Although molecular $N_2$ is most abundant in nature, nitrogen is the major limiting factor for crop productivity. In this context, microorganisms in terrestrial and aquatic environments play a major role in fixing molecular $N_2$. Thus, the long term maintenance of fertility in flooded rice soils, as reflected in steady, but low yields for several years without fertilizer application, was attributed essentially to microbial fixation of molecular $N_2$ (Grist, 1965). Many microorganisms are involved in this process, but this review is mainly concerned with heterotrophic $N_2$ fixation, the subject matter chosen for the present study. The importance of non-symbiotic $N_2$ fixation in soils is well documented (Jensen, 1965; Knowles, 1965; Moore, 1966; MacRae and Castro, 1967; Dobereiner, 1968; Mishustin, 1970; Becking, 1971; Spiff and Odu, 1972; Koch and Oya, 1974; Rao, 1974, 1978a).

2.1. Nitrogen-fixing microorganisms in rice soils

The contribution and importance of free-living heterotrophic $N_2$-fixing bacteria is well established (Nutman, 1971; Mulder and Brotoñegoro, 1974; Matsuguchi et al., 1975; Yoshida, 1977; Rao, 1978a; van Berkum and Bohlool, 1980). Free-living $N_2$ fixers in rice ecosystem,
involving both autotrophic and heterotrophic microorganisms include aerobic, anaerobic, associative, photosynthetic bacteria and blue-green algae. The autotrophs, in particular, blue-green algae predominant in soil and flood water, fix appreciable amount of \( \text{N}_2 \) in the fields in the absence of \( \text{N} \) fertilizers (El-Nawawy et al., 1958; Watanabe, 1962; Venkataraman and Goel, 1968; Watanabe et al., 1978).

2.1.1. Aerobic bacteria

For a long time Azotobacter and Clostridium were considered to be the only \( \text{N}_2 \) fixers. As the rice fields are waterlogged for most of the time, the role of obligate aerobic \( \text{N}_2 \)-fixing organisms and their contribution to the \( \text{N} \) economy of rice soils is questionable under evidently unfavourable conditions of \( \text{O}_2 \) supply. But, the rice plant supplies molecular \( \text{O}_2 \) to its roots in a flooded soil (Van Raalte, 1941; Arikado, 1961; Barber et al., 1962) and rice roots can oxidize a thin layer of the rhizosphere (Mitsui, 1960). Further, the transport of substantial quantities of \( \text{N}_2 \) from aerial plant parts to the rice rhizosphere was demonstrated employing \( ^{15}\text{N}_2 \) (Yoshida and Broadbent, 1975). Thus, waterlogged soil could favour the growth of aerobic heterotrophic \( \text{N}_2 \)-fixing bacteria as indeed reflected in the reported occurrence of

Azotobacter is the most extensively studied of all aerobic N$_2$ fixers. Azotobacter chroococcum, A. indicus, A. lacticogenus, A. agilis, A. beijerinckia and A. vinelandii were isolated from rice soils of India (Rangaswamy and Subbaraja, 1962). Their population was highest in the top layers of the soil and decreased with increasing depth (Rangaswamy and Sadasivam, 1964; Jensen, 1965). Likewise, A. vinelandii, A. agilis and A. chroococcum were isolated from paddy soils of Russia and the distribution was mainly restricted to the rhizosphere (Mishustin, 1970). The ubiquitous occurrence of Azotobacter in the soils of Egypt and Iraq was reported (Abd-el-Malek, 1971). Several reports indicate that A. chroococcum is the most common and predominant organism in most of the tropical rice soils (Rangaswamy and Subbaraja, 1962; Ishizawa and Toyoda, 1964; Balandreau, 1975; Rao, 1977). The population density of Azotobacter was as high as $10^{11}$ in rice soils of France contributing substantial amounts of N to the paddy soils (Rouquerol, 1962, 1964). Fixation of N$_2$ to the magnitude of 16 to 17 mg N by A. chroococcum isolated from rice plants was reported (Purushothaman et al., 1979). Significant
yield increases occurred after application of *Azotobacter* alone or in combination with mineral fertilizers in paddy soils of USSR (Shende and Kokorina, 1964; Shende, 1965). However, N uptake and yield were little affected by inoculation of the soils of Madras (Nair et al., 1972).

Besides *Azotobacter*, other aerobic organisms belonging to *Beijerinckia*, *Arthrobacter* and *Mycobacterium* were isolated from the rhizosphere of rice (IRRI, 1970; Rao, 1974; Balandreau et al., 1975a). *Derxia* is also found in tropical soils (Jensen et al., 1960; Dobereiner, 1968; Dobereiner and Campelo, 1971).

2.1.2. Anaerobic bacteria

*Clostridium* sp. were the principal *N₂* fixers found in waterlogged soil-straw and sand-clay-straw systems (Rice et al., 1967; Rice and Paul, 1971, 1972). The products of cellulose decomposition like lactic and butyric acids in submerged soils may act as energy source for *N₂* fixation by anaerobic heterotrophs such as *Clostridium* sp. (Magdoff and Bouldin, 1970). The occurrence of *Clostridium* sp. in several soils of Egypt and Iraq was reported (Abd-el-Malek, 1971). Periodical fluctuations in *N₂*-fixing clostridial population in waterlogged soils have also been observed (Rao et al.,)
The population density of Clostridium was more than that of Azotobacter in many neutral soils (Balandreau et al., 1975a).

Organisms belonging to the genera Klebsiella, Pseudomonas, Aerobacter and Bacillus were isolated from rice soils (IRRI, 1970; Rao, 1974). The population dynamics of a facultative anaerobe, Bacillus polymyxa have been investigated in flooded rice soils at different stages of plant growth (Rao et al., 1973). However, little information is available on the occurrence and $N_2$ fixation by anaerobic and facultative anaerobic bacteria in Indian soils.

2.1.3. Photosynthetic bacteria

The occurrence and distribution of photosynthetic bacteria were extensively studied in several soils of south east Asia (Kobayashi et al., 1967). Rhodopseudomonas sp., Rhodospirillum sp. and Chromatium sp. occur frequently in soils. Rhodopseudomonas capsulatus, in symbiotic association with heterotrophic bacteria, utilized pyruvic acid excreted by the heterotrophs and fixed appreciable amounts of $N$ even under apparent aerobic conditions (Kobayashi and Haque, 1971). The complex symbiotic relationship between photosynthetic and
heterotrophic bacteria with their possible contributions to the soil fertility have been extensively investigated by Japanese workers (Okuda and Kobayashi, 1961; Kobayashi et al., 1966; Katayama et al., 1967; Haque et al., 1969). Application of photo-autotrophic bacteria to soil at the reproductive stage of rice plants resulted in a significant increase in yield of paddy (Katayama et al., 1967).

The possible effects of photosynthetic microorganisms on the fertility of soils have been investigated (Yoshida et al., 1973; Habte and Alexander, 1980). Although several aspects of \( N_2 \)-fixing photosynthetic bacteria have been thoroughly investigated, the actual contribution by these bacteria is far from clear since factors such as light and anaerobiosis required for their growth and activity seldom coexist on the surface of a submerged soil.

2.1.4. Facultative symbiotrophic \( N_2 \) fixers

In nature the growth of microorganisms in soil is always associative. Mixed cultures of oligonitrophilic bacteria often fix \( N_2 \) even if none of the implicated bacteria fix \( N_2 \) in pure culture. Kalininskaya (1967a) suggested the term 'facultative symbiotrophic nitrogen fixers' for microorganisms with a limited ability for
$N_2$ fixation in pure culture but which can fix $N_2$ readily in mixed cultures with other microorganisms. Subsequently it was demonstrated that such $N_2$-fixing bacterial associations are widespread in the most diverse types of soils (Kalininskaya, 1967b, 1967c). The complex nutritional requirements of these organisms are achieved in association with a non-$N_2$ fixer. Thus, the non-$N_2$ fixer may provide an environment favourable for $N_2$ fixation.

Beijerinck and van Delden (1902) first demonstrated that *Azotobacter chroococcum* fixed more $N_2$ in association with other bacteria like *Agrobacterium*, *Aerobacter* and *Clostridium* than in pure culture. *Beijerinckia indica* fixed 5 times more $N_2$ in the presence of a heterotrophic organism (*Lipomyces starkeyi*) than in pure culture (Dommergues and Mutaftschiev, 1965). Fedorov and Kalininaskaya (1961a) isolated a new bacterium (later identified as *Mycobacterium flavum*) which was able to fix appreciable amounts of $N_2$ in mixed culture with species of *Escherichia*, *Pseudomonas* or *Flavobacterium* whereas only a little $N_2$ was fixed in pure culture. *Mycobacterium flavum* could grow well in a medium with ethanol or organic acids as carbon substrate, but $N_2$ fixation was still improved in the presence of other
bacteria (Fedorov and Kalininskaya, 1961b). Enhanced $N_2$ fixation by free-living $N_2$ fixers as a result of associated growth with non-$N_2$ fixers has also been reported in waterlogged paddy soils (Katayama et al., 1965; Kobayashi et al., 1965). The formation of stable association between facultative symbiotrophic $N_2$ fixers and cellulose decomposers was also reported (Rao, 1980). Although the importance of facultative symbiotrophic $N_2$-fixing organisms in the ecosystems has been recognised recently (Jurgensen and Davey, 1970; Rao, 1974; Jensen and Holm, 1975), no information is available on the participation of these organisms in $N_2$ fixation in rice soils of India.

2.1.5. Associative bacteria

$N_2$ fixation by Spirillum (later renamed as Azospirillum) in association with many crop and forage plants has, of late, come to light in view of its high $N_2$-fixing potential and ubiquitous occurrence in diverse natural ecosystems (Dobereiner et al., 1976; Tyler et al., 1979). The discovery of grass-bacteria associations contributing to the $N$ economy of several forage and agricultural crops prompted the attention of many investigators (Dobereiner et al., 1976; Neyra and
Dobereiner, 1977; Reynders and Vlassak, 1977; Watanabe et al., 1978; Watanabe and Cholitkul, 1979; van Berkum and Bohlool, 1980). Although Azospirillum has been isolated from soils and the roots of a wide variety of plants from temperate and tropical regions (Bulow and Dobereiner, 1975; Dobereiner et al., 1976; Okon et al., 1977), most investigators consider this organism to be of special significance only in the tropics (Day, 1977; Neyra and Dobereiner, 1977). The occurrence and N₂ fixation by Azospirillum sp. in rice has recently been established (Lakshmikumari et al., 1976; Nayak and Rao, 1977). △¹⁵N₂ incorporation by Spirillum lipoferum cultures was demonstrated (Okon et al., 1976a). Similarly N₂ fixation in two tropical grasses was confirmed by △¹⁵N-tracer technique (De-Polli et al., 1977). Substantial information on the physiology and biochemistry of A. lipoferum is now available (Burris, 1976; Day and Dobereiner, 1976; Okon et al., 1976a, 1976b, 1977; Burris et al., 1977, 1978; Albrecht et al., 1977). Besides N₂ fixation, Azospirillum exhibited a capacity to produce growth promoting substances (Tien et al., 1979).

Several crop plants indicated yield increases following inoculation of forage and grain crops in the
field (Sloger and Owens, 1976; Smith et al., 1976, 1977, 1978). But, several other studies conducted under greenhouse and field conditions with maize, sorghum, and wheat have failed to show any dry matter or total N increase due to inoculation although the excised roots of the inoculated plants showed higher N\textsubscript{2}-ase activity (Barber et al., 1976, 1979). In most of these studies there was no significant interaction between N fertilization and inoculation. Studies from Belgium indicated that the effect of inoculation with Azospirillum varied with different plants (Reynders and Vlassak, 1978) and each plant species had its preference for specific strains (Vlassak and Reynders, 1978). Inoculation of a rice soil with Spirillum lipoferum increased the N\textsubscript{2}-ase activity significantly (Rinaudo et al., 1977). Seed inoculation of rice with Azospirillum brasilense resulted in increased grain yield at 40 and 60 kg N/ha over the corresponding uninoculated controls (Subba Rao et al., 1979).

2.1.6. Rhizosphere N\textsubscript{2} fixation

The rhizosphere is an ideal habitat for heterotrophic N\textsubscript{2}-fixing bacteria. N\textsubscript{2} fixation in rice fields was certainly enhanced in the presence of rice
plants (Willis and Green, 1948; De and Mandal, 1956; Rao et al., 1973; Yoshida and Ancajas, 1973a). Free-living heterotrophic N$_2$-fixers are quite competent rhizosphere organisms. The stimulatory effect of the rice plant on N$_2$ fixation in paddy soils was attributed essentially to the activities of N$_2$-fixing microorganisms within or around the roots (Rinaudo et al., 1971; Rao et al., 1973; Yoshida and Ancajas, 1973a; Balandreau et al., 1975a,b). Several groups of N$_2$-fixing microorganisms isolated from rice roots include Beijerinckia (Diem et al., 1977), Enterobacter, Clostridium (IRRI, 1976; Watanabe et al., 1977), Spirillum lipoferum (Lakshmikumari et al., 1976; Nayak and Rao, 1977; Silva and Dobereiner, 1978). The magnitude and factors governing rhizosphere N$_2$ fixation in rice have been extensively studied (Yoshida and Ancajas, 1971, 1973a, 1973b; Balandreau et al., 1975a; Trolldenier, 1977; Lee and Yoshida, 1977a, 1977b; Rinaudo et al., 1977; Dommergues and Rinaudo, 1979; Rao, 1980; Yoshida and Yoneyama, 1980).

In a fertility trial with wetland rice, N dressing (140 kg N/ha) inhibited N$_2$ fixation temporarily (Trolldenier, 1977). On the contrary, addition of 40 ppm N to rice rhizosphere stimulated N$_2$ fixation while higher concentrations were inhibitory (Balandreau et al.,
Application of ammonium sulphate (100 kg N/ha) to a flooded paddy soil suppressed N$_2$ fixation even in the presence of 5 t/ha rice straw, but when the level of rice straw was increased to 10 t/ha, ammonium sulphate was not inhibitory (Rao, 1980). However, no information is available on these aspects in Indian rice soils under flooded and nonflooded conditions.

2.2. Evaluation of N$_2$ fixation in rice soils

In view of the inherent variations in the soil properties and fluctuations in the environment, it is difficult to predict and attribute the contribution of N in rice soils exclusively to a particular group of microorganisms. However, several investigators tried to evaluate the relative contribution of different groups of microorganisms to paddy soils such as blue-green algae (Singh, 1961; Venkataraman, 1975; Watanabe et al., 1977), free-living heterotrophs (Yoshida and Ancajas, 1973b; Rao et al., 1973; Rao, 1976, 1978a) and a symbiotic blue-green alga associated with the water fern *Azolla* (Becking, 1975; Singh, 1977; Watanabe, 1977). These investigators employed different methods and sampling techniques for estimating N$_2$ fixation.

The first evidence for significant N$_2$ fixation in paddy soils was by MacRae and Castro (1967) and the
values for $^{15}$N gains in soils were equivalent to annual rates of 10-55 kg N/ha of which a major proportion of $\text{N}_2$ fixed in dark-incubated soil samples was due to the activity of heterotrophic bacteria rather than to the activities of blue-green algae and photosynthetic bacteria. Fixation rates, in soil, equivalent to 13 kg N/ha were reported (Delwiche and Wijler, 1956). $^{15}$N studies indicated annual gains of 20 to 70 kg N/ha under anaerobic conditions and as low as 10 kg N/ha under aerobic conditions (Chang and Knowles, 1965). Paddy soils exhibited highest $\text{N}_2$-fixing ability among several soils (Kalininskaya, 1972). Similarly many investigators employed $^{15}$N-tracer technique to evaluate $\text{N}_2$ fixation in several rice soils (Rao et al., 1973; Rao, 1973a, 1973b, 1974, 1980; Yoshida and Ancajas, 1973a, 1973b; Ito et al., 1980; Yoshida and Yoneyama, 1980). However, no information is available on the use of $^{15}$N in Indian rice soils. Besides the high cost of $^{15}$N$_2$, a major hindrance to more extensive use of $^{15}$N-tracer technique has been the high cost of the equipment needed for $^{15}$N analysis and problems encountered in maintenance and operation of this equipment (Hauck and Bremner, 1976).

The acetylene reduction assay, although indirect, is a sensitive method for the estimation of $\text{N}_2$-fixing
activity. Most investigators have employed this method for estimating $N_2$-ase activity in different systems (Dommergues et al., 1973; Nayak and Rao, 1981). This method, however, poses problems such as the conversion factor between $C_2H_2$ reduction and $N_2$ fixation, the different diffusion rates and solubilities of $C_2H_2$ and $N_2$ and an incomplete recovery of the formed $C_2H_4$. Several improvements in the assay of this method have been suggested (Balandreau et al., 1975a, 1977; Watanabe et al., 1977; Trolldenier, 1977; Boddy et al., 1978; Watanabe, 1978; Watanabe and Cholitkul, 1979). Burris (1972, 1974) has been highly critical of converting acetylene reduction values to amounts of $N_2$ fixation. According to him, to interpret acetylene reduction data in terms of $N_2$ fixation, it is essential to measure $^{15}N_2$ and acetylene reduction for the same exposure times under identical conditions. Further it has been emphasized that conclusions on $N$ gains based on exclusively one technique can often be misleading and for meaningful estimation of $N_2$-fixing potential in a system, assay by both techniques is essential (Rao, 1978a).

However, no comparative data on $N_2$ fixation in rice soils of India are available employing the sensitive and reliable techniques such as acetylene reduction and $^{15}N$-tracer techniques.
2.3. Factors affecting $N_2$ fixation in rice soils

The distribution and efficiency of $N_2$-fixing bacteria in nature depend on a multiplicity of factors. Based on the available information, $N_2$ fixation was affected mainly by the following factors:

2.3.1. Soil type

Large variations in the magnitude of $N_2$ fixation were noticed in soils of diverse geographic regions. Soils rich in organic matter generally supported better growth of heterotrophic organisms while soils rich in phosphorus stimulated the growth of heterotrophs and blue-green algae (Matsuguchi et al., 1975). Clostridium sp. and blue-green algae were more abundant in soils rich in available $N$.

$N_2$ fixation ranged from 13 to 44 kg/ha in rice fields over a period of 6 weeks (De and Mandal, 1956). Most of the paddy soils in Thailand had the fixation rates of less than 10 kg N/ha although some marine and fresh water alluvial soils fixed around 20 kg N/ha. Of the Philippine soils, the N gain in Maahas clay soil was 10 kg N/ha while Luisiana and Calabanga clay soils exhibited fixation values as high as 19 and 55 kg N/ha respectively (MacRae and Castro, 1967). Annual gains
of 20 to 70 kg N/ha under anaerobic conditions and as low as 10 kg N/ha under aerobic incubation were observed in some Quebec soils of Canada (Chang and Knowles, 1965). Relatively high efficiency (8.0 to 11.5 mg N fixed/g cellulose consumed) of N$_2$ fixation was noticed in paddy soils of the USSR (Kalininskaya, 1972) while a flooded soil from Louisiana exhibited fixation rates ranging from 57 to 61 µg/g soil/year (Reddy and Patrick, 1979). The total estimated value of atmospheric N$_2$ fixed in the soil by acetylene reduction was 52 to 64 kg N/ha in wet and dry seasons respectively (Yoshida and Ancajas, 1973b). The total N contribution as a result of the activities of blue-green algae and photosynthetic bacteria amounted to 10 to 200 mg/kg soil/day (Ishizawa et al., 1970). N$_2$ fixation by free-living bacteria amounted to 7 kg N/ha/season in rice soils of U.S.S.R. (Rao, 1980) while the rates of N$_2$ fixation ranged from 50 to 60 kg N/ha in Philippine soils (Yoshida and Ancajas, 1973a).

The N$_2$ fixation rates varied widely in soils from diverse geographic regions. But comparative studies on N$_2$ fixation in Indian rice soils differing widely in their properties are less numerous.
2.3.2. Water regime

Nitrogen fixation appears to be sensitive to water stress. Soil submergence provides favourable environment for microorganisms. Flooded soil had a greater capacity to fix atmospheric $\text{N}_2$ than upland soil (Yoshida and Ancajas, 1973a, 1973b). $\text{N}_2$ fixation was considerably enhanced under flooded conditions while at low moisture contents (24 to 35 per cent) $\text{N}_2$ fixation was negligible (Rao, 1976). Similarly, high rates of $\text{N}_2$-ase activity were associated with soils from wet areas and were positively correlated with soil moisture (Day et al., 1975). A similar correlation existed between soil moisture and $\text{N}_2$ fixation in soil cores of grasslands (Vlassak et al., 1973). Anaerobically incubated soil exhibited higher $\text{N}_2$-fixing activity (Chang and Knowles, 1965). Soil moisture had a differential effect on acetylene reduction when butyrate and succinate were provided as carbon source; succinate at low moisture level and butyrate under flooded conditions stimulated $\text{N}_2$ fixation (Rao, 1978b). $\text{N}_2$-ase activity increased several fold following a shift from nonflooded to flooded conditions while the activity decreased when the flooded soil was returned to nonflooded condition by draining (Nayak and Rao, 1981). The low $\text{N}_2$ fixation
under nonflooded conditions could be attributed to high $O_2$ tension which is known to inhibit $N_2$-ase activity (Brouzes et al., 1971). On the other hand, submerged soils provide adequate moisture, nutrients supply and favourable aeration conditions for the activity of $N_2$ fixers (Rao, 1976). Further, alternate flooded and nonflooded cycles in upland (rain fed) cropping systems may play a significant role on important microbiological processes including $N_2$ fixation. The comparative influence of water regimes on $N_2$ fixation in paddy soils is less well understood.

2.3.3. Organic matter

Carbon is an essential substrate for heterotrophic $N_2$ fixers. Their population and potential depend on the availability of this substrate (Knowles, 1977; Rao, 1978a). Root exudates, lysates and litter form an important fraction of organic carbon under field conditions. The incorporation of organic matter into soil stimulates the population of $N_2$-fixing bacteria especially under waterlogged conditions (Barrow and Jenkinson, 1962; Rice et al., 1967; Kobayashi and Haque, 1971; Rice and Paul, 1971, 1972; Rao, 1973a, 1976, 1977, 1978b; Mayfield and Aldworth, 1974; Matsuguchi and Shimomura, 1977). The resultant energy obtained through
the decomposition of organic matter would be utilized by \( \text{N}_2 \)-fixers and other microorganisms. Since \( \text{N}_2 \)-fixing bacteria constitute a large percentage of heterotrophic \( \text{N}_2 \) fixers, \( \text{N}_2 \) fixation under such situations can be of considerable importance (Rao et al., 1973; Yoshida and Ancajas, 1973a, 1973b; Trolldenier, 1977; Dommergues and Rinaudo, 1979; Watanabe and Cholitkul, 1979).

Several reports indicate that the addition of rice straw significantly enhanced \( \text{N}_2 \) fixation in submerged soils (MacRae and Castro, 1967; Kalininskaya et al., 1973; Saito et al., 1975; Rao, 1976, 1978b; Yoneyama et al., 1977; Watanabe, 1978; Matsuguchi, 1979; Reddy and Patrick, 1979). Likewise, addition of cellulose to paddy soils also enhanced \( \text{N}_2 \) fixation (Kalininskaya, 1972; Yoneyama et al., 1977; Rao, 1978b, 1980). Further, rice straw application with or without fertilizer \( \text{N} \) considerably increased the crop yield of rice (Rao, 1973a, 1973b). Thus the stimulation of \( \text{N}_2 \) fixation by organic matter application revealed a close association between organic matter decomposers and \( \text{N}_2 \) fixers.

2.3.4. Combined \( \text{N} \)

The reported inhibitory nature of combined \( \text{N} \) is based largely on laboratory studies with pure cultures
or purified enzyme systems (Strandberg and Wilson, 1968; Biggins and Postgate, 1969; Becking, 1971). The degree and type of inhibition by combined N depend upon its concentration (Stewart, 1969). Moreover, the levels of combined N present in natural ecosystems are not sufficient enough to inhibit N₂ fixation and can only suppress the process temporarily (Becking, 1971). NPK fertilizers promoted heterotrophic N₂ fixation in paddy soils (Matsuguchi, 1979; Cholitkul et al., 1980). N₂ fixation in soil was stimulated by ammonium sulphate up to 40 ppm and inhibited by concentrations above 40 ppm (Balandreau et al., 1975a). Estimations of ammonium content performed at the same time showed that all the ammonium added had been used up by plants or immobilized by microorganisms. The slight stimulation of the N₅-ase activity at low levels of N was attributed to an increase in the root exudates (Balandreau et al., 1975a). Also, application of NH₄⁺-N at levels of 50 ppm and above inhibited N₂ fixation in the rice rhizosphere (MacRae, 1975). A similar inhibition occurred when inorganic N was applied as NO₃⁻-N. According to another study (Knowles and Denike, 1974) N₂ fixation decreased following application of 50 ppm NH₄⁺-N and 20 ppm NO₃⁻-N to soil incubated under anaerobic conditions.
$N_2$ fixation was completely inhibited when fertilizer was applied to a paddy soil at 160 ppm N (Yoshida et al., 1973). On the contrary, $N_2$ fixation was inhibited, but not completely, even at concentrations as high as 320 ppm (Rao, 1976). Even combined application of ammonium sulphate (100 kg N/ha) and rice straw (5 t/ha) suppressed $N_2$ fixation, but inhibitory effect of ammonium was alleviated by the level of rice straw at 10 t/ha (Rao, 1980). Also, the concentration of combined N required for suppression of $N_2$-ase activity was proportional to the concentration of added carbohydrate (Knowles and Denike, 1974).

These results suggest that in soil there are organisms which can fix $N_2$ even in the presence of abnormally high concentrations of N and/or these concentrations of N were not inhibitory to $N_2$-ase activity.

2.3.5. Pesticides

There has been little progress in understanding the influences involved in soil-pesticide-microflora relationships (Johnen and Drew, 1977). The beneficial effects of pesticides as pest combatants could be nullified by their detrimental effects on microbial
processes having a major influence on plant growth, crop productivity, and eventually soil fertility.

Application of gamma BHC resulted in significant increase in \( N_2 \) fixation in two Philippine soils (Raghu and MacRae, 1967b) and this was attributed to \( N_2 \) fixation by algae and heterotrophic \( N_2 \)-fixing bacteria (Raghu and MacRae, 1967a). Interestingly, gamma BHC application to pure culture of \textit{Azotobacter vinelandii} had no significant influence on growth or acetylene reduction (MacKenzie and MacRae, 1972). The effects of several pesticides on the growth and \( N_2 \) fixation was extensively studied in pure culture systems (Venkataraman and Rajyalakshmi, 1972; Wood and MacRae, 1974; Tu, 1975, 1978). Dinoseb (DNBP) when applied at a concentration of 6 ppm inhibited the reduction of \( C_2H_2 \) by 90 per cent in an anaerobic soil system (Vlassak et al., 1976). However, investigations on the effects of pesticides on \( N_2 \) fixation in rice soils are limited. The differential effects of pesticides on \( N_2 \) fixation in rice soils were attributed to the alterations in the specific groups of \( N_2 \) fixers (Nayak and Rao, 1980). However, application of pesticides exerted differential response on the rhizosphere nitrogenase and on the \( N_2 \)-fixing activity of root inhabitants (Nayak et al., 1980).
2.4. Transfer of fixed nitrogen from soil to the rice plant

Although the magnitude and the probable influences of certain amendments on N\textsubscript{2} fixation in paddy soils were known, estimates of the availability, transfer and distribution of biologically fixed N to the rice plant are rather few and conflicting. \textsuperscript{15}N\textsubscript{2} incorporation studies on rice plants under water culture conditions revealed that less than 10 per cent of the fixed N was translocated to the plant tissues (Ito et al., 1980). However, 19 to 25 per cent of the atmospheric N\textsubscript{2} fixed in the rice rhizosphere was found in the roots, leaves, stems and ears of the rice plants during \textsuperscript{15}N\textsubscript{2} exposure period (Yoshida and Yoneyama, 1980). Considerable losses of N can occur in anaerobic waterlogged rice soils than in aerobic soils (Jansson, 1963; Becking, 1971).

From the foregoing, it is clear that several aspects of N\textsubscript{2} fixation by heterotrophic bacteria in rice soils under Indian conditions are not understood. Moreover, reliable estimates of the magnitude and extent of N\textsubscript{2} fixation in rice soils by using sensitive techniques are not available.