INTRODUCTION

At the beginning of civilization man was a minor component within the biosphere. His activities and wastes had little effect on the quality of the environment. The trend towards high density community systems then brought about significant waste disposal problems, in the form large quantities of domestic and industrial waste materials in a localized environment. Over the past 20 years, rapid industrialization has led to the production of a vast range of waste materials. The waste of industries involved in the manufacture of these chemicals, contain a large variety of toxic, inhibitory and poorly degradable (biorefractory) compounds.

1.1 The petrochemical Industry and its wastes

One such industry, involved in the manufacture of a large variety of organic compounds, is the petrochemical industry. Petrochemicals, by broad definition, are normally understood to mean chemicals or chemical products such as synthetic fibers, intermediates, synthetic rubber etc. Today these petrochemicals have not only penetrated into all walks of life but have also secured a place at all levels in the social structure. From morning to night, we come across a host of utilities such as tooth brushes, soap cases, plastic utensils, cloth, radio cases etc. which are a few of the numerous petrochemical products manufactured.

The petrochemical industry in India was officially notified as a thrust industry for the first time in 1985. The national effort was to catch up with the world average consumption of 13 kg. per capita, while currently India consumes only 0.65 kg. per capita. This is an indication of the magnitude of the waste disposal problems that would arise from this industry in the next few years.

From the outset, the situation in the petrochemical industry has been paradoxical. On one hand, it is the core industry for the development and manufacture of strategic chemicals used in plastic, dyes, fiber intermediates etc. which increase the quality of life, while conversely, it also poses a significant waste disposal problem with majority of the wastes being xenobiotic in nature.

The term xenobiotic, (stranger to life) is derived from Greek and to the environmental chemist it usually applies to a chemical which is foreign to the biosphere. The other descriptions often used are anthropogenic, manmade and synthetic. Thus, all possible chemical compounds found in the environment can be classified as those which would be, without the presence of man (Natural compounds), and those added by man (Xenobiotic compounds).

A natural compound may be the product of the living organism or formed by other natural processes. The important fact is that they have been components of the biosphere. Therefore the organisms have adapted themselves to their presence. Xenobiotic compounds are substances which are normally absent in the ecosphere and have been introduced by man, often by industrial processes, involving synthetic chemicals. Also xenobiotic compounds are those which have unnatural structural features. Thus, it amounts to the fact that the organisms in their evolutionary history have never been exposed to these compounds. Consequently, these chemicals can be harmful to the biological organism.

A second modern waste disposal problem is concerned with the wide distribution of chemical materials which ultimately have to be considered as waste in the environment. Traditionally, waste treatment has been concerned with collected bulk quantities of materials which are treated *en masse*. However, as a result of present day activities, many compounds which ultimately become wastes in the environment, are widely dispersed into the biosphere, either deliberately, e.g. agrochemicals, or accidentally e.g. oil spillages. Clearly, waste of this nature cannot be collected and treated together.

The Indian Petrochemicals Corporation Ltd. (IPCL) situated near Baroda, is a large scale, integrated petrochemical project

involved in the manufacture of a large variety of synthetic organic chemicals used in dyes, drugs, fibers and intermediates. It feeds almost every major industry either directly or indirectly. The variety of petrochemical compounds manufactured by IPCL is listed in Table 1.

Considering the growth of this industry in the next few years, the magnitude of the waste disposal problems this industry will face is likely to be phenomenal. It is evident that the pollutants generated form such a petrochemical industry are likely to be as diverse as the products it manufactures. The proliferation of these petrochemical industries necessitates the perfection of pollution control technologies in order to minimize the hazards caused by their toxic wastes.

The aqueous wastes from the petrochemical industry, depending on the type of products manufactured may contain a range of organic chemicals (Table 2) viz: organic acids, ketones, aldehydes, cyanides, heavy metals etc. (Chakrabarty *et al.*, 1982). The toxicity of these chemicals to man and other forms of life is well established. Looking at the toxic effects these compounds have on the ecosystem, it has now become imperative to treat these wastes before they are discharged into the environment.

Table 1 : Major products of Indian petrochemicals corporation Ltd. (IPCL) Baroda.*

Products	Production rate (metric tonnes/ annum)
1) Ortho and mixed xylenes	23,500
2) p-Xylene	17,000
3) Dimethyl Terephthalate	40,000
4) Solvent CiX	10,000
5) Poly vinyl chloride	55,000
6) Low density poly ethylene	80,000
7) Ethylene oxide	5,000
8) Ethylene glycol	20,000
9) Di ethylene glycol	2,200
10)Tri ethylene glycol	200
11)Poly propylene copolymer	25,000
12)poly propylene bromopolymer	30,000
13)Atactic polypropylenes	3,000
14)Hydrocyanic acid	3,600
15)Acrylic fiber - Bicomponent	12,000

(Contd.)

16)Acrylic fiber - Monocomponent	12,000
17)Acrylonitrile	24,000
18)Acrylates	10,000
19)Acetonitrile	900
20)Polybutadiene rubber	20,000
21)Poly alkyl benzene	2,500
22)Linear alkyl benzene	30,000
23)Heavy n-paraffins	25,000
24)Benzene	10,000
25)Petroleum Resins	5,000
26)Toluene	15,800
27)Carbonblack feedstock	17,900

---

* IPCL products bulletin

Table 2. Chemicals present in petrochemical wastewaters.

Compounds	Quantity (Kg/ day)	Concentration (in ppm)
1) Acetic acid	1872	55.000
2) Formic acid	1008	30.000
3) Acrylonitrile	95	2.800
4) Acetonitrile	95	2.800
5) Acetamide	22	0.650
6) Ethyl methyl benzoate	125	3.700
7) Ethylene glycol	3402	100.000
8) Acetaldehyde	127	4.400
9) Formaldehyde	192	5.650
10)P.T Ester	125	3.700
11)Heptane	95	2.800
12)Butanol	600	17.500
13)Fatty acid	528	15.000
14)Misc.Org.Substances (Insoluble)	48	1.410
15)Misc.Org.Substances (soluble)	528	15.000

16)Propylene dichloride	392	11.500
17)Naphtha	1400	41.200
18)Phenol	7.25	0.175
19)Benzene	667	20.000
20)Other Hydrocarbons	90	2.650
21)Methanol	150	4.400
22)Deenex	65	2.000
23)T.B.C	132	4.0

INORGANIC CONSTITUENTS

1) $Na NO_2$	11	0.322
2) $NaHSO_3$	1350	40.000
3) $Na_2 S$	250	40.000
4) NaCN	1935	40.000
5) $NaNO_3$	6860	200.000
6) $K_2 SO_4$	12000	350.000
7) HCl	768	25.000
8) $H_2 SO_4 / Na_2 SO_4$	1300	38.000
9) $NH_4 OH$	1687	49.500
10)NaF	0.3000	0.090
11)$H_3 PO_4$	95.0000	2.800

---

* Baroda Effluent Channel Report (1975)

1.2 Methods of Treating industrial waste waters

There are several methods available for the treatment of industrial wastes. They can be broadly classified as :
a) Physical b) Chemical and c) Biological treatment procedures. The physical and chemical methods consist of the following units:

1) Neutralization
2) Oxidation
3) Reduction
4) Reverse osmosis
5) Ion exchange
6) Carbon adsorption
7) Recovery of acids from pickling liquor
8) Phenol destruction
9) Evaporative stripper

These techniques are mainly used for pollutants which contain dissolved solids and liquids. The removal of these compounds are accomplished by one or a combination of the afore mentioned procedures. The second major category of pollutants may be classified as suspended solids or liquids. Such pollutants occur as particles larger than a single molecule. Suspended solids and liquids also contain settleable solids. The control of these include the following treatment procedures:

1) Filtration using
   a) Activated carbon filters
   b) Activated carbon plus polymeric chemical filters
   c) Moving bed sand filters
   d) Diatomaceous earth filters
   e) Fiber glass screen
   f) Micro straining
   g) Reverse flow
   h) In-depth filters

2) Electrodialysis or dialysis
3) Microfloc
4) Centrifugation
5) Concentration
6) Vibrating screens
7) Incineration
8) Atomized suspension technique
9) Oil water separator

Although physico-chemical processes are able to upgrade the effluent quality to some extent, these processes are ineffective in the removal of highly toxic organics which are present only in very small amounts. Also the maintenance, energy requirements and operative costs of these processes are extremely high.

1.3 Significance of the biological treatment procedure

Of late, biological treatment processes are gaining popularity due to the ease of operation, low cost, and their low energy consumption. Moreover, the efficiency of the biological treatment in the removal of organics and certain recalcitrant organic and inorganic compounds has been demonstrated.

Although the technology for biological waste disposal has been available for some time now, pollution control specialists have remained somewhat hesitant regarding the application of these systems for hazardous and toxic wastes. It should be stressed that one advantage of biological treatment systems is that they tend to be the most cost effective as compared to any physical or chemical waste treatment technology (Rozich and Gaudy, 1987). Additionally the operation of the biological system can also be modified in a manner that realizes no net production of organic sludges resulting in the total oxidation of all toxic organic material in the effluent. Thus the physical or chemical operations are eliminated or in any event drastically reduced.

The parameter by which the effluent quality and the operational capabilities of the treatment systems are judged are the Biological oxygen demand (BOD) and chemical oxygen demand (COD). In view of the complexity of the petrochemical waste water systems it has been observed that these parameters are

not a realistic indicator of the quality of the wastes. Many of the organics chemicals present in the waste waters of the petrochemical industry, may not exert BOD or else do so at a very slow rate. Short chain alkanes and/or other volatile organics have also been shown to produce inaccurate COD values. Furthermore, these organics though present in small amounts, may be detrimental to the ecosphere. It is therefore unfortunate that the quality of the waste waters are not judged by the amount of individual organic components present in them (Chakrabarty *et al.*, 1982).

Biological treatment plants are in operation in many industries all over the world. However, till today, a perfect biological waste treatment technology is yet to be developed. At first, bioreactors were designed to deal almost exclusively with domestic wastes. This is basically a simple operation since such wastes have a relatively simpler composition and contain readily degradable material. However, since the 19th century waste treatment has increased in complexity with a number of novel problems as well as logistic difficulties associated with large increases in waste production (Slater and Sommerville, 1979). Considering the fact that industrial waste waters are complex, a systematic and scientific approach is needed in order to effectively evolve an appropriate biological waste treatment procedure. Thus the waste treatment technology has to

be designed in such a way that it ensures the total removal of toxic organic compounds prior to their discharge into the environment.

1.4 Microbial degradation of Xenobiotics

The problem of biological treatment of petrochemical waste is indeed that of microbial biodegradation of the aforesaid organic compounds. Biodegradation is a general term applied to partial or complete breakdown of organic compounds by microorganisms. The term implies cleavage of covalent bonds with resulting transformation to one or more products. This may happen with or without a concomitant exploitation of the compound as a carbon or energy source for the growth of the transforming population. It can also describe complete mineralization where all of the original compound is converted to basic products such as carbon dioxide, water and ammonia. Typically, during aerobic growth 70% of the substrate is mineralized whereas 30% is utilized for production of new biomass. However, there may be degradation products which are not further utilized by the growing population.

It is now beyond doubt that microorganisms play an important role in the disappearance of many xenobiotics from natural environments. The significance of these microbes has been assessed in different ways (Alexander, 1977). These studies

have indicated that some compounds disappear rather rapidly while other chemicals persist for considerably longer periods (Slater and Somerville, 1979).

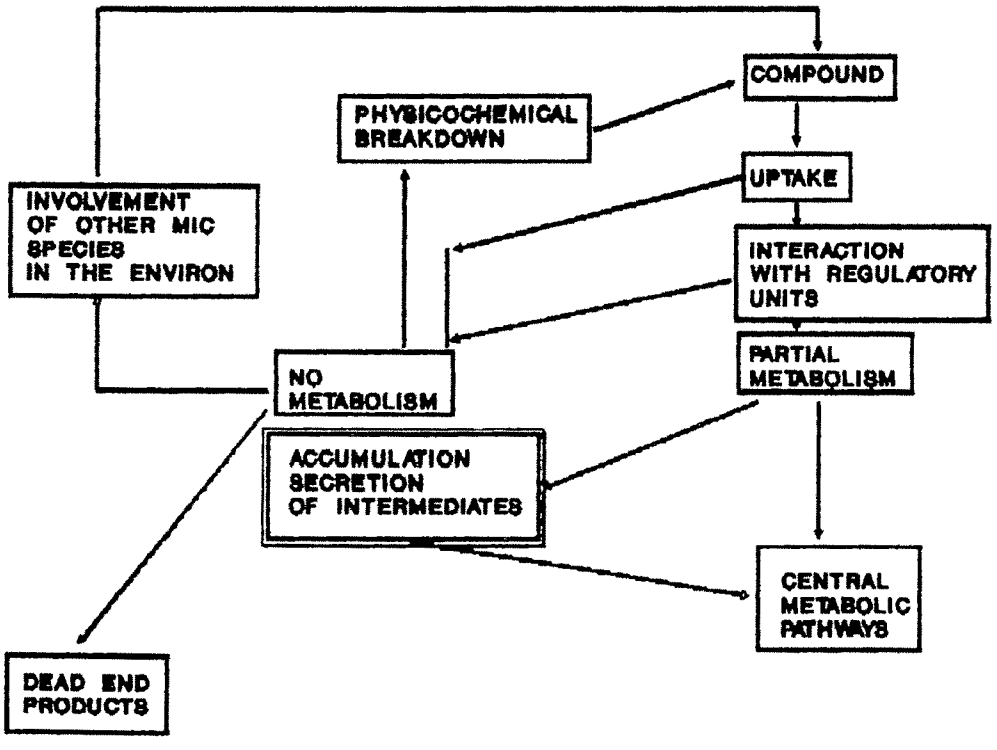
Thus, for biodegradation to occur at all the environment must contain at least one population with the appropriate catabolic mechanism. The rate of such biodegradation depends on the initial interaction between the compound and the organism, the kinetic properties of the metabolic processes and the physicochemical conditions. Every organism does not contain all the necessary mechanisms for the degradation of all the compounds. Therefore,the successful treatment of multicomponent waste depends on the combined activities of a wide range of microorganisms with diverse metabolic capabilities.

A significant portion of the total biodegradative activities towards xenobiotic compounds may involve cometabolism (Horvath, 1972). This is a process in which the microorganisms growing at the expense of one substrate, also have the capacity to transform another compound without deriving any direct benefit from its metabolism.

Fig. 1 shows the series of steps involved when the microorganisms or the microbial community interact with a xenobiotic compound. Despite the diversity of biodegradative processes, it is a common observation that many compounds,

Fig 1

Interaction of Xenobiotics with microbial communities



especially environmentally foreign compounds, apparently are not degraded in a given habitat, despite adequate conditions. Thus not all compounds known today are readily biodegradable and these are classified as recalcitrant. The term recalcitrant is used with reservation as a particular habitat may lack the population with the appropriate metabolic capacities.

1.5 Detoxification of Organic nitriles (cyanides)

One such waste classified as toxic, non-biodegradable, or recalcitrant are the wastes containing organic and inorganic cyanide compounds. The toxic and inhibitory effects of inorganic cyanide is well known. The organic cyanides, otherwise known as organo-nitriles, are the major wastes produced by plants involved in the manufacture of acrylonitrile and acrylates. The toxicity of acrylonitrile and other nitriles have been well documented (Milvy and Wolff, 1977; Vennit *et al.*, 1977). One such plant exists in IPCL.

The characteristics of acrylonitrile/acrylate plant waste water is shown in Table 3. As of date, there is no efficient biological method of treatment for these wastes. These wastes are currently being treated by incineration (Dave *et al.*, 1987). As mentioned earlier, this method is expensive and can result in the leakage of these compounds into the atmosphere, if the incineration is not complete.

Table 3 Characteristics of Acrylonitrile Acrylate plant (ACN/ACR) waste waters

PARAMETER	VALUE
chemical oxygen demand (COD)	25,000 to 110,000 mg/L
Biological oxygen demand (BOD)	10,000 to 20,000 mg/L
Total solids	800 to 1850 mg/L
Suspended solids	80 to 250 mg/L
pH	7.5 to 9.5
color	brown
Chemicals normally found	Acrylonitrile acetonitrile acrylamide acetamide acetic acid propionitrile acrylic acid

However if a biological treatment of these waste waters has to be worked out, one should remember that it is necessary to pre-treat these wastes before they are mixed with the general wastes, as the organonitriles are toxic even to the microorganisms involved in the biodegradation of other organic compounds present in these waste waters. In this context, studies sponsored by the Department of Environment (DOE) Government of India, were initiated in the Department of Biochemistry., M. S. University of Baroda. in collaboration with Indian Petrochemical Corporation Ltd. (IPCL). This was done in order to evaluate the possibility of a viable biological treatment procedure for these wastes.

A flow diagram of the biological treatment facility of IPCL is given in Fig. 2. Microorganisms were isolated form different points at this waste treatment facility. Out of more than a 100 isolates, of bacteria, fungi and algae, 15 of the organisms were found to be dominant (Goud *et al.*, 1985). These organisms when tested showed that they could also tolerate a wide variety of organic chemicals. A mixture of 4 organisms, namely, *Pseudomonas, Aeromonas, Arthrobacter, and Corynebacterium* were used in a mixed culture to treat the waste waters of acrylonitrile and acrylate (ACN/ACR) plant under laboratory conditions. Fig. 3 shows that the BOD and COD of these waste waters were reduced by 85 and 95% respectively in 48 hr. This

Fig. 2 Flow diagram of the Biological treatment facility at IPCL

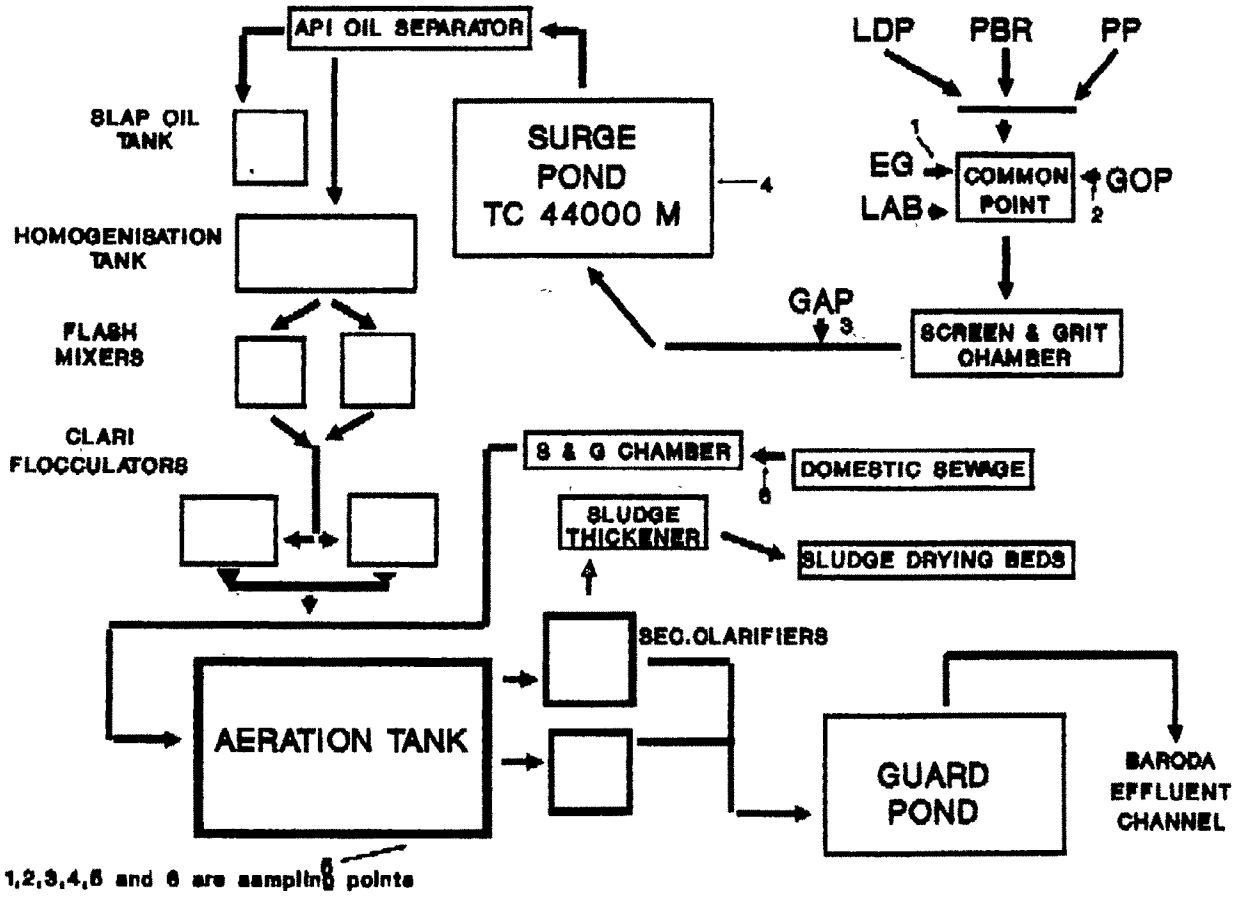
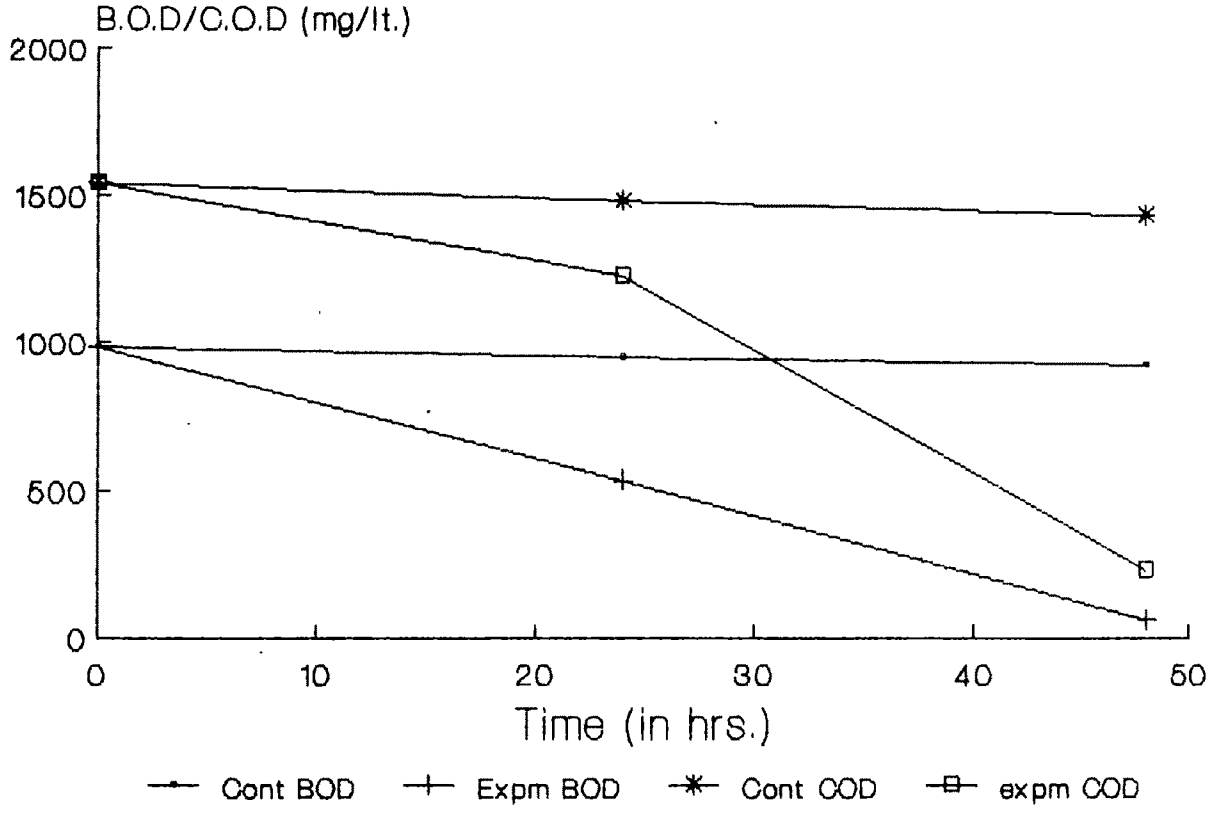


Fig. 3 **Treatment of ACN/ACR Plant wastes By mixed culture**



Values are Mean of Three Observations

study indicated that a viable biological treatment procedure could be worked out.

Organonitriles though found in small amounts, in these waste waters, are still toxic enough to cause severe environmental concern. A mere reduction in the BOD an COD does not necessarily indicate the removal of these organonitriles. Therefore, studies should also be conducted to ensure that the microorganisms present in these waste waters are capable of degrading the organonitriles present. This will enable us to ensure that these compounds are totally removed at the end of the treatment procedure.

1.6 Microbial metabolism of organo nitriles

Nitriles are cyanide containing organic molecules of the formula R-CN. They are found to occur naturally in a wide variety of plants, fungi, etc. The examples of naturally occurring nitriles are summarized in Table 4. Various reasons have been put forth to explain the existence of cyanide substituted compounds in certain plants and microorganisms. They are :

1. The organic cyanides may well be produced as a matter of fixing free cyanide from the environment. The plants or microorganisms could use cyanides to inhibit the growth of other microorganisms nearby,thus increasing the competitive

Table 4. Naturally occuring nitriles

Name	Source
1) Cyanoglucosides	*Basidiomycetes*
2) Cyanohydrins	*Basidiomycetes*
3) ∝-Amino nitriles	*Basidomycetes* *Refectonia solani*
4) Cyanoglucosides	Cassava 800-1000 species representing 70-80 Families
5) Isobutronitrile	*Thiobroma cacao*
6) Indole acetonitrile	*Cruciferae* *Lycopersicum esculentum*
7) Ricinine	*Ricinus communis*
8) B-Cyanoalanine	*Leguminosae*
9) Cyanolipids	*Sapindaceae*
10) Cyanohydrins	*Diplopoda* *Alpheloria corrugate* *Harpaphe haydeniana*

* Jallageas *et al.* (1978)

edge of the cyanide excreting microorganism.

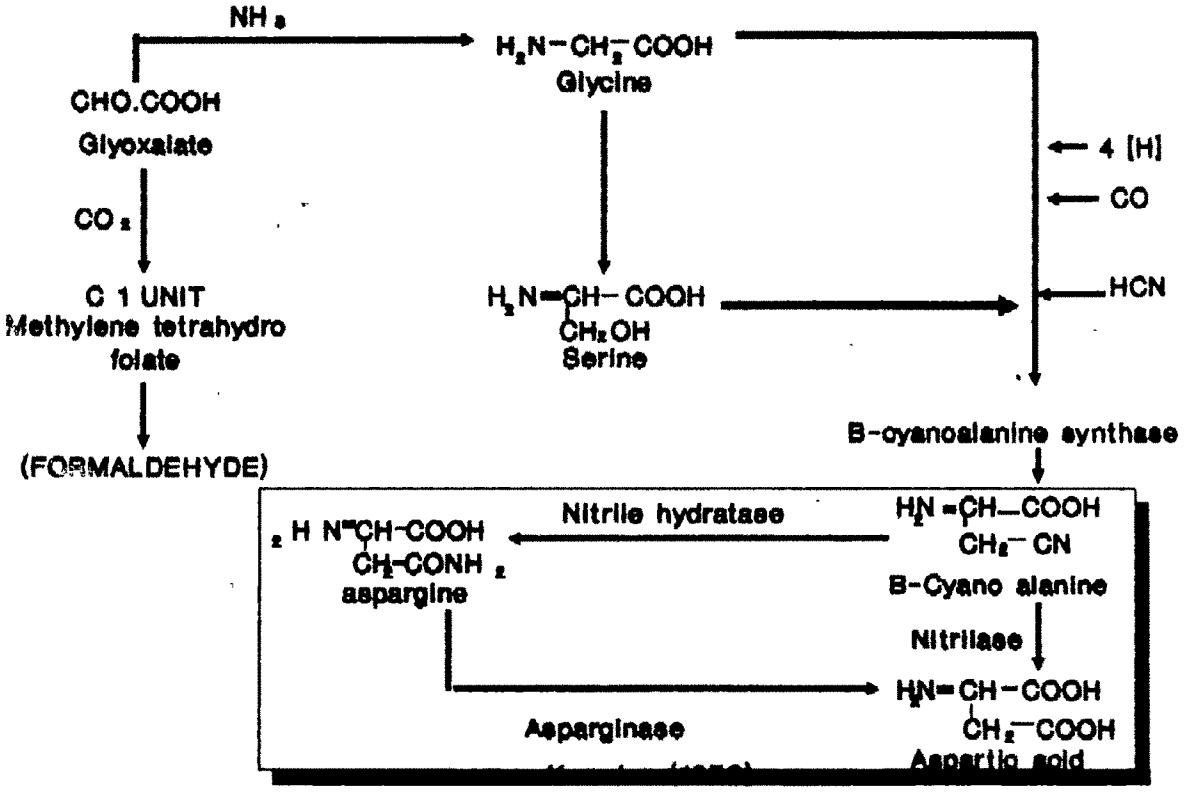
2. Preventing the predatory activity on the affected organism or enabling parasitism in other plants (Knowles, 1976).

Many plants are able to assimilate cyanides into asparagine (Bluementhal-Goldschidmt *et al.*, 1963), or the dipeptide -glutamyl ß-cyanoalanine (Fowden and Bell, 1965). In plants fed with ^{14}CN the label enters the amide of asparagine (Bluementhal-Goldschmidt *et al.*, 1963). The formation of both dipeptide and asparagine occurs via ß-cyanoalanine. The pathway of cyanide detoxification/ assimilation is shown in Fig. 4. It is quite clear that the inorganic cyanide is fixed to a relatively less toxic organic cyanide and then degraded to more useful components of the cell when required.

This existence of nitrile detoxification or degradation in plants has been known for quite sometime. Thiman and Mahadevan (1964) purified and characterized an enzyme, nitrilase, from barley leaves and showed its catalytic action on indole acetonitrile converting it to indole acetic acid and ammonia. It was the first enzyme involved in nitrile metabolism to be studied. Since then considerable attention was focused on the enzymes involved in the breakdown of naturally occurring and synthetic nitriles.

Fig. 4

Cyanide detoxification In plants and microorganisms



Robinson and Hook (1964) were able to show that a *Pseudomonas sp.* was capable of growing on ricine as a nitrogen source. They characterized and enzyme, ricine nitrilase which broke down ricine to the corresponding acid. In the case of synthetic nitriles, Mimura *et al.*, (1969) showed that a soil microorganism *Corynebacterium nitrophiles C-42* could breakdown acetonitrile to acetamide and then to acetic acid and ammonia, indicating the involvement of two independent enzyme systems viz of nitrile hydration and subsequently of hydrolysis of the amide thus formed. It was earlier shown that *Pseudomonas aeruginosa* produced an inducible enzyme amidase which could act on acetamide and convert it into acetic acid and ammonia (Kelly and Clarke, 1962). Continuing the work on amidases, Betz and Clarke (1972) showed that *Pseudomonas aeruginosa* was capable of hydrolyzing a wide range of amides. Firmin and Gray (1976) showed that a *Pseudomonas sp.* which could convert acetonitrile to acetamide, apparently could also use acetonitrile as the sole source of nitrogen.

DiGeronimo and Antonie (1976) showed that *Nocardia Rhodochrous LL-100-21* was capable of utilizing acetonitrile as the sole source of carbon and nitrogen. This was probably the first organism which was shown to utilize acetonitrile as a sole source of carbon/energy and nitrogen. It was also clearly established that the organism converted acetonitrile to acetic

acid and ammonia sequentially, through the formation of an intermediate, acetamide. Formation of amide, in small amounts was also detected in the partially purified enzyme ricine nitrilase (E.C. 3.5.5.2) by Hook and Robinson (1964), inspite of the fact that the enzyme nitrilase converted ricinine directly to the corresponding acid and ammonia.

On the other hand. the formation of the amides during the growth of microorganisms on aliphatic nitriles have been reported by many workers (Mimura *et al.*, 1969; Firmin and Gray, 1976; Digeronimo and Antonie, 1976; Kuwahara *et al.*, 1980; Asano *et al.*, 1980). The question which thus remained unsolved was whether the enzymatic breakdown of nitriles was catalyzed by one or more than one enzyme.

Brevibacterium sp. R312 was shown to possess two enzymes involved in the metabolism of nitriles (Arnaud *et al.*, 1977). These enzymes had a wide substrate spectrum. The enzymes were a nitrilase, named acetonitrilase (Arnaud *et al.*, 1977), which hydrates the water soluble nitriles into the corresponding amides, and an amidase named acetamidase (Arnaud *et al.*, 1976; Jallageas *et al.*, 1978), which hydrolyses the amides into the corresponding acids and ammonia. This pathway was further confirmed by isolating a mutant strain of *Brevibacterium A4* which had lost its amidase activity but retained the nitrile hydrating activity (Bui *et al.*, 1984b). Asano *et al.* (1982a)

purified the enzyme which catalyzed the hydration of aliphatic nitriles to the corresponding amides, from *Arthrobacter J1* cells grown on acetonitrile as the sole source of carbon and nitrogen. The enzyme till then called nitrilase by other workers, was renamed as aliphatic nitrile hydratase. By now it was clear that the aliphatic, water soluble, nitriles were broken down by microorganisms which involved two independent enzymes, nitrile hydratase and amidase.

Harper (1974) showed that a number of microorganisms isolated from the soil were capable of utilizing benzonitrile or 4-hydroxy benzonitrile as a sole source of carbon and nitrogen. Harper (1977a) showed that the breakdown of benzonitrile proceeded by the direct conversion of benzonitrile to benzoic acid and ammonia in *N. rhodochrous NCIB 11216* group and in *Fusarium solani* Yamada *et al.*, (1979) isolated an *Arthrobacter sp. I9,* from acclimatized activated sludge which could grow on acrylonitrile (vinylcyanide) as a sole source of carbon and nitrogen. It was found that *Arthrobacter sp. 19* converted acrylonitrile to acrylic acid and ammonia without the formation of amide as an intermediate. This enzyme nitrilase was then purified and characterized (Bhandyopadyay *et al.*, 1986) and was found to act only on aliphatic, unsaturated nitriles like acrylonitrile, and had no activity on saturated aliphatic nitriles such as acetonitrile. The enzyme performing these

direct conversions were named as nitrilases.

McBride *et al.,* (1986) isolated an organism *Klebsiella ozanenae* capable of metabolizing bromoxynil (3,5-dibromo-4-hydroxy benzonitrile) to the corresponding acid and ammonia without the formation of intermediary amides. The organism also expressed nitrilase activity which was specific to bromoxynil.

Microbial metabolism of organonitriles is now hypothesized to be brought about by two independent enzyme systems. Benzonitrile and related aromatic nitriles, heterocyclic and certain aliphatic unsaturated nitriles like acrylonitrile are catabolized directly to the corresponding acid and ammonia via a nitrilase system (E.C.3.5.5.2. nitrile amino hydrolase). On the other hand saturated aliphatic nitriles are catabolized in two stages by conversion first to the corresponding amide and then to the corresponding acid and ammonia. The enzyme that catalyses the hydration of nitrile to amide, a nitrile hydratase is quite distinct biochemically from the nitrilase previously described. The second step is catalyzed by `amidase' (Nagasawa and Yamada, 1989).

1.7 Enzymes involved in the metabolism of Nitriles :-

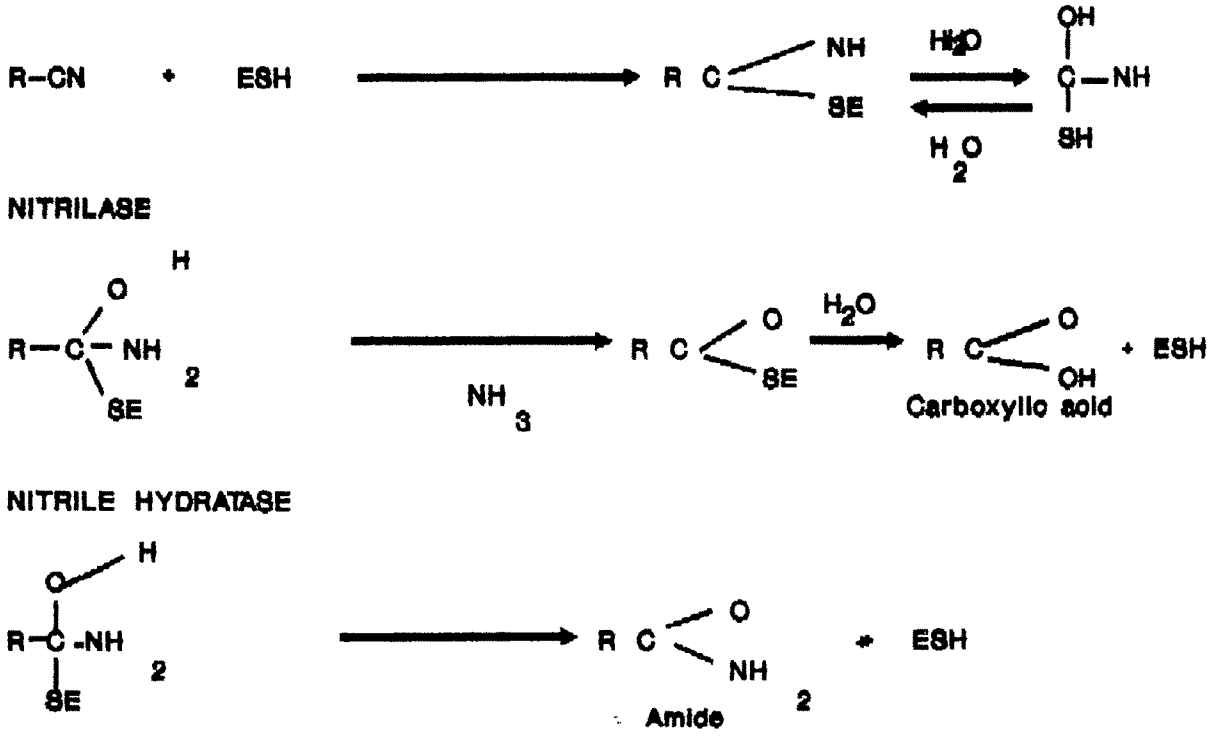
The enzymes nitrile hydratase, amidase and nitrilases have been studied in many systems and nitrile hydratase has been purified from *Arthrobacter J1* (Asano *et al.,* 1982a), *Pseudomonas*

chloraphis B-23 (Nagasawa *et al.*, 1987) and *Brevibacterium R312* (Nagasawa *et al.*, 1986). These enzymes are active towards various aliphatic nitriles. Nitriles with 3 to 6 carbon atoms serve as the most suitable substrates. The enzymes are strongly susceptible to inhibition by sulfhydryl reagents like PCMB, Hg etc., indicating the presence of a sulfhydryl group at the active center, responsible for the catalytic activity of the enzyme. The mechanism of enzymatic nitrile hydrolysis, based on the hydrolysis of nitriles by acid or base (Killipatric, 1947), is shown in Fig. 5.

An important factor in nitrile assimilation is the ability to utilize nitrile as a carbon and/or nitrogen source. Saturated aliphatic nitriles like acetonitrile break down to form acetic acid and ammonia both of which can be easily assimilated. On the other hand, benzonitrile is broken down to benzoic acid and the ability to utilize benzonitrile as both carbon and nitrogen source would depend on the ability of the microorganism to further utilize the benzoic acid. Similarly, the utilization of acrylonitrile as the sole source of carbon and nitrogen would depend on the ability of the microorganisms to further metabolize acrylic acid which is itself known to be toxic to the cell. Hence many acetonitrile utilizing organisms can break down acrylonitrile to acrylic acid and ammonia as the enzyme nitrile hydratase and amidase are quite active towards

Fig. 5

Reaction mechanism of Nitrile hydratase and nitrilase



acrylonitrile and acrylamide respectively. Acrylonitrile in all these cases however cannot support growth as a carbon or nitrogen source, the reason being the inability of these microorganisms to further utilize acrylic acid as a carbon source. Table 5 summarizes the kinetic parameters of these enzymes from various sources.

1.8 **Regulation of the enzymes involved in nitrile metabolism** :-

The regulation of the enzymes involved in the metabolism of nitriles is not fully established, as there are more than one unsolved questions. *Brevibacterium R312* and several other bacteria have been shown to form nitrilases and amidases of very broad substrate specificity (Bui *et al.*, 1984a; Arnaud *et al.*, 1977; Jallageas *et al.*, 1978). However *Pseudomonas* and several other microorganisms form amidases that utilize only a small range of amides (Clarke, 1980). The amidases, where studied have been shown to be inducible. Nitrilases have also been shown to be inducible and specific for a small range of aromatic and (Harper, 1977 a;b) heterocyclic (Robinson and Hook, 1964) and aliphatic (Fakuda *et al.*, 1971, 1973; DiGeronimo and Antonie, 1976; Yamada *et al.*, 1980; Kuwahara *et al.*, 1980). Arnaud *et al.*, (1977) claimed that *Brevibacterium R 312* possessed a constitutive nitrile hydratase whereas as the amidase was inducible. It was shown that in *Nocardia rhodochrous* LL 100-21 both the nitrile hydratase and amidase

Table. 5 Kinetic properties of a few nitrile hydratase, amidase, nitrilase isolated from various sources

Enzyme	Source	Km	Specificity	Mol. Wt (KD)	Subunits/MolWt (KD)	Ref
nitrile hydratase	*Arthrobacter*	5.78 mM	Aetonitrile;	420	2, 24 27	Asano *et al.* (1980)
amidase	"					
nitrile hydratase	*Bravibacterium* R - 312	25 mM	Acetonitrile; Acrylonitrile (Broad)	85	2 26 27.5	Nagasawa *et al.* (1986)
amidase						
nitrile hydratase	*Bravibacterium* A4		Broad	Not Charecterised		Arnaud *et al.* (1976)
nitrile hydratase	*N.rhodochorus*		Actonitrile	Not Charecterised		Linton & Knowles (1986)
amidase	LL 100-21		Acrylonitrile Propionitrile			
nitrile hydratase	*Pseudomonas chloraphis* B 23		Actonitrile Acrylonitrile	10	4 25	Nagasawa *et al.* (1987)
nitrile hydratase	*Rodococcus rhodochrous*		Actonitrile Acrylonitrile	50.5	2 29 26	Nagasawa *et al.* (1987)
nitrile hydratase	*Corynebacterium nitrophilus*	9.1mM	Acetonitrile Acrylonitrile			Amarant *et al.* (1989)
nitrilase	*Klebsiella ozaenae*	.031mM	3,5-dibromo-4-hydroxy benzo nitrile(Bromoxynil)	72	2 37	Stalker *et al.* (1988)
nitrilase	*Arthrobacter* I-9	1.43 mM	Acrylonitrile	40	not available	Bandhopadhyay *et al.* (198
nitrilase	*Nocardia rhodochrous NCIB 11216*	4.0 mM	Benzonitrile	560	12 45	Harper (1977a)
nitrilase	*Fusarium solani*	.039 mM	Benzonitrile	620	8 76	Harper (1977b)

were inducible. These enzymes were induced both by nitriles and amides. This results indicated the possibility of the enzymes nitrile hydratase and amidase being a part of a single operon and if so amide being the most likely inducer (Linton and Knowles, 1986).

In the case of *Rhodococcus sp.* it was observed that nitrile hydratase and amidase were induced by acetonitrile, acetamide and a few other alkyl saturated nitriles and amides. In the presence of succinate or ammonium sulfate acetamide or acetonitrile were not able to induce these enzymes to that extent. When acetamide was omitted from the medium the suppression was complete. The cellfree extracts of *Rhodococcus sp.* could convert acrylonitrile and acrylamide to the corresponding acid and ammonia,but neither acrylonitrile or acrylamide supported growth nor induced these enzymes (Miller and Gray, 1982). Similarly in the case of *N. rhodochrous LL 100-21* although acrylonitrile was not an inducer it served as a good enzyme substrate (Linton and Knowles, 1986). However, in this case it was observed that at extremely low concentration acrylonitrile could support growth as a nitrogen source only. As mentioned earlier in *Arthrobacter I9*, the only organism known to utilize acrylonitrile as a sole source of carbon and nitrogen, the nitrilase was inducible by acrylonitrile. In this case interestingly an amidase was also coinduced. The enzyme

acrylonitrilase converts acrylonitrile directly to acrylic acid and ammonia, and amidase has no role to play in the metabolism of acrylonitrile. The reason for this coinduction still remains unclear.

1.9 Characteristics of Nitrile hydratase, Nitrilase and amidase

Nitrile hydratase has been purified from *Arthrobacter J1* (Asano *et al.*, 1982a), *Brevibacterium R 312* (Nagasawa *et al.*, 1986) and *Pseudomonas chlororaphis B23* (Nagasawa *et al.*, 1987). The *Pseudomonas* enzyme has a molecular mass of about 100 KD and consists of 4 subunits of identical mass. The *Brevibacterium* enzyme has a molecular mass of about 85 KD and is composed of 2 subunits of 26 KD and 27.5 KD. The *Arthrobacter* J1 nitrile hydratase has a molecular mass of about 420 KD and is composed of 2 subunits of 27 and 24 KD.

Concentrated enzyme solutions of both *Pseudomonas* and *Brevibacterium* exibited a characteristic green color and produced a broad absorption peak at 720 nm. These enzymes are immunologically different. Nitrile hydratases were the first non heme iron enzymes found to contain typical low spin ferric active sites. The electron spin resonance (ESR) features are charecrteristic of rhombic low spin ferric type (g max.= 2.284 min.= 1.971) (Sugiura *et al.*, 1988). Ferric ions are tightly coordinated possibly by four nitrogen atoms of an imidazole

ring system. It was also shown that nitrile binds to the iron sites in the enzyme. However it is also seen that not all nitrile hydratases contain iron. The nitrile hydratases of *Rhodococcus rhodochrous J1* contains cobalt (Nagasawa *et al.*, 1986). The enzyme has a molecular mass of about 505 KD and is composed of two subunits of 29 and 26 KD. It is to be noted that without any exception nitrile hydratases are multimeric in nature forming either homomeric or heteromeric multimers.

Apart from metal cofactor nitrile hydratases also contain an active carbonyl cofactor probably pyrrole quinoline quinone (PQQ) (Nagasawa and Yamada, 1987). It is believed that PQQ participates in the hydration of the nitrile group. This form of the enzyme and the presence of PQQ raises the possibility of a biological oxidation reduction reaction being involved.

On the other hand nitrilase from *N. rhodochrous* NCIB 11216 showed that the molecular mass of the active enzyme was about 560 KD, and was composed of 12 identical subunits of 45 KD. The most interesting feature of this enzyme was the time dependent, substrate (Benzonitrile) induced aggregation of the 12 subunits to form the multimeric active enzyme. It has been postulated that the phenomenon of aggregation will be helpful in the regulation of the products, of the enzyme reaction, which may cause an imbalance in the cellular environment due to their accumulation. Similar observation was made in the case of

nitrilase obtained from *F. solani* where the multimeric enzyme of 620 KD was seen to be formed by benzonitrile induced aggregation of 8 subunits of 76 KD (Harper, 1977 a;b). Although, slow aggregation disaggregation of subunits to form an active multimeric enzymes have been observed earlier (Frieden, 1968), this was the fist time that such a behavior was observed in nitrile metabolizing enzymes. The benzonitrilase reported here was succeptible to thiol group reagents indicating a thiol group in the active site, but did not express any metal ion requirement.

In the case of nitrilase isolated from *Arthrobacter I9* the molecular weight of the active enzyme could not be calculated because the enzyme protein was reported to form various aggregates of subunits, each of which had a individual molecular mass of about 45 KD. The interesting feature about this enzyme was was the temperature optima at 50 ^{o}C, something which has not been the characteristic feature of nitrile metabolizing enzymes. However, the activity was lost rapidly when the enzyme was kept at 50 ^{o}C for a long period of time (Bandyopadyay *et al.*, 1986). Another interesting feature of the *Arthrobacter I9* was that, an amidase, which had no role in the metabolism was coinduced. As mentioned earlier nitrilases convert nitriles directly to the corresponding acid and

ammonia. Thus the role of the coinduced amidase has been cryptic.

The nitrilase, obtained from *K. ozanae*, which converted bromoxynil to the corresponding acid and ammonia, was seen to be composed of 2 subunits of 37 KD and the active enzyme had a molecular weight of about 72 KD (Stalker *et al.*, 1988). This enzyme was observed to be closer to *Arthrobacter* acrylonitrilase than to the *N.rhodochrous* benzonitrilase. Recently a novel nitrilase has been isolated *Rhodococcus rhodochrous*. This enzyme preferentially catalyses the conversion of aliphatic nitriles to the corresponding acid and ammonia. The substrates for this enzyme include aliphatic olefinic nitriles such as acrylonitrile, crotononitrile and saturated aliphatic nitriles such as valeronitrile, 4-chlorobutronitrile, and glutaronitrile. The finding of this enzyme contradicts the hypothesis that aliphatic saturated nitriles and unsaturated nitriles have different metabolic pathways. This enzyme had a molecular mass of about 604 KD consisting of 14 to 15 identical subunits of about 41 KD each (Kobayashi *et al.*, 1990).

On the other hand comparatively little is known about the amidases that take part in nitrile degradation (Nagasawa and Yamada, 1989). Aliphatic amidases have been shown to be present in different genera of bacteria. The best documented enzyme are

those of *Pseudomonas aeruginosa* (Clark, 1970, 1984; Ambler *et al.*, 1987; Bremmer *et al.*, 1987; Clark and Drew, 1988) and *Brevibacterium R 312* (Masteracci *et al.*, 1984; 1986; Thiery *et al.*, (1986). The amidase from *Arthrobacter sp.* grown on acetonitrile as a sole carbon and nitrogen source was purified and characterized. The enzyme was found to be active towards acetamide acrylamide and propinamide (Asano *et al.*, 1982a). The *Brevibacterium R 312* produces an amidase that has a wide substrate spectrum (Thiery *et al.*, 1986a). These amidases are inhibited by thiol specific reagents and also catalyze acyl transfer reactions (Thiery *et al.*, 1986b). These properties are apparently similar to aliphatic acrylamide amido hydrolase (E.C. 3.5.1.4) (Clark, 1970). The Active amidases of *Methlophilus* has 4 identical subunits of 38 KD each (Silman *et al.*, 1989). Other than this the subunit structures of other amidases have not been well documented.

In recent years, the major focus has been the possible application of biological systems to chemical reactions. This is because of the fact that the reactions catalyzed by cells or enzymes are far more superior to the conventional chemical reactions. In all the cases it is imperative to fully understand these reaction mechanisms and their regulation. The enzymes involved in nitrile metabolism, other than its application as a potent system for the removal of toxic

nitriles from the environment, have important applications in the field of nitrile bioconversions which will be discussed later.

Fairly limited work is reported in the area of nitrile metabolism, especially that of acrylonitrile. Further studies are required in this area considering the environmental and industrial importance of this system. This is one of the objectives of this present study.

1.10 Genetics of Microorganisms involved in the degradation of xenobiotics:-

The use of microorganisms in the biodegradation and removal of environmental pollutants is of obvious choice as microorganisms play and important and crucial role in nature (Dagley, 1978). In the light of recent knowledge and developments, it is also clear that the microorganisms can be subjected to genetic manipulation so as to improve their degradative capacities. This potential makes biotechnological applications of *in situ* biodegradation attractive :

a) On a cost effective basis

b) as one of the few technologies for the total degradation of environmental pollutants.

Given both, the potential of innovative technology for control of hazardous wastes and environmental contaminants, the

regulatory constraints on the environmental release of genetically engineered microbes, the capabilities and hazards of such work has still to be thoroughly scrutinized (Sayler *et al.*, 1990).

It has been demonstrated that the ability of microorganisms to breakdown xenobiotic compounds is often coded by extrachromosomal genetic elements called as plasmids (Plemberton *et al.*, 1977). The first degradative phenotypes, attributed to catabolic plasmids, involved in the utilization of octane were found in *Pseudomonas sp.* (Chakraborty and Gonsalves, 1971), and later in the assimilation of salicylate Chakraborty, 1972). Bacteria carrying catabolic plasmids have been isolated from a wide variety of environments, although the principal source has been soil (Table 6). The majority of the work done, has been restricted to the catabolic plasmids of *Pseudomonas*. The predominance of *Pseudomonas* like species, although may not be the true representation, is attributed to a number of factors. First, the term *Pseudomonas* is commonly used to describe rod shaped, gram negative, non-sporulating, polar flagellated bacteria, and such a taxon consists of a vast and heterogeneous group of organisms (Pellerони, 1986). Moreover predominantly, the work is carried out on specific compounds like toluene and related compounds where *Pseudomonas* is found to be predominant.

Table 6

Naturally occuring catabolic plasmids

Primary Substrate	Plasmid	Size (Kb)	Bacterial Strains	Trans missible	Inc	Reference
Alkylbenzene sulphonates	ASL	91.5	P.testosteroni	+	--	Cain (1981)
Benzoate	pCB1	17.4	Alcaligenes xylosoxidans subsp.denitrificans PN-1	+	--	Blake *et al* (1987)
Bromoxynil	pBRX1	81	K.ozaenae	--	--	Stalker *et al.* (1986)
Camphor	PpG1(CAM)	500	Pseudomonas.sp	+	P-2	Rheinwald *et al* (1973)
2,4-Dichlorophenoxy acetate	pEML159 pRC10	88	Alcaligenes.sp	+	--	Amy *et al.* (1985)
Nicotine	AO1	160	Arthrobacter.oxidans	--	--	Brandsch *et al.* (1982)
Octane	OCT	500	P.oleovorans	+	P-2	Chakraborty *et al.* (1973)
Phenol	pPGH1	200	P.putida H	+	--	Herrmann *et al* (1987)
Salicilate	sal1	84	P.putida R 1	+	P-9	Chakraborty, (1972)
Toluene	pWWO1(TOL)	117	P.putida (arvilla)mt2	+	P-9	Duggleby *et al* (1977)
3,5 Xylenol	pRA500	500	P.putida p35X,p25X	+	--	Hopper & Kemp, (1980)

Catabolic plasmids are wide spread in nature, and their increase within a community is often observed in pollutant stressed atmospheres. Ecologically, the plasmid coded pathways are an advantage, because they provide a genetic flexibility and can be maintained and transferred within a species. Evolution of the plasmid has been shown to be by the movement of chromosomal genes into plasmids and by duplication (Keil *et al.*, 1985; Perkins and Lurquain, 1988) and by the modification of the genes already present (Farrel and Chakraborty, 1979; Timmis *et al.*, 1985). These plasmids have been demonstrated to be transmissible in nature to either the same species, or under certain conditions, between species, although these transmissions are not well established (Kolenc *et al.*, 1988; Sayler and Stacey, 1986; Trevors *et al.*, 1987). Also the same catabolic plasmids are isolated from different genera (Ghosal *et al.*, 1985; Shields *et al.*, 1985). The modes of transmission of such catabolic plasmids are thought to be similar to that of resistance plasmids and include conjugation of broad host range plasmids, transduction and transformation (Levy, 1986; Levin, 1986; Renny *et al.*, 1982; Saye *et al.*, 1987; Trevors *et al.*, 1987).

The most important factors in plasmid transfers from and within or across the species is the stability of these plasmids. The plasmids transferred to another host may not be maintained and

if maintained there adequate chances that the phenotypes are not expressed. Catabolic plasmids of the *Pseudomonas* incompatibility group (INC) P1 such as pKT230 are broad host range. INC P1 resistance plasmids are shown to be transferable among a large group of gram negative hosts such as *Pseudomonas, Acetobacter and Enterobacteriacae* and in some cases at low frequency to gram positive type (Jacoby *et al.,* 1986; Thomas and Smith, 1987).

Reports regarding the genetic locus of nitrile metabolism has been scarce. Yanese *et al.* (1985) showed by curing experiments that isobutyronitrile in *Pseudomonas sp.* was metabolized by the involvement of the enzymes nitrile hydratase and amidase. These enzymes were observed to be coded by the chromosomal DNA, The organism contained plasmid DNA, which was observed to confer mercury resistance to this organism.. In *Azotobacter sp.* isolated from IPCL waste waters, the enzyme nitrile hydratase was seen to be coded by the plasmid DNA present and the amidase by the chromosomal DNA (Goud, 1987). This is contrary to the hypothesis that the nitrilase nitrile hydratase amidase will mostly be a part of a single operon. A soil microorganism *Klebsiella ozanae* capable of assimilating bromoxynil was isolated and was found to contain plasmids. The enzyme nitrilase was found to be coded by genes located in the plasmid (McBride *et al.,* 1986). Further 2.6 KB of the gene locus was

cloned in *E. coli* (Stalker and McBride, 1987). The enzyme nitrilase was expressed in *E.coli* and found to be similar to that of the wild type. Subsequently the 1212 bp structural gene was sequenced (Stalker *et al.*, 1988).

Unfortunately there is no data available on either the amino acid sequence or the gene sequence of nitrile hydratases, nitrilases or amidases in order to compare their homologies. If available this would give an insight regarding the evolution of these enzymes. There are concrete reasons as to why different nitriles have to be metabolized differently. There are enough reasons to believe that the nitrile hydratase and amidase genes could have fused to give a single nitrilase gene. The products of this fused gene could have different specificities and affinities for substrates. Detailed genetic studies will enable us to answer these anomalies.

Another interesting feature regarding the metabolism of nitriles is, that this capacity to assimilate nitriles are predominant in a restricted genera of bacteria e.g. *Nocardia rhodochrous LL 100-21* (Digeronimo and Antonie, 1970), *Corynebacterium nitrophilus C-42* (Mimura *et al.*, 1969), *Arthrobacter J1* (Asano *et al.*, 1980), *Nocardia rhodochrous* NCIB 11216 (Harper, 1977a), *Arthrobacter I9* (Yamada *et al.*, 1979), *Brevibacterium R 312* (Arnaud *et al.*, 1976), *Pseudomonas chlororaphis B23* (Asano *et al.*, 1982b), *Brevibacterium CH1*

(Hwang and Chang, 1989) and *Klebsiella ozanae* (Mcbride *et al.*, 1986). The majority of these organisms belong to the group actinomycetes and especially to the genera of *Coryneform* group of bacteria. However, this may well be an artifact of the selection procedures which may favour the *Coryneform* group of bacteria. If varied environments are studied, there could be a taxonomic shift which other bacteria coming into the picture.

The Coryneform group includes genera such as *Corynebacterium, Cellilomonas, Brevibacterium, Cleitrobacterium, Microbacterium, and Arthrobacter*. Interest in the genes of the *Arthrobacter sp.* arises from the fact that its members have an extensive secondary metabolism. Relevant features have been assigned to at least one *Arthrobacter sp.* which include a capacity to degrade herbicides, pesticides, chitin, steroid biotransformation ability, ability to produce antibiotics, phytohormones, polysaccharides, riboflavin, etc. (Roberts *et al.*, 1987).

These organisms are also involved in nitrile biotransformation (Nagasawa and Yamada, 1989). In general this group is nutritionally versatile, and can use a diverse range of organic chemicals (Keddie and Jones, 1981). Currently, attention is focused towards the expression of cloned vectors in *Streptomyces sp.* because of their versatile biosynthetic capabilities. *Streptomyces* are also well suited to the

efficient bioconversion of economically important molecules (Chater *et al.*, 1982). The biosynthetic versatility of secondary metabolism is illustrated by the fact that of the 4973 natural antibiotics described till 1978, 2769 came from *Streptomyces* (Bredy 1980). The ability of *Streptomyces sp.*, to produce extracellular enzymes has been used to produce a wide variety of cloned proteins which can be excreted into the culture filtrate.

The difference between *Streptomyces* and *Arthrobacter* is that *Arthrobacter* grows as a single colony, unlike *Streptomyces* which has mycelial growth. This might have distinct advantage over *Streptomyces sp.* (Roberts *et al.*, 1987). It has been seen that *Streptomyces* cloning vector pIJ702 could be maintained and expressed in an *Arthrobacter* which had been made competent. In addition, *Arthrobacter erm A* gene (responsible for erythromycin resistance), was expressed in *Streptomyces*, but not in *E coli*.. By far, this has been the first attempt by workers to produce vectors which could be expressed in *Arthrobacter sp.*. It was observed that *Arthrobacter* promoters were less stringent, and so can be evaluated for their ability to maintain and express broad host range of plasmids. This will be a major advance, if other degradative plasmids such as *Pseudomonas* TOL plasmids could be expressed in organisms capable of degrading nitriles.

Therefore, it will be of interest to study the genetics of nitrile hydrolysis which on one hand provide more information regarding the nitrile metabolism and regulation of the enzymes involved. Moreover, the genetic elements can be mobilized into organisms having other degradative capacity, and increase their degradative capacity. Hydrocarbon wastes are generally nitrogen scarce and if nitriles can be used as the nitrogen source, along with other hydrocarbons, it would be a major breakthrough in producing stable, activated sludge, biotreatment processes for mixed petrochemical waste waters.

In course of the work done by the Department of Biochemistry, we have isolated upto 150 strains from IPCL waste waters out of which, 15 were shortlisted for their ability to grow on the majority of chemical compounds present in the waste waters (Goud *et al.*, 1987). With the diversity in the bacteria, and in their plasmid encoded pathways, their maintenance and transferability needs to be studied. This would provide opportunities to construct a set of microorganisms which would be able to bring about a total degradation of a majority of waste organic compounds present in the waste waters. As mentioned earlier, it has also been observed that a mixture of four organisms namely, *Aeromonas. Pseudomonas, Arthrobacter, and Corynebacterium* were able to reduce the BOD/COD of waste waters. From this we can assume that a good chance exists to

be able to isolate an organism which breaks down nitriles.

1.11 Biotransformation of Nitriles to Amides and Acids :-

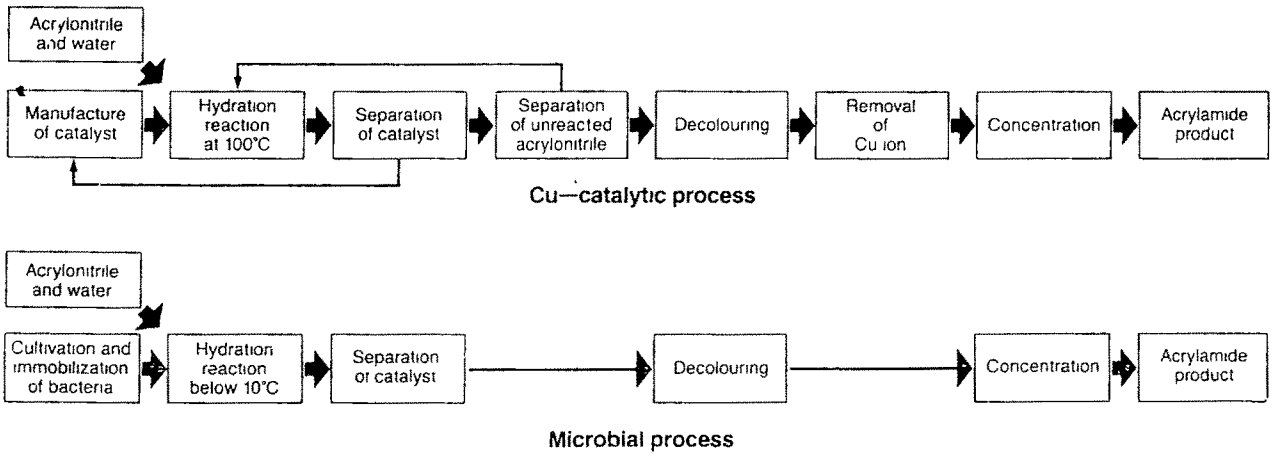
Nitriles are widely used in organic synthesis, as precursors for compounds such as organic acids and amides. However, chemical conversion of nitriles, inspite of being prevalent, is not the procedure of choice as it consumes a large amount of energy, requires highly acid or basic conditions, and the amount of wastes generated in the form of cyanides and salts is also very high. Moreover, at extreme conditions of temperature, in case of acrylonitrile to acrylamide conversion, both the substrate as well as the product tend to polymerize and lower the yield of the reaction (Nagasawa and Yamada, 1989).

Since long, when the metabolism of nitriles was being understood, Galzy and his colleagues in France, recognized the potential of utilizing microorganisms on the industrial scale, to bring about the conversion of nitriles to amides and acids. *Brevibacterium R312* was evaluated for the acrylamide from acrylonitrile. Lot of work has been done on the nitrile biotransformation at the Kyoto University, from where the first report of acrylamide accumulation by wild type and mutant *Pseudomonas chlororaphis B-23* cells were available (Asano *et al.*, 1982b; Ryuno *et al.*, 1988). Upto 400 gms of acrylamide was shown to be accumulated per liter, in about 8 hr. at 10 ^{o}C.,

with a conversion rate of 99% and with almost no acrylic acid formation. Together with the Nittochemicals Company, they proposed and enzymatic procedure for the bioconversion of acrylonitrile to acrylamide, involving the nitrile hydratase of *Pseudomonas chlororaphis B23 and Corynebacterium N774* (Asano *et al.*, 1982; Watanabe *et al.*, 1979; cf. Nagasawa and Yamada, 1989).

For this, a bioreactor system was designed using cells trapped in a cationic polymer gel (Watanabe, 1987) and a compact and efficient bioconversion plant was commissioned. In contrast to the chemical conversion process, shown in Fig 6. the recovery of the unreacted acrylonitrile is no longer necessary as the efficiency of the procedure is almost 100%. The problem of removal of copper was also overcome. The overall transformation was conducted at 10 ^{o}C and so does not require any special energy source. Already this process is producing 6000 tonnes of acrylamide for the Nitto Chemical Company (Nagasawa *et al.*, 1987; Nagasawa and Yamada, 1989). The reason why the cells accumulate such high amounts of acrylamide is that *P. chlororaphis* exhibits much greater nitrile hydratase activity than amidase activity (Nagasawa and Yamada, 1989). Furthermore, acrylonitrile is a powerful nucleophilic agent and inactivates the amidase; this has been observed in *Brevibacterium R312* (Maestracci *et al.*, 1984). However, the reason why acrylonitrile accumulates at 10 ^{o}C is still unclear. These

Fig. 6



Comparison of microbial and conventional process for acrylamide

aspects would be clarified as more work in this area is reported.

These reactions will be able to produce a variety of industrially important chemicals. To name a few : Acrylamide and acrylic acid from acrylonitrile; nicotinamide and nicotinic acid from 3-cyano pyridine; lactic acid from lactonitrile and p-aminobenzoic acid from aminobenzonitrile. A thorough understanding of these reactions will enable us to work out novel bioconversion technologies, which would not only be energy saving but also environmentally benign.

1.12 Aims and objectives of this study

Keeping in view the above information, the objective of this study was to:

1) Isolate and characterize a microorganism capable of utilizing acrylonitrile as a sole source of carbon and nitrogen.

2) Determine the conditions required for the maximum growth and utilization of acrylonitrile.

3) Determine the metabolism of acrylonitrile in resting cells.

4) Determine the conditions for maximum activity of the enzymes, involved in the metabolism of acrylonitrile, in cell free extracts.

5) Characterize the enzymes with respect to their kinetic

properties, cellular localization, inducibilities etc.

6) Purify the enzyme/enzymes involved in the metabolism of acrylonitrile.

7) Determine the genetic locus of the metabolism.

8) Evaluate the capacity of the isolated strain to bioconvert acrylonitrile to acrylamide and acrylic acid.

9) Study the enzymes involved in the assimilation of ammonia which is the product of acrylonitrile breakdown.