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# Characterisation of Grass Fibres

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The elementary grass fibres were isolated from different grass and legumes sorts, i.e. Ryegrass (Lolium hybridum Gumpenstein), Wheat straw, Trefoil (Trifolium pratense) and Lucerne (Medicago sativa). The fibre-samples were obtained in a bio-refinery, after the liquid phase containing proteins and lactic acid was eliminated from the ensiled and green grasses, respectively. For the isolation of elementary grass fibres different processes were used. The morphological characteristics of stems and leaves of different grass species were microscopically observed. On the microscopical stem and leaves cross-section samples the quantification of fibres sclerenchyma cells was performed. The quantitative analysis was carried out in order to obtain basic quantitative data on grass fibres, such as area of the single fibre or group of fibres, diameter of a single fibre or group of fibres and distances between the most distant and least distant points on the area of the fibre. Measurements were made using a Carl Zeiss software KS 300, which runs on a computer connected to the image analysis equipment consisting of a microscope and a digital camera. In addition to, geometrical and mechanical properties of isolated fibres and fibre bundles were determined. Due to the grass history, i.e. deformations and damages caused by the treatment of grasses in the bio-refinery, maturity grade, grass or legumes type and conditions during grass growth, the plant structures vary considerable in their properties.


## 1. Introduction

One new promising utilization pathway for green biomass is offered by Green Biorefineries, the concept of which is similar to that of petroleum refineries, except that it is based on the conversion of biomass feed-stocks. In a Green Biorefinery a flexible mix of high-value products is generated, including bulk chemicals, fuels and materials [1–2]. After bioconversion followed by mechanical treatment of biomass by means of screw presses a fractionation into a liquid and a solid fraction (juice and cake) is performed. The solid fraction contains in rests of grass stems and leafs fibres that could be utilized for different purposes [3–7]. The aim of the study was to investigate the morphology of grass structures.

## 2. Preparation of samples

Several ensiled and green biomass samples have been used for experiments, representing 4 different types of grassland: Hybrid ryegrass (Lolium hybridum Gumpenstein), Wheat grass (Triticumaestivum L.), Lucerne (Medicago sativa) and Trefoil (Trifolium pratense).

For the isolation of elementary grass fibres different processes were used. Fibres were subjected to chemical treatment in acid (10% $H_2SO_4$ at temperature of 90°C, treatment time 3 h), and alkaline medium (1%NaOH at temperature of 100°C treatment time 1 h) and microbial activity, respectively. The last treatment was performed by enzymes, which were developed by the microbes on the plants under wet conditions at room temperature. Thereby the pectine structures connecting fibres with other plant tissues were loosed and the mechanical separation of the elementary fibres or fibre bundles was performed. Separation of fibres under wet conditions was necessary due to very low bending rigidity of dry grasses. A laboratory dyeing apparatus Turbomat was used for the heat treatment of plants.

## 3. Analytical methods

The light microscopical test were performed on whole leaves and stems and on ultimate fibres and fibre bundles. Different structures were observed on cross-sections and on longitudinal views of stems and leaves.

### 3.1. Preparation of microscopical images

Stems or leaves were aligned in a capsule and then embedded in methacrylate resin. Once the specimen was satisfactorily aligned and embedded, the microtome was used to prepare thin sections.

### 3.2. Image processing and quantification

The morphological characteristics of stems and leaves of different grass species were microscopically

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observed. Therefore a Microscope Axiotech 25 HD (+pol) equipped with the CCD SONY video camera model DXC-151AP to resolve images and a frame grabber (grey and "true colour" signal; 8 bit resolution/channel (RGB 8:8:8); image memory 3 MB to digitise the image and a host computer with Kontron KS 300 (Kontron Elektronik) software for image processing were used. On the microscopical stem and leaves cross section samples the quantification of fibres sclerenchyma cells was performed.

For better fibre cells identification in stems and leaves a cellulose coloured complex with ClZnJ solution was prepared. After treating the samples in the test solution lignified cellulose was deeply stained.

The quantitative analysis was carried out in order to obtain basic quantitative data on grass fibres, such as area of the single fibre or group of fibres [micrometer$^2$], diameter of a single fibre or group of fibres [micrometer] and distances [micrometer] between the most distant and least distant points on the area of the fibre. Measurements were made using a Carl Zeiss software KS 300, which runs on a computer connected to the image analysis equipment consisting of a microscope and a digital camera. Quantitative characteristics were determined on photographs, which were previously obtained on image analysis system; only those with fully visible cross sections of leaf and stem fibres were used.

We chose characteristics to be measured and prepared and created a macro in KS 300, which enabled us to carry out the measurements step by step. A certain photograph was selected and loaded in an active window. Then we interactively encircled the whole area of a single fibre or group of fibres and the needed parameters were calculated from the given round shape of the marked area. To evaluate the fibre content in grass plants the cross section area of fibre bundles was measured and compared to the cross section area of the whole stem or leaf.

Lengths of fibres were determined similarly; a photograph with a whole length of a single fibre was loaded and then a line along the centre line of the fibre was drawn interactively. When done, software automatically takes note of value of the measurement.

Several measurements were made in order to achieve as accurate results as possible; an arithmetical value was calculated from the obtained measurements.

## 4. Results

Due to the grass history, i.e. deformations and damages caused by the treatment of grasses in the bio-refinery, maturity grade and conditions during grass growth, the plant structures vary considerable in their properties. This inhomogeneity of samples is confirmed also by very high variation coefficients at measurements. Nevertheless, the mechanical properties of grass stems in axial direction are lower in comparison to the leaves, although the differences are not significant. Stress – strain behaviour of plant structures indicate a rigid character with low elongations (Table II), but especially problematic is high brittleness of leaves and stems.

### 4.1. The morphology of different grass and legume species

The cross-section of an analysed grass is shown in Fig. 1. In addition, Fig. 2 shows the leaf in the cross

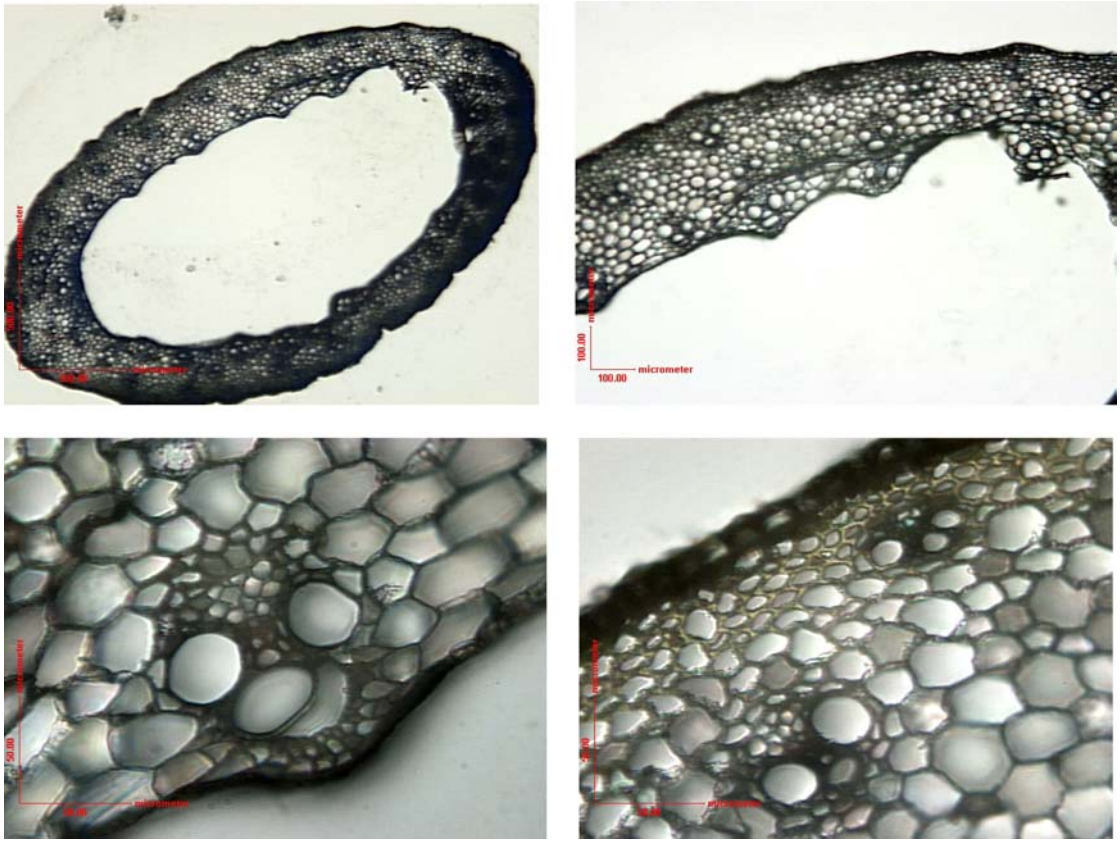


*Figure 1* Cross-sections of the stem of an analysed grass and a detail showing the vascular tissue strengthened by the sclerenchyma fibres.

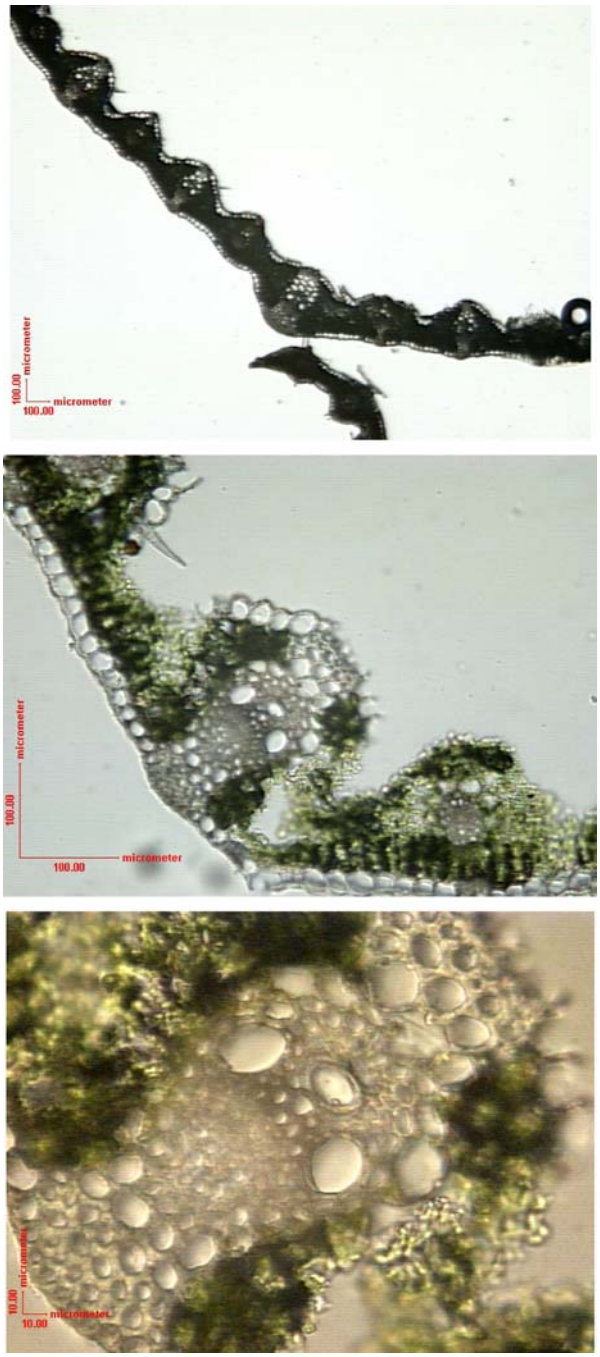


*Figure 2* Cross-sections of the leaf of an analysed grass and a detail showing the vascular tissue accompanied by fibre cells.

section. A longitudinal view of leaf structures is given in Fig. 2.

An even oval cross-section form was observed in the case of grasses, while the surface of legumes is rather folded. The epidermis surrounds the stems. Different structures could be clearly observed on the cross-section views. Especially pronounced are large, compactly arranged parenchyma cells. The parenchyma cells near the centre of the stem are disintegrated during maturation, so that the stem becomes hollow. The hollow occupies about 2/3 of the cross-section; only in Trefoil stem it is smaller (Figs 3–5).

Fibre structures are accompanying the vascular system. Additional fibre layers were found under the epidermis. On the grass samples that were immersed in ClZnJ solution a coloured cellulose complex is

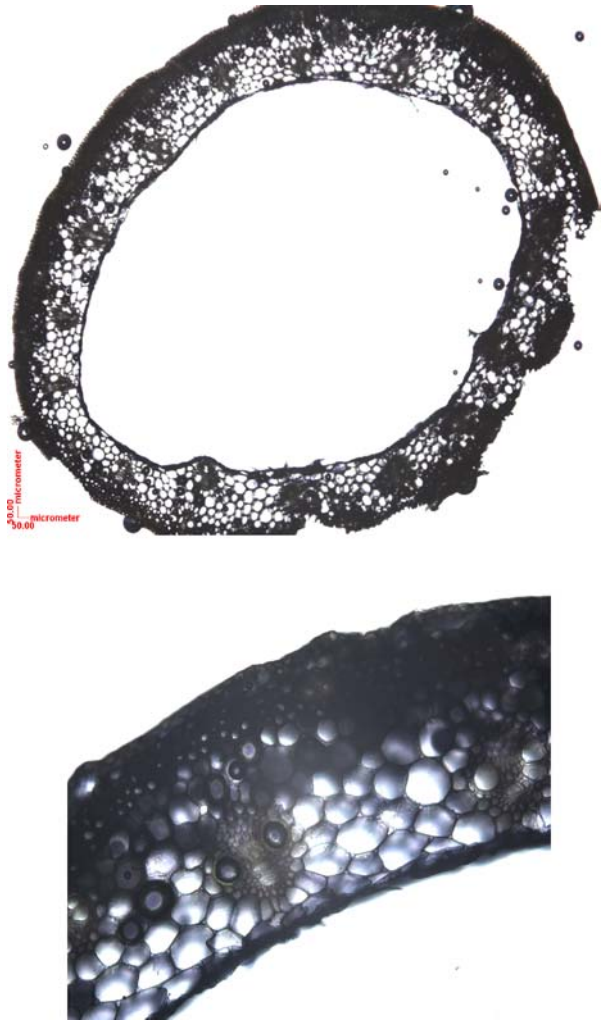


*Figure 3* Cross section of a Ryegrass stem.

obtained and the cellulose fibres can be easily identified. Different tissues are observed in leaves, i.e., epidermis, the vascular system and mesophyll tissue, respectively (Figs 2 and 6).

The cross-section of a Lucerne stem is given in Fig. 4. A layer of epidermis cells covers the stem, below it there are densely packed parenchyma cells. Major vascular bundles are located in folds with smaller bundles between.

Irregular shapes of cross-sections are characteristic for Trefoil stems (Fig. 5). The cross-sections of the vascular structures are nearly triangular with several very dense multi-layers of fibre cells on the outer side of these structures.

The cross-section of a wheat straw leaf is demonstrated in Fig. 6.

A layer of cellulose fibre cells was detected under the epidermis and additional sclerenchyma cells bundles are strengthening vascular systems. Grasses and legumes are belonging to the group of monocotyledon and dicotyledon plants. The fibre structures are differently organized in various grass and legume species as confirmed on microphotographs.

## 4.2. Fibre content in grasses and legumes

In the process of evaluation of the percentage of fibres in stem in leaf cross-section the first step was to

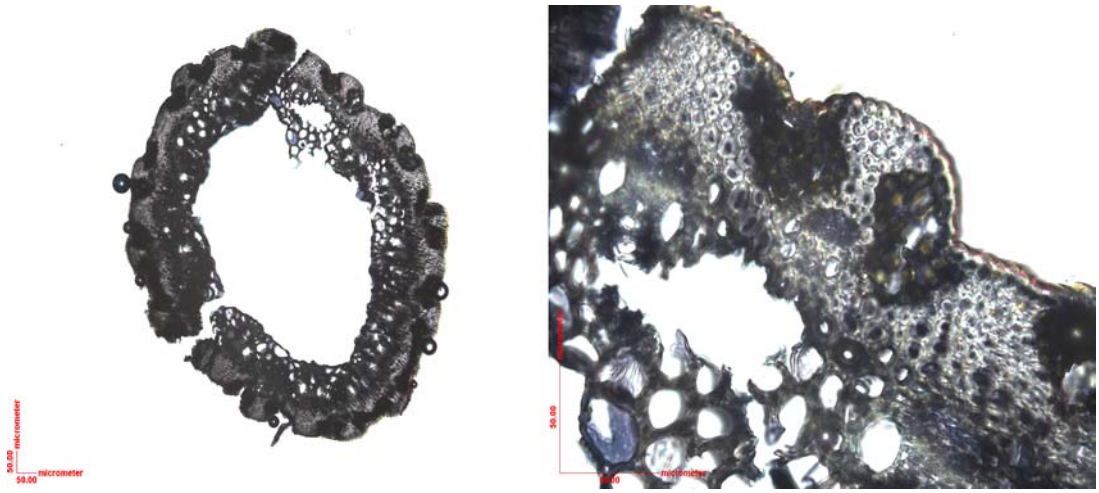

*Figure 4* Cross section of a Lucerne stem.

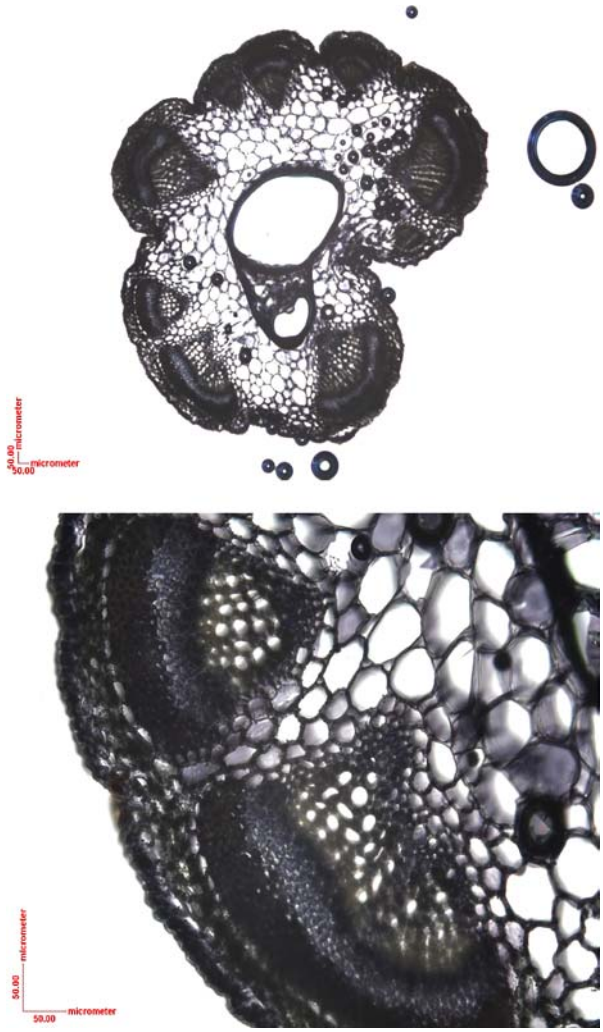

*Figure 5* Cross section of a Trefoil stem.

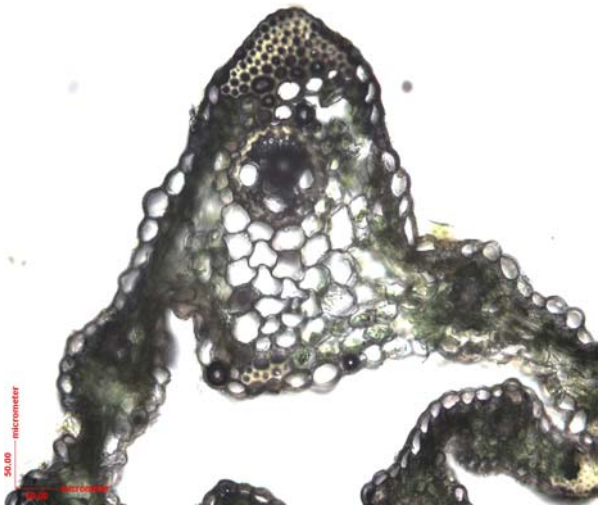

*Figure 6* Cross section of a Wheat leaf.

determine the area of the irregular stem or leaf cross-section. For the better recognition of fibre structures in the cross sections the ClZnJ treated stems and leaves were observed and analysed. Due to the deviations of the stem cross-section forms from the regular geometrical forms the diameter was determined at several positions.

After determining the stem diameter maximum and minimum (feretmin and feretmax) the software calculated the average area. In the same way the area of the lumen and fibre surfaces were calculated. The fibre content represents the ratio between the difference of the whole area and the lumen area and the fibre area, respectively as shown in Table I.

The procedure of determining the fibre content in grass leaves was very similar. However, microscopical photographs of the leaf cross-section were prepared and a part of the leaf was approximated by a rectangle, which dimensions were measured and the leaf area was calculated. Previously the leaves were treated in ClZnJ solution. In this marked leaf area the surface covered by fibre cells was measured. The results are given in Table II. It was not possible to measure the whole leaves as they were heavily damaged due to

TABLE I Fibre content in stems

| Grass species | Ryegrass | Trefoil | Lucerne |
|---|---|---|---|
| Whole stem area ($\mu m^2$) | 4 542 047 | 1 901 115 | 540 298 |
| Lumen area ($\mu m^2$) | 2 440 239 | 131 793 | 176 306 |
| Stem area ($\mu m^2$) | 2.101 808 | 1 769 322 | 363 992 |
| Fibre area ($\mu m^2$) | 829 884 | 357 378 | 125 341 |
| Fibre content (%) | 39.5 | 20.2 | 34.5 |

TABLE II Geometrical and mechanical properties of ultimate and technical grass fibres

| Grass species | Rye-grass | Wheat grass | Trefoil | Lucerne |
|---|---|---|---|---|
| Fibre content stem (%) | 39.5 | – | 20.2 | 34.5 |
| Fibre content leaf (%) | 7.9 | 10.1 | – | 6.9 |
| Length of elementary fibres, stem ($\mu$m) | 900–1100 | 800–1300 | 2100–3200 | 1200 |
| Length of elementary fibres, leaf ($\mu$m) | 400–700 | 1300 | 600–1000 | 600–1300 |
| Linear density, fibre bundles (dtex) | 12–100 | 37–45 | 20–105 | 19–65 |
| Tenacity, fibre bundles (cN/tex) | 6–21 | 9–17 | 6–21 | 7–14 |
| Elongation, fibre bundles (%) | 1–5 | 1–5 | 2–4.5 | 1–6 |

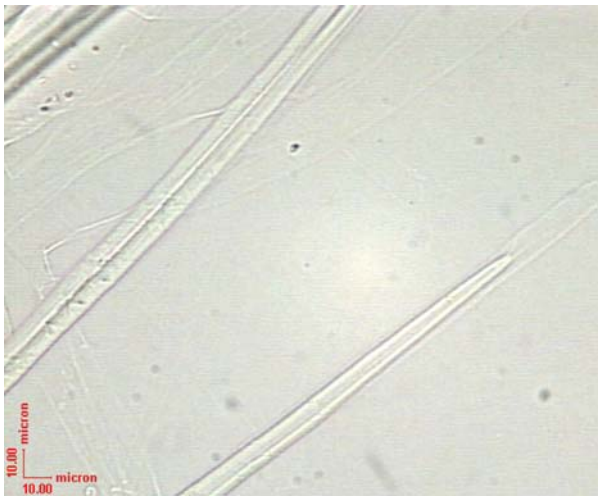


*Figure 7* Elementary fibres isolated from Lucerne leaf by NaOH treatment.

the process of plant pressing in the bio-refinery. Even more, some grasses, e.g. Trefoil leaves and Wheat straw stems, were so badly damaged that it was impossible to prepare suitable cross sections at all.

A high content of fibres was detected in stems regardless the fibres origin. The highest fibre content was determined in Ryegrasses, Lucerne followed and the lowest content of fibres was observed in the cross-section of Trefoil. Due to damaged stems the determination of fibre content in Wheat straw was not possible. Comparing the fibre content in leaves the differences between different grass and legumes species were not relevant, however, in Wheat straw leaves fibres cover approximately 10% of cross-section surface, while the percent in Ryegrass and Lucerne is about 7%. After pressing in bio-refinery the Trefoils leaves were damaged, broken and torn, that's why they were not measured.

### 4.3. Physical and mechanical properties of ultimate and technical fibres

Ultimate fibres and bundles of fibres were isolated from analysed grasses and legumes chemically and biologically. The influence of different isolation processes on elementary fibres was studied and the geometrical properties of ultimate fibres were determined on microphotographs using the image analysis. The longitudinal views of fibres isolated by different procedures are given in Fig. 7.

The characteristical geometrical and mechanical properties of ultimate and technical grass fibres are summarized in Table II. The length of the elementary cells in grasses and legumes is between 0.5 and 3 mm, slightly shorter fibre cells are present in leaves when compared to the cells from stems. The diameter of the isolated cells is approximately 15–18 $\mu$m.

## 5. Conclusions

It was found that grass fibres are comparable to other bast fibres with respect to geometrical and mechanical properties. However, due to their very poor bending characteristics and huge problems by fibre isolation their application possibilities are very limited.

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