Toxic effects of nanoparticles from biomass combustion

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The increased usage of biomass as a fuel for heat generation in private households raises the question of the potentially toxic effects of particulate matter generated during combustion. As it has been demonstrated that fine (<2.5 µm) and ultrafine (<0.1 µm) particles cause stronger adverse health effects than larger particles (ref. 1, 2, 3), we analysed particles with diameters of up to 1 µm, whilst particles larger than 1 µm were removed by a cyclone separator.

Two sources of combustion particles were employed, reflecting the most common modes of residential heating: An automatically-fed log wood cauldron (Fröling FHG Turbo 3000, 30 kW) and a manually-fed wood pellet stove (Buderus Blue Line Pellet 1, 7.6 kW). Both heat generators were shortlisted on a white list of the German federal government, rendering their employment eligible for subsidies. To mimick a typical use of the generators, they were employed either under optimised aeration or under reduced aeration (suboptimal burning conditions).

The nanoparticles generated were analysed according to aerodynamic diameter and to number using a Scanning Mobility Particle Sizer (SMPS), and an elementary analysis was performed using energy dispersive X-ray analysis. Using the automatically fed log wood cauldron, only minor differences were observed in the particle size and composition between optimal and suboptimal aeration conditions. In contrast, the nanoparticles generated from the manually-fed pellet stove showed an increase in the aerodynamic diameter by a factor of approx. 5, which was reflected by a dramatic increase of carbon in the elementary analysis.

To analyse the toxic potential of these nanoparticles, we employed two different experimental settings: First, we simulate the exposure to inhalation of combustion aerosols in an *in vitro* lung model, employing the human lung cell line A549 at a liquid-air interface in the Karlsruhe exposure system (Fig. 1; ref. 4). Second, we employed suspensions of combustion nanoparticles in classical submersed A549 cell culture.

The exposure at the air-liquid interface resulted in cellular stress at longer exposure times, presumably due to desiccation, limiting the amount of particles to which the cells could be exposed. To measure the pro-inflammatory response, we determined the gene expression of human interleukin 8 (IL-8) by quantitative real time PCR, as well as its release into the culture supernatant by a bead-based immunodetection assay on a Luminex machine. At the exposure time tested (2.5 h), no significant cellular stress response was detectable.

Hence, in order to evaluate the toxicity of the nanoparticles generated, we applied the particles generated from the pellet stove under suboptimal combustion conditions as a suspension in cell culture medium. We analysed the gene expression of the two cytokines interleukin 6 (IL-6) and IL-8 by quantitative real time PCR. Furthermore, we assayed cell membrane integrity as a proxy for cell vitality by measuring the release of the otherwise cytoplasmic enzyme lactate dehydrogenase into cell culture supernatant, which is indicative of disrupted cells.

At concentrations of up to 100 µg/ml, the nanoparticles generated did not influence IL-8 gene expression levels, whereas at 400 µg/ml the expression increased approx. 8-fold. On the other hand, amorphous carbon nanoparticles (Printex® 90, average particle size 14 nm) elicited an approx. 8-fold increase in IL-8 gene expression already at a concentration of 50 µg/ml. Thus, Printex® 90 was roughly ten times more efficient than the combustion nanoparticles in evoking a pro-inflammatory response.

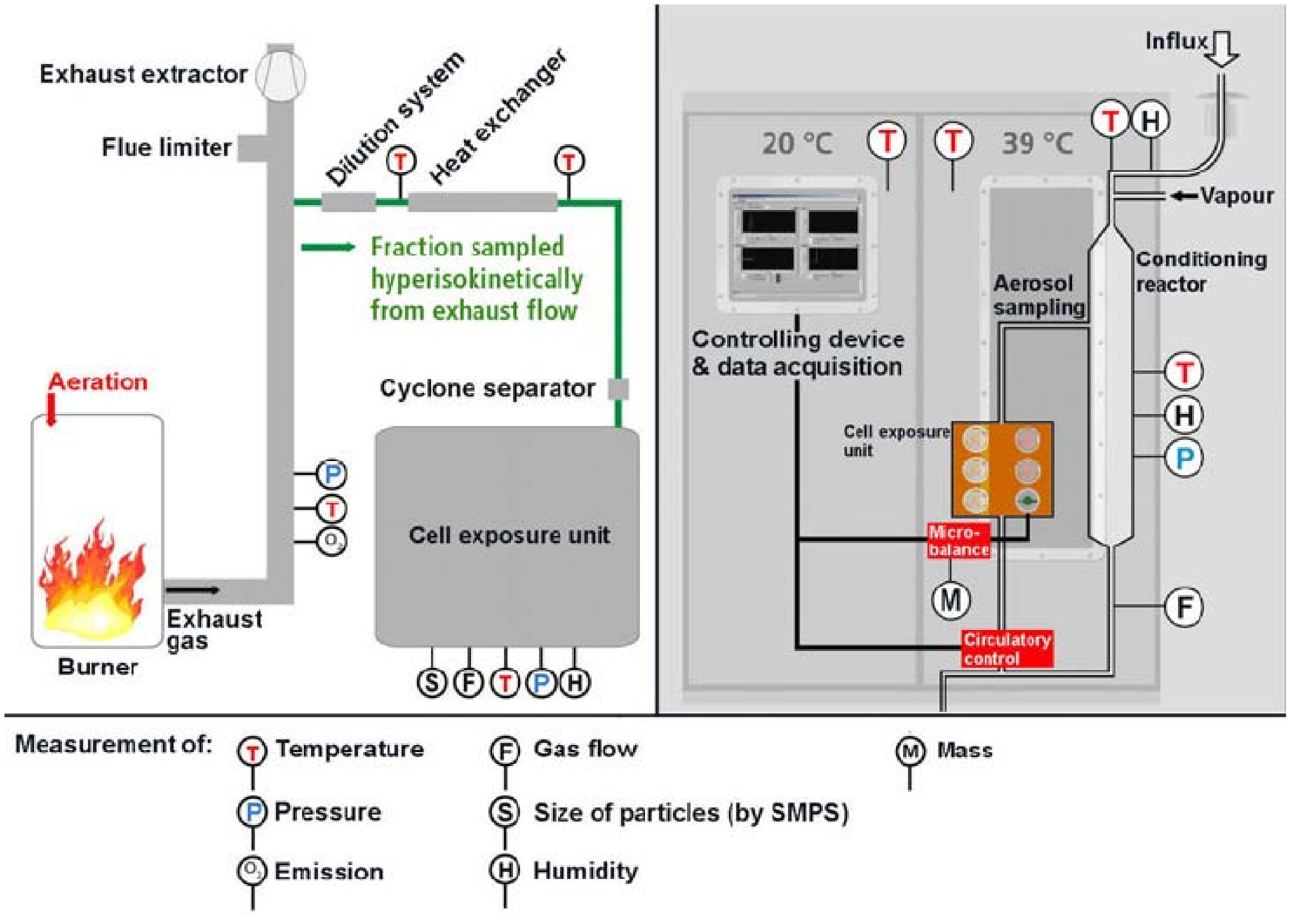

Fig. 1: Karlsruhe exposure system for *in vitro* testing of airborne nanoparticle emissions from combustion processes.

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References:

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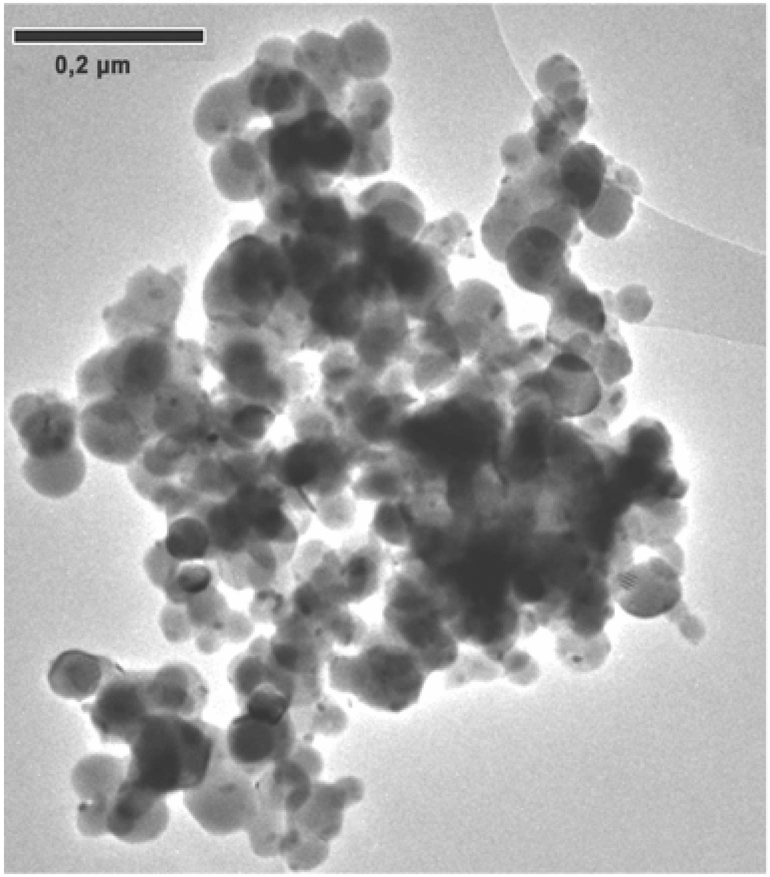


14th ETH Conference
Zurich, August 3rd 2010

Dr. Frank Weise

Toxic effects of nanoparticles from biomass combustion

Natural and Medical
Sciences Institute
at the University of Tübingen

Overview

1) Combustion source: Private-owned heat generators

- subsidised employment of renewable fuels

2) Particulate matter: Nanoparticles in focus

- Karlsruhe exposure system

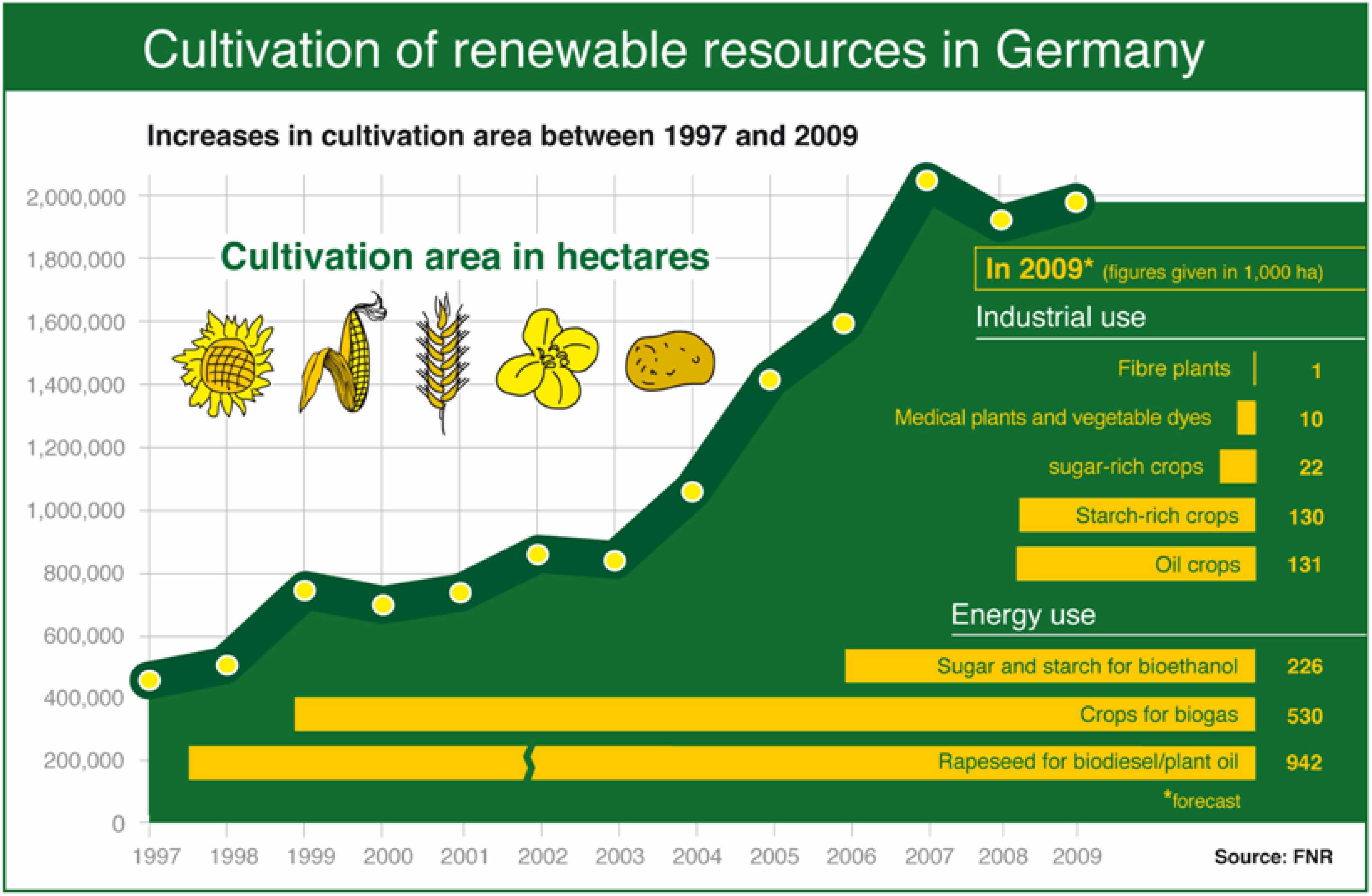
3) Biological model: A549 tumour cell line

- liquid-air interface vs. submerse culture

4) First results & outlook

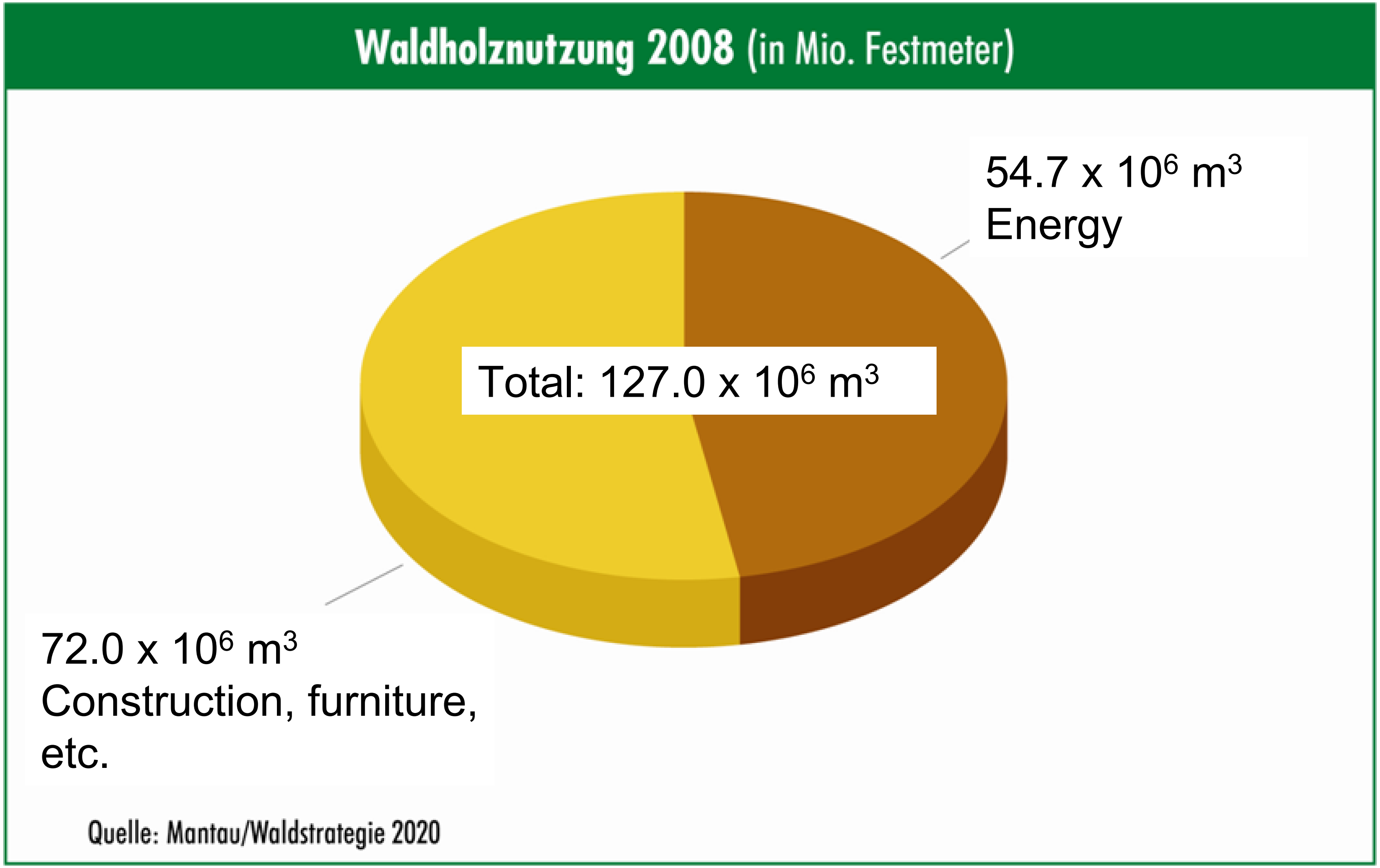
Biofuels – subsidised renewable resources

5-fold increase in acreage (12 % of total land use for agriculture)



Total agricultural land use (2009): 16.9 million ha

Use of wood in Germany (2008):
43 % for energy supply



Waldholznutzung 2008 (in Mio. Festmeter)

Quelle: Mantau/Waldstrategie 2020

Model sources of combustion particles: Two commercially available heat generators

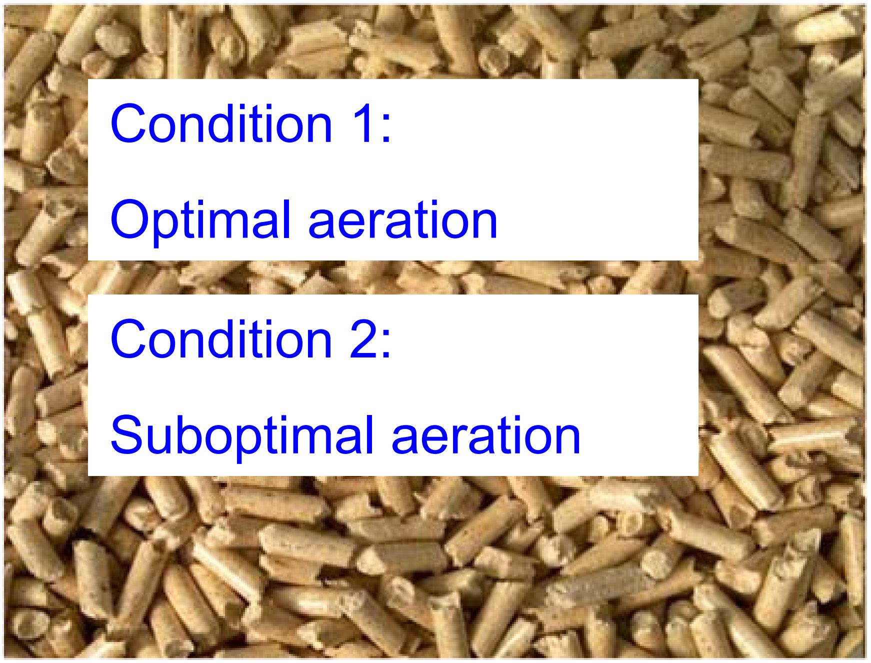
Buderus Blueline Pellet 1
Pellet stove (7.6 kW),
manually fed

Fröling FHG Turbo 3000
Log wood cauldron (30 kW),
automatically fed

Shortlisted by *Bundesamt f. Wirtschaft u. Ausfuhrkontrolle (BAFA)* to be eligible for state subsidies.

Combustion parameters

Pellet stove:



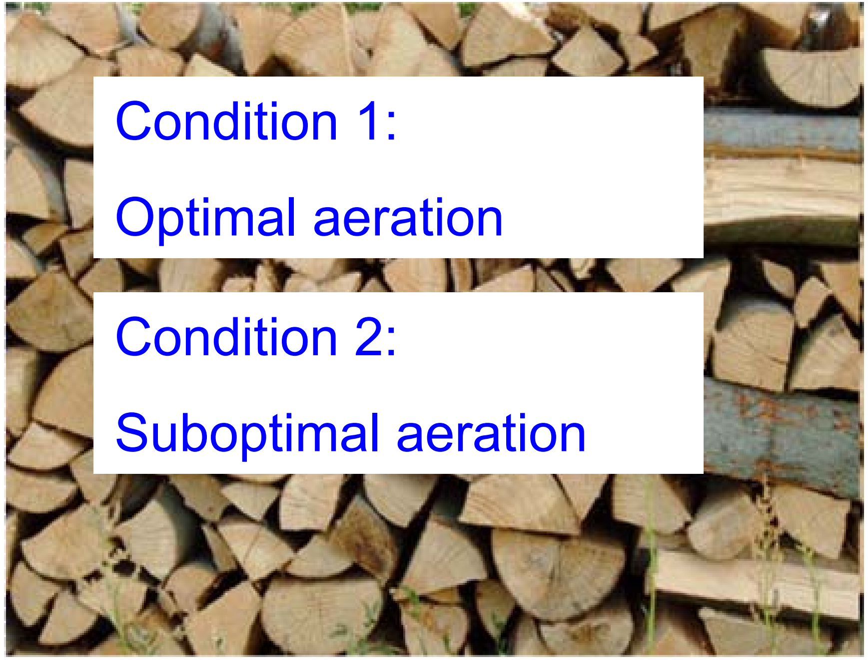
Condition 1:

Optimal aeration

Condition 2:

Suboptimal aeration

Log wood cauldron:



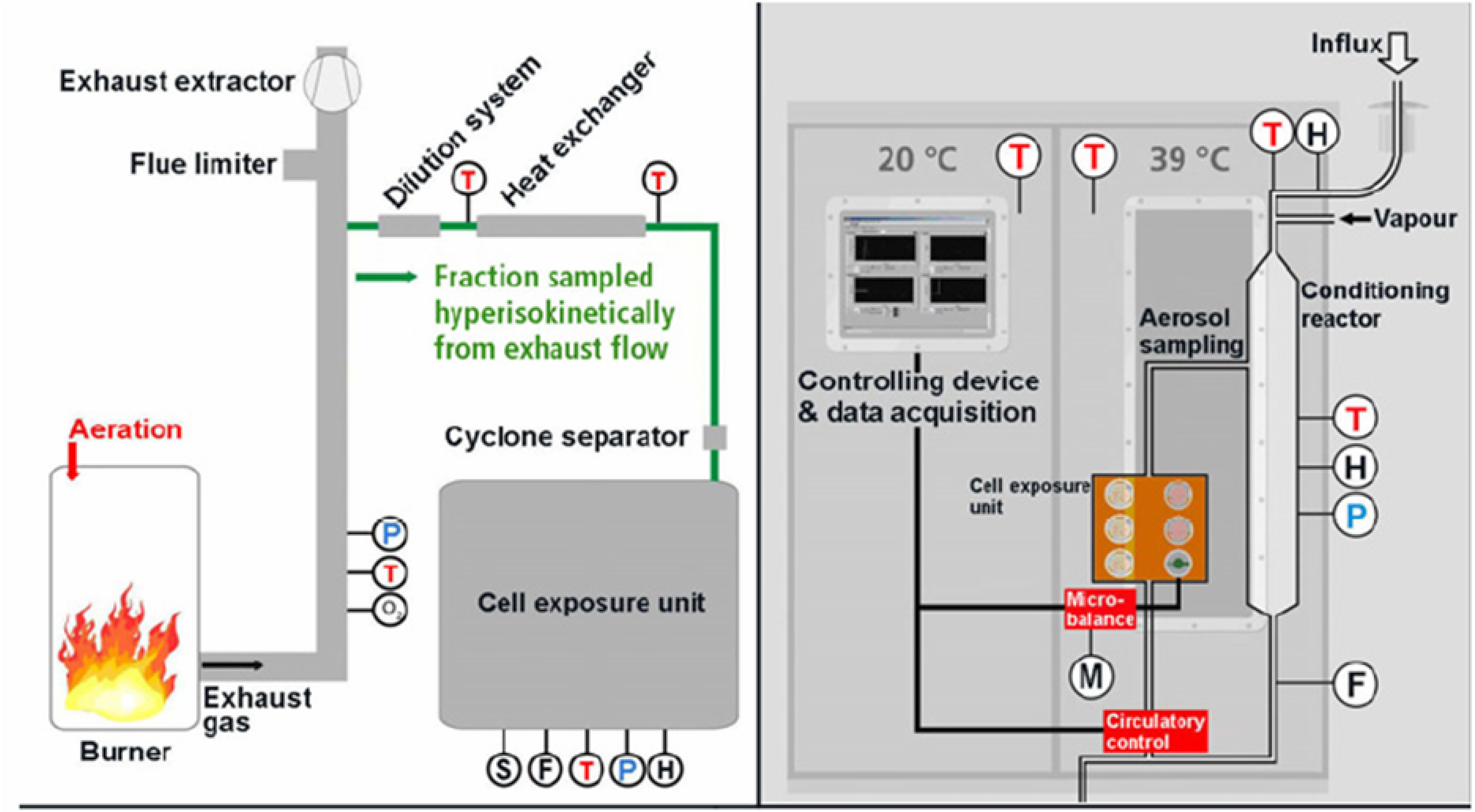
Condition 1:

Optimal aeration

Condition 2:

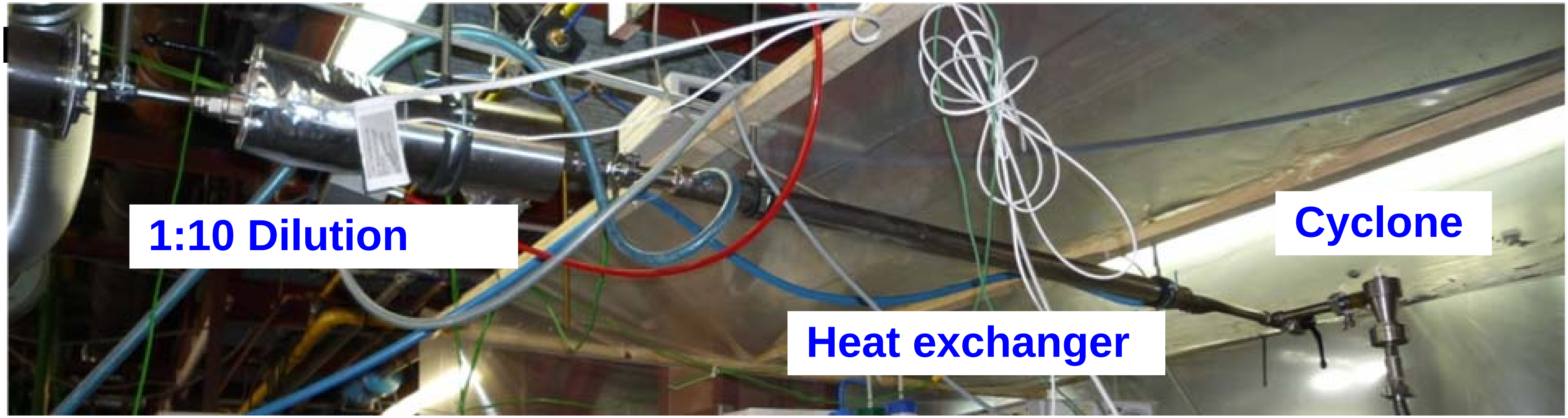
Suboptimal aeration

Nanoparticle selection for exposure: Sketch of the experimental set-up



- Individal mass flow w/ 250 hPa underpressure
- Exposure chamber: 39°C
- Gas flow: 37 °C; 85 % relative humidity
- 6 exposures in parallel
- Three sampling outlets (e.g. for scanning mobility particle sizer (SMPS), impactor)

Nanoparticle selection for exposure : Experimental set-up



1:10 Dilution

Cyclone

Heat exchanger

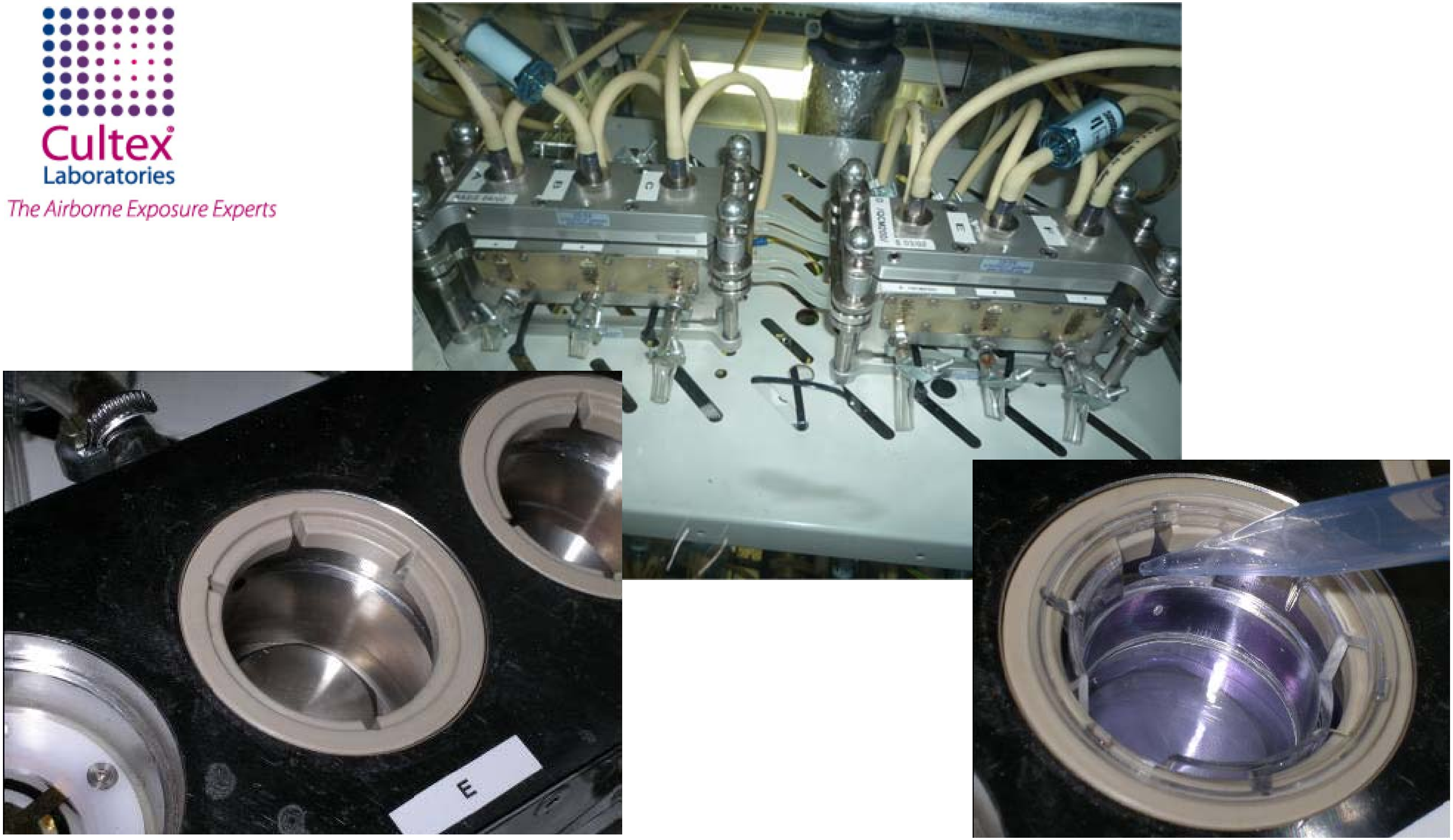
Monitoring unit:
- temperature
- CO_2
- CO
- O_2

Karlsruhe exposure chamber

Cell cultures under exposure

NMI
achieving results

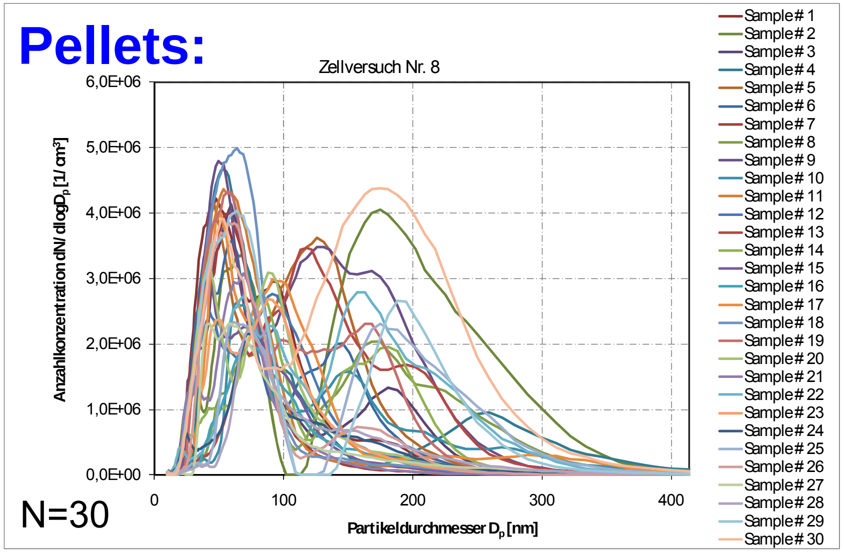
Cultex®
Laboratories
The Airborne Exposure Experts



Number and size distribution of particles (SMPS analysis)

NMI
achieving results

Pellets:



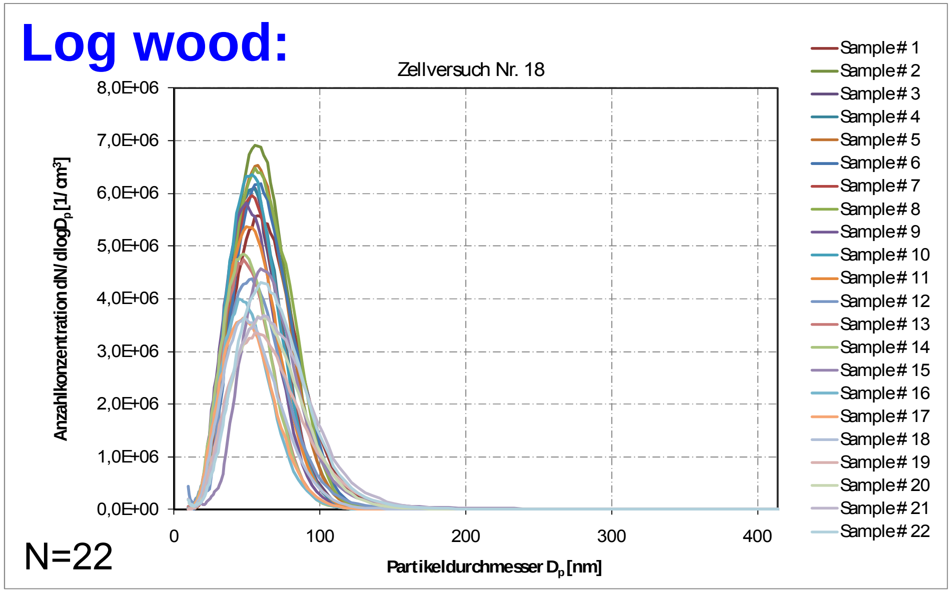
N=30

Optimal combustion: Diameter ca. 55-90 nm

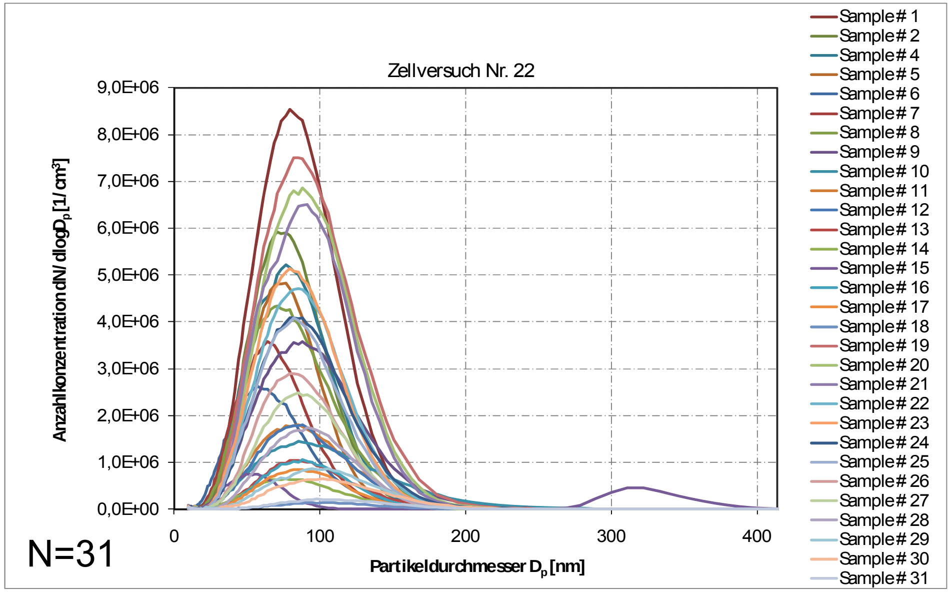
Suboptimal cobustion:
Particle size out of detection range

Suboptimal combustion:
Diameter ca. 250-400 nm

Log wood:



N=22

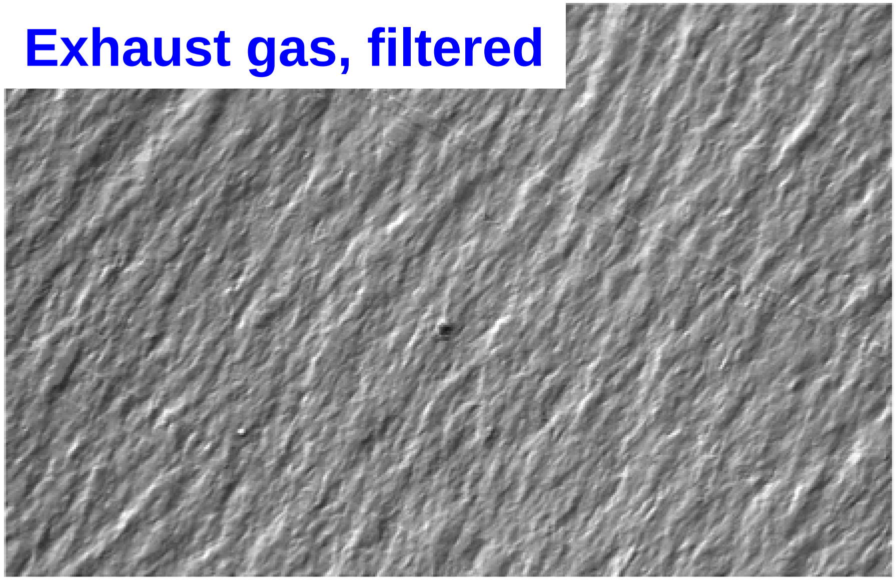


N=31

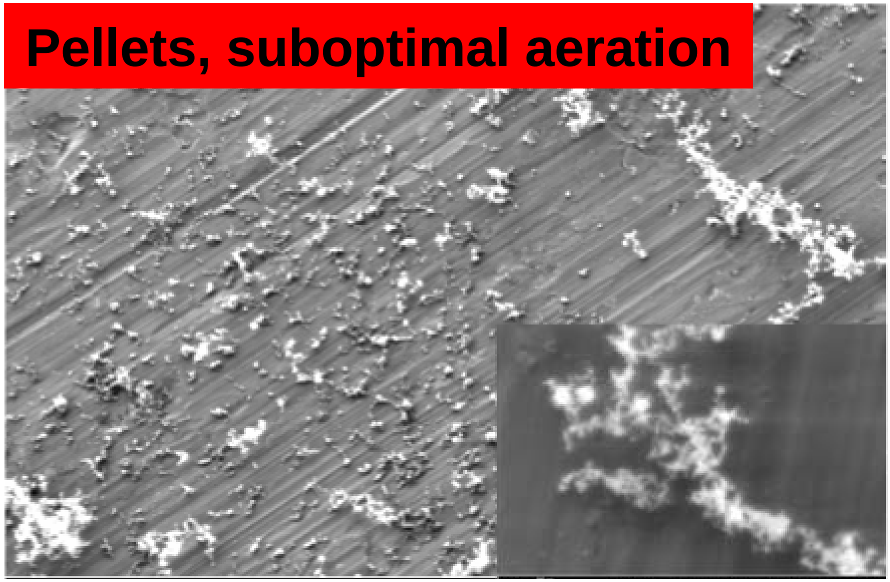
Optimal combustion: Diameter ca. 55 nm

Suboptimal combustion: Diameter ca. 85 nm

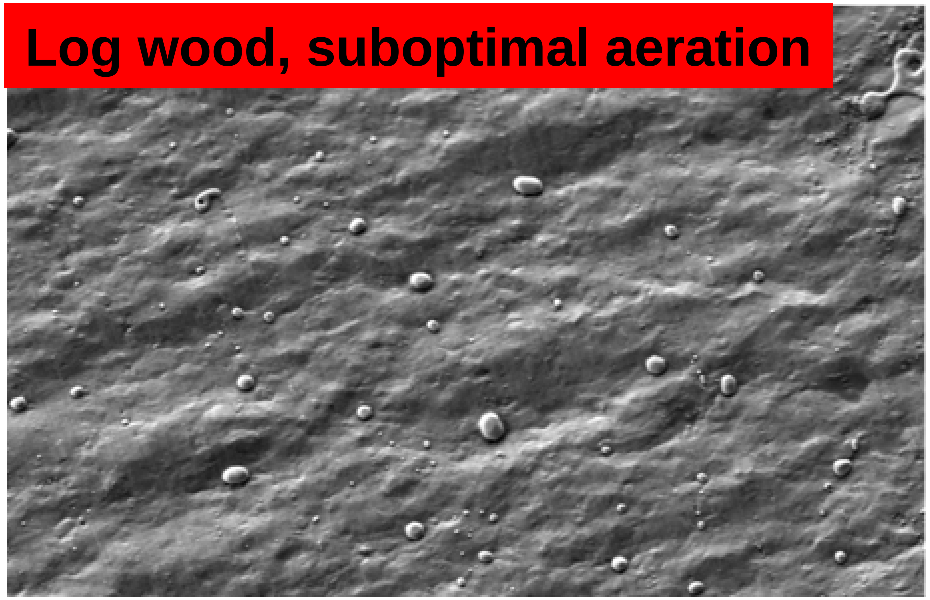
Scanning electron microscopy: Transwell® inserts



Exhaust gas, filtered



Pellets, suboptimal aeration



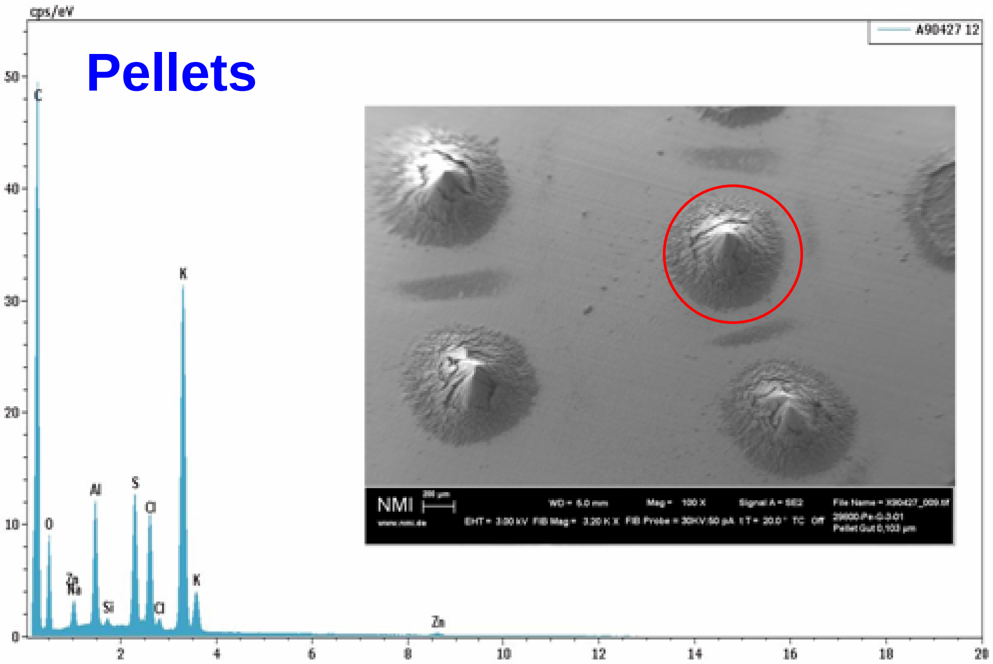
Log wood, suboptimal aeration

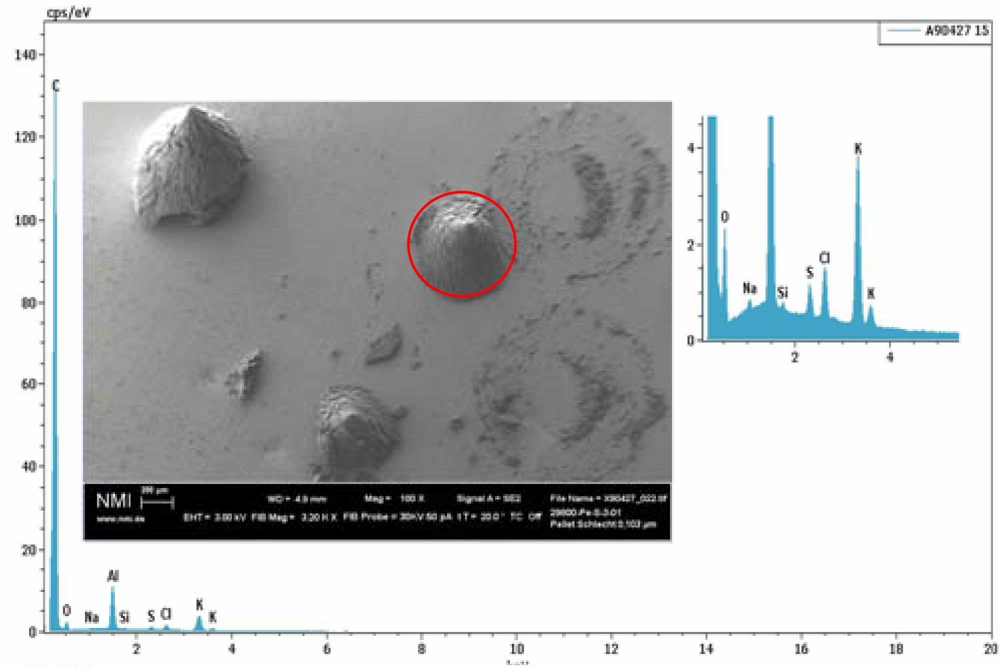
Elementary analysis:

Optimal aeration

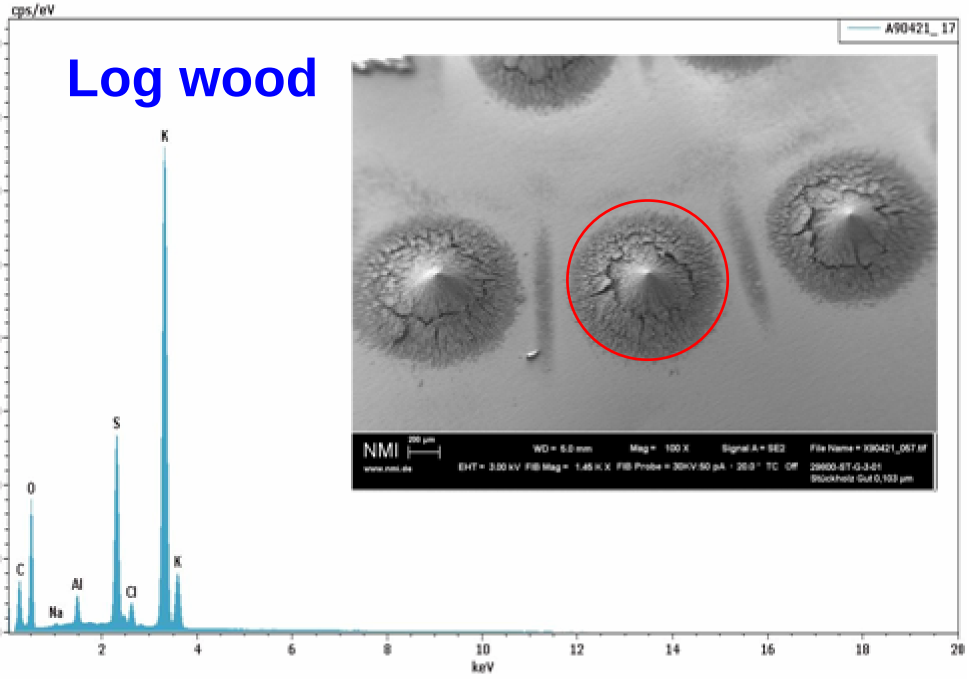
Suboptimal aeration

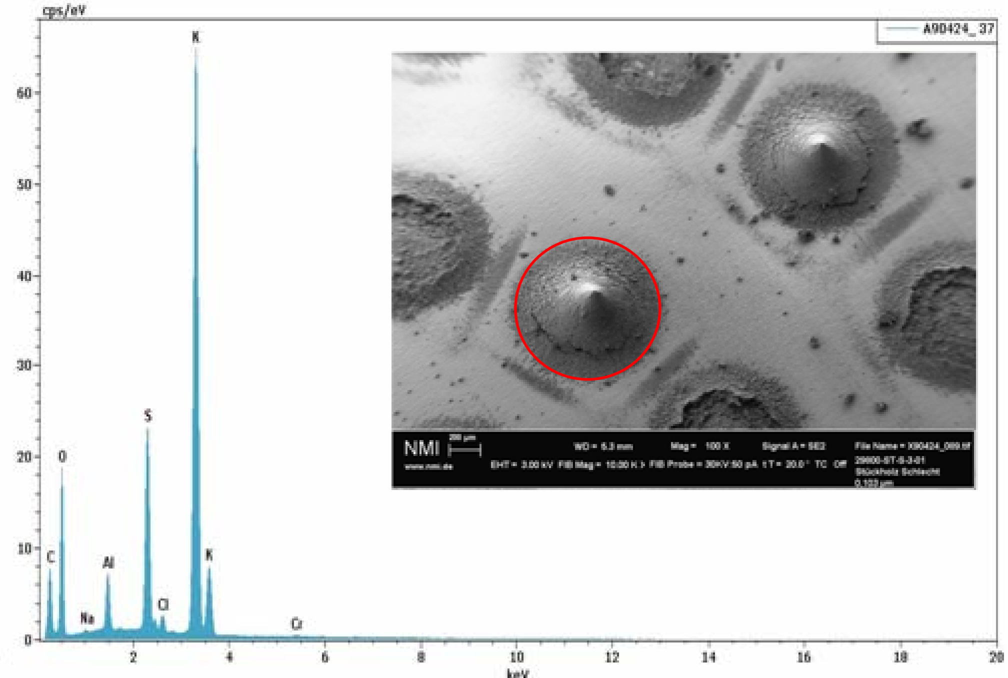
Pellets





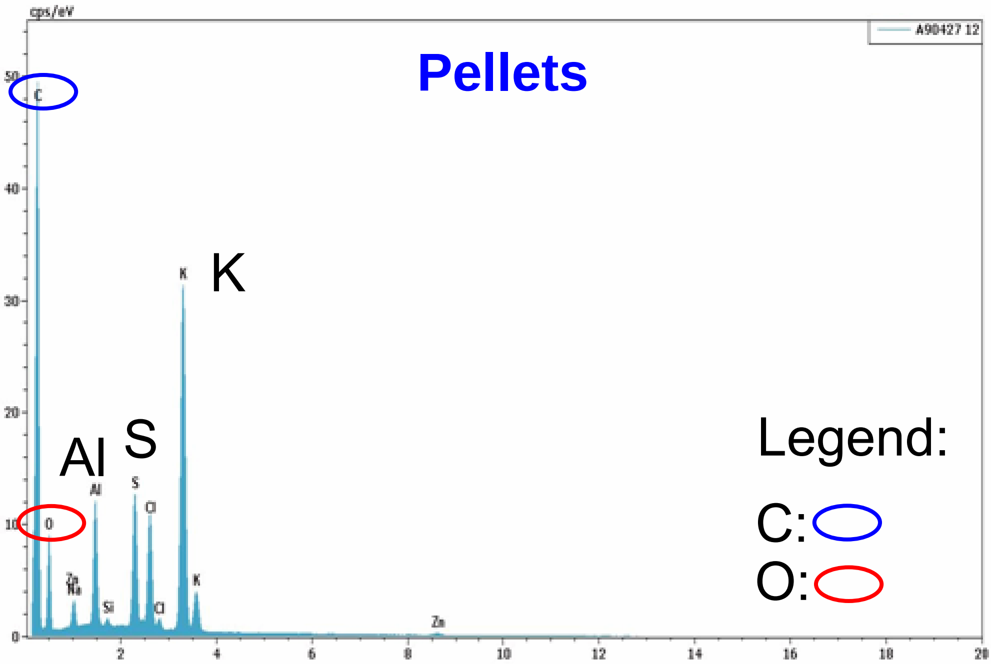
Log wood

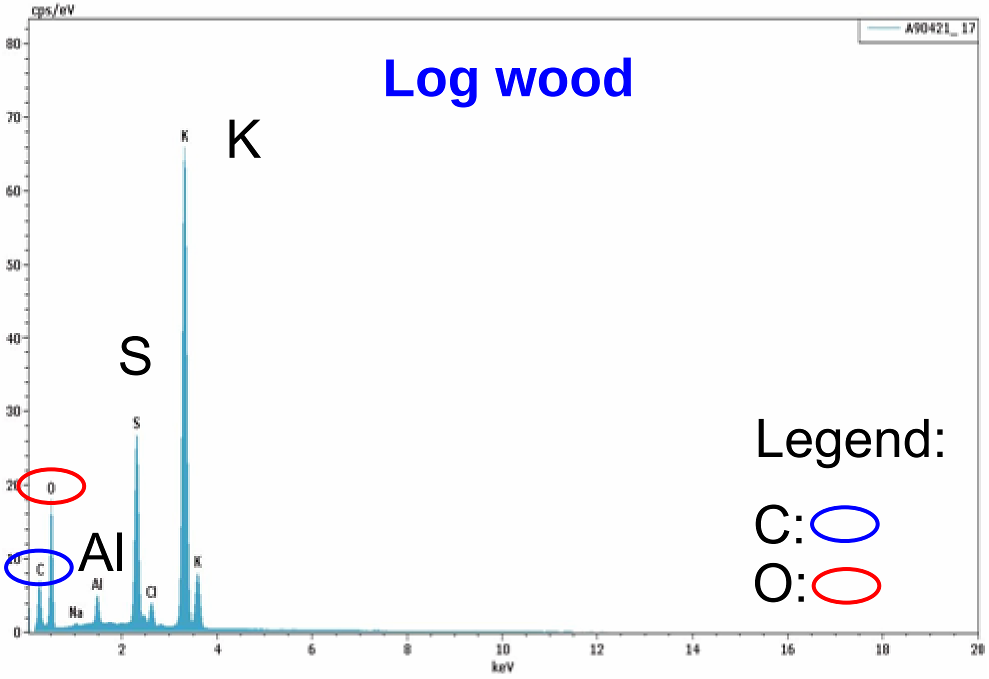




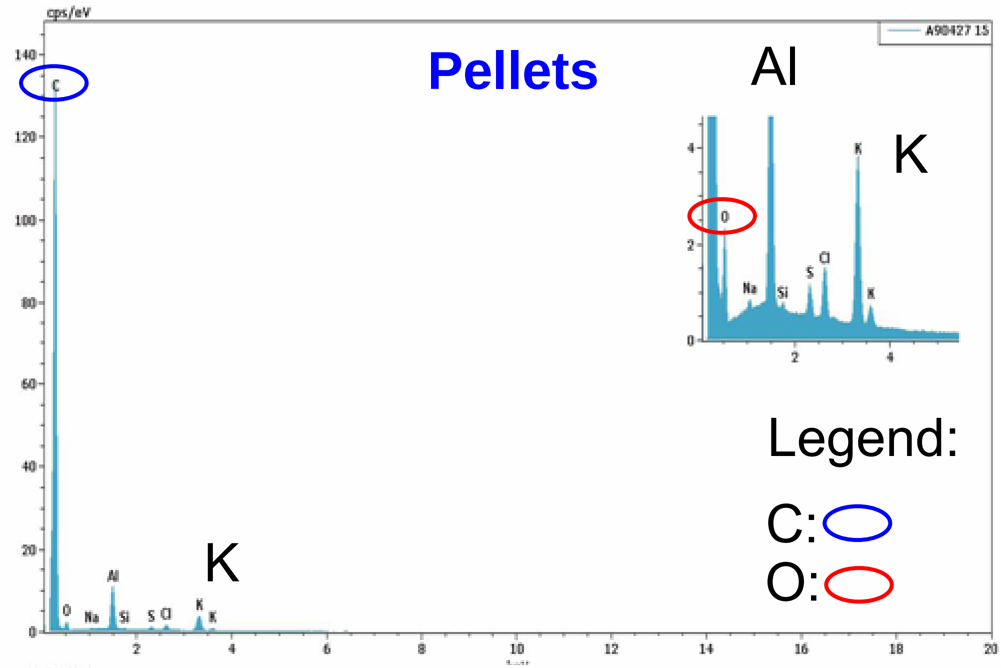
Elementary analysis:

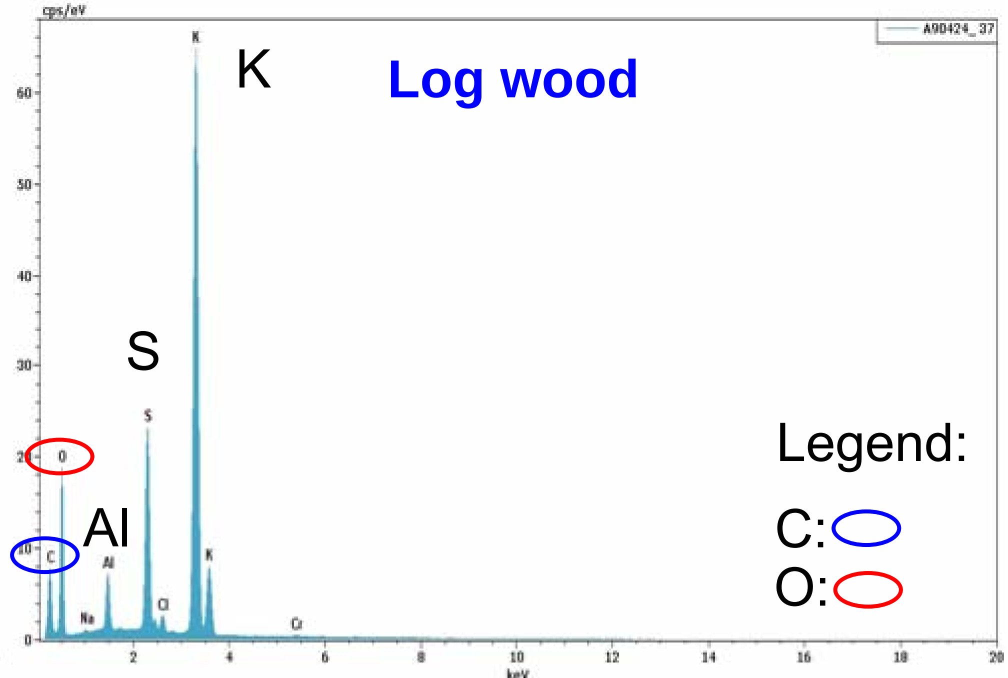
Optimal aeration





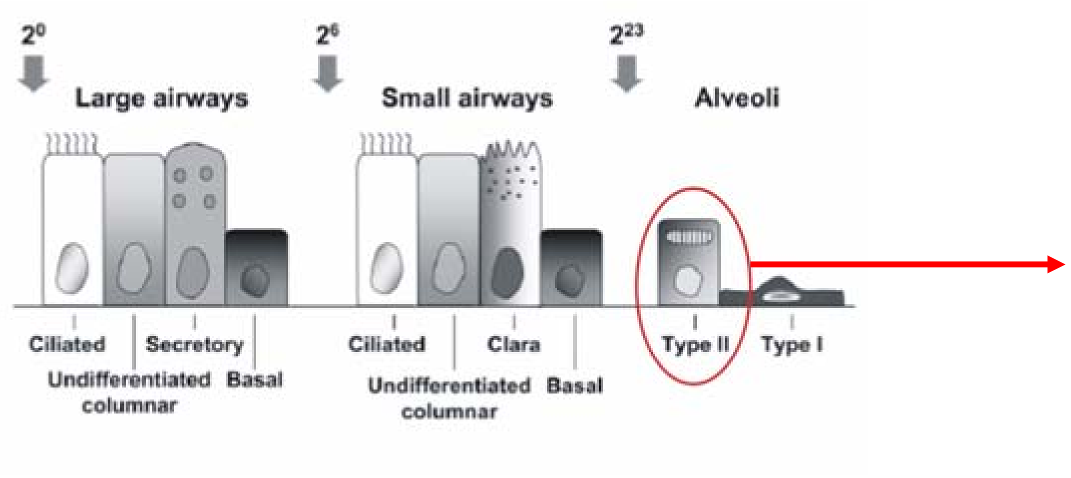
Suboptimal aeration





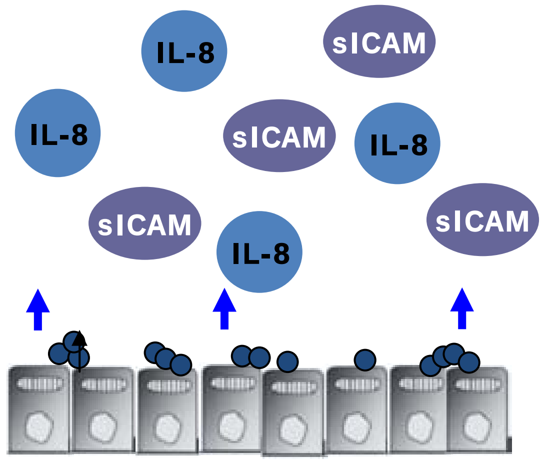
Stress signalling in model lung cells

A549: Type II lung cells (tumour line)



Immunofluorescence microscopy detecting Zonula Occludens 1 (ZO-1)
(*via* Cy3-labelled secondary antibody)

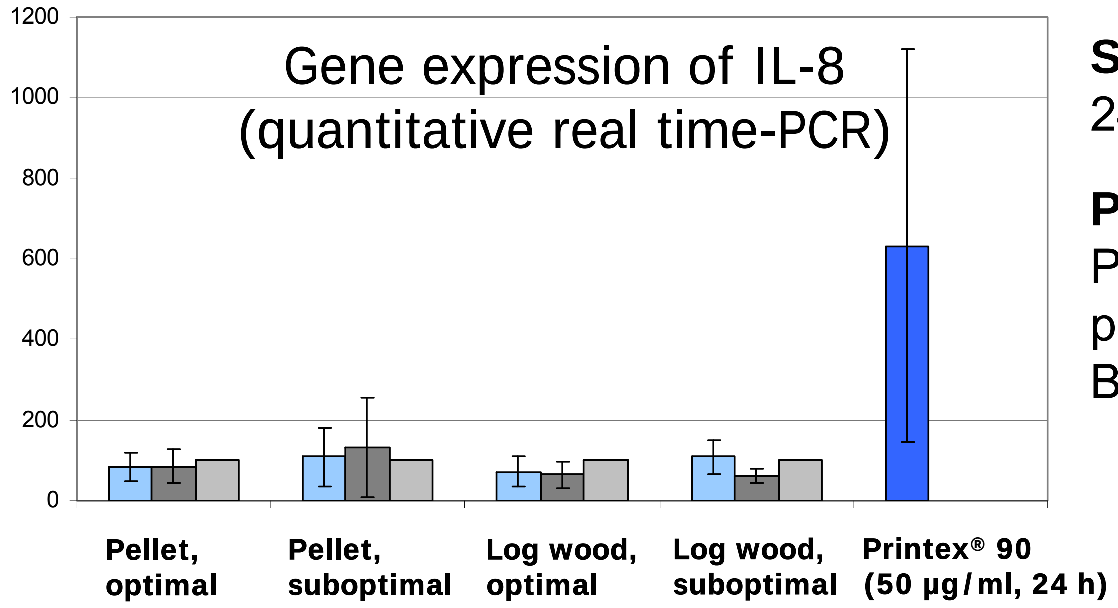
Cellular response to stress: inhibition of growth, decrease in viability



At the protein level: Secretion of
- **interleukin 8 (IL-8)**
- **soluble intercellular adhesion molecule 1 (sICAM)**

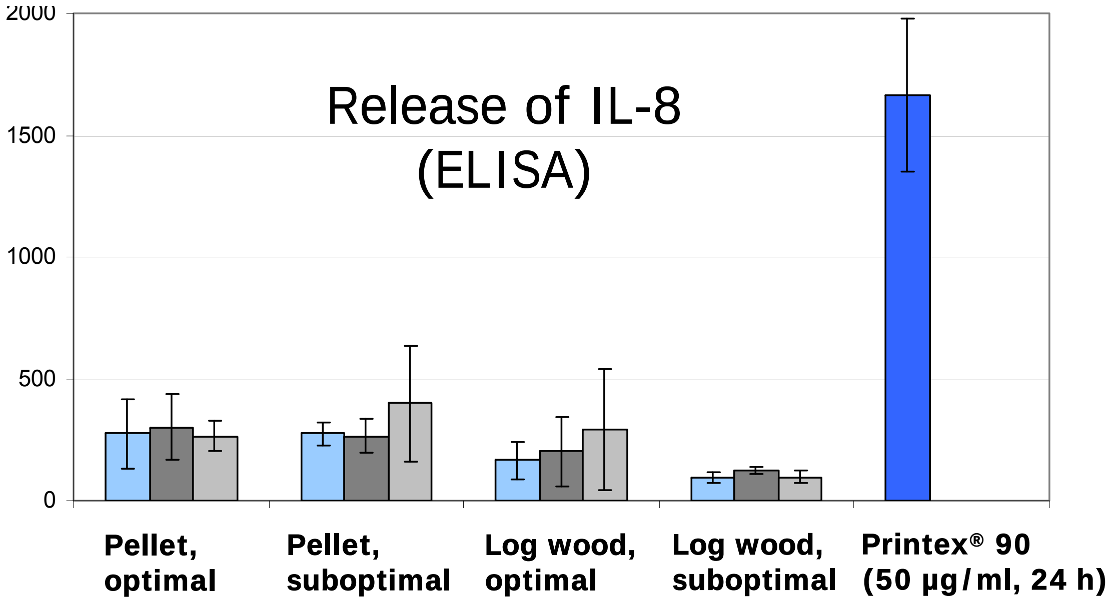
At the level of mRNA: transcription of
- **interleukin 8 (IL-8)**
- **intercellular adhesion molecule 1 (ICAM-1)**

Gene expression and release of IL-8
after 2.5 h of exposure at air-liquid interface



Sampling:
24 h after exposition

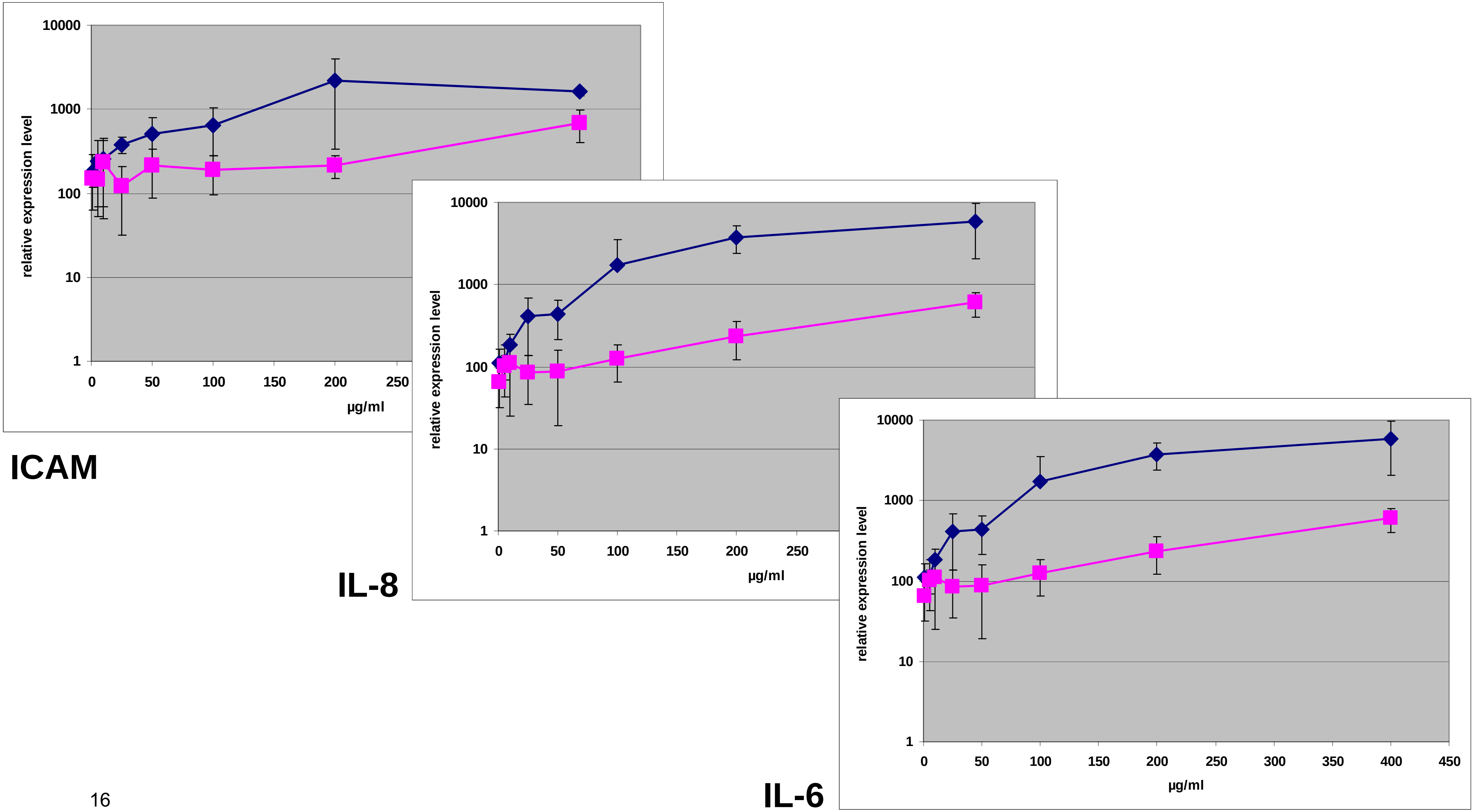
Positive control:
Printex® 90: amorphous carbon,
particle size 14 nm,
BET surface 300 m^2/g



Gene expression after submerse exposure: ICAM, IL-8 and IL-6 analysed by qRT-PCR

Concentration range: 10 - 400 µg/ml; 24 h exposure

Printex® 90 vs. particles obtained from pellet combustion



ICAM

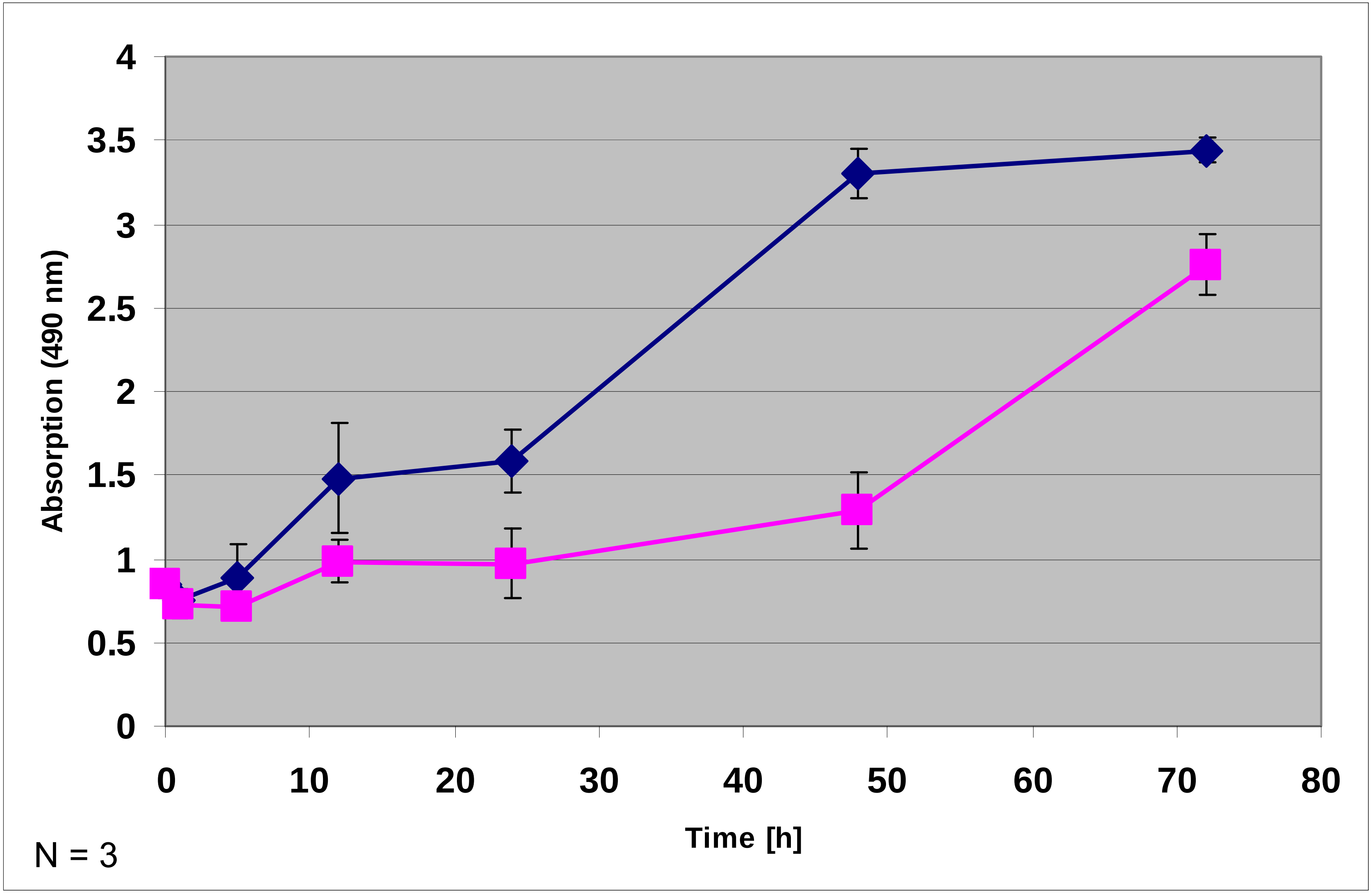
IL-8

IL-6

A549 cell viability after submerse exposure: Kinetics of LDH release

Concentration: 100 µg/ml; exposure range: 0-72 h

Printex® 90 vs. particles obtained from pellet combustion



N = 3

Summary

- **Except for the pellet stove under suboptimal conditions, the particles are mainly composed of inorganic matter (salts).**
- **As little carbonaceous material reaches the cells, no stress response was detectable at air-liquid interface.**
- **Submerse exposure of cells to particles from suboptimal pellet combustion resulted in a response comparable to Printex® 90, though weaker by one order of magnitude.**

Outlook

- **Less sophisticated burners will be included in further studies, better reflecting the real market.**

- **Different cells lines will be employed, widening the basis for evaluation.**

- **The scope of the study will be broadened including alternative biofuels.**

Acknowledgments

NMI
achieving results

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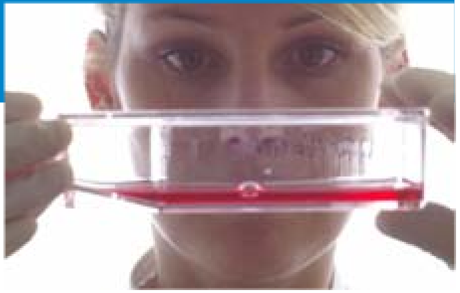

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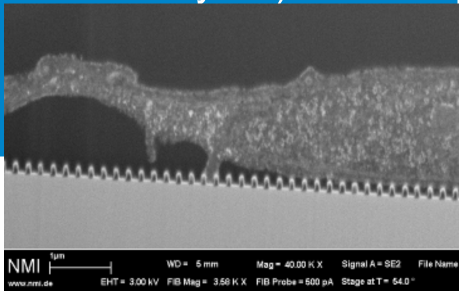

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