Biochemical engineering

Biochemical Engineering is the application of chemical engineering methods and approaches to industrial processes based on biological elements such as living cells or their components. Microbes and enzymes for example are used to produce useful chemical compounds such as antiobiotics, other medically useful chemicals, detergents, amino acids, etc.

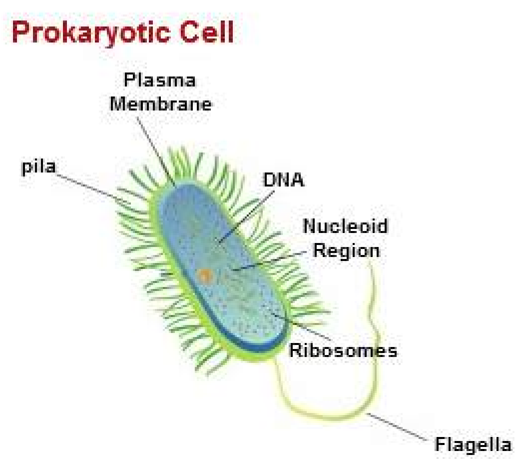
The Cell

The cell can be defined as a basic functional unit of life. The term cell is derived from the Latin word and was first observed by a scientist named Robert Hook in the year 1665. All living organisms are composed of one or many cells to perform their individual functions. A cell is a smallest unit of a life, which is able to control and perform several functions in all living organisms. All unicellular organisms (single cell) including bacteria and archae, which are composed of a single cell, are examples of prokaryotic cells. All multicellular organisms (many or more than one cell) including humans, which are composed of complex or many cells, are examples of eukaryotic cells. Both prokaryotic cells and eukaryotic cells have cytoplasm, cell membrane and genetic material in common

Every living organism can be categorized as either a prokaryote or a eukaryote.

Procaryotes

Prokaryotic cells are simpler and smaller than the eukaryotic cells. The term prokaryote is derived from the Greek word- ***"prokaryote"*** meaning before nuclei. These cells lack membrane bound organelles. Prokaryotic cells are unicellular organisms, which reproduce through binary fission. In some cases few prokaryotic organisms also reproduce by budding. Prokaryotic cells have a cell envelope, which generally consists of a capsule, cell wall, cytoplasm, plasma membrane, cytoplasm region or nucleiod region, ribosome, plasmids, pili and flagella.



Example: Bacteria, blue green algae, E.coli, etc.

Parts of Prokaryotic Cell and their Functions

Cell wall: It is made from the glycoprotein murein. Cell wall provides strength and rigidity to the cell and it is permeable to solutes.

Cytoplasm: It helps in cellular growth, metabolism and replication. Cytoplasm is the storehouses for all types of chemicals and components that are used to sustain the life of a bacterium.

Plasma membrane: It is also known as a cell membrane. It is mainly composed of proteins, phospholipids and carbohydrates, which forms into a fluid-mosaic. Plasma membrane surrounds the bacteria and it is a most important organelle and plays a vital role in controlling the movement of substances in the cell.

Cytoplasm region (or) nucleiod region: An area of the cytoplasm that contains the single bacterial DNA molecule.

Ribosome: They are the smallest part of cell organelle. Ribosome plays a vital role in protein synthesis as they consist of protein and RNA. They are located freely in the cytoplasm of attached to the rough endoplasmic reticulum.

Mesosomes: They are the folding, present inside the plasma membrane. Mesosome plays a vital role in cellular respirations, replication of DNA, cell division, separation of chromosomes during cell division and also performs the role of Golgi bodies and mitochondria.

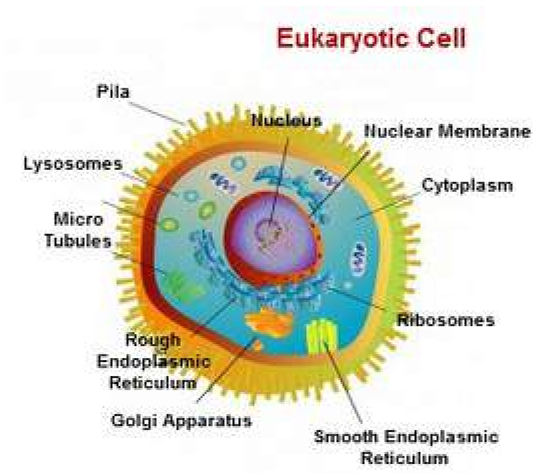
Plasmids: They are a small circle of DNA. Plasmid plays a vital role in exchanging DNA between the bacterial cells. Bacterial cells have many plasmids.

Pili: They are short protein appendages, which fixes bacteria to surfaces. These pili are smaller than those flagella and are used in conjugation to exchange the genetic information.

Flagella: They are rigid rotating tail. The clockwise rotation moves the cell forward and anticlockwise rotation helps the cell to spin. The rotation is powered by H+ gradient across the cell membrane.

Eukaryotic Cell

Eukaryotic cells are those cells, which are complex and larger than the prokaryotic cells. Eukaryotic cells can be easily distinguished through a membrane-bound nucleus. The life, which is present and visible by our naked eye, is all made up of these cells. Eukaryotic cells are membrane-bound organelles, which have a multiple membrane-bound organelles to carry out specific cell tasks. They have different internal membranes, which are known as organelles. These organelles play a vital role in cell maintenance and other functions. These organelles generally consist of cell wall, plasma membrane, nucleus, mitochondria, chloroplasts (plastids), endoplasmic reticulum, ribosome, Golgi apparatus, lysosomes, vacuoles, cytoplasm and chromosomes.



Parts of Eukaryotic Cell and their Functions

Cell wall: It helps in protecting the plasma membrane and plays a vital role in supporting and protecting the cells. It is a thick outer layer made of tough cellulose. Cell walls are present in plant cells and are absent in animal cells.

Plasma membrane: The plasma membrane is present in animal cells, plant cells and even in eukaryotic cells. It is a double layered, thin barrier, surrounding the cell that controls the entry and exit of certain substances. It also refers to a thin, fluid entity that manages to be very flexible and it is stable. It is also called as cell membrane. It is the living ultra thin biological membrane ranging from 6 to 8nm and composed of a dynamic layer that chemically comprises a molecule of lipids and proteins that are arranged in a fluid mosaic pattern. It acts as a protective barrier. This membrane plays a vital role in:

It acts as a boundary and separates the internal and external organelles of a cell.

- Transportation of materials.
- Cell to cell recognition.
- Enzymatic activity.
- Signal transduction.

Nucleus: It is present both in animal cell and in plant cells. It is large and present in the center of a cell. It contains DNA and stores all the necessary information, which is required to control all the activities within the cell. Hence it is also called as a brain of the cell.

Nuclear membrane: It is a double layered, which surrounds the nucleus and helps in the entry and exits of material into the nucleus. It also separates the nucleus from the other parts of the cell.

Nucleolus: It is present in nucleus of both plant cell and animal cell. It plays a vital role in the synthesis of RNA and in the formation of the ribosome.

Mitochondria: The organelles that convert energy into usable forms, which are used by the cell to perform their cellular functions. They are double membranes, semi-autonomous organelles. This organelle plays a vital role in generating and transforming the energy. Albert von Kolliker recognized the structure of the mitochondria in the year 1880. It is a powerhouse of the cell, which produces energy by breaking down fats and carbohydrates. Mitochondria play a vital role in:

- The most important function of the mitochondria is to produce energy.
- It converts glucose to ATP.
- Helps in cellular respiration.
- It synthesizes ATP from the breakdown of sugars, fats and other fuels in the presence of oxygen.
- It plays a vital role in oxidative phosphorylation.

Chloroplast: They are the sub cellular sites of photosynthesis. Chloroplasts were discovered early in the 17th century and were identified by a scientist named Antony van Leeuwenhoek and by another scientist named Nehemiah Grew. Chloroplasts are important because, if there were no chloroplasts, plants cannot produce oxygen, sugars and starches, which other animals use and eat. They also produce energy in the daylight. They are present only in plant cells and are absent in animal cells. Chloroplasts are also found in chlorophyll bacteria, blue-green algae, etc. It is a site for photosynthesis.

Endoplasmic reticulum: It helps in movement of materials around thecell. Its main functions are storage and secretion. It is of two types:-

Rough endoplasmic reticulum - it manufactures proteins.

Smooth endoplasmic reticulum - it contains an enzymes that helps to build molecules.

Ribosome: They are biological molecule, which are composed of proteins and RNA. It is complex and smallest organelle in the cell. It plays a vital role in synthesis millions of protein, which are required for cells to perform several activities. These organelles are present in all animal cells and absent in plant cells.

Golgi Bodies: They are sac like structures, which are specifically used for storing or preserving all the substances made by the cell. It helps in the movement or transportation of materials within the cell and in synthesis of plant cell wall; hence it is also called as the post office of the cell. It also plays a vital role in the modification, transportation and processing of macromolecules, which includes proteins and lipids. These organelles are present in all animal cells and absent in plant cells.

Lysosomes: They are spherical organelles, which contains enzymes that help in maintaining the physiologic turnover of cellular constituents. These organelles are present in all animal cells and absent in plant cells. They play a vital role in breaking the food materials and making it easier to digest. The size of these Lysosomes varies from 0.1 to 1.2 μm. It is also called as suicidal bags as it helps in cell renewal and break down old cell parts. Lysosomes play a vital role in:

- Removal of dead cells, hence they are named as a suicide bags.
- They protect the cell by ingestion of other dying cells or larger extracellular material like-foreign invading microbes.
- Lysosomes attacks the foreign or disease-causing agents such as bacteria, viruses, fungi, food and old organelles and break them into small pieces that can hopefully be used again.

- Lysosomes play a vital role in protecting the cell from being digested by surrounding the cell membrane.

Vacuoles: They are vesicle that helps in the digestion. They are present both in plant cells and in animal cells. In plant cells it helps in maintaining its shape and it also stores water, food, enzymes, wastes, etc.

Cytoplasm: It refers to the jelly like material with organelles in it. It is present both in plant and in animal cells. They consist of inner region of the plasma membrane and also the outer region of DNA. The cytoplasm is made of components, which benefits the cell by keeping the organelles separate from each other. This helps to keep a cell in stable. Cytoplasm also contains some important organelles like endoplasmic reticulum, lysosomes, mitochondria and Golgi apparatus. Along with these organelles, it also contains chloroplast in plant cells. Every organelle is bound by a fatty membrane, which have some specific functions. A cytoplasm plays a vital role in:

- Storage and manufacturing of energy.
- Maintains the cell shape and its consistency and provides suspension to the organelles.
- All types of cell functions like: cell expansion, growth and replication are carried out in the cytoplasm of a cell.
- All types of cellular activities take place in this cytoplasm.
- Cytoplasm also helps in the movement of different elements or molecules present within the cell.

Chromosomes: Chromosomes are small, colored thread like structures present in the nucleoplasm of living cells, which helps in the inheritance or transmission of characters in form of Genes from one generation to another generation. It is made up of DNA and stored in the nucleus, which contains the instructions for traits and characteristics. A chromosome plays a vital role in:

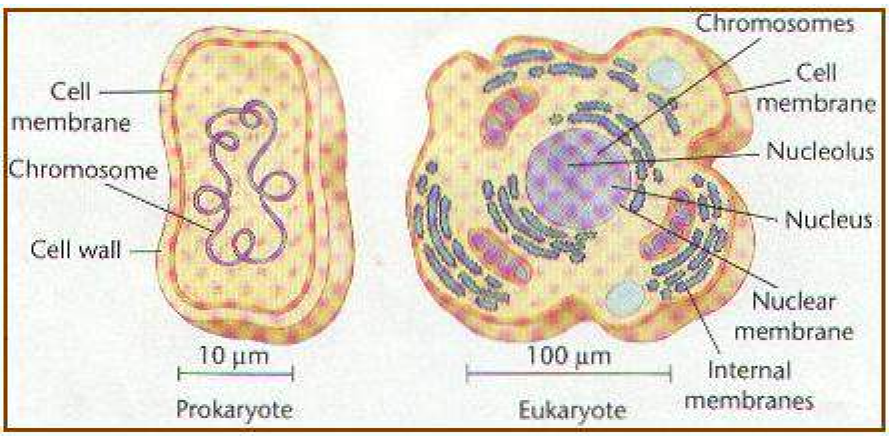
- Self-duplication.
- They help in transmitting or transferring the characters from one generation to another generation (or) from parents to offspring.
- Controls the biological processes in the body of an organism.
- They control cell metabolism by directing the formatting of necessary proteins.
- They help in cell differentiation during development.
- A chromosome also helps in determining a sex of an individual.

Centrosomes: They are the small hollow cylindrical shaped organelles, which are composed of nine bundles of micro tubules. They play a vital role in cell division or in the cell cycle.

Centrosomes are present only in animal cells and are absent in plant cells. Centrosomes are also called by centrioles.

Difference between Prokaryotic and Eukaryotic Cells

The difference between the prokaryotes and eukaryotes is said to be the most important disticnction among the groups of organisms. The main difference is that the eukaryotic cells contain cellular organelles that are membrane bound, like the nucleus. The prokaryotic cells do not have membrane bound cellular organelles. The cellular structure of the prokaryotes and eukaryotes differ in the presence of cellular structures like the mitochondria and chloroplasts, cell wall and the structure of chromosomal DNA.



Differences between Prokaryotic cells and Eukaryotic cells

Prokaryotic Cells	Eukaryotic Cells
They are very minute in size.	They are comparatively larger in size.
Nuclear region (nucleoid) is not enveloped by a nuclear membrane.	Nucleus is surrounded by a double membrane layer.
Single chrmosome present.	More than one chromosome are present.
Nucleolus is absent.	Nucleolus is present.

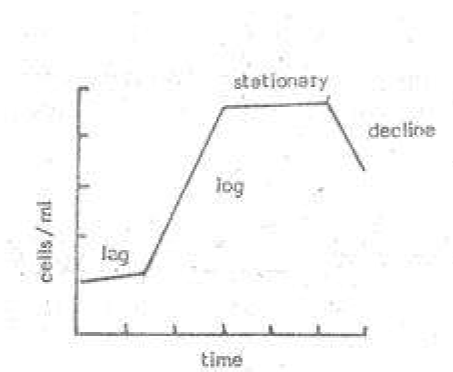
Membrane bound organelles are absent.	Membrane bound organelles are present.
Multiplication of cell is by fission or budding.	Cell division by mitosis or meiosis.
Cell Walls presnt, which are chemically complex.	Cell walls seen in only plant cells, which are chemically simpler.
Cell type is usually unicellular.	Usually multicellular cells.
Cell size is 1-10µm	Cell size 10 - 100µm.
Example: Bacteria, archaea	Example: animal cells and plant cells.

Batch culture:

Growth of microorganisms in a limited volume of liquid medium is generally termed as a batch culture. This culture is helpful in studying the growth characteristics mainly of bacteria. When bacteria are transferred to a known volume of batch culture, their population undergoes a characteristic sequence in its rate of increase in cell number. Four recognizable 'phases' are seen when the increase in cell number is determined in relation to time (Fig.10). These are:

i. Lag phase:

In this phase there is no increase in the number of viable cells but the cells increase in- size due to extensive synthesis of macromolecules. Lag phase represents a period of active growth without cell division; the cells being ready for division.



<u>Growth curve of bacteria in batch culture</u>

ii. Log phase:

In the log phase the cell population increases logarithmically and the cells divide at the maximum rate; growth rates are measured during this period since growth occurs at a maximum rate.

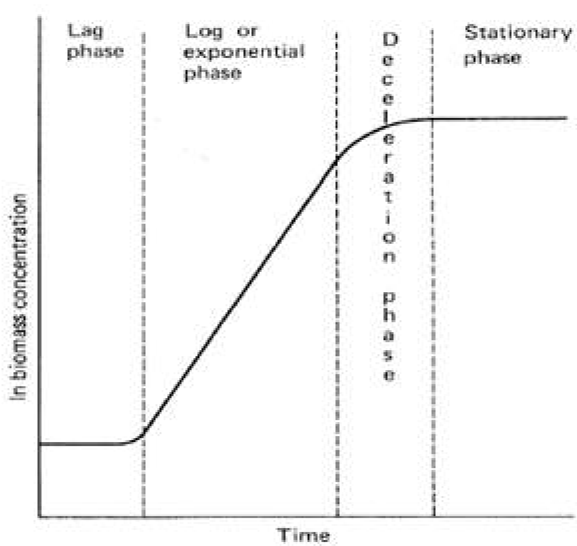
iii. Stationary phase:

In this phase the total number of viable cells remains constant as a result of no increase in viable cell number due to stopage of their division.

iv. Death or Decline phase:

This phase is characterized by exponential decrease in the number of their viable cells.

Batch culture kinetics:



Kinetics

For batch culture , $dx/dt = \mu x$

Where x is the concentration of microbial biomass

time is t

and μ is specific growth rate in time-1

on integration of the above equation gives

$x_t = x_o e^{\mu t}$

where x_o is the original biomass concentration

x_t is the biomass concentration after time interval t hours

e is the base of natural logarithm

On taking natural logarithm equation becomes

$\ln x_t = \ln x_o + \mu t$

Thus a plot of natural logarithm of biomass concentration against time should yield a straight line, the slope of which equal to μ.

Continuous Culture :

Batch and continuous culture systems differ in that, in a continuous culture system, nutrients are supplied to the cell at a constant rate and in order to maintain a constant volume of biomass in the reactor, an equal volume of cell culture is removed. This will allow the cell population to reach a *steady-state* condition.

Therefore, to keep the culture always in log phase, fresh medium is added continuously to the fermentor (before diminishing the nutrients) at the time of removal of medium. Here the rate of supply of nutrients in the form of raw material and removal of products/cells should be volumetrically the same i.e. volume added is equal to volume removed.

It means that volume of the medium always remains constant. This should be optimised with different microbial cultures and different growth media. If the working volume of the fermentor is V m^3, and the rate of flow in and out is F m^3h^{-1}, then the dilution rate (D) will be
D = F/V

Or F = DV ...(1)

The unit of D is per hour (h^{-1}).
The output of biomass from a continuous culture system is given by the rate at which medium passes out of the out flow (i.e. the flow rate, F) multiplied by the concentration of biomass in that out flow (i.e. X).

Thus, output = FX ...(2)

Putting the value of F of equation (1) in equation (2), we get

Output = DVX ...(3)

The productivity of this system (output per unit volume) is thus as below:

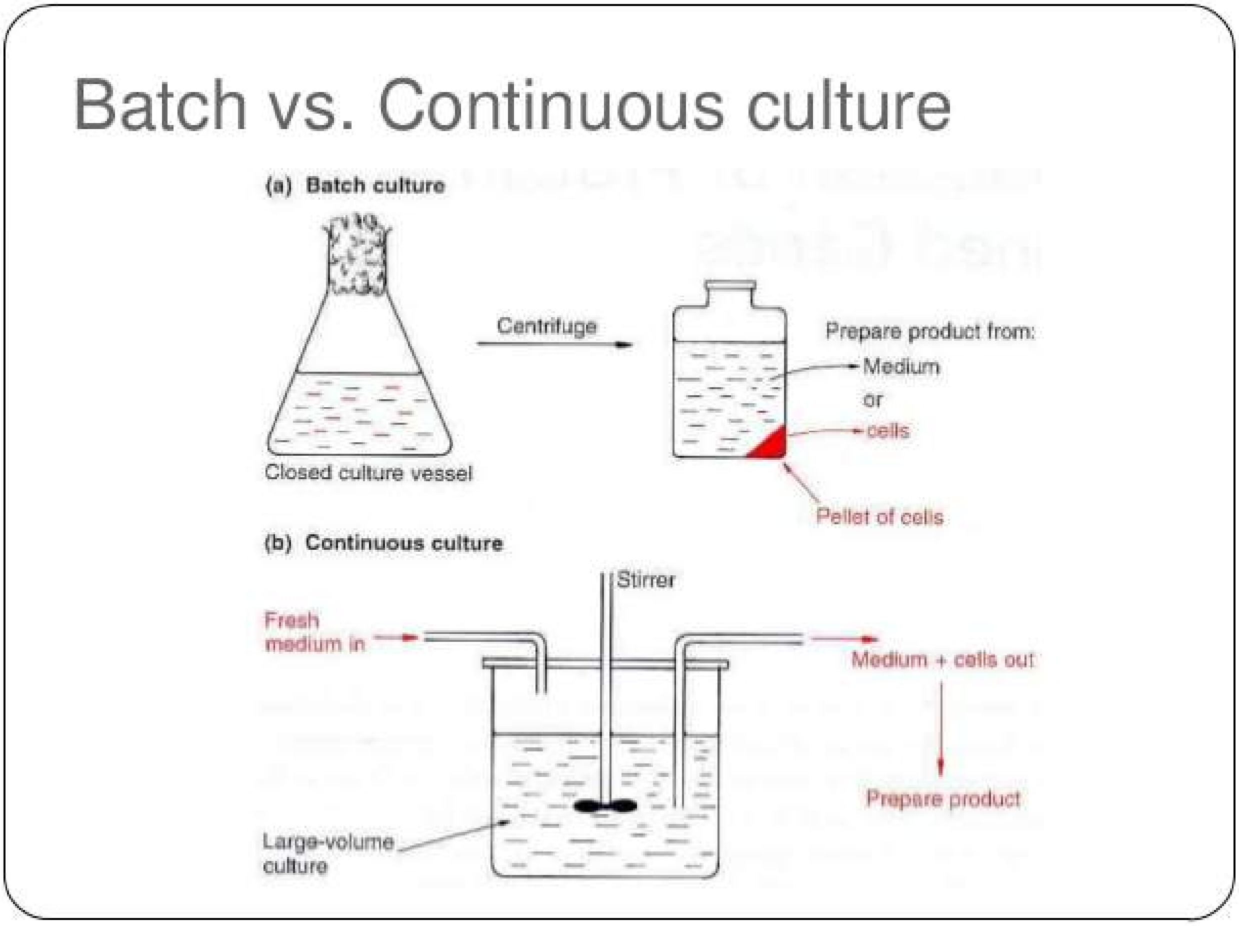
DVX =DX ... (4)

Productivity = DVX/V = DX

In continuous culture cells are grown at a particular growth rate. Then it is maintained for a long time. Most often the continuous culture is used for production of biomass of metabolites.

Besides, liquid wastes are treated by using continuous culture. Microorganisms utilize organic materials of liquid wastes.

Thus microbial biomass is produced in high amount. When such system is in equilibrium, cell number and nutrient status remain constant. At this stage the system is said to be in steady state



Advantages of batch culture versus continuous culture

1) Products may be required only in a small quantities at any given time.
2) Market needs may be intermittent.
3) Shelf-life of certain products is short.
4) High product concentration is required in broth for optimizing downstream processes.
5) Some metabolic products are produced only during the stationary phase of the growth cycle.
6) Instability of some production strains require their regular renewal.
7) Compared to continuous processes, the technical requirements for batch culture is much easier.

Medium formulation for optimal growth and product formation

Medium for fermentation contains all essential nutrients required for growth. Without medium, microbes can neither grow nor multiply.

1.It will produce the maximum yield of product or biomass per gram of sybstrate used.

2. It will produce the maximum rate of product formulation

3. It will produce the maximum concentration of product or biomass.

4. There will be minimum yield of undesired products.

5. It will be of consistent quality and be readily available throughout the year.

6. It will cause minimal problems during media making and sterilization

7. It will cause minimal problems in other aspects of the production process particularly aeration and agitation, extraction ,purification and waste treatment.

Medium optimization methods.

Medium optimization by the chemical method of changing one independent variable (nutrient ,antifoam, temp. etc.,) while fixing all the others at a certain level can be extremely time consuming and expensive for a large no.of variables. When more than 5 independent variables are to be investigated, the Plackett-Burman design may be used to find the most important variables in systems which are then optimized in further studies. This technique allows for the evaluation of X-1 variables by X experiments. X must be a multiple of 4 e.g.8,12,16,20,24 etc.,. Normally one determines how many experimental values need to be included in an investigateion and then selects the Plackett-Burman design which meets the requirement most closely in multiples of 4.Alternately ,factors known not to have any effect may be included and designated as dummy variable.

Trial	**Variables**							**Yield**
	A	**B**	**B**	**D**	**E**	**F**	**G**	
1	H	H	H	L	H	L	H	1.1
2	L	H	H	H	L	H	L	6.3
3	L	L	H	H	H	L	H	1.2
4	H	L	L	H	H	H	L	0.8
5	L	H	L	L	H	H	H	6.0
6	H	L	H	L	L	H	H	0.9
7	H	H	L	H	L	L	H	1.1
8	L	L	L	L	L	L	L	1.4

The stages in analyzing the data using Nelson's method are

1. Determine the difference between the average of H(high) and L(low) responses for each independent and dummy variable.

   $\Sigma A(H) - \Sigma A(L)$

2. Estimate the mean square of each variable

   For A the mean square will be $= [\Sigma A(H) - \Sigma A(L)]^2 /8$

3. The experimental error can be calculated by averaging the mean square of the dummy effects of E and G. Thus, the mean square for error = 0.045+0.020/2=0.0325.this experimental error is not significant.
4. The final stage is to identify the factors which shows large effects.This was done using F-test
   FACTOR MEAN SQUARE/ERROR MEAN SQUARE
   This gives the following values
   A=15.125/0.0325 = 465.4
   B =13.005/ 0.0325 =400.2
   C=0.05 /0.0325 =3.255
   D=0.000/0.0325 =0
   F= 10.580 /0.0325 =325.6

5. When probability tables are examined it is found that factors A,B and F show larger effects which are very significant whereas C shows a very low effect which is not significant and D shows no effect.

ANALYSIS OF YIELD

	FACTOR						
	A	B	C	D	E	F	G
Σ(H)	3.9	14.5	9.5	9.4	9.1	14.0	9.2
Σ(L)	14.9	4.3	9.3	9.4	9.7	4.8	9.6
diff	-11.0	10.2	0.2	0.0	-0.6	9.2	-0.4
Mean Square	15.125	13.005	0.005	0.000	0.045	10.880	0.020

Mean square for error = 0.045+0.020/2 =0.0325

The next stage in medium optimization is to determine the optimum level of each key independent variable. This can be done by response surface technique introduced by Box and Wilson . They are represented by contour plots or topographical plots. Whilst topographical plots show lines of constant value , the contours of response surface optimization plot show lines of identical response. An example is shown below.