Lecture 1: Biotechnology: A Brief Introduction

Introduction

Plant, animal and microbes have been used by humans for nutrition and development of products for consumption such as beer or bread. Understanding of Physical phenomenon has allowed the invention of different types of electronic gadgets, machines, devices and altogether these have been used to increase the efficiency of human activities. Technological advancement has also allowed him to exploit plant, animal and microbial wealth to provide products of commercial or pharmaceutical importance. All these activities (research and development) fall under the big umbrella of biotechnology. In simpler word, Biotechnology is the summation of activities involving technological tools and living organism in such a way that it will enhance the efficiency of the production. The ultimate goal of this field is to improve the product yield from living organism either by employing principles of bio-engineering/bio-process technology or by genetically modifying the organisms. For example, production of bread or other bakery items from wheat flour after adding yeast as fermenting organism (**Figure 1.1**). In India, from ancient times wheat flour has been used to prepare “Roti” but yeast has been added to the wheat flour to make it porous by CO_2 generation during fermentation. Since then this process has been very popular in bakery industry and is responsible for prepration of bread, cakes, pizza, etc.

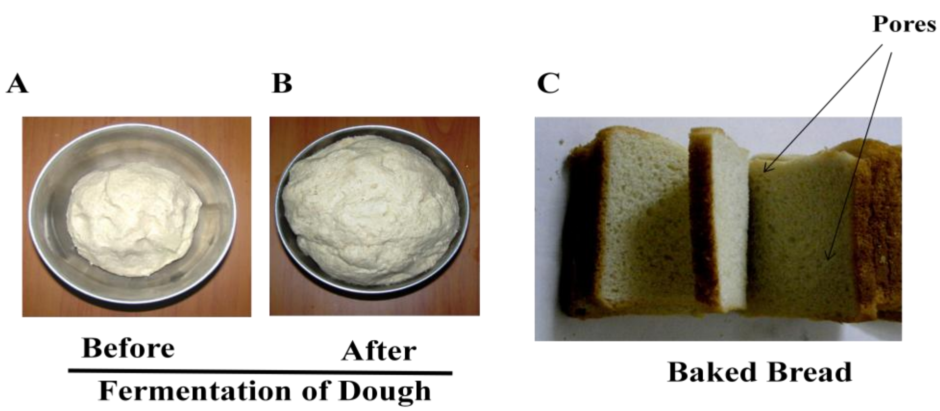

Figure 1.1: Making of Bread from wheat flour. (A) & (B) Dough before and after fermentation. (C) Cross section of baked Bread.

Please note the increase in volume of the dough after fermentation and formation of pores in cross section of bread. Yeast mixed in dough utilizes sugar present in it and produces CO_2 through fermentation, exit of gas causes formation of pores and is responsible for sponginess of bread.

Needs of Biotechnology-The population of india is more than 1 billion and as per projection it may cross 1.5 billion by 2030. This will bring huge burden on biological resources (animal/plant) to provide food for all. Naturally occurring animal, plant or microbial strains have few limitations for them to be utilized for desired products due to following reasons-

1. Purity of the living stock

2. Production of undesired products

3. Secretion of toxic metabolic by-products

4. Inability to withstand harsh biochemical processes/treatments.

5. Higher production cost

6. Susceptible to disease and other environmental conditions

The existing technology today enables us to engineer plants and animals makeing them suitable for maximum production. Living organism has a complex cellular structure, metabolic pathways, genetic make-up, behavior in the synthetic growth media and understanding these processes can help us to modulate specific process/environmental condition or metabolic pathways to achieve the goal of biotechnology. Advancement in different fields of science has paved ways to solve several issues responsible for lower yield of products. Few of the selected science research areas contributing into the development of biotechnology are given in the Figure 1.2. The foundation of biotechnology relies on the research & development activities in different areas of science and interaction of interdisciplinary areas. The research in the field of plant biotechnology allowed us to produce plants through micro-propagation but with the evident advancement of genetic engineering, it is now possible to produce plant with predefined characteristics imprinted at genetic level through genetic engineering. The similar relationship may also exist for many other overlapping areas and as a result biotechnological operation output is amplified several folds.

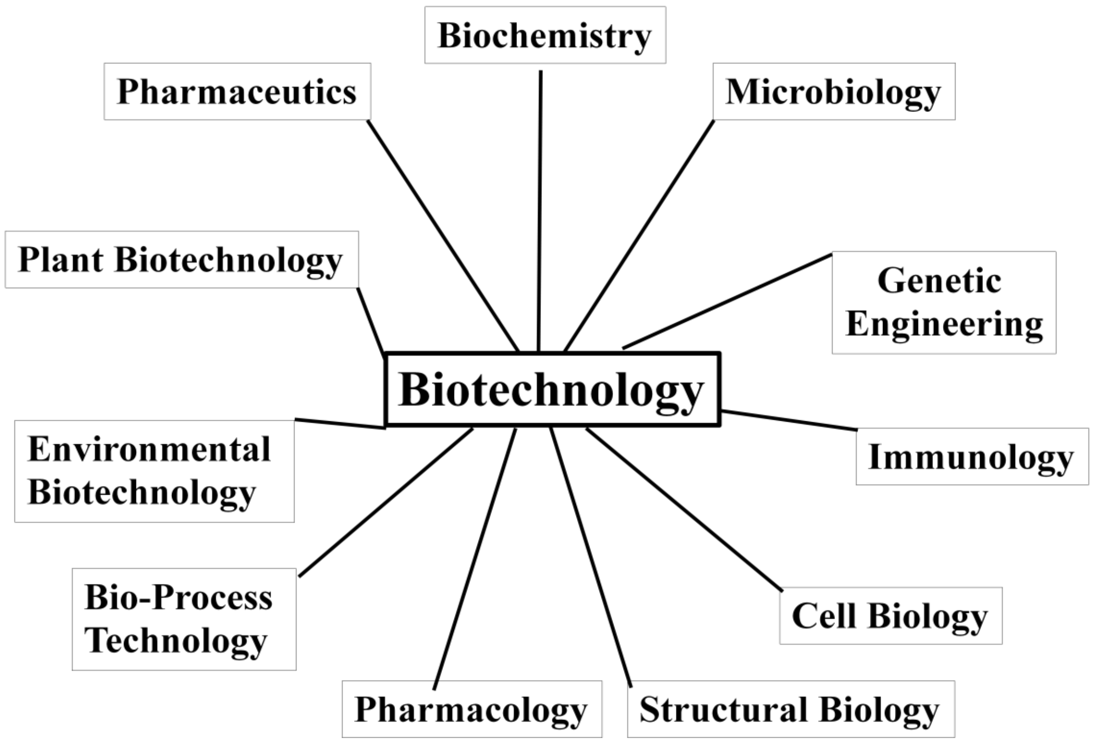

Figure 1.2: Different Science fields contributing into the advancement of biotechnology

Historical Advancement of Biotechnology-Biotechnology related activities depend on two parameters: technological advancement and knowledge of available biota. Technological upgradation goes parallel with the over-all understaning of physical and chemical phenomenon in different time periods. Hence, Biotechnology starts as early as human have realized the importance of organism (animal/plants or microbes) to improve their life-style. A systematic chronological description of biotechnological adavancement over the course of different time periods is given in Table 1.1. The earliest biotechnology related activities are selection and cross breeding of high yielding animals, cross breeding of plants to acquire specific phenotype and preserving the seeds of high yielding crop plant for next sowing season. These were few initial scientific experiments and based on the results, human have made significant modification in available biota. In last century, the systematic and scientific study of living objects with advanced technology has given immense potential to human imagination to either genetically manipulate living organism with desired phenotype or mimic metabolic reactions in an in-vitro system (either in test tube or in cells) to produce molecules with therapeutic importance. Such as “Humulin” is the insulin being produced in bacterial expression system and it is now been making life of millions of diabetic patients easier. Similarly during this era, drought, pest or abiotic resistant plants, high milk yielding animals, transgenic bacteria to produce biofuel, degrade environmental hazard or chelation of heavy metal have been developed. In addition, the historical advancement of biotechnology will not be complete without mentioning development of procedure for artificial insemination and test-tube baby for thousands of couples.

Table 1.1: Important milestones of Biotechnology

S.No.	Time Period	Major break-through
1.	7000 BC-100CE	• Discovery of fermentation • Crop rotation as a mechanism to improve soil fertility. • Animal and plant products as a source of fertilizer and insecticide respectively.
2.	Pre-20th Century	• Identification of living cell and bacteria • Discovery of small pox vaccine, rabies vaccine. • Process development to separate cream from milk, • Discovery of artificial sweetners, “invertase”. • Discovery of DNA and chromosome responsible for genetic traits.
3	20th Century	• Discovery of Pencillin. • 3-D Struture of DNA. • Fabrication of artificial limb and arms, • Production of human insulin in bacteria “Humulin”. • Discovery of PCR. • Gene therapy, • Procedure for artificial insemination and test-tube baby. • Cloning of first mammal “Dolly”.
4	21st Century	• Vertebrate, invertebrate and bacterial genome sequences. • Completion of Human Genome sequence. • Sequencing of Rice genome. • Discovery of Nano radio. • Invention of Bionic leg.

Applications of Biotechnology- Biotechnology has influenced human life in many ways by inventions to make his life more comfortable. Many scientific fields contribute to biotechnology and in return it gives product for their advancement. Few of the biotechnology applications are given in Figure 1.3. The brief description of application of biotechnology in different field is as follows-

Plant sciences- Genetic Engineering has allowed us to produce genetically modified plants with diversified properties such as resistance against pest, drought and abiotic stress. It has enabled us to produce ediable plants with short life-span or ability to grow in different season to increase the number of crops in a year to ultimately increase the food production. Horticulture has used biotechnology tools to produce plants with multiple color, shades, aroma to increase the production of natural colors and scent. A detail description of other biotechnology application in plant sciences is discussed in next lecture.

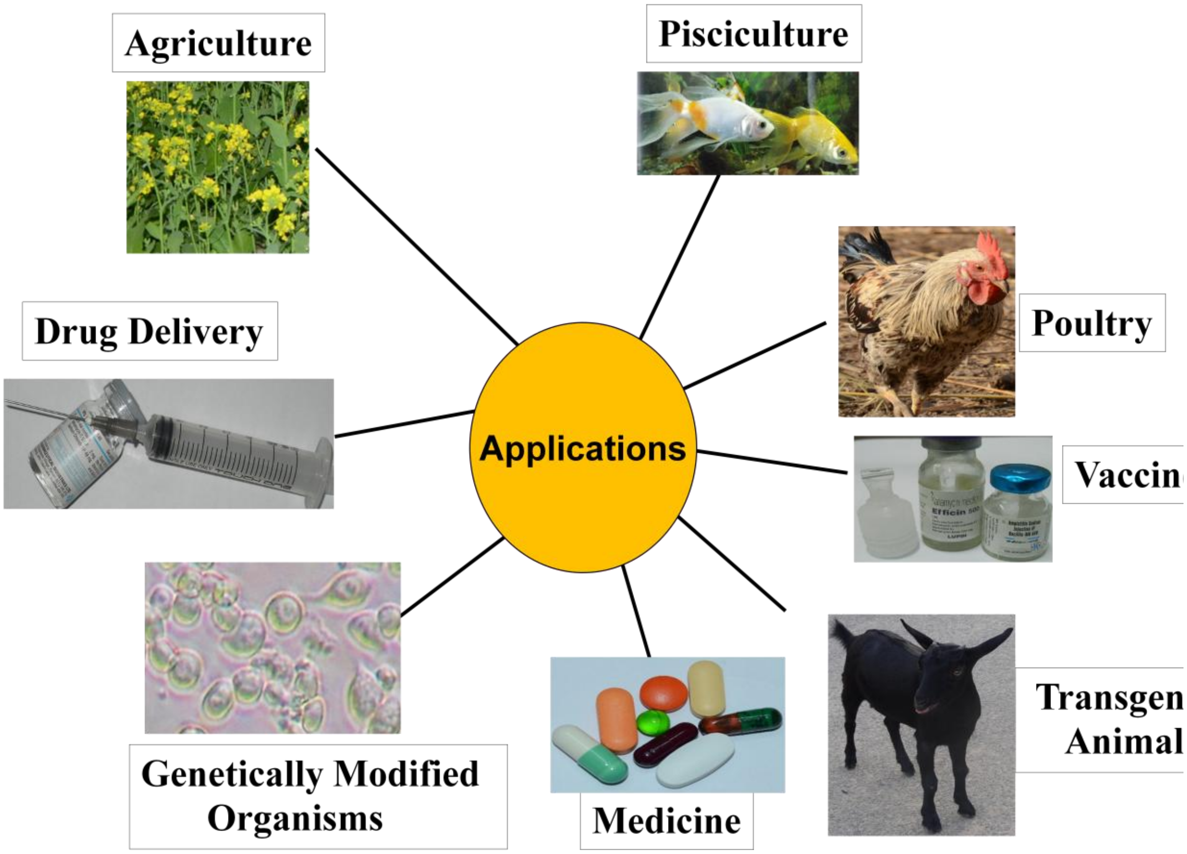

Figure 1.3: Impact of Biotechnology on different fields & human life.

Animal sciences- One of the early application of biotechnology in animal science is developing method to separate cheese and other food products from milk by enzyme and microbes. Genetic engineering in conjugation with cell biology and biochemistry has developed multiple products of animal origin. Transgenic animal strains with desired phenotype such as high milk yielding animals, fishes and hens with more fat content. A detail description of other biotechnology application in animal sciences is discussed later.

Medicine and Medical Sciences-Biotechnology helped identification of drug like molecules, antibiotics and other medicines. At present a number of antibiotics are being produced by fermentation or in cell based systems. Apart from antibiotic, vaccine, diagnostic kits and other immunotherapy are gift of biotechnological advancement. Development of artificial limb, arms, heart and medical procedures to perform open-heart operation, dialysis, artificial insemination, test-tube baby and other medical procedures.

In the current lecture I have put effort to briefly discuss about biotechnology, its scope and impact on human life with several customized products. The Development of technology and generation of product has multiple steps and understanding these steps are being covered in this course with a discussion of biotechnology application at the end. By the end of this course, student will be able to understand following aspects of biotechnology:

1. Basic metabolic pathways and their regulation.

2. Microbial growth kinetics with an emphasis on fermentation

3. Basic molecular biology tools used in biotechnology.

4. Basic methodology for product recovery and analysis.

Lecture 2: Water and its role in controlling biological processes

Life on earth originates in ancient ocean after water has reduced the temperature on earth to the permissive level to support vital biological activities. Most of the biological reactions operate in aqueous environment and use water as one of the reactant. It is making ~70% content of the organism and controls the enzymatic activity of major metabolic pathways. In higher mammals, it has been used as a medium for material transfer between different organs and a reactant to neutralize and facilitates excretion of toxic metabolites out of the body. The enormous capacity of water to perform multitasking, is the sole basis of life activities and lies in its unique structure and ability to interact with biological molecules.

Structure of Water: Water has a chemical formula of H_2O with central oxygen covalently attached to two hydrogen atoms (Figure 2.1, A). It has two lobes of unpaired electron opposite to the hydrogen atom called as "lone pairs". The H-O-H bond angle is 104.5^0 and over-all molecule adopt partial tetrahedral geometry. In the water molecule, oxygen is more electronegative than hydrogen, as a result sharing electrons are placed towards oxygen and gives partial positive charge to hydrogen (δ^+) and negative charge to oxygen ($2\delta^-$). Hence, water molecule develops a dipole and form hydrogen bonding involving electronegative oxygen of first molecule and positively change hydrogen of next molecule. In this manner, each water molecule can be in hydrogen bonding interactions to 4 neighboring molecules (Figure 2.1, B). As a result, water molecules exist in three different state of matter; liquid, solid (Ice) and gas (vapor). In liquid state, water molecules are under dynamic hydrogen bonding interaction to the neighboring molecules where as in solid state (Ice) molecules present in one plane are in hydrogen bonding interaction with molecules present in same plane and neighboring plane giving a quasi crystal like packing (Figure 2.1, C).

THINK TANK??

Why H_2S Exists as gas ?

Water Ionization and pH: Water weakly ionizes reversibly into hydrogen ion (H^+) and hydroxide ion (OH^-) as shown in Eq. 2.1. But H^+ thus formed gets hydrated to form hydronium ion ($H3O^+$) due to hydrogen bonding between two water molecule which allows instantly hydration of ionized proton (Eq 2.2).

$$H_2O \leftrightharpoons H^+ + OH^- \ldots\ldots(2.1)$$

$$2H_2O \leftrightharpoons H_3O^+ + OH^- \ldots\ldots(2.2)$$

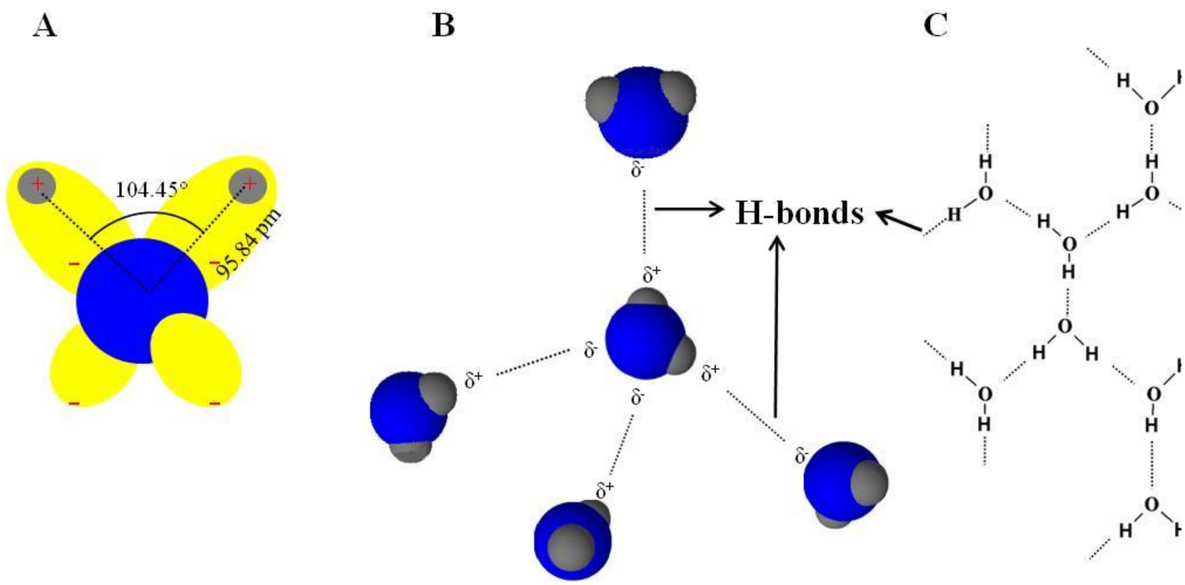

Figure 2.1: Structure of Water. (A) Chemical structure of water molecule. (B) Hydrogen Bonding between water molecules and (C) Hydrogen bonding pattern observed in Ice.

Ionization of water and pH: The equilibrium constant, K_{eq} for the reversible ionization of water is:

$$K_{eq} = [H^+][OH^-]/[H_2O] \ldots\ldots 2.3$$

The Concentration of pure water, $[H_2O]$=55.5M and K_{eq} calculated from electrical conductivity measurement, $K_{eq}=1.8 \times 10^{-16}$M

$$\text{Hence, } [H^+][OH^-]=1.8\times10^{-16}M/[55.5] \ldots\ldots 2.4$$

$[H^+][OH^-]=K_w=1.0\times10^{-14}\ M^2$, Here K_w=Ion product of water at 25^0C.

In pure water, $H^+=\sqrt{K_w}=\sqrt{1.0\times10^{-14}}$ or $[H^+]=[OH^-]=10^{-7}$M

Ion product is a constant and allows us to calculate $[H^+]$, if $[OH^-]$ is known or vice-versa. Hence, it is used to develop pH scale to define concentration of H^+ or OH^- in any aqueous solution. pH is defined as "**negative logarithm of hydrogen ion concentration**" and it ranges from 1-14 (Figure 2.2, A). Solution with pH=7 is neutral whereas solution with pH lower than 7 is acidic and pH above 7 are considered as alkaline. Biological samples of different origin and products of daily use are examples of solution with variable pH (Figure 2.2, B).

$$\mathbf{pH = -\log [H^+] \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2.5}$$

Hence ability of any acid to dissociate to give H+ ion or a base to give OH- ion define the acid or alkali as strong acid or weak acid.

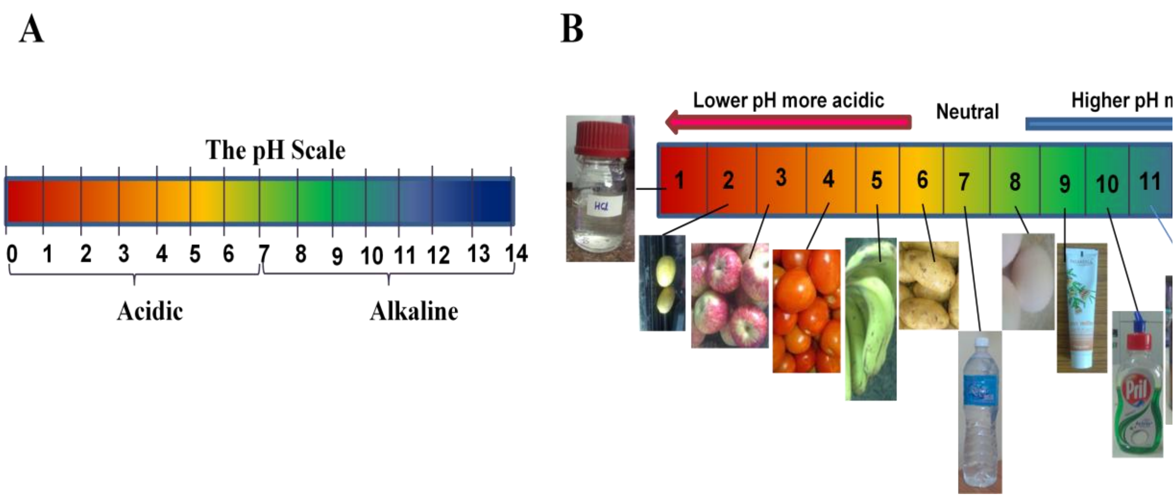

Figure 2.2: pH Scale and pH of different fluids. (A) pH Stripe, (B) pH in different biological fluids and daily use items.

Handerson-Hasselbalch Equation-Titration is the method to determine the content of acid or base present in a solution. In a typical titration experiment, a fixed amount of acid is titrated with a solution of strong base, such as NaOH in the presence of pH indicator dye. The purpose of adding pH indicator dye is to monitor the change in pH during the course of titraton. A typical plot of change in pH against the amount of NaOH added for acetic acid is given in Figure 2.3. In the beginning, acetic acid is present majorly as CH_3COOH but with the addition of NaOH, OH- will neutralize H^+ to form H_2O and promotes further dissociation of acetic acid to give H^+ (Figure 2.3). At the mid point of where 0.5 equivalent NaOH has been added, solution has a equal concentration of

CH_3COOH (proton donor) and CH_3COO^- (proton acceptor). At the end of titration, all undissociated CH_3COOH will be convereted into the CH_3COO^-.

During a titration experiment, two reversible equilibrium exists.

$$H_2O \leftrightharpoons H^+ + OH^- \ldots\ldots (2.1)$$

$$CH_3COOH \leftrightharpoons H^+ + CH_3COO^- \ldots\ldots (2.6)$$

$$K_a = [H^+][CH_3COO^-]/[CH_3COOH] \ldots\ldots (2.7)$$

pKa= -log K_a, Strong acid will have lower pK_a. it is calculated by titration experiment and pH at the mid-point of the titration curve for acid or base (Figure 2.3).

After rearranging, $[H^+] = K_a [CH_3COOH]/[CH_3COO^-] \ldots\ldots (2.8)$

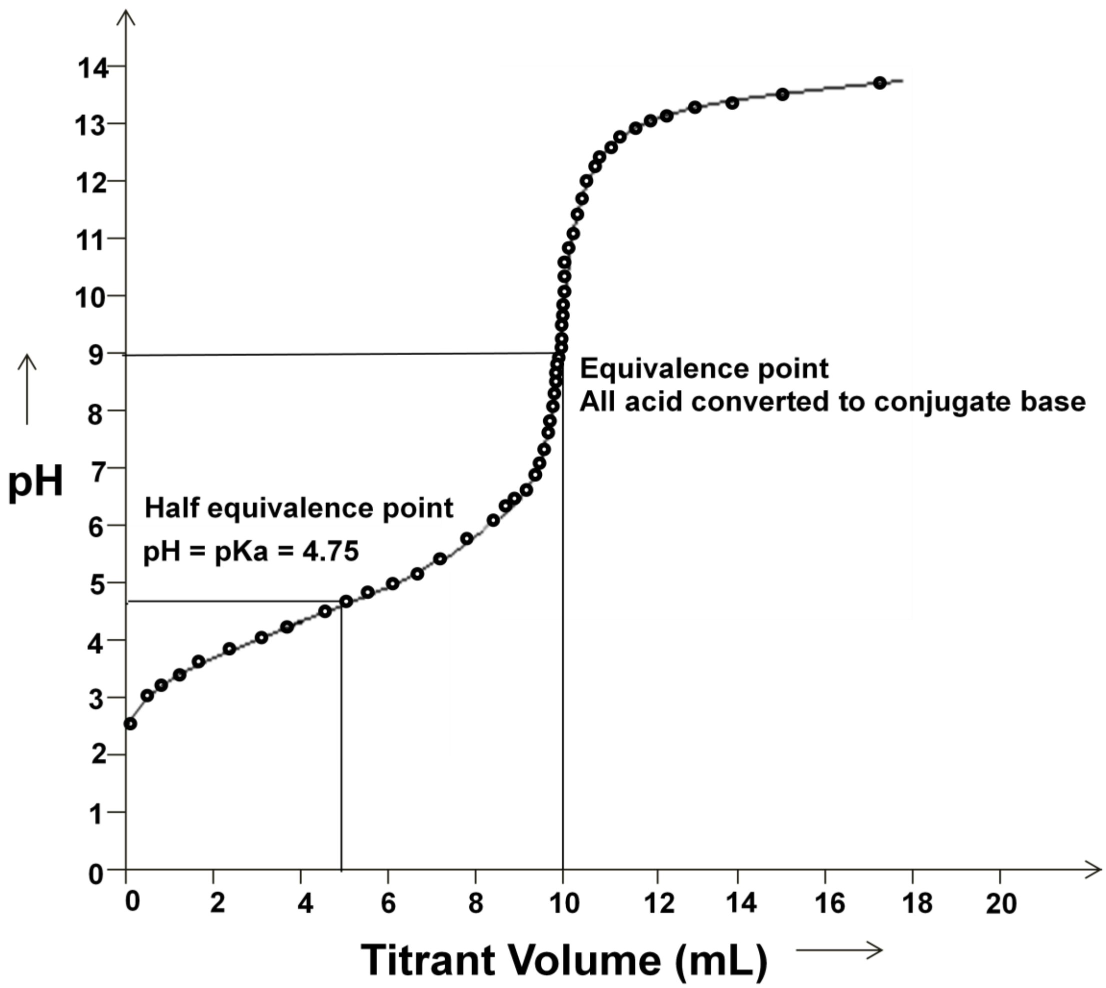

Figure 2.3: Titration Curve of acetic acid.

Taking negative log on both side, **$-\log [H^+] = -\log K_a - \log [CH_3COOH]/[CH_3COO^-]$**

$\because -\log [H+] = pH$ and $-\log K_a = pK_a$

$\therefore pH = pKa - \log [CH_3COOH]/[CH_3COO^-]$ or

$pH = pKa + \log [CH_3COO^-]/[CH_3COOH]$

or in general for any acid

$pH = pK_a + \log$ [proton acceptor]/[proton donor] ---------------------------------------(2.9)

The equation 2.9 is known as **Henderson-Hasselbalch equation** and it relates molar ratio of proton donor/acceptor with pKa at a given pH. It is also used to calculate the amount of proton donor, acceptor needed to prepare the solution of given pH, if pKa is given.

Buffer: The aqueous solution of weak acid or base and conjugated strong base (salt) and it resists to the change in pH on addition of small amount of acid or base. In biological system and in the in-vitro reactions are performed in buffer to keep the pH constant during the process. The degree of buffer solution to resist towards change in pH is calculated as **buffering capacity**, β. It is directly related to the pKa of the particular acid used in the buffer and buffering capacity is maximum at pKa and a safe range to use any buffer is pKa $\pm$1 unit.

THINK TANK??

What is the role of salt in buffer solution?

Role of buffer in biological system: each and individual organism keeps a constant pH to maintain homeostasis and growth. A number of biological buffers (including proteins) are known to maintain a constant pH within different tissues of human body. Few Buffers are specific to the organ or tissue where as many buffers are universally present. Biological reactions as well as macromolecules are sensitive to the change in pH and therefore buffer plays vital role for their optimal activity.

Few examples of role of pH in controlling biological processes:

1. Enzymes are proteinous in nature and madeup of amino acids with ionizable side chains such as histidine. In addition, active site of enzyme has amino acid residues and a particular ionization state is important for substrate binding, formation of catalytic intermediates and release of products. For example, Pepsin is a serine protease present in stomatch and has a optimum pH of 1.5 where as trypsin has a pH optimum of 7.4.

2. In many pathological condition such as diabetes, body utilizes stored fat as an alternate energy source. The similar condition exist in the case of starvation or fasting and under these conditions a large amount of acid (β-hydroxybutyric acid) from fat is generated leading to the lowering of blood pH to cause "acidosis". It disturbs activity of several enzymes present in blood and ultimately lead to headache, nausea and convulsions.

3. Blood pH is maintained by bicarbonate buffer system and play vital role in respiration.

4. Bacterial growth in mouth causes disturbance in mouth pH (acidity) and as a result it affects the mineral content of teeth resulting into the tooth decay.

QUIZ

Q1: The pH and concentration of $[H^+]$ in 0.1M NaOH solution?

Ans: pH=13 and $[H^+]= 10^{-13}$

Q2: What is the pH of a mixture of 0.032M NaH_2PO_4 and 0.018M Na_2HPO_4 ? Given pKa=6.86

Ans: pH=6.61

Q3: What will be the $[OH^-]$ in a 0.1M NaOH Solution?

Ans: $[OH^-]=10^{-1}$

Q4: Enlist the different types of biological buffers and their locations in the body?

Q5: Calculate the ratio of salt and acid to prepare the 0.1 M phosphate buffer with pH 6.8, Given pKa=6.8 ?

Ans=1.0