

Review

Biodegradation of catechols by micro-organisms - A short review

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Many aromatic hydrocarbons and catechols are known to be toxic and carcinogenic for humans, and their contamination of soils and aquifers is of great environmental concern. Soil microorganisms, like *Pseudomonas* spp. and *Mycobacterium*, were found to be capable of transforming and degrading toxic catechols to easily absorbable TCA metabolites. These abilities may be useful in removal of toxic organic compounds from the environment. The successful application of microorganisms to the bioremediation of contaminated sites requires a deeper understanding of how microbial degradation proceeds. In this review, the microorganisms involved and the metabolic pathways for the degradation of many aromatic hydrocarbons are summarized and the biological aspects of catechol bioremediation are discussed.

Key words: *Pseudomonas* spp., catechol metabolic pathways, microbial degradation, bioremediation.

INTRODUCTION

The question of recalcitrant organic compounds first surfaced as a real public and scientific issue with the publication of 'Silent Spring' (Carson, 1962). This book raised awareness of the actual and potential problems caused by the accumulation in the environment of organic compounds which were not readily destroyed by biological activity and so built up to concentrations which were toxic to man or wildlife or had some other unacceptable effects (Alexander, 1965). Perhaps the most infamous of these compounds are the polychlorinated insecticides such as DDT, aldrin and dieldrin which are subject to bioaccumulation in the food chain and which caused devastating effects on the populations of predatory birds in the 1950s and 60s. A more visible example was the problem of water pollution by so-called 'hard' detergents during the same period. The realisation of the consequences of the release of these chemicals in the environment led to much research, which is still on-

going, into the fate of these materials in the wider environment.

In the 1950s there was a strongly held view amongst microbiologists, often called the 'principle of microbial infallibility', that all chemicals were susceptible to microbial degradation if the right organism and conditions could be identified. The metabolic capabilities of microbes were 'all powerful'. The growing realisation that many chemicals which found their way into the environment were not being degraded and were persisting for many years puts an end to such thinking (Alexander, 1994). Half-lives of polychlorinated organic insecticides in the order of 10s of years were reported and there was considerable fear that such compounds could be with us forever. With these rather frightening statistics in view, Martin Alexander reviewed biodegradation in 1965 and raised the possibility that microbes were in fact fallible and that, through a combination of a chemical's structure and the environment it found itself in, a chemical might survive in the environment for prolonged periods. Since then there has been intensive research into this problem world-wide in both academia and industry. Although there are still many causes for serious concern there is

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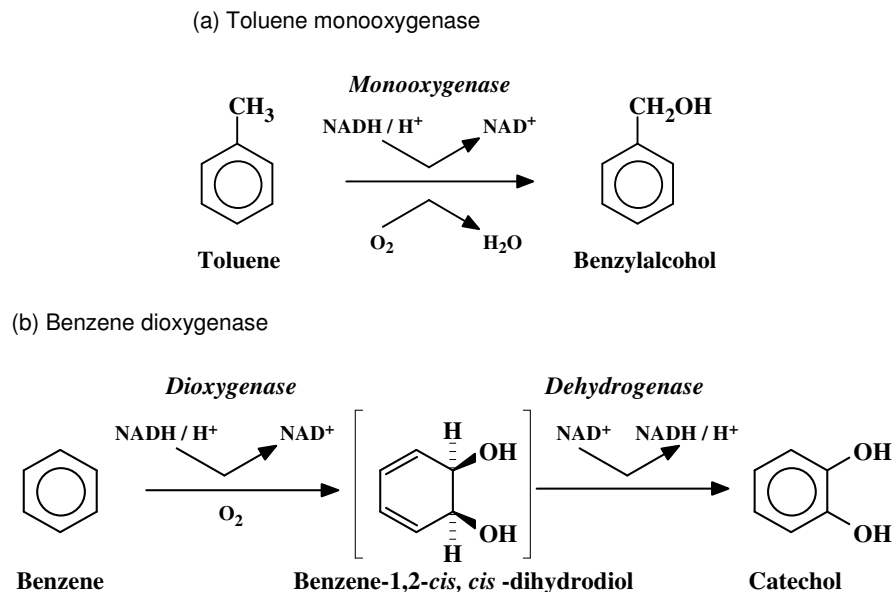


Figure 1. Examples of monoxygenase and dioxygenase reactions.

now hope that some of the mistakes of the past will not be repeated and indeed that some old pollution problems may be cleared up. Particularly important developments have included:

- 1) An understanding of how catechols are degraded and what structural factors make them recalcitrant (Carson, 1962). This has allowed the design of important chemicals with degradability in mind.
- 2) The discovery of co-metabolism.
- 3) The realisation of the role of microbial communities rather than pure cultures in degradable processes.
- 4) The realisation that degradation of many chemicals can occur in anaerobic environments.

EVOLUTION OF DEGRADATIVE ABILITIES

Assuming that it is not toxic, the ability of an organism to degrade a compound depends on the ability of the compound to come into contact with an enzyme or a series of enzymes which can degrade it (Chaudhry, 1994). This is affected by three things: access of the compound to the enzymes, ability of the enzyme to catalyse a degradative reaction, and production of the enzyme in suitable quantities. All three of these may be subject to evolutionary change (Chaudhry, 1994). Figure 1 shows examples of monoxygenase and dioxygenase reactions.

Evolution may involve the mutation of pre-existing genes and thus the production of new, altered proteins. It can also involve the acquisition of new genetic information from other organisms. This new information may come via plasmid transfer, transposons (jumping genes) or by uptake of DNA from the environment.

Sometimes extracellular enzymes are involved in a biodegradation, in which case the ability of compounds to enter the cell is not a problem (Karsa et al., 1995). If enzymes are intracellular, then the compound needs to cross the cell membrane. This can involve free diffusion or use of a permease or other transport system (Pitter et al., 1990). If the compound cannot easily enter the cell, there is the possibility for improvement in access to the cytoplasm due either to alterations in the structure of the cell membrane (this could involve changes in either proteins or lipids) or to changes in the specificity of the permease proteins which catalyse translocation of compounds across membranes (Daly, 2000).

Enzymes may already exist that catalyse metabolism of similar compounds. Small changes in the structure of the protein may alter its substrate specificity such that a new substrate can be metabolised (Daly, 2000). Such changes can occur either in the regions which catalyse reaction or in regions responsible for binding of the substrate into the enzyme/substrate complex.

Where an organism already possesses genes for the production of an enzyme which can metabolise a target compound, degradation may not occur due to a failure of the organism to produce the requisite enzymes. This can be overcome by mutations that lead to constitutive production of the enzyme (i.e. the enzyme is produced all the time) or to a change in the inducer specificity for the enzyme such that the target compound is now an inducer.

It should be noted here that there are often large differences in the specificity of transport systems and enzymes, and also of the control systems which regulate their production. Thus when a new metabolic activity arises in an organism, it may be due to mutation in one or

several of the functions mentioned above. Studies on the evolution of new metabolic activities in organisms have shown that often the early stages of evolution involve selection of constitutive mutants or mutants that produce very large amounts of an enzyme and this is then followed by the selection of mutants with altered enzyme activity (Daly, 2000). Alterations in enzyme activity are more likely to involve changes in the regions of the protein that involve substrate binding than changes in catalytic sites.

One stage in the evolution of an organism to degrade a toxic compound may involve the organism becoming tolerant to the toxic effects. This tolerance may be due to a range of different adaptations depending on the mechanism by which the compound causes inhibition.

It is interesting to observe that although some microbes are much specialised in the type of compounds they can degrade, or utilise as growth substrates, others may not be. This is particularly so for bacteria in a few genera which show extreme nutritional versatility i.e. the ability to degrade many organic compounds. It is not uncommon to find individual bacterial strains (not species) that can utilise over 100 defined organic compounds as sole source of carbon and energy. This trait is most famous in the aerobic pseudomonads. The old genus *Pseudomonas* has now been dismantled by taxonomists and split into a range of new genera. Some of the most versatile species include *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Pseudomonas stutzeri*, *Burkholderia cepacia* and *Comamonas testosteroni*. However the Pseudomonads are not the only group possessed of nutritional versatility. Other noted Gram-negative bacteria include *Acinetobacter*, *Alcaligenes*, *Moraxella*, *Achromobacter* and *Flavobacterium* spp. For many years the qualities of the Gram-positives were rather overlooked - possibly because the nutritionally versatile species are often rather slow-growing in comparison to the Pseudomonads. The Gram-positives most noted for their ability to degrade recalcitrant chemicals are all in the actinomycete line and include *Mycobacterium*, *Nocardia*, *Rhodococcus* and *Arthrobacter* spp. The organisms most frequently identified as agents of biodegradation tend to belong to the genera named above, although other genera e.g. *Streptomyces* are occasionally encountered.

Clearly with the plethora of different recalcitrant chemicals, a very wide range of metabolic pathways are involved in biodegradation. If a compound is to serve as a carbon and energy source then it has to be converted into a form that can enter central metabolism. Normally this involves converting it into one, or more, low molecular weight intermediates of the tri-carboxylic acid (TCA) cycle (otherwise known as Krebs's or the citric acid cycle) or compounds that feed into it. The means by which this is done obviously varies considerably typical intermediates include acetate (or acetyl CoA), acetaldehyde, pyruvate, succinate or fumarate. The length of metabolic

pathways varies enormously according to the complexity of the target compound; the number of steps ranges from one or two to over a dozen. In some cases target compounds require very little modification before they can enter a pre-existing pathway.

It should be stressed that the metabolic pathways used for degradation may vary not only according to the environmental conditions (e.g. aerobic or anaerobic) but also according to the type of organism. There may be a wide range of pathways available some of which differ from each other subtly and others markedly (Young et al., 1995). It is quite likely that different strains of the same species will employ different metabolic pathways for the same degradative process (Young et al., 1995). For this reason it is of little value to reproduce here details of lots of metabolic pathways, as no assumptions can be made as to what pathway will be used for a particular degradative process without proper testing being done. Generally there is no obvious reason why one strain uses one pathway and another strain a different pathway. Indeed in effluent treatment plants there do not appear to be any evidence as to which pathways are in practice used by the microflora.

One of the best examples of multiple pathways is found with toluene degradation. Two pathways have been proposed for anaerobic degradation and at least five for aerobic degradation (Diaz et al., 2000). Two of the five are from different strains of *P. putida*.

Extensive work of biodegradation of methylcatechols were done by Cain et al. (1989) on the mechanistic aspects of β -keto adipate pathway and also on carboxy derivatives by Kirby et al. (1975, 1977) (Figure 2, Table 1). Deuterium labelled methylcatechols was fed to the microorganisms and the corresponding Deuterated muconolactones were isolated and the enzyme mechanisms on this pathway were established (Cain et al., 1989). The stereochemistry of the intermediates on this pathway, the methyl muconolactones were established by x-ray studies on the naturally occurring 3- and 4- methyl muconolactones of the biodegradation of methyl-catechols on this pathway (Cain et al., 1989). This revealed the absolute configuration of the intermediates and thus clearly understanding the enzyme mechanism of microbial degradation of aromatic hydrocarbons, phenols and catechols (Figure 3). Catechols are aromatic and it is perhaps useful to look briefly at how aromatics are degraded aerobically (DeBont et al., 1986). There are basically three different sets of pathways. To begin with, the aromatic compound is converted to a ring cleavage intermediate which normally has two hydroxyl substituents. These may be next to, or opposite, each other. This intermediate is then cleaved by a dioxygenase to give a straight chain intermediate. If the hydroxyl groups are next to each other then the ring is either cleaved between them (*ortho* cleavage) or to one side of them (*meta* cleavage). Where the hydroxyls are opposite each other the ring is cleaved immediately to one side of

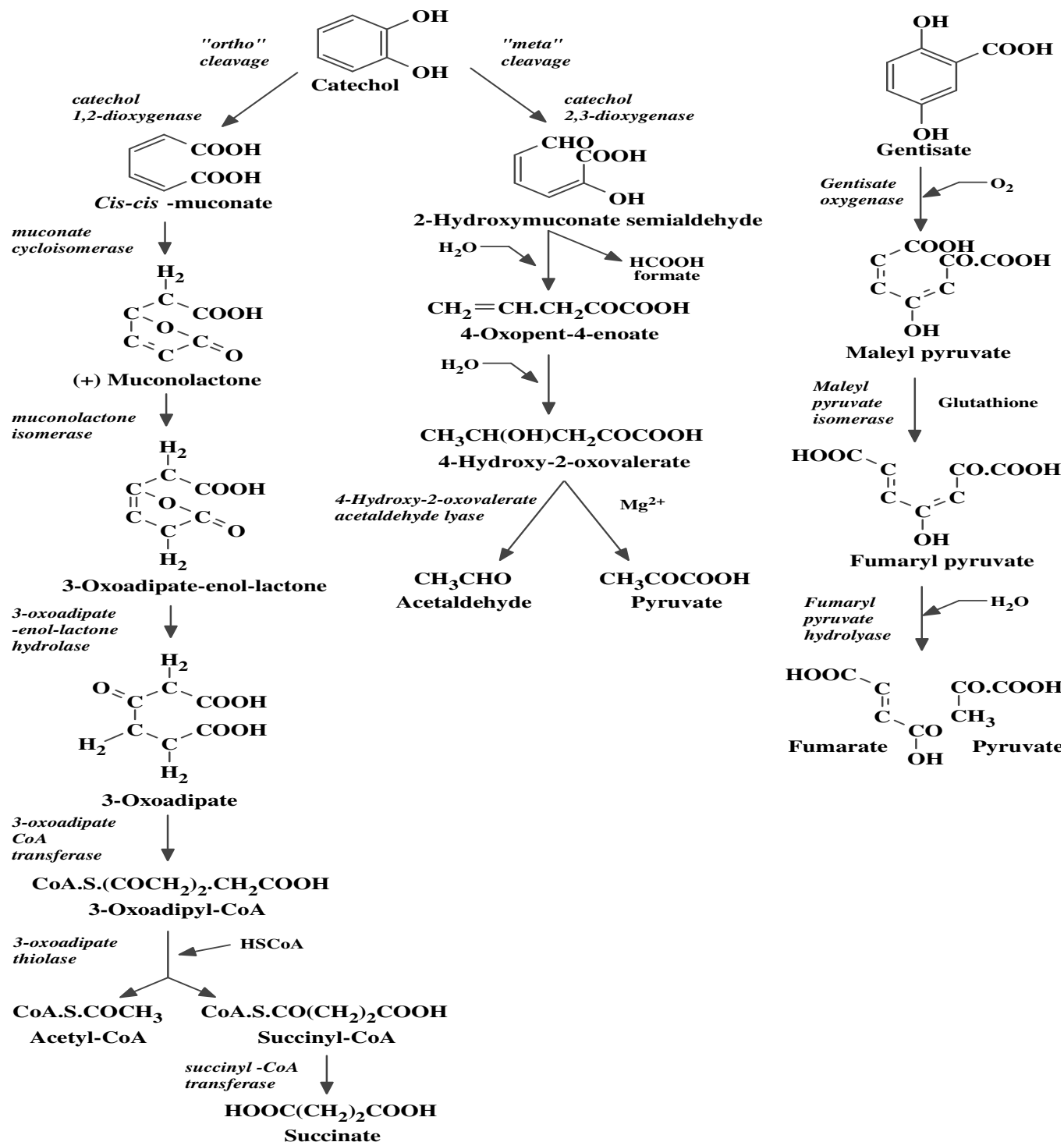


Figure 2. Major metabolites involved in aerobic aromatic degradation pathways.

one of the hydroxyl groups. The straight chain ring cleavage product is degraded to give smaller units which will enter the tri-carboxylic acid cycle. The initial pathway which produces the ring cleavage substrate may be simple, involving only one step (e.g. hydroxylation of phenol to

produce catechol) or complex involving many steps. It should be noted that a range of analogues may act as ring cleavage substrates but often (not always) distinct, specific enzymes are involved. So, for example, in *P. putida* catechol 1,2-oxygenase employed to cleave cate-

Table 1. Major metabolites involved in aerobic aromatic degradation pathways.

Name of pathway	Ortho-cleavage intra-diol cleavage; β -ketoacid pathway	Meta-cleavage; extra-diol cleavage	Gentisate/homogentisate pathway
Typical ring cleavage substrate	Catechol; protocatechuate; 3 or 4 methyl catechol	Catechol; protocatechuate; 3 or 4 methyl catechol	Gentisate; homogentisate
Typical ring cleavage product	<i>cis,cis</i> -Muconate; β -carboxy- <i>cis,cis</i> muconate	2-Hydroxymuconic semialdehyde; 2-hydroxy-4-carboxymuconic semialdehyde	Maleylpyruvate; maleylacetoacetate
Typical degradation products	Acetyl CoA, succinate		Fumarate; pyruvate; acetoacetate; acetate

chol is distinct from protocatechuate 3,4-oxygenase (Dorn et al., 1978), which cleaves its carboxylated analogue in the *ortho* pathway (DeBont et al., 1986).

Anaerobic degradation of aromatics proceeds in a very different way since it is not possible to activate the benzene ring by the addition of oxygen. Instead the ring is activated by converting alkyl side chains, or adding carbon dioxide, to give a carboxylated ring which is then converted to a Coenzyme A ester. Benzoyl CoA or its *para* hydroxy derivative appears to be a common intermediate and this is progressively reduced to a cyclohexyl derivative which is primed for ring cleavage by the addition of water across a double bond next to the carboxyl group. After ring cleavage the straight chain intermediate is degraded to acetyl CoA by β -oxidation. Thus in this type of attack the oxygen required is introduced from water and oxidation is accomplished by removal of hydrogen/electrons. These are generally disposed of by the reduction of nitrate or sulphate or the generation of hydrogen which is utilised in consortia by methano- or sulphido-gens.

GENETIC ASPECTS

The genes that encode for biodegradation of many recalcitrant organic chemicals are often encoded on plasmids. Sometimes only one or two enzymes are encoded on a plasmid with the rest on the bacterial chromosome while in others an entire pathway is plasmid encoded.

Plasmids are small genetic elements separate from the chromosome and are often unstable. There is a range of causes for their instability but often a plasmid will be lost from an organism if it is cultivated in the absence of the substrate which degradation it encodes. Plasmids tend to remain stable in a population when their presence confers a selective advantage (e.g. ability to utilise a substrate which is present) on individual cells which carry them. When no advantage accrues to the organism, plasmids can be lost because their maintenance in the population represents a drain of resources which can impose (at least in the short term) a selective disadvan-

tage.

Plasmids can often be transferred easily between organisms, even ones that are not closely related, and this transfer offers a means by which genetic recombination can occur. This happens in nature but it can also be made to happen in the laboratory giving scientists the potential to develop new bacterial strains with altered and enhanced degradative ability (Dorn et al., 1978). Various researchers have genetically engineered so-called 'super bugs' which can degrade a wider range of, for example, hydrocarbons. The potential application of these strains in clearing up oil spills or other pollution incidents has been patented and much 'hyped'. In practice it seems unlikely that most of these genetically engineered organisms will really offer much advantage over mixed cultures developed by conventional enrichment culture, or indeed over the native microflora. Such strains are more likely to be of use in the more carefully controlled conditions of special treatment plants. Genetic engineering may have a role in creating bacterial strains with entirely new metabolic activities by combining genes from different organisms to produce an entirely new metabolic pathway. An example of this is the creation of a new strain of *Pseudomonas* to degrade chlorobenzoates by using part of the *ortho* pathway from one organism with part of the *meta* cleavage pathway from another. It is likely that such strains will also arise as a result of 'natural' genetic recombination.

Conclusions

After the gloom of impending disaster and environmental calamity which was present in the 1960s, the threat from recalcitrant organic chemicals such as catechol has diminished to some extent. Extensive microbiological and biochemical research has explained the reasons for recalcitrance and suggested routes by which problem compounds may be degraded. New processes of biodegradation have come to light and methods of utilising microbial activity have been developed or improved. An understanding of the effects of chemical structure on biodegradability means that we now have the knowledge

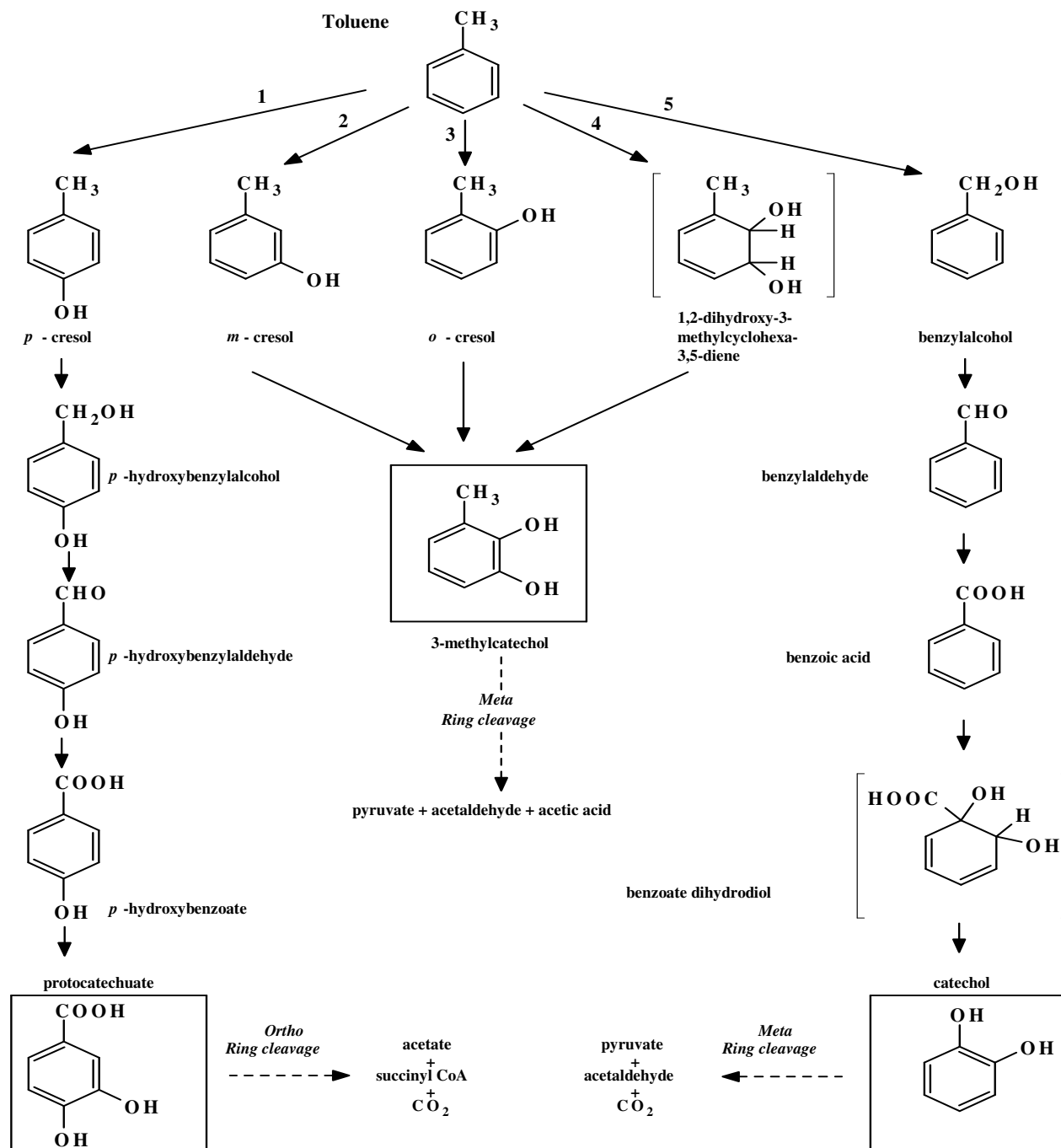


Figure 3. Different pathways for the aerobic biodegradation of toluene. Toluene is degraded via 5 different routes by the following bacteria; *Pseudomonas mendocina* KR1 (1), *P. pickettii* PKO1 (2), *Burkholderia* (formerly *Pseudomonas*) *cepacia* G4 (3), *P. putida* F1 (4), *P. putida* mt-2 (5). Postulated intermediates are shown in parentheses. Intermediates immediately prior to ring cleavage are shown in boxes. ----- indicates several steps.

to design chemicals that are more amenable to biodegradation in the environment (Evans et al., 1971). If progress is to be made it will be essential to gain an improved knowledge of the microbiology and biochemistry of the treatment of catechol, and the biodegradation of

their components, so that it can become more effective. If tighter environmental protection legislation is to be applied effectively and fairly then research will be required to ensure that industry can comply with more stringent limits on the COD of effluents and direct toxicity assessments.

As we progress into the 21st century, further scientific investigation is essential but its beneficial effects will be nullified without a trans-national legal framework to outlaw serious pollutant chemicals and generous financial aid to make it effective across the globe.

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