

## **Microbial Degradation of Biaryl Structures: Relationships between Fluorene, Dibenzofuran and Biphenyl Pathways**

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### **Introduction**

In an investigation on the bacterial degradation of polycyclic aromatic hydrocarbons (PAH), fluorene was used as a model compound for a PAH containing a methylen bridge. Fluorene is also a structural element of coal and therefore of interest for coal biotechnology.

After enrichment on fluorene, dibenzofuran (DBF) and biphenyl as sole sources of carbon and energy, several strains have been isolated. Two strains, DPO 1361 (kindly provided by Dr. Rast, Bayer AG) and DPO 360, were further investigated.

The results show that the degradative pathways for fluorene, dibenzofuran and biphenyl are closely related, and that fluorene is productively metabolized via initial angular dioxygenation.

### **Results**

Growth characteristics of strain DPO 1361 and DPO 360 are listed in Tab. 1.

**Tab. 1: Growth characteristics of strain DPO 1361 and DPO 360**

<b>Substrate</b>	<b>DPO 1361</b>	<b>DPO 360</b>
Fluorene	+	+
9-Fluorenone	+	+
9-Fluorenol	+	+
Biphenyl	+	+
Dibenzofuran	+	+
Naphthalene	-	-
Anthracene	-	-
Phenanthrene	-	-
Salicylate	+	+
Benzoate	n.d.	+
Phthalate	-	*

**+** = growth substrate

**-** = no growth substrate

**n.d.** = not determined

**\*** = growth only after adaption

Growth on fluorene, dibenzofuran and biphenyl suggests that these substrates constitute a physiologically defined group, which are degraded by similar mechanisms. In contrast no growth is observed with phenanthrene, anthracene and naphthalene.

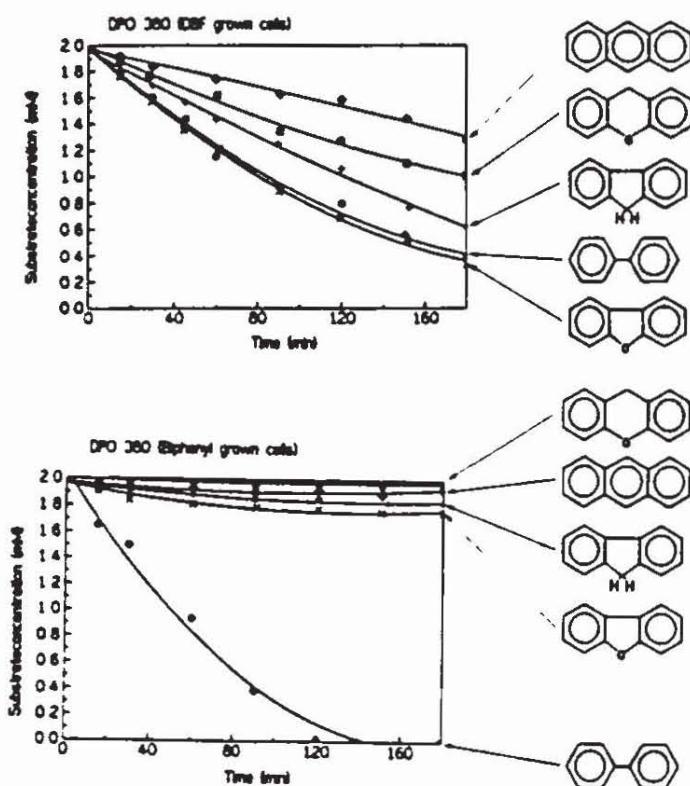
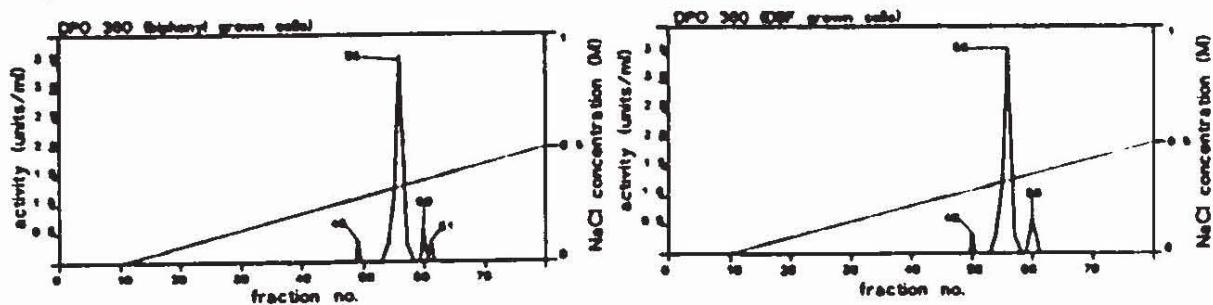


Fig. 1: Degradation kinetics of initial dioxygenases in strain DPO 360

The result of the experiment outlined in Fig. 1 demonstrates that strain DPO 360 expresses two different types of initial dioxygenases, depending on the growth substrate. The experiment was performed by incubating resting cells of strain DPO 360, cultivated on DBF or biphenyl, with biaryl ethers or related compounds in a two-liquid phase system, consisting of phosphate buffer and an organic phase (2,2,4,4,6,8,8-heptamethylnonane). The substrates were completely dissolved in the organic phase (2 mM, referred to the total culture volume). Turnover of substrates was monitored by following their concentrations in the organic phase by use of normalphase HPLC.

After growth on Biphenyl or DBF, crude extracts of strain DPO 360 were separated by Mono-Q-ion exchange chromatography (Fig 2.). Enzyme activities were determined in each fraction, using catechol and 3-phenylcatechol as substrates for the metapyrocatechases,

2,3-dihydro-2,3-dihydroxy-biphenyl for the dehydrogenase and 2-hydroxy-6-oxo-6-phenyl-2,4-hexadienoic acid (HOPDA) for the hydrolase activity.



Fraction 49: HOPDA-hydrolase, fraction 56: Phenylcatechol-2,3-dioxygenase (metapyrocatechase type 1), fraction 60: Catechol-2,3-dioxygenase (metapyrocatechase type 2), fraction 61: 2,3-Dihydro-2,3-dihydroxybiphenyl-dehydrogenase

Fig. 2: Enzyme pattern of crude extracts of strain DPO 360.

Two different metapyrocatechases and a HOPDA-hydrolase were detected in the crude extract of the biphenyl grown cells. This enzyme pattern could also be found in crude extracts of DBF grown cells. In contrast, 2,3-dihydro-2,3-dihydroxy-biphenyl-dehydrogenase was only induced in biphenyl grown cells.

After growth on fluorene, dehydrogenase activity was also found in fraction 61, which catalyzes dehydrogenation of 2,3-dihydro-2,3-dihydroxy-biphenyl and 1,10-dihydro-1,10-dihydroxyfluoren-9-one.

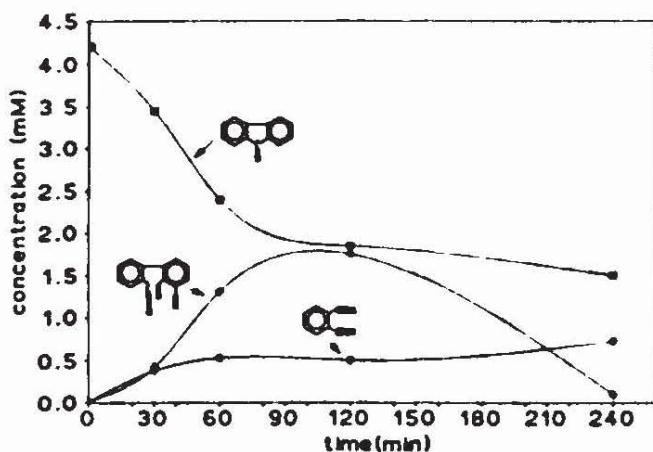


Fig. 3: Turnover of fluoren(-9)-one by resting cells of strain DPO 1361, grown on fluorene, in the presence of 3-chloro-catechol.

In order to elucidate the degradation pathway of fluorene, fluoren-9-one was metabolized in the presence of 3-chlorocatechol (1mM), a well-known inhibitor of metapyrocatechases. Substrate consumption and metabolite accumulation were analyzed by HPLC.

Insoluble substrates were dissolved by adding four volumes of dioxane.

The respective fluorenone, 2-(3-carboxyphenyl)catechol (CPC) and phthalic acid concentrations after 60 minutes were determined as 2.4, 1.3 and 0.5 mM (see Fig. 3). 72% of the substrate transformed is thus accumulated as CPC, showing that CPC is the key intermediate in the degradation pathway.

The inhibitor 3-chlorocatechol is also turned over slowly, as soon as its concentration falls below 0.1 mM, accumulated CPC is further metabolized .

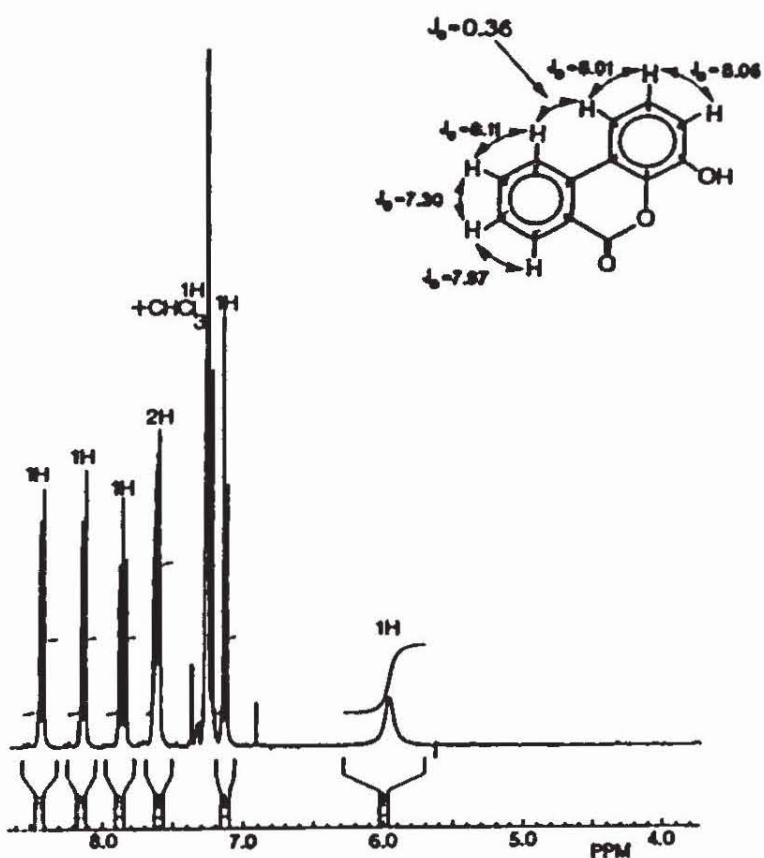
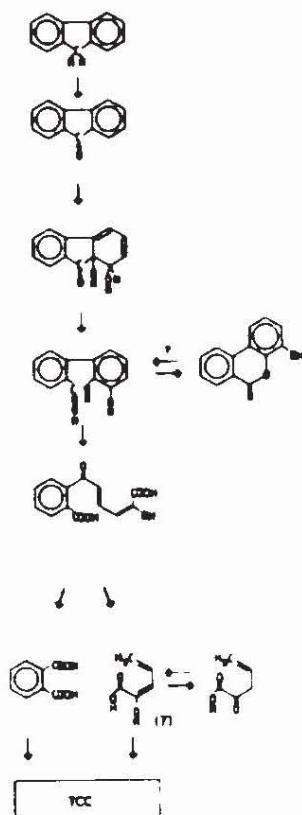


Fig. 4:  $^1\text{H}$ -NMR-Spectrum of the lactonized form of 2-(3-carboxyphenyl)catechol.

Using this technique it was possible to isolate the lactonized form of CPC by the use of preparative HPLC. The  $^1\text{H}$ -NMR of the molecule is shown in Fig. 4. Lactonization is probably an artefact due to acidic conditions during isolation.

Summarizing the results obtained, the following degradation pathway for fluorene is proposed:



**Fig. 5:** Proposed pathway for the bacterial degradation of fluorene.

#### Conclusions

Initial dioxygenation of fluorene by dibenzofuran degrading strains occurs in the unusual angular position. The resulting dihydrodiol is converted to 3-(2-carboxyphenyl)catechol by action of a dehydrogenase. This is a novel activity for a dehydrogenase causing a C-C-bond cleavage.

After growth with dibenzofuran and biphenyl respectively two different initial dioxygenases are expressed. The first enzyme shows a broad substrate range, the second enzyme only converts biphenyl.

Strains degrading fluorene, dibenzofuran and biphenyl may constitute a unique physiological group.

#### Literature:

1. Engesser et al., FEMS Microbiology Lett. 65(1989), 205-210
2. Strubel et al., J. Bact. 173(1991), (in press)