

ADVANCES IN BIOCHEMICAL ENGINEERING

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Managing Editor: A. Fiechter

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Eidgenössische Technische Hochschule
Hönggerberg
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Bioconversions of Nitriles and Their Applications

Jean-Claude Jallageas

Laboratoire de Chimie Organique

Université des Sciences et Techniques du Languedoc

F-34060 Montpellier Cedex, France

Alain Arnaud and Pierre Galzy

Chaire de Génétique et Microbiologie

Ecole Nationale Supérieure Agronomique

F-34060 Montpellier Cedex, France

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The bioconversions of nitriles and primary amides have a practical interest for the production of optically active α -hydroxy - or α -amino acids and for the preparation of highly pure amides and acids. The appropriate chemical hydrolyses are generally not suitable for such syntheses.

The literature concerning the reactions of nitrile catabolism by living organisms is analyzed. Among these reactions, only hydrolyses are involved in industrial processes.

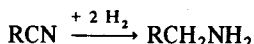
A large number of economically important products may be obtained by hydrolyzing nitriles or primary amides: acrylamide, lysergic acid, DL- or L-lactic acid, DL- or L-alanine, DL- or L-methio-

nine, DL- or L-phenylalanine, and D- or L-phenylglycine, to name a few. The descriptions of the principal known processes and the possibilities for their improvement are presented and discussed.

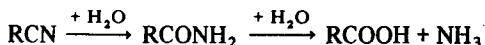
1 Introduction

A number of economically important organic compounds are industrially produced from nitriles by chemical synthesis (Table 1). The main processes are based on three chemical properties of nitriles:

- hydrogenation to amines



- acid or basic hydrolysis to amides or organic acids resp.



- action of bicarbonates on α -aminonitriles (reaction of Bücherer-Berg).

The production of amines from nitriles involves a rather complex methodology due to the presence of hydrogen under consuming pressure appreciable amounts of energy.

All reactions based on hydrolysis of nitriles or the Bücherer-Berg-reaction share some common disadvantages: formation of large quantities of salt, critical separation and isolation resp. of reaction products and prolonged heating. In addition, α -hydroxylic acids or optically active α -amino acids are never obtained.

Catalytic reactions represent a useful tool for hydration of nitriles replacing acids or alkali and simplifying technology by reducing the energy demand. The catalytical hydrolysis by the copper salts of acrylonitrile to acrylamide is the sole example of an industrial process of this type. Nevertheless, some side reactions are taking place in these reactions and the proper regeneration of the catalyst is difficult. On the other hand, laboratory experiments have been accomplished for optimization of the Strecker reaction.

Nitriles may be biologically transformed in order to avoid some of the disadvantages associated with all these chemical processes. The bioconversions occur under milder conditions, at pH values close to neutrality and at moderate temperatures. They also can lead to the synthesis of optically active α -hydroxy- and α -amino acids. In general biological reactions are of lower economy compared to chemical processes. Generally, they represent the only selection for the production of optically active compounds.

Considering the interesting possibilities offered by the bioconversions of nitriles, it is appropriate to examine the literature which deals with living organisms containing nitriles and which describes these natural compounds. These data are summarized in Table 2, where it may be seen that there are in fact relatively few known natural nitriles. It is true that published reports have preferentially treated organisms which liberate

Table 1. Industrial chemical processes leading to amides or acids from nitriles

Nitrile	Reaction	Product	Application of product
Adiponitrile $\text{NC}(\text{CH}_2)_4\text{CN}$	Pressure hydrogenation, 150°C	Hexamethylene-diamine	Polymers
Nitriles of fatty acids (stearic acid, oleic acid, palmitic acid, lauric acid)	Pressure hydrogenation, 150°C	Amines of fatty acids	Antiagglomerants, surfactants, emulsifiers, detergents, flotation agents
Acrylonitrile $\text{CH}_2 = \text{CHCN}$	Acid hydrolysis, hot	Acrylamide	Polyacryl- amide
Phenylacetone nitrile $\Phi\text{CH}_2\text{CN}$	Acid hydrolysis, hot	Phenylacetic acid	Perfumes
Lactonitrile $\text{CH}_3-\text{CH} \begin{array}{l} \text{CN} \\ \text{OH} \end{array} \text{DL}$	Acid hydrolysis, hot	DL-Lactic acid	Therapeutics, dyes, tanning, lacquers
Mandelonitrile $\Phi\text{CH} \begin{array}{l} \text{CN} \\ \text{OH} \end{array} \text{DL}$	Acid hydrolysis, hot	DL-Mandelic acid	Antiseptics
α -Hydroxymethylthio- butyronitrile, DL $\text{CH}_3\text{SCH}_2\text{CH}_2\text{CH} \begin{array}{l} \text{CN} \\ \text{OH} \end{array}$	Acid hydrolysis, hot	α -Hydroxymethyl- thiobutyric acid DL or MHA	Chicken feed
Acetone cyanohydrin $(\text{CH}_3)_2\text{C} \begin{array}{l} \text{CN} \\ \text{OH} \end{array}$	Acid hydrolysis, hot	α -Hydroxyisobutyric acid then methacrylic acid	Plastics
α -Amino nitriles $\text{RCH} \begin{array}{l} \text{CN} \\ \text{NH}_2 \end{array}$	Acid hydrolysis or acid/basic hydrolysis, hot (Strecker reaction)	α -Amino acids	Animal feed, therapeutics, cosmetics, surfactants, polymers
β -Aminopropionitrile $\text{NH}_2\text{CH}_2\text{CH}_2\text{CN}$	Acid/basic hydrolysis, hot	β -Alanine	Precursor of panthotenic acid
Malonitrile $\text{CH}_2 \begin{array}{l} \text{CN} \\ \text{CN} \end{array}$	Basic hydrolysis, hot	Malonic acid	Medicaments
α -Amino nitriles $\text{R}-\text{CH} \begin{array}{l} \text{CN} \\ \text{NH}_2 \end{array}$	Bucherer-Berg reaction	α -Amino acids	

Table 2. Natural nitriles

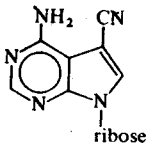
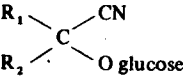
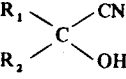
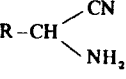

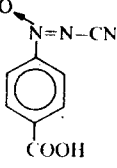
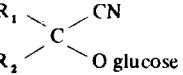
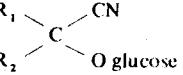
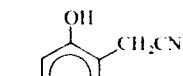
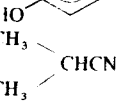
	Formula	Name	Organism ^a [Ref.]
Bacteria		Antibiotic 1037 or E-212 or naritheracin or toyocamycin or unamycin B or vengicide	<i>Streptomyces</i> ¹⁻⁴⁾
Fungi	   	Cyanoglucosides Cyanhydrins α -Amino nitriles Acetylenic nitriles	<i>Basidiomycetes</i> ⁵⁻⁶⁾ <i>Basidiomycetes</i> ⁷⁾ <i>Basidiomycete</i> W ₂ ^{8, 9)} <i>Rhizoctonia solani</i> ¹⁰⁾ <i>Clitocybe diataeta</i> ¹¹⁻¹³⁾ <i>Lepista diemii</i> ¹⁴⁾ <i>Lepista glaucona</i> ¹⁵⁾
		<i>p</i> -Carboxyphenylazoxycyanide	<i>Calvatia lilacina</i> ^{16, 17)}
Algae		Cyanoglucosides	<i>Chlorella</i> ^{5, 6)}
Plants	  	2,4-Dihydroxyphenylacetonitrile Isobutyronitrile	<i>Erica scoparia</i> ¹⁸⁾ <i>Theobroma cacao</i> ¹⁹⁾

Table 2 (continued)

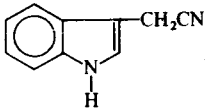
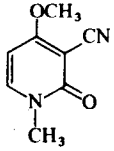
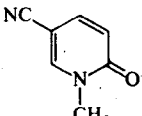
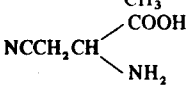
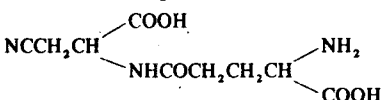
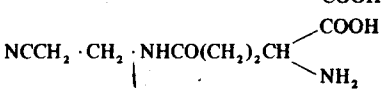
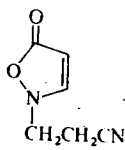

	Formula	Name	Organism ^a [Ref.]
Plants	$\Phi \text{CH}_2\text{CH}_2\text{CN}$	3-Phenylpropionitrile	<i>M. sturtium officinale</i> ^{20, 21)}
	$\Phi \text{CH}_2\text{CN}$	Phenylacetonitrile	<i>Theobroma cacao</i> ¹⁹⁾ <i>Tropaeolum majus</i> , <i>Lepidium sativum</i> . ²⁰⁻²²⁾ <i>Leptactina senegambica</i> , <i>Codonocarpus cotinifolia</i> . ²³⁾
		Indoylacetonitrile	<i>Cruciferae</i> , <i>Lycopersicum esculentum</i> , etc. ²⁴⁻²⁹⁾
		Ricine	<i>Ricinus communis</i> ³⁰⁾
		Nudiflorine	<i>Trewia nudiflora</i> ³⁰⁻³²⁾
		β -cyanoalanine	Leguminosae ³³⁾
		N(γ -L-glutamyl)- β -cyanoalanine	
		N(γ -L-glutamyl)- β -aminopropionitrile	
		2-(2-Cyanoethyl)-3-isoxalin-5-one	<i>Lathyrus pusillus</i> , <i>Lathyrus odoratus</i> ³⁴⁻³⁶⁾
			<i>Lathyrus odoratus</i> ³⁶⁾

Table 2 (continued)

	Formula	Name	Organism ^a [Ref.]
	$\begin{array}{c} \text{OCOR}' \\ \diagup \\ \text{CH} \\ \diagdown \\ \text{CH}_2=\text{C} \quad \text{CN} \\ \quad \quad \quad \diagdown \\ \quad \quad \quad \text{CH}_2\text{OCOR} \end{array} \quad \text{I}$	Cyanolipids	Sapindaceae ³⁸⁻⁴⁰⁾
	$\begin{array}{c} \text{ROOCCH}_2 \quad \text{C=CHCN} \\ \diagup \\ \text{R}'\text{OOCCH}_2 \end{array} \quad \text{II}$		
	$\begin{array}{c} \text{CH}_3 \quad \text{C=CHCN} \\ \diagup \\ \text{ROOCCH}_2 \end{array} \quad \text{III}$		
	$\begin{array}{c} \text{CH}_3 \quad \text{CN} \\ \diagup \\ \text{CH}_2=\text{C} \quad \diagdown \\ \quad \quad \quad \text{CH} \quad \diagdown \\ \quad \quad \quad \text{OCOR} \end{array} \quad \text{IV}$		
	(with R, R' saturated or unsaturated, 14-22 carbons)		
Insects (arthropods)	$\begin{array}{c} \text{R}_1 \quad \text{CN} \\ \diagup \\ \text{C} \\ \diagdown \\ \text{R}_2 \quad \text{O glucose} \end{array}$	Cyanoglucosides	<i>Polydesmus vicinus</i> , <i>Paropsis atomaria</i> . ^{5, 6, 41-45)}
	$\begin{array}{c} \text{R}_1 \quad \text{CN} \\ \diagup \\ \text{C} \\ \diagdown \\ \text{R}_2 \quad \text{OH} \end{array}$	Cyanhydrins	<i>Diplopoda</i> , <i>Alpheloria corrugata</i> , <i>Harpappe haydeniana</i> ⁴⁶⁻⁵³⁾
	$\begin{array}{c} \text{R}_1 \quad \text{CN} \\ \diagup \\ \text{C} \\ \diagdown \\ \text{R}_2 \quad \text{OCO } \Phi \end{array}$	Cyanhydrin benzoate	<i>Polydesmus collaris</i> ⁴⁶⁾
Sponges		Aerophlysinin	<i>Lanthella</i> , <i>Verongia</i> ⁵⁴⁾

^a Species containing the cited product or the group to which the species belongs

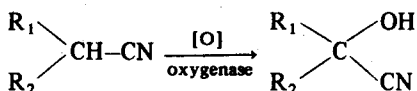
HCN (cyanogenic organisms), a phenomenon found primarily in the plant kingdom. Furthermore, nitriles are difficult to isolate and identify. It is thus probable that a large number of natural nitriles exist, awaiting discovery. Table 2 also shows that nitriles are found in various families of organisms, except mammals for which they are toxic.

Natural cyano compounds and toxic nitriles must be catabolized by organisms and degraded to conventional products which are not toxic to cellular metabolism. The present review on the bioconversions of nitriles will thus initially treat their catabolism and biodegradation, particularly hydrolysis reactions. We will then consider the isolation and identification of bacterial strains with nitrilase activity. The possibilities of using these strains for bioconverting nitriles to organic acids and amides will be discussed. After a description of the uses of nitrilase-containing bacteria for the production of optically active α -amino acids, we will finally analyze the various biological processes for the preparation of acids from nitriles and primary amides described in the literature.

2 Catabolism and Biodegradation of Nitriles

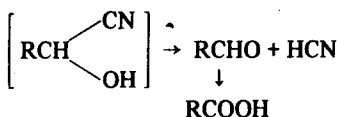
2.1 α -Hydroxylation of Nitriles

Nitriles may be oxidized to cyanhydrins by oxygenases:



This enzymatic pathway appears to be present in a large variety of organisms, including plants, fungi, insects, algae, sponges, and mammals. In spite of the fact that the corresponding enzymes and the intermediate α -hydroxynitriles have never been isolated, several proofs have been advanced for the existence of this reaction.

Some nitriles of the type RCH_2CN are transformed by plants to carboxylic acids RCOOH^{55-58} . The most probable explanation for the synthesis of an acid with a shorter carbon chain is an initial α -oxidation of the nitrile, followed by the decomposition of the cyanhydrin to an aldehyde, which is then oxidized to an acid:



This hypothesis is supported by the finding that the metabolism of 3-indolylacetoni-trile by wheat tissue involves the formation of 3-indolealdehyde⁵⁵.

In addition, it is probable that nitrile toxicity to mammals and insects results from the formation of HCN after α -hydroxylation⁵⁹⁻⁶¹. Indeed:

- the administration of nitriles, as that of cyanide, causes the excretion of nontoxic thiocyanate in the urine; this thiocyanate arises from the action of CN^- on thiosulfate in the presence of rhodanese⁶²;

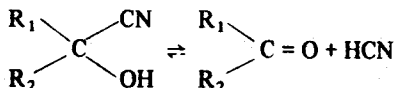
- a study of the action of a mouse liver extract on benzyl cyanide $\text{C}_6\text{H}_5\text{CH}_2\text{CN}$ *in vitro* demonstrated the synthesis of benzaldehyde $\text{C}_6\text{H}_5\text{CHO}$, which must have arisen after the α -hydroxylation of the nitrile⁵⁹;

- in the special case of ω -fluorinated nitriles, it has been shown that nitriles with an odd number of carbon atoms $2n + 1\text{C}$ are much more toxic than those with an even number $2n\text{C}$ ^{63, 64}. This difference can be understood only by an α -hydroxylation of the nitrile, followed by an oxidation of the ω -fluorinated aldehyde to the corresponding acid and then by a β -oxidation degradation of the ω -fluorinated acid to acetic acid and to toxic fluoracetic acid (for $2n + 1\text{C}$ nitriles), or to nontoxic fluoropropionic acid (for $2n\text{C}$ nitriles).

Finally, the mechanism generally admitted for the biosynthesis of cyanoglucosides and cyanhydrins involves an intermediate α -oxidation of the nitriles obtained from α -amino acids⁶⁵⁻⁶⁹. This hypothesis is supported by the finding that HCN liberation by a large number of organisms very often depends on the presence of α -amino acids^{70, 71}.

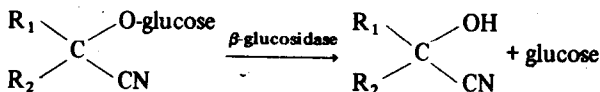
2.2 Formation of Aldehydes from Cyanhydrins

Cyanhydrins give rise to ketone group and cyanhydric acid, either spontaneously or after the action of specific enzymes, oxynitrilases or hydroxynitrilelyases⁷²⁻⁸⁰. The enzymatic degradation of cyanhydrins has been demonstrated by UV-measurements due to a ketonic function in presence and absence of the enzyme.



This reaction is found in fungi, plants, and certain insects (antropods). The sorghum hydroxynitrilelyase has been extensively studied, particularly concerning its isolation and purification⁷².

Natural cyanhydrins arise from the α -hydroxylation of nitriles (cf. 2.1) or from the enzymatic hydrolysis of cyanoglucosides, which liberates glucose and an aglycone moiety⁸¹.



2.3 Reduction of Nitriles

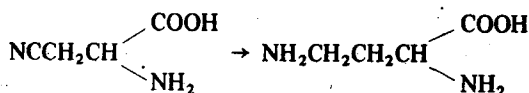
The nitrogenase present in algae and bacteria⁸²⁻⁸⁵) is catalyzing the reduction of numerous important types of substrates like nitrogen, alcyynes, allenes, cyanides, nitriles,

isonitriles, cyanogen, azides, N_2O , H^+ . Certain nitriles are similarly transformed to hydrocarbons (Table 3) releasing ammonia and probably also forming intermediately primary amines. Nitrogenase is found in procaryotes like bacteria, blue-green algae and actinomycetes considered as less developed organisms. It is possible that these types of organisms were involved in the formation of hydrocarbons from plant material. It is noteworthy that acetonitrile has been found in the lighter fractions of far distillates⁸⁶.

Table 3. Reduction of nitriles by nitrogenase

Nitriles	Products formed
$R-CN$	$R-CH_3 + NH_3$ ($R = CH_3, C_2H_5, C_3H_7$)
$CH_2=CH-CN$	$CH_3CH=CH_2 + CH_3-CH_2-CH_3 + NH_3$
$CH_3-CH=CH-CN$ (<i>cis</i>)	$CH_3-CH_2-CH=CH_2 + CH_3-CH_2-CH_2-CH_3 + CH_3-CH=CH-CH_3$ (<i>cis</i>) + NH_3
$CH_3-CH=CH-CN$ (<i>trans</i>)	$CH_3-CH=CH-CH_3$ (<i>trans</i>) + NH_3

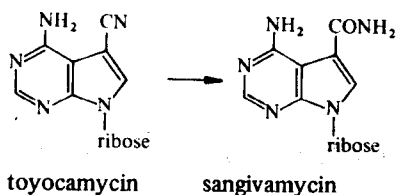
Besides reduction of nitriles by nitrogenase the hypothetical enzymatic reduction of β -cyanoalanine to α,γ -diaminobutyric acid from *Lathyrus odoratus* has been never confirmed⁸⁷)



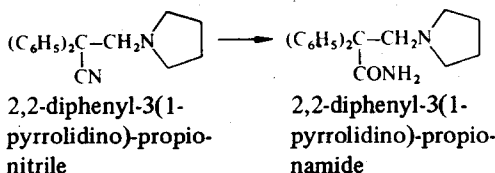
2.4 Hydrolysis of Nitriles

This reaction is the most common nitrile transformation. Thus, it is normal that different authors have attempted to demonstrate it in numerous and varied organisms. The nitriles tested belong to highly divers chemical types. Nevertheless the bibliographic data can be classed on the basis of the chemical type of the starting product and on the pathway employed beginning with the nitrile.

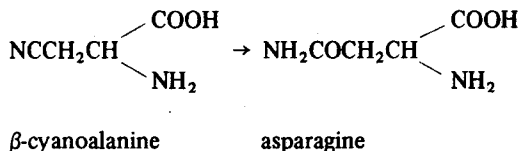
Nitrile hydrolysis may form amides with an arrest of the reaction at this point. There is a relatively limited number of examples of this type of reaction:



* The transformation of a cyanopyrrolopyrimidine nucleoside has been described only in *Streptomyces rimosus*⁸⁸). It is nonetheless probable that it also exists in other *Streptomyces* sp. which produce toyocamycin and sangivamycin.

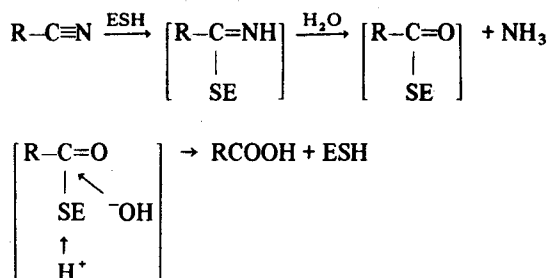


The bioconversion of 2,2-diphenyl-3(1-pyrrolidino)-propionitrile found in *Penicillium*⁸⁹) must also be present in other organisms. Indeed, it has been reported that about 300 molds, basidiomycetes, and actinomycetes are capable of transforming this nitrilated substrate.



This hydrolysis reaction has been described in numerous plants such as wheat, sorghum, etc.⁹⁰⁻⁹²). β -cyanoalanine is a natural nitrile found primarily in *Leguminosae*³³).

Nitrile hydrolysis can also give rise to acids without passing through the amide stage. This is particularly the case of the nitrilase described by Thimann and Mahadevan⁹³⁻⁹⁵) and found in a certain number of plants. This enzyme converts 3-indolylacetonitrile and analogous compounds to the corresponding acids; it is not possible to demonstrate the presence of the corresponding amide at any moment of the reaction. The authors advanced the following reaction mechanism:



ESH represents the enzyme, since essential sulfhydryl groups were demonstrated at the active site(s).

Direct hydrolysis of benzonitrile and of other aromatic nitriles has been described by Harper⁹⁶⁻⁹⁸⁾ in a *Fusarium* and a *Nocardia*. It was not possible to show the intermediate formation of an amide. In these two studies, the nitriles hydrolyzed had aromatic rings and thus the enzymes could have been particular nitrilases, specific for aromatic compounds.

In most cases, nitriles are hydrolyzed to acids with the formation of an amide intermediate. Literature references to this type of transformation appear in Table 4.

Table 4. Nitrile hydrolyses with amides as intermediates

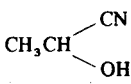
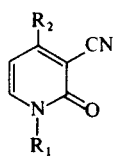
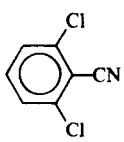
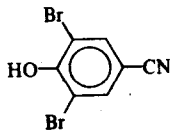
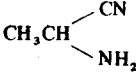
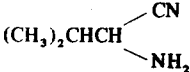
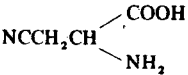
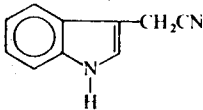
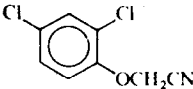
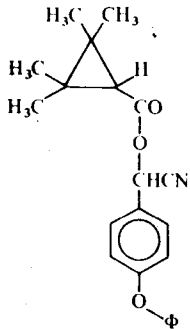
Nitriles	Formulas	Organisms [Ref.]
Aliphatic Nitriles		
Acetonitrile	CH_3CN	<i>Corynebacterium nitrilophilus</i> , <i>Corynebacterium</i> sp. ⁹⁹⁾ , <i>Corynebacterium pseudodiphtheriticum</i> ¹⁰⁰⁾ , <i>Pseudomonas</i> sp. ¹⁰¹⁾ , <i>Nocardia rhodochrous</i> ¹⁰²⁾
Propionitrile	$\text{CH}_3\text{CH}_2\text{CN}$	<i>Nocardia rhodochrous</i> ¹⁰²⁾
n-Butyronitrile, adiponitrile, butene-1-nitrile	$\text{CH}_3(\text{CH}_2)_2\text{CN}$, $\text{NC}(\text{CH}_2)_4\text{CN}$, $\text{CH}_2=\text{CHCH}_2\text{CN}$	<i>Corynebacterium pseudodiphtheriticum</i> ¹⁰⁰⁾
α-Hydroxynitrile		
Lactonitrile		<i>Corynebacterium pseudodiphtheriticum</i> ¹⁰⁰⁾
Aromatic Nitriles		
Ricinine and analogs		<i>Pseudomonas</i> sp. ^{103, 104)}
Benzonitrile	ΦCN	<i>Corynebacterium pseudodiphtheriticum</i> ¹⁰⁰⁾
Dichlobenil		<i>Trichoderma</i> sp., <i>Penicillium</i> sp., <i>Fusarium</i> sp., <i>Geotrichum</i> sp. ¹⁰⁵⁾ , <i>Soil bacteria</i> ¹⁰⁶⁾
Bromoxynil		<i>Flexibacterium</i> sp. ¹⁰⁷⁾

Table 4 (continued)

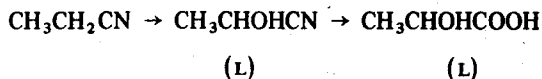
Nitriles	Formulas	Organisms [Ref.]
α-Amino Nitriles		
α -Aminopropionitrile		<i>Corynebacterium</i> sp. ¹⁰⁸⁾
α -Aminoisovaleronitrile		<i>Corynebacterium</i> sp. ¹⁰⁸⁾
Diverse		
β -Cyanoalanine		<i>Escherichia coli</i> ¹⁰⁹⁻¹¹¹⁾ , <i>Neurospora crassa</i> , <i>Lathyrus sylvestris</i> , <i>Lathyrus odoratus</i> , <i>Vicia villosa</i> , Guinea pig ¹¹²⁾
Indoylacetoneitrile		Various plants ^{55, 113, 114)}
2,4-Dichlorophenoxy-acetonitrile		Various plants ^{55, 113, 114)}
α -Cyano-3-phenoxy-benzyl-2,2,3,3-tetramethylcyclopropane-carboxylate		Undetermined soil organism ¹¹⁵⁾

Finally, in considerable research on the hydrolysis of nitriles to acids, no studies on the demonstration of an amide intermediate were performed. This is especially true in work on the toxicity of aromatic nitriles and aminoacetonitrile to mammals¹¹⁶⁻¹¹⁸⁾ and in research on waste water treatment by activated sludge¹¹⁹⁾. In addition, no mention of passage through an amide intermediate was made in studies of the mechanism of DL-cyanhydrins hydrolysis to α -hydroxyacids by *Torulopsis candida* and of DL- α -amino nitriles hydrolysis to α -amino acids by fungi⁸⁻¹⁰⁾.

2.5 Possibilities of Using Pathways of Nitrile Catabolism for their Bioconversions

The production of simple hydrocarbons by nitrogenase action on compounds as elaborate as nitriles is not economically interesting. The same is true for obtaining aldehydes from cyanhydrins.

Bioconversions using the other two nitrile catabolic pathways, however, seem to be promising. Biological α -hydroxylation could yield optically active cyanhydrins which could be hydrolyzed to yield the corresponding D- or L- α -hydroxyacids. The most economically interesting acid, L-lactic acid, could thus be synthesized from propionitrile:



The above literature search also shows the numerous possibilities of applying biological hydrolyses of nitriles for the production of economically important amides and acids.

Relatively few amides are commercially available: acrylamide, benzamide, formamide, chloroacetamide, propionamide, salicylamide, nicotinamide, and phenylacetamide. Among them, only acrylamide is chemically obtained by nitrile hydrolysis; the others are prepared from acids or their derivatives by the action of ammonia. The corresponding nitriles are generally obtained by dehydration of amides and they are more expensive than the amides and acids. Thus, the only current important application of the biological hydrolysis of nitriles to amides is for acrylonitrile. This bioconversion could eliminate the production of secondary products present during chemical hydrolysis and reduce the energy requirement of the reaction.

There is a larger number of possibilities in the field of biological hydrolysis of nitriles to acids. Indeed, certain nitriles are much less expensive than the corresponding acids, e.g., α -amino acids, lactic acid, malonic acid, mandelic acid, methacrylic acid, phenylacetic acid, β -alanine, methylmercaptohydroxybutyric acid, and nitrilotriacetic acid. The biological production of these acids would have several advantages: avoid the production of a large quantity of salts, limit energy consumption and, above all, produce optically active acids.

We will now examine the research we have performed in an attempt to apply the biological hydrolyses of nitriles to the production of economically important amides and acids.

3 Study of Bacterial Strains with Nitrilase Activity

The above literature search showed that nitrile hydrolysis was found primarily in bacteria and fungi. Since the multiplication and utilization of bacteria are more manageable than those of fungi, it is logical to attempt the selection of bacterial strains with nitrilase activity.