CHAPTER I

INTRODUCTION
I. INTRODUCTION

"WATER IS LIFE-CHERISH IT"

Our earth is the only watery planet known so far of the solar system. Water, support life on earth, waters either as fresh or sea water sustain the umpteen lives. It is difficult to imagine a world without water. Added to the many benefits with which water blesses life and around which the entire fabric of life is woven, further enhances the utility as rainfall and the rains nourish life both plants and animals.

Water receives microorganism from air, soil, sewage, organic wastes, dead plants and animals. It is obvious that at times almost any organism may be found in water, those finding unfavourable conditions die, and the other finding favourable conditions grow and multiply to increase their population. Water contains microbes and cause diseases of various kinds. Physical and chemical characters of water are changed due to addition of organic, inorganic substances and organisms to water. Thus changes are harmful to the health and hence reduce the usefulness of water. The main water polluting agents are household detergents, sewage and other wastes (industrial and agricultural) release from nuclear reactors etc. added to water in large quantities. Many microbes are capable of producing products of human value if they are produced in large quantities. Microbiologists, engineers, businessman have come together for this purpose and have developed the field in industrial microbiology.
Lake in general and pond in particular may be considered an evanescent feature of earth's surface because of their shallowness and a relatively short life in geological time. Every lake basin forms a bed into which the sediment carried out by inflowing water is deposited. Natural lakes and impoundments of small streams are attractive environmental resources that create a demand for shared uses ranging from water supply to community development of shorelines for housing and recreational activities (Geldreick 1989, 1990). As a result of increasing population pressure and urbanization, the disposal of sewage in large amount is becoming a major global problem. Each day about a million-gallon sewage is generated in India, out of which 30% is produced in cities having 20% treated and 80% remain untreated. On an average, the sewage of Indian towns contains 52 ppm nitrogen, 16 ppm phosphorous, 45 ppm potassium and 350 ppm biodegradable organic matter. Biochemical oxygen demand (BOD) and dissolved oxygen (DO) values are extremely high in sewage water.

Temperature plays a very important role in wetland dynamism affecting the various parameters such as alkalinity, salinity, dissolved oxygen, etc. In an aquatic system these parameters affect the chemical and biological reactions such as solubility of oxygen, CO$_2$, bicarbonate equilibrium, increase in metabolic rate and physiological reactions of organisms etc. Water temperature is important in relation to fish life. The temperature of drinking water has an influence on its taste.
The effect of pH on the chemical and biological properties of water makes it determination very important. It is thought to be important factor in maintaining the bicarbonate and carbonate system of fresh water and provides an important meance to understand the chemical conditions prevailing in natural water. The pH range may vary 2 to 12 units in different fresh water systems. The reciprocal concentration of hydrogen ions controls the production of flora and fauna (Davis 1954) in aquatic system.

The chloride a dominant anionic constituent of salinity is normally found in very low quantities in fresh water, when present in higher concentration it indicates the extent of pollution due to sewage (Aboo and Manuel 1967) and play metabolically active role in photolysis of water and photophosphorylation reaction in autotrophs. High concentration considers being indicator of pollution, which is either due to organic wastes of animal origin or industrial effluents. Sodium chloride is common article of diet and passes unchanged through the digestive system. Chloride does not precipitate and sediments and cannot be removed biologically during treatment of the waste. The high concentration of chloride in fresh water is assigned to human sewage (Moyle 1949) and undesirable taste to water and beverages (Dhanaselvan and Lakshmanaperumalsamy 1991).

Alkalinity of water refers to the quantity and kind of compounds present. The alkalinity shifts the pH to the alkaline side of neutrality. It is usually imparted by the presence of bicarbonates, carbonates, hydroxides, borates, silicates and phosphates (Wetzel 1975).
In most natural water, bicarbonates and sometimes carbonates are present in appreciable amounts. Their salts get hydrolyzed in solution and produce hydroxyl ions, consequently raising the pH.

The term hardness was frequently used to assess the quality of water. The hardness of water governed by the contents of calcium and magnesium salts, as bicarbonate and carbonate (temporary hardness), sulphate, chloride (permanent hardness) etc. According to Wetzel (1975) hard water contains large concentration of alkaline earth derived from the drainage of calcareous deposits.

Calcium is essential for all living organisms being important cell wall constituents and regulates various physiological functions in animal too. It has a direct effect on pH, carbonate and calcium ions which contribute to hardness of water. Sulphates are generally present in appreciable concentration and impart hardness to water (Sharma 1983).

Water reservoirs always maintain a balance between available oxygen and decomposition of organic matter. This capacity is lost due to addition of excessive sewage and other wastes, hence recycling and self-regulation of the system is disturbed. The rate of reoxygenation becomes slower than the rate of deoxygenation. Biochemical oxygen demand is the amount of oxygen required for biological oxidation by microorganisms present in water. Lesser value of biochemical oxygen demand indicated comparatively clean water while the values of biochemical demand are higher in polluted water.
The atmospheric air contains about 20.9% oxygen and 79.1% nitrogen. Oxygen is comparatively more soluble in water. The zone of organic pollution is characterized by low dissolved oxygen concentration and higher oxygen demand. When conditions are especially severe, directly down stream of the discharge, the zone may be almost devoid of all living beings except for certain bacteria that tolerate the extremely low dissolved oxygen content and high toxicity of effluents (Dhanaselvan and Lakshmanaperumalsamy 1991, Nagaratha and Hosmani 2003).

Nitrogen and phosphorous are major components of runoff and domestic sewage. These substances are added to the water in large quantities where phosphorous as phosphate is most useful algal nutrients. It along with nitrogen (nitrates) stimulates algal growth and covers almost the entire water surface called water bloom. Algal blooms compete amongst the constituent algae for light and oxygen. At the same time, algae release toxins harmful for aquatic organisms. This results in the death of organisms and as a result, organic matter accumulates and called eutrophic and the process as eutrophication.

The organism and its environment together constitute an ecosystem. Both the components of ecosystem maintain a balance. Disturbances in the ecosystem are mostly the result of excessive use, misuse and mismanagement of biosphere resources. The human activities aimed at development are responsible for disturbance and thus are the major cause of pollution.
Bacteria are ubiquitous in distribution, they are found in all material habitats, i.e. soil, water and air. They occur in all situations except in the pits of volcanoes, deep strata, or rock and the blood of normal animals. The majority of bacteria found in water belong to groups: (i) **Fluorescent bacteria** (*Pseudomonas, Alginomonas* etc). (ii) **Chromogenic rods** (*Xanthomonas* etc.), (iii) **Coliform group** (*E. coli, Aerobacter* etc.), (iv) **Proteus group**, non-gas forming, non-chromogenic and spore forming rods, spore formers of the genus *Bacillus* and pigmented and non-pigmented cocci (*Micrococcus*). It is generally accepted that the planktonic bacteria play an important role in the carbon flow and nutrient recycling of lower trophic levels in aquatic ecosystems.

**CELLULOSE**

A prominent carbonaceous constituent of higher plants and probably the most abundant organic compound in nature is cellulose and the estimated synthesis is approximately $4 \times 10^7$ tons per year. In structure, cellulose is a carbohydrate composed of glucose units bound together in a long, linear chain by $\beta$ linkages at carbon atoms 1 and 4 of the sugar molecule. About 280 to 800 chains of cellulose molecules are bound laterally by hydrogen bond and other physical forces to give rise to microfibrils which in turn aggregate to form the fibrils. The polymer chains at some places in the fibrils show maximum orientation being very tightly packed, while in other places they are relatively loosely arranged. The compact orientation places are called as **crystalline areas** while regions with loose chains are known as **amorphous areas**. A single cellulose chain passes through several crystalline and amorphous areas in the
fibril. Crystalline areas are resistant to enzyme attack as high orientation prevent entry of big enzyme molecules.

Native cellulose is insoluble but can be made soluble in a variety of ways. The chain length of native cellulose vary greatly. The longest chain measures 3-4 microns (30,000-40,000 Å) and have a molecular weight of more than a million. Such chain consists of about 6,000-8,000 glucose molecules (Agrios 1969). The cellulose molecule is built up of units of β-glucose. Two molecules of β-glucose are combined through a 1,4 linkage to give celllobiose. The cellulose molecule is a simple linear polymer of 1000 to 10,000 units of celllobiose linked end to end through 1,4 β-glucosidic linkages.

Mark (1969) considered the basic protofibril of native cellulose as a helical spring of diameter \(2R = 35\) Å, pitch \(h = 40\) Å and rectangular cross section of \(a = 7.86\) Å and 35 Å.

Cellulose is present in woody parts, seed hairs, bast fibres, straws, stalks and hulls in major quantity. Purest natural form of cellulose is in cotton fibres and also in fibrous plants like flax, jute, ramie etc. Generally cellulose occur in close association with many other compounds, such as hemicellulose, lignin, pectin, chitin, and is present in the primary and secondary cell wall of higher plants in the form of microfibrils. Cellulose microfibrils are considered to provide the structure and support for all the other components of plant cell walls (Carpita and Gibeaut 1993) and their degradation by fungal cellulases may contribute to the weakening of the cell wall (Copper 1983). Cellulase is also found in few microorganisms and lower organisms. It is not digested by the
digestive enzymes of man and therefore forms the bulk and roughage of food. Cellulose is an organic polymer containing:

- 44.4 % of carbon
- 6.2 % of hydrogen
- 49.4 % of oxygen.

It becomes soluble when the hydrogen of the primary and secondary hydroxyl group is replaced with methyl, ethyl, carboxymethyl or other groups. On the complete hydrolysis cellulose yield glucose units while partial hydrolysis yield mixture of polysaccharides such as cellobextrins, oligosaccharides and a disaccharide cellobiose.

Out of 30 billion tons of carbon that is transformed into organic compounds by plants annually, one third is made insoluble cellulose (Bonner and Varnes 1965). In contrast to starch and glycogen, it is cellulose which is insoluble in ordinary solvents and boiling with dilute acids does not hydrolyse it. It gives no colour with iodine. Both alkalies and acids are known to bring about the swelling of cellulose. Sodium hydroxide reacts on the amorphous region only upto 8% concentration while higher concentration initiate the breakdown of the crystalline regions of cellulose (Sharma 1980). Cellulose is a complex enzyme containing chiefly exo \( \beta \)-glucanase (C\(_1\)) [EC-3.2.1.91], endo \( \beta \)-glucanase, C\(_x\) [EC-3.2.1.4] and cellobiase or \( \beta \)-glucosidase [EC-3.2.1.21]. As recognized today cellulases are widely distributed in plants, bacteria, actinomycetes and fungi. Cellulase is attacked by a wide variety of bacteria including, aerobic mesophilic sp. and anaerobic thermophilic types. Almost all the aerobic mesophilic cellulolytic bacteria so far isolated have been placed in
the following genera: Cellfalicula, Cellulomonas, Cellvibrio, Cytophaga, Pseudomonas rods, Sporocytophaga and Vibrio. Cellulolytic enzymes isolated from various sources differ in their molecular characteristics: molecular weight, amino acid composition and sequence, isoelectric point, carbohydrate content, adsorbidity on the cellulose, catalytic activity and substrate specificity.

The enzymatic breakdown of cellulose results in the final production of glucose molecules. This is brought about by a series of enzymatic reactions. There are two theories to explain the mechanism of cellulose degradation. Cellulose degradation: (i) the unienzyme theory (Whittaker 1953, 1957; Aitkin et al., 1956) and (ii) the multienzyme theory (Reese 1959). Wood (1967) proposed a scheme of cellulolytic enzymes when native cellulose is degraded.

Native cellulose

\[ \text{Enzyme C}_1 \]

\[ \text{Linear chain} \quad \text{Modified chain} \]

\[ \text{Enzyme Cx} \quad \text{Cx Enzyme} \]

\[ \text{Celllobiose} \]

\[ \text{Cx or } \beta\text{-glucosidase} \]

\[ \text{Glucose} \]
Reese (1954) has also given the following scheme for the degradation of native cellulose to glucose molecule:

\[
\text{Native cellulose} \xrightarrow{C_1 \text{ Exo enzyme}} \text{Relative cellulose} \xrightarrow{Cx \text{ Enzyme (hydrolytic)}} \text{shorter cellulose chains} \xrightarrow{Cx \text{ Enzyme (hydrolytic)}} \text{Cellobiose} \xrightarrow{\text{Cellobiase (Hydrolytic)}} \text{Glucose}
\]

In both the schemes, glucose is the final product of enzymatic degradation of cellulose, which is produced by the action of Cx enzyme or the products liberated by the action of C_1 enzyme on native cellulose. It was also agreed by some workers that Cx enzyme produces cellobiose, which is degraded to glucose by the action of cellobiase or β-glucosidases. Manners (1982) suggested that the group of enzyme referred to as Cx might be regarded as endo β-1,4-glucanase enzyme. The cellulase enzyme produced by most microorganisms consists of at least 4 components:

(i) Enzyme C_1 which releases single chain from microfibrils,
(ii) Enzyme C_x breaks cellulose fibrils to short fibres,
(iii) Enzyme C_x which hydrolysis the chain to cellobiose and
(iv) Enzyme cellobiase hydrolyses cellobiose to glucose.

There are again different views regarding the mode of hydrolysis (random or end wise). Many evidences have been accumulated in favour of random action:
(a) Reduction in the viscosity of soluble cellulose before appreciable increase in favour of random action.

(b) Formation of oligosaccharides during hydrolysis.

(c) Rapid loss in tensile strength of cellophane without corresponding increase in reducing sugar produced.

The cellulase enzyme complex is divisible into $C_1$, $C_x$ and cellulobiase or $\beta$-glucosidase upon the stages of cellulose undergoing degradation. The activity of isolated exoglucanases may be determined by measuring the release of reducing sugar or more specifically of cellulobiose or dextrose from the oligosaccharide. This inducible enzyme loosens cellulose fibrils of the crystalline area. An important feature of this enzyme is that it is not recovered from the culture medium. The $C_1$ cellulase enzyme acts on cross linkages between chains of the microfibrils which is then cleaved by $C_x$ enzyme into low molecular cellosaccharides with some amount of glucose. The best substrate for assaying $C_1$ activity is cotton fibre which is unaffected by $C_x$ enzyme. Most exoglucanases are glycoprotein and exist as single polypeptide. The range of molecular weight being remarkably narrow as in the case with the other components of cellulolytic systems. These enzymes are acidic and are most active and stable under such conditions.

The enzymatic breakdown of cellulose results in the final production of glucose molecules. This is brought about by a series of enzymatic reactions. There are two theories to explain the mechanism of $C_x$. It is the $C_x$ enzyme where the 'x' subscript refers to its multiple forms.
β-GLUCOSE

β-CELLOBIOSE

CELLULOSE CHAIN

Cellulose is composed of glucose bonded in β-1, 4 bonds that produce linear, lengthening chains of polysaccharides (a polymer of 5 or more monosaccharides)
The enzyme is able to catalyse the hydrolysis of amorphous cellulose and soluble derivatives such as CMC (Reese 1956). The $C_X$ group of enzymes hydrolyse the cleavage of $\beta$-1,4-glycoside linkage but a prior modifying action of $C_1$ enzyme is essential for their activity. They can not attack native cellulose directly. Several enzymes have been characterized in the $C_X$ group of enzymes, which attack the glycoside bonds randomly. The endo $C_X$ enzyme breaks the long cellulose chains into soluble, low molecular weight of glucose units. The $C_1$ exo-enzymes, by acting on terminal bonds further break these smaller chains into cellobiose, which may further be hydrolyzed by another enzyme cellobiase to liberate glucose units. $C_X$ activity was assayed by measuring the loss of viscosity or the production of reducing sugars from carboxymethyl cellulose.

The enzyme $\beta$-glucosidase ($\beta$ D-glucoside glucohydrolase) occurs widely in prokaryotes and eukaryotes. It catalyses the hydrolysis of aryl and alkyl $\beta$ D-glucosides as well as glucosides with only a carbohydrate (cellobiose) moiety (Esen 1993). Literature indicates that almost all $\beta$-glucosidases have subunit molecular weights of 55-65 kD and acidic pH optima (pH 5 to 6). Human acid $\beta$-glucosidase has potential in the development of therapeutic and diagnostic procedure that will be useful in the treatment of Gaucher's disease, an inherited disorder caused by the deficiency of acid $\beta$-glucosidase localized in the lysosome. Plant $\beta$-glucosidases have been implicated in the variety of growth and productivity.
These enzymes have a vital role in the saccharification of cellulose because they hydrolyse the products of cellulase action and because of their glycosyl transfer abilities may be involved in the induction of cellulase synthesis (Woodward and Wiseman 1982). They catalyse the removal of dextrose from the non-reducing ends of oligosaccharides up to cellohexose and affect the cleavage of celllobiose. Many of the β-glycosidases are glycoproteins with molecular weight ranging from 35,000 to 44,000.

After having established that cellulase enzyme has three main components, namely exoglucanases, endoglucanase and β-glucosidase, the isolation and purification of the individual components of cellulase systems is a necessity for complete understanding of the relative importance and mechanism of action of each enzyme in the hydrolysis of cellulose.

Cellulolytic enzymes involved in the cellulose degradation is given in the following table:

<table>
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<tr>
<th>Assay of cellulolytic enzymes involved in cellulose degradation</th>
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<tbody>
<tr>
<td><strong>Enzyme</strong></td>
</tr>
<tr>
<td>Endo1,4-β-glucanase</td>
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<tr>
<td>Exo1,4-β-glucanase</td>
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<tr>
<td>1,4 β-glucosidase</td>
</tr>
<tr>
<td>Cellobiase, quinone-oxidoreductase</td>
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<tr>
<td>Cellobiose oxidase</td>
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</table>
Many species of microbes are responsible for producing improved quality and variety of products for betterment of life of human beings while on the other hand urbanization, industrialization and increase in human growth/population rate are responsible for degradation of environment. Microbes are able to degrade solid waste (lignocellulosic) into compost, some are able to degrade pesticides and also those generated in industries such as heavy metals present in industrial effluents and from thermal power stations, sewage sludge and in other sources.

Pollution as related to water has over the years been variously defined depending upon the different interests involved. Earlier definitions especially by biologists referred to "reduction in diversity of aquatic life and eventually destroying the balance of life in stream" (Patrick 1953) or adverse change in plants and animals communities (Hawker 1962).

Sewage consists of waste water containing human excreta, waste waters, industrial and agricultural wastes. In general sewage contains about 95.5% water and 0.1 to 0.5% organic and inorganic materials. The solid remains in suspended form in water. The other inorganic materials are dissolved and are found in ionic forms.

The observable effects on the biota associated with organic pollution are the results of a number of interacting factors, the most important of which are:

- Change in nutrient status.
- Change in concentration of the dissolved oxygen and CO₂, sewage and industrial effluents, which are the most common cause of organic pollution.
Many constituents of the organic matter present in aquatic environment serve as an energy and nutrient source for metabolism of heterotrophic microorganisms (Bell and Kuparin 1984, Munster and Chrost 1990). Organic matter, either particulate or dissolved, present in the aquatic environments consists of an innumerable amount of high molecular weight, organic compound, among them are proteins, peptides, polysaccharides, lipids, nucleic acids and other organic acids, phosphoric esters, humid substances, cellulose and hemicellulose (Chrost et al., 1991, Munster and Albert 1994, Raunkyaer et al., 1994). It has also been long recognized that bacteria cannot directly assimilate these high molecular weight organic compounds. (Rogers 1961, Billen 1984, Chrost et al., 1986 and Paul 1992).

Application of Cellulases

Cellulose is the most abundant carbohydrate polymer in the biosphere. An estimated synthesis rate of cellulose is approximately $4 \times 10^7$ tons per year for a long range solution to our resources problems of energy, chemicals and food. Cellulose is the most promising renewable carbon source that is available in large quantities. Enzymatic hydrolysis of cellulose has been widely studied during the last 20 years as a way to provide fermentable sugars, which in turn, could be converted into various value added chemicals and fuels.

Cellulases are used in textile processing, in improving the nutritive value and digestibility of animal feed, in clarification of fruit juice, in minimizing mechanical beating of paper pulp, in pharmaceutical
preparation as digestive aid in efficient disposal of solid cellulosic waste and in metropolitan areas to control pollution. The $C_X$ types of cellulytic enzymes are used in the pharmaceutical industry (Conder 1971).

The efficiency of cellulytic enzymes requires improvement in most cases. Considerable progress in our understanding of the role of various enzymes component in cellulose degradation has recently been provided by studies involving the powerful tools of molecular biology, genetic engineering techniques, monoclonal antibodies, and protein chemistry. In recent years, recombinant DNA technology has provided a means for isolating, characterizing and manipulating the genes for a number of different proteins. Therefore, the use of r-DNA technology applied to the cellulase system will facilitate not only a better understanding of catalytic functioning, cooperative interactions, between different enzyme components and regulation of these enzymes but also the development of practical systems for the utilization of native cellulose.

The advent of recombinant DNA technology has dramatically accelerated research in the field of cellulase enzyme systems. Efforts have been directed to clone genes from cellulytic organisms with the desired molecular properties. In the past few years a large number of cellulase genes have been isolated, characterized and expressed in a variety of hosts. Cloning of genes for individual components of the cellulase system would allow the biosynthesis of pure components and also provide information on their relative importance in the enzymatic hydrolysis of cellulose (Din et al., 1990).
Understanding the enzymology of cellulose hydrolysis appears to be more difficult than anticipated earlier. This is mainly because of the very complex and variable nature of the natural substrate cellulose. The development of an efficient transformation system opens the possibility, constructing improved strains producing cellulases with higher specific activities on particular substrates. An equally important aspect would be by improvement in the thermoresistance of these enzymes.

Cellulases have most impact on textile processing in recent years. Current commercial applications include ‘biostoning’, ‘biopolishing’ and as laundering ‘brightness’ of cotton fabrics (Hamlyn 2000). The most widely used application of cellulases, (neutral pH cellulases) is the replacement of pumice stones in the ‘stone washing’ process to produce the aged appearance of denim garments. Using cellulases in replacement of pumice stones prevents damage by abrasion to washing machines and the garments, eliminates the need for disposal of the used stones and improves the quality of the waste water. Depending on the finishing effect required, a mixture of cellulases and pumice may be used. The finishing of denim jeans has also become a popular application for cellulases in the textile industry.

In the modern textile industry enzymes are used increasing in the finishing of fabrics and cloths. The main component of cotton and other natural fibres is cellulose, while most of the fibres are arranged as long straight chains, some small fibres can protrude from the yarn or fabric. The correct application of a cellulase enzyme can remove these
rough protuberances giving a smoother, glossier, brighter colored fabric. This technique has become 'Biopolishing' and results not only as a softer fabric but also improved colour brightness.

Research has been carried out to replace the acid treatment by incubation with a mixture of cellulases and pectinases to hydrolyse the cellulose and lignin of the vegetable contaminants to water-soluble substances, which would easily be removed from the wool fabric. This process would be harmless to the wool fibres and require lower operational costs.

Industrial ethanol production is currently based on corn starch that is first liquefied and saccharified. The oligosaccharide syrup is than used as a feastock for ethanologenic yeast fermentation. The use of cellulases to increase the yield of starch liquefaction and saccharification has been described recently (Lewis 1996).

There is also an increasing use of cellulases in domestig washing products, where they are claimed to aid detergency and to remove damaged fibrillar material, improving fabric appearance, softness and colour brightness. Cellulases are frequently used together with proteases and lipases. Proteases help removing protein based stains (such as egg and blood) from clothing, while lipases are responsible for removing fatty substances. The discovery of alkaline cellulases created a new industrial application of cellulases as a laundry detergent additive (Ito et al., 1989). Cellulases are industrially important enzymes and its demand is growing with the growth of industry. Dey et al., (2002) reported
that groundnut shell can also be utilized as a substrate for large scale production of cellulase with the help of microorganisms having good cellulase production potential. Cellulases are also being used to replace singeing (a rather hazardous physical process), which is applied to many cotton goods prior to scouring and bleaching.

Recently, it was found that the treatment of jute, flax and ramie with cellulases improves the mechanical properties of the fibres. A treatment with a mixture of cellulases and xylases prior to peronide bleaching was found to enhance significantly the brightness of jute fibres. reducing therefore the peronide requirement for bleaching. This treatment was also found to produce softening benefits.

Cereals have been adopted as a major source of animal feed. They contain primarily starch, other polysaccharides than starch, so-called non-starchy polysaccharides (NSP) and proteins. The animal digestion system does not include enzyme able to cleave NSP. Thus the animal without help cannot use a considerable part of the cereals therefore, cellulases are used for degradation of feed components for improved feed utilization and nutrient digestion especially by monogastric animals.

Cell wall of cereals and grains contain a complex carbohydrate structure consisting of cellulose and hemicellulose (beta-glucans, beta-xylans and others). This structure can cause serious gumming problems in the brewing process. They can also cause filtration problems during starch processing. High activity cellulases, beta-glucanases and beta-xylanases are being used for efficient degradation of these non-starchy polysaccharides.
The commercial use of cellulases is based on the following:

1. High liter and good enzymatic activity
2. Low production costs and
3. Feasible mass production

Looking into the need of the present problem, this investigation has been undertaken for the study of Sagar lake where no microbiological work has been done so far. This work can be further extended by genetically manipulating the present bacterial isolates either by mutation or protoplast fusion. Media containing cheap substrates and inducers can enhance the enzyme production thus reducing the enzyme cost. This work can also give an overview of biodiversity of bacterial species with special reference to cellulolytic bacteria and obtaining stable enzyme preparations needed for the conversion of cellulose into glucose.

The main objectives of this study are as follows:

1. Selection and survey of study sites
2. To study the physico-chemical characteristics of Sagar lake i.e. pH, Temperature, Chloride, Alkalinity, Total hardness, Calcium hardness, Magnesium content, Free CO₂, DO, BOD, COD, Phosphate and Nitrate.
3. Identification and maintenance of bacterial isolates.
4. Screening and assay of cellulase producing bacterial isolates.
   a. Screening of extracellular cellulase producing bacteria.
   b. Production of cellulase enzyme
c. Measurement of cellulases
   i  Exo β-glucanase
   ii Endo β-glucanase
   iii β-glucosidase

5. Studies on factors affecting enzyme production and activity
   i  Effect of pH on enzyme activity
   ii Effect of pH on enzyme stability
   iii Effect of temperature on enzyme activity
   iv Effect of temperature on enzyme stability
   v  Effect of metals on enzyme activity
   vi Effect of inhibitors on enzyme activity