2. REVIEW OF LITERATURE

Studies dealing with interactions between PNP and its reduction metabolites, PNSP and PAP towards cyanobacteria and microalgae are very limited (Venkateswarlu, 1993). The review of literature presented below is therefore an account on the effects of other toxicants, in general, and PNP and its metabolites, in particular, when tested alone and in combination towards microalgae and cyanobacteria, isolated from soil and water.

2.1. Toxicity evaluation

In microbial toxicity tests, the response measured is typically a continuous variable, and the associated error is an experimental error related to the physical measurement of the response and to handling differences between experimental units (Walsh et al., 1987). The traditional statistical theory for treating quantal data from tests with animals is based on the concept of a tolerance distribution for individual test subjects that is not appropriate for microbial tests. Data from microbial tests should be dealt with by conventional curve-fitting techniques without any confusion with tolerance distributions (Nyholm et al., 1992). The linear regression model has been recommended over other conventional models.
of probit and logit while estimating effective concentrations (EC) that cause a certain percentage of inhibition in microbial toxicity tests (Millie and Hersh, 1987).

2.2. Toxicity towards microalgae and cyanobacteria

The entry of pollutants into soil might have far-reaching consequences, as they would disturb the delicate equilibrium between a microorganism and its environment, both involved in important biological processes. Thus, interference of the heavily used pesticides with the normal activities of soil populations of cyanobacteria and microalgae may be expected to have potentially serious effects on the overall fertility of soils. Following is the available literature pertaining to the toxic effects of organic and inorganic chemical pollutants towards the isolates of microalgae and cyanobacteria.

2.2.1. Insecticides

Investigations concerning nontarget effects of environmental pollutants on cyanobacteria dealt primarily with growth and related phenomena of the pure cultures as toxicity criteria. It is evident from the literature that conflicting reports on the toxic effects of aldrin, dieldrin and endrin are available (Vance and Drummord, 1969). For instance, Schaubberger and Wildman (1977) reported that aldrin and dieldrin at 1 ppm inhibited the growth of *Anabaena cylindrica*, *Anacystis nidulans* and *Nostoc muscorum* without affecting their morphology. Dieldrin inhibited the growth of *Anacystis nidulans* in laboratory cultures (Lazaroff and Moore, 1966). Endrin was more toxic than its metabolite, ketodieldrin, to *Agmenellum quadruplicatum* (marine alga) and *Anacystis nidulans* (Batterton et al., 1971). Singh (1973) reported that *Cylindrospermum* sp. tolerated 60 ppm of endrin. However, endrin at 10 ppm suppressed the growth of *Aulosira*
fertilissima in rice field, which is higher than field application dose of 1 to 2 ppm (Ahmed and Venkataraman, 1973). Plectonema boryanum and A.fertilissima tolerated endrin up to 600 ppm in laboratory conditions (Singh, 1973). Clegg and Koevenig (1974) reported that aldrin, dieldrin and endrin inhibited the growth of Microcystis aeruginosa, but many other forms were resisted even much higher concentrations without any apparent effect on population densities.

The growth of Anacystis nidulans (Gregory et al., 1969), M. aeruginosa and Anabaena cylindrica (Vance and Drummond, 1969) at 1000 ppb of DDT was adversely affected incorporating higher p,p'-DDT concentrations up to 20 ppm in cultures of cyanobacterial forms. The growth and morphology of Synechococcus elongatus was not adversely affected at 99 ppb of DDT (Worthem, 1973). DDT and its metabolites, DDD and DDE, inhibited the growth of A. nidulans; DDD was toxic, followed by DDE and DDT. DDT at 10 ppm inhibited growth of M. aeruginosa (Boush and Matsumura, 1975), while at concentration up to 20 ppm hindered the growth of two strains of Aphanocapsa as well as of Anabaena variabilis and Nostoc (Hutber et al., 1979). Likewise, Goulding and Ellis (1981) reported that in A. variabilis 1 ppm DDT inhibited the chlorophyll content causing reduction in size of cells.

Marco et al. (1990) reported a decrease in the content of nitrogen compounds, increase in carbohydrates, and alterations in cell division and morphology in Anabaena pcc 7119 under the influence of trichlorofon. The decrease in contents of phycobiliproteins, chlorophyll and total proteins in trichlorofon-treated cultures of Anabaena pcc 7119 was observed by Orus et al. (1990). Lindane at 200 ppm concentration completely eliminated blue-green algae (Singh, 1973). Ankistrodemus brauni and A. nidulans when exposed to
Lindane at 0.1 to 100 ppm, the chlorophyll content, dry weight, photosynthetic capacity, and the formation of autospores were all reduced at the concentrations used (Kopeck et al., 1976).

Less attention has been paid in determining the toxic effects of organophosphorus insecticides when compared with organochlorines toward cyanobacteria (Anderson, 1978), although organophosphates are widely used in recent agriculture. The cultures of Anacystis nidulans, Euglena gracilis and Scenedesmus obliquus were not affected by 1 ppm parathion despite the accumulation of parathion at levels up to 72 ppm (Gregory et al., 1969). Parathion treatment even at 1 to 100 ppm did not affect the growth of Euglena gracilis till 24 h, and the growth was even stimulated at the end of 7 days after treatment (Poorman, 1973). Inhibition in cell number, packed cell volume, pigments, and protein content were observed in a green alga, Chlorella protothecoides, when grown autotrophically in the presence of methyl parathion (Saroja and Bose, 1982). Megharaj et al. (1994) reported that two strains of microalgae and four species of cyanobacteria completely hydrolyzed methyl parathion to PNP (with traces of PAP) after 30 days.

Malathion at 1 ppm reduced the chlorophyll content in Oscillatoria sp.; however, chlorophyll production was stimulated at low concentrations of 0.01 to 0.55 ppb (Torres and O'Flahaty, 1976; Tiwari et al., 1979). Megharaj et al. (1986a) reported differential response to monocrotophos and quinalphos of Synechococcus elongatus, Nostoc linckia and Phormidium tenue. Monocrotophos was toxic to P. tenue at 50 and 100 μg/ml, and to N. linckia at 100 μg/ml, while the growth of S. elongatus was enhanced even at 100 μg/ml. Quinalphos, at 100 μg/ml, showed significant enhancement in growth of P. tenue.
but inhibitory effects on *S. elongatus* and *N. linckia* were observed with concentrations above 5 or 10 \( \mu g/ml \). Nitrogen fixation by *N. linckia* was inhibited by monocrotophos and quinalphos (Megharaj *et al.*, 1988a).

The growth of *Aulosira fertilissima* was not inhibited at 10 ppm carbaryl (Ahmed and Venkataraman, 1973). However, this concentration enhanced the growth of *Cylindrospermum* sp. (Padhy, 1985). Vaishampayan (1985) reported high sensitivity of *N. muscorum* grown on solid nitrogen-free medium containing carbaryl.

The EC50 of permethrin toward growth of *Anabaena cylindrica* and *A. variabilis* was less than 10 ppm, but the EC50 values of various phenoxybenzyl degradation products of permethrin ranged from 1.4 to 8 ppm (Stratton and Corke, 1982). The toxic effects of permethrin and ten of its degradation products on the growth of ten fungi were determined. Permethrin was relatively nontoxic with an EC50 of 100 mg/l, but six of its degradation products were significantly more inhibitory (Stratton and Corke, 1982). The growth of *Synechococcus* stimulated or only slightly inhibited at 10 to 50 \( \mu g/ml \) cypermethrin. Growth response in terms of chlorophyll a concentration was significantly enhanced in *Nostoc linckia* but not in *Phormidium tenue*, by cypermethrin and fenvalerate at 10 to 50 \( \mu g/ml \). In a strain of *N. linckia*, nitrogen fixation was stimulated by fenvalerate up to 20 \( \mu g/ml \) (Megharaj *et al.*, 1987).

Megharaj *et al.* (1986b) also reported that *N. linckia* was highly sensitive to PNP, MNP, DNP and catechol compared to the green alga *C. vulgaris*. The toxicity of PNP and MNP toward *N. linckia* and *Synechococcus elongatus* could not be reversed even after the addition of 0.5% glucose to culture medium (Megharaj *et al.*, 1989). The nitrogen-fixing activity of *N. linckia*
was inhibited at 5 \( \mu g/ml \) of PNP, MNP and DNP (Megharaj et al., 1988a). At concentrations from 5 to 50 \( \mu g/ml \) of \( p \)-aminophenol (PAP), the product of nitro group reduction of PNP, inhibited cell number, chlorophyll a, total carbohydrates, \( ^{14}CO_2 \) uptake, nitrate reductase, nitrogenase activity in \( N. \) \textit{linckia} and \( N. \) \textit{muscorum} (Megharaj et al., 1991a). The cell constituents (such as chlorophyll a, protein and carbohydrates), \( ^{14}CO_2 \) uptake, and the activity of nitrate reductase, nitrogenase in \( N. \) \textit{linckia} were significantly inhibited also by phenol (Megharaj et al., 1991b). The toxicity exerted by MNP could be reversed by the addition of 10 \( \mu M \) ATP. Transmission electron microscopy revealed the secretion of mucous around the filament, and induction of spore formation in the culture subjected to nitrophenol toxicity. Megharaj et al. (1991a) reported that the toxicity of PNP towards \( N. \) \textit{linckia} was alleviated in the presence of 2 to 5 \( \mu g/ml \) of PAP.

### 2.2.2. Herbicides

The impact of herbicides on a number of cyanobacteria has been thoroughly understood. 2,4-Dichlorophenoxy acetic acid (2,4-D) at 250 ppm was found ineffective in controlling the growth of bloom-forming \( \textit{Microcystis aeruginosa} \) (Cook and Conners, 1963). Hamdi \textit{et al.} (1970) reported a decrease in dry matter, chlorophyll content, and nitrogen-fixing activity in \( \textit{Tolypothrix tenuis} \) at 0.045 to 4.5 ppm of 2,4-D. Low doses \( (10^{-4} \text{ M}) \) of 2,4-D stimulated the growth of \( \textit{Nostoc muscorum}, \textit{N. punctiforme} \), and field application rates \( (1.1 \times 10^{-2} \text{ M}) \) inhibited both growth and nitrogen fixation (Inger, 1970). Inhibition in nitrogen-fixing activity of \( \textit{N. muscorum}, \textit{N. punctiforme} \) and \( \textit{Cylindrospermum} \) sp. was observed at \( 1.1 \times 10^{-2} \text{ M} \) of 2,4-D (Lundqvist, 1970). However, 440 ppm of 2,4-D had no adverse effect on the growth of \( \textit{Cylindrospermum licheniforme} \) in an agar plate culture (Arvick \textit{et al.}, 1971).
Noll and Bauer (1973) reported that triazines at 0.3 to 15 ppm inhibited trichome migration in *Phormidium* sp. Atrazine at 0.2 ppm significantly inhibited photosynthesis in *Anabaena cylindrica*, but 1 ppm atrazine inhibited nitrogenase activity, oxygen production, and growth (Rohwer and Fluckiger, 1979). Concentration of atrazine above 100 ppm inhibited C$_2$H$_2$ reduction in species of *Anabaena* (Kallio and Wilkinson, 1977; Holst et al., 1982). The EC50 values for atrazine ranged from 0.1 to 0.5 ppm for growth, and 40 to 70 ppm for C$_2$H$_2$-reducing activity in three species of *Anabaena* (Stratton, 1984). Cyanobacteria are more resistant than microalgae to monuron (Maloney, 1958). The growth of *Anabaena variabilis*, *A. spiroides*, *Nostoc* sp. and *Plectonema boryanum* was inhibited by monuron and diuron (Shilo, 1965). Schluter (1965) reported that *Anabaena flos-aquae* was sensitive to 2 ppm monuron. Nitrogen fixation in cyanobacteria was initially inhibited by monuron and linuron; however, the inhibition was followed by subsequent recovery and/or stimulation (Dasilva et al., 1975).

*Anabaena cylindrica* and *Tolypothrix tenuis* were more resistant to propham and chlorpropham than *Anacystis nidulans* and *Gloeocapsa alpicola*, but lower concentrations of propham, chlorpropham, or barban stimulated the growth of both *A. cylindrica* and *T. tenuis* (Wright, 1978). The growth of cyanobacteria was inhibited by chlorpropham, and was attributed to its adverse effects on photosynthesis, respiration, and synthesis of RNA, protein and lipid (Ashton et al., 1977).

### 2.2.3. Fungicides

Oxine at 5 ppm totally inhibited a bloom-forming strain of *Microcystis aeruginosa* (Fitzerald et al., 1952). Venkataraman and Rajyalakshmi (1971) reported that...
zineb at higher levels (100 kg/ha) was not inhibitory to most strains of *Anabaena* and *Nostoc*. On the contrary, zineb at 1 and 30 ppm was toxic to growth of *Cylindrospermum* sp. and *Nostoc muscorum*, and 0.5 ppm ziram inhibited the growth of these cyanobacteria (Padhy, 1985).

Growth of *Anacystis nidulans* was suppressed at 140 ppb dichlone (Whitton and MacArthur, 1967), whereas even low concentrations (10 ppb) of dichlone inhibited the growth of *Anabaena* sp. (Bisiach, 1970). Captan, zineb, blitone, blitox and rovral, all at 5000 ppm, reduced the formation of heterocysts in *Nostoc* sp. (Gangawane et al., 1980). Venkataraman and Rajyalakshmi (1972) reported that 5 to 50 ppm mancozeb did not affect twenty-seven strains of cyanobacteria. Heterocyst frequency in *Cylindrospermum* was not affected by ziram, zineb mancozeb, but nitrogenase activity was adversely affected (Padhy, 1985). Paraschiv et al. (1972) reported that two of *Oscillatoria* species were sensitive to maneb.

Topsin-M at concentrations of 5 to 1000 ppm showed a differential sensitivity towards species of *Calothrix, Westiellopsis, Aulosira, Tolypothrix* and *Nostoc* (Gangawane and Kulkarni, 1979). Species of *Nostoc* and *Tolypothrix* were resistant to rovral than to those of *Westiellopsis, Aulosira* and *Calothrix*, but thiram was inhibitory to the growth of the first two genera. Thimet at 100, 500, 500, and 300 ppm had harmful effects on species of *Westiellopsis, Aulosira, Tolypothrix* and *Calothrix*, respectively, whereas species of *Nostoc* were sensitive to even 1 ppm of the fungicide (Gangawane, 1980). The growth of *Nostoc, Anabaena, Lyngbya*, and *Mastigocladus laminosus* was completely inhibited by thiram at concentrations from $10^{-3}$ to $5 \times 10^{-1}$ M (Cameron and Julian, 1984).
2.2.4. Heavy metals

Very limited number of studies were conducted on the nontarget effects of heavy metals towards cyanobacteria and microalgae. Nickel significantly inhibited the growth and $^{14}$CO$_2$ fixation in Chlorella pyrenoidosa (Ajay et al., 1996). Aluminium highly affected physiological processes and ATP content at low pH in Nostoc linckia (Yasmin and Rai, 1992). Murthy and Prasanna (1993) reported that mercury ions inhibited photosynthetic electron transport chain at multiple sites in the cyanobacterium Synechococcus 6301.

2.3. Siderophore production

Iron is essential to the growth of virtually all organisms. However, iron is not readily available in most biological systems. Iron is tightly sequestered by host carrier proteins. Microorganisms have evolved various mechanisms to acquire iron when confronted with iron-limiting conditions. Many microorganisms respond to iron limitations by secreting low-molecular-weight iron chelators known as siderophores (Goldman et al., 1983). Solubilized iron can then be scavenged from these siderophore complexes through the use of specific membrane transport systems (Neilands, 1981). A number of cyanobacteria have been found to produce hydroxamate-type siderophores (Armstrong et al., 1979; McKnight and Morel, 1979; Murphy et al., 1983), but the only one which has been structurally identified is schizokinen (Simpson and Neilands, 1976). This siderophore coordinates a ferric ion via two hydroxamate groups and the hydroxycarboxylate group of a citrate moiety (Plowman et al., 1984).

Schizokinen is known to facilitate iron uptake in Anabaena sp. strains 6411 and 7120, and has also been observed as an extra-cellular product of
Anabaena flos-aquae and of rice field cyanobacteria (Akers, 1983). The production of siderophores has been implicated for the noted bloom-formers Microcystis, Nostoc linckia and Lyngbya, as well as the marine cyanobacteria Agmenellum quadruplicatum and Coccochloris elabens. Although cyanobacteria siderophores are synthesized in response to iron starvation, they are capable of binding other metal ions such as copper (Mcknight and Morel, 1979, 1980). This adventitious metal complexations could be an important factor in the well-establishing tendency of algal exudates to moderate copper toxicity (Fitzgerald and Faust, 1963). A number of studies have indicated that it is mainly free cupric ions which govern the toxic effect of copper towards phytoplankton and that copper ion activity is diminished in natural systems by materials such as humic acids (Anderson and Morel, 1978). Accordingly, the cyanobacterium Aphanizomenon flos-aquae is considerably less sensitive to copper in the presence of EDTA or a sediment extract (Wurtsbaugh and Horne, 1982). However, there are instances in which binding to organic ligands appears to enhance metal toxicity (Laegrid et al., 1983).

2.4. Biodegradation

Pesticide use has benefited modern society by improving the quantity and quality of the world’s production while keeping the cost of food supply reasonably. The use of pesticide has become an integral part of modern agricultural systems. Because of continuous pest problems, their usage possibly cannot be discontinued in the near future. Increased use of these chemicals has already caused considerable environmental pollution and problems of human health. It is well established that microorganisms are the major or frequently the only means of degradation/detoxification of several pesticides in the
environment. In addition, microbial degradation is advantageous because a wide variety of pesticides can be degraded completely even under mild conditions compared with degradation through chemical and physical means.

A thorough perusal of literature on the degradation of xenobiotics reveals that by and large, microbial degradation studies have two objectives. First, they are aimed at gaining a basic understanding of how biodegradation activities arise, evolve, and are transferred among members of the soil microflora. This information provides the basis upon which the environmental fate of a wide array of compounds can be predicted. The second objective is to devise bioremediation methods for removing or detoxifying dangerously high concentrations of pesticide residues.

Organophosphorus (OP) pesticides such as diazinon, fenitrothion, methyl parathion and parathion are perhaps the most extensively used insecticides in many agricultural practices in India. Microbial degradation through hydrolysis of \( p \)- or \( o \)-aryl bond are considered as the most significant steps in the detoxification of OP compounds. The hydrolase, responsible for catalyzing this reaction, referred to as an esterase or phosphotriesterase, is the most important enzyme in the bacterial metabolism of OP pesticides (Kumar et al., 1996).

Many microorganisms are able to "adapt" to one of a variety of substances added to the culture medium by forming an enzyme system that is not already present when the organism is grown in the absence of the added substance. This phenomenon is termed "enzyme induction" and the substance that initiates the response is the enzyme "inducing agent". Andus (1960)
suggested that microorganisms can develop the ability to degrade pesticides either by enzyme induction or by chance mutations.

All the OP hydrolysis studies deal with the primary hydrolysis of a parent molecule. This is an important step in the mineralization of pesticides because such degradation makes the compounds vulnerable to further metabolism. For example, PNP the major metabolite formed by hydrolysis of parathion, methyl parathion or paraxon is readily metabolized by numerous microorganisms (Spain, 1995; Kumar et al., 1996). Microorganisms have an inherent capacity to metabolize many naturally occurring chemicals by virtue of their comprehensive enzyme systems. However, they may lack the necessary enzymes required for the metabolism of synthetic compounds, such as OP insecticides that occur in the environment in very minute quantities.

Nitrophenols are very much susceptible to biodegradation, since the presence of additional polar groups such as NO₂, OH, NH₂, N-C-(O)- and COO- in the benzene nucleus provides a new focal point for microbial attack (Helling et al., 1971). However, the type, position and the number of derivatives in the benzene ring also contribute in determining the rates of microbial degradation of several organic compounds (Alexander and Lustigman, 1966). The three biotransformation processes that have been observed for nitrophenols are reduction of the nitro group (McCormick et al., 1976), hydroxylation of the aromatic ring (Raymond and Alexander, 1971), and displacement of the nitro group by a hydroxy group (Raymond and Alexander, 1971; Siddaramappa et al., 1973; Munnecke and Hsieh, 1974).

Polar nitroaromatic compounds are more accessible to electrophilic attack by oxygenases than nonpolar molecules. Consequently, a
considerable amount of information is available about the oxidation of nitrophenols by aerobic bacteria (Spain, 1995). Biodegradation of nitroaromatics can be initiated by both reductive and oxidative mechanisms. The reductive pathway involves initial reduction of the nitro substituent to an amino group, which may subsequently be released as ammonium. The action of nitroreductases has been demonstrated in cell-free systems under both aerobic and anaerobic conditions (Villanueva, 1964; McCormick et al., 1976; Kinouchi and Ohnishi, 1983). It has been shown that a number of nitroreductases have a broad substrate specificity and suggested that many nitroaromatic compounds are rapidly reduced to aromatic amines in a natural environment (Golab et al., 1979; Hallus and Alexander, 1983). In the oxidative pathways, nitrite is directly released from the aromatic ring, and this mechanism has been demonstrated with nitrotoluenes, nitrobenzenes and nitrophenols (Brian et al., 1984).

Spain and Gibson (1991) reported that PNP was converted to hydroquinone with simultaneous release of nitrite through a NADH-dependent monooxygenase reaction in a Moraxella sp.; hydroquinone is then degraded via $\gamma$-hydroxymuconic semialdehyde, maleylacetic acid, and $\beta$-ketoacidipic acid (Figure 1). But, Jain et al. (1994) described an alternative pathway for the degradation of PNP by an Arthrobacter sp., yielding 1,2,4-benzenetriol through the formation of 4-nitrocatechol, 4-nitroresorcinol or both (Figure 2). Very recently, Venkateswarlu and Spain (1997) proposed a complete pathway in Bacillus sphaericus JS905 for PNP degradation via 1,2,4-benzenetriol with 4-nitrocatechol as an intermediate.

Several reports on microbial metabolism of nitroaromatic compounds including picric acid (trinitrophenol) and trinitroresorcinol (Erikson, 1941), DNOC
Figure 1. Proposed pathway for biodegradation of PNP by *Moraxella* sp.
Figure 2. Proposed pathway for biodegradation of PNP by *Arthrobacter* sp.
(Gundersen and Jensen, 1956), 2,4-dinitrophenol (Madhosingh, 1961; Teuteberg, 1964) are available. But in recent years, the bacterial metabolism of PNP has drawn much more attention. Earlier Simpson and Evans (1953) isolated two species of *Psuedomonas* from the filter beds of an industrial effluent which decomposed ONP and PNP to nitrite. A culture of *Corynebacterium simplex*, obtained from DNOC-treated soil, utilized DNOC as sole carbon and energy source, liberating more than 70% of the nitrogen in DNOC as nitrite (Gundersen and Jensen, 1956; Jensen and Gundersen, 1955). This bacterium utilized also PNP, 2,4-dinitrophenol and 2,4,6-trinitrophenol as the sole carbon source with the formation of nitrite. Various strains of *Psuedomonas* sp., *Flavobacterium* sp., and *Nocardia* sp. utilized o- and p-nitrobenzoic acid, ONP and PNP as nutrient sources (Jensen and Gundersen, 1955). Several pseudomonads isolated from parathion-treated soils degraded PNP but did not use parathion as sole source of carbon (Griffiths and Walker, 1970). Raymond and Alexander (1971) reported that a soil bacterium utilized PNP as the sole source of carbon and energy with a stoichiometric formation of nitrite.

Barik and Sethunathan (1978) reported the complete disappearance of PNP, ONP and m-nitrophenol in a basal salts medium when inoculated with a parathion-hydrolyzing enrichment from a flooded alluvial soil. A similar enrichment obtained from another soil type was very specific in degrading only PNP. However, the substrate specificities of this enrichment culture were least pronounced when tested under flooded soil conditions. Enhanced evolution of $^{14}$CO$_2$ from uniformly labelled $^{14}$C-PNP from the uninoculated flooded soils under aerated (stirred) conditions was also demonstrated (Barik and Sethunathan, 1978).
Two bacterial cultures, a *Psuedomonas* sp. and a *Corynebacterium* sp., isolated from parathion-amended flooded soil (Siddaramappa *et al.*, 1973), utilized PNP as the sole carbon and energy source and metabolized it to nitrite (Siddaramappa *et al.*, 1973) and CO₂ (Barik *et al.*, 1976). However, these two bacterial isolates differed markedly with regard to their specificities in metabolizing various other nitrophenols. While *Psuedomonas* sp. utilized only 2,4-dinitrophenol, *Corynebacterium* sp. metabolized only *m*-nitrophenol (Barik *et al.*, 1976). Similarly, Munnecke and Hsieh (1976) obtained a *Psuedomonas* sp. from a parathion-hydrolyzing enrichment which utilized PNP as a carbon source and formed nitrite via hydroquinone metabolism.

Cells of a soil bacterium, *Psuedomonas* sp. ATCC 29353, readily hydrolyzed parathion and then liberated nitrite from PNP (Barik and Sethunathan, 1978). This isolate metabolized 157 µg of PNP completely in 24 h, liberating 52 µg nitrite with a consequent increase in cell number from 14 x 10² at start to 126 x 10² after 24 h. But, in cell-free suspension, the reaction ceased at the PNP stage. Bacteria capable of degrading PNP were isolated from river samples pre-exposed to methyl parathion or PNP but not salt marsh samples, and PNP degradation was observed with pure cultures of bacteria (Spain *et al.*, 1980). In presence of glucose, a *Psuedomonas* sp. converted PNP to *p*-aminophenol, and nitrite was released from PNP in the absence of glucose (Adhya *et al.*, 1981).

*Psuedomonas putida* that could degrade ONP and MNP was isolated from a soil supplemented with 1 mM ONP or MNP as sole source of carbon and nitrogen (Zeyer and Kearney, 1984). Two bacteria, identified as *Arthrobacter auresens* TW17 and *Nocardia* sp. strain TW2, capable of degrading PNP and numerous other phenolic compounds, were isolated from PNP-enriched soil.
(Hanne et al., 1993). The degradation of PNP by A. auresens TW17 was induced by pre-exposure to PNP, 4-nitrocatechol, 3-methyl-4-nitrophenol, or MNP, whereas PNP degradation by Nocardia sp. strain TW2 was induced by PNP, 4-nitrocatechol, phenol, p-cresol, or MNP. In all cases, PNP was degraded to hydroquinone or 4-nitrocatechol, depending on the inducing compound.

Two nitrophenol monoxygenases oxidizing PNP or ONP have been isolated (Zeyer and Kearney 1984, Spain et al., 1979, Zeyer and Kocher, 1988), and the one for ONP oxidation from P. putida B2 has been purified and characterized (Zeyer and Kocher, 1988). A two-component PNP monoxygenase was shown to catalyze the initial two reactions of hydrolysis of PNP to yield 4-nitrocatechol and subsequent removal of the nitro group from 4-nitrocatechol to produce 1,2,4-benzenetriol (Venkateswarlu and Spain, 1997).

2.5. Immobilized technology for biodegradation

The current environmental problems associated with pesticide usage and contamination require serious attention. It is estimated that up to 800,000 individuals each year are affected by pesticide poisoning in U.S. (Feldman, 1983). Of particular interest are the organophosphates since they comprise the major proportions of the agricultural industry. This class of agricultural insecticides dominates as the leading cause of pesticide poisoning cases in the U.S. Thus, the effective minimization and disposal of pesticide wastes are the major practical problems facing now-a-days (Karns et al., 1987). There are several methods of disposal currently available, but most of these have some undesirable limitations (Munnecke et al., 1976). However, biological methods such as the use activated sludge and anaerobic digestion have proved successful. But these methods require the maintenance of a biological system for detoxifying contaminants.
There is a growing interest in the use of immobilized bacteria technology (IBT) for the cost-effective biological treatment of chemical wastes. IBT utilizes highly selected, chemical-degrading bacteria in bioreactors designed to provide optimal conditions for microbial activity (Heitkamp et al., 1990). Algal cells have a natural tendency to adhere, therefore immobilization helps in obtaining viable cells without depending on their natural tendencies to adhere or flocculate.

In general, immobilized algal systems currently offer various advantages over free systems although little is known on the changes in cell function that occur when cells are immobilized. Much work still remains to be done in order to elucidate these effects or provide a sound scientific basis for an exploitation of this technology. Development of bioreactors with good mixing, gas flow and light penetration are also important (D'Souza, 1989). Transformation of organic compounds by immobilized microbial cells has been reviewed by Chibata and Tosa (1977). Parathion-hydrolysing enzyme from mixed bacterial culture, covalently bound to either porous glass or silica beads was examined for its use in detoxification of pesticides in industrial effluents (Munnecke, 1979). A phosphotriesterase purified from *Pseudomonas diminuta* (Dumas et al., 1989) has been successfully immobilized onto trityl agarose in a fixed bed reactor and was shown to hydrolyse a wide range of OP pesticides (Cladwell and Raushel, 1991). The parathion hydrolase from *Streptomyces lividus* (Stiert et al., 1989) had been used to treat a cattle dipping liquid containing the pesticide which is used to kill a disease-causing tick (Smith et al., 1992). Three species of *Pseudomonas* immobilized onto diatomaceous earth bioreactor have been shown to be more efficient in PNP removal from aqueous waste streams (Heitkamp, 1990).
It is imminent that like many other areas of biotechnology, algal cell immobilization has considerable commercial potential in the near future. Although the cell immobilization process has emerged as an important tool for increasing the longevity of photosynthetic cells as biocatalysts, a very limited number of studies have used algal cells, particularly those of unicellular freshwater algae or macroscopic forms (D'Souza, 1989). There have been virtually no studies on immobilization of soil isolates of microalgae and cyanobacteria in PNP degradation.