NORTH MAHARASHTRA UNIVERSITY,

JALGAON (M.S.)

Third Year Biotechnology

Faculty of Engineering and Technology

Teacher and Examiner's Manual

SEM – V

W.E.F 2014 – 2015

Bioprocess Instrumentation & Analysis

Teacher, Paper setter and Examiner should follow the guidelines as given below.

Unit – I

Teacher should facilitate learning of temperature measurement techniques and elements of instruments.

1.			**Lecture required**	**References**
	a	Qualities of Measurement: The meaning of measurement, the elements of instruments	02	1,2
	b	Expansion Thermometers: Introduction, Constant volume gas thermometer, Bimetallic Thermometer, Industrial pressure spring thermometer, Response of Thermometer. Thermoelectric	02	1,2
	c	Temperature Measurement: Introduction, Simple thermocouple circuit	02	1,2
	d	Industrial thermocouples, Thermocouple lead wires, thermal wells, Response of thermocouples. Resistance temperature detector	02	1,2
Guidelines for the examiner and paper setter. 1) Numerical should not be asked on above topics.				

Unit – II

Teacher should facilitate learning of pressure and level measurement techniques.

2.			**Lecture required**	**References**
	a	Pressure and Vacuum Measurement: Introduction, Indicating pressure gage, Bellows pressure element	02	1,2
	b	Useful ranges of absolute pressure measuring gages, Mclead vacuum gage.	02	1,2
	c	Measurement of Level: Float and tape liquid level gage, Float and shaft liquid level unit	02	1,2
	d	Level measurement in pressure vessels, Gamma ray method, Ultrasonic method and resistive method.	02	1,2
Guidelines for the examiner and paper setter. 1. Numerical may be asked on above topics.				

Unit – III

Teacher should facilitate learning of pH measurement, Infrared Spectroscopy, X- ray diffraction.

3.			**Lecture required**	**References**
	a	pH measurement: Introduction , Method of P^H Indicator, Application of P^H Measurement.	02	1,2,4
	b	Potentiometric Method	02	1,2,3
	c	Infrared Spectroscopy: Introduction, Instrumentation, Application of Infrared spectroscopy.	02	1,2,4
	d	X- ray diffraction: Introduction, Application of X- ray diffraction	02	1,2,4
Guidelines for the examiner and paper setter. 1) Numerical should not be asked on above topics.				

Unit – IV

Teacher should facilitate learning of Refractrometry, UV Spectrophotometer, and Colorimetry.

4.		Refractrometry	Lecture required	References
	a	Refractrometry: Introduction, Abbe refractometer, Application of refractometer	02	1,2,3
	b	UV Spectrophotometer: Introduction, Instrumentation, Application of UV Spectrophotometer	03	1,2,4
	c	Colorimetery: Introduction, Theory.	03	1,2,4

Guidelines for the examiner and paper setter.

1) Numerical should not be asked on above topics.

Unit - V

Teacher should facilitate learning of Flame photometry, Scanning Electron Microscope, Transmission Electron Microscope.

5.		Flame photometry	Lecture required	References
	a	Flame photometry: Introduction, Instrumentation, Application of Flame photometry	02	1,2,3
	b	Scanning Electron Microscope: Introduction, Instrumentation, Application of Scanning Electron Microscope	03	1,2,4
	c	Transmission Electron Microscope: Introduction, Instrumentation, Application of Transmission Electron Microscope.	03	1,2,4

Guidelines for the examiner and paper setter.

1. Numerical should not be asked on above topics.

References:

1. D.P.Eckman, Industrial Instrumentation, Willey Eastern Ltd., New Delhi
2. Gurdeep Chatwal and Sham Anand, Instrumental methods of Chemical analysis, Himalaya publication House, Mumbai.
3. Patranabis D. Industrial Instrumentation, Tata – Mcgraw Hill Publications, New Delhi.
4. Nakra B.C. and K.K. Chaudhary, Instrumentation Measurement and Analysis, Tata – McGraw Hill, New Delhi.

Molecular Biology

Teacher, Paper setter and Examiner should follow the guidelines as given below:

Unit- I
Teacher should facilitate learning of genetic materials and their properties.

I	Introduction to Genetic Material	Lectures required	Reference
	Nucleic acids, DNA Chemical Composition, Chargoffs Equimolar Base Ratio	01	1-2
	Molecular Structure of DNA, Watson and Crick Double Helical Model of DNA	02	1-2
	Forms of DNA (B-DNA, A-DNA, C-DNA, D-DNA, E-DNA, Z-DNA)	01	1-2
	RNA: Occurrence, Types of RNA: rRNA, tRNA, mRNA	02	1-2
	Structure of ribosomes. Central Dogma, One Gene – One Polypeptide Hypothesis.	02	1-2

Unit- II:
Teacher should facilitate learning of DNA replication.

II	DNA Replication	Lectures required	Reference
	Replication: Overview, Basic rules and requirements of Replication	01	1,2,4
	Types of DNA replication: Generalized Model for the DNA replication, Semi conservative method of replication	02	1,2,4
	Meselson and Stahl experiment, bidirectional DNA replication, Molecular mechanism of DNA replication	02	1,2,4
	Enzymes and proteins involved in DNA replication: Structure and functions of DNA polymerase I,II,III, primase, polynucleotide ligase, endonuclease, helicase, single stranded binding proteins, topoisomerase	01	1,2,4
	Replication Models: Theta replication model, Rolling circle Model, D-Loop Model.	02	1,2,4

Unit- III
Teacher should facilitate learning of Transcription mechanism of prokaryotes and in Eukaryotes.

III	Transcription	Lectures required	Reference
	Transcription, Mechanism of Transcription in Prokaryotes, RNA polymerase of prokaryotes (structure, types and function)	01	1,2,4
	Transcription Unit, Promoter Site, Molecular Mechanism of Transcription in Prokaryotes	02	1,2,4
	Molecular Mechanism of Transcription in Eukaryotes, RNA polymerase of Eukaryotes (structure, types and function), Transcription Factors, Eukaryotic promoters	02	1,2,4
	RNA processing/Post transcriptional modification: Introduction, processing of the pre rRNA, tRNA, and the mRNA transcript(eukaryotic), RNA splicing (mechanism).	02	1,2,4

Unit- IV

Teacher should facilitate learning of Genetic code and protein synthesis.

IV	Genetic Code and Protein Synthesis	Lectures required	Reference
	Genetic code: Nature and characteristics of Genetic Code, Reasons for degeneracy, Biological Significance of Degeneracy of Genetic Code	01	1,2,3
	Mechanism of protein synthesis: Transcription Overview	01	1,2,3
	Translation: Activation of the amino acids, attachment of activated amino acids with tRNA, stages during translation,	02	1,2,3
	Translation in Prokaryotes and Eukaryotes, Translocation of proteins	02	1,2,3
	Post translational processing of Proteins (Protein Folding and Biochemical Modifications)	02	1,2,3

Unit- V

Teacher should facilitate learning of regulation of gene expression and DNA Damage and its repair.

V	Regulation of gene expression & DNA damage and repair	Lectures required	Reference
	Gene regulation in prokaryotes, Mechanisms of gene regulation at Transcription level, Induction and repression,	02	1,2
	Lac Operon System, Tryptophan Operon System,	02	1,2
	Gene regulation and Translation level, Gene regulation in eukaryotes,	02	1,2
	Types of damages, damaging agents,	01	1,2
	Repair mechanisms - photoreactivation, dark repair, post-replicational recombination repair, SOS repair.	01	1,2

Reference books:

1. Fundamentals of Molecular Biology by Veer Bala Rastogi; Ane Books Pvt. Ltd
2. Cell and Molecular Biology by P.K.Gupta, Third Edition, Rastogi Publications
3. Molecular Biology of cell – Lodish et al
4. Genes and Genomes – Singer M and Berg P.

Chemical Reaction Engineering

Teacher, Paper setter and Examiner should follow the guidelines as given below.

Unit – I

Teacher should facilitate learning of Classification of chemical reaction, reaction mechanism

1.		**Introduction to chemical reaction engineering**	**Lecture required**	**References**
	a	Introduction to chemical reaction engineering: Classification of chemical reaction, comparison of theories	02	1,2,4
	b	rate of reaction, order and molecularity of reaction, rate constant	02	1,2,4
	c	Activation energy, transition state theory and temperature dependency	02	1,2,4
	d	Reaction mechanism.	02	1,2,4
Guidelines for the examiner and paper setter. 1) Numerical based on above topics.				

Unit – II

Teacher should facilitate learning of kinetic study of different reactions and its applications in process industries.

2.		**Collection and interpretation of kinetic data**	**Lecture required**	**References**
	a	Collection and interpretation of kinetic data	02	1,2,4
	b	Integral and differential method of analysis of data	02	1,2,4
	c	Half life method , Constant volume batch reactor	01	1,2,4
	d	Variable volume batch reactor	01	1,2,4
Guidelines for the examiner and paper setter. 1) Numerical based on above topic.				

Unit – III

Teacher should facilitate learning of types of reactors and its application in process industries.

3.			**Lecture required**	**References**
	a	Ideal reactors, mixed flow reactor	02	1,2,3
	b	Plug flow reactor, space time and space velocity	02	1,2,3
	c	Holding time and space time , comparison in mixed and plug flow reactors	01	1,2,3
	d	Recycle reactor, Autocatalytic reaction.	02	1,2,5
Guidelines for the examiner and paper setter. Numerical based on above topics.				

Unit – IV

Teacher should facilitate learning of Residence time distribution.

4.			**Lecture required**	**References**
	a	Residence time distribution of fluid in vessel	02	1,2,4
	b	Conversion directly from tracer information, Models for non-ideal flow	02	1,2,3
	c	Dispersion models, Tank in series model	02	1,2,3
	d	Concept of micro and macro mixing.	02	1,2,4
Guidelines for the examiner and paper setter. 1. Numerical should be asked on above Topics. 2. No numerical should be asked on micro and macro mixing.				

Unit – V

Teacher should facilitate learning of heterogeneous systems and its application in process industries.

5.			**Lecture required**	**References**
	a	Introduction – Rate equations for heterogeneous systems	03	1,2,5
	b	Contacting patterns in Two –Phase system ,Introduction to fluid particle reaction non-catalytic reactions	01	1,2,5
	c	Un reacted core model for Spherical particle of unchanging size	01	1,2,4
	d	Rate of reaction for shrinking spherical particles, Determination of rate controlling step	02	1,2,4
	e	Various contacting patterns in fluid solid reactors for fluid-particle non-catalytic reactions.	01	1,2,5
Guidelines for the examiner and paper setter. 1. Numerical should be asked on above Topics.				

References:

1. H. Scott Fogler, Elements of chemical reaction engineering, Prentice Hall New, Jersy.
2. Octave Levenspiel, Chemical reaction engineering, John Wiley and sons.
3. J.M. Smith, Chemical engineering kinetics, McGraw Hill
4. S.D. Dawande, Principles of reaction engineering, Central Techno publication, Nagpur.
5. Lanny D. Schimdt , Chemical reaction engineering, Oxford University Press.

Enzyme Engineering

Teacher, Paper setter and Examiner should follow the guidelines as given below:

Unit- I

Teacher should facilitate learning of basics of enzymes, its classification and structure.

I	Enzymes	Lectures required	Reference
	Classification, nomenclature, International units and types of enzymes	01	1,2,4
	General characters of enzymes: characters such as specificity, catalysis and regulation and localization of enzymes in the cell	03	2
	Structure of enzymes: Primary, secondary and tertiary structure of enzyme	01	1,2,4
	Models of enzyme activity: Lock and key model, Induced fit, Substrate Strain model	02	1,2
	Isoenzyme, with example and its application.	01	1,2

Unit- II

Teacher should facilitate learning of Enzyme kinetics equations.

II	Enzyme Kinetics	Lectures required	Reference
	Introduction to kinetics: activation energy, transition state theory and energy, consideration	01	1,2
	Enzyme kinetics, rate equation, Rate of reaction, First order and second order reaction	01	1,2
	Michaelis – menten equation (Steady state kinetics) and Haldane relationship, Significance of Km	03	1,2,3
	Lineweaver – Burk or Double – reciprocal plot, Eadie-Hofstee plot, Hanes plot	02	1,2,3
	Turnover number, Specificity constant, Bisubstrate reaction.	01	1,2,3

Unit- III

Teacher should facilitate learning of Enzyme inhibition, its kinetics and Catalysis.

III	Enzyme inhibition, its kinetics and Catalysis	Lectures required	Reference
	Types of inhibition- Reversible and irreversible inhibition, Kinetics of inhibition	01	2
	Catalytic efficiency- proximity and orientation effects, distortion or strain	01	2
	Different mechanisms of enzyme catalysis, acid base and covalent catalysis and metal-ion catalysis	02	2
	Molecular mechanism of action of chymotrypsin, Lysozyme	02	2
	Chemical modification of enzymes, Bisubstrate or Multisubstrate reaction: Ping – Pong mechanism, sequential mechanism	02	2

Unit- IV

Teacher should facilitate learning of Allosteric and regulatory enzyme, enzyme production and purification

IV	Allosteric and regulatory enzyme, enzyme production and purification	Lectures required	Reference
	Binding of ligands to Protein, Co-operativity models- MWC and KNF model	01	2
	Regulations by allosteric enzymes, other mechanisms of enzyme regulation-enzyme induction and repression and covalent modification	01	2
	Sources of enzymes-animal plant and microbial sources, large scale production of enzymes	02	1,2,4
	Basic methodology of production, extraction and purification of enzymes	02	1,2,4
	Enzyme production and recombinant DNA technology	02	1,2

Unit- V

Teacher should facilitate learning Enzyme immobilization and Enzyme applications

V	Enzyme immobilization and Enzyme applications	Lectures required	Reference
	Methods of immobilization - ionic bonding, adsorption, covalent bonding (based on R groups of amino acids), and microencapsulation and gel entrapment	02	1,2,4
	Properties of immobilized enzymes, Applications of immobilized enzymes.	02	1,2,4
	Applications of enzymes in food, sugar, leather, detergent industries etc.,	02	1,2
	Uses of enzymes in drug, medicine, industries	01	1,2
	Uses of enzymes to make amino acids and peptides, Legislative and safety aspects.	01	1,2

References:

1. Lehninger, Nelson and cox. Principles of Biochemistry –Macmillan publishers..
2. Palmer, Enzymes, Oxford University press.
3. Pauline M. Doran, Bioprocess Engineering Principles, Academic Press an Imprint of Elsevier.
4. Textbook of Biotechnology by B.D.Singh, Kalyani Publication.

Bioprocess Industrial Economics and Management

Teacher, Paper setter and Examiner should follow the guidelines as given below:

Unit- I

Teacher should facilitate learning of Bio process Design Considerations

I	Bio process Design Considerations	Lectures required	Reference
	Technical feasibility survey, process development, flow diagram, equipment design and specifications	01	1,2,3
	Marketability of product, availability of technology, raw materials, equipments, human resources, land and utilities, site characteristics	02	1,2,3
	Waste disposal, government regulations and other legal restrictions	01	1,2,3
	Community factors and other factors affecting investment and production cost	02	1,2,3
	Indian Bioprocess Industry - Current Status and Trends	02	1,2,3

Unit- II: Teacher should facilitate learning of Cost Estimation methods

II	Cost Estimation	Lectures required	Reference
	Factors affecting investment and production cost	01	2,3
	Capital investment, fixed investment and working capital	02	2,3
	Estimating equipment cost by 6/10 factor rule	02	2,3
	Method of estimating capital investment. Different costs involved in total product cost	02	2,3
	Computer automization in costing.	01	2,3

Unit- III: Teacher should facilitate learning of Investment Cost and Profitability of the different processes and products.

III	Investment Cost and Profitability:	Lectures required	Reference
	Interest and investment cost, type of interest	01	2,3
	Types of taxes and tax returns, types of insurance and legal responsibility	01	2,3
	Depreciation, types of depreciation, and methods of determining depreciation	02	2,3
	Profitability, mathematical methods of profitability evaluation	02	2,3
	Cash flow diagram, break even analysis, balance sheet, pricing issue method and income statement.	02	2,3

Unit- IV: Teacher should facilitate learning of Fermentation Economics and waste treatments

IV	Fermentation Economics	Lectures required	Reference
	Introduction, isolation of microorganisms of potential industrial interest	01	4
	Strain improvement, market potential, effects of legislation on production of antibiotics and recombinant proteins	02	4
	Plant and equipment, media, air sterilization, heating and cooling, aeration and agitation	02	4
	Batch process cycle times, continuous culture, recovery costs	02	4
	Water usage and recycling, effluent treatment.	01	4

Unit- V: Teacher should facilitate learning of Bioproduct Economics

V	Bioproduct Economics	Lectures required	Reference
	Bioproduct regulation, Fermentation process economics: A complete example	01	5
	Economic consideration of commercial Bioproduct: Enzymes, Proteins via rDNA	02	5
	Antibiotics, Vitamins, Alkaloids, Nucleosides, Steroids, Monoclonal antibodies	02	5
	Brewing and wine making, Fuel Alcohol Production, Organic and Amino acid manufacture	02	5
	Single cell protein, Anaerobic methane production	01	5

References:

1. Peter M.S. Timmerhaus K.D. Plant Design and Economics for Chemical Engineers. McGraw Hill.
2. T.R. Banga and S.C.Sharma, Industrial Organization and Engineering Economics, Khanna Publications, New Delhi.
3. O.P.Khanna Industrial Engineering and Management, Dhanpat Rai Publications Pvt. Ltd. New Delhi.
4. P. F. Stanbury, A. Whitaker and S. J. Hall, Principles of Fermentation Technology, Aditya Book Private Limited.
5. Murray moo-young, Comprehensive Biotechnology Pergemon Press (Vol. 2)

Molecular Biology

LAB COURSE CONTENT

Teacher should facilitate learning of following lab experiments:

(Note: Minimum EIGHT Experiments from the following.)

List of Experiments		Lab hours required
1	Isolation of genomic DNA from bacteria.	04
2	Isolation of RNA from yeast.	04
3	Isolation of total plasmid DNA from bacteria.	04
4	Calculation of molecular weight by using DNA marker with agarose gel electrophoresis	04
5	DNA extraction from Blood.	04
6	Spooling of chromosomal DNA from onion cells.	04
7	Determination of melting temperature (Tm) and base composition of DNA from thermal denaturation characteristics	04
8	Principles and practice of agarose gel electrophoresis.	04
9	Quantitation of Nucleic acids..	04

References:

1. Biochemical Methods, Second Edition by S. Sadasivam, A. Manickam, New Age International Ltd,Publishers
2. S. Harisha. An Introduction to Practical Biotechnology. Laxmi Publications (P) Ltd. New Delhi.
3. Aneja K.R.(2nd Edn., 1996). Experiments in Microbiology, Plant pathology, Tissue Culture and Mushroom Cultivation. Wishwa Prakashan, New Age International (P) Ltd.
4. Plummer David T. “An Introduction to Practical Biochemistry”, Tata McGraw-Hill Pubblishing Co. Ltd., New Delhi.
5. Jayraman J. A Laboratory Manual in Biochemistry. New Age International Publishers

Guide lines for ICA:

ICA shall be based on continuous evaluation of student performance throughout semester and practical assignment submitted by the student in the form of journal.

Guide lines for ESE:

ESE will be based on practical assignment submitted by the student in the form of journal. Evaluation will be based on oral examination.

Bioprocess Instrumentation & Analysis

LAB COURSE CONTENT

Teacher should facilitate learning of following lab experiments:

(Note: Minimum EIGHT Experiments from the following)

List of Experiments		Lab hours required
1	To study the response of bimetallic thermometer.	02
2	To study Calibration of thermocouple.	02
3	To measure the pH of given solution.	02
4	To determine concentration of given solution by colorimeter	02
5	To study Flame photometry	02
6	To study Abbey's refractometer	02
7	To study Infra red spectrophotometer	02
8	To study UV spectrophotometer.	02

References:

1. D.P.Eckman, Industrial Instrumentation, Willey Eastern Ltd., New Delhi.
2. Gurdeep Chatwal and Sham Anand, Instrumental methods of Chemical analysis,Himalaya publication House, Mumbai.
3. Patranabis D. Industrial Instrumentation, Tata – Mcgraw Hill Publications, New Delhi.P. Kudesia and S.S. Sawhaney,
4. Instrumental methods of chemical analysis Pragati Prakashan, P.O.Box No. 62, Begum Bridge, Meerut 250001, U.P.

Guide lines for ICA:

ICA shall be based on continuous evaluation of student performance throughout semester and practical assignment submitted by the student in the form of journal.

Guide lines for ESE:

ESE will be based on practical assignment submitted by the student in the form of journal. Evaluation will be based on oral examination.

Chemical Reaction Engineering

LAB COURSE CONTENT

Teacher should facilitate learning of following lab experiments:

(Note: Minimum EIGHT Experiments from the following.)

List of Experiments		Lab hours required
1	To determine the reaction rate constant {k} for given reaction. (CSTR / BATCH / SEMIBATCH / PFR)	02
2	To determine the effect of temperature on reaction rate constant. .(CSTR / BATCH / SEMIBATCH / PFR)	02
3	To determine the activation energy {E} for the given reaction.	02
4	To draw C [t], E [t] and F [t] curve and to calculate the mean residence time {tm} variance {σ2} and skewness {S3} for plug flow reactor.	02
5	To draw C [t], E [t] and F [t] curve and to calculate the mean	02
6	To draw C [t], E [t] and F [t] curve and to calculate the mean residence time {tm} variance {σ2} and skewness {S3} for packed Bed reactor.	02
7	To study the cascade CSTR.	02
8	To study the kinetic in tubular flow reactor [coiled tube] for the given reaction.	02

References:

1. H. Scott Fogler, Elements of chemical reaction engineering, Prentice Hall New, Jersy.
2. Octave Levenspiel, Chemical reaction engineering, John Wiley and sons.
3. J.M. Smith, Chemical engineering kinetics, McGraw Hill
4. S.D. Dawande, Principles of reaction engineering, Central Techno publication, Nagpur.
5. Lanny D. Schimdt , Chemical reaction engineering, Oxford University Press.

Guide lines for ICA:

ICA shall be based on continuous evaluation of student performance throughout semester and practical assignment submitted by the student in the form of journal.

Guide lines for ESE:

ESE will be based on practical assignment submitted by the student in the form of journal. Evaluation will be based on oral examination.

Tissue Culture Engineering

LAB COURSE CONTENT

Teacher should facilitate learning of following lab experiments:

(Note: Minimum EIGHT Experiments from the following.)

List of Experiments		Lab hours required
1	Laboratory Setup & Introduction to PTC techniques	02
2	General Sterilization techniques	02
3	Preparation of culture medium sterilization of explants	02
4	Initiation of callus culture	02
5	Micropropogation/ Multiple shoot induction	02
6	Embryo culture	02
7	In-vitro seed germination	02
8	Meristem culture	02
9	RAPD (DEMO)	02
10	Lab design, sterilization procedures, media preparation for ATC and cryopreservation	02
11	Hardening and acclimatization of in vitro raised rooted shoots	02
12	Encapsulate the shoot buds/ seeds to demonstrate the production of synthetic seeds	02
13	Primary culture from chick embryo.	02
14	Laboratory Setup & Introduction to PTC techniques	02

References:

1. R.A.Dixon and Gonzales, Plant cell culture: A Practical Approach, IRL Press.
2. S.S.Purohit, Biotechnology Fundamentals and Applications, Agrobios (India), 4th Edition, 2005.
3. S.S.Bhojwani and M.K.Razdan, Plant Tissue Culture : Theory and Practical, (1996) Elsevier, Amsterdam.
4. S.B Primrose and R.M.Twyman, Principles of Gene Manipulation and Genomics, Blackwell publishing, 7th edition, 2006.
5. Plant Biotechnology: The genetic manipulation of plants; A. Slater, N. Scott, M. Fowler; Published by Oxford University press, New York (2003).
6. Methods in Plant Tissue Culture; U Kumar; AgroBios India, (2003).

Guide lines for ICA:

ICA shall be based on continuous evaluation of student performance throughout semester and practical assignment submitted by the student in the form of journal.

Industrial Training/EDP/Special Study

COURSE CONTENT

Teacher should facilitate learning of following real life working environment, new knowledge, skills, and current technologies.

Industrial Training	• Student shall undergo industrial training for a minimum period of **two weeks** during summer vacations between fourth semester and fifth semester. • The industry in which industrial training is taken should be a medium or large scale industry • The paper bound report on training must be submitted by the student in the beginning of Fifth semester along with a certificate from the company where the student took training. • Every student should write the report separately. • Institute / Department/T&P Cell have to assist the students for finding Industries for the training. • Students must take prior permission from Department before joining for Industrial Training.
EDP (Entrepreneurship Development Program)	• Student has to participate in Entrepreneurship Development Program for a minimum period of **One week** during summer vacations between fourth semester and fifth semester. • Every student must submit the paper bound report based on the program in the beginning of Fifth semester along with a certificate (Course / Program completion) from the program organizers. • Every student should write the report separately. • Institute / Department may arrange Entrepreneurship Development Program at their campus. • Students must take prior permission from Department before attending any Entrepreneurship Development Program.
Special Study	• Student has to submit name of three topics of his interest to the department. • Special study in a group shall not be allowed. • The three-member committee appointed by Head of Department shall allot one topic out of the three topics submitted by the student. • Every student must submit the paper bound report based on special study at the end of Fifth semester. • Department should allot guide to all such students, for monitoring their progress and guide them for literature survey / report writing etc. • Evaluation of special study shall be done based on presentation made by student, followed by brief question answer session.

Guide lines for ICA:

Assessment shall be based on the active participation of the students in the Industrial Training / EDP / Special study and based on knowledge / skill acquired by the student. The three-member committee appointed by Head of Department in consultation with the Principal shall assess the reports and award marks based on following:

(a) Report	10 marks.
(b) Presentation	10 marks.
(c) Viva-voce at the time of presentation	05 marks.
Total:	**25 marks.**

NORTH MAHARASHTRA UNIVERSITY, JALGAON (M.S.)

Third Year Biotechnology

Faculty of Engineering and Technology

Teacher and Examiner's Manual

SEM –VI

W.E.F 2014 – 2015

Bioprocess Engineering

Teacher, Paper setter and Examiner should follow the guidelines as given below:

Unit- I
Teacher should facilitate learning of Introduction to Fermentation process & Media for Industrial Fermentation Process

I	Fermentation process & Media for Industrial Fermentation Process	Lectures required	Reference
	Upstream Process, Downstream Process	02	1,2
	Range of fermentation process, Component parts of fermentation process	02	1,2
	Medium Sterilization, Batch Sterilization, Continuous sterilization	02	1,2
	Fermentor Sterilization, Feed Sterilization, Filter sterilization.	02	1,2

Unit- II: Teacher should facilitate learning of Design of Bioreactors

II	Design of Bioreactors	Lectures required	Reference
	Introduction, Basic objective in design of a reactor, aseptic operation and containment	02	1,2
	Body construction, aeration and agitation	01	1,2
	Stirrer glands and bearings, baffles design, sparger system	01	1,2
	Achievement and maintenance of aseptic conditions	02	1,2
	Valves and steam traps, types of valves and pressure control valves	01	1,2
	Scale up of fermenters, design condition for scale up, scale-up methods.	01	1,2

Unit- III: Teacher should facilitate learning of types of Bioreactors

III	Types of Bioreactors	Lectures required	Reference
	Batch bioreactors, Continuous bioreactors, Semi continuous bioreactors	02	1,2
	Stirred tank bioreactors, Airlift bioreactor systems, Trickle bed bioreactor	02	1,2
	Airlift external loop bioreactors, waldhof-type fermenter	02	1,2
	Tower fermenter, Cylindro- conical vessel, Deep jet fermenter	01	1,2
	Cyclone column, Rotating disc fermenter, Reactor dynamics: Dynamic models and stability	01	1,2

Unit- IV: Teacher should facilitate learning of Solid state & Submerged Fermentation, Process monitoring & Control

IV	Solid state & Submerged Fermentation, Process monitoring & Control	Lectures required	Reference
	Introductions, types of solid state fermenter	02	1,2
	Submerged Fermentation	02	1,2
	Brief introduction to pipe joints	02	1,2
	Physical and chemical sensors for medium and gases, Online/ Offline sensors	02	1,2

Unit- V: Teacher should facilitate learning of Bioreactor Design Considerations

V	Significance of rDNA technology and Human Welfare	Lectures required	Reference
	Design consideration: Design codes, maximum working pressure	01	1,2
	Design pressure, design temperature, design stress, factor of safety, and selection of factors of safety	02	1,2
	Design of wall thickness, corrosion ratio, Poisson ratio, criteria of failure	02	1,2
	Materials of construction: mechanical properties	02	1,2
	Materials Corrosion, protective coating, choice of materials, corrosion prevention.	01	1,2

References:

1. Principles of Fermentation Technology (1995) (2/e) Stanbury PF, Whitaker A and Hall SJ, Butterworth-Hienemann Ltd., UK.

2. Bioprocess Engineering Principles (1995) Doran PM, Academic Press Ltd, USA.

Genetic Engineering

Teacher, Paper setter and Examiner should follow the guidelines as given below:

Unit- I
Teacher should facilitate learning of recombinant DNA technology.

I	Recombinant DNA technology	Lectures required	Reference
	The recombinant DNA concept, Principles of cloning	02	1,2,3
	Important Discoveries	02	1,2,3
	Principles of cloning	02	1,2,3
	Biohazards and Bioethics of Genetic Engineering.	02	1,2,3

Unit- II: Teacher should facilitate learning various enzymes useful for construction of rDNA.

II	The Tools: Enzymes	Lectures required	Reference
	Nucleases, The Restriction Endonucleases Type I, II, III	02	1,2,3
	Star activity, isoschizomers	01	1,2,3
	Phosphodiesterase, Polynucleotidekinase	01	1,2,3
	DNA ligase, DNA polymerase-I, Reverse transcriptase	02	1,2,3
	Terminal deoxynucleotidyl transferase, Poly A polymerase.	02	1,2,3

Unit- III: Teacher should facilitate learning of various vector systems for the transfer of rDNA to the host.

III	The Tools: Vector Systems	Lectures required	Reference
	E. coli systems – the host cells , *E. coli* – Plasmid Vectors , *E .coli* – Bacteriophage vectors	02	1,2,3
	E. coli systems – Plasmid-Phage combination vectors , Other Prokaryotic Host-Vector systems	02	1,2,3
	Eukaryotic Host-Vector Systems: Yeast	02	1,2,3
	Eukaryotic Host-Vector Systems: Animals, Eukaryotic Host-Vector Systems: Plants.	02	1,2,3

Unit- IV: Teacher should facilitate learning of various Molecular research procedures

IV	Molecular research procedures	Lectures required	Reference
	DNA sequencing techniques	02	1,4,5,6
	PCR, Blotting Techniques	02	1,4,5,6
	Gene silencing techniques, RNAi	02	1,4,5,6
	Knockout Technology, SAGE	02	1,4,5,6

Unit- V: Teacher should facilitate learning of Significance of rDNA technology and Human Welfare.

V	Significance of rDNA technology and Human Welfare	Lectures required	Reference
	Gene therapy	02	1,4,5,6
	Restriction fragment length polymorphism (RFLPs), Random amplified polymorphic DNA (RAPD)	02	1,4,5,6
	SNPs, AFLP, microarray	02	1,4,5,6
	DNA fingerprinting.	02	1,4,5,6

References:

1. Fundamentals of Molecular Biology by Veer Bala Rastogi; Ane Books Pvt. Ltd
2. Genes VIII – Benjamin Lewin
3. Genes and Genomes – Singer M and Berg P
4. Textbook of Biotechnology by R.C.Dubey, S. Chand & Co. P Ltd, New Delhi.
5. Textbook of Biotechnology by B.D.Singh, Kalyani Publication.
6. Textbook of Biotechnology by U.Satyanarayana, Books and Allied Pvt.Ltd.

Fermentation Technology

Teacher, Paper setter and Examiner should follow the guidelines as given below:

Unit- I
Teacher should facilitate learning of Introduction to Fermentation Process & Media Formulation

I	Introduction to Fermentation Process & Media Formulation	Lectures required	Reference
	An introduction to fermentation process, Isolation methods for Industrial microorganisms	01	1,5
	Culture preservation and stability, the improvement of industrial microorganisms	02	1,5
	Media for Industrial fermentation, Introduction ,typical media	02	1,5
	Medium fermentation: Water, Energy sources, Carbon sources, Nitrogen sources, Minerals	02	1,5
	Growth factors, Nutrient recycle, Buffers, Precursors, Metabolic regulators, Oxygen requirement and antifoams, pH	01	1,5

Unit- II: Teacher should facilitate learning of various types of sterilization processes.

II	Types of Sterilization Processes	Lectures required	Reference
	Sterilization: Introduction, Medium sterilization, Design of Batch sterilization process	02	1,2
	Calculation of Del factor during heating and cooling, Calculation of holding time at constant temperature	02	1,2
	Richard's rapid method for the design of sterilization cycles, the scale up of batch sterilization processes	02	1,2
	Filter sterilization: Filter sterilization of fermentation media, air and fermenter exhaust air	01	1,2
	The theory and design of depth filters.	01	1,2

Unit- III: Teacher should facilitate learning of various inoculum development processes

III	Inoculum Development Processes	Lectures required	Reference
	The development of Inocula for industrial fermentation: Introduction	02	1
	Criteria for the transfer of inoculums, The development of inocula for yeast processes	02	1,5
	The development of inocula for bacterial processes, The development of inocula for mycelial processes	02	1
	The aseptic inoculation of plant fementers, Solid state fermentation.	02	1

Unit- IV: Teacher should facilitate learning of Fermentative production of Beverages, Industrial Chemicals and Biomolecules.

IV	Fermentative production of Beverages, Industrial Chemicals and Biomolecules.	Lectures required	Reference
	Beer, Wine, Rum, Gin, Whisky, Brandy, Champaign	02	3,4
	Fermentative production of citric acid, acetic acid, lactic acid, ethanol	02	3,4
	acetone and butanol, gluconic acid, steroid biotransformation	01	3,4
	Enzyme production- Amylases, Proteolytic enzymes, Invertase enzyme, Pectinases, Lipases	01	3,4
	Vitamins: Vitamin B12, Riboflavin, Vitamin A,	01	3,4
	Amino acid production: L-Glutamic acid, L-Lysine, L-Threonine	01	3,4

Unit- V: Teacher should facilitate learning of regulation of gene expression and DNA Damage and its repair.

V	Fermentation of food products and Antibiotics.	Lectures required	Reference
	Fermentative production of food products: cheese and types of cheese	02	3,5
	Fermented soyabean foods, biomass production (single cell protein, baker's yeast)	02	3,5
	Fermented dairy products like yogurt, cultured buttermilk	02	3,5
	Production of penicillin, B-Lactum antibiotics	01	3,5
	Streptomycin, Cephalosporin, Tetracycline.	01	3,5

References:

1. P. F. Stanbury, A. Whitaker and S. J. Hall, Principle of Fermentation Technology, Aditya Books (P) Ltd, New Delhi.
2. Pauline M. Doran, Bioprocess Engineering Principles, Academic Press an Imprint of Elsevier.
3. Text Book of Biotechnology by U. Satyanarayana, Books and Allied Pvt.Ltd.
4. Murray moo-young, Comprehensive Biotechnology Pergemon Press (Vol. 2)
5. L. E. Casida, Industrial Microbiology, New Age Industrial Publishers.

Mass Transfer

Teacher, Paper setter and Examiner should follow the guidelines as given below.

Unit – I

Teacher should facilitate learning of mass transfer coefficient and application of mass transfer process.

1.		Introduction to mass transfer	Lecture required	References
	a	Introduction to mass transfer, Equilibrium for mass transfer process: Local two phase mass transfer.	02	1,2,4
	b	Local overall mass transfer coefficient, Use of local overall coefficient.	02	1,2,4
	c	Material balances for steady state co current, countercurrent, cross flow cascade, counter flow cascade	02	1,2,4
	d	Application of mass transfer processes.	02	1,2,4
Guidelines for the examiner and paper setter. 1) Numerical based on mass transfer coefficient.				

Unit – II

Teacher should facilitate learning of distillation process and its applications in process industries.

2.		Distillation Operation	Lecture required	References
	a	Distillation: Introduction to distillation process, Vapor liquid equilibrium.	01	1,2,4
	b	The methods of distillation (Binary mixture)	02	1,2,4
	c	McCabe Thiele & Lewis Sorel method	02	1,2,4
	d	Batch distillation, Azeotropic, extractive and steam distillation.	01	1,2,4
	e	Introduction to distillation equipments.	01	1,2,3
Guidelines for the examiner and paper setter. 1) Numerical based on McCabe Thiele & Lewis Sorel method Rayleigh Method.				

Unit – III

Teacher should facilitate learning of Extraction & Leaching and its application in process industries.

.

3.		Extraction & Leaching Operation	Lecture required	References
	a	Extraction & Leaching: Introduction to extraction process, Liquid equilibria,	02	1,2,3
	b	Material balances for stage wise contact methods	02	1,2,4
	c	Leaching: General principles of leaching	01	1,2,4
	d	working principle of moving-bed leaching equipments: Bollman extractor, Hildebrandt extractor	02	1,2,4
	e	Stage contact and continuous contact type extractors	01	1,2,4
Guidelines for the examiner and paper setter. 2) Numerical based on Extraction. 3) No numerical should be asked on leaching operation.				

Unit – IV

Teacher should facilitate learning of adsorption operation and its application in process industries

4.		Adsorption Operation	Lecture required	References
	a	Introduction to adsorption operation, Type of adsorption operation.	01	1,2,3
	b	Nature of adsorbents, Adsorption equilibria, Adsorption of vapor, gas mixture and liquids,	02	1,2,3
	c	Material balances for stage wise for operation, Continues contact process for adsorption	02	1,2,3
	d	Principle of ion exchange operation, Equilibria for ion exchange operation, Rate of ion exchange operation	02	1,2,3
	e	Application of ion exchange operation.	01	1,2,4

Guidelines for the examiner and paper setter.

2) Numerical based on Extraction.
3) No numerical should be asked on leaching operation.

Unit – V

Teacher should facilitate learning of Crystallization and its application in process industries.

5.		Crystallization	Lecture required	References
	a	Crystallization: Introduction to crystallization, Growth and properties of crystals.	03	1,2,4
	b	Effect of impurities in crystallization, Effect of temperature on solubility	01	1,2,4
	c	Fractional crystallization, Caking and yield of crystals	01	1,2,4
	d	Different type of crystallizers.	03	1,2,4

References:

1. R. E. Treybal , Mass transfer operation ,McGraw Hill Publication
2. Coulson and Richardson Chemical Engineering (Vol. I and II), Pergamon Press
3. Christie J. Geankoplis ,Transport Processes and Unit Operations ,Prentice Hal inc
4. P. Chattopadhayay , Unit operation in Chemical Engg. (Vol. I and II), Khanna Publications Delhi.

IPR & Entrepreneurship

Teacher, Paper setter and Examiner should follow the guidelines as given below:

Unit- I
Teacher should facilitate learning of IPR, Patents and copyrights.

I	IPR, Patents and copyrights	Lectures required	Reference
	General Overview of Intellectual Property Rights, WIPO, WTO	02	1,2,3
	Trade Related Intellectual Property Rights, Patent- Basic requirements of Patentability, Patentable Subject Matter,	02	1,2,3
	Procedure for Obtaining Patent, Provisional and Complete Specification	02	1,2,3
	Copyright-Objectives of copyright, Rights conferred by registration of copyright, Infringement of copyright.	02	1,2,3

Unit- II: Teacher should facilitate learning of various types of IPR.

II	Trademarks, GI and other types of IPR	Lectures required	Reference
	Trademarks-Basic Principles of Trademark, Rights conferred by Registration of Trademark, Infringement of Trademark	02	1,2,3
	Geographical Indications-Objectives of Geographical Indications, Rights conferred	02	1,2,3
	Infringement of Geographical Indications, International Position, Indian Position, Bioprospecting and Biopiracy	02	1,2,3
	GATT Farmers rights, plant breeders right	02	1,2,3

Unit- III: Teacher should facilitate learning of Biosafety and Bioethics

III	Biosafety and Bioethics	Lectures required	Reference
	Biosafety and Bioethics Management-Key to environmentally responsible use of biotechnology	02	1,2,3
	Cartagena Protocol on Biosafety, Ethical implications of Biotechnological products and techniques	02	1,2,3
	Contempory ethics of healthcare	02	1,2,3
	Ethical aspects of hazardous worte and toxic substance. Ethical aspects of scientific publishing.	02	1,2,3

Unit- IV: Teacher should facilitate learning of various aspects of Entrepreneurship.

IV	Entrepreneurship	Lectures required	Reference
	Need, scope and characteristics of entrepreneurship management of self and understanding human behavior	03	1,2,3
	Business ethics, performance appraisal, and (SWOT) analysis.	03	1,2,3
	Market survey techniques - Criteria for the principles of product selection and development	02	1,2,3

Unit- V: Teacher should facilitate learning of various aspects of marketing management.

V	Marketing	Lectures required	Reference
	Elements of Marketing and Sales Management - Nature of product and market strategy	02	1,2,3
	Packaging and advertising, After Sales Service, Pricing techniques	02	1,2,3
	Financial institutions, financial incentives. Technical feasibility of the project, plant layout & process planning for the product	02	1,2,3
	Quality Control, Critical Path Method (CPM) and Project Evaluation Review Techniques (PERT) as planning tools for establishing SSI.	02	1,2,3

References:

1. Entrepreneurship: New Venture Creation, David H. Holt.
2. Patterns of Entrepreneurship: Jack M. Kaplan.
3. Entrepreneurship and Small Business Management: C.B. Gupta, S.S. Khanka,Sultan Chand.

Bioprocess Engineering & Fermentation Technology

LAB COURSE CONTENT

Teacher should facilitate learning of following lab experiments:

(Note: Minimum EIGHT Experiments from the following.)

List of Experiments		Lab hours required
1	Introduction to the fermenter.	04
2	Feed Sterilization	04
3	Fermenter Sterilization	04
4	Growth kinetics of microorganisms using shake flask method	04
5	Determination of specific thermal death rate constant (Ka).	04
6	Determination of Volumetric oxygen transfer coefficient (KLa), effect of aeration and agitation speed	04
7	Preparation of Immobilized enzymes and cells and evaluation of kinetic parameters.	04
8	Kinetics study of Product formation.	04
9	Effect of substrate and product concentration on biomass yield for baker's yeast production	04
10	Studies on settling characteristics of various microbial cultures	04
11	Study of Physical and chemical sensors for medium and gases.	04
12	Fermentative production of Sauerkraut.	04

References:

1. R.A.Dixon and Gonzales, Plant Cell Culture : A Practical Approach, IRL Press.
2. S.S.Purohit, Biotechnology: Fundamentals and Applications, Agrobios (India), 4th Edition, 2005.
3. P.F.Stanbury, A.Whitkar and S.J.Hall, Principles of Fermentation Technology, Aditya Book House, New Delhi.
4. B.D.Singh, Biotechnology: Expanding Horizons, Kalyani Publishers, New Delhi, Second Revised Edition, 2008.
5. Biochemical Methods, Second Edition by S. Sadasivam, A. Manickam, New Age International Ltd,Publishers.

Guide lines for ICA:

ICA shall be based on continuous evaluation of student performance throughout semester and practical assignment submitted by the student in the form of journal.

Guide lines for ESE:

ESE will be based on practical assignment submitted by the student in the form of journal. Evaluation will be based on oral examination.

Mass Transfer

LAB COURSE CONTENT

Teacher should facilitate learning of following lab experiments:
(Note: Minimum EIGHT Experiments from the following.)

List of Experiments		Lab hours required
1	To determine mass transfer coefficient for dissolution of benzoic acid without chemical reaction.	02
2	Simple Distillation: To verify Rayleigh's equation for simple distillation	02
3	Bubble Cap Distillation.	02
4	Liquid – Liquid Extraction: To study and determine the efficiency of cross Current liquid- liquid extraction.	02
5	To construct ternary for acetic acid –water –benzene	02
6	To plot Tie line diagram for acetic acid –water –benzene	02
7	To determine the percentage leaching of NaOH from a mixture of NaOH and $CaCo_3$.	02
8	Adsorption: To study adsorption of acidic acid on activated charcoal	02
9	To calculate percentage yield of crystals obtained with and without seeding in saturated solution of solute.	02
10	To determine mass transfer coefficient for dissolution of benzoic acid without chemical reaction.	02
11	Simple Distillation: To verify Rayleigh's equation for simple distillation	02

References:

1. R. E. Treybal , Mass transfer operation ,McGraw Hill Publication
2. Coulson and Richardson Chemical Engineering (Vol. I and II), Pergamon Press
3. Christie J. Geankoplis ,Transport Processes and Unit Operations ,Prentice Hal inc
4. P. Chattopadhayay , Unit operation in Chemical Engg. (Vol. I and II), Khanna Publications Delhi.

Guide lines for ICA:
ICA shall be based on continuous evaluation of student performance throughout semester and practical assignment submitted by the student in the form of journal.

Guide lines for ESE:
ESE will be based on practical assignment submitted by the student in the form of journal. Evaluation will be based on oral examination.

Genetic Engineering

LAB COURSE CONTENT

Teacher should facilitate learning of following lab experiments:

(Note: Minimum EIGHT Experiments from the following.)

List of Experiments		Lab hours required
1	Restriction digestion of genomic DNA of bacteria.	04
2	Ligation of bacterial DNA.	04
3	Plasmid Preparation	04
4	DNA fingerprinting (by RFLP)	04
5	DNA mapping using restriction enzymes	04
6	Transformation of *E.coli* with plasmid pBR 322	04
7	Transduction	04
8	Southern Blotting	04
9	Northern Blotting	04

References:

1. Biochemical Methods, Second Edition by S. Sadasivam, A. Manickam, New Age International Ltd,Publishers.
2. S. Harisha. An Introduction to Practical Biotechnology. Laxmi Publications (P) Ltd. New Delhi.
3. Aneja K.R.(2nd Edn., 1996). Experiments in Microbiology, Plant pathology, Tissue Culture and Mushroom Cultivation. Wishwa Prakashan, New Age International (P) Ltd.
4. Plummer David T. “An Introduction to Practical Biochemistry”, Tata McGraw-Hill Pubblishing Co. Ltd., New Delhi.
5. Jayraman J. A Laboratory Manual in Biochemistry. New Age International Publishers.

Guide lines for ICA:

ICA shall be based on continuous evaluation of student performance throughout semester and practical assignment submitted by the student in the form of journal.

Minor Project
COURSE CONTENT

Following should be considered:

1	Every student shall undertake the Minor Project in semester VI. It is expected that the broad area of major project shall be finalized by the student in the beginning of the VI semester and Minor project undertaken may be a part of Major Project
2	Each student shall work on an approved project, a group of 05 students (maximum) shall be allotted for the each minor project and same group may be continued for major project
3	Minor project may involve fabrication, design or investigation of a technical problem that may take design, experimental or analytical character or combine element of these areas. The project work shall involve sufficient work so that students get acquainted with different aspects of fabrication, design or analysis
4	Each group of students is required is required to maintain separate log book for documenting various activities of minor project
5	The three-member committee appointed by Head of the department shall be constituted for finalizing the topics of minor project. Maximum four minor project groups shall be assigned to one teaching staff

Guide lines for ICA: Assessment of the project for award of ICA marks shall be done jointly by the guide and departmental committee as per the guidelines given in Table-A.

Assessment of Minor Project
Name of the Project: _____
Name of the Guide: _____

Table-A

Sr.No	Exam Seat No	Name of Student	Project Selection	Documentation	Innovation/Technology/Design/Simulation	Laboratory Work	Result Verification	Presentation	Total
			5	10	10	10	10	5	50

Guide lines for ICA: Assessment of the project for award of ICA marks shall be done jointly by the guide and departmental committee.

Seminar-I
COURSE CONTENT

Seminar-I
COURSE CONTENT

Teacher should facilitate learning of communication ability of an individual and to improve technical knowledge through study of specific topic. Teacher should also facilitate understanding ability, ability to listen, proper language, oral presentation skill amongst students.

Following should be considered:

1	For Seminar-I every student will individually study a topic assigned to him / her and submit a report and shall deliver a short lecture / Seminar on the topic during the term
2	The three-member committee appointed by Head of the department shall be constituted for finalizing the topics of Seminar-I. Seminar shall be related state of the art topic of his choice approved by the committee
3	Seminar topic should not be repeated and registration of the same shall be done on first come first serve basis
4	Topic of Seminar shall be registered within a two week from commencement of VI Semester and shall be approved by the committee
5	Maximum six seminar supervision shall be allotted to each teacher
6	At the end of semester, student should submit the seminar report (paper bound copy)in following format: a. Size of report shall be of minimum 25 pages. b. Student should preferably refer minimum five reference books / magazines/standard research papers. c. Format of Report i. Introduction ii. Literature survey iii. Theory 1) Implementation 2) Methodology 3) Application 4) Advantages, Disadvantages iv. Future scope v. Conclusion

Guide lines for ICA: ICA shall be based on evaluation of student performance by a seminar presented by the student. Every student shall be required to present a seminar in presence of Panel of teachers constituted by the Head of Department in consultation with the Principal. The evaluation shall be based as per the guidelines given in Table- B

Title of Seminar: _____
Name of Guide: _____

Table-B

SN	Exam Seat No	Name of Student	Topic Selection	Literature survey	Report writing	Depth of understanding	Presentation	Total
			5	5	5	5	5	25