

## A New Method for Labeling Humic Substances with Tritium: Future Prospects for Biological Studies

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# Contents

- \* Introduction
- \* How to produce  $^3\text{H}$  labeled HS
- \*  $^3\text{H}$ -HS for studying biological properties of HS
- \*  $^3\text{H}$ -HS for quantification of interaction of HS with biota
- \* Visualization of humics-biota interaction using  $^3\text{H}$ -HS
- \* Future prospects

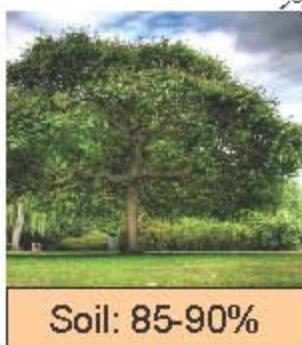
# What humics are?

Humic substances

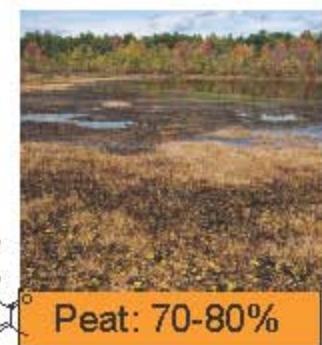
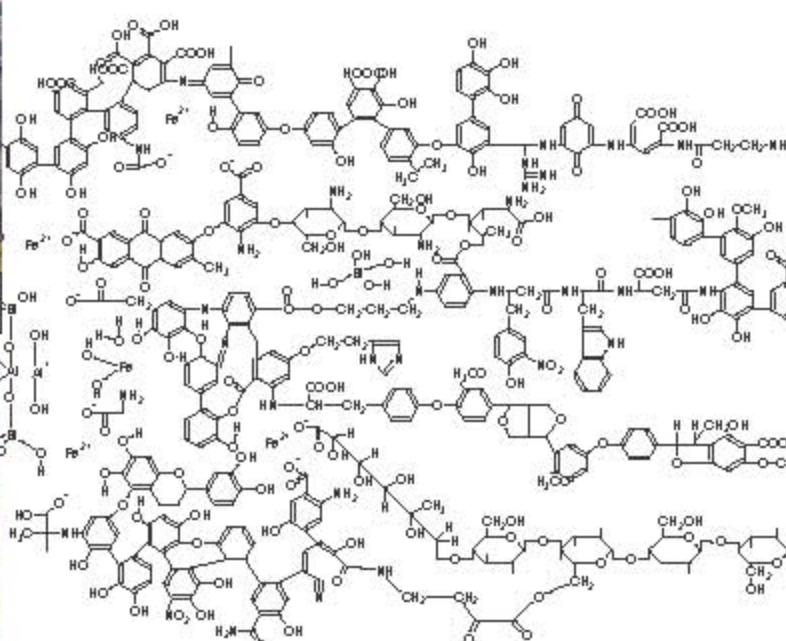
are high-molecular-weight polymers originating from decomposition of organic matter



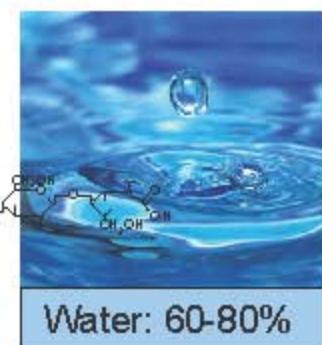
Coal: 15-60%



Soil: 85-90%



Peat: 70-80%



Water: 60-80%

## HS properties

- ★ Polyfunctionality:  $-\text{COOH}$ ,  $\text{C}_{\text{Ar}}-\text{OH}$ ,  
 $>\text{C}=\text{O}$ ,  $-\text{CH}_n$
- ★ Presence of hydrophilic and hydrophobic moieties
- ★ High molecular weight
- ★ Surface activity
- ★ Redox activity

# Humics: prerequisites for usage

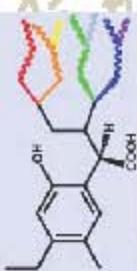
HS can be found  
everywhere

HS are  
polyfunctional

HS can interact  
with biota

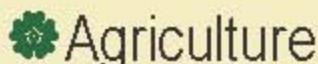
HS possess  
bioactivity

\* HS are promising agent for usage in  
agriculture, veterinary, and medicine

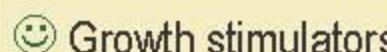


# Humics as biological agents

## Positive



Agriculture



Growth stimulators



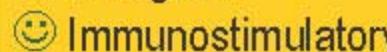
Veterinary



Antidiarrheal



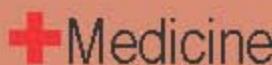
Analgesic



Immunostimulatory



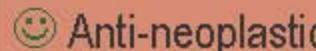
Antimicrobial



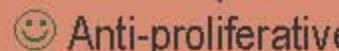
Medicine



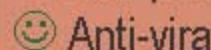
Anti-inflammatory



Anti-neoplastic



Anti-proliferative



Anti-viral

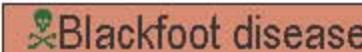
## Negative



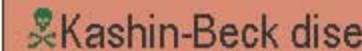
Inhibiting activity



Negative effects



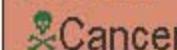
Blackfoot disease



Kashin-Beck disease



Goiter

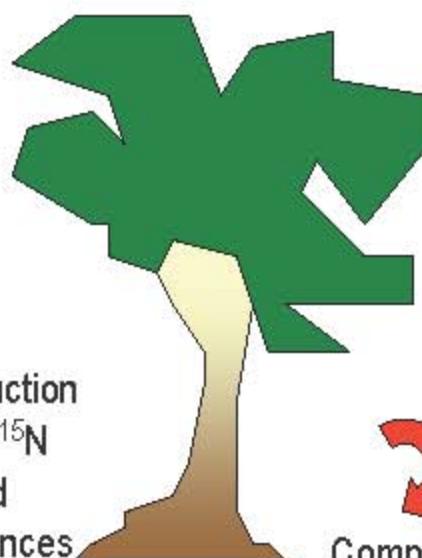


Cancer

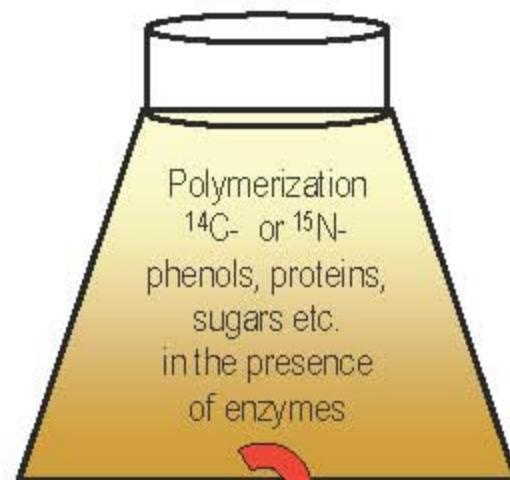
\* HS possess bimodal activity

# How to trace humics?

Isolation from  
labeled substrate



Synthesis from  
precursors



$^{14}\text{C}$  or  $^{15}\text{N}$  analogues of humic substances

\* Direct radioisotope labeling of HS is deadly needed

# Why tritium?

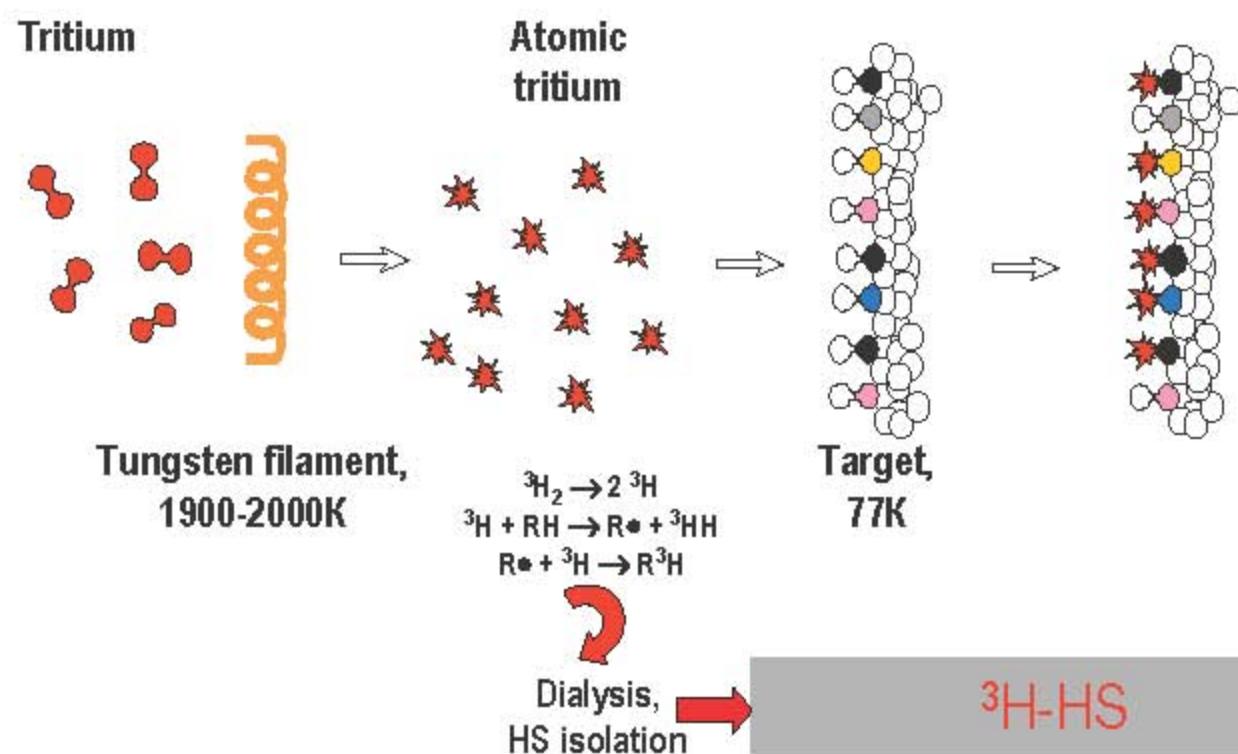
- \* The decay is a simple one-step process



- \* Energy of  $\beta$ -radiation is 18 keV
- \*  $\beta$ -particles path length in condensed media reaches 1.6  $\mu\text{m}$
- \* Tritium that is bound to a substance is best measured by liquid scintillation counting

# Preparation of $^3\text{H}$ -HS

## Tritium thermal activation method



Radiat. Phys. Chem. 98, 1–5 (2013). DOI 10.1016/j.radphyschim.2013.06.160  
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A new technique for tritium labeling of humic substances

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# SEC analysis of $^3\text{H}$ -HS

Wrong conditions of tritium introduction

The figure displays two SEC chromatograms side-by-side. The left chromatogram, labeled 'Parent HS' at the top right, shows a single sharp green peak at A254 absorbance, indicating a single homogenous population of high-molecular-weight hyaluronic acid. The right chromatogram, labeled 'Labeled HS' at the top right, shows a broad red peak at Radioactivity, indicating a heterogeneous population where the label has been introduced under incorrect conditions, leading to lower molecular weight fragments.

A254

Parent HS

Radioactivity

Labeled HS

V, ml

V, ml

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\* SEC analysis can be used to study  $^3\text{H}$  distribution

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# SEC analysis of $^3\text{H}$ -HS

Wrong conditions of tritium introduction

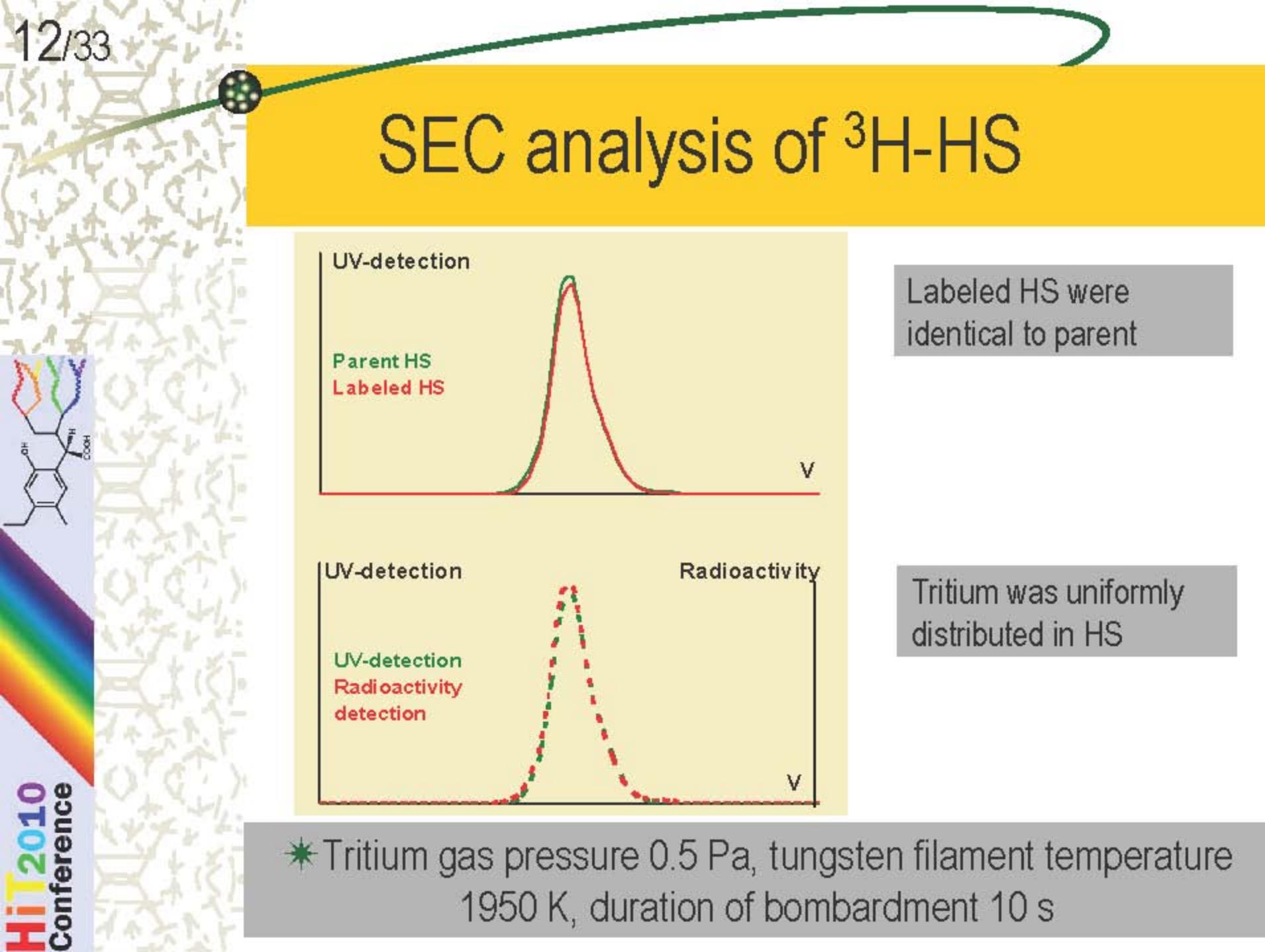
The figure displays two SEC chromatograms side-by-side. Both plots have 'A254' on the y-axis and 'V, ml' on the x-axis. The left plot, labeled 'Parent HS', shows a single sharp green peak. The right plot, labeled 'Parent HS' and 'Labeled HS', shows two overlapping peaks: a broad red peak on the left and a sharp green peak on the right. A large yellow arrow points from the text 'Wrong conditions of tritium introduction' towards the right plot.

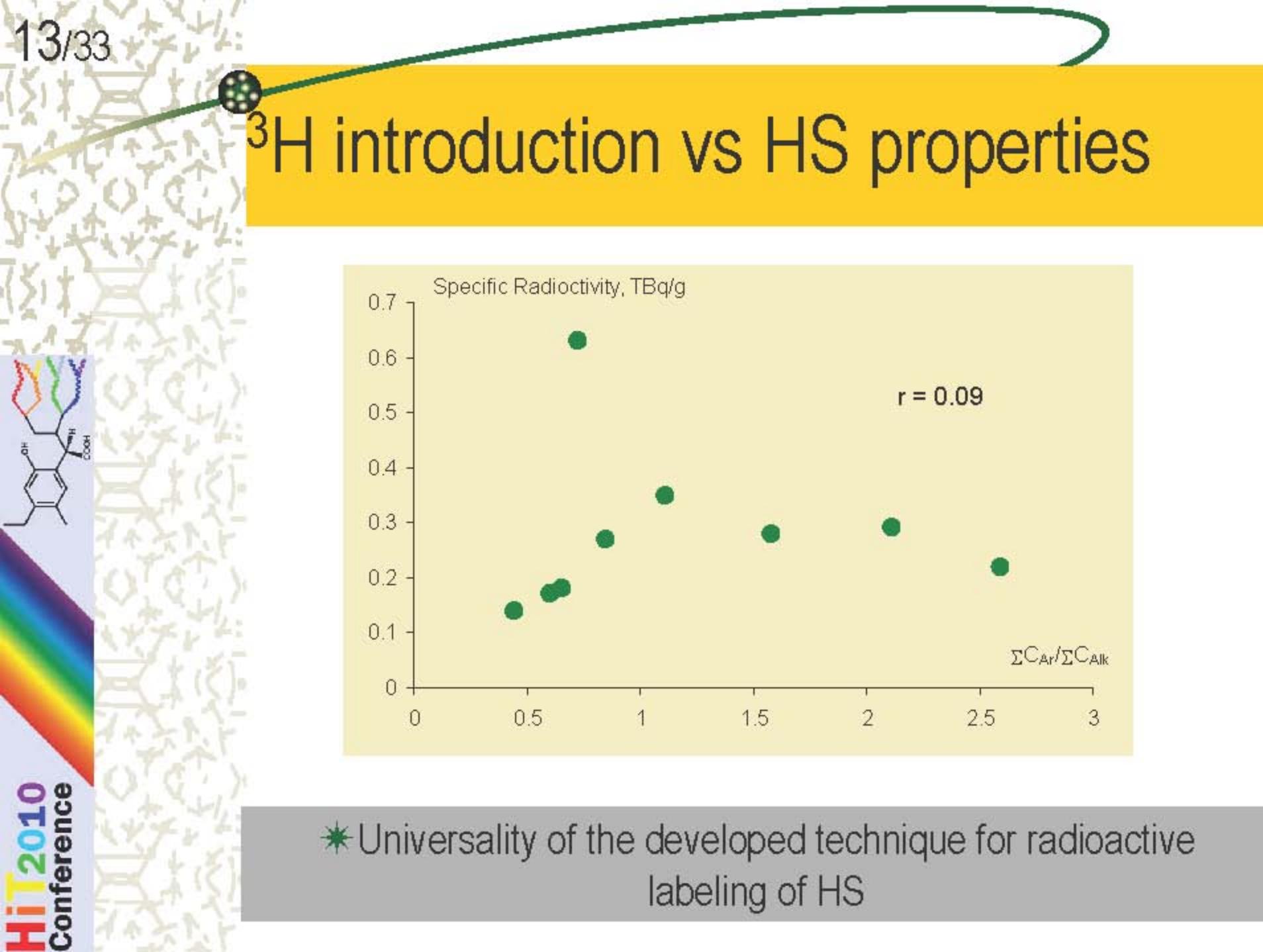
$^3\text{H}$ -HS structure:

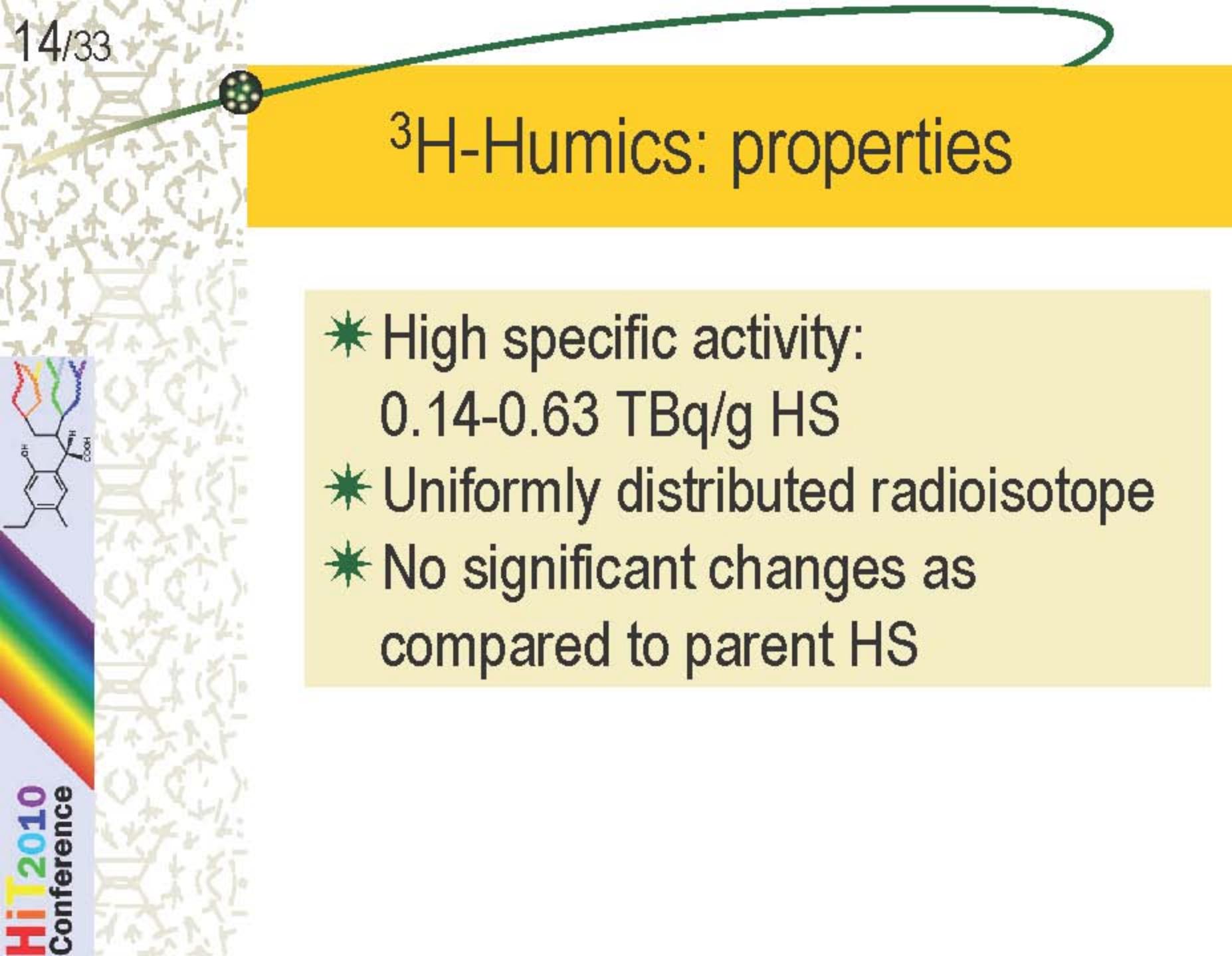
The structure shows a repeating disaccharide unit. It consists of a pyranose ring with a sulfonate group (-SO<sub>3</sub><sup>-</sup>) at the C6 position and a glucuronic acid residue linked at C3. The glucuronic acid has a carboxylate group (-COO<sup>-</sup>) and a sulfate group (-OSO<sub>3</sub><sup>-</sup>) at its C6 position.

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\* SEC analysis can be used to study HS alteration







## $^{3\text{H}}$ -Humics: properties

- ★ High specific activity:  
0.14-0.63 TBq/g HS
- ★ Uniformly distributed radioisotope
- ★ No significant changes as compared to parent HS

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# <sup>3</sup>H-HS: to learn new about humics

**Scintillation phase method**

Diagram illustrating the Scintillation phase method:

- Three stacked layers represent the detector components:  $I_V$  (top),  $I_S$  (middle), and  $I_W = 0$  (bottom).
- The middle layer ( $I_S$ ) contains a radioactive sample.
- Wavy lines indicate light emission from the sample, which is detected by the top layer ( $I_V$ ).
- A yellow starburst indicates the total signal:  $I = I_S + I_V$ .
- A graph shows the signal count ( $I_{Count}$ ) versus time (in microseconds). The signal rises rapidly and plateaus around 30,000 counts.

**Liquid scintillation counter**

$K_{OW} = \frac{I_V / \varepsilon_V}{V \cdot a_V \cdot C_{OW}}$

$\Gamma = \frac{I_S}{\varepsilon_S \cdot a_S \cdot S}$

$\varepsilon$  - counting efficiency;  $a$  - specific radioactivity of HS

DCI.10.1007/s10657-008-0517-5  
Journal of Radioanalytical and Nuclear Chemistry, Vol. 256, No. 2 (2008) 303–306

RADIOCHEMISTRY

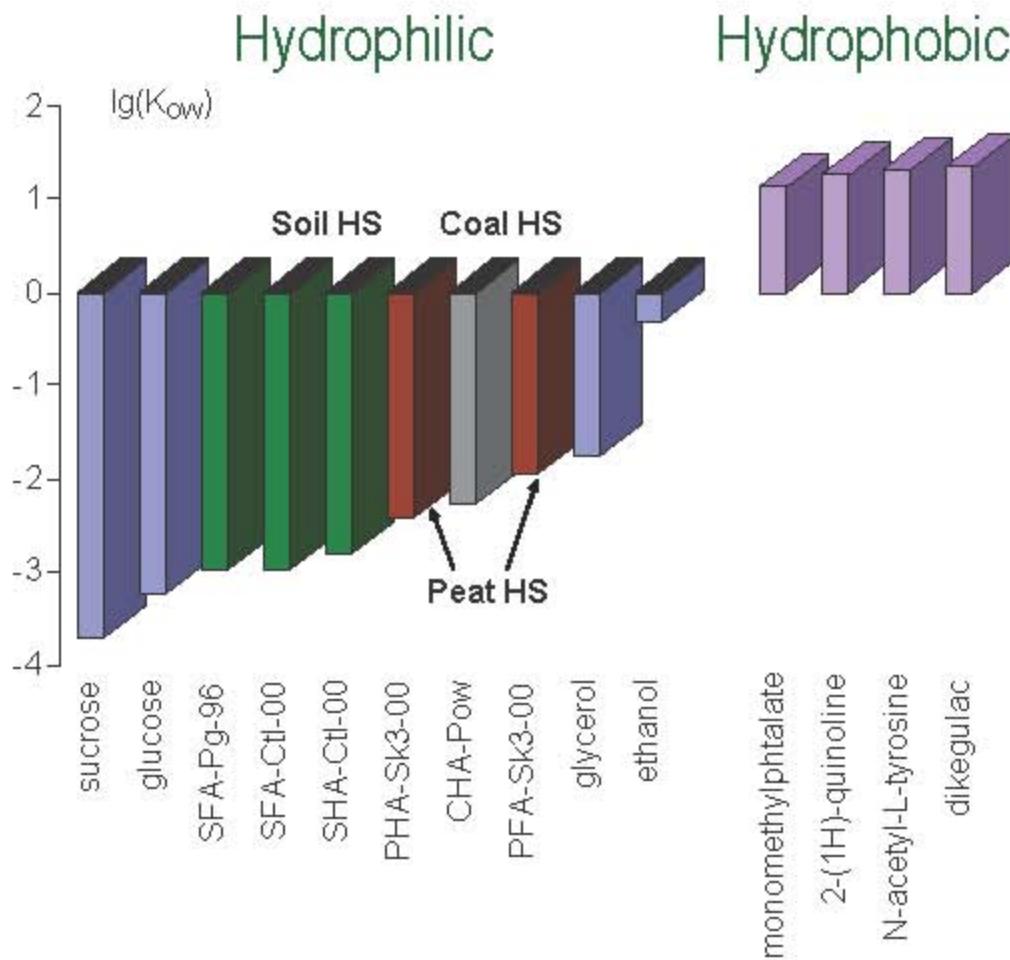
Scintillation phase method:  
A new approach for studying surfactant behavior at liquid/liquid interface

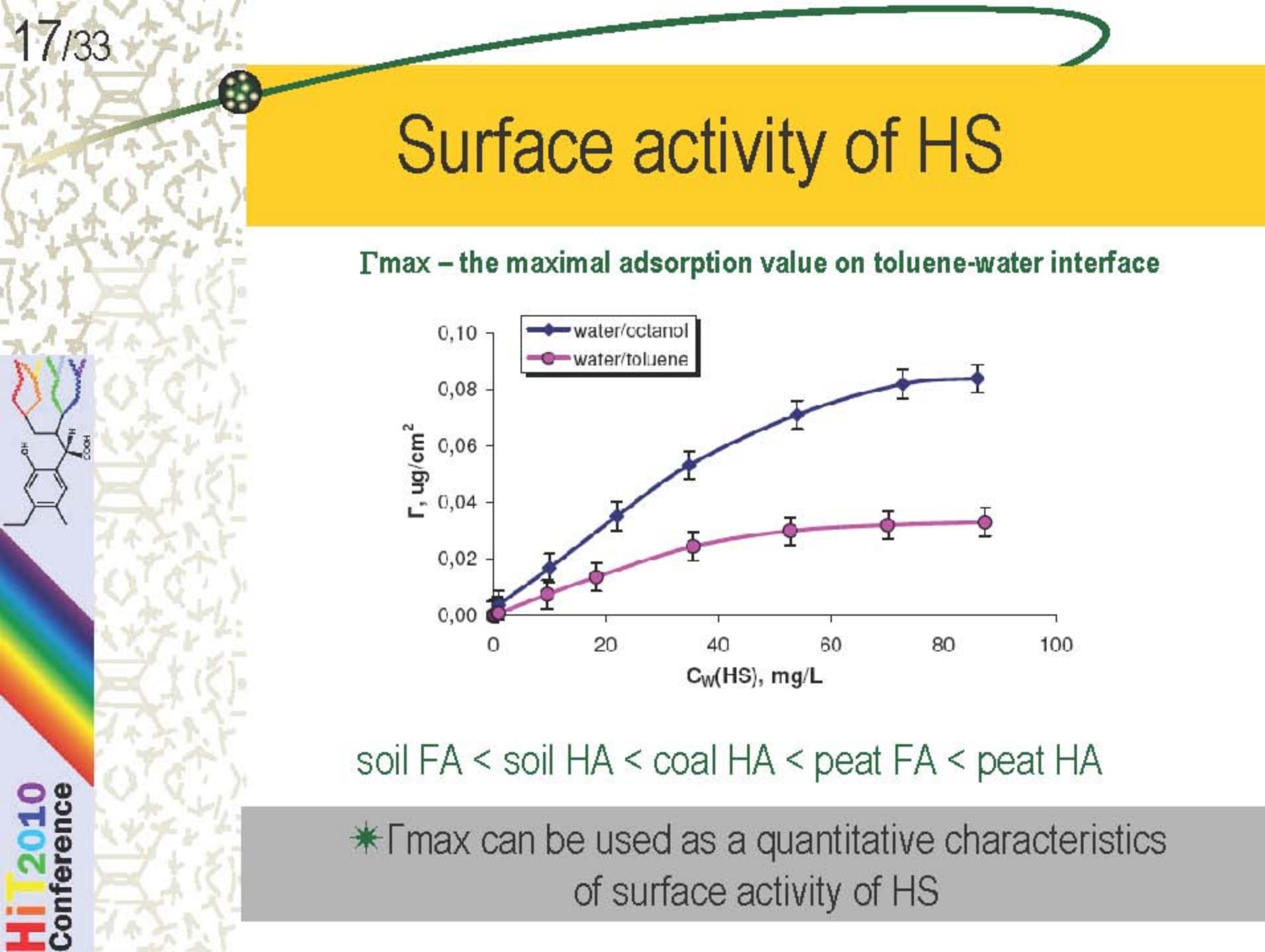
M. G. Chernyshova, Z. A. Tyasto, G. A. Badun

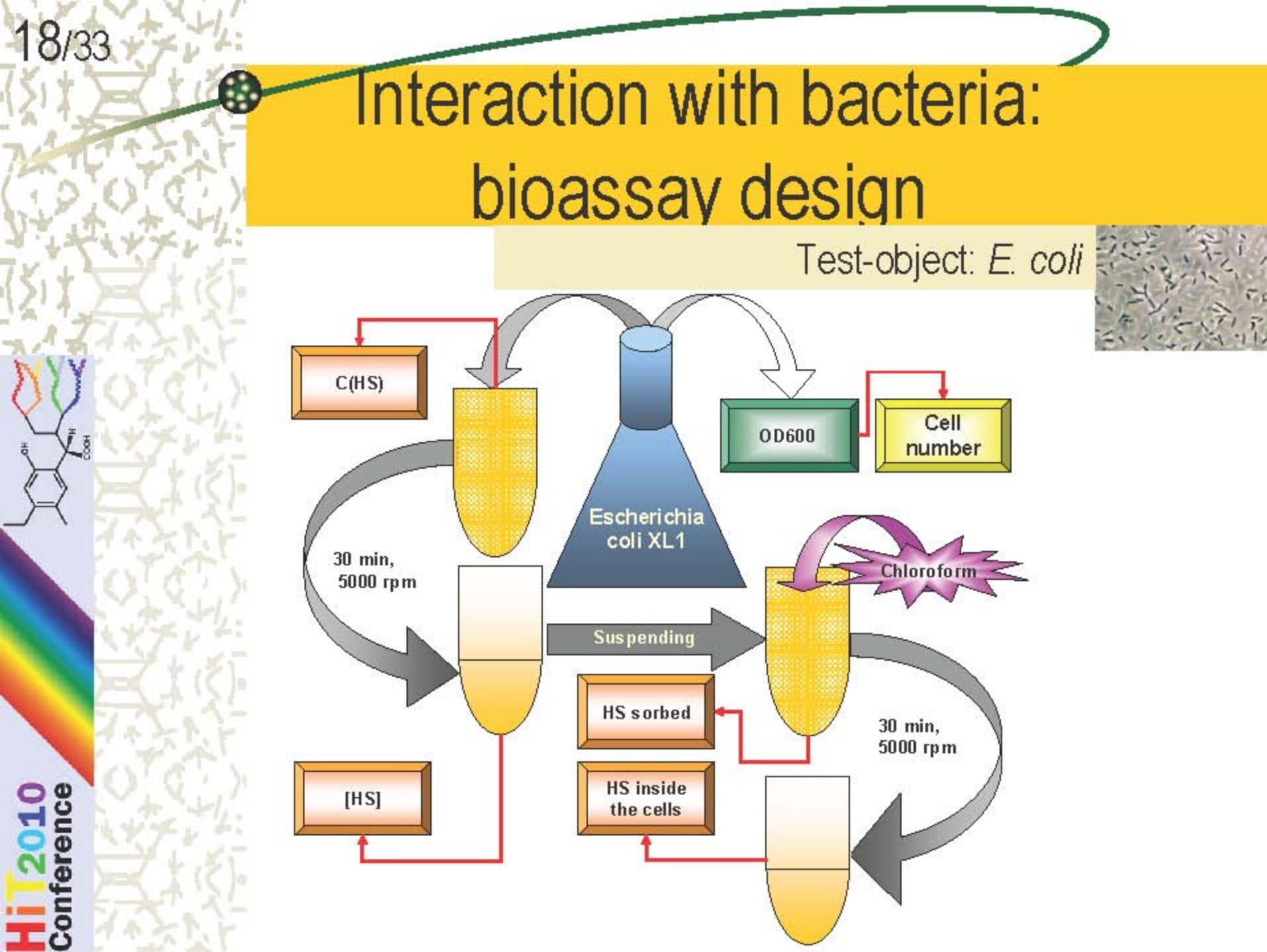


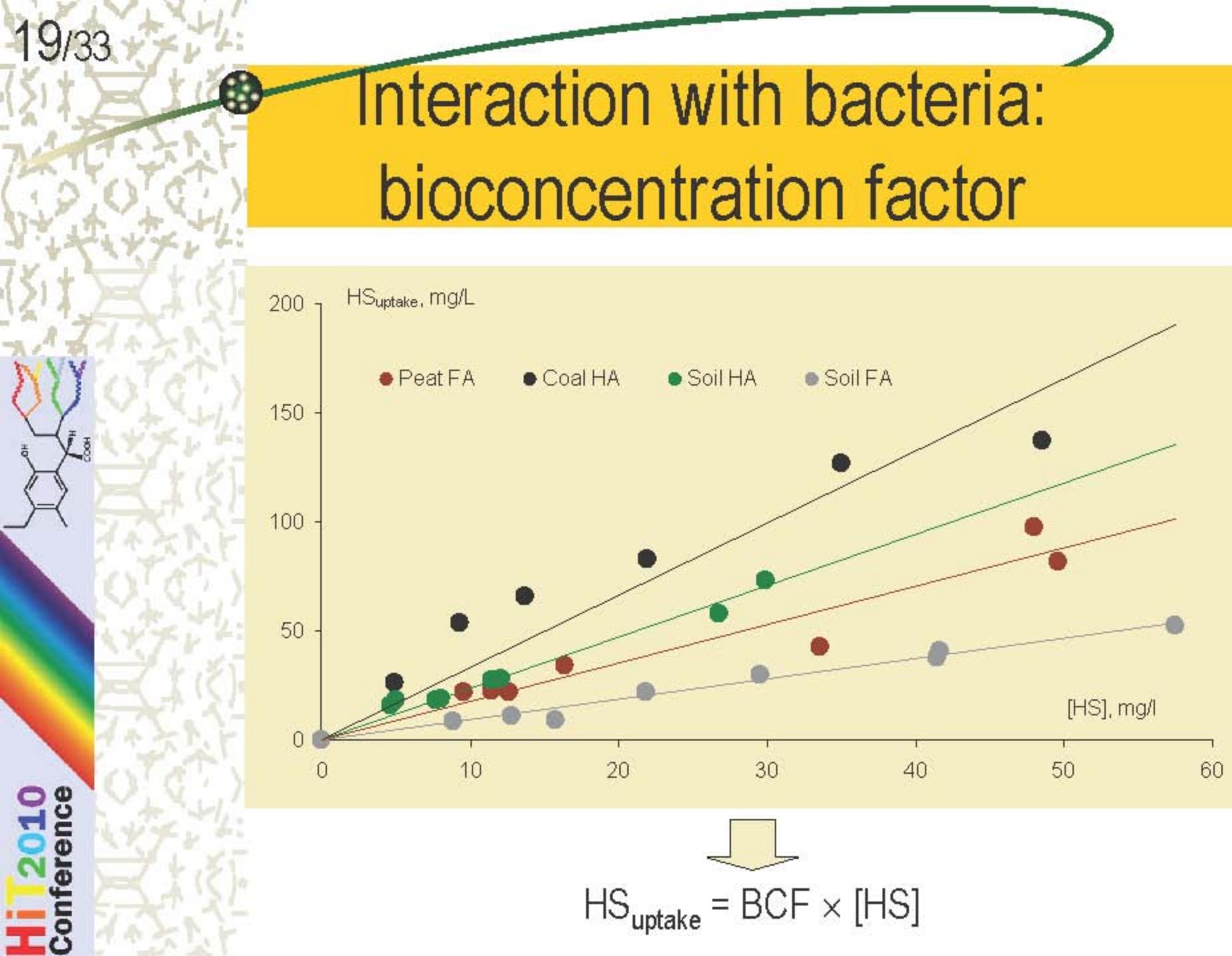
# Hydrophobicity of HS

Kow – distribution coefficient between octanol-water phases









# Interaction with bacteria: quantitative estimation

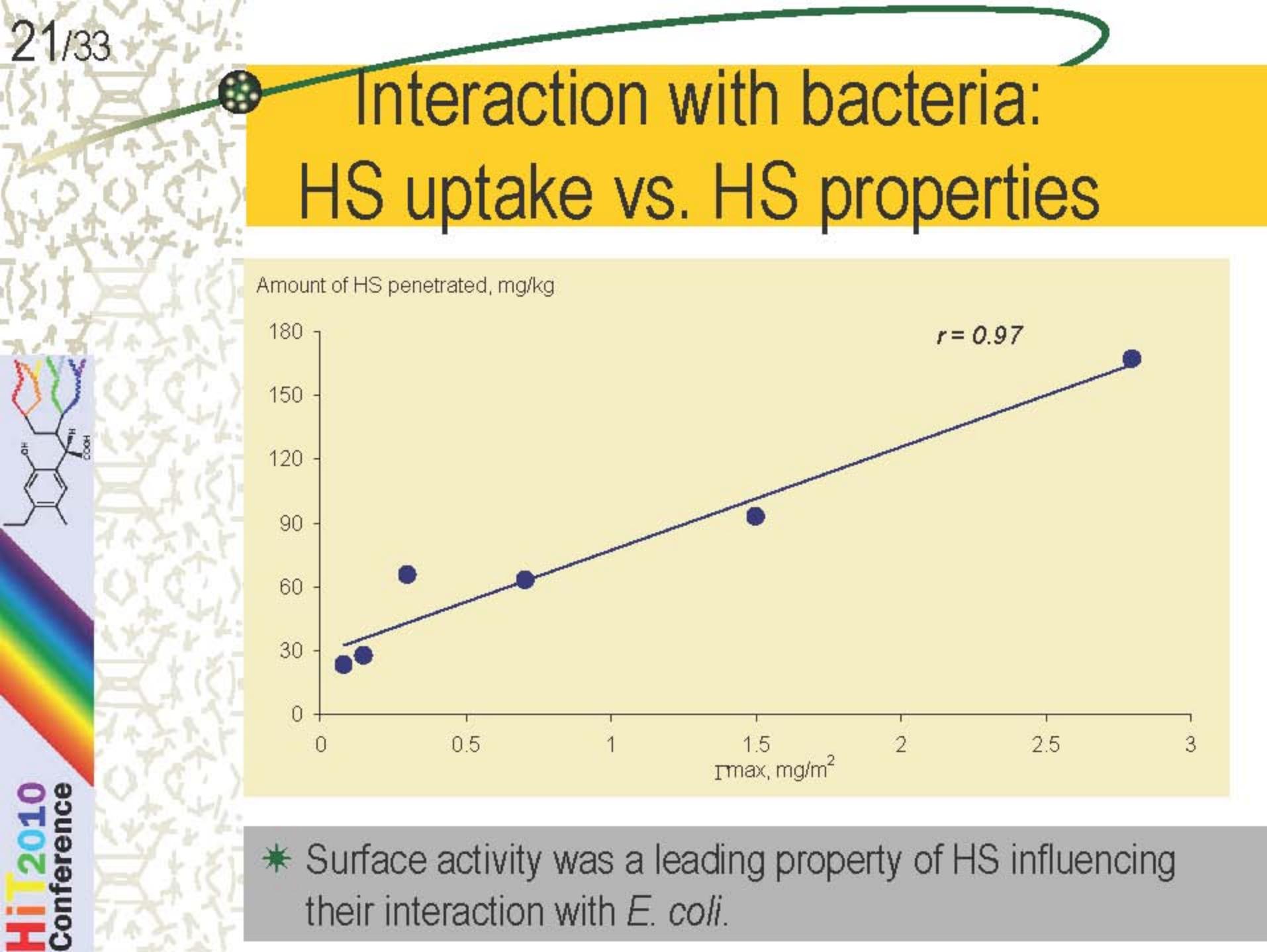
BCF, L/kg	0.9 – 13.1
HS sorbed at 50 mg/L, mg/m <sup>2</sup>	1 – 62
HS sorbed at 50 mg/L, mg/(kg cells)	33 – 720
HS penetrated at 50 mg/L, mg/(kg cells)	23 – 167

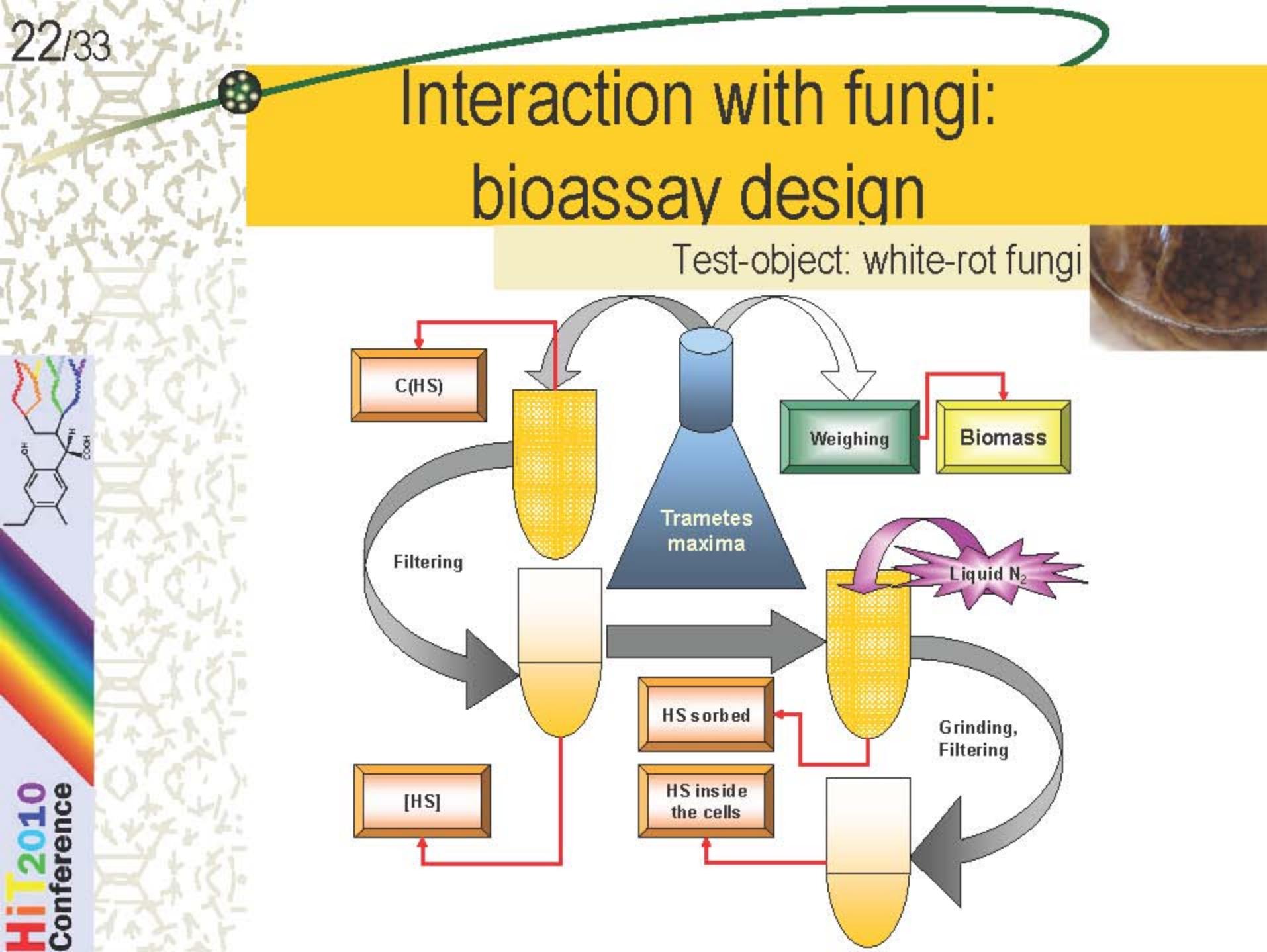
APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 2010, p. 622–626  
0093-2700/10/061220-06 \$15.00/0  
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Estimation of Uptake of Humic Substances from Different Sources by *Escherichia coli* Cells under Optimum and Salt Stress Conditions by Use of Tritium-labeled Humic Materials<sup>1</sup>

Natalia A. Kalikova,<sup>2,3\*</sup> Irina V. Pernina,<sup>1</sup> Gennady A. Dabur,<sup>1</sup> Maria G. Chernyshev,<sup>1</sup> Olga V. Krasilova,<sup>2</sup> and Eugenia A. Tsvetkova<sup>3</sup>





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## Interaction with fungi: quantitative estimation

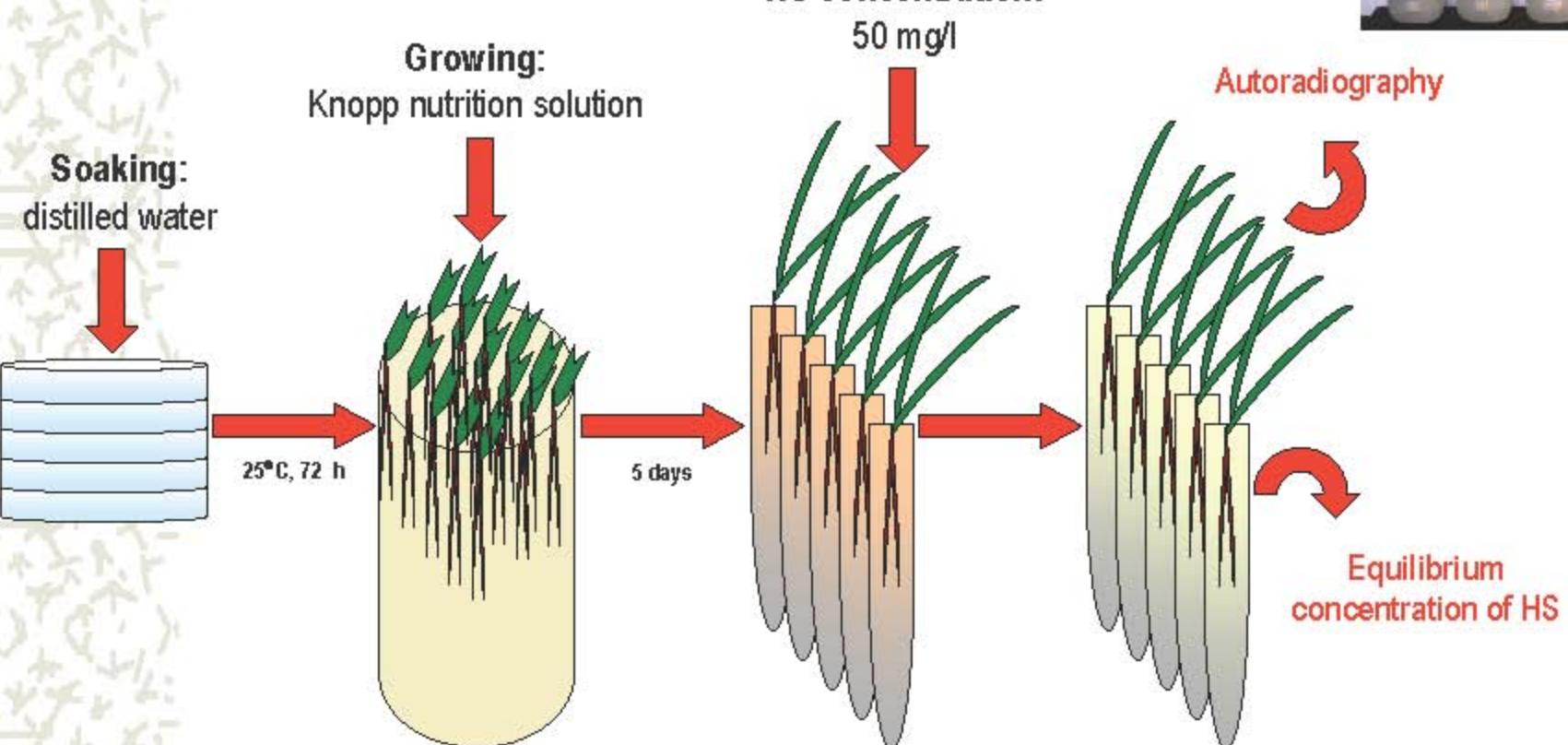
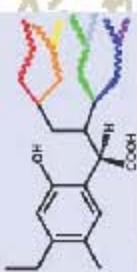
Sorbed, mg/kg	190 – 800
HS penetrated at 50 mg/L, mg/kg	70 – 150

C[C@H](CCCC(=O)c1ccc(cc1)C(=O)N2Cc3ccccc3C2)C(=O)N3Cc4ccccc4C3

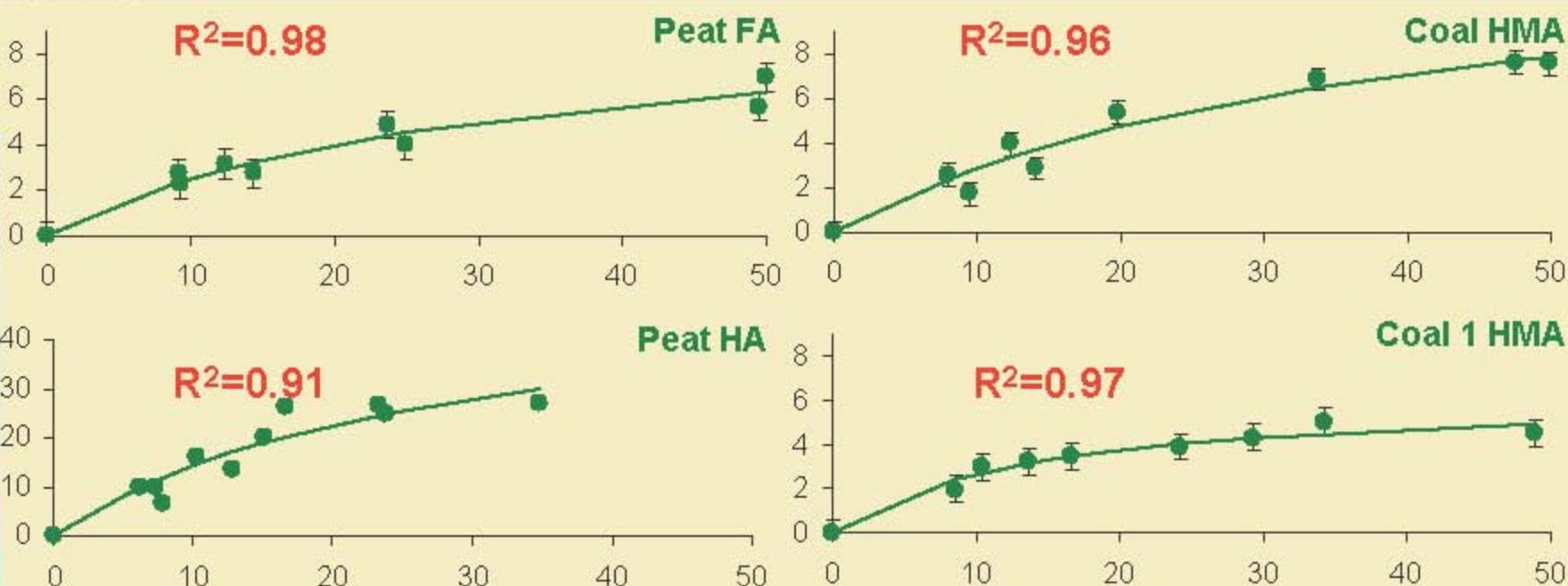
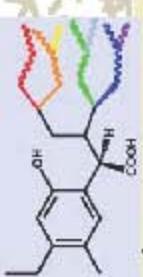
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# Interaction with plants: bioassay design

Test-object: wheat

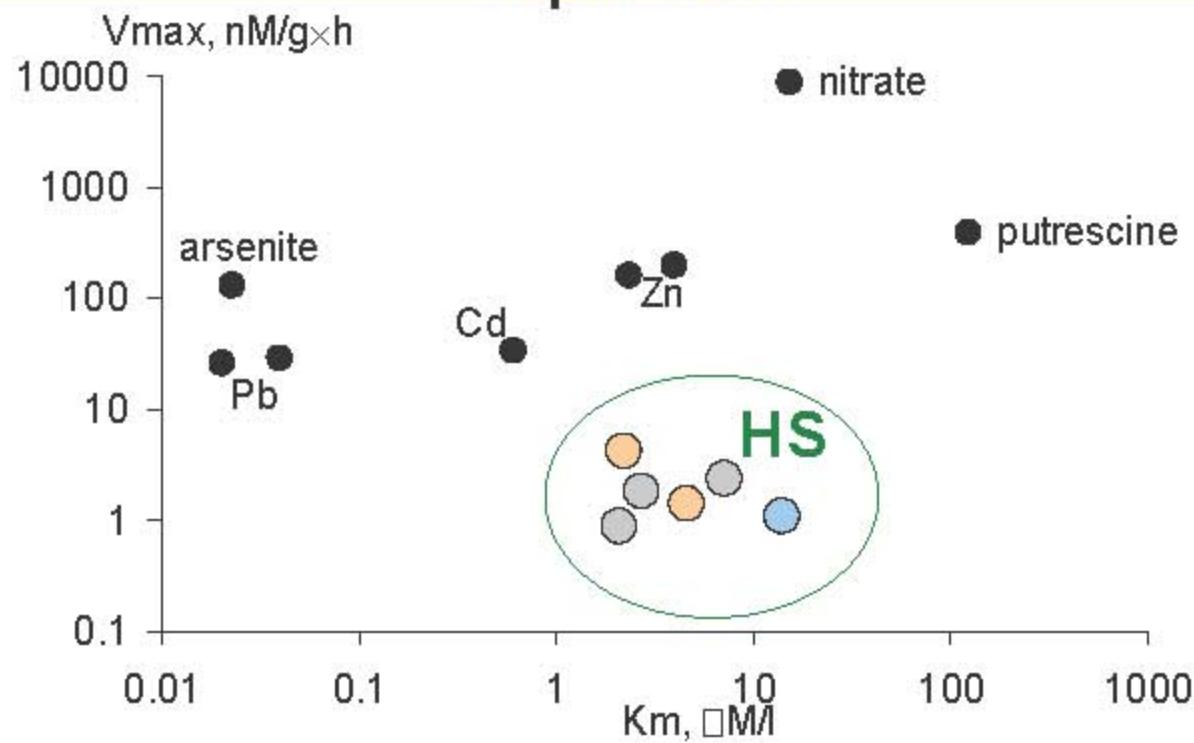


# Interaction with plants: kinetics



- \* Kinetics of HS uptake by plants can be described using Michaelis-Menten equation
- \* Uptake of HS by plants is hypothesized to be carrier-mediated (transporter-limited)

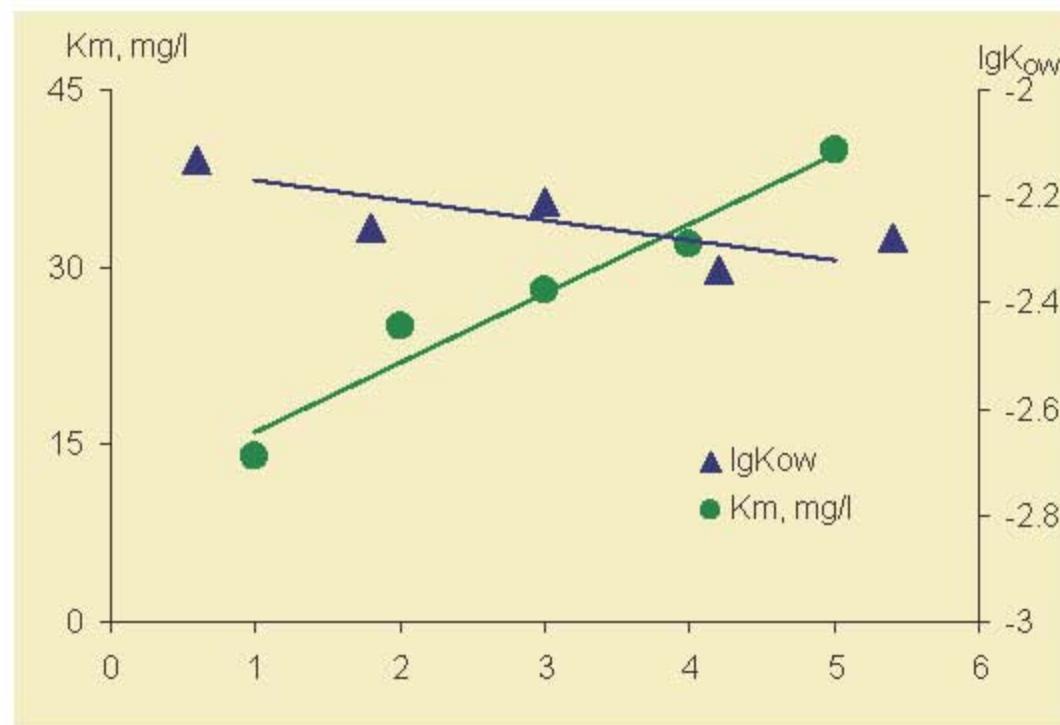
# Interaction with plants: uptake



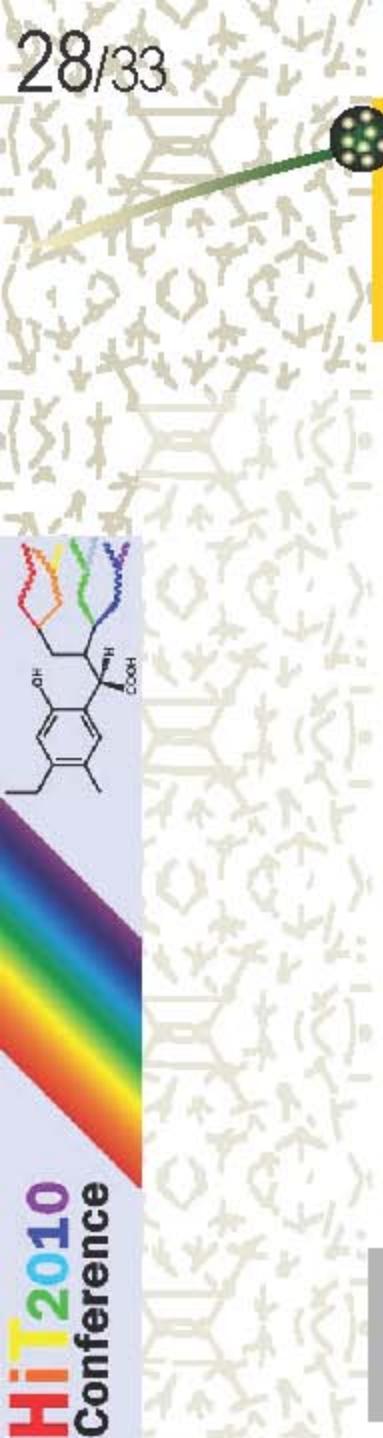
- \*  $V_{max}$  of HS <<  $V_{max}$  of individual compounds and ions
- \*  $K_m$  of HS  $\approx K_m$  of individual compounds and ions



# Interaction with plants: HS uptake vs. HS properties

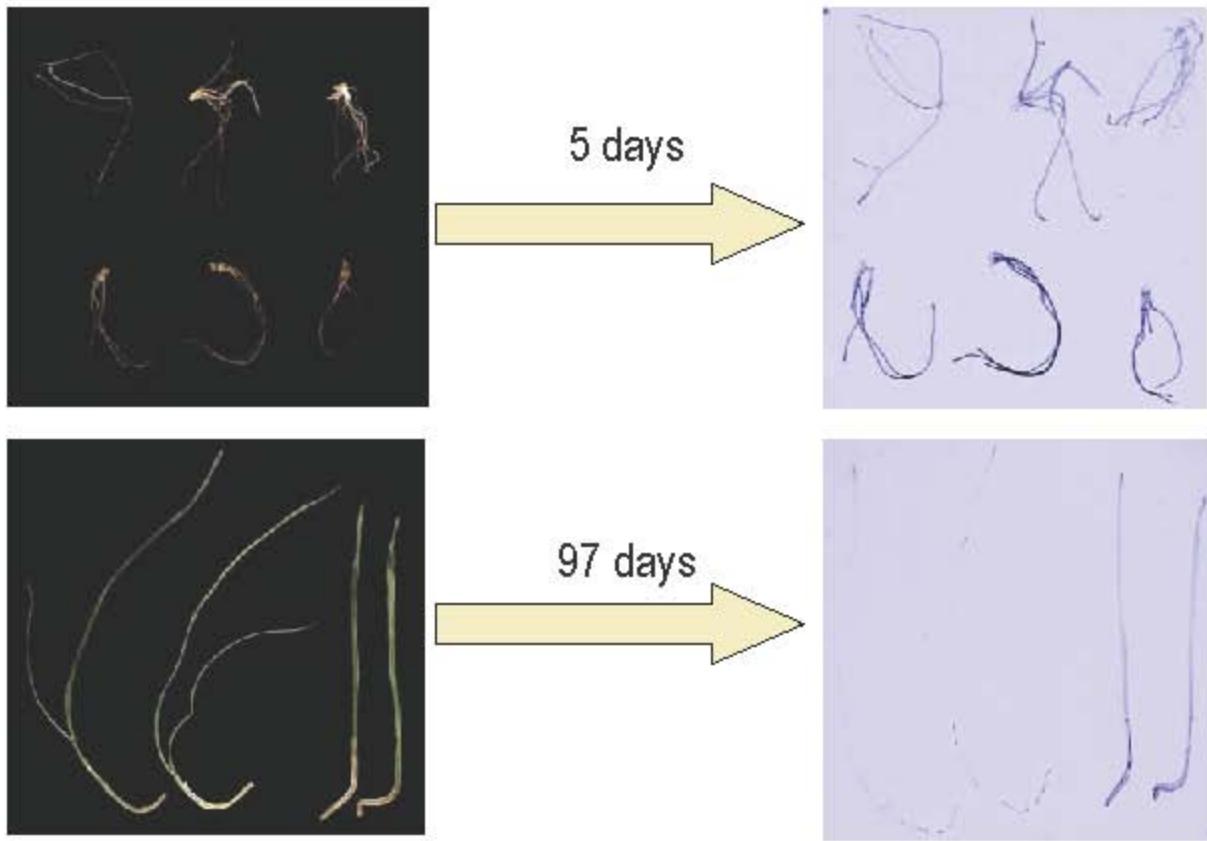


- \* Hydrophobicity was a leading property of HS influencing their interaction with wheat plants

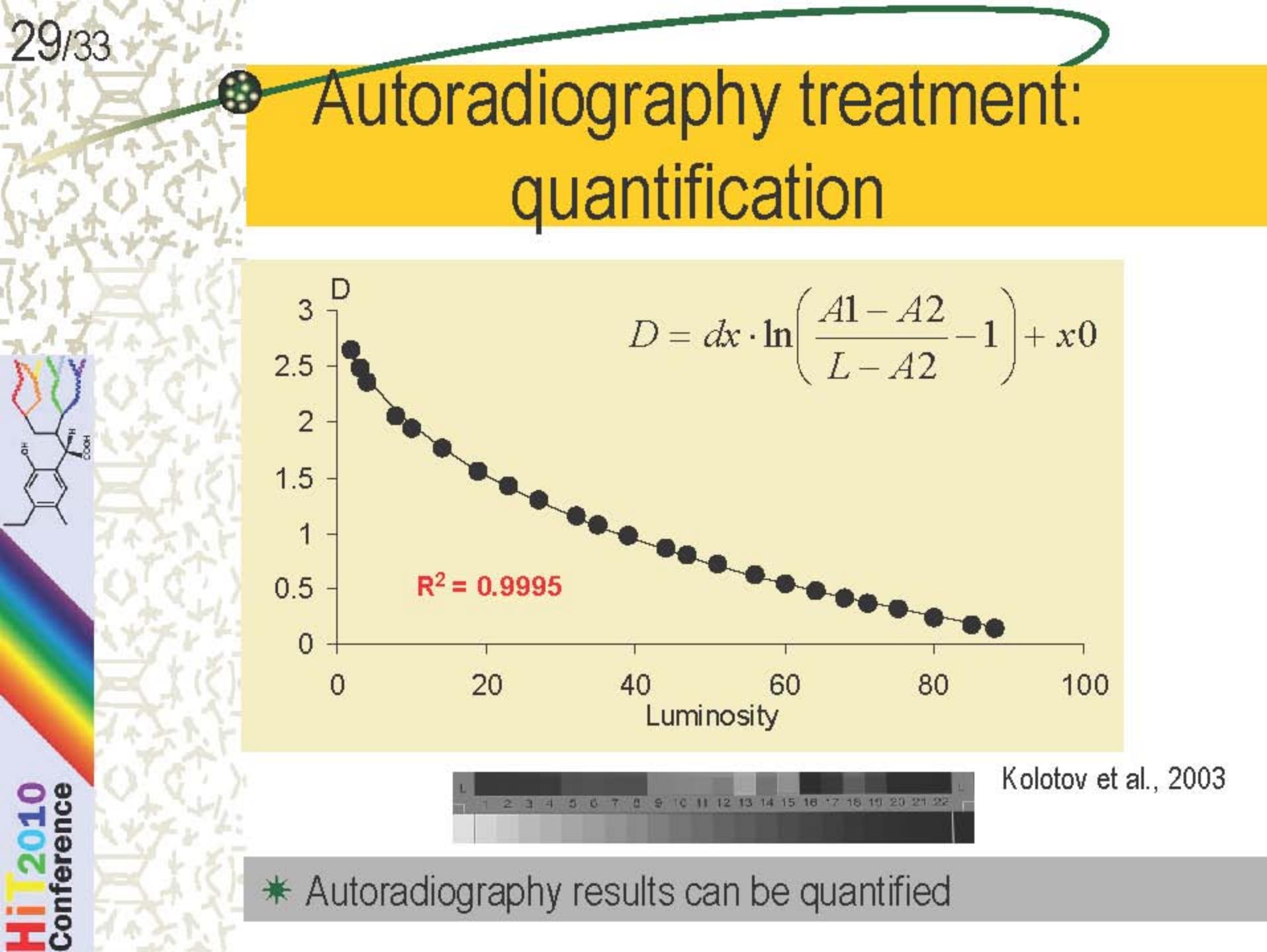


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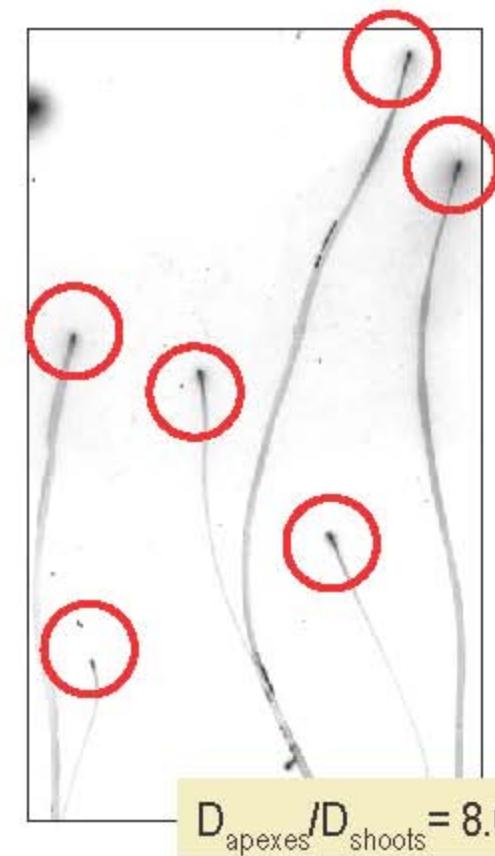
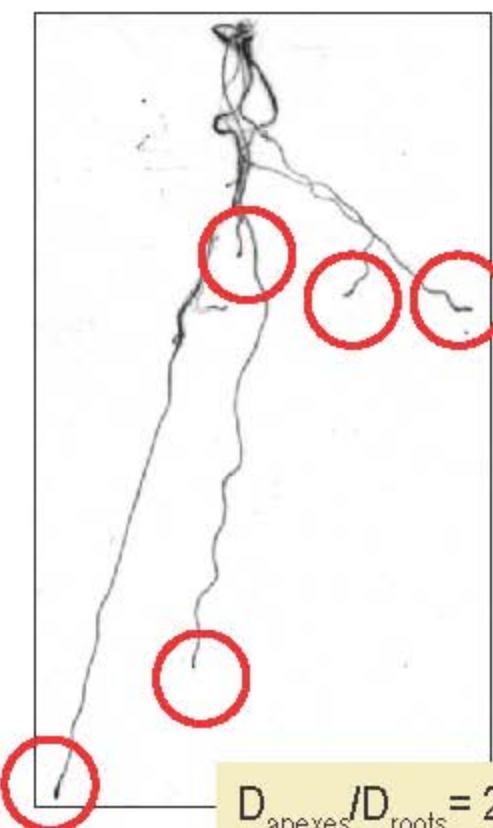
# Interaction with plants: visualization



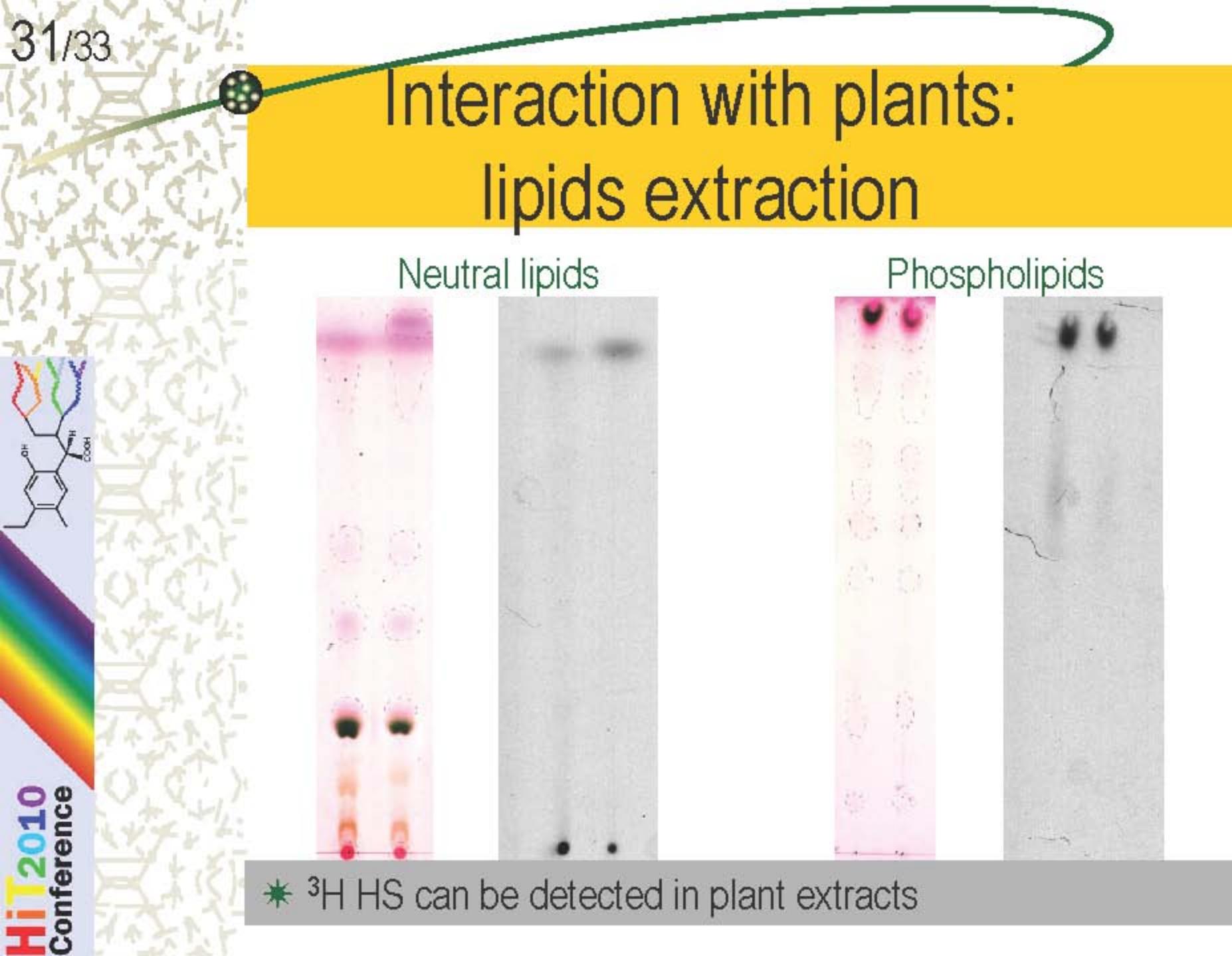
\* Tritium autoradiography can be used for visualization of HS interaction with plants



# Interaction with plants: detailed visualization



- \* HS are mainly accumulated in the apexes of the both shoots and roots



# $^3\text{H}$ -HS: future prospects for medicine studies

## $^3\text{H}$ -HS in medicine studies

Autoradiography  
Microautoradiography

Pharmacokinetics  
Pharmacodynamics

Study of HS targeting

Mechanisms of absorption  
and distribution

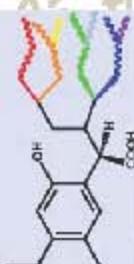
Mechanism of HS biological activity?

The last!

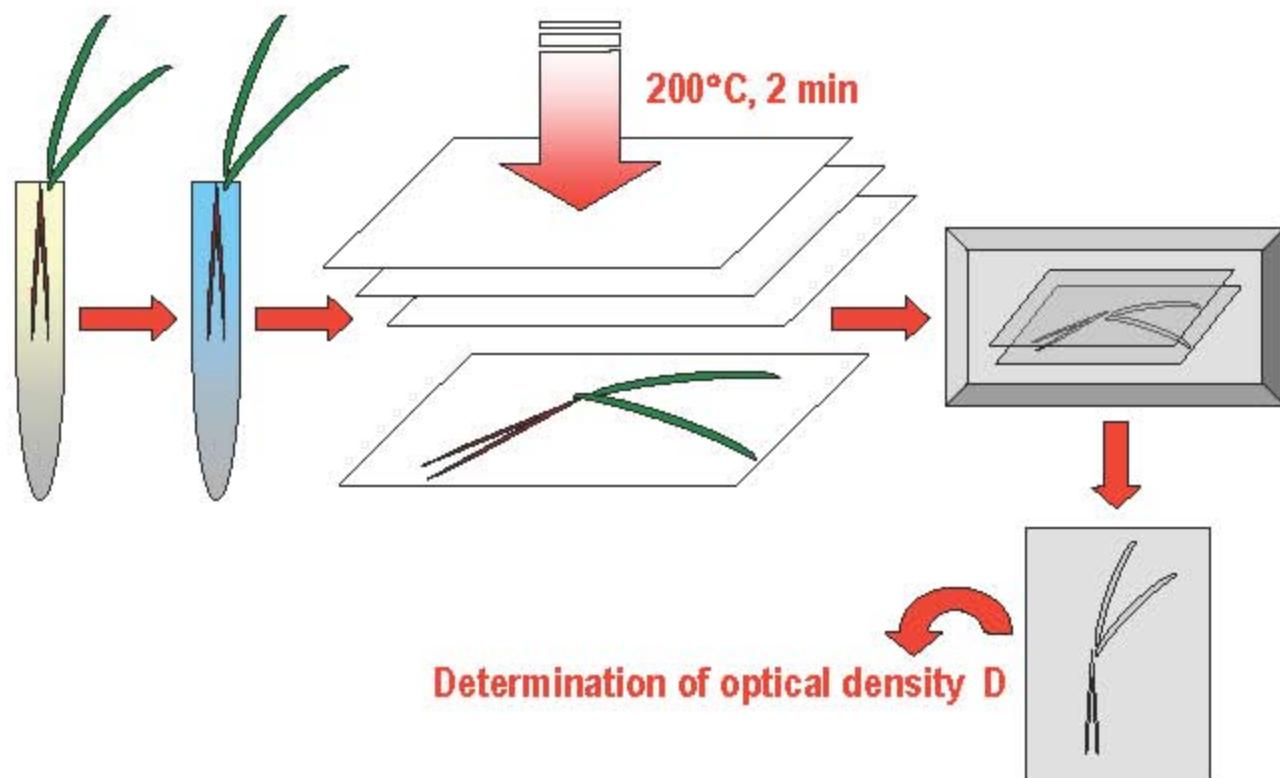


Thank you very much for attention!





# Tritium autoradiography



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Original Russian Text © G.A. Badun, N.A. Kullikova, M.G. Chernysheva, Z.A. Tyasto, V.I. Korobkov,  
V.M. Fedosov, E.A. Tsvetkova, A.I. Konstantinov, A.V. Kudryavtsev, I.V. Perminova.

**Tritium Labeling: A Unique Tool for Studying the Behavior  
of Humic Substances in Living Systems**

G. A. Badun<sup>a</sup>, N. A. Kullikova<sup>a</sup>, M. G. Chernysheva<sup>a</sup>, Z. A. Tyasto<sup>a</sup>, V. I. Korobkov<sup>a</sup>,  
V. M. Fedosov<sup>a</sup>, E. A. Tsvetkova<sup>a</sup>, A. I. Konstantinov<sup>a</sup>, A. V. Kudryavtsev<sup>a</sup>, I. V. Perminova<sup>a</sup>