Through carbon recycling, microorganisms carry out their most important function in the maintenance of life on earth. They affect the mineralization of the organic carbon compounds produced by photosynthesis and thereby maintain a delicate equilibrium. Atmospheric air contains hardly more than 0.03% carbon dioxide (12 μmol/l). The photosynthetic activity of green plants is of such magnitude that the whole carbon dioxide content of the atmosphere would be exhausted in about twenty years. This is relatively a short period in our time span. However, it is estimated that the energy and carbon sources of the earth should suffice for another 1000-3000 years. The green plants would soon have to replenish the carbon dioxide reserves by mineralization of organic compounds. However, life on earth continues and would exist for a long period of time only because of the four major cycles in nature, i.e., carbon, nitrogen, sulfur and phosphorous.

Most of the organic matter present in soil originates as leaves, trees, decaying roots and other plant tissues. Soil bacteria and fungi are largely responsible for recycling this carbon. Insoluble dead vegetable matter is first acted on by extracellular microbial enzymes to release
soluble products, thus making them easily available to microbes and plants. While bacteria participate in both cycling of carbon and mineralization of nitrogen, phosphorous and sulfur, fungi contributes mainly to the cycling of carbon. Undigested organic plant material and plant matter becomes part of the soil humus. Coal also originates from plant material buried in primeval swamps eons ago. Both coal and peat complete the cycle (get converted to carbon dioxide), when they are burned as fuel.

1.2.1 Role of termites in carbon recycling:

A fascinating and frequently cited example of nutritional symbiosis is the association between certain xylophagous termites and their intestinal microflora. Of all the intriguing activities and properties of termites, none seems as widely recognized (or as often quoted) as the ability of termites to utilize wood as a food resource. Indeed, many species of termites thrive on sound, decay free wood which contains as little as 0.03-0.1% nitrogen (dry weight bases - Cowling and Merrill, 1966). However, it is also important to recognize that there are nearly 2000 species of termites and their biology and behaviour are quite varied (Krishna, 1969). For example, many species prefer wood that is partially decayed by fungi (Lee and Wood, 1971), whereas some termites (subfamily
Macrotermiteinae) actually cultivate fungi in elaborate gardens for use as a nutrient resource (Sands, 1969). Still others, depending on the species, feed on leaves, roots, grasses, dung of other herbivorous animals, humus or soil (Lee and Wood, 1971). Clearly the diet of termites as a group is quite diverse, but is basically one rich in cellulose, hemicellulose and lignin or lignin derivatives. Since their diet is also relatively poor in combined nitrogen, termites may be thought of as oligonitrotrophic saprovores. This trait places termites in an important position ecologically, particularly in tropical regions where their activities can dominate the processes of decomposition and nutrient cycling. Further, the biomass density of termites can be so large (10-20 g/m) that their impact is similar to, and may surpass, that of grazing mammals (Wood and Sands, 1978).

1.2.2 Lignocellulolytic microflora of the termite gut/mound soil

Digestion of cellulose, hemicellulose and lignin, and its utilization as a carbon source by termites requires the presence of a variety of lignocellulolytic gut microorganisms. These microbes are probably involved in many important aspects of termite nutrition (Breznak, 1975; Breznak and Pankratz, 1977). These microbes have been found
important for the survival of the host (Breznak, 1982; O'Brien and Slaytor, 1982) and are generally obligate i.e., many of the microbes do not survive for long outside the termite gut and the termites can not survive without the microbial community.

The hindgut of termites host many species of protozoa, fungi, bacteria and actinomycetes. Among the heterogenous microbial community it appears, anaerobic flagellate protozoa are the major, if not sole, agents of wood cellulolysis. Some of the protozoans isolated from Trichonympha chula, T. sphaerica and Metadevescovina polyspira from hindgut of Zootermopsis, Cryptocercus punctulatus and Pterotermes accidentalis (Yamin, 1980). Hoingberg (1970) detected hydrolytic activity by Oxymonad, Trichomonad and Hypermastigote protozoa. Trichonympha agilis, Spirotrichonympha sp., Pyrosonympha vertens, P. major, Dinenympha gracilis, Microjoenia falax, Holomastigotes sp., Arthromitus and other forms of protozoa were isolated from Reticulotermes (Grosovsky and Margulis, 1980). Partial or complete defaunation proved that protozoa are essential for normal cellulose catabolism, lipid synthesis and survival of the Coptotermes worker termites.

Fungus serves as the essential diet of termites (source of vitamins and nitrogen) and helps in degradation
of lignin in the comb thereby facilitating subsequent digestion of cellulose by bacteria. Reports state that fungi play their role both as ecto as well as endosymbionts. While Rajagopal and Varma (1980) and Rajagopal et al. (1981) isolated several fungi from termite guts producing cellulases, actively solubilizing cellulose, Rossi and Blackwell (1986) found fungi only on the external body of several workers and soldiers of Amitermes evuncifer and termites belonging to the families of Macrotermiteidae and Hodotermitidae. However, Breznak (1984) found neither stable population nor a specific type of fungi in termite guts.

Bacteria were found living both intracellularly (within protozoa) and freely in the termite gut fluid. Thayer (1976) isolated cellulose depolymerizing Bacillus cereus, Serratia marcescens, Arthrobacter and Alcaligenus species from the lower termite Reticulotermes hesperus. In higher termites (Termitidae) xylophagous protozoa were absent. Hungate (1946a,b) isolated a Clostridium sp. capable of cellulolysis from the gut of Amitermes minimus. Although all termite species tested had cellulose degrading bacteria in the hind gut, the number and species type depends on the condition and mode of cultivation. Mishra and Ranganathan (1954) demonstrated cellulase and cellobiase activity by bacteria in gut of Odontotermes obesus. Four
cellulolytic strains were isolated from the gut of Odontotermes obesus by Sarkar (1987). They included Bacillus circulans, Staphylococcus saprophyticus, Micrococcus luteus and M. roseus. However, existence of cellulolytic bacteria was doubted by Eutick et al. (1978) who isolated Staphylococcus, Bacillus, Streptococcus, Enterobacter and Flavobacter species which were noncellulolytic in nature from four lower and one higher termite. Sarkar (1987) screened the microflora of termite (Odontotermes obesus) infested mound soil and isolated sixteen strains of bacteria. Out of them only four strains were cellulolytic. Apart from this report literature seems silent on the microflora of termite mound soil.

1.2.3 The genus Bacillus

Bacillus is the first genus of family Bacillaceae belonging to Part fifteen (Endospore-forming rods and cocci) as per the standard reference for bacterial classification and taxonomy, Bergey's Manual of Determinative Bacteriology (Gibson and Gordon, 1975). Rod shaped Gram-positive cells normally straight with dimensions 0.3-2.2 x 1.2-7.0 μ capable of aerobically producing endospores, which are located in the centre of the cell or subterminally or terminally comprise the genus Bacillus. These cells are aerobic and generally motile with long peritrichious
flagella. Catalase is produced by most strains while pigmentation is rare. This genera constitutes strictly chemoorganotrophs with molecular oxygen (in some cases replaced by nitrate) as terminal electron acceptor in the respiratory metabolism. The G+C content of DNA ranges from 32.0-62.0 moles% (Tm and bouyant density).

The refractile endospores are resistant than vegetative cells to heat, drying and other destructive agencies. Sporangia do not differ from vegetative cells, except when bulged by spores larger than the cell diameter, such spores are spindle shaped when spores are central and wedge or drumstick shaped when spores are terminal or subterminal.

These bacteria are widely distributed in nature. They occur mostly in soil. The resistant nature of the spores allows them to survive long even under unfavourable conditions.

1.2.4 Extracellular enzymes of the genus Bacillus

Microbial enzymes have become increasingly important in the advancement and better utilization of diverse fields like medicine and industry. The genus Bacillus has played a major role in this development. Reasons for the predominance of these bacteria in this area
of study are several. Firstly, they comprise a group of chemoorganotrophs that can be easily maintained and cultivated and yet are markedly heterogenous in character. Psychrophiles, mesophiles and thermophiles, in addition to alkalophilic, neutralophilic and acidophilic species are well represented. Furthermore, virtually all 48 species of the genus listed in Bergey's Manual of Determinative Bacteriology (Gibson and Gordon, 1975) secrete a variety of extracellular enzymes, which reflects the diversity of the parental habitats. To site a couple of extreme examples of enzyme adaptation, certain Bacillus sp. are known to excrete amylases that can liquefy starch under pressure at 110°C (Madeson et al., 1973) and proteases that are stable and active at pH 12.0 (Aunstrup et al., 1972).

A large variety of exoenzymes synthesized by the genus Bacillus is summarized in Table 1. Most of the truly extracellular enzymes synthesized by bacilli have no cellular substrates and it would seem probable that their sole function is to degrade polymers in the environment and supply the bacterium with an assimilable source of nutrients. However, exoenzymes that possess cellular substrates like proteases, nucleases, cell wall lytic enzymes etc., have a number of physiological processes including sporulation, cell wall turnover, growth and genetic transformation.
<table>
<thead>
<tr>
<th>Species</th>
<th>Enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. amyloliquejaciens</td>
<td>α-Amylase, Galactanase, Isomylasse, mannanase, Xylanase, Metal &amp; Serine Protease, Alkaline Phosphatase, DNAase</td>
</tr>
<tr>
<td>B. licheniformis</td>
<td>α-Amylase, Aminopeptidase, Metal and Serine protease, Metal-Serine protease, blactamase, Endo-N-acetyl glucosaminidan lipase, nacetyl-muramyl-L-alanine, amidase</td>
</tr>
<tr>
<td>B. cereus</td>
<td>β-Amylase, Metal protease, β-Lactamase, Alkaline phosphatase, DNAase, 5' Nucleotidase, phospholipase C.</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>α-Amylase, Arabinase, Cellulase, Dextranase, β-1, 3-glucanase, Levanosucrase, Maltase, Pectatolyase, xylanase, Aminopeptidase, Esterase, Alkaline phosphatase, DNAase 5' Nucleotidase</td>
</tr>
<tr>
<td>B. polymyxa</td>
<td>β-Amylase, Cellulase, Isomylase, Pectatolyase, Xylanase, Metal protease.</td>
</tr>
<tr>
<td>B. stearothermophilus</td>
<td>α-Amylase, Pectatolyase.</td>
</tr>
<tr>
<td>B. megaterium</td>
<td>β-Amylase, Dextranase, Metal Protease, β-Lactamase, 5'-Nucleotidase</td>
</tr>
<tr>
<td>B. pumilus</td>
<td>Cellulase, Lichenase, Pectatolyase, Serine protease, Serine-Metal Protease.</td>
</tr>
</tbody>
</table>

Data compiled from Priest, 1977.
Maximal synthesis of extracellular enzymes normally occurs before sporulation in the late exponential and early stationary phases of growth (Schaeffer, 1969). This situation is greatly affected by the media used. In media containing commercial nutrient sources of varying and unknown composition, the exponential growth is often followed by a lengthy period of deceleration, extending from 12 to 36 hrs, during which the bacteria gradually enter stationary phase and ultimately sporulate. The metabolic activity and stable RNA content of these cells are radically different from those grown in minimal medium containing a single carbon source and in which the transition from exponential to stationary phase is rapid (Herbert, 1961). Variations in exoenzyme synthesis are therefore to be expected from bacteria grown in such different environments.

1.2.5 **Secretion of extracellular enzymes**

Number of observations suggest exoenzymes from prokaryotes do not exist in their native configuration inside the cytoplasm, thus supporting the view that they are secreted as they are synthesized. Blobel and Sabatini formulated the 'signal' hypothesis for eukaryotic cells (Blobel and Sabatini, 1971; Blobel and Dobberstein, 1975), and evidence is now emerging to suggest that a similar system may also operate in prokaryotes. The signal
hypothesis proposes that mRNA's for secretory proteins possess a unique sequence of codons located immediately to the right of the initiation codon. These codons, the signal codons, are not present in the mRNA's coding for cytoplasmic proteins. Translation of the signal codons results in a unique amino acid sequence on the amino terminal of the polypeptide chain, the signal sequence. When the nascent polypeptide chain bearing the signal sequence emerges from the large ribosomal subunit, it is recognised by two or more membrane receptor proteins, causing their loose association to form a tunnel in the membrane. At the same time, the sequence may dissociate one or more proteins on the large subunit revealing an attachment area. The ribosome would then bind to the membrane receptor proteins stabilizing the tunnel and providing a confluent passage from the ribosome through the membrane. After completion of the nascent polypeptide chain, ribosomal detachment from the membrane would eliminate the cross-linking effect of the ribosome on the membrane receptor proteins, and these would be able to diffuse freely in the plane of the membrane. After the nascent polypeptide has traversed the membrane, it is proposed that an endopeptidase specifically removes the signal sequence, thus allowing the enzyme to assume its native conformation.
1.2.6. **Importance of thermophilic bacteria in biotechnology**

Thermophilic microorganisms have been isolated from many prokaryotic groups of microorganisms including cyanobacteria, photosynthetic bacteria, the spore formers—*Bacillus* and *Clostridium*, lactic acid bacteria, methane producers, methane utilizers, sulfur oxidizers and reducers, mucoplasma, psuedomonads, actinomycetes and Gram negative aerobes (Brock, 1978). While a number of definitions or classification systems for thermophilic microorganisms have been proposed, the most commonly accepted definition is that of Williams (1975). Organisms with a maximum growth temperature of more than $60^\circ C$ and an optimum of more than $50^\circ C$ were described as thermophilic, whilst those with a maximum growth temperature of more than $90^\circ C$ and an optimum of more than $65^\circ C$ were described as cladoactive.

Thermophilic microorganisms have been isolated from a wide range of environments including solar heated environments such as soil, ground litter, self heating organic rich materials such as compost heaps, seaweed piles, hay, straw, saw dust and coal refuse piles, domestic and industrial hot water and cooling systems and steam lines and steam condensate lines.
The use of renewable resources and of thermophilic organisms for industrial processes is not new. In the early 1930's as well as in the 1950's, biomass utilization and thermophilic microorganisms were used in patented processes (Langwell, 1939; Veldhuis et al., 1936; Owen, 1960). Most of these processes ceased to operate due to unfavourable economics. The rising prices of crude oil, its slow depletion and also the immense increase in our understanding of thermophilic microbes and their biochemistry have brought back and broadened the possibilities of the use of thermophiles and extreme thermophiles in industrial processes.

As a consequence of thermal stability of their enzymes as well as of a number of other significant processing advantages thermophiles have been the subject of intense investigation. Some of the major advantages of thermophilic microorganisms are as follows:

* The costs of cooling large scale thermophilic fermentations are reduced. Expensive heat exchange and refrigerated equipment required for mesophilic cultures is not needed, since cooling water at ambient temperature is sufficient. Most of the heat required for the fermentation is self generated by exothermic growth of the microorganism.
* The reduced viscosity of the medium increases the efficiency of mixing, thus reducing the power input to stirrers and aid harvesting by centrifugation.

* The solubility of reactants is increased and permits the use of higher concentrations of certain relatively insoluble compounds.

* Volatile products, such as ethane, may be removed from the fermentation broth by applying a mild vacuum or by spraying with carbon dioxide. This reduces the build up of inhibitory levels of end product in broth.

* Operation at elevated temperatures reduces the possibility of contamination by mesophilic microorganisms. However, this does not eliminate contamination by thermophilic spore formers.

* The decreased solubility of oxygen is advantageous when culturing strict anaerobes.

* Thermostable enzymes are more resistant to the denaturing activities of detergents and organic solvents.

* Theoretically, catalytic activity and hence growth rate should increase with temperature.

* Enzyme isolation and purification can be carried out at room temperature.

* Higher enzyme recoveries may be obtained owing to the enhanced enzyme stability.

[Compiled from Sharp and Munster, 1985; Wiegel and Ljungdahl, 1986].
1.2.7. **Importance of alkalophilic bacteria in biotechnology**

Microorganisms from alkaline environments represent a selectively new unexplored and unexploited area of microbiology. Krulwich and Guffanti (1983) state that almost all alkalophilic bacteria are bacilli, although a small exceptional numbers are other Gram positive isolates. The earliest report on microbial life in an alkaline environment concerned alkaline-tolerant nitrifying bacteria of the *Nitrosomonas* and *Nitrobacter* species (Meek and Lipman, 1922) and the alkaline tolerant enteric bacterium *Streptococcus faecalis* (Downie and Cruickshank, 1928). Later Gibson (1934) and Vedder (1934) studied the growth patterns of alkalophilic bacilli, *Bacillus oasturii* (growing well at pH 11.0) and *B. alcalophilus* (growing well at pH 8.6-10.0), respectively. There was a long gap of 25 years in the study of alkalophilic microorganisms until Kushner and Lisson (1959) reported a strain of *B. cereus* changed to grow at pH 10.3 by repeated transferring of cultures in alkaline environment. In 1960, a highly alkalophilic bacterium was isolated from the fermenting indigo leaves, which was identified as *B. alcalophilus* in 1962 (Takahara and Tanabe, 1960, 1962). The growth optimum lies in the pH range of 10.0-11.5. Since 1969, Horikoshi and his coworkers have isolated a great number of
alkalophilic bacteria from soil and purified many alkaline enzymes, a new type of enzymes.

Alkalophilic bacilli are aerobic and usually motile. Some strains, which we call obligately alkalophilic, grow well only at pH values above 8.5 or 9.0 and do not grow at near neutral pH values. Other strains, called facultatively alkalophilic grow well at the very alkaline pH values, but are also capable of growth at near neutral pH (Mitchell, 1961).

Horikoshi and Akiba (1982 have described a new microbial world of alkalophilic microorganisms comprising of aerobic or anaerobic spore forming bacteria, aerobic nonspore forming bacteria, actinomycetes fungi, yeasts and phages. The mechanism of alkalophilicity is not yet clear and investigations are on. The isolation of alkalophilic phages and more recently several plasmids from alkalophilic Bacillus sp. has opened a new DNA source for genetic engineering. New novel products, enzymes, antibiotics and so on, have been found one after another and many more are yet to be discovered from alkalophilic bacteria. Alkaline microbial world and its applications can be summarized as in Fig. 1.
Fig. 1. New Alkaline Microbial World and Its Application

Adapted from Horikoshi and Akiba, 1982.