency test for stain removal	i. Efficiency of amylase, Alkaline protease and lipase in cleaning or removal of the stain will be determined by placing on small square white cotton cloth pieces (5x5 cm) stained with a) Gravy/ ketchup b) baby food / chocolate c) Blood stain d) Grease/oil e) Vegetable/ fruit pulp The stained cloth pieces will be allowed to sit overnight then 1) each cloth piece will be taken in a separate100 ml conical flask, to which commercial detergents will be added and incubated 2) stained cloth pieces + individual enzyme – commercial detergent complex 3) Stained cloth pieces+ Mixture of enzyme-commercial detergent complex 4) Stained cloth pieces+ Indegeneous detergent-enzyme complex All the flasks will be incubated from 10 – 60 min at different temperatures and pH The test will be performed for different fabrics and water samples

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	The wash performance will be analyzed on reflectance meter. The relative reflectance of all pieces will be examined to test the efficiency of enzymes to remove the stains
Reusability: In order to test the reusability of enzymes entrapped in calcium alginate beads, the beads will be repeatedly used for the hydrolysis reaction and enzyme activity will be determined.	

Phase-V- commercial formulation of the enzyme based soap and detergents, labelling, packing	
Objectives	**Details**
Manufacture of enzyme based soaps and detergents	Based on the skill training, manufacturing of the enzyme based detergent Preparation of the SOP Quality, safety analysis Costing analysis, yield estimation market analysis
Labelling of the product	Formulation of ingredients Labelling techniques stickering
Packaging	Selection of suitable packing materials Packaging techniques

Phase-VI- Industrial Internship, Project dissertation and Business proposal write-up	
Objectives	**Details**
Industrial Internship	10 days industrial internship programme with the collaborated industries
Project	**Students will be assigned projects based on the skill development training**
Business proposal write up	Students will be asked to write up a business proposal to evaluate the overall outcome of the training and encouraged to submit for a funding agencies

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