

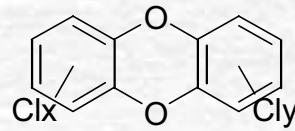
Degradation of dioxin-like compounds by microorganisms

장윤석

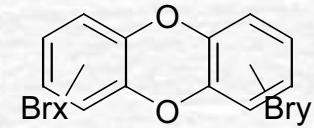
2001. 03.27

Chemical structures of Polychlorinated Dibenzo-p-dioxins (PCDD), and related compounds

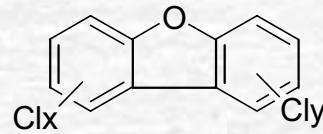
PCDD [75]



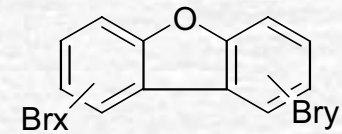
PBDD



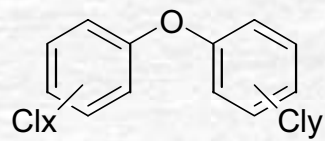
PCDF [135]



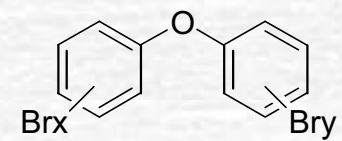
PBDF



PCDE[209]



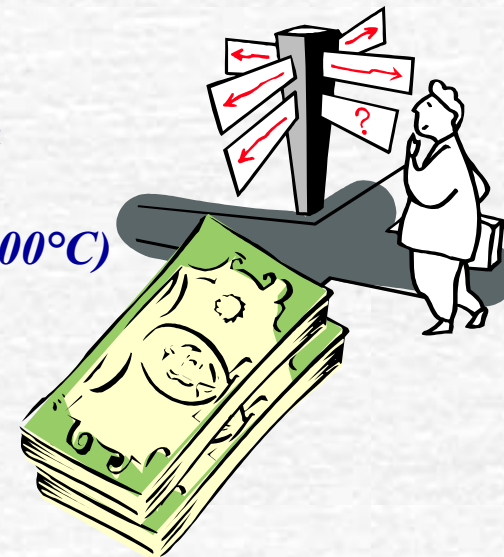
PBDE





Removal of Dioxins

1. *Catalytic destruction --- TiO_2 , V_2O_5 , Pt, Pd*
2. *Chemical --- Metal Na, Active carbon*
3. *Physico-Chemical – SWO & Hot electron*
4. *Thermo destruction --- Incineration ($>800^\circ C$)*
5. *Photo destruction --- UV (250-340 nm)*
6. *Ozone destruction – O_3 , H_2O_2*
7. *Biological treatment – Fungi, Bacteria*



Factors most likely responsible for the environmental persistence

- 1. The organisms may lack the enzymatic or biological potential to degrade the compound.*
- 2. The compound may be unable to penetrate the cell.*
- 3. The concentration or the physical nature of the compound may be inhibitory to organisms or their enzymes.*
- 4. The steric configuration of the compound, including the position and degree of substitution, may prevent or hinder enzymatic attack.*
- 5. The environment may be toxic or deficient in some factor essential to support the growth of degradative organisms.*

History

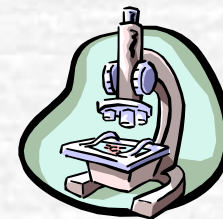
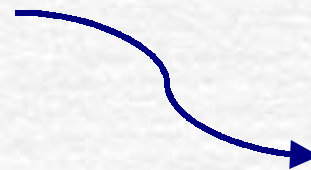
The first investigation : Kearny et .al (1972)

-Persistence and metabolism of chlorodioxins in soils.

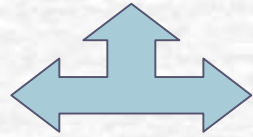
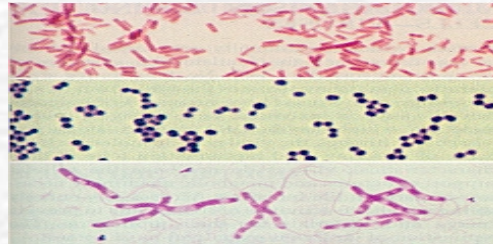
-ES & T 6:1010-1017.



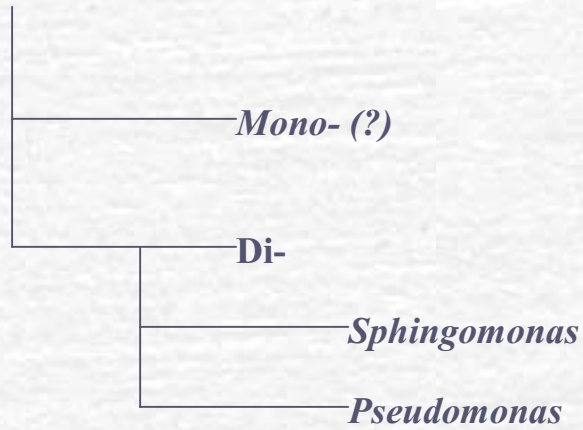
**Active research after explosion in chemical plant
belonging to the ICMESA firm near **Seveso**,
Northern Italy (July, 10th 1976)**



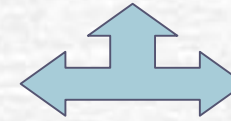
Prokaryotic cells
(Bacteria)



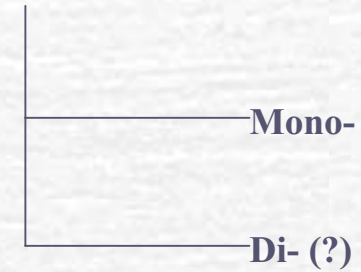
Aerobic ***Anaerobic (?)***



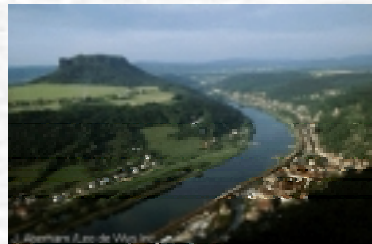
Eukaryotic cells
(Fungi)



Aerobic ***Anaerobic (?)***



By White Rot Fungus
(Phaanerrochaete chrysosporium
Science 1985)



Elbe river



쓰레기 소각장에서 나오는 **다이옥신**을 효율적으로 분해하는 버섯을 후쿠오카현 보건환경연구소와 규슈대학 농학부의 공동팀이 발견했다. 26일부터 도쿄에서 열리는 일본 약학회에서 발표될 예정이다. 부식된 나무에 번식하는 버섯의 일종이다. 오염 토양의 정화나 소각장의 처리 플랜트 등에 실용화 시도를 해볼 계획이다. 발견된 것은 [백색 부수균]라 불리는 흰버섯의 일종이다. 목재가 부식한 부분에 부착해, 나무의 주성분인 리그닌을 분해한다. 이제까지는 미국의 연구원이 살충제인 DDT 등을 분해하는 능력을 확인한 바 있으며, 이번에는 **다이옥신**에도 응용을 시도해 본 것이다. ◎ 배지에서 육성한 2mg의 균에, 소각재로부터 모은 약 2ng의 **다이옥신**을 투입, 1개월간 배양한 결과, 50~75%의 **다이옥신**이 이산화탄소와 물로 분해되었다고 한다. 후쿠오카현 보건환경연구소의 다카다 전문 연구원은 "버섯이 내는 효소가 **다이옥신**을 분해하는데 높은 효능을 가진 것은 아닐까?"라고 추측했다고 한다. ◎ **다이옥신**은 발암성이 지적되고 있으나 분해가 어려워 처리에 곤란을 겪고 있다. 발견된 버섯은 분해 속도는 늦으나, 간단한 설비로 분해가 가능할 것으로 보여져 폭넓은 사용이 기대된다

◎ 일본 에히메(□□)대학 농학부의 다찌바나(橘) 교수그룹은 쓰레기소각장에서 발생하여 암이나 기형의 원인이 되는 유해물질인 **다이옥신**을 효과적으로 분해하는 균을 발견했다. 이는 버섯의 일종인 목재 부패균으로, 실험에 의하면 고농도의 **다이옥신**을 90% 가까이 분해한다고 한다. 앞으로 세균융합 등을 통해 더욱 분해능력이 높은 균을 개발, **다이옥신** 처리를 위해 실용화할 생각이다.



From : KORDIC news, 2000

*White rot fungus*의 다이옥신 분해율

화합물	분해율(%)		
	<i>P.sordida</i>		<i>P.chrysosporium</i> (글루코스 첨가)
	(글루코스첨가)	(글루코스무첨가)	
2,3,7,8-TCDD	22.1±0.0	26.5 ±1.5	37.1 ±5.9
1,2,3,7,8-PeCDD	24.3 ±1.9	26.7 ±6.5	53.9 ±6.8
1,2,3,4,7,8-HxCDD	35.3 ±5.0	43.7 ±4.0	64.9 ±5.7
1,2,3,4,6,7,8-HpCDD	10.3 ±0.6	38.1 ±4.3	53.6 ±7.9
OCDD	6.20 ±2.0	14.2 ±4.1	41.1 ±7.6
2,3,7,8-TCDF	28.6 ±5.6	38.8 ±1.2	27.3 ±8.0
1,2,3,7,8-PeCDF	22.0 ±1.5	24.2 ±3.3	38.5 ±5.7
1,2,3,4,7,8-HxCDF	41.5 ±1.6	50.4 ±2.6	54.7 ±6.1
1,2,3,4,6,7,8-HpCDF	22.0 ±1.2	39.5 ±3.2	51.9 ±7.1
OCDF	13.7 ±1.0	35.0 ±2.8	58.6 ±12.3

Fungi VS Bacteria

Advantages of fungal metabolism

1. No adaptation to specific chemicals.
2. Acting on whole compound classes.

Disadvantages of fungal metabolism

1. Complex nutritional requirement.
2. Low specificity (Low yield).
3. High oxygen demand.
4. Difficult industrial application.

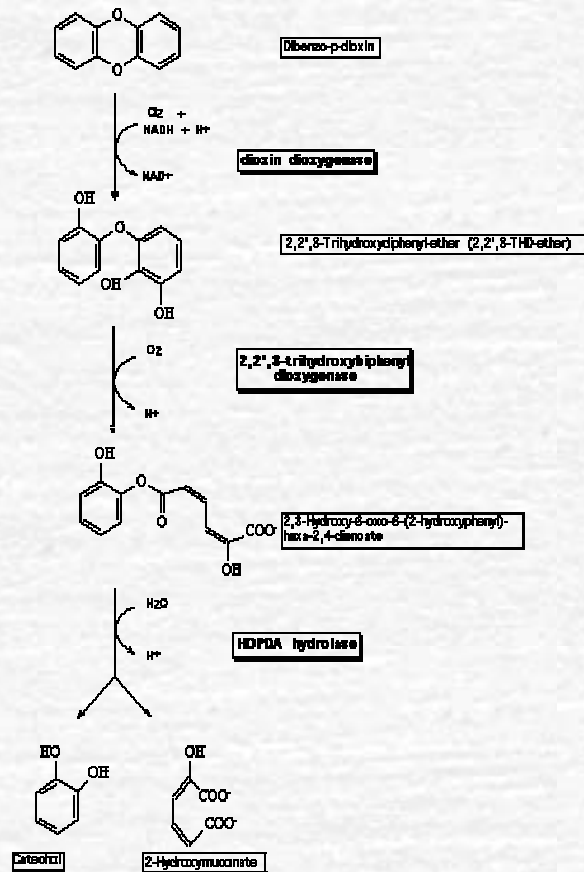


Aerobic microorganisms able to degrade dioxins

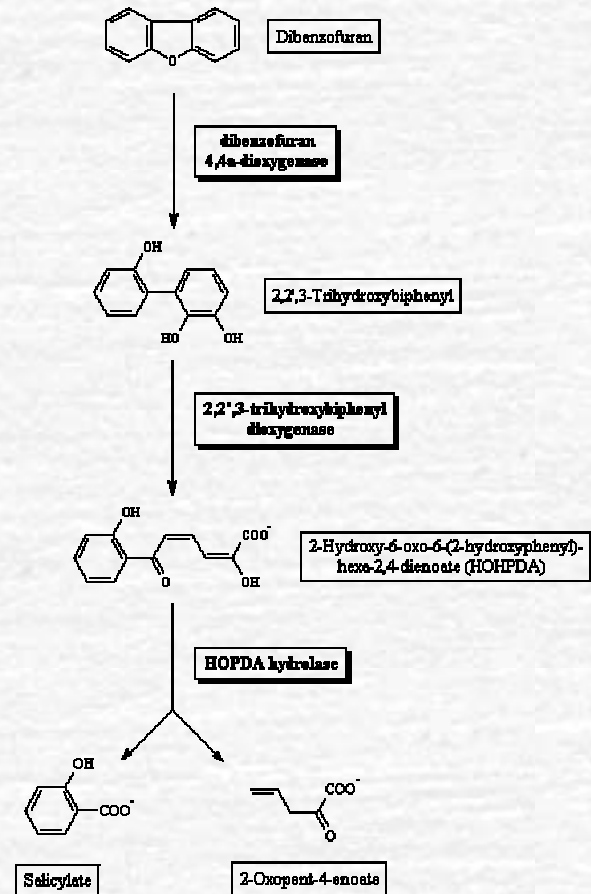
<i>Strain</i>	<i>Substrate</i>
<i>Bacillus megaterium</i>	TCDD (?)
<i>Escherichia coli</i>	4-nitro-DE
<i>Pseudomonas cepacia</i>	DE
<i>P. cruciviae</i>	DE
<i>P. delafieldii</i>	3-POB,4-POB
<i>P. pseudoalcaligenes</i>	3-POB,4-POB
<i>P.sp.</i>	DD,DF
<i>Sphingomonas sp.</i>	DD,DF
<i>Staphylococcus auriculans</i>	DD
<i>Terrabacter sp.</i>	DF
<i>White Rot Fungus.</i>	TCDD

Non-Chloro

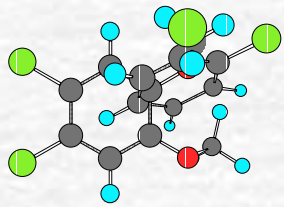
Dibenzo-p-dioxin



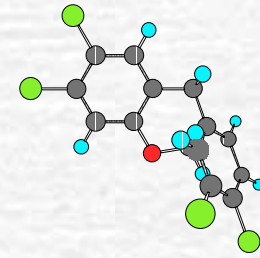
Dibenzofuran



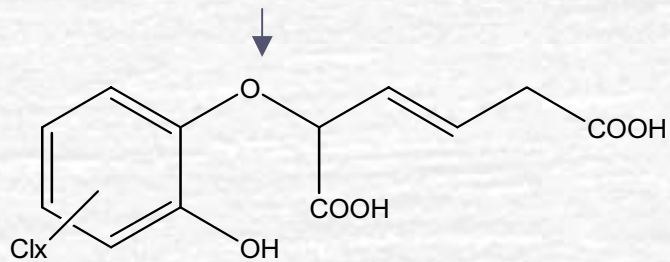
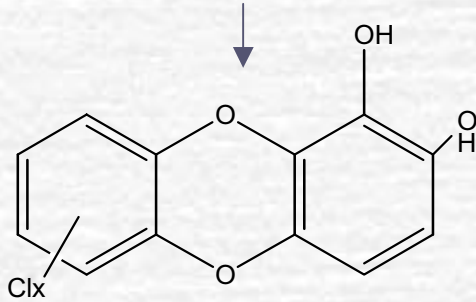
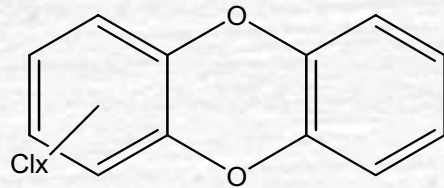
Mono, di, tri-Chloro



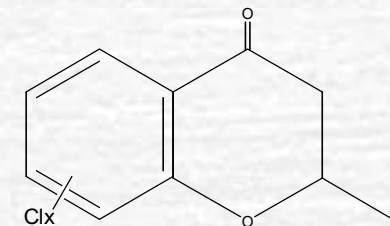
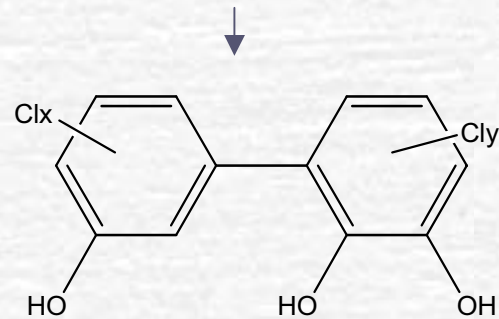
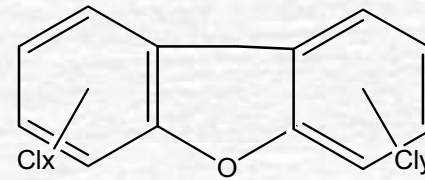
Dibenzo-p-dioxin



Dibenzofuran



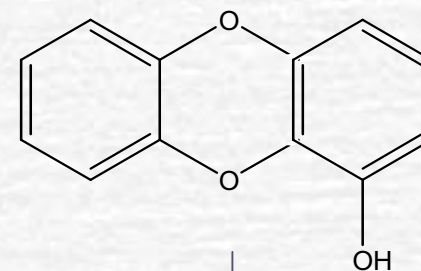
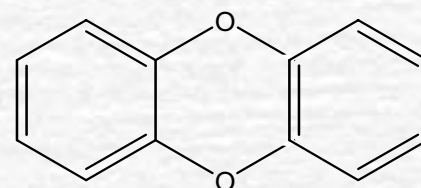
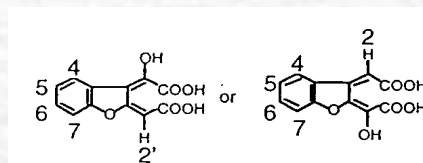
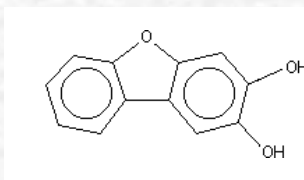
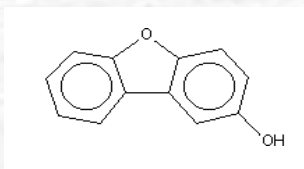
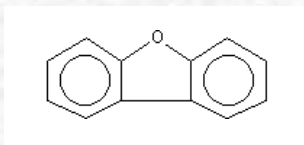
Chlorocatechol



Chlorocatechol



Mono-oxygenation



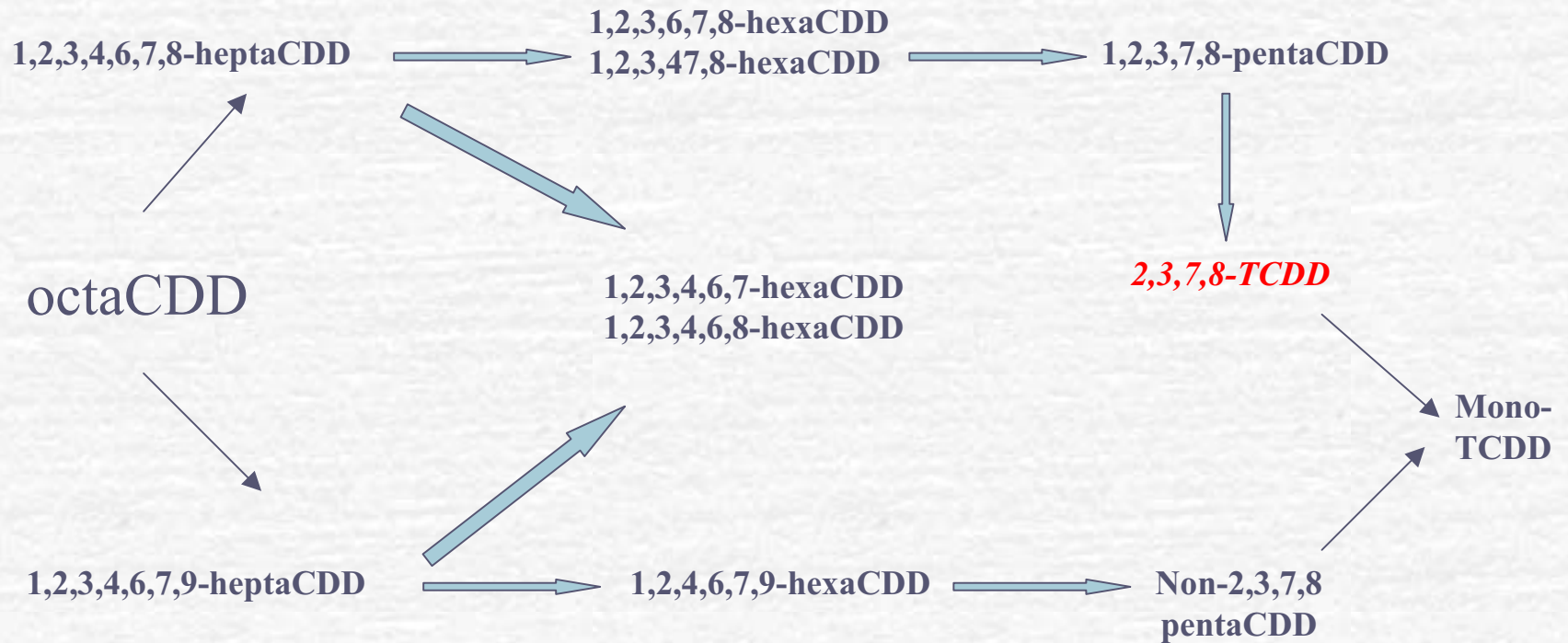
Dead end product
(*Pseudomonas* and Fungi)



By *S.auriculans* DBF63



Observed not explained



Branched pathway of PCDD microbial dechlorination

** Reproduced from : AEM, 1996, 4556-4562*

Conclusion and prospects

- 1. The attack by bacterial angular dioxygenase seems to be restricted to all mono- and some di or tri chlorinated DD and DF.*
- 2. Extension of the substrate range of DD- and DF- mineralization has to be achieved.*
- 3. A process based on anaerobic dehalogenation of PCDD/F and subsequent degradation of less halogenated products in aerobic should be recommended.*
- 4. Provided that biodegradation rates can be increased significantly by the use of (bio-) emulsifiers.*

SEAL

포항공대 환경공학부 환경분석연구실
Scientific environmental analysis lab

DIOXIN LAB



Biosorption vs. Biodegradation (for the treatment of inert organic compounds)

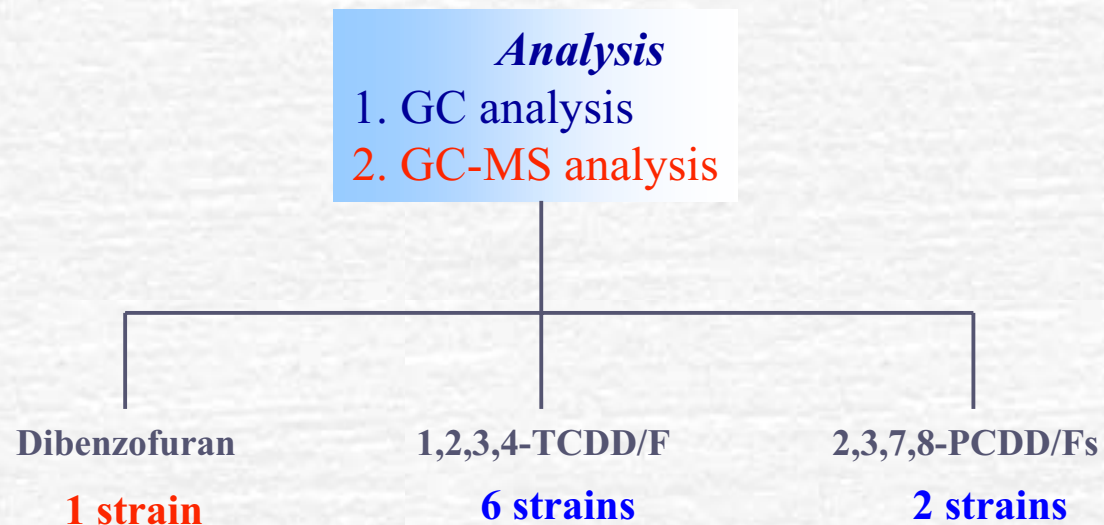
	Biosorption	Biodegradation
Advantage	<ol style="list-style-type: none">1. Nontoxic byproducts2. Rapid	<ol style="list-style-type: none">1. Ultimate goal2. No secondary treatment
Disadvantage	<ol style="list-style-type: none">1. Secondary treatment required	<ol style="list-style-type: none">1. Slow2. Toxic byproducts in some cases



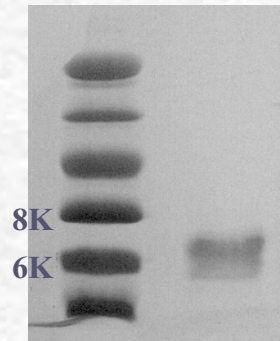
A. Bio-degradation

Isolation of Microorganisms

1. Nutrient broth (180 colonies from soil, waste drain and hood)
2. Minimal salts Medium with 1,2,3,4-TCDD/F or 2,3,7,8-PCDDs (9 strains)



B. Bioadsorption

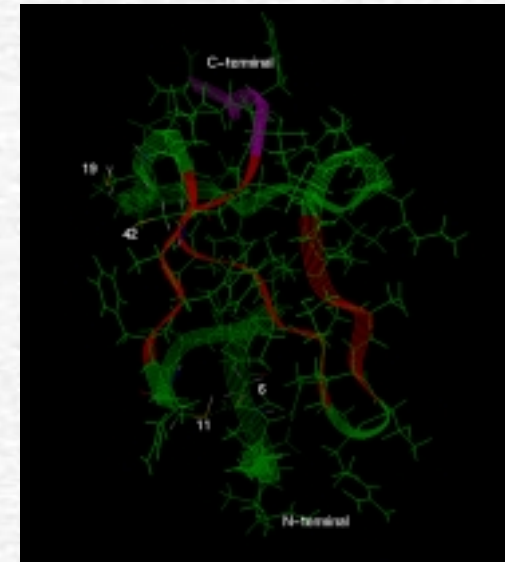


From *Bacillus Pumilus* PH-01

Protein Sequence

n-AQNVLCEVNSCR
FWADNSCTASTIK
IGK
STMTDVTR
NAETDCETFETQ-c

Results of Protein Molecular Modeling



Adsorption of another dioxin isomers

Research on going

Bio-adsorption

**Complete structure
determination**

Kinetic Study

**Mass production through
molecular biology**

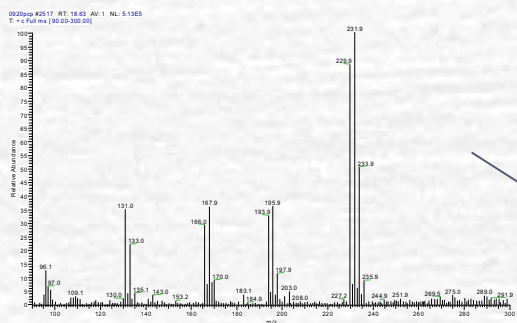
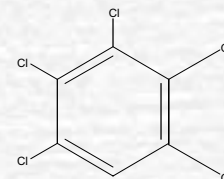
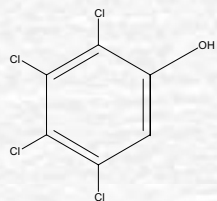
Bio-degradation

**Development of field situation
applicable Microorganisms**

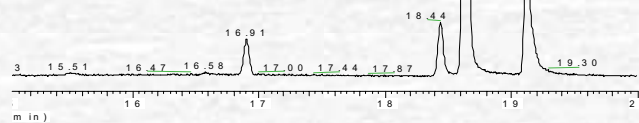
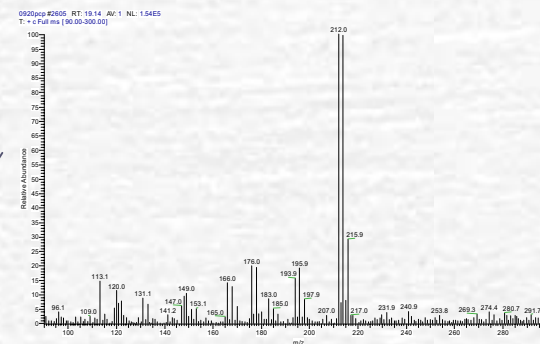
***Development of
Hybrid system***

```
graph LR; A[Complete structure determination] --> C[Development of field situation applicable Microorganisms]; B[Kinetic Study] --> C; C --> D[Development of Hybrid system]; E[Mass production through molecular biology] --> D;
```

Bio-transformation of PCP by mixed culture



N L :
3 7 4 E 6
T I C M S
0 9 2 0 p c p



After 3 months of incubation

다이옥신 관련 화합물을 분해 할수 있는 호기성 세균의 초기 효소, 기질, 공동 기질

세균	초기효소	기질(공동기질)
<i>Acinetobacter</i> sp. PE7	N.D	4-클로로-DE
<i>Alcaligenes</i> Jb1	N.D	2-클로로-DD
<i>Bacillus denitrificans</i> (ATCC 13368)	N.D	TCDD
<i>Beiferinckia</i> sp. B8/B6	디옥시게나제	DF,DD
DPO220	DF 4,4a-디옥시게나제	DF
<i>Erwinia</i> (mutated) CU3614	N.D	4-니트로-DE
<i>E.coli</i> JM107i	N.D	4-니트로-DE
<i>P. cepacia</i>	N.D	DE
<i>P. cruciviae</i> 593B1	N.D	DE
<i>P. delafieldii</i> ET1	N.D	3,4-POB
<i>P. Pseudoalcaligenes</i> POB310	POB 1,2- 디옥시게나제	3,4-POB
<i>P.</i> B13-D5	POB 1,2- 디옥시게나제	3,4-POB
<i>P.</i> B13-D5C	POB 1,2- 디옥시게나제	3,4-POB
<i>P.</i> HL7B	N.D	DF
<i>P.</i> N.C.I.B 9816	디옥시게나제	DD
P. NSS2	POB 1,2- 디옥시게나제	3-POB, 4-POB
<i>P.</i> pBS3	N.D	DF
<i>Rhodococcus</i> sp. HH19K	N.D	DF
<i>S.</i> HH19K	DF 4,4a-디옥시게나제	DF
<i>S.</i> HH69	DF 4,4a-디옥시게나제	DF
<i>S.</i> RW1	DF 4,4a-디옥시게나제	DD,DF
<i>S.</i> SS3	DE 1,2-디옥시게나제	DE
<i>S.</i> SS31	DE 1,2-디옥시게나제	DE
<i>S.</i> SS33	DE 1,2-디옥시게나제	DE
<i>Staphylococcus auriculans</i> BDF63	DF 4,4a-디옥시게나제	DF
<i>Terrabacter</i> sp. DPO360	DF 4,4a-디옥시게나제	DF
<i>Terrabacter</i> sp. DPO1361	DF 4,4a-디옥시게나제	DF

참고: 첨단 환경기술 2000.12

약어

N.D : Not Detected DE: 디페닐에테르 DD: 다이벤조-p-다이옥신 DF: 다이벤조퓨란 POB : 페녹시안식향산

P : *Pseudomonas* S: *Sphingomonas*