II. REVIEW OF LITERATURE

Lakes and rivers are the major fresh water bodies which are used for potable water. However due to growth of phytoplanktons and decomposition of organic matter nutrient status vary throughout the year. In addition, animal activities including men also influence the nutrient level of lakes and rivers.

The nutrient poor lakes are known as oligotrophic whereas the nutrient rich lakes are called eutrophic lakes which contain high amount of bottom sediments containing organic matter. Eutrophication (enrichment of lake due to high concentration of nutrients) of lakes occurs by multifarious ways where anthropogenic activities are of much importance. The eutrophic lake support luxuriant growth of bacteria and algae.

In natural water pH is governed by the equilibrium of carbon dioxide/bicarbonate/carbonate ions which ranges between 4.5 to 8.5. The high buffering capacity in the tropical systems was also noticed (Moore 1981, Ganapati 1960, Singh 1965, Verma 1967 and 1969). Hutchinson (1957) found that when bicarbonate alkalinity is high and the tropogenic zone is productive, a relatively small lowering of pH occurs in well-buffered water. The hydrogen ion concentration (pH) appeared to be chiefly dependent on the productivity of macrophytes and phytoplanktons. Many workers have reported freshwater to be alkaline and also had seasonal variation of pH (Ganapati 1962, Verma 1964, 1969, Singh 1963.)

Among various physical factors, the influence of water, temperature and availability of light energy are, of course, over riding. The heat budget, storage of heat by sediments and thermal stratification significantly control the metabolic characteristics of lakes in temperate as well as in tropical regions (Welch 1935, Ganapati 1957, 1960, Hutchinson 1957, Sreenivasan 1964). Surface water temperature closely reflects to ambient air temperature. This is particularly true for shallow lakes and ponds (Efford 1967). Decreased water temperature was due to frequent clouds, high humidity, high velocity, increased turbidity and high water level (Vyas 1968, Bhattacharya et al., 1997, Chandrawati 2002).

The presence of chlorides in natural water can mainly be attributed to dissolution of salts deposits in the form of ions (Cl⁻). High concentration may indicate pollution by sewage, industrial wastes, intrusion of seawater or other saline water (Owney and Kee 1967, Moyle 1949, Singh 1960, Sreenivasan 1965, Wetzel 1966, 1975, Aboo and Manuel 1967). Salinity of fresh water is usually dominated by Ca, Mg, Na, K, carbonates, sulphates and chlorides (Livingstone 1963, Benoit 1969, Bhattacharya et al., 1997, Banerjee and Pathak 1991). The salinity of surface water have a world average concentration of these ions which is about 120 mg/L⁻¹ which varies appreciably with the lithology of the land mass (Wetzel 1975).
The chloride contents showed an increase during early winter reaching its peak during late summer followed by a subsequent decline in rainy season (Sharma 1980). Lowering of chloride level during rains and early winter season may be due to dilution, precipitation and declined human disturbances (Verma 1969, Adoni 1975, Hussainy 1965, Bhargava and Sewani 1996).

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). The significant interrelationships were shown by total CO$_2$, bicarbonate and carbonate alkalinity. Total CO$_2$ and bicarbonate alkalinity were positively related and the bicarbonate and carbonate alkalinitities were found to be inversely related to each other (Adoni 1975, Ghosh 1986, Joshi 1987, Gupta 1987). The increase in alkalinity with decreasing water level was observed by many workers (Michael 1969, Rao and Govind 1964, Banerjee and Pathak 1991, Mohanty et al., 1991, Portielje and Vandermolen 1998, Sansalone et al., 2002).

The term hardness is frequently used to express the quality of water. The hardness of water is mainly due to the content of calcium and magnesium salts, bicarbonates, carbonates, sulphates, chlorides etc. (Moss 1973, Wetzel 1975). According to Ruttner (1953) the total hardness is the total amount of alkaline earth present. The hardness (calcium and magnesium) started increasing from July onwards probably due to initial
phase of leaching and drainage following first monsoon showers (Joshi 1987). According to APHA (1976) total hardness of water is the sum of the concentration of metallic cations, other than the alkali metals. In most fresh water all the hardness imparted is by the calcium and magnesium ions. The hardness imparted by these metals in combination with carbonates and bicarbonates is known as temporary hardness while that due to sulphates, chlorides and nitrates as permanent. Prasad et al. (1991) reported higher values of hardness from more polluted than the less polluted ponds.

In Sagar lake, the total hardness followed an increasing trend from monsoon to winter where it reaches to its peak followed by a decline in summer. The lower values of hardness were also reported by many workers (Tucker 1958, Brown and Austin 1973, Sahu et al., 1991). Calcium is essential for metabolic processes in all living organisms and forms the main skeletal component of animals and plants. It is important in buffering of lake water (Adoni 1975, Ghosh 1986, Joshi 1987, Gupta 1987 and Singhal 1980).

The values of calcium hardness were high during monsoon and winter season while low during summer. The value during winter correlated with less produce whereas low summer values correlated with higher phytoplanktonic productivity indicating the utilization of Ca\(^{++}\) ions by autotrophs. The higher values with lower concentration were also recorded (Tucker 1958, Brown and Austin 1973, Sharma 1983 and Prasad et al., 1985).
Calcium is one of the important components of the plant tissues and participates in various cellular functions. Calcium pectate is a constituent of cell membranes. It is also required as a nutrient for various metabolic processes and assists in the proper translocation of carbohydrates and facilitates the availability of other ions (Ruttner 1952, Wetzel 1975, Shaw et al., 1991, Bhargava and Sewani 1996).

Magnesium content is vital for energy transfer in any living cell system. It catalyses the change from ATP to ADP. Plants also require magnesium to form the active center of their major pigment chlorophyll a'. The metallic action is usually present in excess amounts to the requirement of the existing biota of the aquatic system (Joshi 1987, Adoni 1975). The metabolic requirement for magnesium is much less than the quantities generally available in fresh water. The magnesium salts are more soluble in water in comparison to calcium (Wetzel 1975, Joshi and Parashar 1992).

The importance of free CO$_2$ as a biological factor has been recognized by many workers. The source of CO$_2$ dissolved in water, in air, in flowing water, decomposition of organic matter, respiration of organisms, fixed or bound CO$_2$ in the form of insoluble monocarbonates of Ca and Mg and half bound CO$_2$ as bicarbonates of Ca and Mg were studied by many workers (Sreenivasan 1965, Adoni 1975 and Joshi 1987).

Absence of free CO$_2$ in water attributed to its complete utilization in photosynthetic activity (Sreenivasan 1965, Sahai and Sinha
1969) or it is inhibited by the presence of appreciable amount of carbonate in the water (Sahai and Sinha 1969, Bhargava and Sewani 1996). Vyas (1968) reported the direct relation of free CO$_2$ with visibility, temperature, pH and inverse correlation with oxygen.

Dissolved oxygen (DO) a factor of paramount importance in aquatic ecosystem is essential for the metabolism of autotrophic organisms of lake system. Cold water is found to have more solubility of oxygen and is further influenced by latitudinal changes of atmospheric pressure (Hutchinson 1957, Ohle 1952, Mortimer 1969, Hussainy 1967 Singh 1960, Vyas 1968).

In temperate and tropical lakes the amount of oxidizable substances and temperature influence the consumption of oxygen (Ruttner 1953, Wetzel 1975, Banerjee and Pathak 1991). Various types of vertical oxygen systems have been recognized by limnologists depending upon the trophic status, climatic condition and productional pattern of lake ecosystem. i.e. orthograde, olinograde and heterograde oxygen curves (Wetzel 1975). Orthograde type of oxygen curve is usually found in eutrophic waters. Low oxygen content in surface water during summer months when compared to winter month may be due to low transparency and high turbidity of water (Quasim et al., 1969). Yentsch and Ryther (1957) observed low O$_2$ content to be due to decline in chlorophyll content through photooxidation of pigments in increased light (Sinde et al., 1991). Biochemical oxygen demand is defined as the amount of oxygen required by microorganisms for
stabilizing biologically decomposable organic matter in water under aerobic condition (Bhattacharya et al., 1997, Ghosh 1997, Banerjee and Pathak 1991). Chemical oxygen demand is defined as the measure of oxygen equivalent to the organic content of the sample that is susceptible to oxidation by a strong chemical oxidant (Sansalone et al., 2002).

Carbon, hydrogen, oxygen, nitrogen, sulphur and phosphorous are important nutritional and structural components of the biota. Out of these C, N and P are generally considered as key elements. The C, N and P ratio in plants is roughly 40 : 7 : 1 by weight. In tropical water nitrogen is observed to limit metabolic processes (Talling and Talling 1965, Sioli 1980). Phosphorous generally plays a key role in temperate water. Phosphorous is least abundant and most commonly limits the biological productivity in a vast majority of lake systems (Wetzel 1975). Odum (1971) reports that nitrates and phosphates seem to be limiting to some extent in nearly all the fresh water ecosystems. Duthie (1972) recognized N and P as limiting factors. An excess of phosphorous to the actual need is stored in planktons. If the supply is available it allows the growth of organisms in the absence of an external source of given nutrient (Kaur et al., 1996). Inorganic phosphates are taken up by phytoplankton and macrophytes for their synthesis and growth which are released after the death and decay (Singh 1979). Sediments also release phosphate to the lake system (Wetzel 1975, Ruttner 1953, Somasekhar et al., 1991, Vandermolen et al., 1998 and Vanluijn et al. 1998).
Nitrogen occurs in fresh water in various forms, i.e. dissolve molecular nitrogen, inorganic nitrogen in the form of ammonia, nitrite, nitrate and organic nitrogen in the form of amino acids, proteins and various organic compounds (Adoni 1975, Ghosh 1986, Joshi 1987). The role of nitrogen in the metabolism of lake has drawn a considerable attention due to its importance in eutrophication. Some notable contributions in this field are from Vollenweider (1968), Landner (1976), Schindler (1974), Forsberg (1975) and Schindler (1978). The values of nitrate nitrogen during rains are higher than in other seasons, primarily due to addition of nitrates into the lake water by run off water (Adoni 1975). Similar observations were made by Vyas (1968), Bhargava and Sewani (1996), Somasekhar et al., (1991), Hefting and Klein (1998), Vandermolen et al., (1998) and Billore (1990).

Microbes are essential for recycling biomass and maintaining the biosphere chemistry. Microorganisms possess several characteristics which make them most ideal organisms. These microbes possess variety of enzymes which make array of chemical conversions possible. They have a relatively high metabolic activity which allow conversion to take place rapidly. They possess a large surface area for quick absorption of nutrients and release the end products. Moreover, they have a high rate of multiplication. The microbes should be easily cultivated, maintained and should have genetic stability during frequent mutations.
There exists several species of bacteria which are found virtually everywhere even in some harsh or adverse environments. Some are pathogenic but as a group, the benefits of bacteria to mankind are truly enormous. In clinical microbiology proper phenotypic characterization play an important role. Identification based on growth factor requirements and assay based on metabolic pathways, staining behaviour morphology and colony appearance, although useful, usually involve much subjectivity. Indeed, it is not generally possible for any single laboratory to identify correctly all possible bacterial species. Therefore, diagnostic microbiologists have devised methods based on simpler and more objective test. Some of these methods identify bacteria on the basis of chemical compounds present in the cell. These methods speed up analysis and usually can work even on non-viable cells (Mathieu and Sonia 1995).

Some classes of compounds used to identify bacteria include nucleic acids, proteins, lipopolysaccharides, carbohydrates and lipids. The relative utility of these classes depend on taxonomic differences in their occurrence, speed and simplicity of the method used. Nucleic acid sequencing and fatty acid analysis have proved to be the most promising and versatile methods (Bottger 1996).

Excellent reviews are available on the enzymology of fungal and bacterial cellulases (Srinivasan and Seetalaxman 1988, Robson and Chambliss 1989, Coughlan 1985,1989). Cellulase capable of degrading crystalline forms of cellulose is composed of essentially of three enzymes.
(i) exo β-glucanase (ii) endo β-glucanase and (iii) β-glucosidase. The three enzyme groups work synergistically to hydrolyse crystalline cellulose. The net effect is a rapid decrease in polymer length coupled with a slow increase in reducing group. Cellulose is the most abundant organic source of food, fuel and chemical. However, its usefulness is dependent upon its hydrolysis to glucose (Spano et al., 1975).

Cellulases are enzymes which hydrolyse cellulose. The enzymatic mechanism involves certain microorganisms which can quite rapidly and completely degrade cellulose which is not yet understood. Reese et al., (1950) proposed a scheme in which at least two steps are involved: (i) a prehydrolytic step occurs where anhydrous glucanase chains are swollen or hydrated and (ii) hydrolytic cleavage of the new susceptible polymers either randomly or endwise. The first enzyme is designated C₁ and the second, hydrolytic enzyme termed Cₓ. A third type of enzyme is β-glucosidase (cellobiase) has been studied in detail (Emert et al., 1974, Gould 1969, Mendal and Webr 1969, Baker and Panow 1991, Beguin et al., 1992, Esen 1993). Cellulase system of the three enzyme complex have been extensively reviewed by Chan and Wayman (1991), Nihalini and Satyanarayana (1992), Joshi and Ball (1993), Jay et al. (1992), Klesov (1990), Maes et al., (1991a), Ooshima et al., (1991), Park et al., (1992), Paul and Verma (1992), Reese et al., (1950), Mathew and Rao (1992), Matsushita et al., (1991), Mayer (1988), Meinke et al., (1991), Meinke et al., (1992), Nakamura et al., (1991), Ogawa et al., (1992)
Ooshima et al., (1991), Ozaki et al., (1990), Ozaki and Ito (19911), Pilz et al., (1990), Poole et al., (1992), Romaniec et al., (1992), Soole et al. (1993), Terri et al., (1992), Tiwari (1990), Bezukladnikov et al., (1992), Fauth et al., (1991), Paul and Verma (1990) and Harchand and Singh (2001). The C₁ component attacks highly ordered (crystalline) cellulose, i.e., cotton fibres or avicel but has little effect on soluble derivatives such as carboxymethyl cellulose (CMC). According to Spano et al. (1975) C₁ ‘decrystallizes’ or hydrates cellulose chains whereas Cₓ consists of exo and endo β1,4-glucanases that attack soluble derivatives or cellulose (Wood and Philips 1969).

The individual cellulose chain molecules differ in degree of polymerization but may have an average of about 3000 glucose units. Carefully isolated chain may have 100,000 or more glucose units with molecular length of 0.05 mm (Siegel 1963) and the entire chain lies in a single plane. The cellulose molecules are strongly hydrogen bonded to each other resulting in substances of extreme insolubility and low chemical reactivity. An individual cellulose may pass through several regions of high crystalline (micelles) as well as several amorphous regions where the chains are more loosely ordered. The crystalline micelle is about 600 Å long which is equal to about 60 cellobiose units.

The cellulose elementary fibril consists of about 100 cellulose molecules of a cross sectional area of 3000 Å². A cellulose fibre contains about $7.5 \times 10^6$ elementary fibrils. The specific gravity (Byrde
1963) of the dry fibre indicates relatively little vacant space upon hydration and the inter and intra fibrillar spaces increase to form a system of fluid filled cavities averaging $10 \, \text{Å}$ in width with some spaces upto $100 \, \text{Å}$ (Siegel 1963). Evidence that amorphous cellulose is more rapidly degraded than crystalline cellulose, includes the increase in degree of crystallinity as enzyme action proceeds and the inverse correlation of degree of crystallinity with the rate and extent of hydrolysis (Reese 1959 and Walseth 1952). The introduction of substitute groups (methyl, carboxymethyl, hydroxymethyl, sulphate etc.) at one or more of the free hydroxyls on carbons 2,3 and 6 of the anhydroglucose units prevent chain aggregation thus conferring water solubility on the cellulose and making the glucosidic linkages freely accessible to the enzyme. Mountenocourt et al., (1979) proposed a model of cellulose degradation where cellulase acts in a co-operative and sequential manner.

The importance of cellulolytic microbes for the production of glucose from cellulose is well recognized by Sharma et al., (1995). The products of cellulose hydrolysis is used as carbon and energy sources by microorganisms which inhibit cellulose rich environment (Leschine 1995). Cellulosic materials can be hydrolyzed into soluble sugars by acid treatment but the process is uneconomical and yields poor products (Haper and Lynch 1981). Banu and Ramasamy (1997) studied the role of Clostridial cellulases in the digestion of food by wood boring grab.
Malik et al., (1988) observed a correlation between in situ cellulose degradation, cellulolytic bacteria and enzyme activities. About 1/3 of the cellulose in the lignocellulose material is degraded within a week and found the subsequent slow rate of cellulose degradation in lignocellulosic materials which is due to the recalcitrant nature of the substrate. The importance of enzymatic hydrolysis of lignocellulosic substrate over acid hydrolysis, availability of raw materials and their various uses to achieve practical success in biotechnology has been discussed by (Srinivasan and Seetalaxman 1988).

The production of cellulolytic enzymes by microorganisms is affected by various environmental conditions. It has been suggested by various workers that culture medium, pH of the substrate and incubation period greatly influence the type and activity of enzymes produced (Ghosh 1987, Lamed and Bayer 1986 and Srinivasan and Seetalaxman 1988).

The production and activity of enzyme cellulas by fungi and bacteria is greatly dependent on factors such as nutritional salts, pH, temperature, substrate, concentration, aeration and agitation (Mandels and Reese 1957). Economic hydrolysis of cellulose with maximum saccharification in a short interval is essential to determine the optimum conditions. Enzymes are sensitive to heat and having its own optimal temperature for activity (Bhatt and Bhatt 1997).

Nimalini and Satyanarayana (1992) reported that the production of cellulase from Aeromonas and Bacillus was active at pH 8.0.
and 9.0 which was used in detergents and sewage processing. Joshi and Shalika (1991) reported that *Bacillus firmus* and *Bacillus licheniformis* species showed maximum C$_1$ enzyme production at pH 4.8 and at 40°C while C$_X$ enzyme production was maximum at pH 5.0 and at 40°C. Estimation of the enzymatic capacity of the two cellulolytic isolates revealed that *Bacillus firmus* is more active in its ability to degrade cellulosic material than *Bacillus licheniformis*.

Joshi and Ball (1993) reported the production of cellulase enzyme from *Aeromonas sp.* and *Bacillus sp.* at pH 9.0. The alkaline cellulases (C$_1$ and C$_X$) could be produced by *Bacillus sphaericus*. Best C$_1$ activity was recorded at pH 10 on 8 d of incubation while best C$_X$ activity was observed with the same bacterium at pH 12 on 6 d. Siddique *et al.* (1997) reported the identification of the active site residue of CMCase from *Aspergillus niger* and *Cellulomonas biazotea*. This is the first report on the kinetic effect of pH on the CMCase as both were measured at different temperatures and in the presence of an organic solvent.

Chemical inhibition of cellulase has been covered in three reviews (Gascogine and Gascoigne 1960, Mandels and Reese 1963 and Norkrans 1963). Cellulases are inhibited by mercury, silver, copper, chromium, lead and zinc salts at about $10^{-3}$ M (Mandels and Reese 1963). Cysteine, glutathione, cyanide and sodium sulphide have occasionally been reported as cellulase inhibitors at $10^{-2}$ to $10^{-3}$ M (Gascogine and Gascoigne 1960, Hanstein 1960, Lyr 1961 and Mandels and Reese 1963). Cellulases are remarkably stable to changes in pH, temperature and chemical inhibitors. They are competitively inhibited by cellobiose and methacel and inactivated by protein reactants (halogens, heavy metals and detergents).

Applications of cellulase producing microorganisms include treatment of garbage, agricultural residues, live stock feeds, factory effluents and in textile processing. Current commercial application include ‘biostoning’ and ‘biopolishing’ and as laundering ‘brightness’ of cotton fabrics (Hamlyn 2000). The discovery of alkaline cellulases created a new industrial application of cellulases as a laundry detergent additives (Ito et al., 1989).

Cellulases have also recently been introduced as laundry enzymes, although they are more widely accepted in Japan than elsewhere. Cellulase from an alkalophilic Bacillus sp. has already achieved a 40 % market penetration in the Tokyo area. Cellulases exhibit fabric softening and colour brightening properties besides removing oil
(Christensen et al., 1987 and Satoh 1989). Current research on \( \beta \)-glucosidases has significant scientific, medical and economic implications. Human acid \( \beta \)-glucosidases has potential in the development of therapeutic and diagnostic procedures that will be useful in the treatment of Gaucher's disease and inherited disorder caused by the deficiency of acid \( \beta \)-glucosidases localized in the lysosome. Plant \( \beta \)-glucosidases have been implicated in a variety of growth and productivity (Esen 1993).

Successful isolation of the first cellulase gene was reported in 1982 from *Cellulomonas fimi* (Din et al., 1990) since then cellulase genes have been isolated from different prokayotes and eukaryotes. Cellulase genes pertaining to all the three components i.e. endoglucanase, exoglucanases and \( \beta \)-glucosidase has been cloned in recent years (Liobertas et al., 1991, Lo et al., 1988, Maglione et al., 1992, Mantyla et al., 1992, Mishra et al., 1991, Navarro et al., 1991, Navas and Beguin 1992, Ohmiya et al., 1990, Ozaki et al., 1991, Romaniec et al., 1991, Schimming et al., 1992, Schlothermeier et al., 1992, Wang et al., 1993, Yague et al., 1990, Din et al., 1990, Hall et al., 1992, Jorgenson and Hansen 1990, Beguin 1990).

The water resources consist of open water bodies (lakes and rivers), ground water and rain water. These sources of water are used in several ways (human consumption in both rural and urban areas, industrial use, livestock). Water quality may also be affected by the discharge of untreated sewage into the water bodies. Sanitary practices
have the greatest impart of all human activities. These introduce high level bacteria into the water. Bacteria have been found to play a major role in biodegradation of organic polymers in aquatic environments (Rubblee and Roman 1982, Brock 1984; Benner et al., 1986, Gaur et al., 1992, Ramteke and Bhattacharya 1992, Sinha 1991, Gupta and Kumar 1991). Rao (1998) reported the treatment of industrial effluents by the indigenous bacteria.

Biodegradation can be defined as the degradation and assimilation or organic polymers by the action of living organisms, primarily bacteria and fungi. Earlier biologists defined biodegradation as 'reduction in diversity of aquatic life and eventually destroying the balance of life in the stream' (Potts 1984).

Microorganisms are being used to speed up the biodegradation of organic halogen compounds, metal and sulphur containing compounds, inorganic and organic acids. Bioleaching has been used for the recovery of metals from low grade ores, particularly copper and uranium, cobalt, gold, lead and nickel. It can also be used for the treatment in situ of high sulphur coal and oil without which problems of acid rain may be caused when the sulphur is oxidized (on combustion) and discharged into the atmosphere with the formation of sulphuric acid (Kumar and Kumar 1997).

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 DO, high BOD, sewage and industrial effluents which are the most common cause of organic pollution. On the other hand, COD is the amount of chemical oxidation required to convert organic matter in water and waste water in to carbon-di-oxide (Montgomery 1985).

All the three bacterial strains *Bacillus cereus*, *Bacillus megaterium* and *Xanthomonas fragarie* are capable of removing COD and colour from distillery waste water (Jain *et al.*, 2001). Microorganisms have also been isolated that can degrade different organic compounds like benzene (Dagley *et al.*, 1975), phenol (Feist and Hageman 1969), naphthalene (Davis and Evans 1964), salicylate (Chakrabarty 1972), toluene (Chakrabarti 1976) and p- and m-hydrobenzoiates (Johnson and Stanier 1971).

Rubio and Molina (1989) described the use of mixed culture of *Cellulomonas species* and *Bacillus subtilis* for the treatment of the potato waste effluent. Sivaji and Srikanth (1996) described the use of *Bacillus* and *Pseudomonas* sp. for the treatment of domestic waste water pollutants while Jain *et al.*, (1997) described the use of *Acinetobacter calcoaceticus* for the treatment of pulp mill waste water.

Biodegradation of hazardous nitro and chloroaromatic compounds is completed only when the nitro and chloro substituents are removed and the carbon skeleton is broken down to intermediate compounds which will finally be oxidized to CO$_2$ (mineralization). Diverse microorganisms capable of mineralizing some, but not all, chloro and nitro substituted aromatics have been isolated and characterized (Piper *et al.*, 1996).