Biodegradation of Benzene-d_6 Associated with Various Black Carbon Materials by *Pseudomonas Putida* strain F1: A Static Solid State ^{2}H NMR study

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Black Carbon

- BC: strong adsorbent for Aromatic Compounds

	Diesel Soot (SRM 1650)	**Charcoal** (bark, <150 µm)	**Activated Carbon** (peat based, 4-14 mesh, Sigma-Aldrich)	**MWCNT** (OD:8-15 ID: 3-5 nm)
Phenanthrene	**5.41[a]**	**7.45[a]**	**8.76[a]**	**6.42[b]**
Anthracene	**5.72[a]**	**7.61[a]**	**8.96[a]**	
Fluoranthene	**6.30[a]**	**7.82[a]**	**9.06[a]**	
Naphthalene				**4.68[b]**
Pyrene				**5.33[b]**

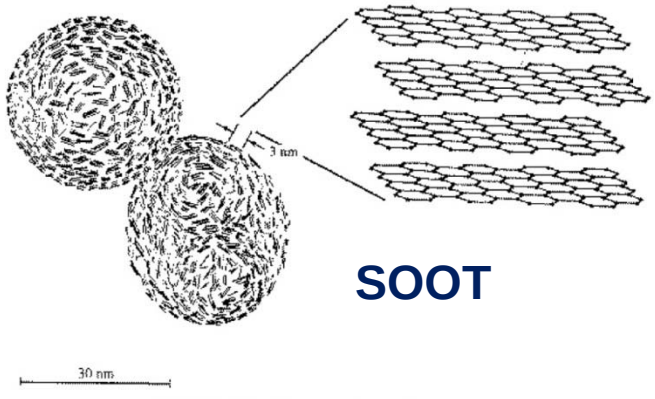
Logarithmic Sorbent-Water Distribution Coefficients (Log K_d, L/kg sorbents)

Research Question:

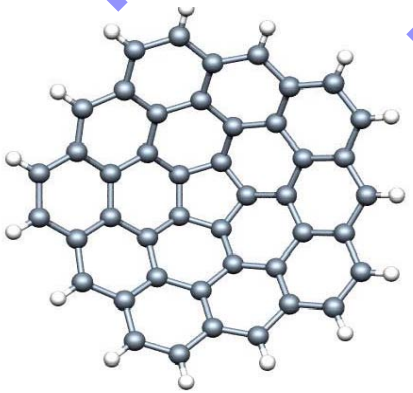
What controls the bioavailability of aromatic compounds associated with BC?

*(a) Jonker, M.T. & Koelmans, A. Environ. Sci. Technol. **2002**, 36. 3574-3734.*
*(b) Xing, B. et al.. Environ. Sci. Technol. **2006**, 40. 5804-5810*

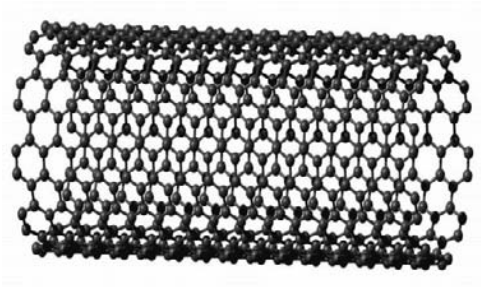
Black Carbon



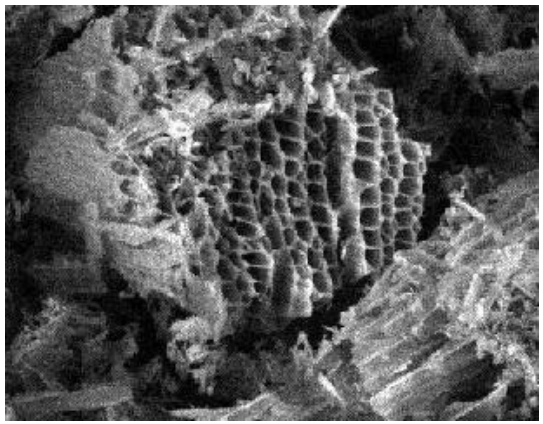
SOOT



GRAPHENE LAYER



CARBON NANOTUBES



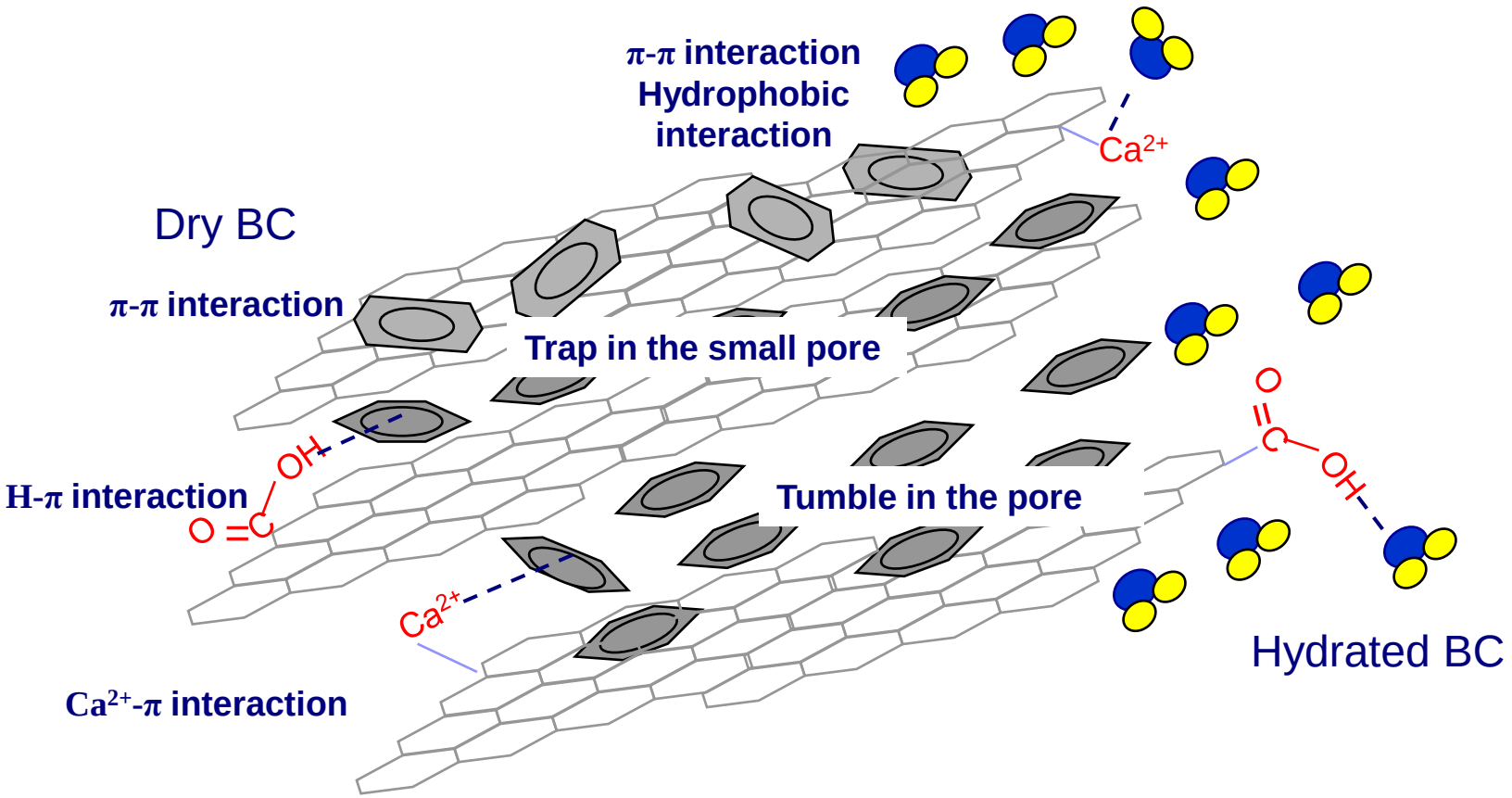
ACTIVATED CARBON

- Same unit structure: Graphene sheet
- Different bulk structure
- Porosity
- Polar functional group: -OH, -COOH…

http://www.atmos.umd.edu/~pedro/soot2.jpg
www.ewels.info/img/science/graphite/index.html

Hypothesis

The bioavailability of benzene associated with BC is a function of the BC structure because of the different molecular interaction between benzene and BC



Experimental Design

How bioavailable is benzene associated with different BC materials?

Variability in biodegradation of benzene as a function of BC

Biodegradation Experiment

Loss of benzene associated with BC over time

Molecular interaction of benzene influences the biodegradation

Solid state ^{2}H NMR Experiment

Change of molecular motion after incubation

Experimental

Sample preparation: Biodegradation Experiment

- Sample: Black Carbon + Benzene-d_6 + Minimal Media + Bacteria

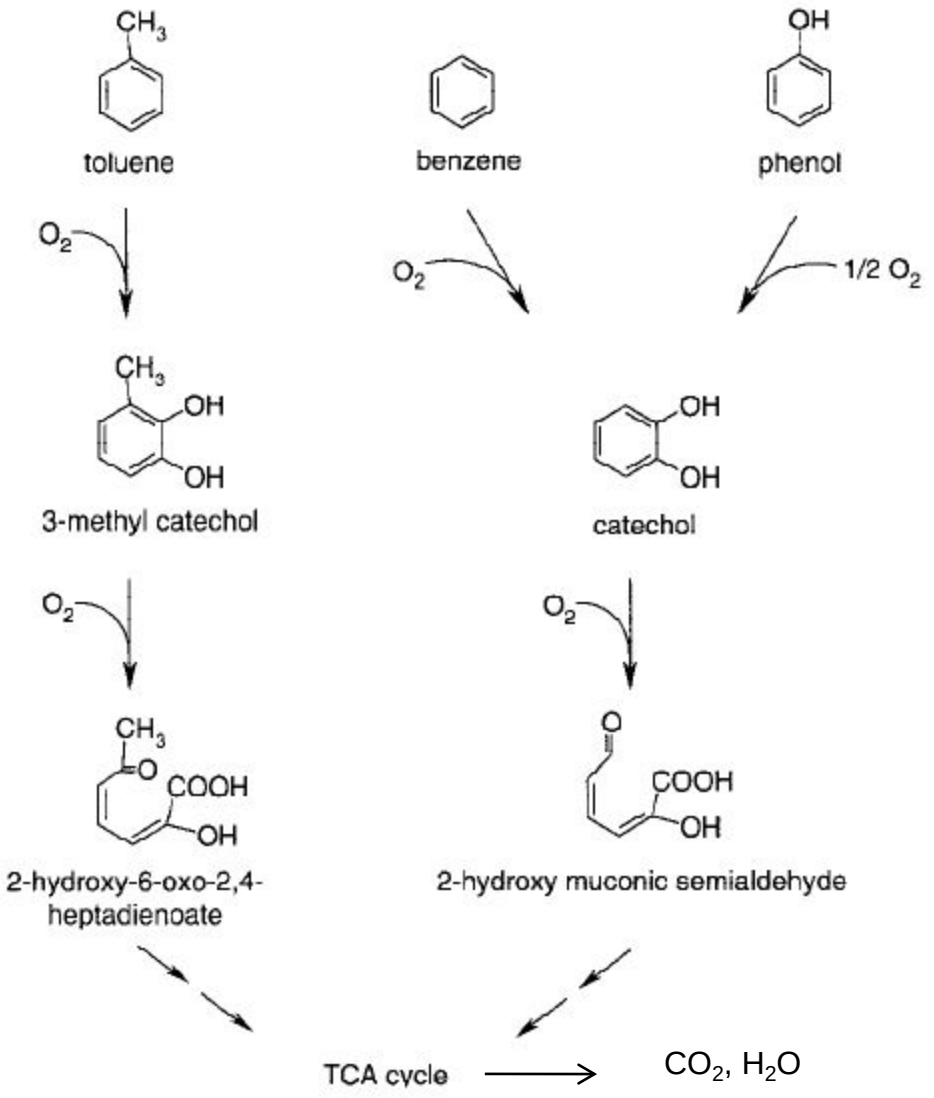
Sample	Surface Area (m^2/g)	Pore size (nm)	SA coverage by 1% benzene w/w
Graphite (325 mesh, 44µm dia.)	4.5		697.0%
Carbon nanoparticle (amorphous, OD = 60-100 nm)	100	<< 30	30.7%
Multi-walled Carbon nanotube (OD = 40-70 nm, L= 0.5-50 µm)	105	5-10	26.8%
Soot (NIST #2975**)**	91	20	34%
Activated Carbon (Darco G-60, 100 mesh)	776	4-50	3.9%

- Hydration time: 48 hours; Equilibrium time: 8-10 hours
- Incubation time: 60 hours
- Benzene concentration is analyzed by headspace GC-FID

Experimental

- ***Pseudomonas putida* strain F1**:
  - ✓ a fast-growing, gram-negative bacterium
  - ✓ contains a plasmid that codes for all the enzymes necessary for it to degrade toluene, benzene, and other aromatic hydrocarbons.
  - ✓ uses the hydrocarbons as a food source.

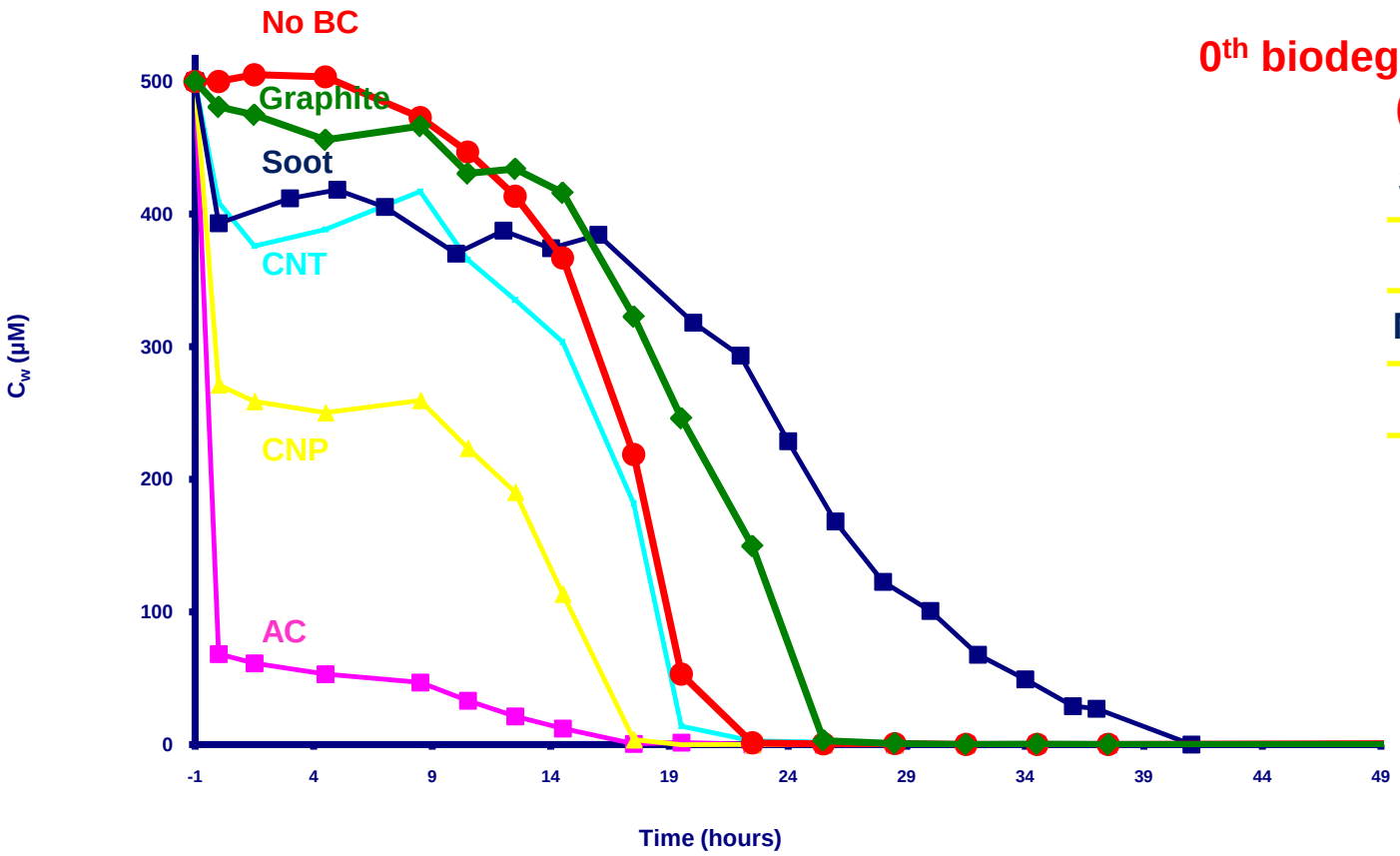
SEM image of Putida F1.



P. Putida F1 catabolic pathways for toluene, benzene and phenol

Reardon et al., Biotechnology and Bioengineering, 2000

Biodegradation Results



0th biodegradation rate constant (µmoles/hrs)

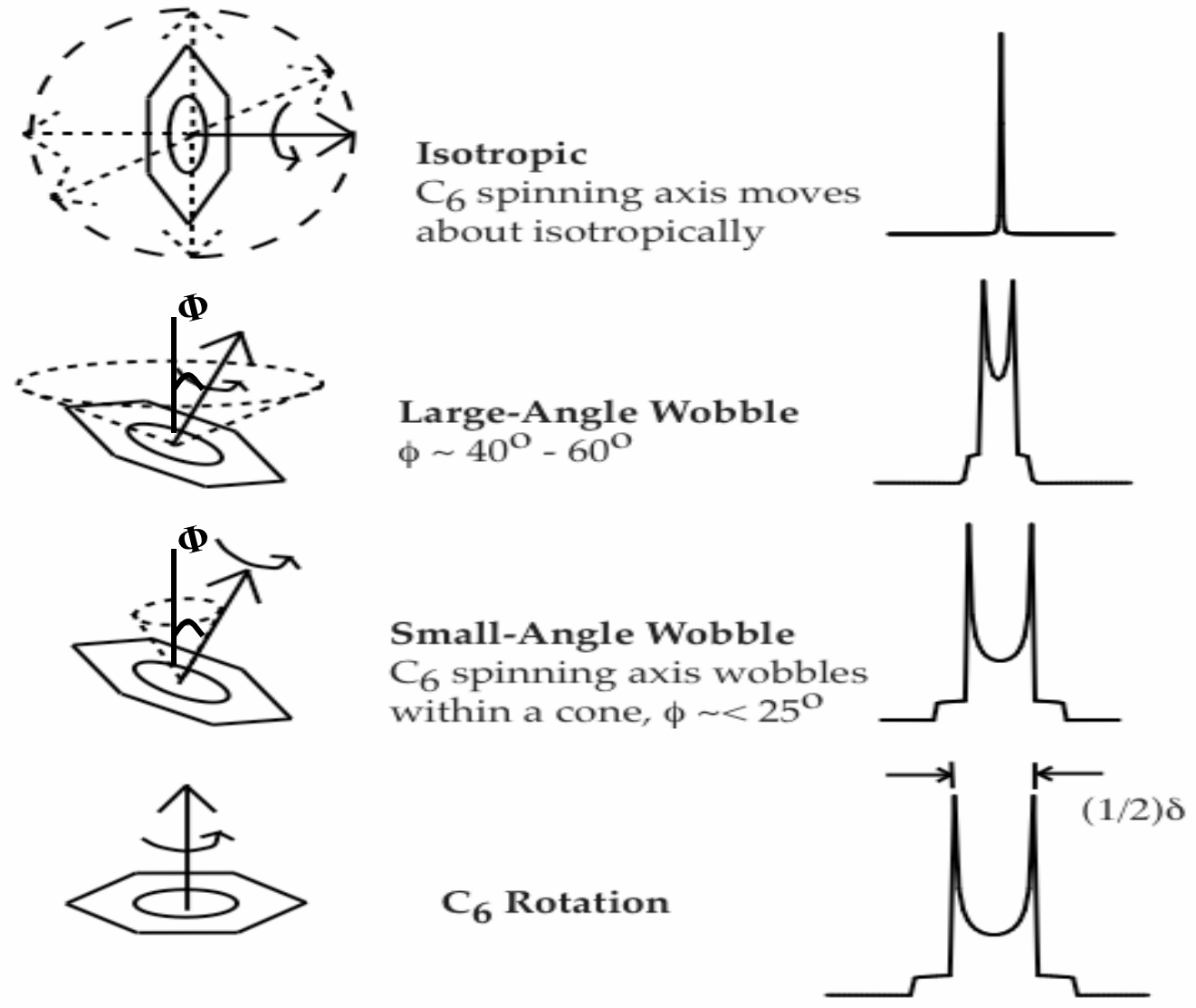
Sample	µmoles	%
Graph	0.000	0.0
MWCNT	0.000	0.0
CNP	0.085	17.0
AC	0.234	46.8

Benzene remained in BC after 60 hrs

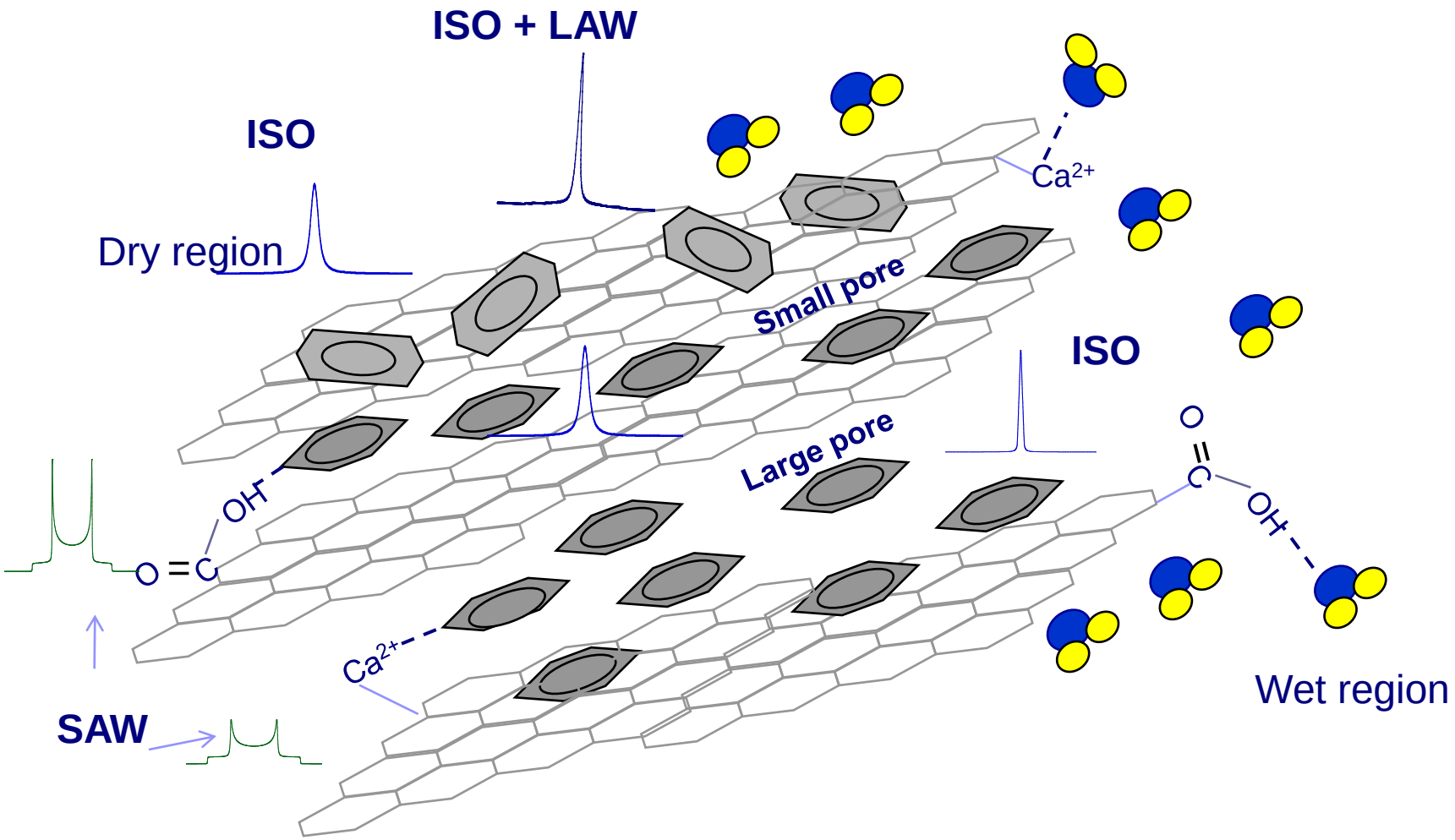
Soot	18.18
CNP	28.64
Graph	29.69
MWCNT	37.56
No BC	43.10

Biodegradation of benzene varies with black carbon materials

Static Solid-state ^{2}H NMR Benzene-d_6 Spectra for Common Motional Models

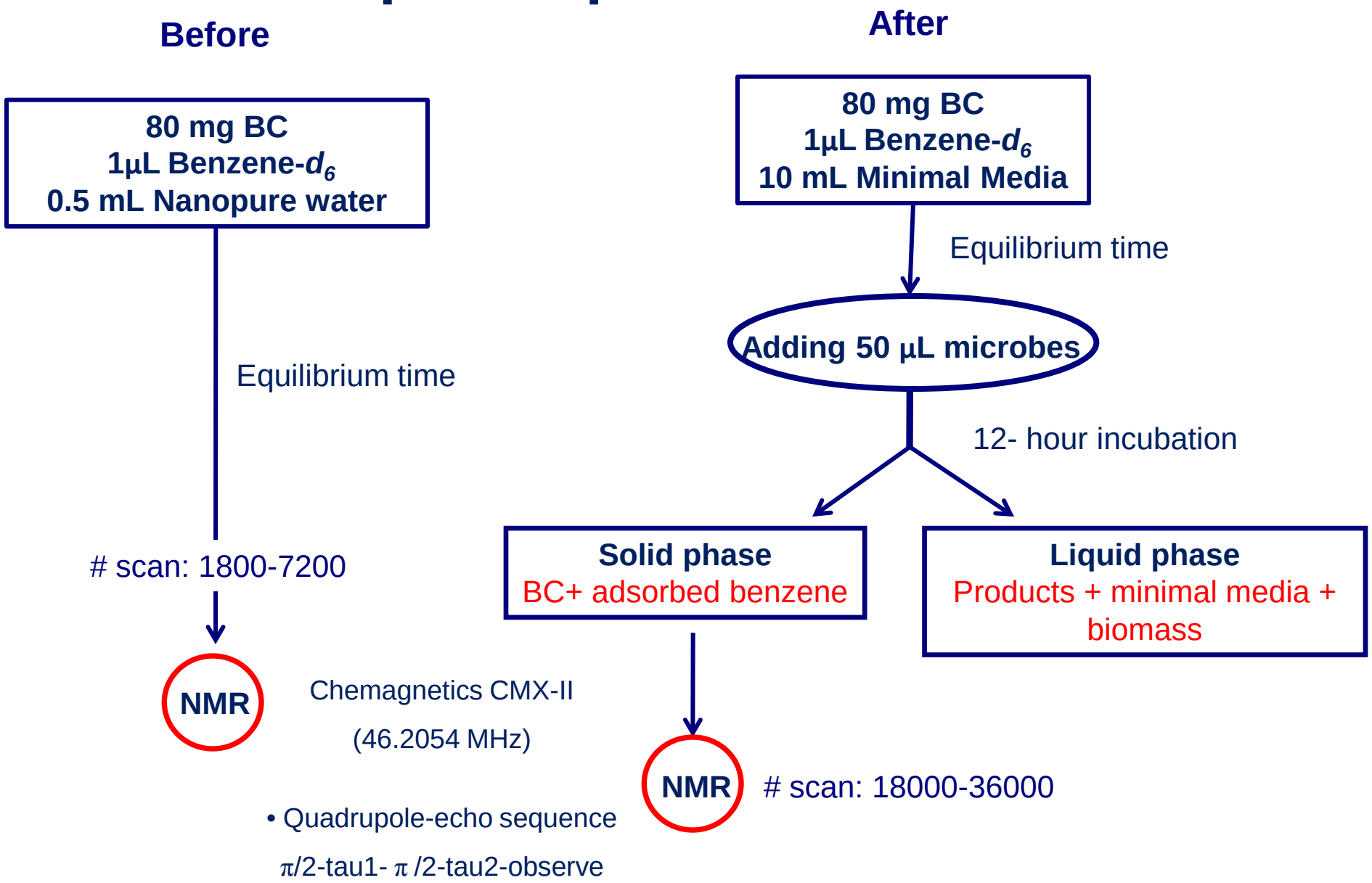


Expected NMR Spectral Pattern



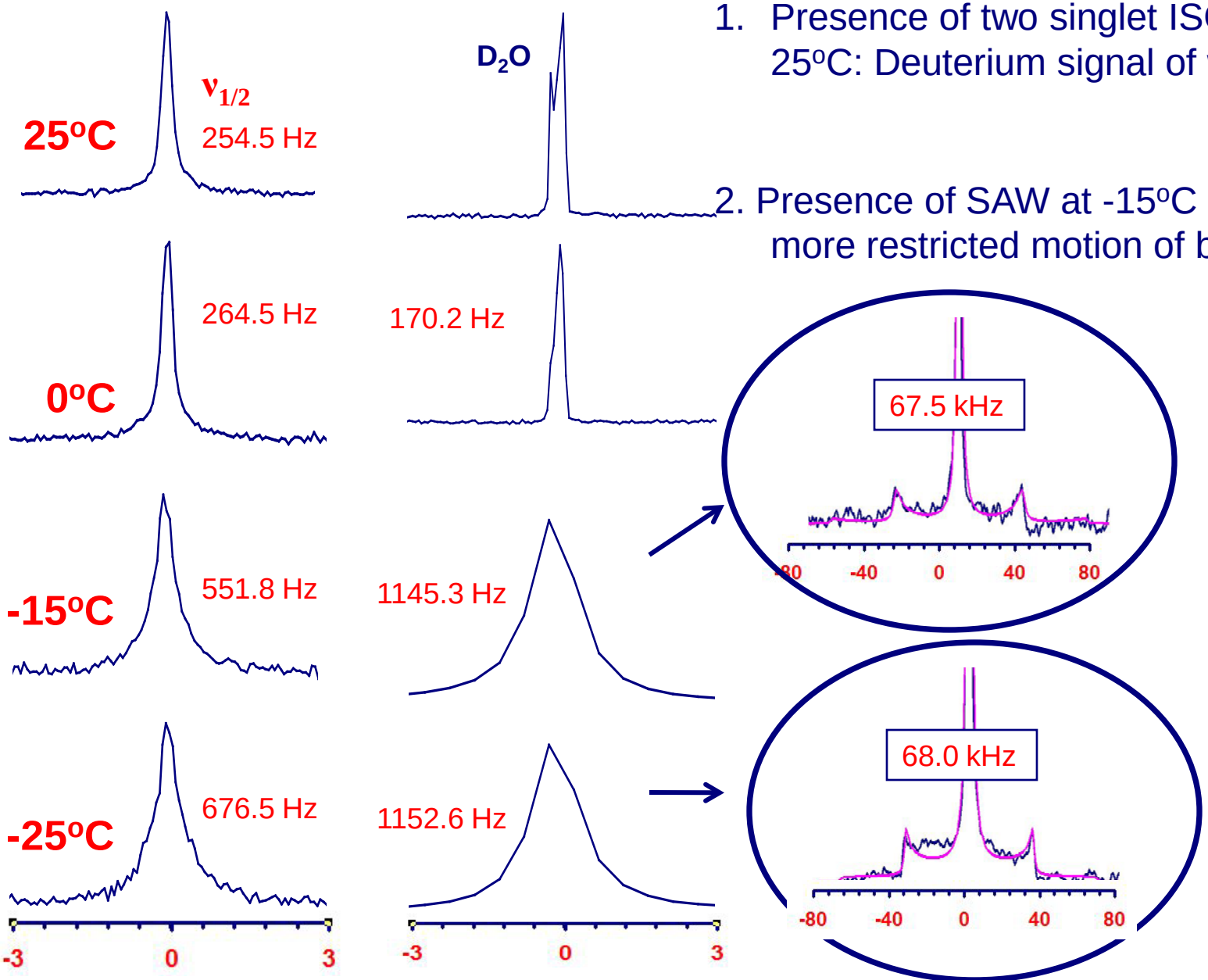
Nanny and Eastman. J. Magn. Reson. 2007

NMR Sample Preparation



Vold et al. J. Magn. Reson. 69,1986

SOOT

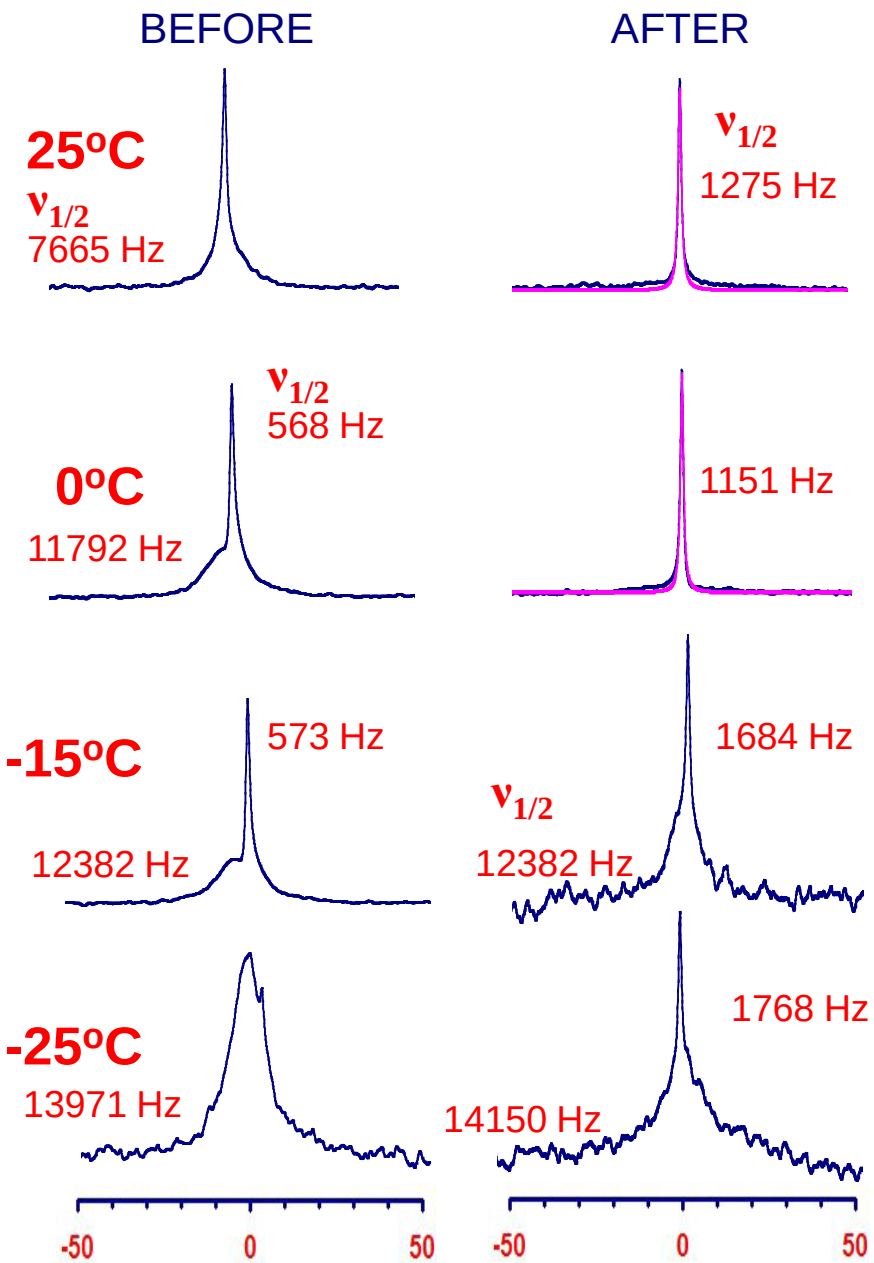


After

1. Presence of two singlet ISO peaks at 25°C: Deuterium signal of water

2. Presence of SAW at -15°C and -25°C: more restricted motion of benzene

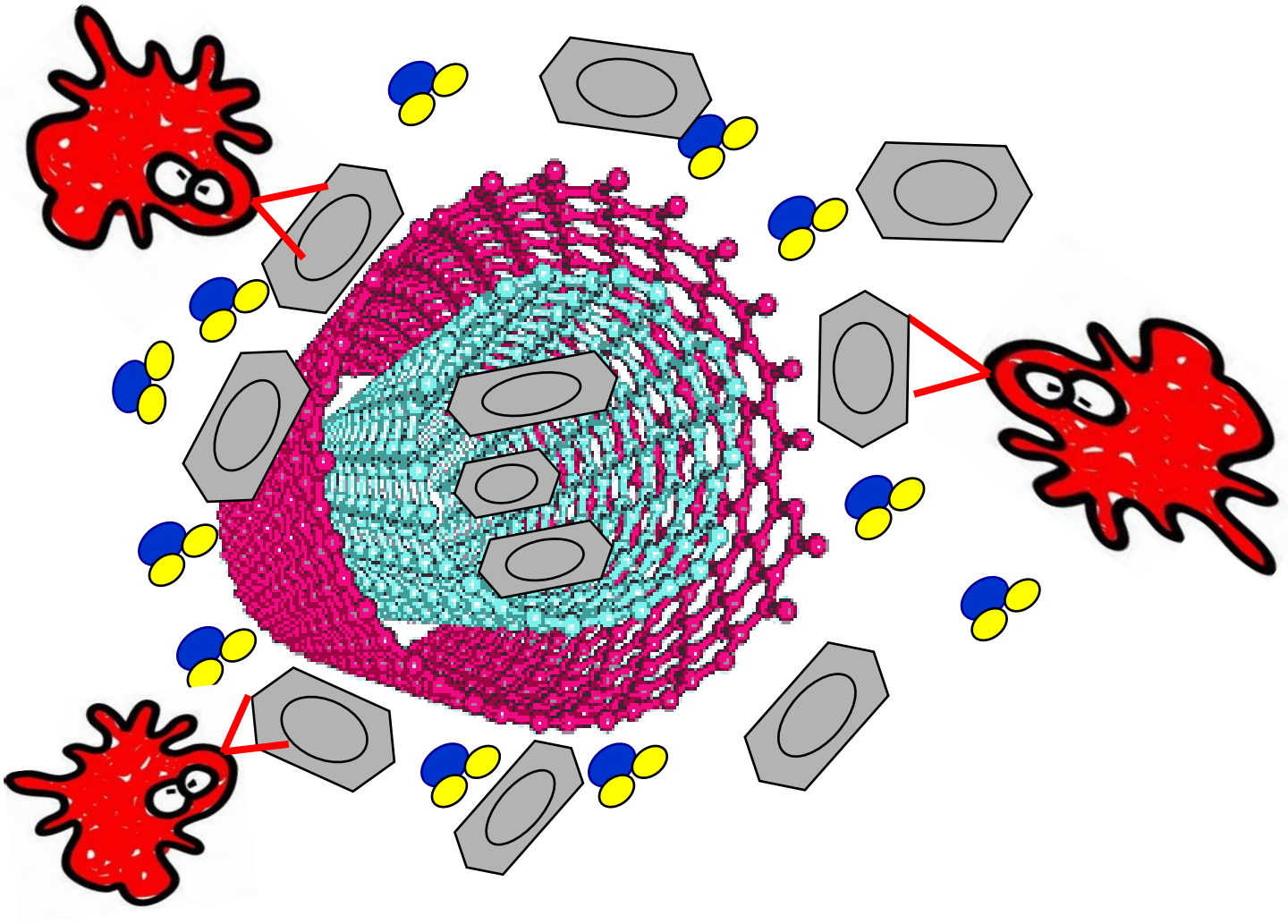
CARBON NANOTUBES



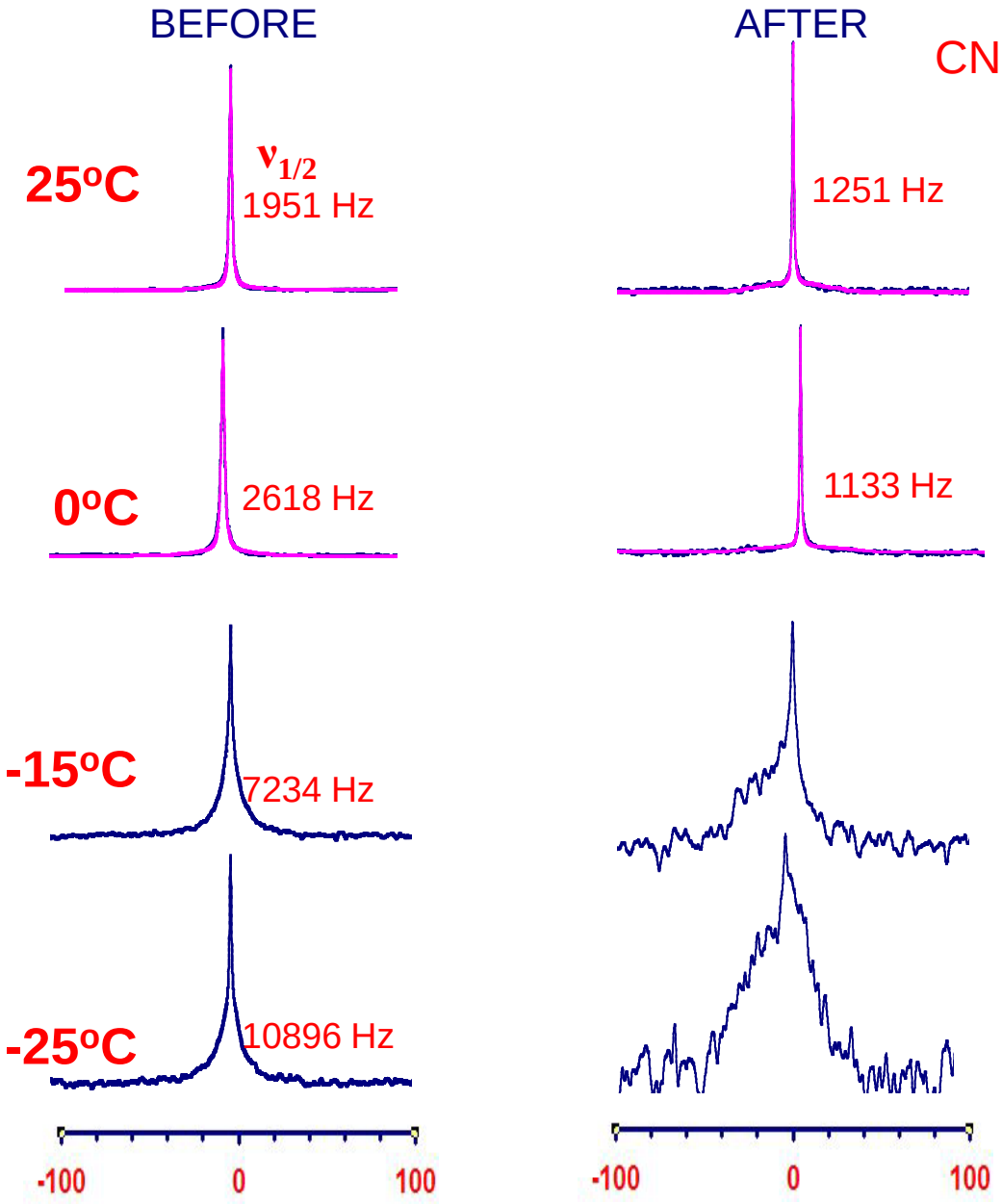
After:

1. Freezing point depression
2. Decrease of broad peaks: remaining benzene in nanopores

CARBON NANOTUBES



CARBON NANOPARTICLES



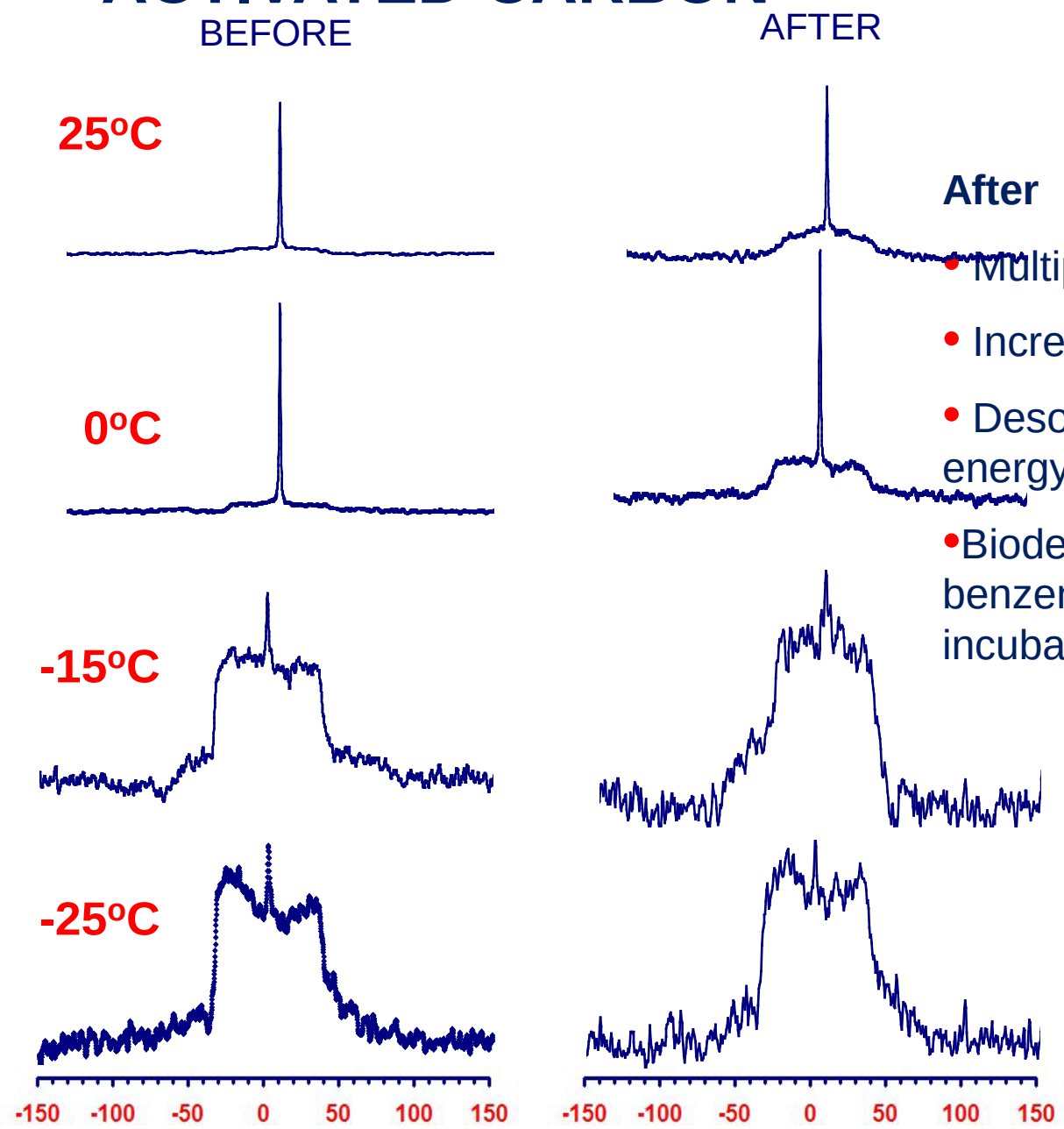
CNP: laser ablation, amorphous structure

$[Ca^{2+}]$ = 767 ppm

After:

- Freezing point depression
- More restricted motion at -25°C and -15°C: Benzene interacts with Ca^{2+} through Ca^{2+}-π interaction
- Biodegradation experiment: 17% of benzene remaining after 60-hour incubation with microbe

ACTIVATED CARBON



After

- Multiple associations: SAW, LAW, ISO
- Increase of SAW+LAW
- Desorption of benzene from low site energy for biodegradation
- Biodegradation experiment: 45% of benzene remaining after 60- hour incubation with microbe

CONCLUSIONS

1. **The biodegradation of benzene associated with BC varies with BC materials**
2. **The molecular interaction of benzene influence strongly the bioavailability of benzene in different ways**
    - Benzene which associates with BC surface through π-π interaction or hydrophobic force are readily accessible and degraded by microbes
    - Benzene which penetrates and tumbles in the pore can diffuse and becomes accessible for biological degradation
    - Benzene which strongly associates with high energy site of BC by forming π-H or π–Cation bonding is not very accessible to microbes

Acknowledgement

- **National Science Foundation (**NSF NIRT 0210839) *"Bioavailability of Aromatic Hydrocarbons and Their Interactions with Natural Organic Matter: Linking Molecular- and Microbial-Scale Interactions"*
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QUESTIONS AND COMMENTS ?