

Turbidity Identification

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Approach to Differentiate the Nature of Beer and other Beverages Deposits

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Causes for haze formation

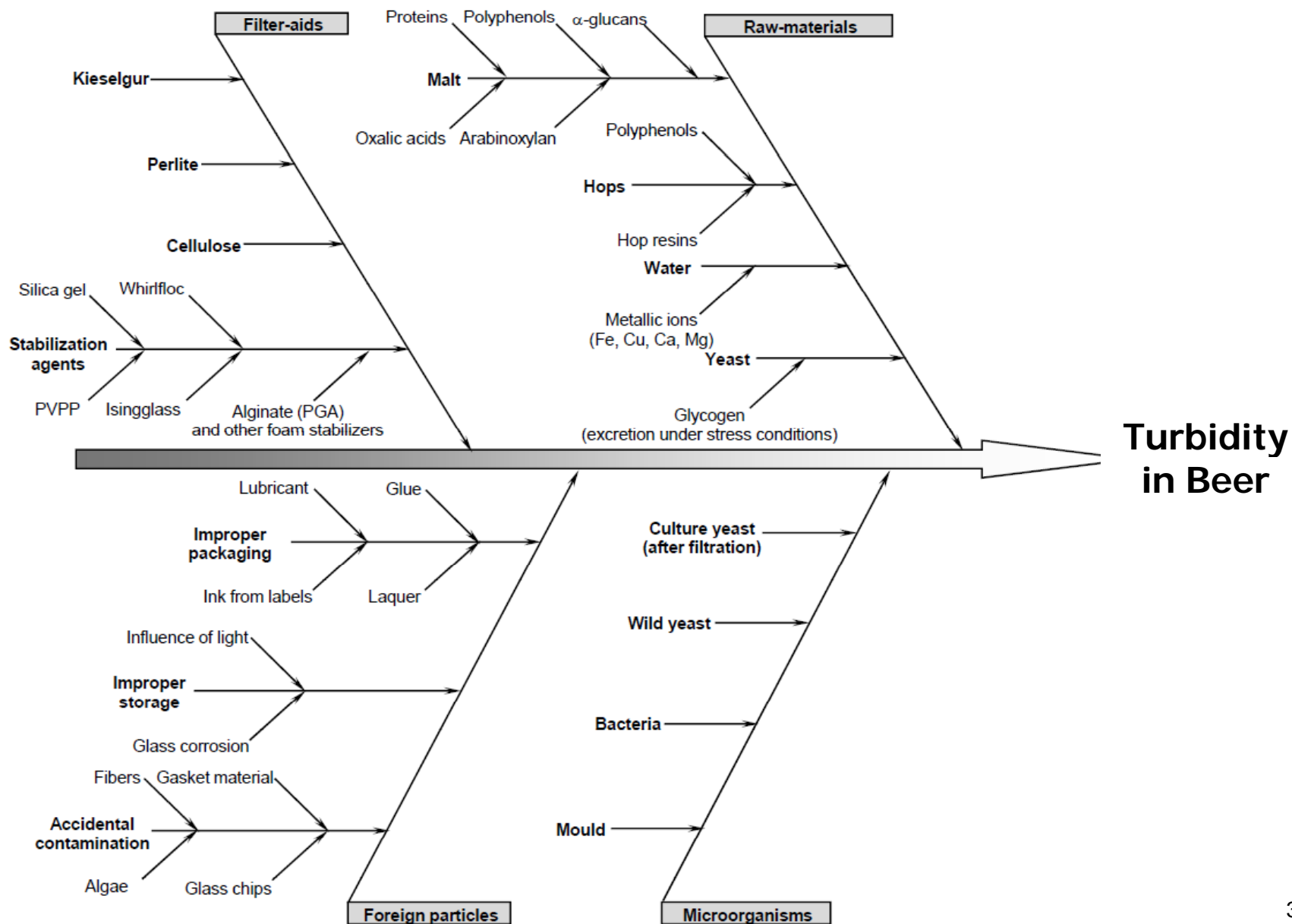


Many substances are „blamed“ with causing or inducing haze, e.g.:

- **Raw materials** (malt + adjuncts, yeast, water)
- **Process aids**
 - Kieselgur
 - Stabilization aids
- **Microorganisms**
- **Other (foreign) material**



TURBIDITY IN BEER

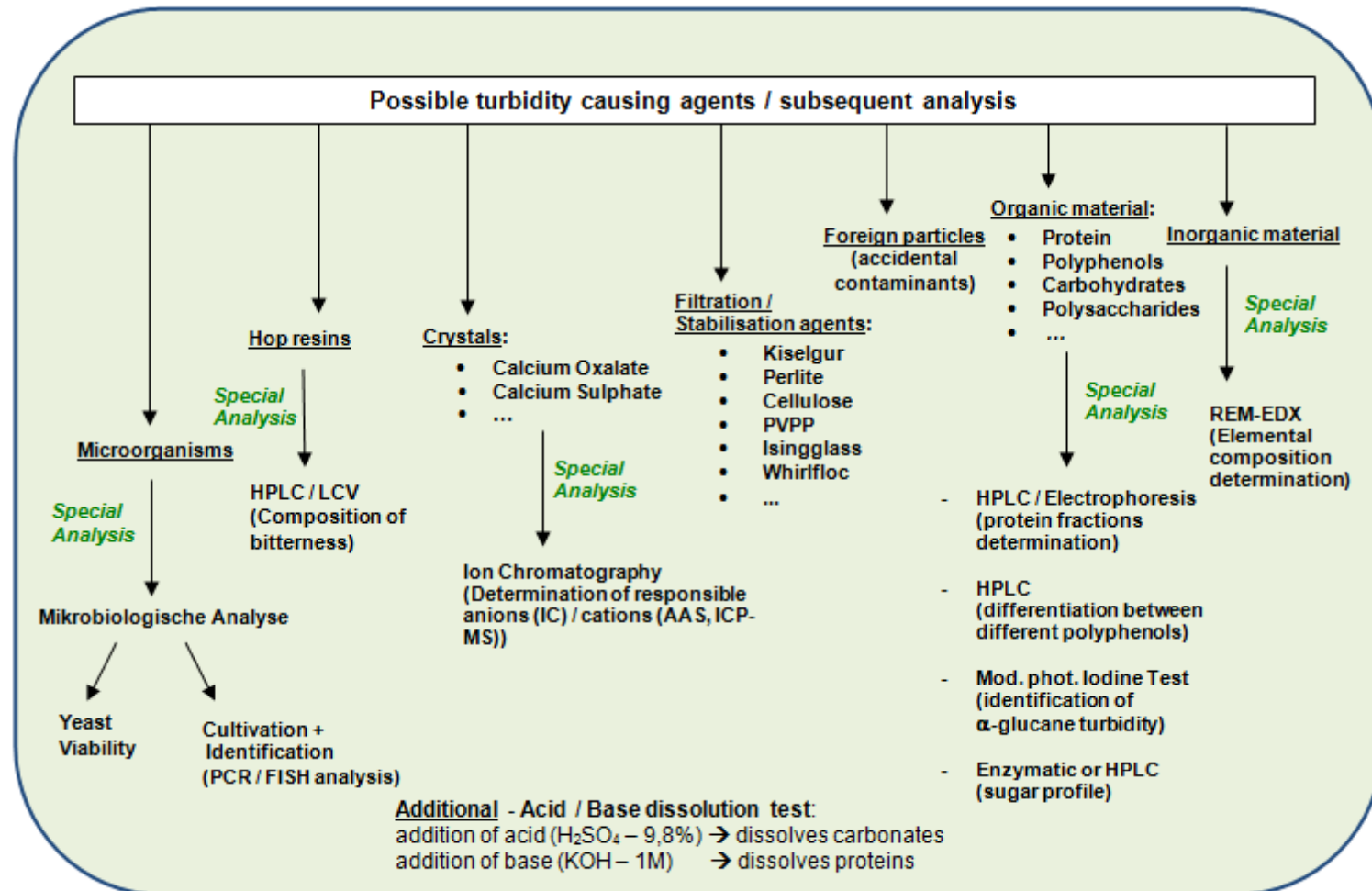


Turbidity/Particle Identification



Microscopic ID & staining, incl. comparison with VLB data bank

Eosine yellow (proteins); Iodine (starch); Thionin (neutral and acidic polysaccharides); Methylene Blue (polyphenols); Ruthenium Red (fruit pectin); Congo Red (cellulose structures, β -glucan)

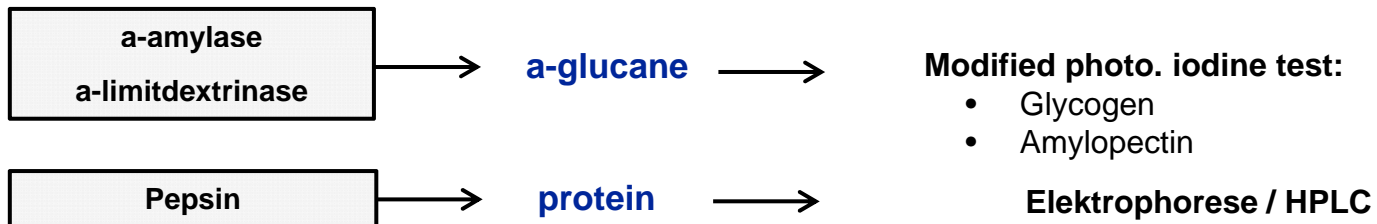


Turbidity Identification



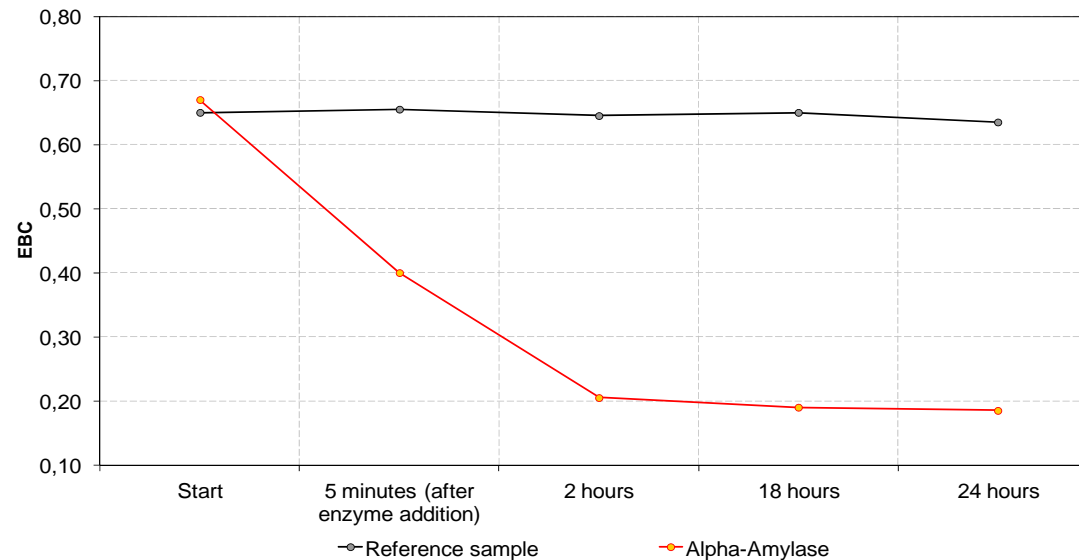
Enzymatic ID

- Sample treatment with different enzymes
- Key Question: Is it possible to reduce turbidity by adding enzymes?



→ Example of a treatment with enzymes

Turbidity development (90 °) after adding of enzymes

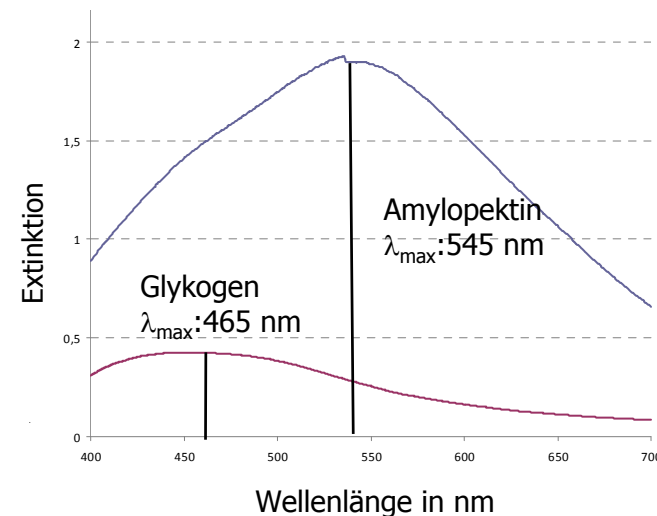


Establishing new approaches



Modified photometric Iodine Test

- Distinction of α -glucane turbidity based on the origin
 - glycogen: process related turbidity
 - amylopectin: raw material related turbidity
- Separation of macromolecular turbidity units of beer
- Staining with iodine test: blue coloring agent - „iodine- starch“
- Differentiation with spectroscopic methods possible
- Improvement approach: complete separation of amylopectin and glycogen should be achieved

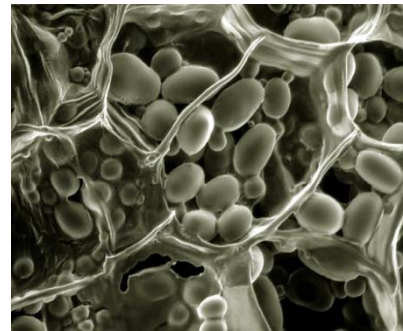
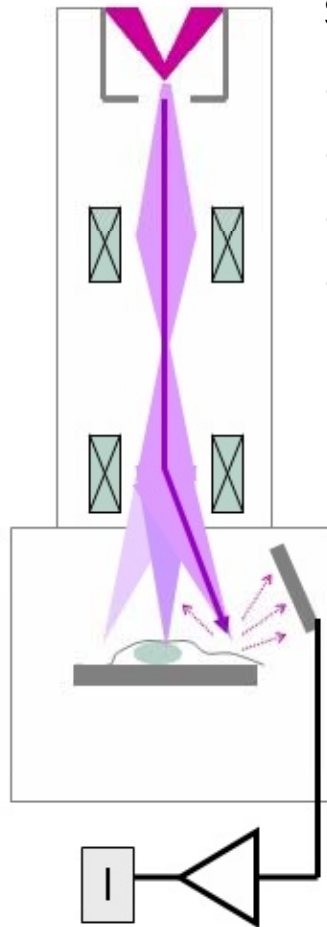


Scanning Electron Microscopy

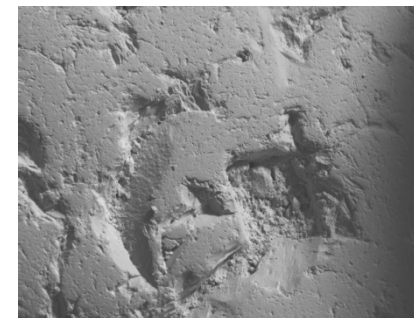


Scanning electron microscopy (SEM)

- resolution $< 10 \mu\text{m}$
- Contrast, „spatial“ depth
- Examination of biol. samples (e.g. starch kernels, yeast cells)
- Identification of submicroscopic structures



Starch kernels



Structure defects

Quelle: wikicommons

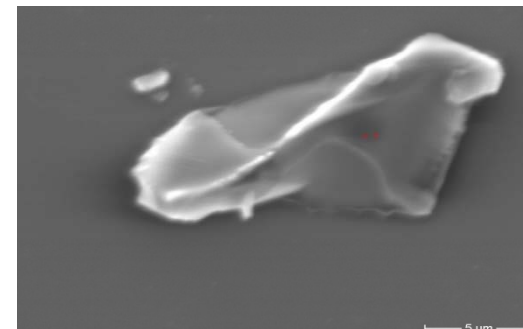
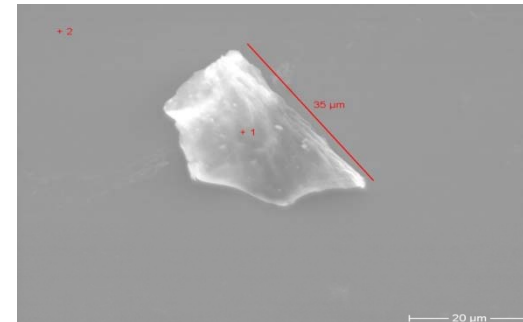
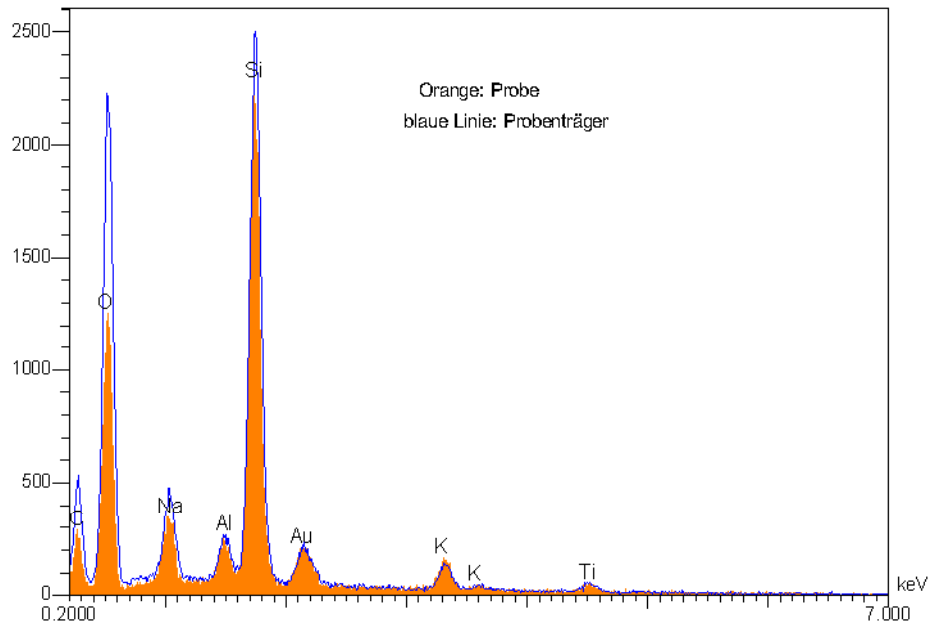
EDX Analysis



➤ Energy Dispersive X-ray Spectroscopy (EDX)

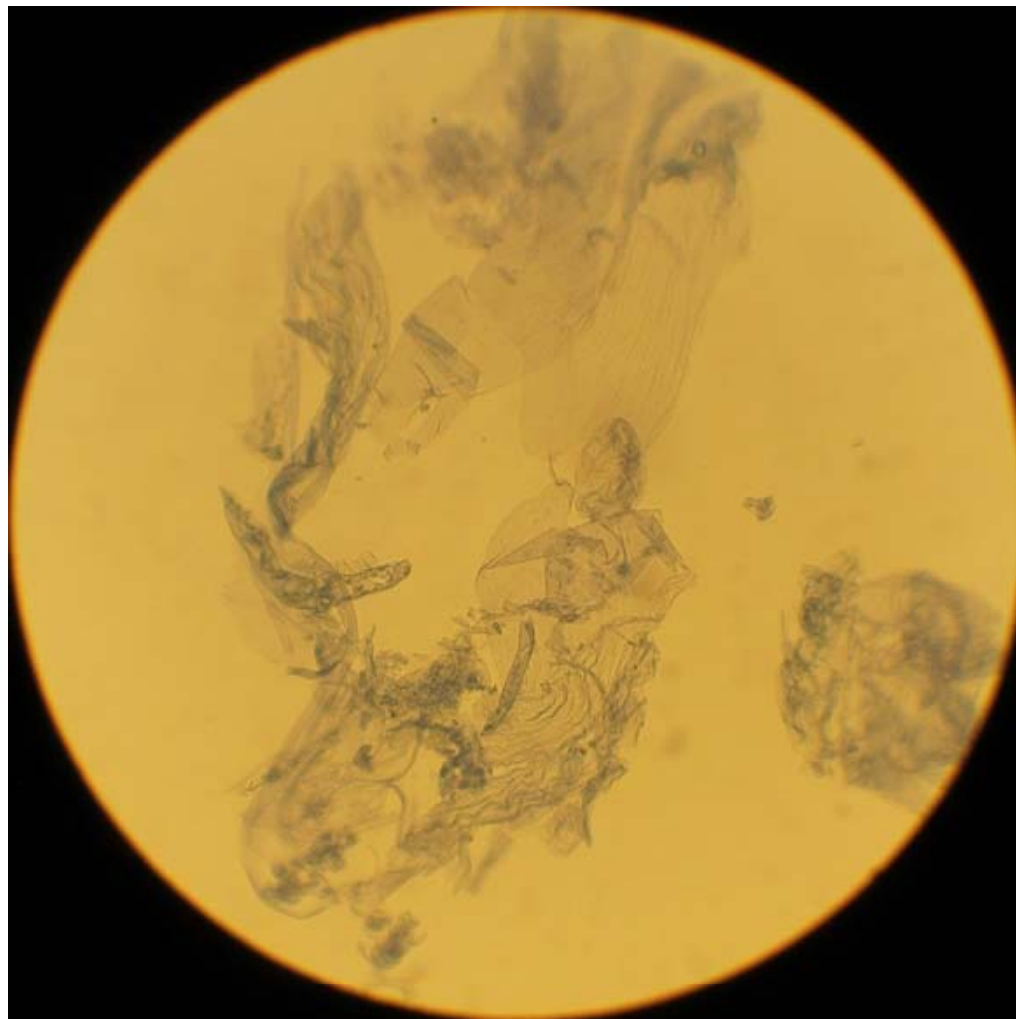
- possible to analyze particles as small as 10 μm
- Determination of elemental composition
- Frequency scale of identified elements allows quantitative assessment
- High-quality electron microscope pictures of particles

Example of EDX-range and identification of glass particles



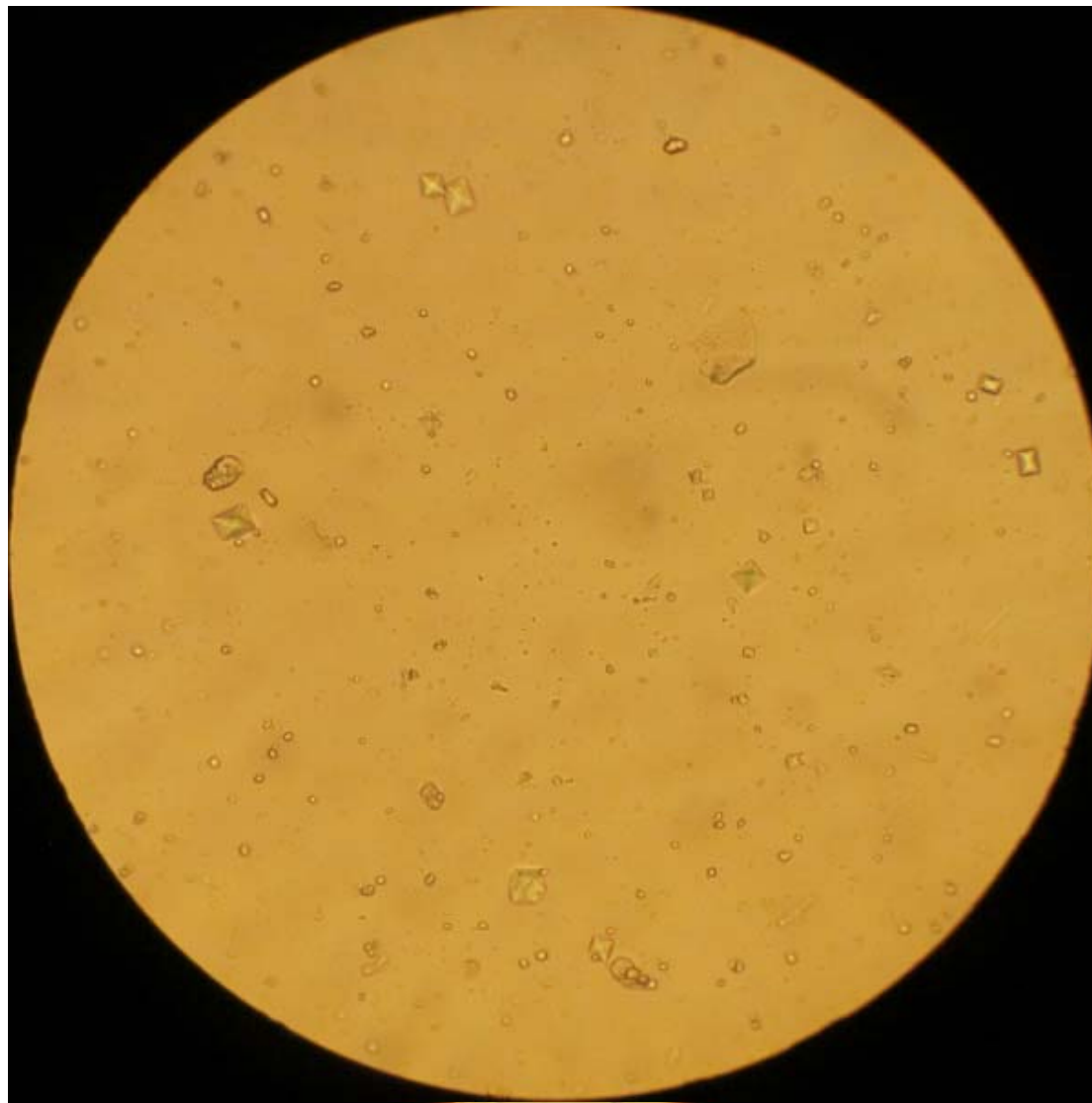
Microscopic images

Organic material



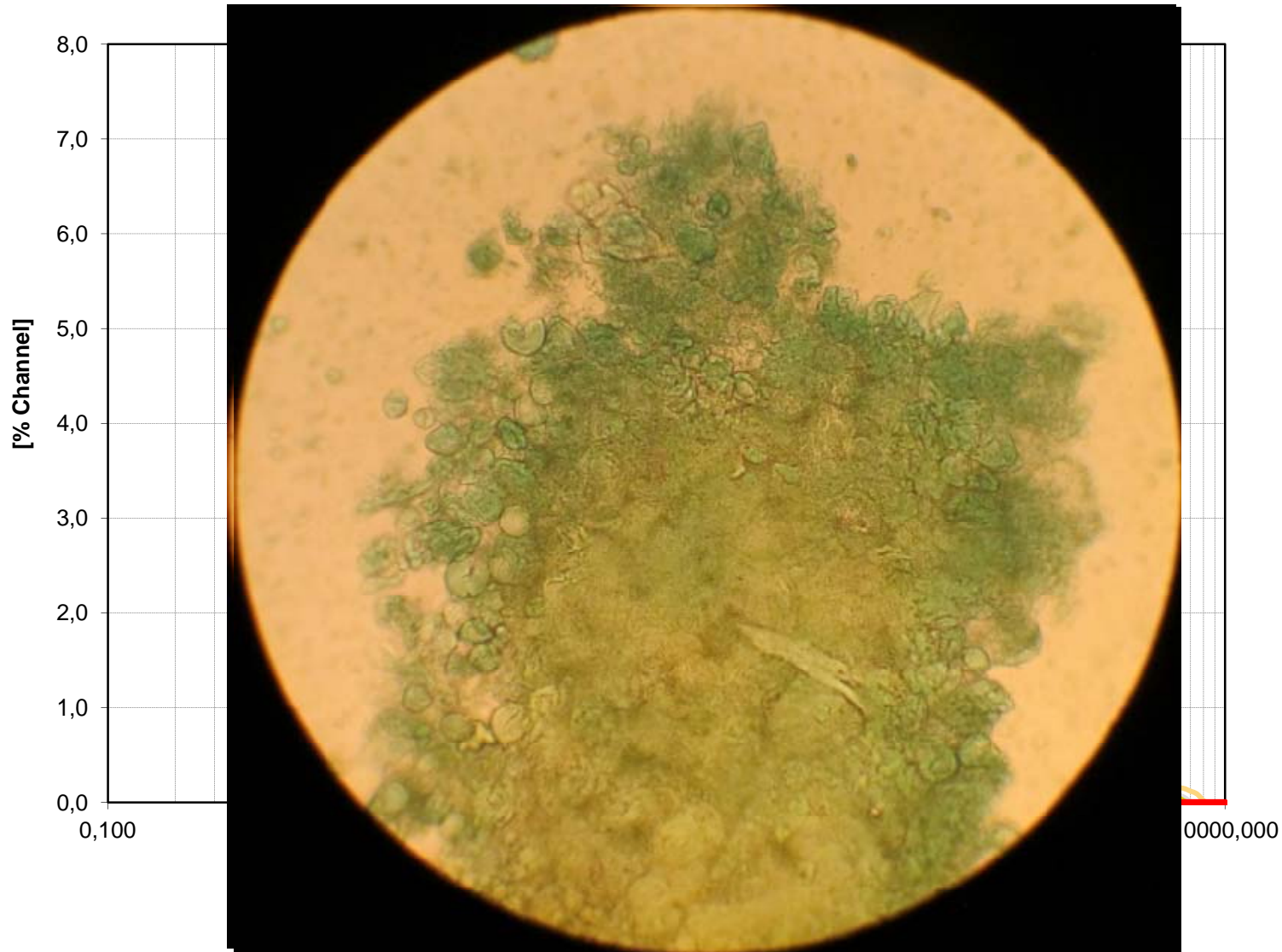
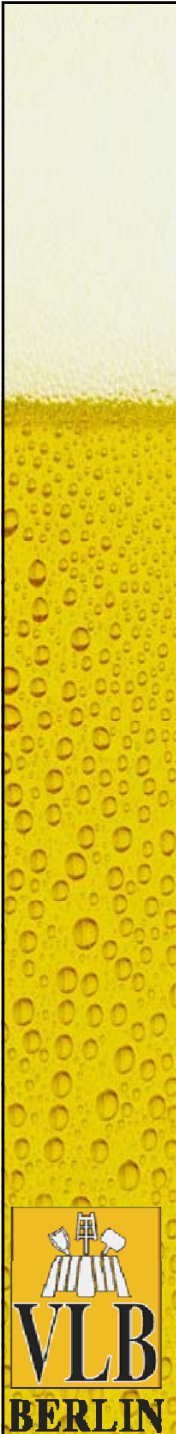
Microscopic images

Filtration aids & oxalate crystals



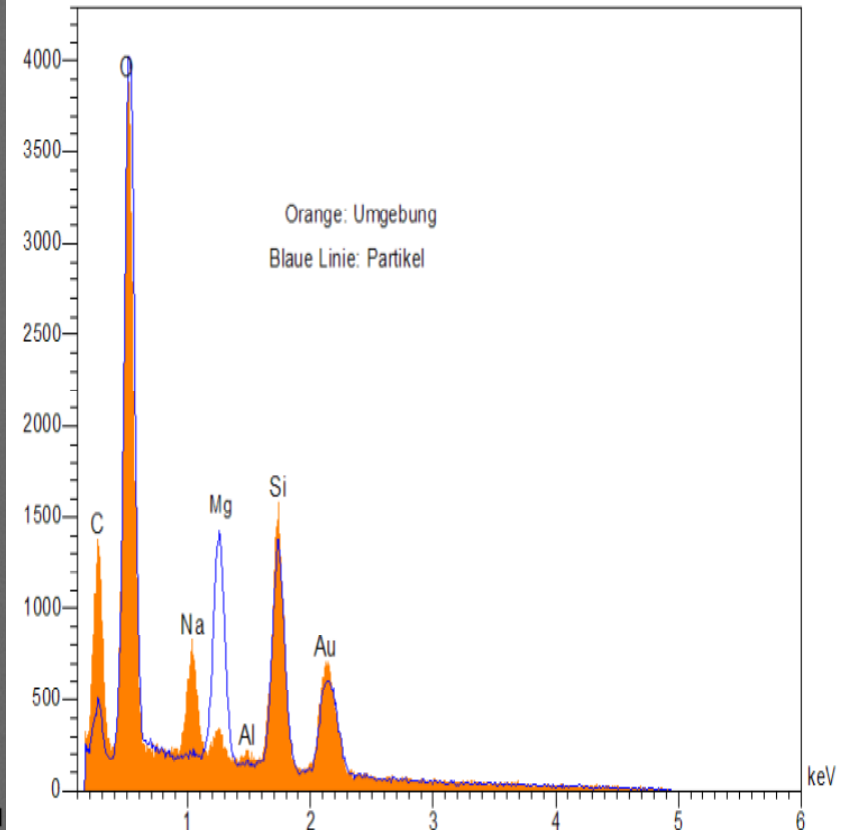
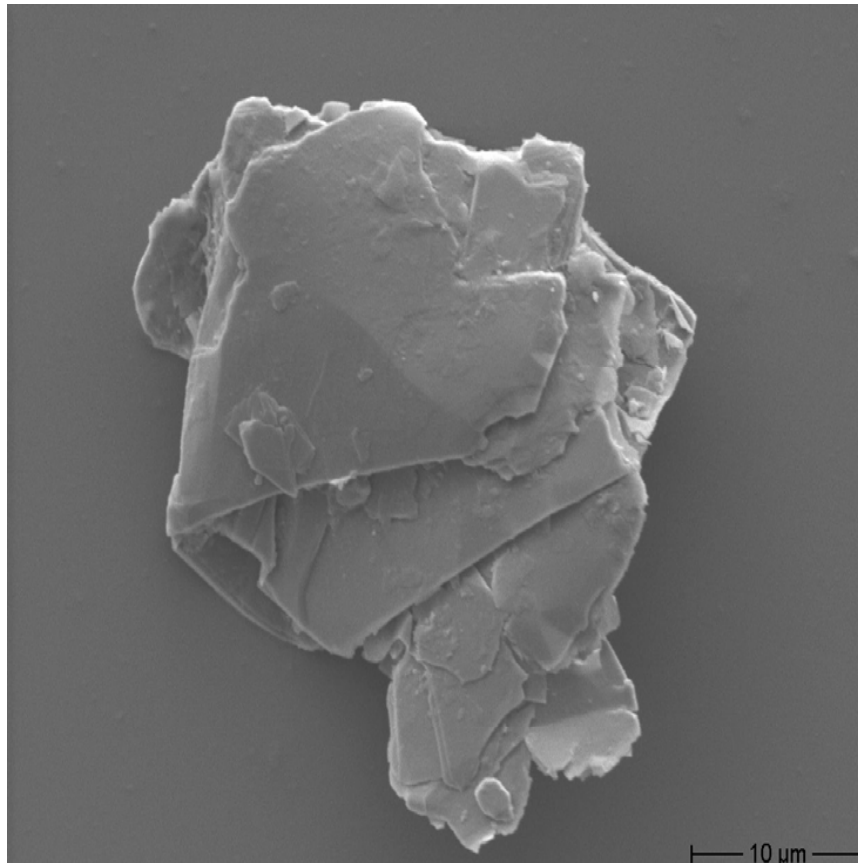
Case Study No. I

Flavored hazy-style beer – sediments



Case Study No. II

Step control



Case Study No. II



Step control to indentify origin of particles

	Yeast	Kieselgur	CaOx.	Organic Particles	Foil Structure
Centrifuge In	XX	0	XX	XX	0
Centrifuge Out	X	0	X	XX	0
Flash Past. In	0	XX	X	X	0
Flash Past. Out 1	0	0	0	X	0
Flash Past. Out 2	0	0	0	X	0
BBT	0	0	0	X	X
Bottle	0	0	0	X	X
Keg (product 1)	0	0	0	X	X
Keg (product 2)	0	0	0	X	X

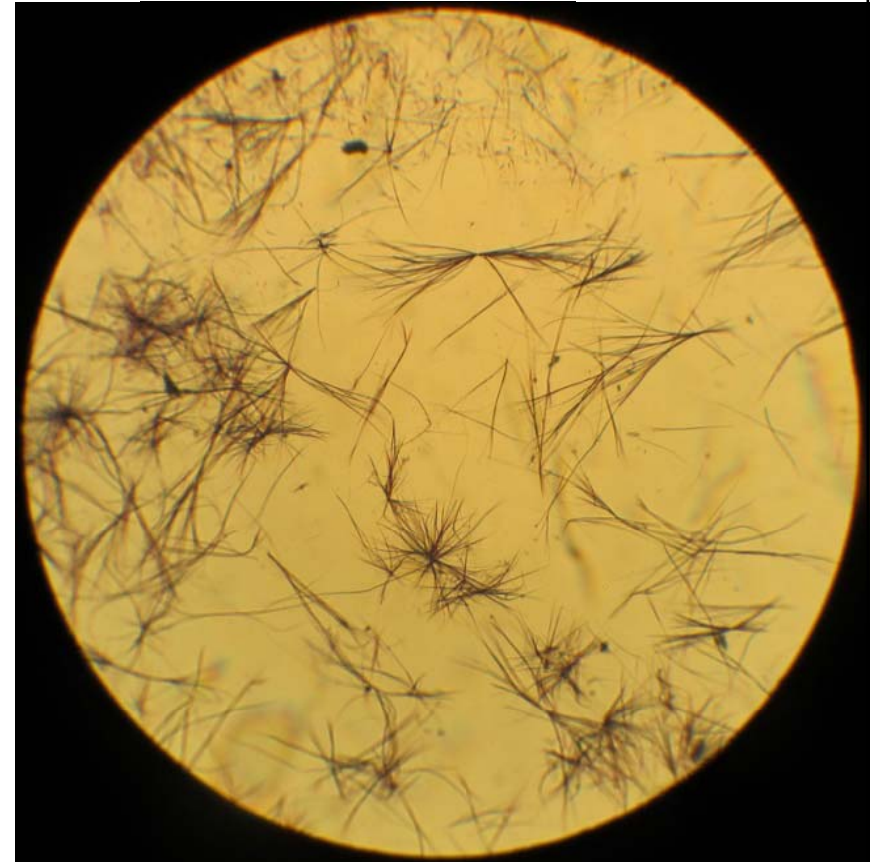
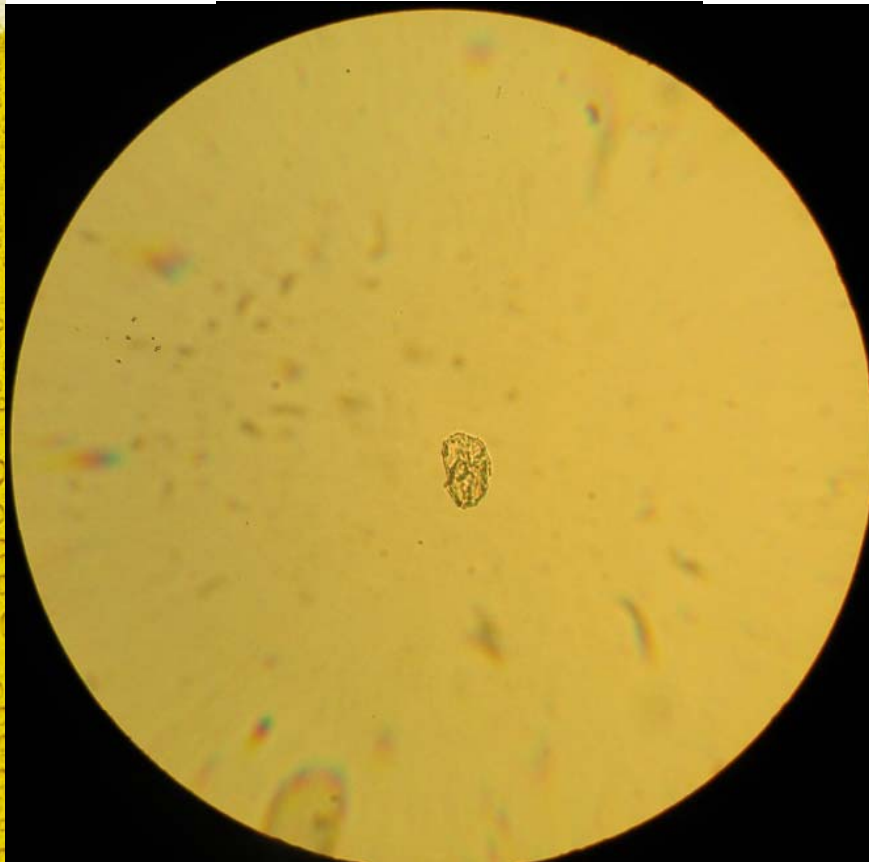
Conclusion:

Further investigation on the origin of the particles is ongoing with focus on the piping to BBT and the BBT itself.



Case Study No. III

Micro problem in water



Case Study No. IV

Carbonized alcoholic beverage



Analyzed sample: Carbonized alcoholic beverage, apple flavored

Order: Identification of turbidity related/ turbidity caused particles

Results:

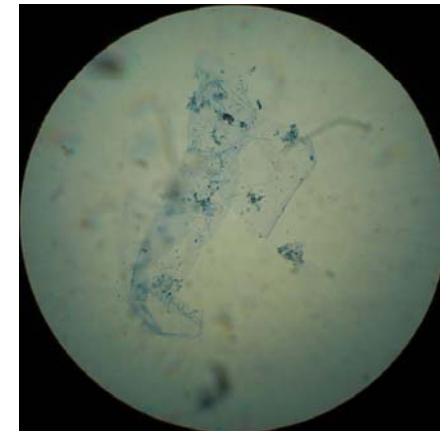
Values of turbidity measurement [EBC]

11°	90°
0,359	0,683

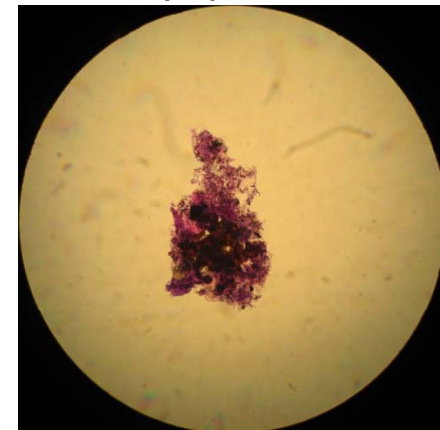
Results of microscopy and staining

Color reagent	Observation
Methylen Blue	<ul style="list-style-type: none"> clearly blue colouring – <u>polyphenols</u>
Thionin	<ul style="list-style-type: none"> Rosy, purple coloring shows presence of acidic polysaccharids staining pectin with thionin leads to same coloring like acidic polysaccharide Because of this and by the particles morphology it is assumed that apple pectin could be involved in the particle's composition.

Microscopic picture– Methylene Blue



Microscopic picture– Thionin



see next slide...

Case Study No. IV

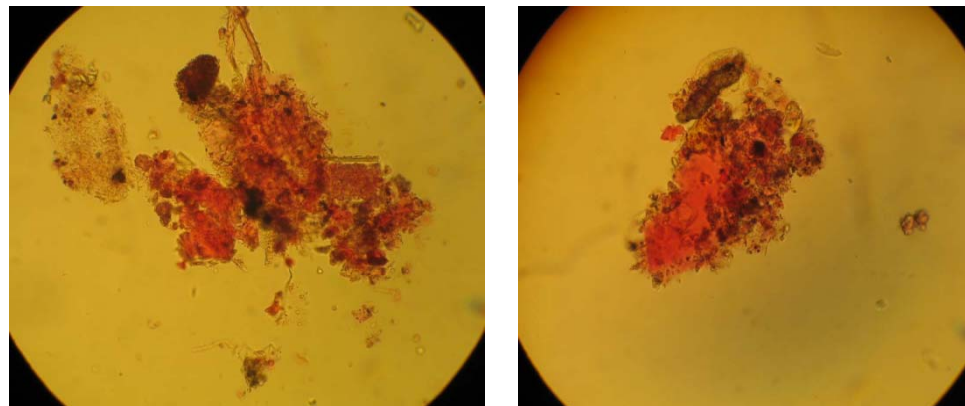
Carbonized alcoholic beverage



■ ■ ■

➤ Confirmation of presence for fruit pectin by:

Staining with ruthenium red and microscopy

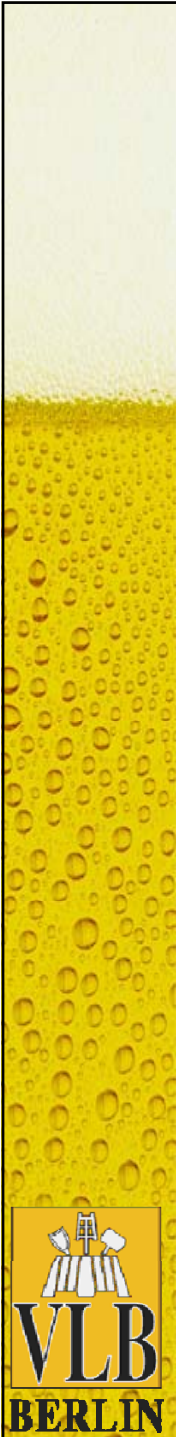


Results of microscopy and staining with ruthenium red

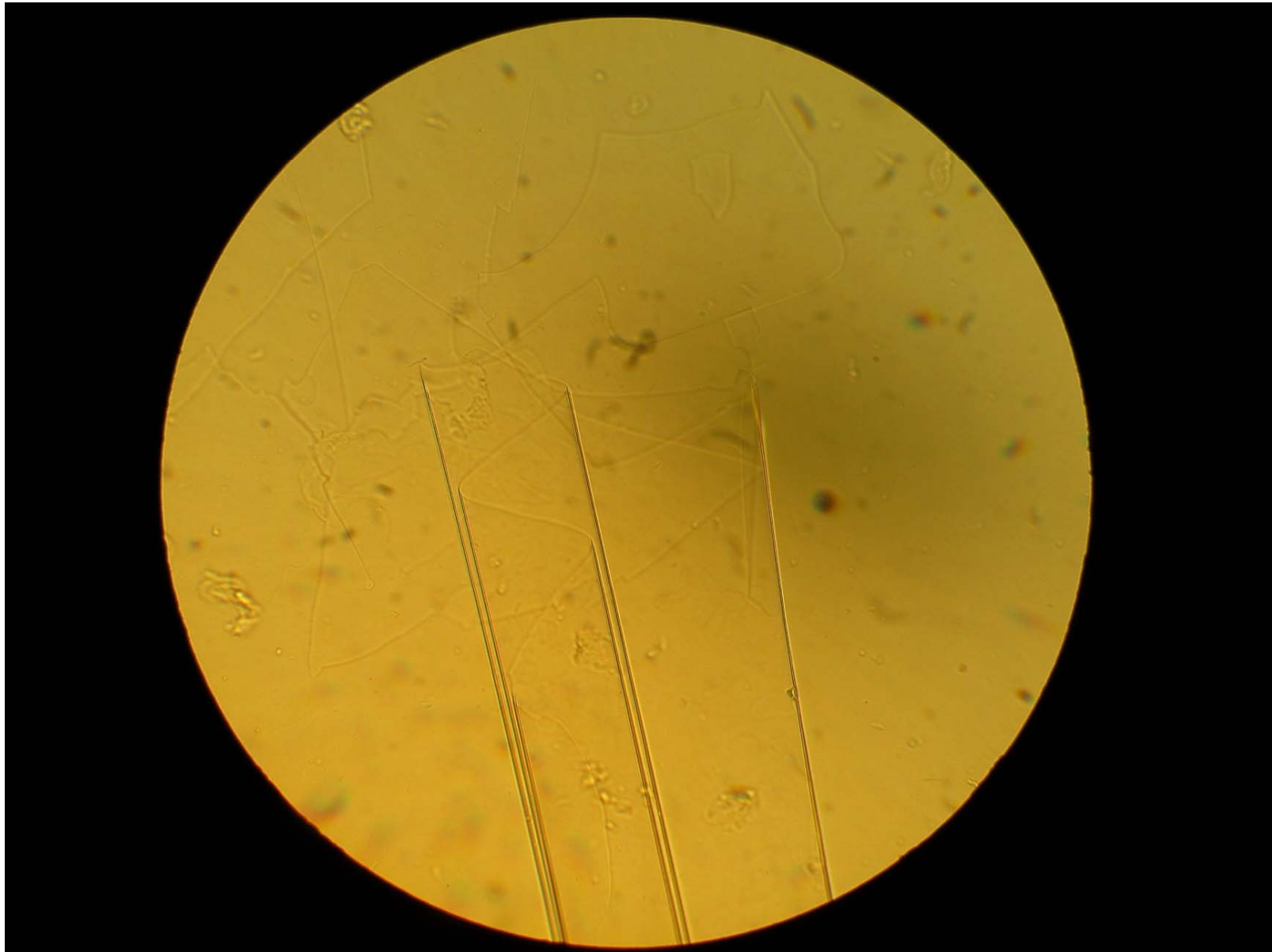
Color reagent	Observation
Ruthenium red	• clearly red coloring of one part of the material unit, which leads to a positive result for pectin

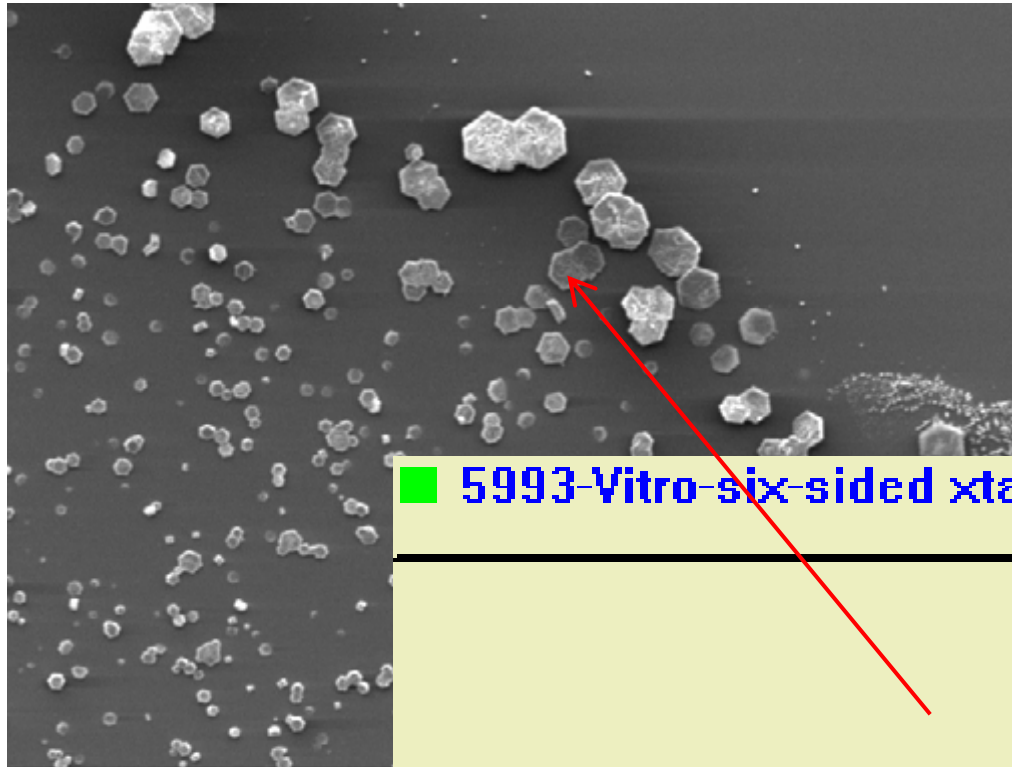
Conclusion:

Turbidity caused material mainly consists of arranged units of apple pectin and polyphenols.



Isolated particles



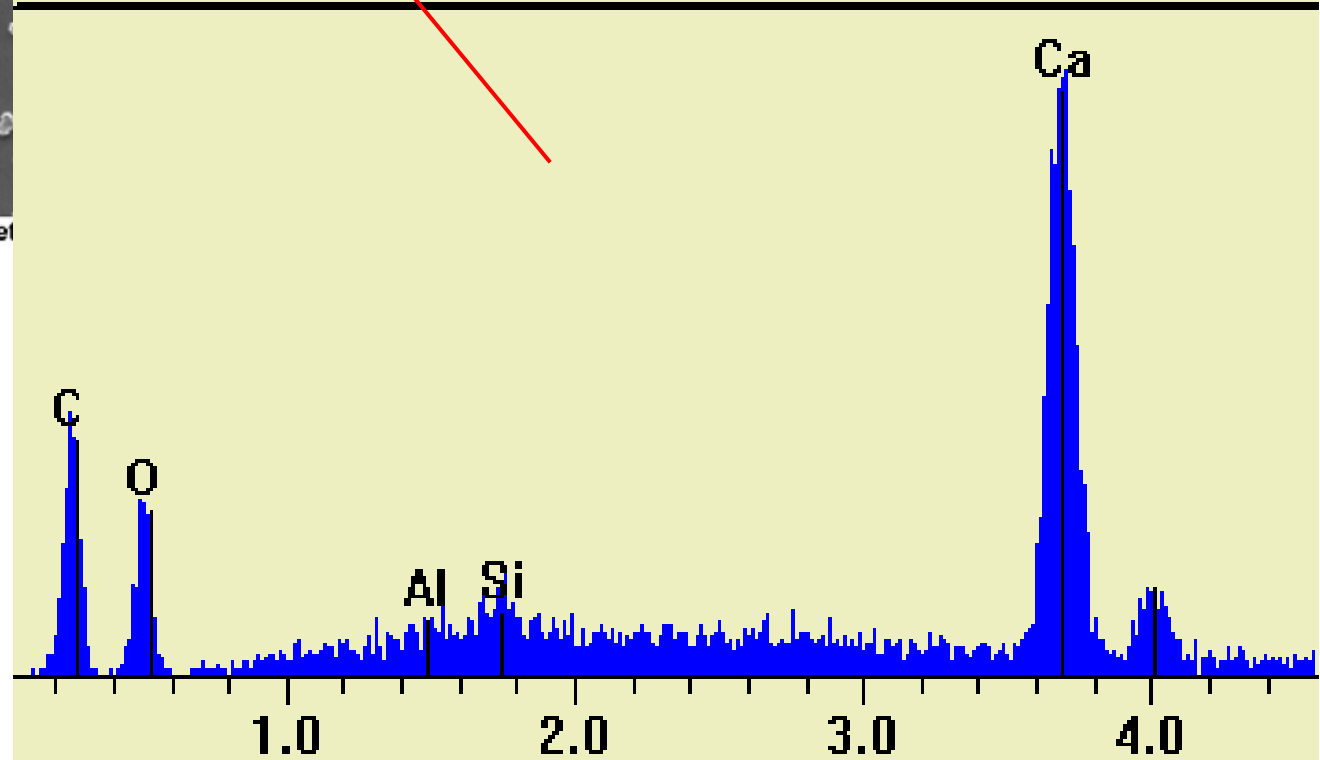


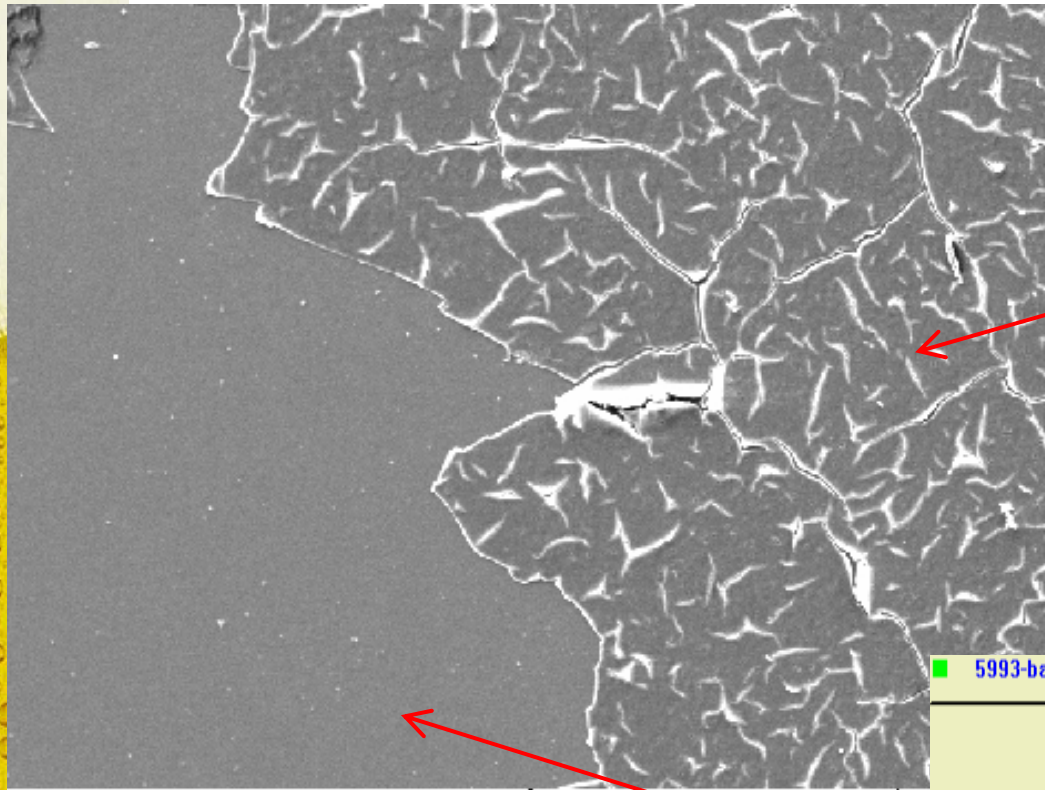
Analysis with EDX
(energy dispersive X-ray
spectroscopy)

5993-Vitro-six-sided xtals-1 a.pgt

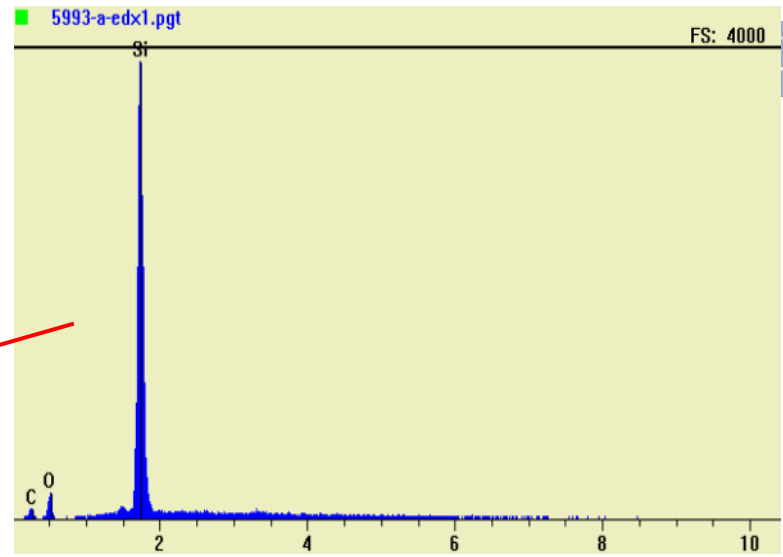
FS: 275

SEM MAG: 861 x
SEM HV: 7.00 kV
PC: 5
Det: SE Det



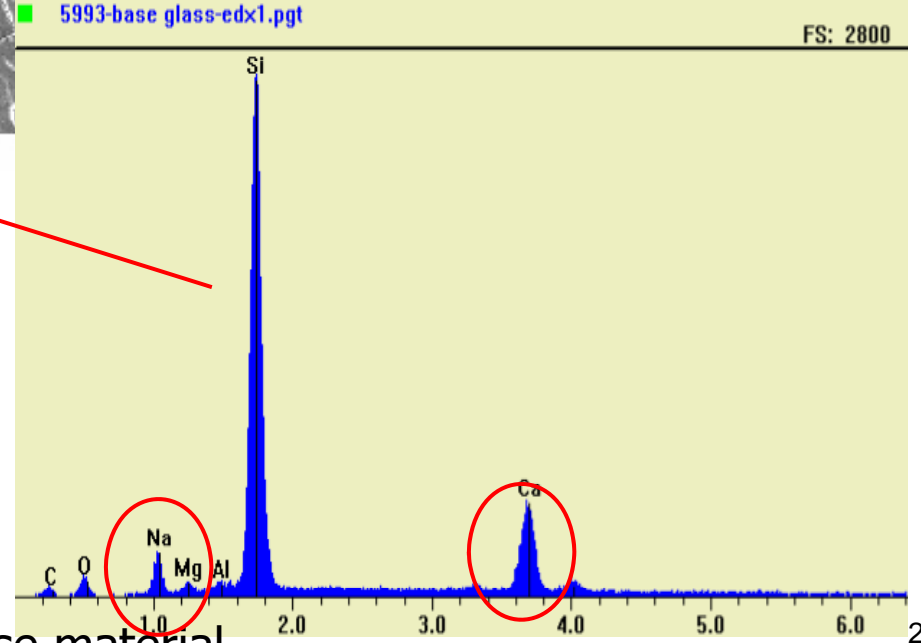


SEM MAG: 796 x
SEM HV: 7.00 kV
PC: 6
Det: SE Detector



EDX „flakes“

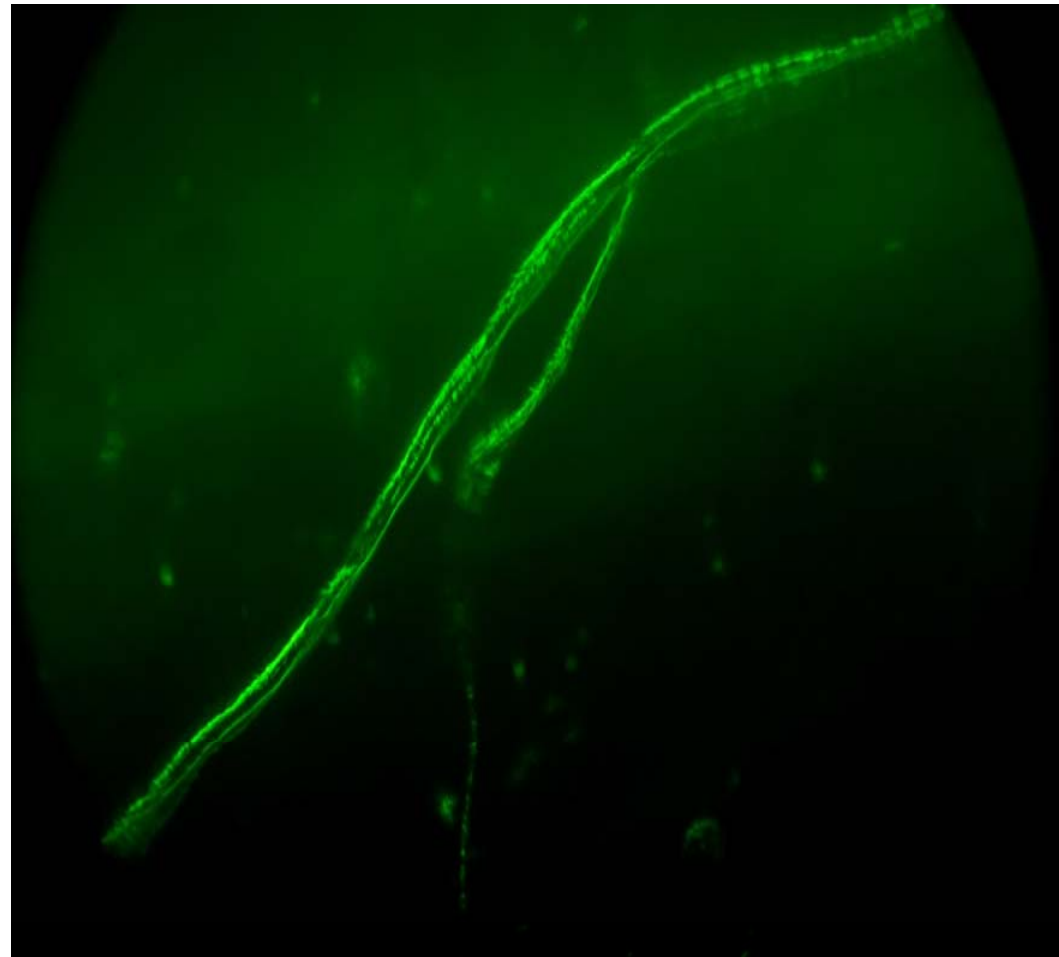
Inside wall of a bottle



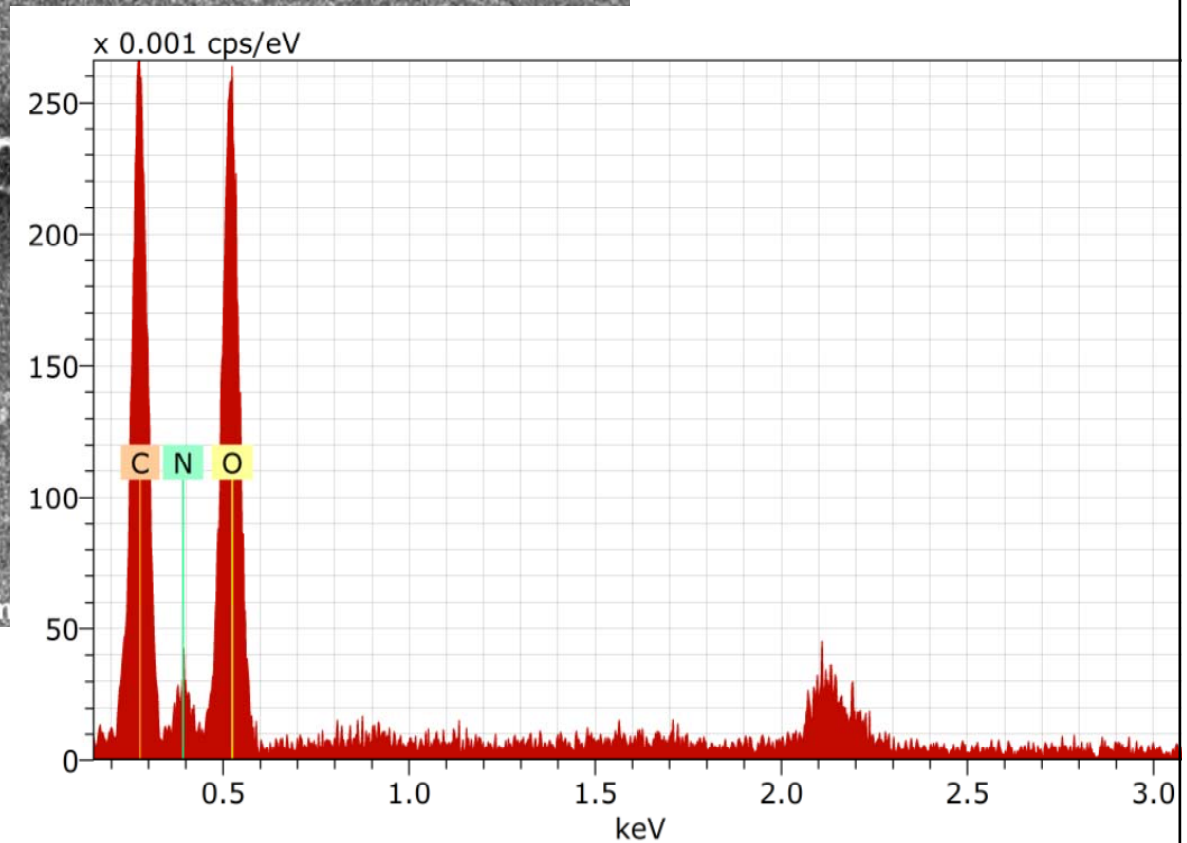
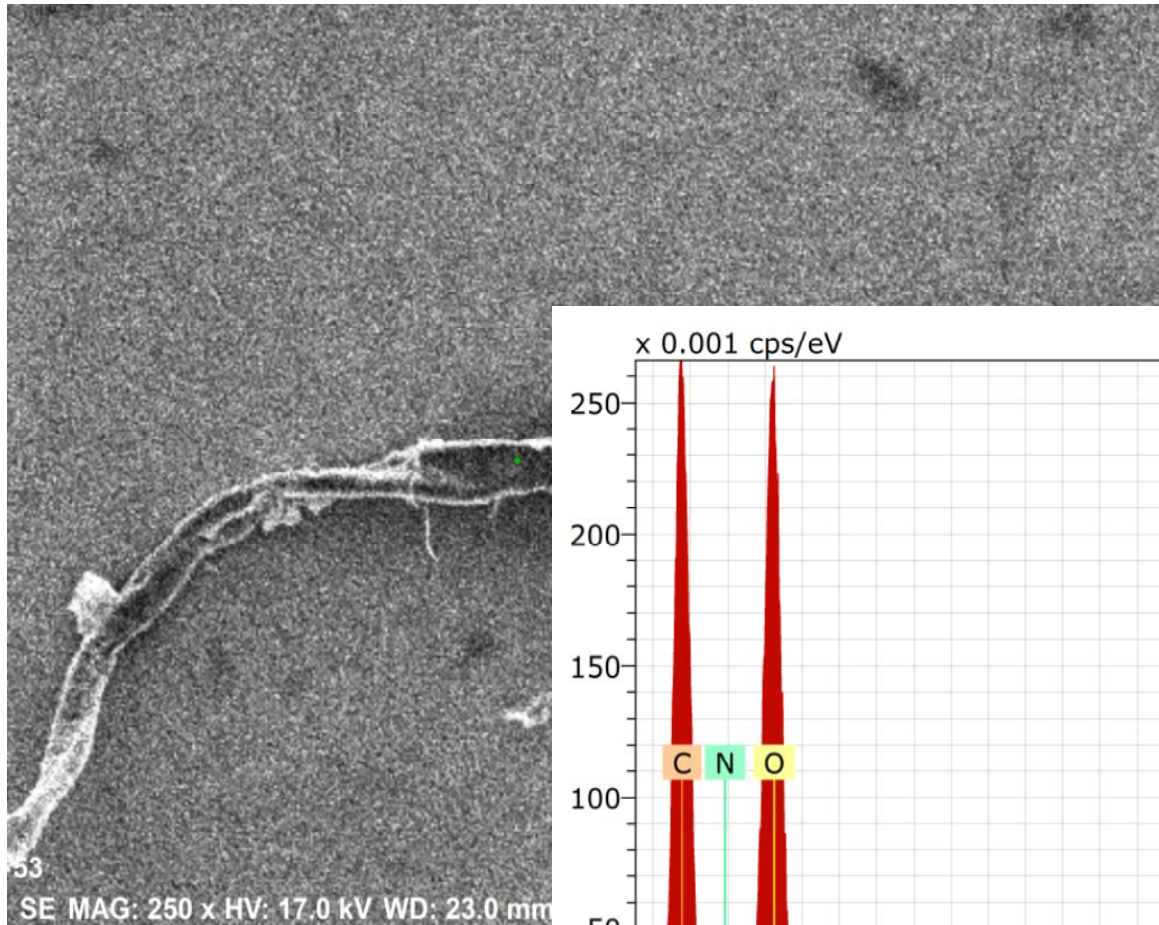
EDX base material



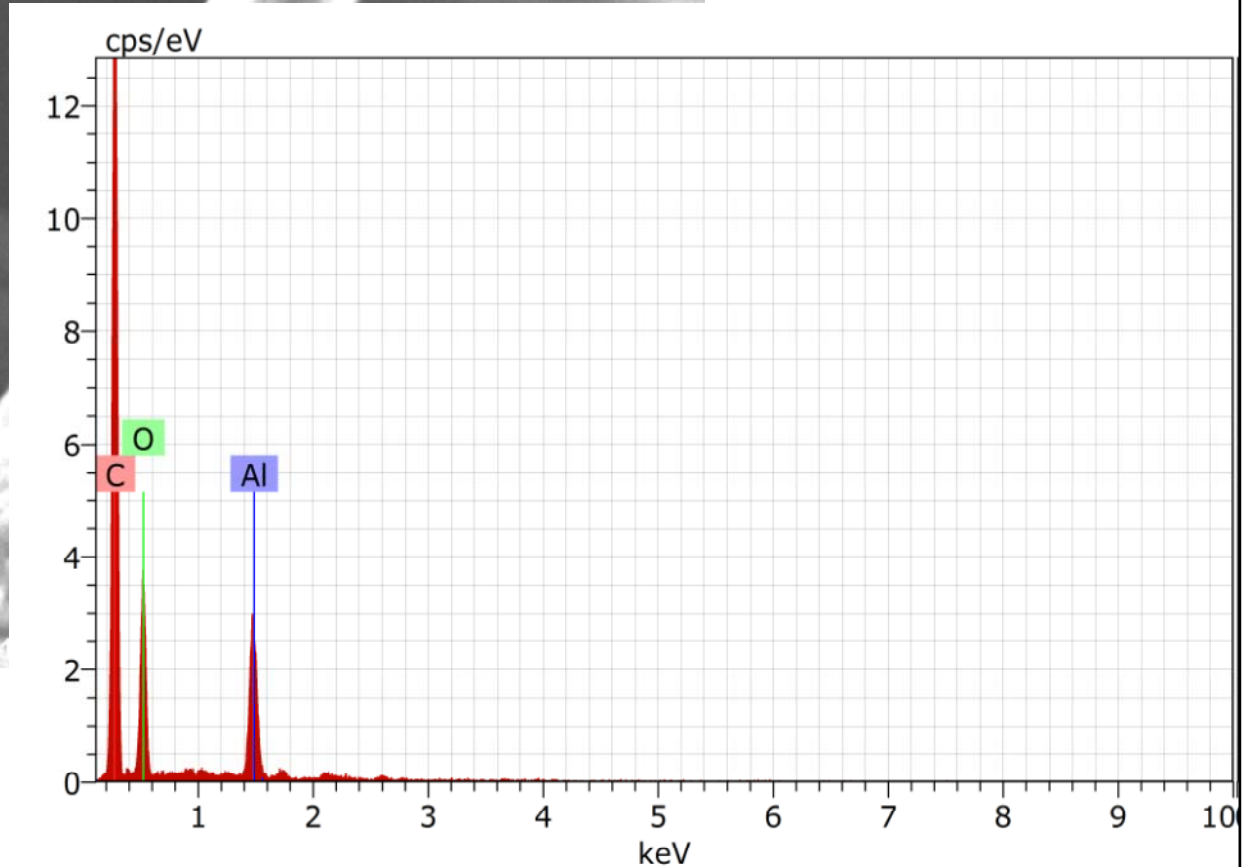
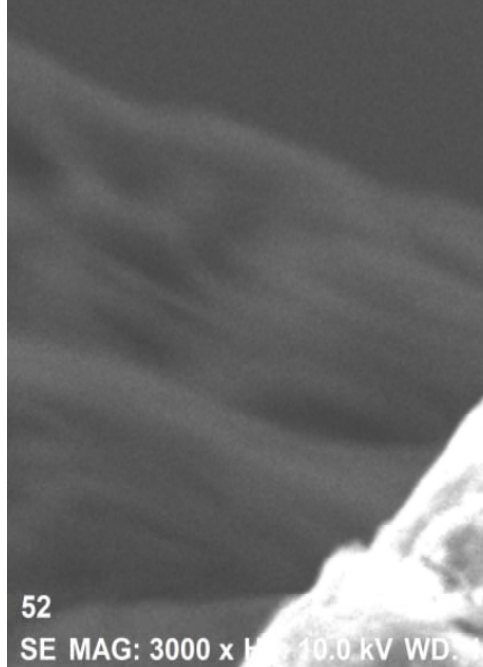
Micro fibres



Micro fibres



Micro fibres



Summary



- Turbidity in beer is mainly generated from the colloidal „classic“ **protein-polyphenol complexes** or **carbohydrate based**.
- It is mainly caused by the **raw-materials, filter-aids, microorganisms** or **foreign particles** that come into contact with the beer.
- The VLB approach on turbidity identification is based on an **initial standard visual-check, turbidity and pH-measurement**, followed by a meticulous **observation of the sample under a light microscope** and a **staining procedure**, using staining agents specific for identification of different organic materials.
- For deeper investigation, special analyses such as **PCR, HPLC, LCV, Electrophoresis, Ion chromatography, Enzyme tests, Iodine tests, Cytometry** or **EDX-analysis** are conducted to the samples, in order to gain more detailed information on the composition and possible origin of the turbidity causing particles.
- Finally, an open discussion with the breweries favorable
 - attempt to find the source of the turbidity problem in the beer – if it is a **product- or a process-related problem**.



Future Prospects



- A continuous search for **new staining agents**:
 - more specific
 - for detection of other organic materials / substances not presently covered
 - possible use of fluorescent staining agents

- A **Scanning Electron Microscope** with EDX was installed last year in the VLB implementing new approaches:
 - Identification of elemental composition of inorganic particles in house
 - faster handling of samples and delivery of results

- Full implementation of the **Modified Photometric Iodine Test** (including separation of the compounds by GPC) which will allow the effective separation of glycogen and amylopectin.

- **Flow Cytometry** as an option for further insights on cell and organic material contents will be installed in 2014.



Acknowledgement



- Patricia Diniz
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- **The Audience!**





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