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NANOFIBRILLATION OF CELLULOSE

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ABSTRACT

Nanofibers of cellulose are nowadays very important for scientific and also of industrial interest. In these days it is possible to produce nanofibers in a laboratory scale and one can achieve good and satisfying results with the use of high-pressure homogenizers. But some problems are still present in the existent methods. The main disadvantage in using this device is its very high energy consumption because of the need of up to several hundreds of passes under high pressure through these devices. Also blocked canals and gaps in the devices can easily happen. Instead of this conventional and well approved method with all its disadvantages it was the aim to find some other options which show a good compromise between energy consumption, high quality of nanofibers and a maximum yield. In best case the usage of toxic chemicals should be avoided.

The first steps in order to characterize the degree of fibrillation were to define some characterization parameters.

The second part of the experiments was the pretreatment of cellulose samples. This includes the premixing conditions of cellulose, measurements of moisture content, rising of temperature and cooling solutions, finding the appropriate cellulose concentration for milling, influences of different cellulose types and reproducibility of all these procedures. Also the pretreatment in ultrasonic bath and its optimal test parameters were part of these pretests.

Third part and main part of the thesis was the milling itself. Therefore a selection of different chemicals was added separately to watch the influence on the cellulose pulp and results of the degree of fibrillation. Also some device parameters for measuring the throughput speed and the water retention value were important to find out in order to establish a constant environment for further experiments.

The fourth part of the experiments contains the measuring of different parameters of cellulose treatment. For example water retention, throughput speed, degree of settlement, optical microscope and SEM/ESEM-observation.

It was found that some methods resulted very effectively in the production of cellulose nanofibers such as simple treatment of cotton linters without any chemicals for the duration of one hour in an ultrasonic bath and subsequent milling for 20 minutes. Also high efficiency in fibrillation could be observed in milling with sodium metaperiodate solution.

The best result was obtained with “in-situ” precipitation in water after dissolving cotton linters cellulose in 9% *N,N*-dimethylacetamide/lithium chloride (DMAc/LiCl) which one of the few non-ionic liquid solvents for cellulose.

Keywords:
Cellulose, Milling, Nano fibrillation, Ultra sound, SEM, ESEM, Throughput speed, Water retention, Sodium metaperiodate, Grade of segregation, Precipitation, DMAc, LiCl, microscopy

ZUSAMMENFASSUNG

Nanofasern aus Cellulose spielen heutzutage eine wichtige Rolle, finden mehr und mehr Beachtung im wissenschaftlichen Bereich und sind auch von wachsendem industriellem Interesse. Heutzutage stellt es kein großes Problem dar, nanofibrillierte Cellulose durch die Verwendung von Hochdruckhomogenisatoren herzustellen und gute und zufriedenstellende Ergebnisse erzielen. Die Probleme der aktuell verwendeten Methode sind jedoch immer noch präsent. Der Hauptnachteil bei der Verwendung dieses Gerätes ist der hohe Energieverbrauch, der durch die Notwendigkeit einiger hundert Umläufe unter sehr hohem Druck im Gerät verursacht wird. Desweiteren können blockierte Kanäle und Spalte zu einer Verstopfung führen. Anstelle dieser konventionellen und gut erprobten Methode mit all ihren Nachteilen war es das Ziel einige andere Möglichkeiten zu finden, die einen guten Kompromiss zwischen Energieverbrauch einerseits, hoher Qualität der nanofibrillierten Zellulose andererseits und einem Maximum an Ausbeute aufweisen. Im besten Fall sollte die Verwendung von toxischen Chemikalien so gering wie möglich gehalten werden

In einem ersten Schritt wurden die Charakterisierungsparameter definiert.

Der zweite Teil der Experimente behandelte die Vorbehandlung der Zelluloseproben. Dies beinhaltete zum Beispiel die Bedingungen für das Aufschlagen der Zellulose im Mixer, Messung des Feuchtigkeitsgehaltes, Messung der Temperaturerhöhung, Möglichkeiten der Kühlung, Ermittlung der geeigneten Zellulosekonzentration für die Mahlung, Einflüsse unterschiedlicher Zellulosearten und die Reproduzierbarkeit der Methoden. Auch die Vorbehandlung im Ultraschallbad und die optimalen Testparameter dafür waren Teil der Vorversuche.

Der dritte Teil, der Hauptteil der Masterarbeit, gilt der Mahlung der Zelluloseproben. Dafür wurde eine Reihe von unterschiedlichen Chemikalien zugesetzt, um deren Einfluss auf die Zellulosemasse sowie den resultierenden Fibrillierungsgrad zu beobachten. Um gleiche Versuchsbedingungen zu gewährleisten, wurden einige Geräteparameter zur Messung der Durchlaufgeschwindigkeit und dem Wasserrückhaltevermögen ermittelt.

Der vierte Teil der Experimente beinhaltet die Messung von unterschiedlichen Charakterisierungsparametern der Zellulosebehandlung. Zum Beispiel das Wasserrückhaltevermögen, die Durchlaufgeschwindigkeit, den Absetzgrad sowie lichtmikroskopische Beobachtungen und SEM/ESEM-Aufnahmen (SEM = Scanning electron microscope; ESEM = Environmental scanning electron microscope).

Es konnte festgestellt werden, dass einige angewendete Methoden zu einer sehr effektiven Nanofibrillierung der Zellulose führten. Ein Beispiel hierfür ist die einfache Behandlung von cotton linters Zelluloseproben ohne jegliche Chemikalien im Ultraschallbad für die Dauer von einer Stunde und anschließender Mahlung für 20 Minuten. Auch eine Mahlung mit Natriummetaperiodatlösung führte mit hoher Effizienz zu einem guten Ergebnis.

Das beste Ergebnis wurde durch "in-situ"-Präzipitation in Wasser erreicht, nachdem cotton linters Zellulose in 9%iger *N,N*-Dimethylacetamid/Lithiumchloridlösung gelöst wurde.

Schlagworte:

Zellulose, Mahlung, Nanofibrillierung, Ultraschall, SEM, ESEM, Durchlaufgeschwindigkeit, Wasserrückhaltevermögen, Natriummetaperiodat, Absetzgrad, Präzipitation, DMAc, LiCl, Mikroskopie

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ABBREVATIONS

AGU	Anhydroglucose unit
ASA	Alkenyl succinic anhydride
Celluclast®	Enzyme obtained from *Trichoderma reesei* strains (Endoglucanases I + II)
CMC	Carboxymethyl cellulose
DMAc	*N,N*-Dimethylacetamide
DMSO	Dimethyl sulfoxide
DP	Degree of polymerization
[EMIM]Ac	1-ethyl-3-methylimidazolium acetate
ESEM	Environmental scanning electron microscope
GS	Degree of settlement
keV	Radiation energy
kGy	Radiation dosage [J/kg]
LiCl	Lithium chloride
MFC	Microfibrillated cellulose
NFC	Nanofibrillated cellulose
PE	Polyethylene
PLA	Polylactide
PP	Polypropylene
S2	Secondary wall (middle layer)
TCF	Totally chlorine free
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl
TS	Throughput speed
U	Enzymatic activity
WRV	Water retention value

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1 MORPHOLOGY, TECHNOLOGY, PRODUCTS

1.1 Morphology of cellulose

Cellulose is a native material and also a renewable resource. One of its main advantages is the good mechanical property, especially its high tensile strength. (Berglund 2005)

This property is the most important for the present thesis because strength provides a wide range of application for cellulosic nanofibrils.

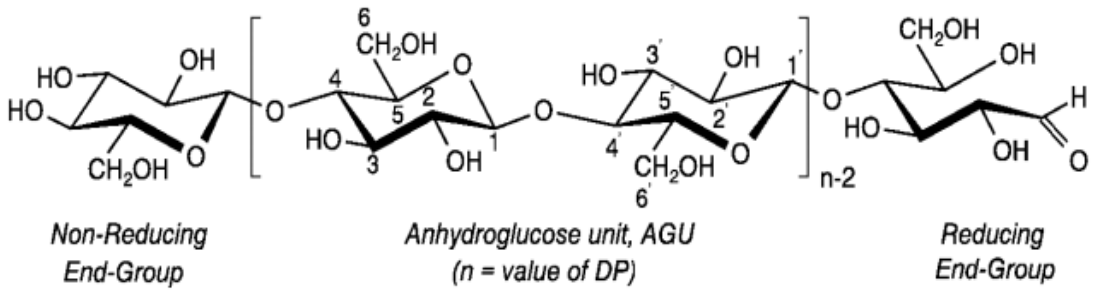

Figure 1. Molecular structure of cellulose (Klemm et al. 2002)

Cellulose is insoluble in pure water and also in pure organic solvents but shows a swelling capability in polar solvents. It is infusible and disintegrates at 180°C (Fischer 2004) and the average degree of polymerization of natural cellulose is assumed to be higher than 10,000. (Krässig 1993)

Cellulose is a polydisperse and linear homopolymer which consists of regio- and enantioselectively β-1,4-glycosidic linked D-glucopyranose units. (Klemm et al. 2002)

Klemm et al. (2002) describe that the polymer contains free hydroxyl groups at the C-2, C-3, and C-6 atoms. The OH groups and the oxygen from the pyranose ring and the glycosidic bond form a couple of supramolecular semi-crystalline structures which are based on hydrogen bond systems. (Klemm et al. 2002)

Both intra- and intermolecular hydrogen bonding occur in cellulose. The presence of intramolecular hydrogen bonds is of high relevance with regard to the single-chain conformation and stiffness. (Klemm et al. 2002)

In native cellulose structures the sheet-like morphology is formed due to intermolecular hydrogen bonding. Cellulose morphology represents a well-organized architecture of fibrillar elements. “It has been considered that the elementary fibril of native cellulose is the smallest morphological unit with a diameter of about 3.5 nm.” (Frey-Wyssling, Mühlethaler (1963); Heyn, 1966; Fengel and Wegener, 1989 as cited in Klemm et al. 2002)

In some cases there exist deviations of the ideal chemical regularity of cellulose like for example in technical cellulose samples (bleached cotton and wood pulp) which are mostly due to carboxylic acid groups and carbonyl groups. Klemm et al. (1998)

The morphology of cellulose can be described as a well-organized architecture of fibrillar elements. Fibrillar networks which have a different tightness characterize the morphology of regenerated cellulose.

Regarding to electron microscope investigations (Chanzy et al. 1986 as cited in Klemm et al. 1998:23) it is probably that the elementary fibrils do have a non-uniform diameter of 3-20 nm (depending on the cellulose source) and many authors (Mühlethaler 1965, Heyn 1966) defined this as the smallest morphological unit. In contrast Klemm et al. (1998:23) sees microfibrils as the lowest well defined morphological entity, although it does exists of non-uniform subunits.

Fink et al. (1990 as cited in Klemm et al. 1998) showed that the diameter of cotton linters was 7-9 nm. Additionally Fengel and Wegener (1989) and Krässig (1993) (as cited in Klemm et al. 1998:23) reported that the lengths of the microfibrils can reach micrometers.

Klemm (et al. 1995 as cited in Fischer 2004) describes that the input of mechanical energy - for example at milling procedures - can go along with depolyimerization when milling dry cellulose.

This case was not part of the experiments which follow, because all of those were done under aqueous conditions where these form of degradation is not that much expected.

1.2 Technologies of microfibrillation

The main techniques of producing microfibrillated cellulose which are used as common methods are milling and using high pressure homogenizers. (Chinga-Carrasco 2011)

Milling is a relatively simple method and was done in the present thesis work. Turbak et al. (1983 as cited in Chinga-Carrasco 2011) introduced the method of homogenization for fibrillation cellulose fibers for commercial purposes.

As cited in Siró and Plackett (2010) some methods of milling were done by Suzuki and Hattori (2004). They patented a method in which a cellulose pulp having 1–6% solid content was repeatedly treated in a disk refiner in order to generate MFC. The authors claimed that their method allows the production of high quality MFC with good stability and efficiency. Vegetable pulps, especially sugar beet pulp, were utilized in a French patent to produce MFC via homogenization followed by passing the cellulose suspension through a small diameter orifice (Dinand et al. 1996b). Taniguchi (2003) utilized natural cellulose fibers derived from different sources (such as cotton, hemp, wood pulp, seaweed, cereals, sea squirts, bacteria, etc.) for the production of MFC by fibrillating raw materials between rotating twin disks while adding shear stress in the vertical direction of fiber long axes.

The main problem of using mechanical refining methods is that they tend to damage the microfibril structure by reducing molar mass as well as the degree of cristallinity or fail to sufficiently disintegrate the pulp fiber. (Henriksson et al. 2007)

As mentioned above the high pressure homogenization method needs very high pressures which can reach up to 300 bar for 10 – 15 cycles when a laboratory homogenizer was used. (Leiter et al. 2007) As cited in Siró and Plackett (2010) it is mentioned that with increasing homogenization cycles, the energy demand increases and can be as high as 30,000 kWh/t. (Nakagaito and Yano 2004; Lindström 2007)

1.3 Used pre-modifications of cellulose

1.3.1 Enzymatic degradation

As done during the thesis the application of enzymes was also a way to pre-modification and pre-treatment. According to Pääkkö et al. (2007) a mixture of enzymes was added to the aqueous and buffered cellulose sample. The optimal result would be if the used cellulases did not degrade the cellulose rather modifying it. (Henriksson 2004)

Later Henriksson et al. (2007) and Pääkkö et al. (2007) found that endoglucanase pre-treatment facilitates disintegration of cellulosic wood fiber pulp into MFC nanofibers. Furthermore the microfibrillated cellulose which was produced from enzymatically pre-treated cellulosic wood fibers showed a more favorable structure than nanofibers produced by subjecting pulp fiber to strong acid hydrolysis.

Testing a low enzyme concentration such as 0.02% resulted in disintegrated fibers while molecular weight and fiber length were well preserved. (Henriksson et al.2007) This is what would lead to the wanted final morphology. Additionally López-Rubio et al. (2007) and Svagan et al. (2007) combined mechanical and enzymatic treatments.

1.3.2 Chemical derivatization

Saito et al. (2006) as cited in Siró and Plackett (2010) did experiments with TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) and used a Waring©-blender afterwards. The modification of the surface with the help of TEMPO mediated oxidation under mild conditions is characterized by introducing carboxyl and aldehyde groups into the native cellulose structure. (Saito et al. 2006).

Saito and Isogai (2005) reported that the native fibrous morphology is mostly maintained even after TEMPO-mediated oxidation. This occurs also under harsh conditions.

The advantage of using TEMPO-mediated oxidation is the negative charging of the microfibrils surface. This charging causes a repulsion of nanofibers and leads to much easier fibrillation. (Saito and Isogai 2005)

Cited in Siró and Plackett (2010) and Saito et al. (2009) established an oxidation system (TEMPO/ NaClO/ $NaClO_2$) under neutral or also slightly acidic condition which showed almost a complete preservation of the original DP and uniform nanofiber distribution (~ 5 nm in width) in contrast to the former system which was performed under alkaline conditions. This material was also free of aldehyde groups and was protected from undesirable alkaline side reactions which could lead to depolymerization. The product was prepared as a film out of TEMPO-oxidized cellulose gels and had high transparency, high toughness and low density. (Saito et. al 2009)

1.4 Products and applications of nanofibrillated cellulose

1.4.1 Compounds

Using MFC in hydrophobic polymers resulting as compounds was studied by Wang and Sain (2007a, b). The polymers used were PP and PE. The aim was to produce MFC-reinforced composites by compression molding. In these experiments MFC was directly incorporated up to 5 wt%. The mechanical properties were slightly improved compared to the ones of pure matrices but did not result in a significant rise of enhancement. Furthermore Cheng et al. (2007) tried if PP composites with cellulose fibers would lead to a more sufficient result after compression molding. They did an ultrasonic pre-treatment to enhance the fibrillation of these fibers. As a result, some gaps between the fibers and the PP matrix occurred. The distribution of fibrils itself was satisfying. Finally it was shown that the tensile tests of the fiber-reinforced composites ended in better properties than the pure PP.

Experiments with polylactide show also promising results. Polylactide could replace synthetic polymers and its physical and mechanical characteristics are useful. (Plankett et al. 2006)

The main advantage is that the property is almost the same as that of fossil fuel-based commodity plastics. (Mathew et al. 2005; Ren et al.2007)

The disadvantages such as brittleness (Boonfaung 2010), low thermal stability (Tsuji and Fukui 2003) and relatively high price (Li et al. 2006 as cited in Boonfaung et al. 2011) stand on the other side.

Another disadvantage is that the solubility of cellulose in organic solvents is poor and it also has a low thermal decomposition temperature, which lies below its melting point. Especially this property makes it difficult to disperse in melted polymers [...]. (Long 2009)

The preparation of MFC-lactide compounds was reported for examples by Jonoobi et al. (2010): "An extrusion device (twin-screw extrusion) gave a non-uniform dispersion of cellulose fillers in PLA matrix when nanocomposites of PLA with 5 wt% cellulose nanowhiskers and MFC were prepared. The reinforcements were fed into the extrusion process by liquid pumping using water as the pumping medium."

But the study showed also some backdraws. Due to the fast evaporation of the water there occurred a re-aggregation of the fillers in the polymer matrix. Poor mechanical properties can result because of the generation of microscale agglomerates together with the non-uniform dispersion of whiskers and fibers. (Mathew et al. 2006)

As a conclusion the development of nanocomposites has some important advantages. As the cellulose fibers are a native grown product it is cheap and renewable. The experiments showed also some efforts in enhancing the mechanical properties of nanocomposites. Increasing the tensile strengths and modulus could be useful for packaging material where polymer films could be used. Especially for food and pharmaceutical applications where a defined oxygen barrier is important combined with high transparency.

Also the electronic industry could take an effort by trying to yield this potential of these new materials. Low thermal expansion of nanocellulosics together with high strength, high modulus and transparency result in a high potential reinforcing material (Roll-to-roll technologies). Examples: fabricating flexible displays, solar cells, electronic paper, panel sensors and actuators. (Siró and Plackett 2010)

Siró and Plackett (2010) expect furthermore that the high number of reactive hydroxyl groups on the surface of cellulose also provides the possibility for fabricating a wide range of functionalized MFC-based materials for future advanced applications.

2 EXPERIMENTS

2.1 Materials and chemicals

2.1.1 Materials

The main sample used in all further experiments was cellulose from cotton linters FEZ1525. Other cellulose samples for benchmark tests used were: beech sulfite KZO3 (LAG) FEZ1085, birch kraft (*m-real)* PULP5 sample with a high xylose content of 24.4% Xylose and 0.5% Mannose), softwood sulfite (*DOMSJÖ)* PULP5 sample FEZ1486, and *kraft* TCF (*Rosenthal)* (FEL 1050).

For the milling and precipitation experiments with electron e-beam irradiation cellulose pulp samples were used.

2.1.2 Chemicals

Furthermore as additional chemicals during the milling treatments 10% NaOH solution and 18% NaOH solution were prepared.

In addition dimethlysulfoxide (DMSO) p.A. in H_2O provided by Sigma Aldrich was used.

For the oxidation experiments (TEMPO-oxidation) 10% sodium hypochlorite solution (purum 10%) and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) were used.

For the next oxidation test 0.1 mol/L sodium metaperiodate (Sigma Aldrich) solution was prepared.

For the dissolution and cellulose regeneration experiments in *N,N*-dimethylacetamide/lithium chloride a 9% (m/w) (DMAc/LiCl) solution was prepared.

The milling procedure was accompanied by the addition of 2% (m/m) alkenyl succinic anhydride (ASA) as sizing agent. Milling procedure with carboxymethyl cellulose required CMC as additive during milling. The used CMC had a molar mass of 250.000 g/mol.

The enzymatic treatment procedure according to the method of Ungurean et al. (2011) was done with 25 µL Celluclast enzyme mixture (enzymatic activity: U=600/g cellulose).

Milling tests with an ionic liquid like EMIMAc were done with 20% solution of 1-Ethyl-3-methylimidazolium acetate.

All chemicals used for analysis were analytical grade and were purchased by Sigma Aldrich Production GmbH, Switzerland.

2.2 Methods

2.2.1 Standard pre-tests

First of all the sort of cellulose appropriate for all following test had to be selected. Because cotton linters FEZ 1525 was available in sufficient amounts and very well characterized it was chosen as the standard cellulose for comparison of different methodical approaches.

In order to determine the water content an IR-balance (Sartorius MA 30) was used.

Repeated measurements resulted in an average water content of 5.8%.

This water content was fundamental for all further initial weight calculations when cotton linters was used.

As a second part of pretests the mixing of cellulose in water was reviewed. Two possible processing steps would be acceptable. First the usage of the IKA magic lab device. Therefore the disperse head (UTC-NK-25G, Ultra-turrax®) was used. Tests give non-satisfying results because of chocking the rotor inside the device with cellulose flocs. This did not happen when using a very low concentration of cotton linters in water (0.02%, 0.04%, 0.06%, 0.08% and 0.1% cotton linters in deionized water). Using a concentration of 0.25% cellulose in 250 mL deionized water turned out as the optimal choice.

Second possibility for premixing the samples was the usage of a kitchen blender (PHILIPS HR2094) for the duration time of 1 min at full speed.

This method turned out to be more robust and was therefore chosen. No blocking of blender rotor occurred. The kitchen blender device was in all further tests the device used as a standard tool for premixing the cellulose sample into a slurry state to provide a homogenous cellulose-water mixture for the milling procedure afterwards.

Transferring the cellulose slurry into the milling device had to be quantitative in any case.

Additionally to the pre-tests the IKA magicLAB UTC-NK-25G Ultra-turrax® was used to test, if treatment with the Ultra-turrax® would give the same results as treatment with the IKA MK/MKO mill as described in 2.2.1.
The results were not satisfying because of poor handling and blocking of the blender rotor especially when the later used standard concentration of 0.25% cellulose in deionized water was used for experiments.
Pre-tests resulted in using the IKA MK/MKO mill as the standard device for all further experiments.

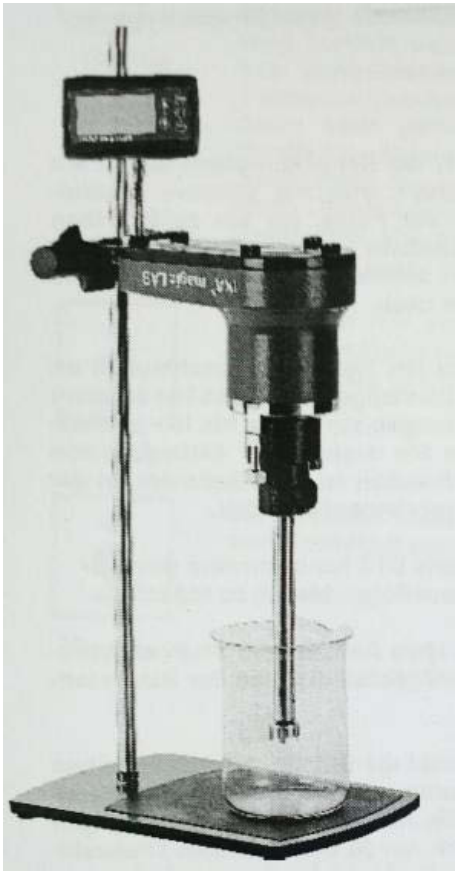

Figure 2. IKA magicLAB with UTC disperser from IKA magicLAB manual, page 63, 09/12

2.2.2 Milling procedure

As a result of pre-tests for the identification of the most suitable device for the following experiments an IKA milling device was used as the standard tool for all milling experiments. Figure 1 shows the milling apparatus as it was used in the experiments. To

provide a sufficient number of passes through it the time was determined to 20 minutes at 15000 rounds per minute.

Cooling was provided through a hose attached to a water-tap which is opened for the milling process time of 20 minutes which limited the temperature to a maximum of 45°C.

Figure 3. IKA magicLAB MK/MKO colloid mill with cooling water hose and reservoir flask on the top

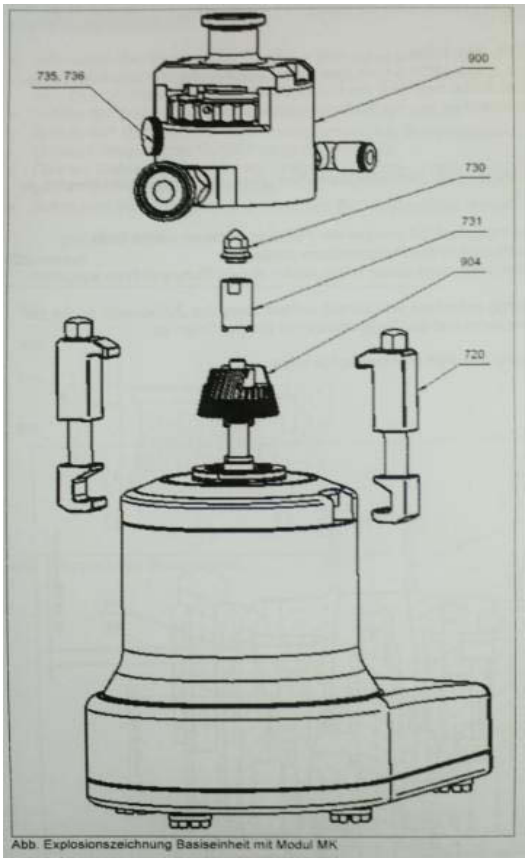

Figure 4. IKA magicLAB MK/MKO colloid mill in exploded view with rotor and stator with adjustment ring for definable refiner gap, IKA magicLAB manual, page 41, 09/12

The optimal refiner gap as shown in figure 4 was the fixed in the position of 180° which describes a distance between the rotor and stator of 0.159 mm. This provides that the temperature is permanently under a maximum of 45°C because of lower shear force which is important for enzymatic tests or other temperature sensitive reactions but is the basic for comparable test settings for all experiments.

2.2.3 Experiments for checking the test-setup

2.2.3.1 Reproducibility of milling (Tests 10,11,12)

As mentioned before the milling parameters had to be tested for 3 different agitator speeds. Therefore 3 samples were prepared with the use of cotton linters with a concentration in deionized water with 0.2%.

The agitator speeds were defined as 15000 rounds per minute for 30 min, 20000 rpm for 20 min and for standard procedure 15000 rpm for 20 min. Therefore 3 cotton linters samples with a concentration of 0.2% of deionized water were prepared.

For all of these conditions the results of the main criteria for the degree of settlement were nearly constant, so the consequence was to choose as standard parameters for milling 15000 rpm and a duration time of 20 minutes. This combination provides also a measured temperature of a maximum of 45°C with an acceptable compromise between duration and agitation speed without overstressing the IKA MK/MKO mill.

2.2.3.2 Dependency of solid content

The solid content of the milling solution was tested by preparing a set of samples beginning from a low concentration of 0.02% rising up to a concentration of 0.25%.

Low concentration under 0.1% cellulose content resulted in slurries difficult to observe the front of segregation because of cellulose flock aggregation.

So, as a consequence a concentration of 0.25% was chosen. Furthermore, a higher concentration in the milling solution provides a bigger amount of milled cellulose which is very desirable for the following test procedures such as water retention value, the degree of settlement and shear forces between the agitator and also between fiber and fiber.

Although a higher concentration results consequently in higher temperatures the experiments showed that an effective cooling system holds down temperature at about 45° maximum.

2.2.3.3 Rise of temperature during milling

Using pure water in the mill the temperature rises within 20 min from 20°C room temperature up to 64°C with at a fixed refiner gap of 180° and 15000 rpm. When a 0.02%

solution of cotton linters is filled in, the temperature rises at same conditions up to 70°C which means that there is no significant increase.

Higher agitation speed means in every case higher temperature because of a higher input of mechanical energy. A bigger or smaller refiner gap on the other hand showed no influence on the temperature level. The comparison of two experiments indicated that temperatures in test setups with 20000 rpm (test 12) are much higher than in those with 15000 rpm (test 13). Even when the duration is 1/3 longer at lower agitation speeds.

Table 1. Comparison of temperatures at different agitation speeds and durations.

Test 12

	Ø Temperature [C°]
Gap=180° c=0.02% n=15000 rpm t=30min	70

Test 13

	Ø Temperature [C°]
Gap=180° c=0.02% n=20000 rpm t=20min	86

As a consequence of the measurements that are showed in table 1 and the choice of a compromise as mentioned in 2.2.3. the standard procedure for all further experiments was determined.

Duration = 20 minutes

Agitation speed = 15.000 rpm

Cellulose concentration = 0.25% in deionized water

2.2.4 Specific Pre-preparations

2.2.4.1 Ultrasonic bath treatment

As an addition to kitchen blender pre-treatment an ultrasonic bath was used to suspend the cellulose fibers before starting the milling procedure.
The parameters in the used ultrasonic device were 25°C temperature and 60 minutes duration time.
In all experiments the ultrasonic treatment began with the initial weighing of cellulose (cotton linter). The sample was placed in a 50 ml Schott flask and 50 ml of deionized water were added. The ultrasonic device itself named EMAG Technologies EMMI 12HC ran at 100% of available power which is equal to 80 W and was tempered constant at 25°C in all tests.
When finished the cellulose was added to the rest of the deionized water volume to ensure that enough volume was available to run the IKA MK/MKO milling device safely.

2.2.4.2 Irradiation of cellulose samples with electron e-beam irradiation

Electron beam irradiation was carried out at NHV Corporation (Kyoto, Japan), using an electron beam irradiator, Curetron® EBC300-60. The cotton linters pulp sheets were directly submitted to the irradiation source. The samples went across the beam flow twice (upside-down) at 300 keV and irradiation doses of 10 kGy, 50 kGy, 100 kGy, and 200 kGy.

2.2.4.3 Dimethlysulfoxide treatment (DMSO)

To test the swelling capability of cellulose fibers tests with dimethlysulfoxide solution DMSO can be divided into 2 different procedures.
On the one hand an over-night-swelling-test which was done in 40% and 50% DMSO in deionized water solutions in advance and the milling afterwards to observe any positive influences by swelling the cotton linters fibers.

On the other hand it was part of further investigations to add the pure DMSO directly into the IKA mill while milling.

To complete the possible combinations, the treatment in ultrasonic bath for 60 minutes and milling in 40 and 50% DMSO in water solution was done at standard parameters.

After treatment the rest of the DMSO has to be washed out and the fibers should be free of any contamination.

This was done by stirring the fibers in a 500 ml beaker and filtrate afterwards the DMSO-soaked fibers on a filter paper over a vacuum flask and wash intensively with at least 500 ml of deionized water. To provide a complete removal of any DMSO remainders the washing procedure with water was done twice. Bubbles are caused by the porous swelling structure of these fibers after washing them through a filter. After this no more bubbles could be observed under the optical microscope as a test for complete purification.

This was achieved through a long diffusion time during stirring steps.

2.2.4.4 Sodium hydroxide treatment (NaOH)

A second swelling test was done with sodium hydroxide was done in 2 different ways.

One treatment consisted in swelling the cotton linters cellulose sample in 10% NaOH during ultrasonic influence for 60 minutes under standard conditions and the IKA MK/MKO mill additionally.

Second method of NaOH treatment was the use of 18% NaOH solution to watch the influence on the swellability of the cellulose fibers. Afterwards an intense washing procedure was made to remove most of the alkaline milieu. To achieve a neutral pH-value a small volume of 10% citric acid was used by rinsing over a cellulose filter.

2.2.4.5 Sodium metaperiodate treatment (periodate oxidation)

Sodium metaperiodate oxidation experiments were one of 2 oxidation tests done during this thesis. In particular 0.1 mol/L sodium metaperiodate solution was prepared. In these tests related to periodate oxidation the defined amount was added according to Sirvio et al. (2011) direct to the cellulose in water in the reservoir during circulation.

To achieve high yields in oxidized cellulose fibers the reservoir tank was covered with aluminum foil. Otherwise quick photo-induced decomposition of periodate can happen. (Sirvio, Hyvakko et al.)

Also the hint that the process temperature should not raise over 85°C what was no problem in this experimental setup because of intense water cooling. After milling the sample in the IKA MK/MKO mill for 20 minutes the cellulose slurry is filtrated and washed with at least 500 ml of deionized water to remove all the residue of periodate.

2.2.4.6 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) - oxidation

TEMPO oxidized cellulose fibers were prepared and treated by standard operation milling procedure afterwards.

In literature good results are described for the production of nano cellulose fibers by the usage of TEMPO oxidation. Saito (2004)

During the experiments a mild oxidation was done in 2 concentrations.

2 mmol/g and 8 mmol/g sodium hypochlorite on dry cellulose gave 2 relatively mild oxidation reactions.

According to the method described by Saito (2004) the 2 oxidation solutions containing 2 different NaOCl-amounts were prepared. The pH value has to be adjusted at 10.5 by means of 0.1mol/L HCl.

An adequate amount of NaBr was added and additionally TEMPO was added by diluting it in a droplet of ethanol. Finally the cotton linters sample amount was added and homogenized. During the reaction in the mill for 20 minutes milling duration the pH value had to be maintained at a level of pH 10.5 by adding 0.5 mol/L NaOH.

To stop the reaction after defined time several milliliters of ethanol were dropped into the solution while circulation.

Before doing the segregation test the milled cellulose fibers had to be washed intensively to get rid of possible TEMPO and NaClO residues.

During the entire process the reservoir bottle was covered with aluminum foil to prevent the samples from UV light influence. (Saito, Kimura, et.al. 2007)

2.2.4.7 Enzymatic treatment (Celluclast®) with cellulase enzyme

The enzyme we used was Celluclast®. Celluclast® is a commercial cellulase complex. It is obtained from Trichoderma reesei strains and contains endoglucanases (EG) I and II, and cellobiohydrolases (CBH) I and II. Celluclast® is used in the paper industry mainly for the improvement of drainage. According to Bhat (1997) a comprehensive review on cellulose degrading enzymes and their potential industrial application was done in 1997.

Some experiments regarding to the enzymatic activity on the cellulose were done by using an enzyme mixture containing Celluclast®, which was already prepared in the institute. The activity amounts 600 U and a volume of 25 µL per g dry cellulose was employed.

According to the method which is described by Ungurean et al. (2011) a 0.05 M pH 4.8 sodium acetate buffer was prepared and tempered to 40°C.

Furthermore 12.5 µL, 25 µL and 50 µL of cellulase enzyme were added.

To complete observations for different conditions the addition of cellulase was done before and after pre-preparation in the ultrasonic bath to watch any possible differences.

After IKA MK/MKO milling the cellulase was inactivated by rising up the temperature up to at least 70°C and stirring for 15 minutes.

The last experiment which was done with enzyme treatment was the incubation of a sample for more than 16 hours and addition of 50 µL enzyme solution while shaking at 40°C.

The rest of the experiment was identical.

2.2.4.8 "In situ" - precipitation during milling procedure

A 9% (m/w) *N,N*-dimethylacetamide/lithium chloride (DMAc/LiCl) solution is able to dissolve cellulose fibers. After putting 0.5 g of cotton linters cellulose into 200 ml of deionized water it was mixed up with a kitchen blender for 1 minute. Afterwards the water was removed by filtration and some milliliters of ethanol were used to dry the fibers. Then 80 ml *N,N*-dimethylacetamide were added to the cellulose and the mixture was shaken over night at room temperature. The DMAc was also removed by filtration over a Büchner funnel. The next step was to add 40 ml of 9% DMAc/LiCl-solution to the cellulose. All this together was homogenized very well. This solution process lasted overnight and the next day it was filtrated again. This time a 0.45µm filter was used. This clear and high

viscose mixture was then added to the adequate amount of water which is circulating in the IKA MK/MKO mill.

After milling under standard conditions the turbid, regenerated cellulose fiber solution was collected in a beaker. By using a centrifuge (HERMLE lab centrifuge Z-206A) the jellylike solution is concentrated at 2700 rpm for 5 minutes and collected before washing to avoid unnecessary volumes because otherwise the washing procedure would last too long. Intense washing over a Büchner funnel with 300ml of deionized water followed.

This internal preparation method to dissolve the cellulose sample consisting of cotton linters followed a standard procedure used for GPC analysis and was slightly altered to fit the experimental requirements.

This preparation was followed by ultrasonic treatment in some cases as described in 2.2.4.1 and milling in the IKA MK/MKO device according to 2.2.2.

2.2.4.9 Alkenyl succinic anhydride (ASA)

A sample of cotton linters cellulose was prepared with the standard procedure using 0.664g cotton linters and 250ml deionized water. Milling in IKA MK/MKO mill followed standard conditions (15000 rpm and 20 minutes duration).

After milling 2% alkenyl succinic anhydride (ASA) on mass of dry cellulose was added and placed on a stirrer for 36 hours at an agitation speed of 250 rpm.

The cellulose fibers were 4 times washed by using each time 200 ml of deionized water. Four passes were needed till the turbidity was removed and the solution was clear.

2.2.5 Milling additives

2.2.5.1 Carboxymethyl cellulose (CMC)

In this test CMC was added to the cellulose/water solution while milling. The concentration was 10% (m/w).

Milled at standard conditions as already mentioned the cellulose slurry was washed 4 times over a Büchner funnel and stirred after each filtration for 3 hours in deionized water.

After preparing a 0.25% cellulose mixture in water the degree of settlement and observation under the optical microscope were done.

2.3 Analysis methods

2.3.1 Optical microscopy

For the first the assessment of pre-treatment and milling procedures, a small amount of sample was taken out of the beaker which contains the homogenous milling mixture and observed under an optical microscope. Using a Pasteur pipette provides the appropriate amount of cellulose fibers. They are placed on a microscope slide.

For the observation of all cellulose samples the magnifications of the used BRESSER LCD microscope were 50x, 100x and 200x.

For most of the photos a magnification of 50x and 100x were optimal whereas 200x resulted in blurred pictures and the focus was difficult to adjust.

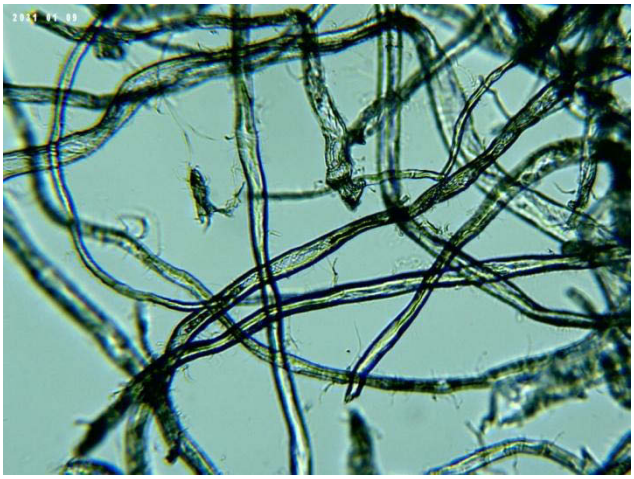

Figure 5. Cotton linters cellulose fibers 100x magnification (Bresser LCD microscope)

2.3.2 Degree of settlement

Another main criterion was defined by the degree of settlement. To find a practical way to measure the degree of settlement value for each sample a relatively easy test setup was found. 4 values per sample were taken.

Main part was a 100 ml graduated cylinder and a stop watch. A vertical cylinder with 100 ml volume was used because it had the optimal distance to take measuring points after 1 and 10 minutes. At low cellulose concentrations such as 0.02% up to 0.1% (prepared and

tested in experiment 5) the cellulose settled too fast and no segregation front was recognizable. A concentration of 0.25% cellulose in water gave best conditions beside good conditions during milling. Due to this fact the standard procedure for measuring the degree of settlement was done with 0.25% cellulose concentration and degree of settlement measured in % was taken after 1 minute and 10 minutes. The homogenized solution directly taken out of the beaker was filled quickly into the measuring cylinder exactly to the 100 ml mark and the distance was noted 2 times as described above (after 1 minute and 10 minutes).

Figure 5. 100 mL graduated cylinder with EMIMAc sample after 10 minutes shows a GS value of 2% after 10 minutes

2.3.3 Throughput speed

The value of the throughput speed is measured in seconds. The time spans describes how long the filtration lasts between 2 marks and correlates with the grade of fibrillation. The device for doing the vacuum filtration named VARIO Vacubrand PC3001 and CVC 3000 microcontroller established a constant vacuum of 800 mbar. The speed of the pump to ensure that the vacuum built up was not too fast was fixed at 30% of possible power. A vacuum flask was connected to the Vacubrand device and on the top a class-3 porosity glass frit was placed with a diameter of 15 mm. A relatively thin glass drip was necessary to achieve a certain height of homogenous cellulose mixture inside. This height of approximately 8 cm guaranteed that the distance between the measuring marks was long enough to achieve measureable time spans for all types of samples. Therefore a volume of 30 ml cellulose suspension as a result of milling procedure described in 2.2.2 was used.

Figure 6. Vacuum flask with class-3 porosity and blue line mark at lower measure point

2.3.4 Water retention value (WRV)

The WRV describes the rest amount of water in cellulose after applying a defined vacuum for a defined time span. (Cheng et.al 2010)
The cellulose sample suspension which remained on the glass filter after the throughput speed experiment was filtrated further. When the filter cake formed, the filtration procedure under certain parameters as described in 2.3.3 went on for further 60 seconds. Then the filtration was stopped abruptly. The cellulose sample was then weighed and the rest water content was calculated. This correlates to the grade of fibrillation.

2.3.5 SEM/ESEM

As one of the most meaningful methods SEM/ESEM-microscopy could be identified. It provides an observation of the prepared fibers at a nanoscale-level. One can describe the lengths and also the diameter of the native and resulted fibers. Also the existing morphology of cellulose fibers is visible in detail with SEM/ESEM-microscopy.
The tests were performed with a row of samples such as experiments with irradiated samples no. 50 (100 kGy) and 51 (200 kGy), the sample no. 67 of “in-situ” precipitated cellulose dissolved in DMAc/LiCl (9%) described in 2.2.4.8.
Furthermore ESEM-pictures were obtained from TEMPO-oxidized sample in the experiment with “in-situ” precipitated cellulose which was dissolved in DMAc/LiCl (9%) described in 2.2.4.8. and treatment of a cellulose sample in experiment no. 69 which was an oxidation experiment with 0.1M sodium metaperiodate solution as described in 2.2.4.5.
Also the sample 68 of cellulose treated in ultrasonic bath was observed per ESEM.
The last cellulose sample no. 60 was photographed as a reference sample for completely untreated cotton linters to get an overview how cotton linters looks like without any milling and chemical procedures.

As SEM/ESEM device a PHILPS XL 30 ESEM was used. The samples themselves were freeze dried and placed in an EDWARDS Scancoat Six for gold sputtering procedure.

3 RESULTS AND DISCUSSIONS

3.1 Influence of IKA magic lab Ultra turrax® device (UTC-NK-18G) on fibrillation result

The degree of settlement should correlate to the throughput speed test because higher fibrillation as obtained after longer treatments in the Ultra-turrax are likely to increase the time of settlement.

In experiment 62 the Ultra turrax® device of IKA was used to check if the result of fibrillation is the same as using the IKA MK/MKO mill as described in 2.2.2.

For this reason the standard parameters were used except the preparation device for the fibrillation.

For the pre-treatment the used cotton linters was treated in the ultra sonic bath of Technologies EMMI 12HC for 60 minutes which ran at 100% of available power and was tempered constant at 25°C as described in 2.2.4.1.

The Ultra turrax® treatment was applied for 20 minutes with an agitator speed of 15000 rpm.

Four measurements of the degree of settlement were done in each experiment and furthermore the value of the throughput speed was measured.

The results of the degree of settlement and the throughput speed are shown in table 2.

Table 2. Influence of IKA magic lab Ultra-turrax device (UTC-NK-18G) on fibrillation result

Test 62 Ultra-turrax® treatment

	Degree of settlement (GS)		**Throughput speed (TS)**
	1 min	**10 min**	**sec**
	30	50	81
	35	55	95
	35	55	85
	35	53	80
Average value	**34**	**53**	**85**
Standard deviation	**2.5**	**2.5**	**7**

The observation with the light microscope (100x) is shown below.

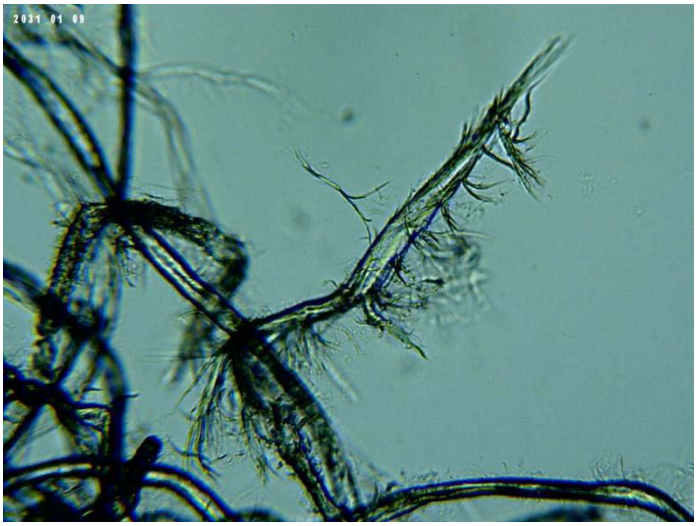

Figure 7. Cotton linters treated according to 4.1. Magnification 100x. (BRESSER LCD microscope)

Despite of pre-treatment in an ultrasonic bath the cellulose fibers show a compact appearence. There are not many fibrils in the surrounding water. According to the picture made via light microscopy the grade of microfibrillation is very low compared to the result of later experiments done with the IKA MK/MKO mill and different treatments.
Also the measured degree of settlement is not higher either. Values of less than 10% show no effective fibrillation treatment by the use of ultrasonic bath and Ultra-turrax®.
After 10 minutes, the degree of settlement has risen up to 53% which is very high. This indicates still compact cellulose morphology even after treatment with deionized water in the Ultra-turrax®. This sounds logical because a lower degree of settlement should correlate with a longer duration time in the throughput speed test as a result of a higher grade of fibrillation.
To compare the ultrasonic bath and Ultra-turrax® treatment to cellulose without any treatments the following figure shows native cellulose fibers also in the same magnification as shown in figure 8, but without any treatment.

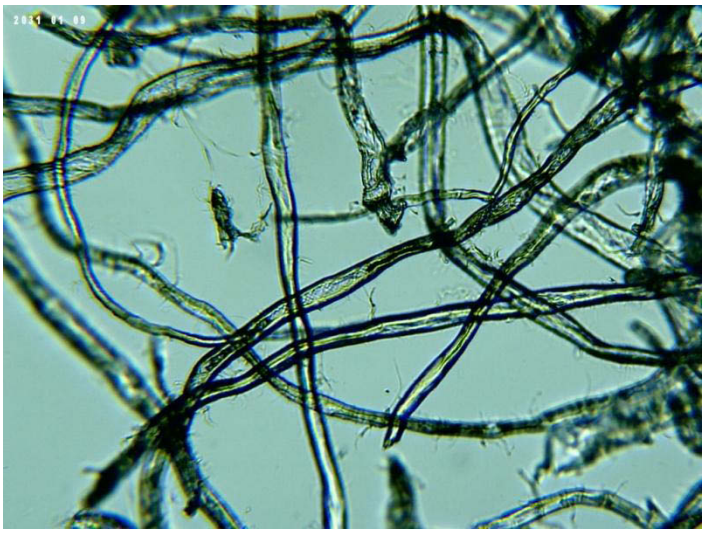

Figure 8. Native cotton linters cellulose via light microscopy 100x (BRESSER LCD microscope)

3.2 Influence of oxidative damage on fibrillation

3.2.1 Oxidation with 0.1 mol/L sodium metaperiodate

Experiment 47 was performed with the standard procedure as described in 2.2.1. and 2.2.2. It was done with the kitchen blender and afterwards by milling in the IKA MK/MKO device according to 2.2.4.5.

Table 3. Influence of oxidation with 0.1 mol/L sodium metaperiodate solution on fibrillation result

Test 47/69 Sodium metaperiodate oxidation

	Degree of settlement (GS)		**Throughput speed (TS)**		**Water retention value (WRV)**
	1 min	**10 min**	**min**	**sec**	
	0	1,5		150	
	0	1		140	
	0	1		165	
	0	1		160	
Average value		**1,1**		**154**	**57.1%**
Standard deviation		**0.25**		**11**	

The oxidation experiment with 0.1M sodium metaperiodate was very efficient at microfibrillation of the used cotton linters sample. The degree of settlement was very low. This means that the front of the turbid cellulose solution sank very slowly and reached a maximum settlement of 1.1% after 10 minutes of precipitation.
Simultaneously the throughput speed value increased up to an average of 154±11 seconds under constant vacuum pressure of 800 mbar and 30% maximum pump speed using a class 3 glass filter as described in 2.3.3. and 2.3.4.

Light microscopy shows also expected results. The IKA MK/MKO milling procedure caused a massive fibrillation of the original cotton linters fibers as can be seen in figure 9. The photo was made at a magnification of 100x.

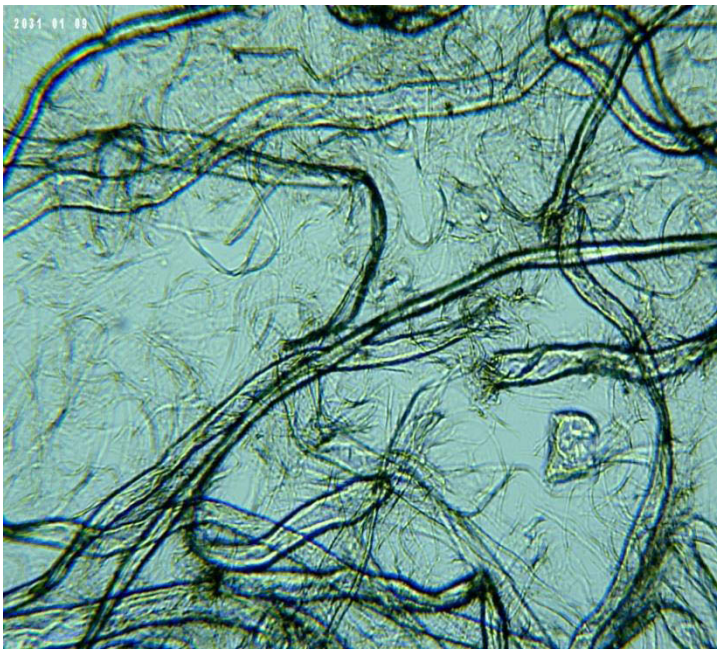

Figure 9. Cotton linters cellulose oxidized for 20 minutes during milling procedure in 0.1 mol/L sodium metaperiodate under standard conditions. 100x (BRESSER LCD microscope)

Additionally a micrograph was made by using the ESEM as figures 10 and 11 show.

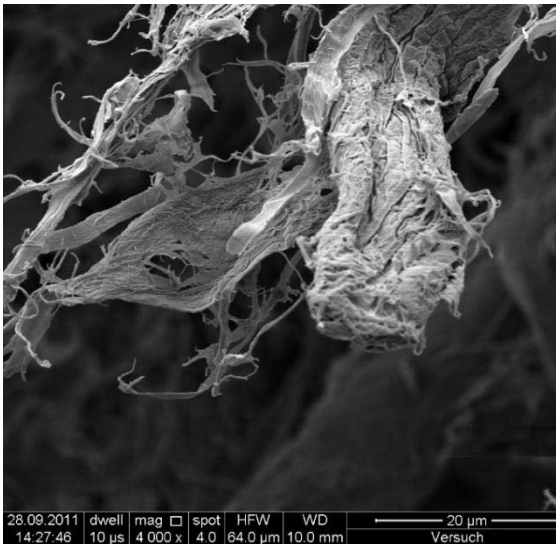

Figure 10. ESEM micrograph of cotton linters after oxidation during milling with 0.1 mol/L sodium metaperiodate solution PHILPS XL 30 ESEM (4000x)

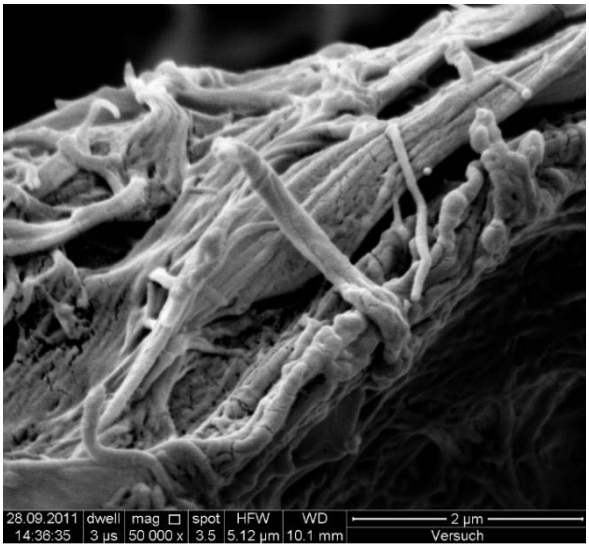

Figure 11. Micrograph of the same procedure as described (50000x)

As can be seen on the ESEM micrographs the fibrils are still faultless and intact and a high grade of fibrillation can be observed.

Oxidation of cellulose with sodium metaperiodate is a very intensively investigated method. According to Sirvio et al. (2011), higher temperatures could be used during the oxidation reaction and the milling in order to achieve higher aldehyde contents without a serious simultaneous periodate decomposition. Temperatures rose up to 45°C in the experiment.

The results found in Sirvio et al. described a value of round about 0.086 mmol/g at RT and 0.364 mmol/g aldehyde content was found at a temperature of 55°C. The oxidation duration in literature was 25 minutes. These conditions resemble the ones in a study by Varma and Kulkarni (2002).

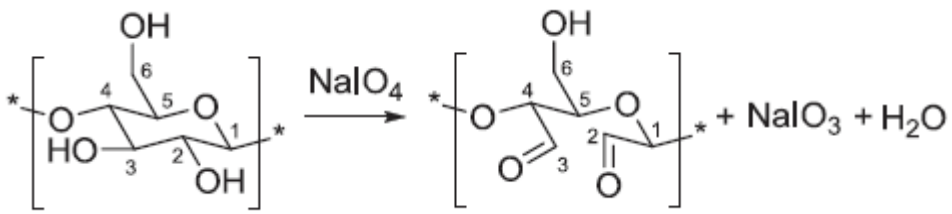

Figure 12. Scheme of periodate oxidation of cellulose. Sirvio et al. (2011)

When doing the oxidation the metaperiodate ions interact with hydroxyl groups at carbon atom 2 and 3. The result is an intermediate complex. Then a second and slower decomposition step leads to breaking of the C-C bond. This reaction leads the two aldehyde groups (see figure 12). (Maekawa et al., 1986)
The result of such an oxidation reaction on cellulose is the formation of dialdehyde cellulose (DAC).
The aim of most chemical treatments which were performed during this thesis had been mainly to introduce stable negative or positive electrostatic charges on the surface of cellulose fibers to obtain better dispersion in the suspension because of induced forces of repulsion between the separated fibers.
In the best case the functionalization affects only the surface of the nanofibers and to prevent changes of the original morphology of cellulose. The cristallinity should be maintained during the procedure. Habibi (2010)

3.2.2 TEMPO-mediated oxidation of cellulose

Second part of the oxidation experiments was the TEMPO mediated oxidation of cotton linters cellulose samples as described in 2.2.4.6. according to Saito, Isogai (2004) and Saito et al. (2007).

Aim of the oxidation procedure was to introduce negatively charged entities at the surface of the fibrils resulting in a separation effect of single fibrils. Saito et al. (2006)
For the oxidation experiments (TEMPO-oxidation) 10% sodium hypochlorite solution (purum 10%) and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) were used. 0.5 mol/L sodium hydroxide solution, ethanol and additionally sodium bromide were used according to the method described by Saito and Isogai (2004).

Table 4. Influence of oxidation after TEMPO mediated oxidation (2 mmol/g dry cellulose) on fibrillation result

Test 63 TEMPO mediated oxidation used 2 mmol NaClO per gram dry cellulose

	Degree of settlement (GS)		**Throughput speed (TS)**	**Water retention value (WRV)**
	1 min	**10 min**	**sec**	
	2	60	183	
	1	60	210	
	2	60	178	
	1	58	200	
Average value	**1.5**	**60**	**193**	**21%**
Standard deviation	**0.6**	**1**	**15**	

These results of GS and TS values diverge strongly to those resulted by periodate oxidation. The reason for that could be that the TEMPO mediated oxidation treatment was very mild and as a consequence the effects of TEMPO oxidation described afterwards were not very intense. Even the treatment with 8 mmol NaClO per gram dry cellulose did not led to an effective fibrillation result due to periodate oxidation. Even the WRV fits to this observation. The value after periodate oxidation is much higher than the one after both - 2 mmol and 8 mmol NaClO - TEMPO oxidations.

Table 5. Influence of oxidation after TEMPO mediated oxidation (8 mmol/g dry cellulose) on fibrillation result

Test 64 TEMPO mediated oxidation using 8 mmol NaClO per gram dry cellulose

	Degree of settlement (GS)		**Throughput speed (TS)**	**Water retention value (WRV)**
	1 min	**10 min**	**sec**	
	1	25	690	
	1	23	675	
	1	27	690	
	1	30	710	
Average value	**1%**	**26%**	**691**	**25%**
Standard deviation	**0**	**3**	**14**	

The degree of settlement decreased after the NaOCl input of 8 mmol from 60% (2 mmol NaOCl) to 26%. When the sample was treated with 8 mmol of NaOCl per gram dry cellulose the degree of settlement was only 26% which means a higher grade of fibrillation.

Also the throughput speed time rose up from 193±15 seconds to an average of 691±14 seconds when using 8 mmol NaOCl. This could be because of clogging the type 3 glass filter plate due to long nanofibers.

The WRV rose from experiment 63 to experiment 64 and the GS and the TS value indicate for lower grades of fibrillation compared with those values of periodate treatment.

In addition the optical microscope was used to take pictures of the milled sample of trial 63 and 64 as following on the next page. Figure 13 shows the influence of the milder TEMPO oxidation of the two samples which were made. One can see a slight difference to the sample of cotton linters in figure 14 which was treated with a 4 times higher concentration of NaOCl. The grade of fibrillation is not as much as the one described in 3.2.1.

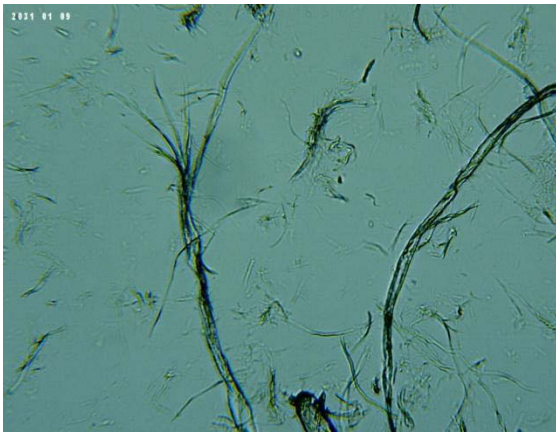

Figure 13. Experiment 63 cotton linters cellulose oxidized for 20 minutes during milling procedure with TEMPO treatment (2 mmol/g cellulose) under standard conditions. 100x (BRESSER LCD microscope)

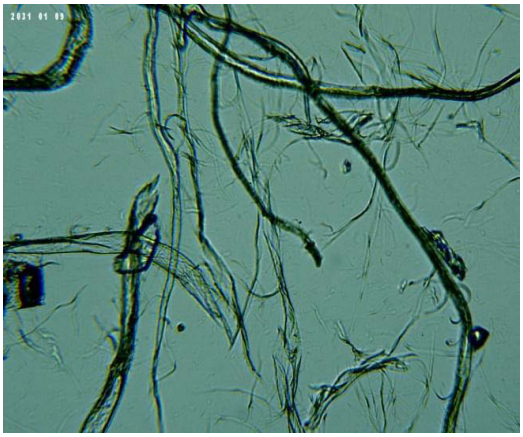

Figure 14. Experiment 64 cotton linters cellulose oxidized for 20 minutes during milling procedure with TEMPO treatment (8 mmol/g cellulose) under standard conditions. 100x (BRESSER LCD microscope)

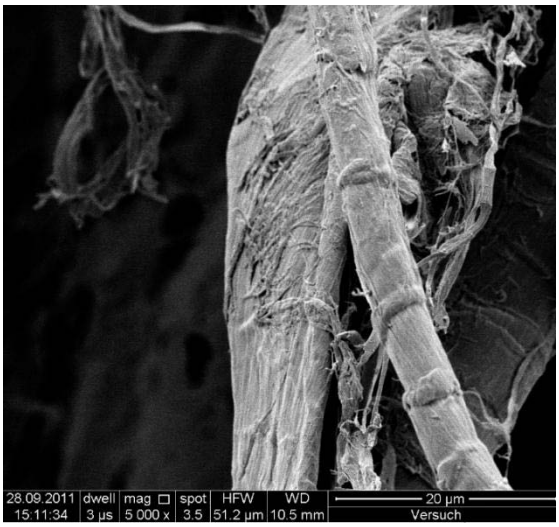

Figure 15. ESEM micrograph of cotton linters after oxidation during milling with 8 mmol/g cellulose NaOCl. PHILPS XL 30 ESEM (5000x)

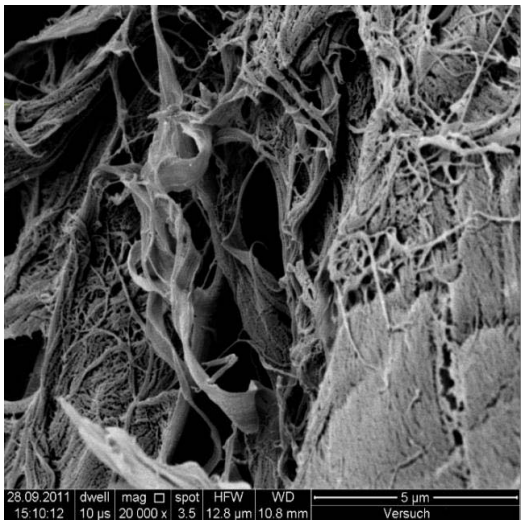

Figure 16. ESEM micrograph of cotton linters after oxidation during milling with 8 mmol/g cellulose NaOCl. PHILPS XL 30 ESEM (20000x)

ESEM micrographs were also made of sample treated in experiment 64 as shown in figures 15 and 16. The grade of fibrillation compared to cellulose treated in 0.1 mol/L sodium metaperiodate is much lower as can be seen when figure 15 and figure 10 are compared with each other.

In my case the oxidation was too mild to observe an intense effect, but increasing fibrillation of the fibers with increased TEMPO concentration from one milling step to the next experiment was definitely measureable.

The results of TEMPO mediated oxidation and its purpose are summarized below:

First negative charges generated by carboxyl groups as already mentioned above introduce repulsive forces between the fibrils. This helps to loosen the cohesions forces between the fibrils which are established by hydrogen bonding. Saito, Nishiyama, Putaux, Vignon & Isogai (2006)

As a second reason the oxidation forwards the hydration and the swelling of the fibers. This effect makes them more flexible and the crystalline zone more accessible according to Dang et al. (2007).

After TEMPO oxidation also the water retention value (WRV) should increase as described by Dang et al. (2007).

Besbes et al. experimented with a high pressure homogenizer to produce nanofibrillated cellulose out of eucalyptus fibers but also when using cotton linters cellulose as in my case during this master thesis the effect should be comparable. The oxidation loosens the

primary S1 cell wall. This makes the S2 layer more accessible and is a very good pre-condition for an effective fibrillation through treatment Besbes et.al. (2004).

As a last effect of the oxidation experiments the scission in the amorphous zone creates weak points within the fiber cell wall.

This enhances the mechanical fibrillation process Besbes et.al. (2004).

3.2.3 Influence of electron e-beam irradiation on fibrillation

Four samples of irradiated cotton linters cellulose no. 3 were milled under standard conditions.

A short summary is shown in table 6 and gives an overview of GS and TS values.

Table 6. GS and TS values of cotton linters samples FEZ 1523 of increasing dosages of irradiation

Irradiated samples

Test 48	**10 kGy**	**300 keV**	**Test 49**	**50 kGy**	**300 keV**
c=0,25% cellulose in deionized water					
Pre-treatment: ultrasonic bath t=60 min (valid for all tests no. 48 – 51)					
GS		**TS**	**GS**		**TS**
1 min	**10 min**		**1 min**	**10 min**	
		sec			**sec**
0,5	3	800	0	2	1080
0	2	780	0	2	1020
0	2	810	0	2	1080
0	3	-	0	2	1050
Standard deviation			Standard deviation		
0.25	0.6	15	0		29

Test 50	**100 kGy**	**300 keV**	**Test 51**	**200 kGy**	**300 keV**
GS		**TS**	**GS**	**TS**	
1 min	**10 min**		**1min** **10min**		
		sec		**min**	**sec**
5,5	60	910	**No measurements possible because of colloidal precipitate**	**20**	
7	55	930		**> 20**	
6	55	900			
6	60	910			
Standard deviation					
0.6	3	13			

The results show that a low irradiation of cellulose can result in a higher grade of degree of settlement which should correlate with the degree of fibrillation. In sample 48 and 49 the degree of settlement was very low which should be good. Also the throughput speed was very slowly which is also an indicator for a high degree of polymerization because of long fibers should block the used glass filter (class 3) as described in 2.3.3.
But the pictures made with the optical microscope and the ESEM device lead to another conclusion in this case. Shortened fibers through irradiation could also block the glass filter and could also cause a low degree of settlement. These two tests alone are not a cogent evidence for an effective production method for nanofibrillated cellulose.

Irradiating the cellulose sample was one of the physical tests made and resulted in shorter, degraded fibrils as described in Discroll et.al (2009).
The first dose was relatively low (10 kGy) and the irradiated cellulose (cotton linter) was milled under standard conditions. The fiber lengths were shortened significantly and milling did not affect any fibrillation of the fiber.
The degree of settlement increased up to almost 60% at a dosage of 100 kGy according to Discroll et.al (2009)
In the case of the present master thesis and the relating research it was not the aim to lower the degree of polymerization. In best case the irradiation treatment should make the fiber more accessible for the following milling in the IKA MK/MKO mill and much more affine for possible further treatments with chemicals. A high irradiation treatment also reduces the degree of cristallinity and leads to a higher yield of sugar in case of hydrolyzation. Kumakura and Kaetsu (1978) Kumakura and Kaetsu (1979), Stepanik et al. (1998), Stepanik et al. (2000)

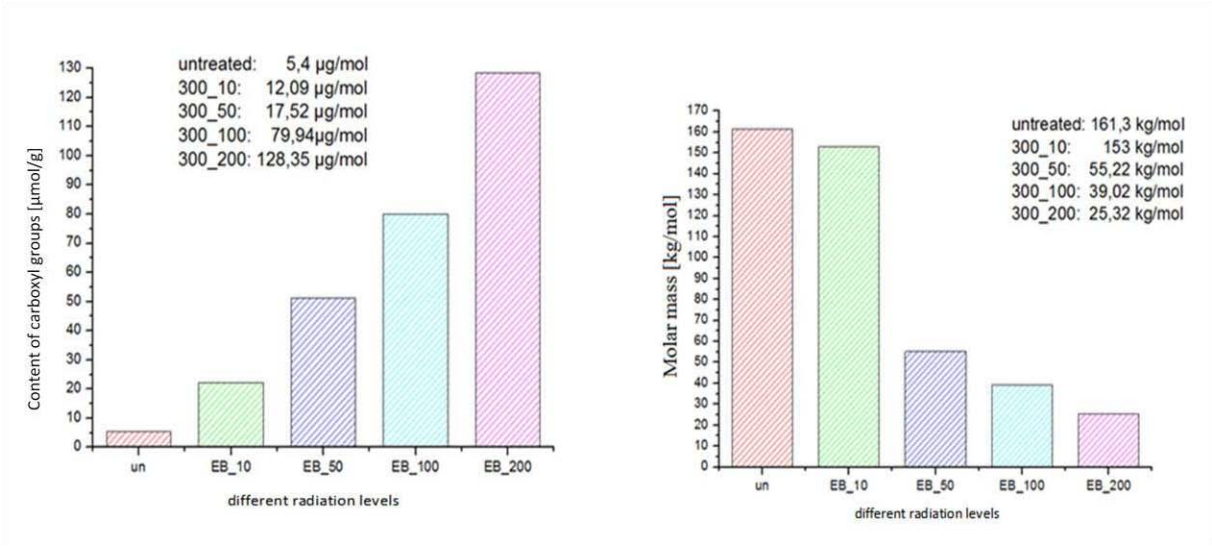

Figure 17. Content of carboxyl groups [µmol/g] and molar masses [kg/mol] on y-coordinate of cotton linters sample FEZ 1523 of increasing dosages of irradiation (10, 50, 100 and 200 kGy). Different radiation dosages on x-coordinate.

Additionally the optical microscope pictures showed very well the consequences of irradiation.

The length of the fibers is very short and furthermore the structures show no fibrils. The fiber morphology is compact and ESEM micrographs show brittle characteristics.

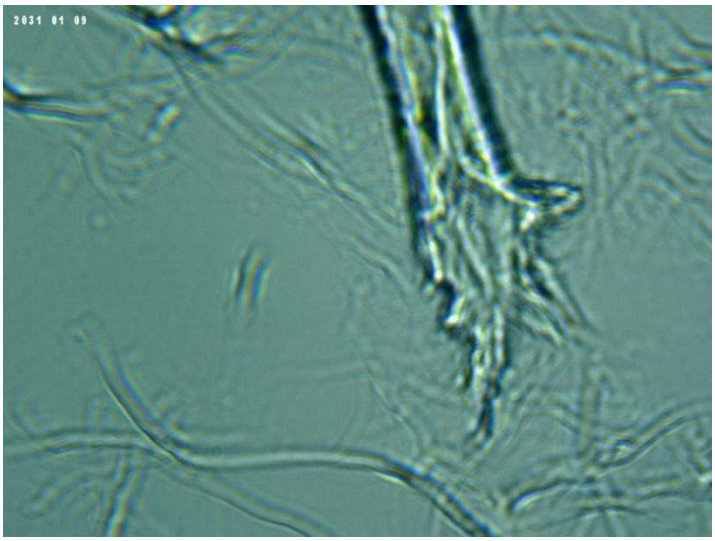

Figure 18. Experiment 48 cotton linters cellulose after milling under standard conditions and irradiation treatment before (10 kGy, 300 keV), 200x (BRESSER LCD microscope)

In figure 19 the cotton linters cellulose sample is shown with 200x magnification. In the background one can see some fibrils but the main part of the sample consists of shortened, non-fibrillated cellulose material. At a dosage of 10 kGy the fiber is already degraded slightly. Increased dosages result in a much higher degradation as can be seen in the upcoming figures.

After the treatment only some cracked cellulose fibers are left. The degree of polymerization seems to be very low according to the researches done by Kumakura and Kaetsu (1978), Kumakura and Kaetsu (1979), Stepanik et al. (1998), Stepanik et al. (2000).

In fact irradiation treatment of cellulose samples can cause an increase of the reactivity because of lattice distortion. This leads to an easier accessibility of the cellulose backbone. (Stepanik et al. 1998; Iller et al. 2007)

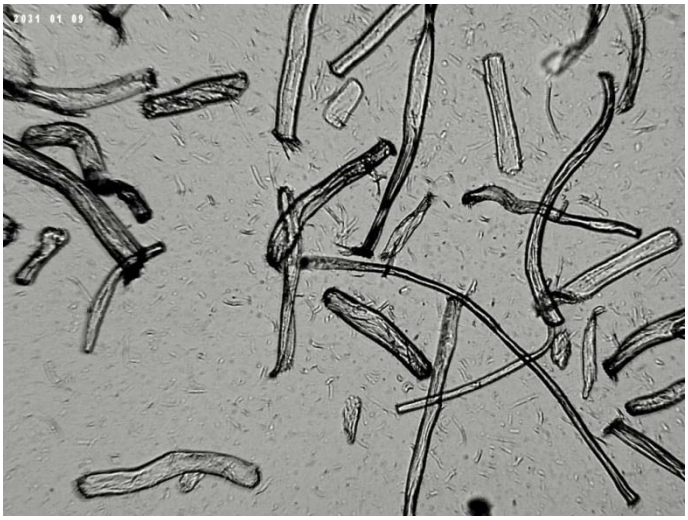

Figure 19. Experiment 48 cotton linters cellulose after milling under standard conditions and irradiation treatment before (10 kGy, 300 keV), 200x (BRESSER LCD microscope)

The ESEM micrographs show the same condition of the sample and it is very obvious that the fibers are brittle as shown in figure 20 and 21.

The ESEM itself causes less damage to the fiber. Furthermore no matrix affects and no cracking due to thermal stress can be observed.

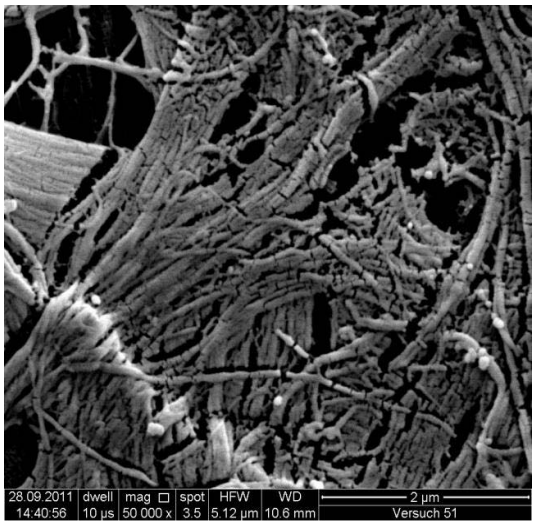

Figure 20. ESEM micrograph of cotton linters after irradiation (300 keV, 200 kGy) and milling procedure under standard conditions. XL 30 ESEM (50000x)

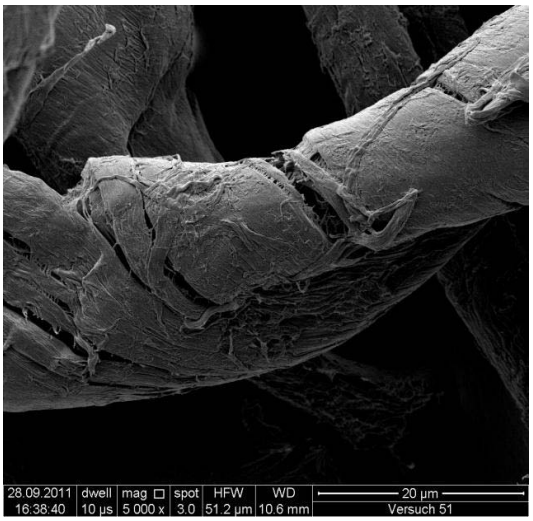

Figure 21. ESEM micrograph of cotton linters after irradiation (300 keV, 200 kGy) and standard conditions XL 30 ESEM (5000x)

3.3 Influence of swelling additives on fibrillation

3.3.1 DMSO

Dimethlysulfoxide solution (DMSO) in H_2O 1:1, short DMSO, was used in several experiments during the thesis practical work. It was used a 50% DMSO / 50% deionized water solution, this mixture was chosen based on experience.

First DMSO was used in different concentrations. In the case described subsequently the 50% solution was used. Also a 40% DMSO / 60% deionized water mixture was used either but did not gave as satisfying results as the experiment with the 50% DMSO did.

The results of two methods done with the help of DMSO will be shown.

The first one was to observe if the swelling treatment in pure DMSO for 1 hour and milling the same cellulose suspension will lead to a higher grade of fibrillation.

The second one was to observe if a mixture of DMSO in water (1:1) for 1 hour will result in a higher grade of fibrillation.

Both samples began with the swelling procedure in 50 ml of pure DMSO on a magnetic stirrer for 1 hour and milling at standard conditions in the IKA MK/MKO mill afterwards.

Table 7. GS and TS values of cotton linters samples FEZ 1525 after treatment with DMSO in deionized water (1:1)

Test 46 **125 ml DMSO plus 125 ml deionized water (x=0.5)**
Pre-swelling an stirring in pure DMSO for 60 minutes, than completion of both mixtures, than milling

	Degree of settlement (GS)		**Throughput speed (TS)**	**Water retention value (WRV)**
	1 min	**10 min**	**sec**	
	0	4	220	
	1	4	210	
	1	5	220	
	0	3	200	
Average value	**0.5%**	**4%**	**213**	**Not measured**
Standard deviation	**0.6**	**0.8**	**10**	

Table 8. GS and TS values of cotton linters samples FEZ 1525 after pre-treatment and milling in pure DMSO

Test 26 **250 ml DMSO Pre-swelling an stirring in DMSO for 60 minutes, than milling**

	Degree of settlement (GS)		**Throughput speed (TS)**	**Water retention value (WRV)**
	1 min	**10 min**	**sec**	
	2	13	90	
	2	8	105	
	1	10	90	
	2	12	110	
Average value	**2%**	**11%**	**99**	**Not measured**
Standard deviation	**0.5**	**2.2**	**10**	

The degree of settlement of the two samples shows that the same pre-treatments and standard milling procedures but once in a mixture of DMSO and water (1:1) and once in pure DMSO make a difference. In both cases the value is compared to most of the other experiments relatively good and describes a certain grade of fibrillation. The treatment in DMSO/water (1:1) mixture shows even better results compared to the one made in pure DMSO.

Using water and DMSO in a mixture for swelling cellulose seems to work very good because of an increased reactivity. (Wagenknecht 1976)

Swelling of cellulose in pure DMSO is delayed and can last for a couple of hours. (Schleicher, Wagenknecht 1973)

Cellulose activation is caused through loosening of supra molecular structure but under maintenance of the basic structure of cellulose. The pore system is widened and separation of the aggregates and disruption of the crystalline order is also caused. (Krässig 1993)

Often used procedures to activate cellulose are swelling treatments with appropriate chemicals such as H_2O, NaOH solution, DMSO and liquid NH_3 under application of energy. (Krässig 1993)

Although the treatment of swelling and milling the used cellulose samples in pure DMSO and diluted DMSO in water (1:1) did not result in a significant fibrillation of cotton linter. It has to be mentioned that in any case the DMSO was removed by rinsing the fibers over a filter with an appropriate amount of deionized water.

The degree of settlement was higher at the treatment with pure DMSO as can be seen in table 8 which concurs with the explanation of Wagenknecht (1976).

Furthermore the photos made by optical microscope give also information about DMSO treatment results.

Figure 22. 125 ml DMSO plus 125 ml deionized water (1:1). Pre-swelling and stirring in pure DMSO for 60 minutes, than completion of both mixtures, than milling. 100x (BRESSER LCD microscope)

Figure 23. 250 ml DMSO after pre-swelling and stirring in DMSO for 60 minutes, than milling. 100x (BRESSER LCD microscope)

As one can see the photos done by the optical microscope seem to be very similar. Fibrils are spliced of the fibers but the solution surrounding the cellulose fibers is still almost free of possible nanofibers.
According to Chitumbo et al. (2007) the temperature has generally a very big influence in the efficiency of swelling on cellulose and in case of DMSO the influence of an increased temperature is dramatic. (Chitumbo et al. 2007)
The temperature during milling procedure did not rise above 45°C in my case and this could be a reason why milling treatment did not affect so much to the sample.

3.3.2 NaOH

After swelling experiments done in different concentrations with DMSO and DMSO in water mixtures the standard samples of cotton linters FEZ 1525 were treated with NaOH.
The concentrations used were 10% (m/v) sodium hydroxide solution and 18% (m/v) sodium hydroxide solution.
The effect on the cellulose fiber is a reduction of crystalline order due to the formation of addition compounds of cellulose with activation chemicals such as aqueous solution of sodium hydroxide in certain concentrations. (Krässig 1993)
The alkaline cellulose underlies chain degradation. (Cheek, Struszczyk 1980)
This effect is not very beneficial for the production of nanofibers but the experiments were within the scientific interest because of the swelling ability of NaOH.
Milled under standard conditions the treatment of cellulose with the two used concentrations of aqueous sodium hydroxide solution lasted for 20 minutes as done in all the other experiments. Even the milling was done in 10% (m/w) and 18% (m/w) solutions and required a secure cooling during procedure to fix temperature at a maximum of 45°C.
During a couple of pre-experiments the cellulose changed color slightly from clear white to light brown but intensive cooling provided that changing the color was almost prevented.

The mercerization process caused by soaking cellulose in aqueous NaOH (17% to 20%, w/v) followed by decomposition of the intermediate by neutralization or washing out the NaOH. (Klemm et al. 2002) In the practical work the neutralization was done by using a 10% solution of citric acid.

Mercerization is used to activate the polymer prior to the production of technical cellulose as already mentioned above. “The transformation process of cellulose I to II is considered to be irreversible.” (Klemm et al. 2002)

NaOH solution effects the cellulose chains in amorphous regions and because of this these regions are rearranged into antiparallel Na-cellulose I. Continuing swelling enhances cellulose chain mobility. (Aravindanath 1986)

Furthermore Kamide et al. (1984) found that soda hydrates can penetrate the amorphous area of cellulose, then solvate to cellulose and destruct the neighboring crystalline regions. Additionally to the possible depolymerisation of cellulose through aqueous sodium hydroxide solution the activation of cellulose through the input of mechanical energy can be attended by depolymerisation. (Klemm et al. 1998).

An increase of the atomic force of the metallic ion (Li < Na < K < Rb < Cs) leads to a decrease of swelling. The bigger the alkali ion, the more difficult is its penetration into cellulose fibers (Petitpas 1948) but more uniform is the swelling (Sreenivasan et al. 1993).

The following tables show the influence of aqueous sodium hydroxide solution on cotton linters cellulose samples in 2 concentrations.

In both cases a pre-treatment in the ultrasonic bath EMAG Technologies EMMI 12HC was done at 100% of available power which is 80 W and was tempered constantly at 25°C.

Milling procedure itself was performed under standard conditions.

Table 9. GS and TS values of cotton linters samples FEZ 1525 after pre-treatment and milling in pure DMSO

Test 44 **250 ml 10% (w/v) NaOH (pre treated in ultrasonic device for 60 minutes)**

	Degree of settlement (GS)		**Throughput speed (TS)**		**Water retention value (WRV)**
	1 min	**10 min**	**min**	**sec**	
	1	5		5	
	1	4		5	
	1	4		4	
	1	5		4	
Average value	**1%**	**5%**		**5**	**Not measured**
Standard deviation	**0**	**0.6**		**0.6**	

Table 10. GS and TS values of cotton linters samples FEZ 1525 after pre-treatment and milling in pure DMSO

Test 36 250 ml 18% (w/v) NaOH (pre-treated in ultrasonic device for 60 minutes)

	Degree of settlement (GS)		**Throughput speed (TS)**	**Water retention value (WRV)**
	1 min	**10 min**	**sec**	
	4	45	5	
	5	50	5	
	5	55	5	
	5	50	5	
Average value	**5%**	**50%**	**5**	**Not measured**
Standard deviation	**0.5**	**4.1**	**0**	

In table 10 the degree of settlement shows a relatively low value which should be an indicator for a certain grade of fibrillation. Unfortunately, fibrillation did not occur in such intensity as wished. Looking at figure 24 one can see that there is almost no fibrillation which can already be seen by light microscopy. The low value of 1% of settlement after 1 minute and only 5% after 10 minutes of observation can also result from swelling and a certain stabilization of the fibers of each other.

Interestingly, the treatment in 18% NaOH solution results in much higher degrees of settlement, meaning that the settlement of fibers is much quicker and does not indicate high efficiency in nano fibrillation either. According to table 10 and figure 25 the values and respectively the morphology of the used sample underline this result. Figure 25 shows almost unfibrillated cellulose after alkali treatment. In comparison to figure 24 the fibers are all swollen and look curled.

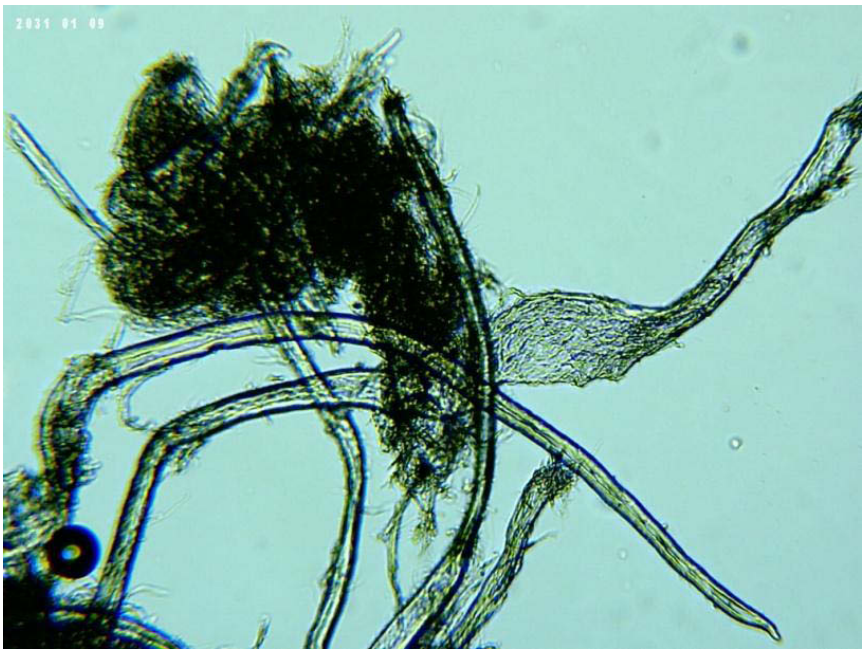

Figure 24. Cotton linters after ultrasonic bath and milling in 10% (w/v) NaOH solution for 20 minutes. (100x BRESSER LCD microscope)

Figure 25. Cotton linters after ultrasonic bath and milling in 18% (w/v) NaOH solution for 20 minutes. (100x BRESSER LCD microscope)

3.4 Influence of enzymatic treatment on cellulose fibers

The cellulose sample used for enzymatic treatment was also cotton linters FEZ 1525. In order to test the influence of disintegration of the cotton linters cellulose through an enzyme a premixed enzyme solution was used as described in Ungurean et al. (2011). Treatment procedure was done with 25 µL Celluclast® enzyme mixture (enzymatic activity: U=600 U/g cellulose).

Three volumes of the same enzyme activity were added (12.5µL, 25 µL and 50µL) in different combinations of treatments. To give an overview of the most meaningful tests the results of addition of 25 µl Celluclast® enzyme mixture before treatment in the ultrasonic bath and then milling as case 1 shall be discussed.

Case 2 describes the addition of 25 µl Celluclast® enzyme mixture after ultrasonic bath treatment and then milling in the IKA MK/MKO mill.

The addition of 50 µl of Celluclast® enzyme mixture resulted in a short, degraded cellulose fiber which precipitated very fast and was well comparable with the degraded cellulose earned after high dosage irradiation as shown in figure 19 in 3.2.3.

Table 11. GS and TS values of cotton linters samples FEZ 1525 milled in 0.05 mol/L sodium acetate buffer solution (pH 4.8) + 25µl Celluclast® (U=600 U/g) addition BEFORE ultrasonic bath

Test 57 **0.25% cotton linters milled in 0.05 mol/L sodium acetate buffer solution (pH 4.8) + 25µl Celluclast® (U=600/g cellulose) addition BEFORE ultrasonic bath**

	Degree of settlement (GS)		Throughput speed (TS)	Water retention value (WRV)
	1 min	**10 min**	**sec**	
	0	1	147	
	0	2	180	
	0	3	190	
	1	3	170	
Average value	**0%**	**2%**	**172**	**50.8%**
Standard deviation	**0.5**	**1**	**18**	

Table 12. GS and TS values of cotton linters samples FEZ 1525 after pre-treatment and milling in pure DMSO milled in 0.05 mol/L sodium acetate buffer solution (pH 4.8) + 25µl Celluclast® (U=600/g cellulose) addition AFTER ultrasonic bath

Test 58 **0.25% cotton linters milled in 0.05 mol/L sodium acetate buffer solution (pH 4.8) + 25µl Celluclast® (U=600/g cellulose) addition AFTER ultrasonic bath**

	Degree of settlement (GS)		Throughput speed (TS)	Water retention value (WRV)
	1 min	10 min	sec	
	4	65	190	
	6	60	168	
	4	53	158	
	5	58	155	
Average value	**5%**	**59%**	**168**	**Not measured**
Standard deviation	**1**	**5**	**16**	

According to Henriksson et al. (2007) enzyme treatment was found to facilitate disintegration and the nanofibrillated nanofibers produced go through lower degradation effect.

During the process of enzyme hydrolysis, the reduction of degree of polymerization (DP) can only be observed at the early stages induced by a random attack of endoglucanases at amorphous regions. Those enzymes can gradually decrease the DP but only slightly. (Cao; Tan 2005)

The test did not lead to results which were expected. The degree of settlement in table 12 is much higher than the one shown in table 11 although the addition of the Celluclast® enzyme mixture was done after the ultrasonic pre-treatment. Normally the enzyme activity is highly decreased after ultrasonic influence because of denaturation. In my case the degree of settlement values should not be as high as shown in table 12 and lead instead to a higher grade of fibrillation of the cotton linters fibers used. Interestingly the throughput speed is almost the same in both cases. This could be explained because of the similar morphology which occurred despite of different treatments. But figure 27 and figure 28 do not show major differences in cellulose morphology.

Observing the photos made by light microscope show a slightly higher fibrillation of cellulose treated in test 57 whereas the fibers in test 58 seem to be compact and relatively unfibrillated despite of identical milling treatment and post-ultrasonic enzyme addition.

One would expect the opposite because of possible enzyme denaturation through ultrasonic treatment for 60 minutes.

On the other hand it can be possible that ultrasonic treatment did not affect the activity of the enzyme and the addition of it before starting the ultrasonic treatment resulted in a better GS value due to better accessibility of the cellulose structure by the enzyme.

So the loss of enzyme activity due to ultrasonic treatment ways less than the much better accessibility of Celluclast® enzyme right during ultrasonic treatment although it lasted for 60 minutes.

The addition of Celluclast® after ultrasonic treatment (as done in test 58) shows much higher GS values. The reason for this can be that after ultrasonic treatment the structure of cellulose is then no more reactive and affine for absorbing additives and reactants. It seems that the accessibility and effectiveness of an enzyme is higher when it is applied direct during the ultrasonic treatment process and that the loss of enzyme activity plays a minor role as already mentioned above.

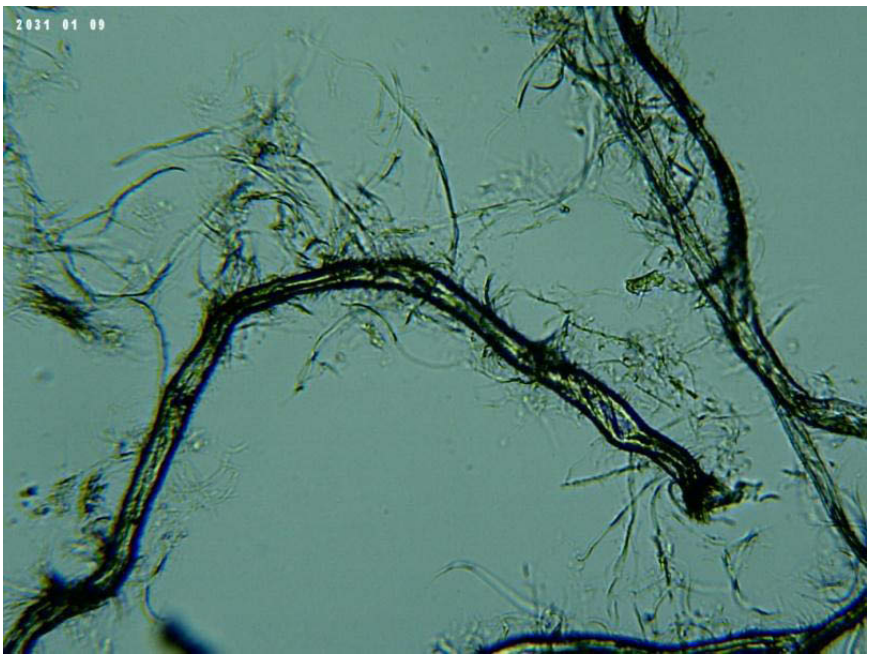

Figure 26. 0.25% cotton linters milled in 0.05 mol/L sodium acetate buffer solution (pH 4.8) + 25µl Celluclast® (U=600/g cellulose) addition before ultrasonic bath (100x BRESSER LCD microscope)

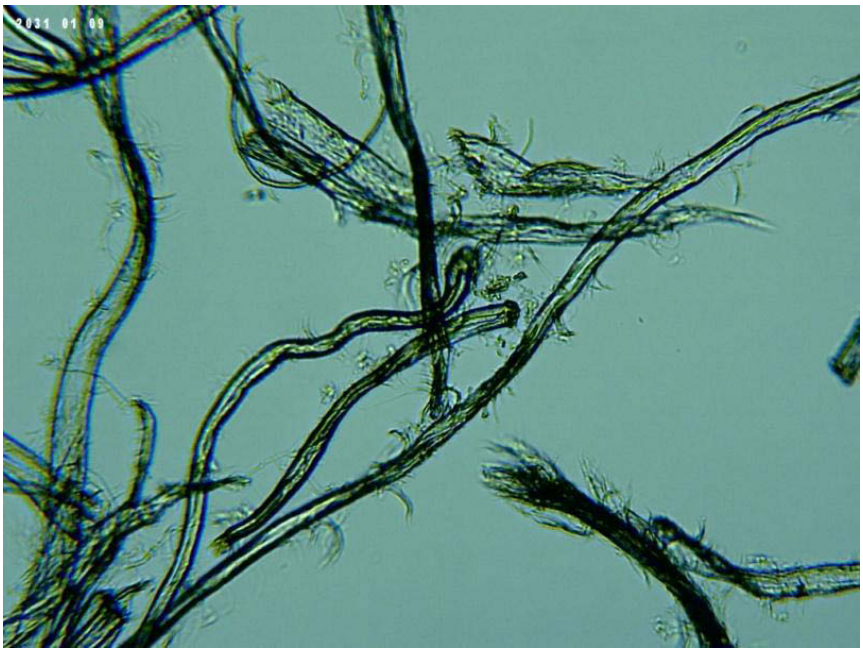

Figure 27. 0.25% cotton linters milled in 0.05 mol/L sodium acetate buffer solution (pH 4.8) + 25µl Celluclast® (U=600/g cellulose) addition after ultrasonic bath (100x BRESSER LCD microscope)

3.5 Influence of other additives on fibrillation

3.5.1 Alkenyl succinic anhydride

Using alkenyl succinic anhydride (ASA) which is used as sizing agent in the paper industry (Gess; Rodriguez 2005) was another method which was done.
By using sizing agents as additives before milling a certain stabilization was wanted. The resistance of the penetration of liquids could support the separation of cellulose fibers during mechanical treatment in the used hydrophilic medium water if one assumes that ASA as shown in figure 28 binds to these cellulose fibers over an esterification reaction.

This test was done to investigate if ASA treatment of cellulose will lead to an enhanced ability to produce nano fibers.

The reaction caused by edition of ASA is shown in figure 29.

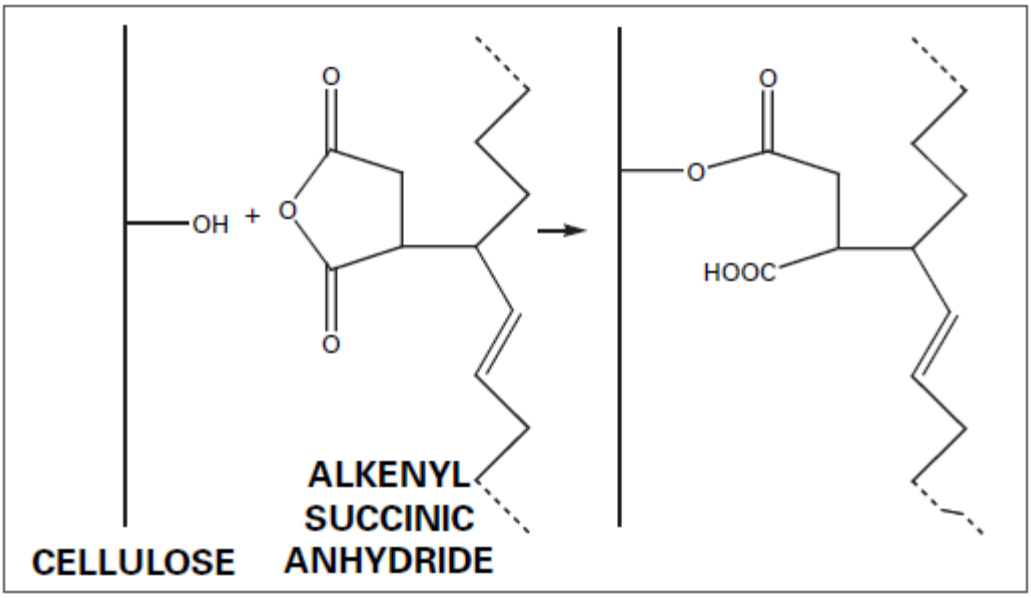

Figure 28. Esterification reaction of ASA with cellulose (Gess; Rodriguez 2005)

According to Dufresne (2010) ASA can be used for acylating the surface of cellulose nanocrystals. It is widely used as a sizing agent in paper industry, where it is added to pulp fibers in aqueous systems. Acylated whiskers disperse in medium- to low-polarity solvents (Dufresne 2010).

It was further determined a concentration of 2% ASA per gram dry cellulose and milling under standard conditions. ASA was added during milling procedure which caused voluminous white foam and small bubbles in the solution. After milling the ASA had to be washed out by rinsing the samples with at least four times 0.5 L of deionized water for each washing step to remove all of the ASA and to clear the turbid milling solution.

The results were not very satisfying as the following table 13 shows. The degree of settlement reached a value of 11% after 10 minutes which is not very good compared to results of other experiments but much better than treatment only in the Ultra turrax® device of IKA without any additives like table 2 shows. The GS value there shows a grade of 53% after 10 minutes.

The time of TS after ASA treatment shows a value which is low and that did not indicate a high fibrillation grade either.

Almost comparable is the TS value and achieved 79±20 seconds (Table 2).

Table 13. GS and TS values of 0.25% cotton linters suspended in 2% aqueous ASA solution (per gram dry cellulose)

Test 52 **0.25% cotton linters aqueous suspension + 2% ASA per gram dry cellulose**

	Degree of settlement (GS)		Throughput speed (TS)	Water retention value (WRV)
	1 min	10 min	sec	
	2	10	104	
	2	10	80	
	2	11	75	
	2	11	56	
Average value	**2%**	**11%**	**79**	**Not measured**
Standard deviation	**0**	**0.6**	**20**	

The following figures made with the light microscope lead to the same conclusion that ASA does not support milling procedure in fibrillation of cotton linters cellulose used.

Figure 29. 0.25% cotton linters suspended in 2% aqueous ASA solution (per gram dry cellulose) (100x BRESSER LCD microscope)

Figure 29 shows no extensive fibrillation effect on used cotton linters samples and correlates with the other observations made in the GS and TS values.

As a conclusion using ASA as a promoting additive for the production of nanofibers with the use of a mill is not necessary because almost the same results in GS, TS and optical observation tests can be achieved also with other tests and chemical additives.

3.5.2 Carboxymethyl cellulose

Using carboxymethyl cellulose was performed with defined parameters to test the influence on the fibrillation efficiency of the standard cotton linters sample.
10% (w/w) CMC was added to dry cellulose and the milling was done at standard conditions.
After the treatment four steps of intensive washing were done. The treated cotton linters was stirred for three hours in deionized water to remove the CMC completely from the milling solution.
According to Beghello (1998) and Yan et al. (2006) carboxymethyl cellulose (CMC) as an anionic cellulose derivate has a dispersing effect on cellulose. Ahola et al. (2008) found that the interaction between CMC and nanofibrils is repulsive because of the anionic behavior of both materials. “At low electrolyte concentrations as it was performed during the thesis experiment CMC is not irreversibly adsorbed onto the cellulose nanofibrils because of the electrostatic repulsion due to the interaction of the polysaccharide backbone.” Ahola et al. (2008)
Normally the adsorption of CMC on cellulose fibers is irreversible under higher temperature and electrolyte concentration (Laine et al. 2000) but even under reversible adsorption the dispersing effect still exists as described by Beghello (1998) and Jokinen et al. (2006).

Table 14. GS and TS values of 0.25% cotton linters suspended in 10% (w/w) aqueous CMC solution

Test 54 **0.25% cotton linters aqueous suspension + 10% (w/w) CMC**

	Degree of settlement (GS)		Throughput speed (TS)	Water retention value (WRV)
	1 min	10 min	sec	
	7	45	62	
	6	40	55	
	7	45	50	
	7	40	53	
Average value	**7%**	**43%**	**55**	**Not measured**
Standard deviation	**0.5**	**2.9**	**5**	

As can be seen table 14 shows the results of the measurement of CMC as additive during milling procedure. GS and TS values do not indicate a significant grade of fibrillation. Furthermore the settlement of cellulose fibers proceeded very fast in cellulose aggregates and did not show a homogenous cellulose fiber suspension. TS value is very low which means that there is almost no fibrous close-meshed network of cotton linters fibrils.

In figure 30, the non-fibrillated morphology of the used cotton linters cellulose can be seen. Almost no separated fibrils with a high expect ratio can be found.

Figure 30. 0.25% cotton linters suspended in 10% (w/w) aqueous CMC solution (100x BRESSER LCD microscope)

3.6 Influence of other pre-treatments on fibrillation

3.6.1 Ultrasonic bath

Treatment in ultrasonic device as used in most of the experiments as a pre-treatment step was also done without any following special treatment or without any additives.
According to Chen et al. (2011) the yield of nanofibrils out of cotton linters cellulose as standard sample material was also tested by using an ultrasonic device. The chemical pre-treatments done by Cheng et al. (2011) were not necessary in this study because the used cotton linters was free of lignin and other impurities.
Therefore a 0.25% suspension of cotton linters in deionized water was placed in a 50 ml flask and swollen for 60 minutes in the ultrasonic device as described in 2.2.4.1.
Milling in the IKA MK/MKO device followed and resulted in following values.

Table 15. GS and TS values of 0.25% cotton linters aqueous dispersion after ultrasonic treatment

Test 31 **0.25% cotton linters aqueous dispersion**

	Degree of settlement (GS)		**Throughput speed (TS)**	**Water retention value (WRV)**
	1 min	**10 min**	**sec**	
	0	2	210	
	0	1	205	
	0	2	205	
	0	1	210	
Average value	**0%**	**2%**	**208**	**57%**
Standard deviation	**0**	**0.6**	**3**	

As one can see the results are quite good. The GS value was almost 0. No settlement of cellulose front was observable. Also very satisfying results were achieved after measuring the GS after 10 minutes which are also at a very low level.
The TS gave an average of round about 208±3 seconds. These two results combined with the high fibrillation which can be seen on following photographs lead to the assumption that ultrasonic treatment of cellulose even in pure deionized water has a high potential in producing nanofibrillated cellulose.

When comparing the resulted WRV to the method using sodium metaperiodate described in 2.2.4.5. the values are almost equally high. Also the GS values after 1 and 10 minutes are quite equal as well. To conclude, both methods work well and both lead to a relatively high degree of fibrillation. The main advantage of method 2.2.4.1. is that no single chemical additive was used. Other advantages – also as a consequence of this, are a shorter production time span, no waste-water production and samples do not have to be washed.

The photos of the cotton linters sample made after milling are shown in figure 32 as followed.
Standard conditions during ultrasonic treatment were applied as done in all experiments before.

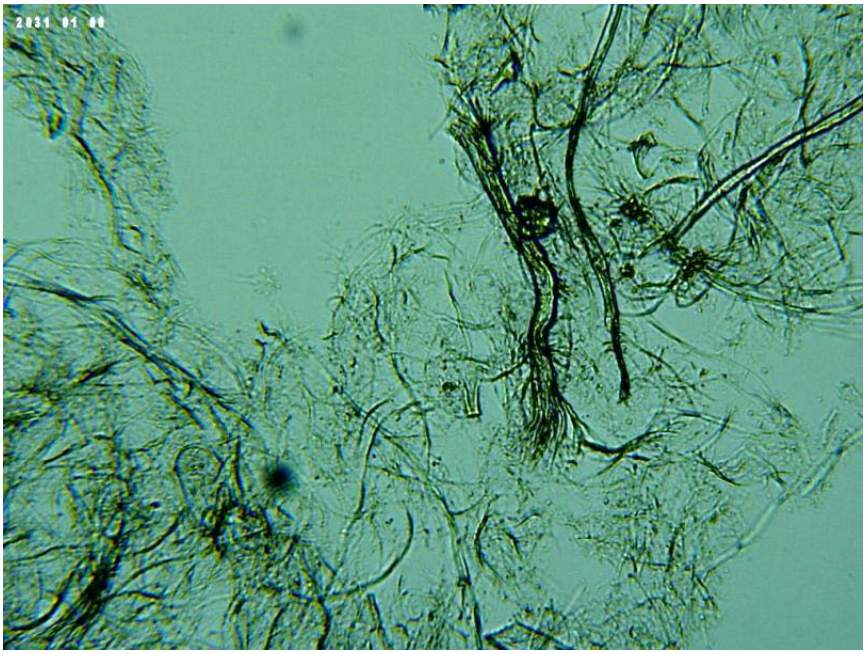

Figure 31. 0.25% cotton linters aqueous dispersion after ultrasonic treatment (100x BRESSER LCD microscope)

Despite of the fact that the device used in this study had much less power than the one Cheng et al. (2011) used the results are quite good. One has to mention that experiment 31 was done completely without any chemicals. This is a big benefit and also one of the general aims of this master thesis: producing nanofibers out of cellulose with a minimum of chemical input and power consumption. In this case good results were achieved without the addition of any chemical substance.

The achieved result is really astonishing because of the obvious potential of making nanofibrils through ultrasonic treatment.

Because of these conclusions and results some SEM/ESEM micrographs were taken from the treated sample and also presented very satisfying achievements. In figure 33 the ability of simple ultrasonic treatment on pure cotton linters cellulose in deionized water is shown and the nanofibrils can be seen.

The magnification is 10000x and the fibrous mesh is clearly visible. The fiber length seems to be much longer than 5 µm which means that the aspect ratio is high enough to describe the sample fibers with nanofibril character.

The fibrils in figure 33 micrographed with a magnification of 30000x look like broken. This is possible caused by the longtime ultrasonic treatment of 60 minutes but more possibly caused due to the electron beam in the ESEM during the focusing and recording.

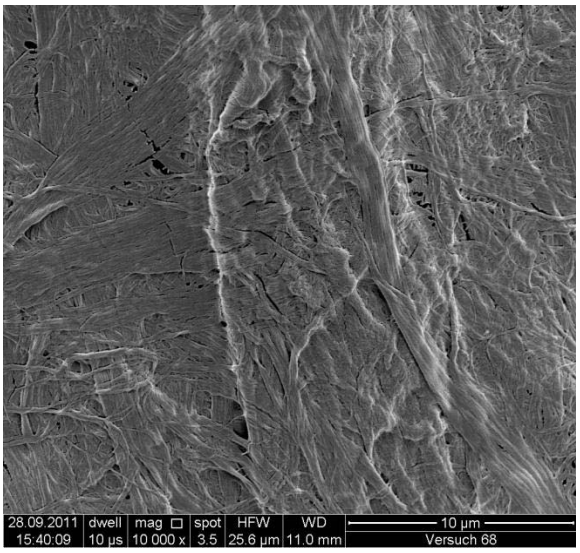

Figure 32. ESEM micrograph of cotton linters after ultrasonic treatment and milling (PHILPS XL 30 ESEM 10000x)

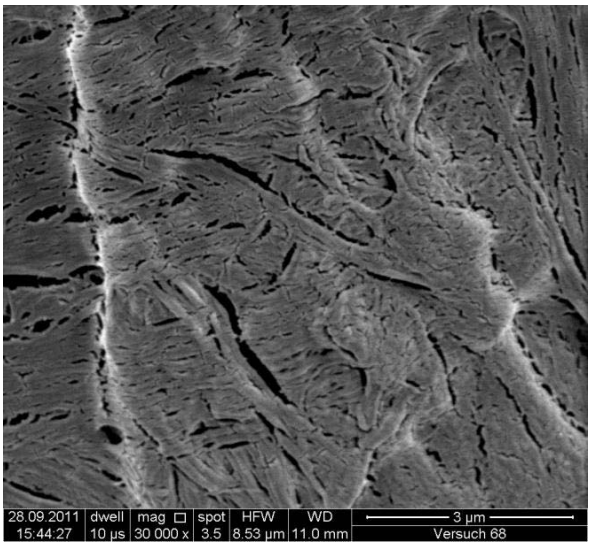

Figure 33. ESEM micrograph of cotton linters after ultrasonic treatment and milling (PHILPS XL 30 ESEM 30000x)

3.6.2 Pre-swelling in water

To compare the cellulose treated with ultrasonic with cellulose treated without any pre-procedure except swelling in water experiment 32 was done.

The only difference to experiment 31 was to pre-swell the cotton linters sample in deionized water at room temperature for at least 72 hours on a magnetic stirrer. This duration is much longer than the swelling time in ultrasonic bath.

The results show that a long time of swelling leads to almost identical values of GS and values for TS which are comparable to those of ultrasonic treatment.

Table 16. GS and TS values of 0.25% cotton linters aqueous suspension swelling time 73h and afterwards milling treatment

Test 32 **0.25% cotton linters aqueous suspension after 72h swelling; milling afterwards.**

	Degree of settlement (GS)		**Throughput speed (TS)**		**Water retention value (WRV)**
	1 min	**10 min**	**min**	**sec**	
	1	2		220	
	1	2		215	
	1	3		200	
	1	2		205	
Average value	**1%**	**2%**		**210**	**Not measured**
Standard deviation	**0**	**0.5**		**9**	

The picture made with the optical microscope is shown below:

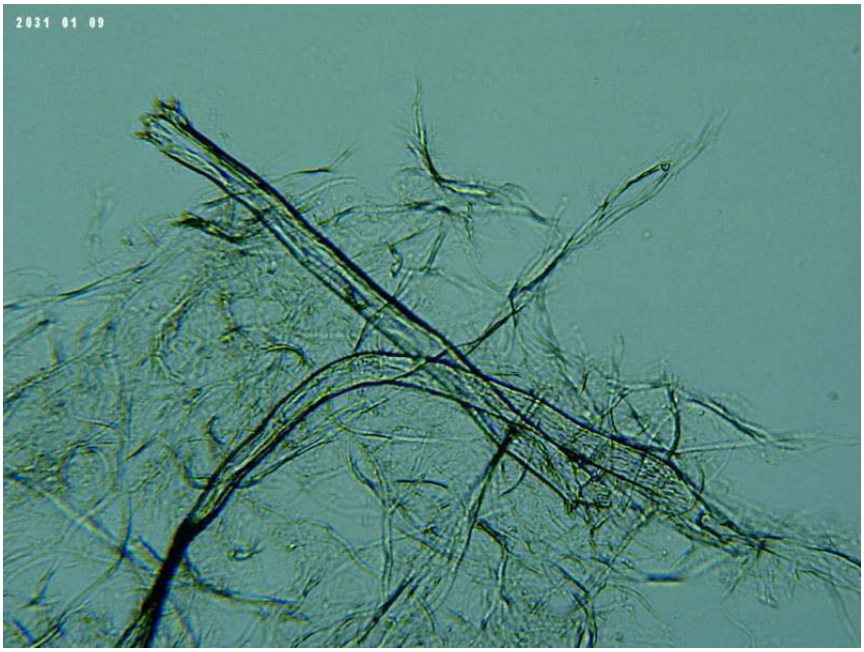

Figure 34. 0.25% cotton linters aqueous suspension after 72h swelling; milling afterwards. (100x BRESSER LCD microscope)

The cellulose fibers show a high grade of fibrillation and the sample which was photographed shows that the fibers are widely not very compact.

This means that without any addition of chemicals and even without ultrasonic treatment the results seem to be very good in order to yield nanofibrils. Because of limited time resources a further observation with ESEM was not performed.

Furthermore the needed duration for swelling to achieve those results is too long. Almost three days of pre-treatment stand in contrast to an efficient method.

3.7 Fibrillation at "in situ"-precipitation during milling after dissolution in 9% DMAc/LiCl

Different cellulose samples were dissolved in a 9% DMAc/LiCl solution. According to an internal dissolution instruction used at the local institute the cellulose samples were regenerated as described in 2.2.4.8. (Stryuk et al. 2005 and Röder et al. 2001)

McCormick et al. (1985) did solution studies of cellulose in lithium chloride and *N,N* – Dimethylacetamide. The mechanism of cellulose dissolution is described in figure 35.

According to McCormick et al. the hydroxyl protons of the anhydroglucose units are associated with the chloride anion by hydrogen bonding. The chloride ion is associated

with a Li^+(DMAc), macrocation. The resulting charge - charge repulsions (Hudson 1980, Spurlin 1955) or a bulking effect (Gruenwald, Price 1964), would tend to allow further solvent penetration into the polymer structure. The rate at which cellulose dissolves appears to be highly dependent on the number of intermolecular hydrogen bonds present in the initial sample. Disruption of hydrogen bonds (by swelling in polar or hydrogen bonding solvents or by heating) increases dramatically the rate of dissolution.

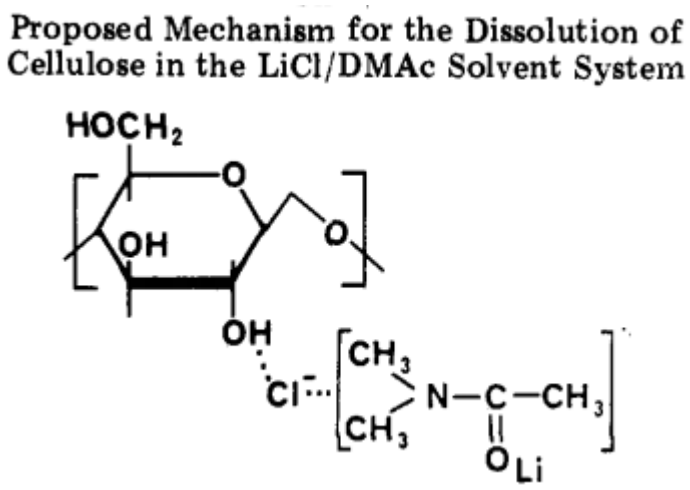

Figure 35. Mechanism for dissolution of cellulose in the DMAc/LiCl solvent system, McCormick et al. (1985)

The dissolution procedure was done for the standard cotton linters sample and also for four other cellulose pulps such as: beech sulfite KZO3 (LAG) FEZ1085, birch kraft (m-real) PULP5 (75% Glucose, 24.4% Xylose, 0.5% Mannose), softwood sulfite (DOMSJÖ) PULP5 FEZ1486, and TCF kraft (Rosenthal), FEL 1050.

As a consequence that row of tests resulted in 5 comparable sets of GS-, TS- and WRV-values.

The procedure used is described in 2.2.4.8.

Table 17. GS and TS values of 0.25% cotton linters aqueous suspension. (After dissolving in 9%DMAc/LiCl, added to deionized water and washed afterwards according to 2.2.4.8 Milling in IKA MK/MKO device)

Test 67 **0.25% cotton linters aqueous suspension; (After dissolving in 9%DMAc/LiCl, added to deionized water and washed afterwards according to 2.2.4.8 Milling in IKA MK/MKO device)**

	Degree of settlement (GS)		**Throughput speed (TS)**	**Water retention value (WRV)**
	1 min	**10 min**	**min**	
	0	2	26	
	1	3	25	
	1	4	-	
	0	3	-	
Average value	**1%**	**3%**	**26**	**51%**
Standard deviation	**0.6**	**0.8**	**0.7**	

The GS values underline a high grade of fibrillation and also the TS values lead to the conclusion that in-situ precipitation of cotton linters cellulose in deionized water during milling results in potential cellulose nano fibers (Table 17). WRV shows a high percentage of absorbed water due to a high fibrillation grade.

The treated samples were also photographed under the optical microscope.

One of the main aims of the thesis was to achieve a maximum of efficiency of a prospective method in producing cellulose nano fibers.

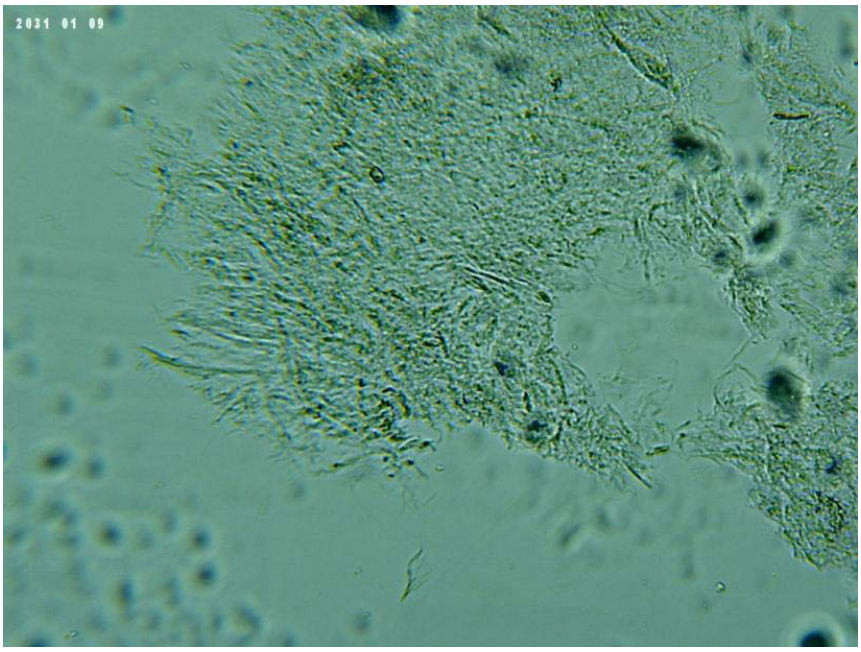

Figure 36. 0.25% cotton linters aqueous suspension; (After dissolving in 9%DMAc/LiCl, added to deionized water and washed afterwards according to 2.2.4.8 Milling in IKA MK/MKO device; 200x BRESSER LCD microscope)

In comparison to cellulose which was treated in a homogenizer (material kindly provided by Prof. Dr. Gindl) the result is quite satisfying especially focused on the desired minimum of length of the fibers. It seems that the resulted fibers are longer and thinner. Cellulose in figure 37 shows more granular type fracture morphology (figure 36).

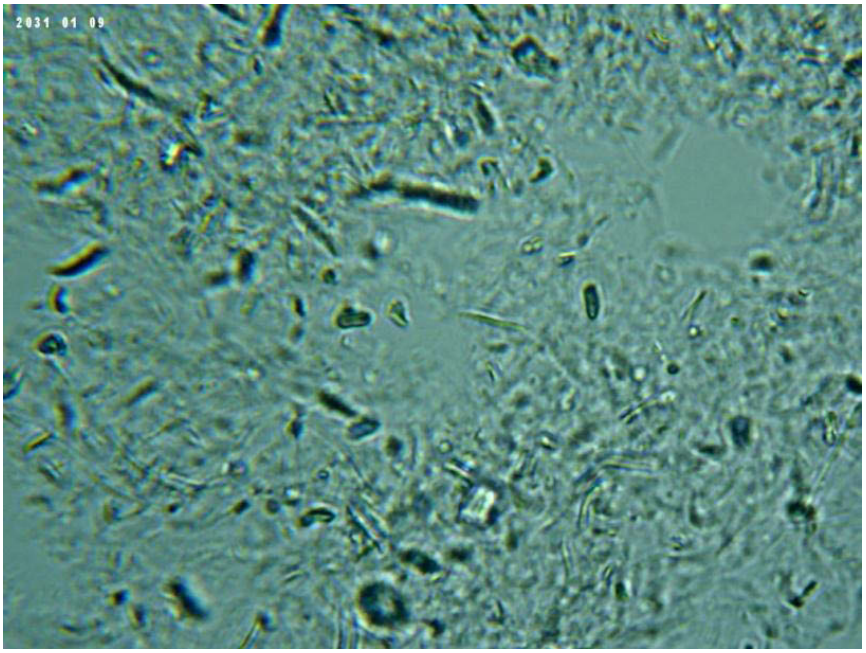

Figure 37. Cellulose treated in high pressure homogenizer provided by Prof. Dr. Gindl. (200x BRESSER LCD microscope)

Without any microscope the samples appearance is widely similar. The main difference is that in case of in-situ precipitation after dissolution in 9% DMAc/LiCl there is almost no mechanical work necessary in contrast to the preparation with a high pressure homogenizer although the method is very effective and the resulted grade of fibrillation is high according to all resulted values shown in table 17.
Furthermore a SEM/ESEM micrograph was taken to complete experiment 67.

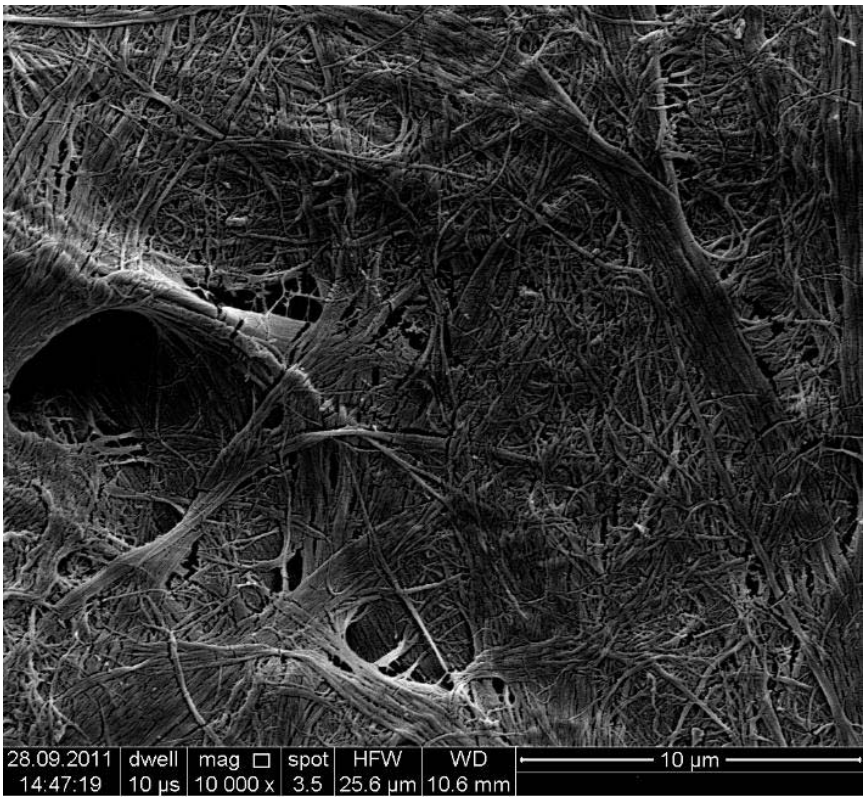

Figure 38. ESEM micrograph of cotton linters after regeneration during milling in deionized water and dissolution in 9% DMAc/LiCl. (PHILPS XL 30 ESEM 10000x)

In figure 38 a highly fibrous meshwork of regenerated cotton linters cellulose is shown. It shows that the cellulose fibers after regeneration do not show granular properties. Instead the length-diameter ratio is relatively high. One can see that most of the fibers have a length of at least 10 µm up to 40 µm or 50 µm whereas the diameter is approximately 0.1µm. As described in Chinga (2011) the definition of the terms nanofibrils and microfibrils are that the term nano fibril refers to fibrils with diameters less than 100 nm. Compared to this definition, it seems obvious that microfibrils can be considered nanofibrils, which also are composed of crystalline and amorphous regions. (Chinga 2011) The difference between microfibrils and nanofibrils is that the former is a well-defined biological structure found in plant cell walls. (Chinga 2011)

As can be seen in the experiments the micrographs of regenerated cellulose look like a skin-core structure. (Klemm et al. 1998:23)

Tables of measured values of 4 other cellulose pulps follow:

72A: Beech sulfite KZ03 (LAG), FEZ1085
73B: Birch kraft (m-real), PULP5 (75% Glucose, 24.4% Xylose, 0.5% Mannose)
74C: Softwood sulfite (DOMSJÖ) PULP5, FEZ1486
75D: TCF kraft (Rosenthal), FEL 1050

Table 18. GS and TS values of 0.25% beech sulfite aqueous suspension. (After dissolving in 9%DMAc/LiCl, added to deionized water and washed afterwards according to 2.2.4.8 Milling in IKA MK/MKO device)

Test 72A **0.25% beech sulfite aqueous suspension; (After dissolving in 9%DMAc/LiCl, added to deionized water and washed afterwards according to 2.2.4.8 Milling in IKA MK/MKO device)**

	Degree of settlement (GS)		**Throughput speed (TS)**	**Water retention value (WRV)**
	1 min	**10 min**	**sec**	
	10	50	11	
	10	50	11	
	10	47	12	
	10	51	12	
Average value	**10%**	**50%**	**12**	**75%**
Standard deviation	**0**	**1.7**	**0.6**	

Table 19. GS and TS values of 0.25% birch kraft aqueous suspension. (After dissolving in 9%DMAc/LiCl, added to deionized water and washed afterwards according to 2.2.4.8 Milling in IKA MK/MKO device)

Test 73B **0.25% *birch kraft* aqueous suspension; (After dissolving in 9%DMAc/LiCl, added to deionized water and washed afterwards according to 2.2.4.8 Milling in IKA MK/MKO device)**

	Degree of settlement (GS)		**Throughput speed (TS)**	**Water retention value (WRV)**
	1 min	**10 min**	**sec**	
	2	12	18	
	2	13	19	
	2	13	21	
	2	12	22	
Average value	**2%**	**13%**	**20**	**46%**
Standard deviation	**0**	**0.6**	**2**	

Table 20. GS and TS values of 0.25% softwood sulfite aqueous suspension. (After dissolving in 9%DMAc/LiCl, added to deionized water and washed afterwards according to 2.2.4.8 Milling in IKA MK/MKO device)

Test 74C **0.25% *softwood sulfite* aqueous suspension; (After dissolving in 9%DMAc/LiCl, added to deionized water and washed afterwards according to 2.2.4.8 Milling in IKA MK/MKO device)**

	Degree of settlement (GS)		**Throughput speed (TS)**	**Water retention value (WRV)**
	1 min	**10 min**	**sec**	
	1	14	35	
	1	12	37	
	1	13	40	
	1	11	40	
Average value	**1%**	**13%**	**38**	**84%**
Standard deviation	**0**	**1.3**	**3**	

Table 21. GS and TS values of 0.25% Rosenthal kraft TCF aqueous suspension. (After dissolving in 9%DMAc/LiCl, added to deionized water and washed afterwards according to 2.2.4.8 Milling in IKA MK/MKO device)

Test 75D **0.25% *Rosenthal kraft* TCF aqueous suspension; (After dissolving in 9%DMAc/LiCl, added to deionized water and washed afterwards according to 2.2.4.8 Milling in IKA MK/MKO device)**

	Degree of settlement (GS)		**Throughput speed (TS)**	**Water retention value (WRV)**
	1 min	**10 min**	**sec**	
	0	1	150	
	0	2	180	
	0	3	190	
	0	2	198	
Average value	**1%**	**2%**	**180**	**100%**
Standard deviation	**0**	**0.8**	**21**	

The results of all 5 samples show that cotton linters used as standard cellulose pulp shows overall the best values regarding the GS values and the TS value. Especially the TS value is relatively high which indicates a good fibrillation. Additionally the measured GS values are low which matches also to the other parameters.

Comparing the results to those of ultrasonic treatment experiments which produced also very satisfying properties it can be shown that the average WRV of ultrasonic treatment (shown at 3.6.1.) is almost the same as the one in case of "in-situ"-precipitation but TS-values are significantly lower. GS-values are almost on equal level.

Table 22. Overall summary of average test results (in situ-precipitation)

	GS 1 min	**GS** 10 min	**TS** [sec]	**WRV [%]**
Rosenthal kraft TCF 75D	1	2	180±21	100
Cotton linters 67	**1**	**3**	**26±0.7**	**51**
Softwood sulfite 74C	1	13	38±3	84
Birch kraft 73B	2	13	20±2	46
Beech sulfite 72A	10	50	12±0.6	75

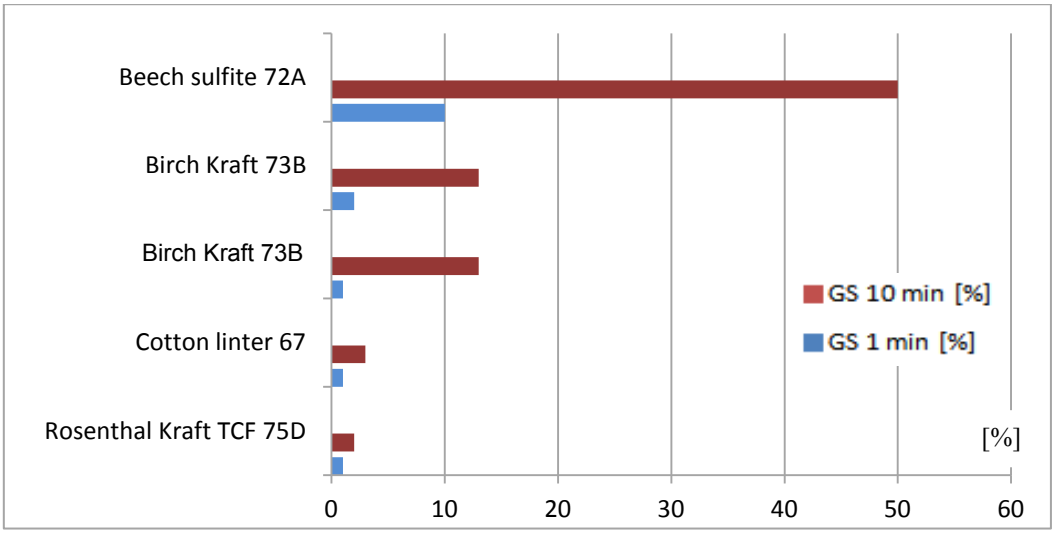

Figure 39. Bar-chart of measured GS values of 5 differently treated cellulose types

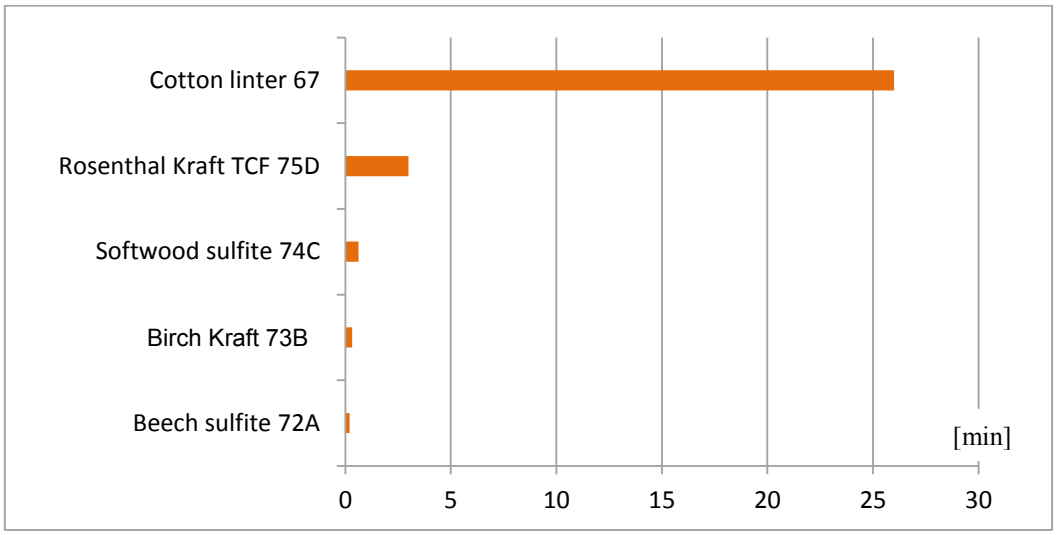

Figure 40. Bar-chart of measured TS values of 5 differently treated cellulose types

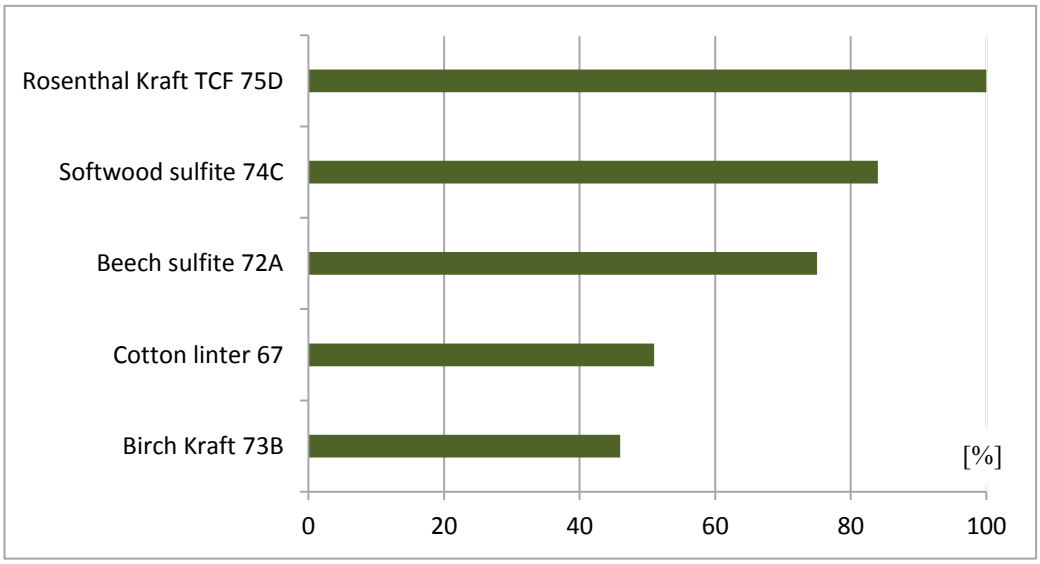

Figure 41. Bar-chart of measured WRV values of 5 differently treated cellulose types

Despite of the identical procedure of dissolving in DMAc/LiCl (9%), milling and "in-situ"-precipitation the results for each of the used cellulose sort samples are different. To compare the results of the dissolution treatment the GS, TS and WRV values are discussed.
Different pulps of cellulose used during the treatment result in very different characterization parameters.
Shortly summarized cotton linters (sample 67) and the cellulose sample 75D Rosenthal kraft TCF (sulfate pulping) gave the optimal results in order to produce nanofibrils.
On the other hand the sample beech sulfite pulping (72A) is not an appropriate material for producing nanofibrils via dissolution in DMAc/LiCl and milling afterwards.
Sample 75D: According to Bachner (1993) cited by Freese (2010) cellulose produced by sulfate digestion can be characterized as very long chained but with short hemicellulose chains. It contains also very short fragments and is also characterized through its high DP and its high milling resistance. These properties are a good foundation for producing long chained nanofibers. One can see that the GS values of 75D are low whereas the TS values are high which indicates a certain high grade of fibrillation. When comparing the TS values one can see that they are significantly higher than those of the other cellulose sorts except the one of cotton linters. The WRV of sample 75D fits in this hypothesis. It is the highest of the dissolution samples.

Sample 72A: The beech sulfite cellulose sample stands on the other side of wished level of GS, TS and WRV values. Bachner (1993) as cited in Freese (2010) comments that the DP of cellulose made in sulfite processing is much lower, which results of the degradation of the polysaccharides because of acid hydrolysis. As a consequence the resistance against milling is much lower than that one of sulfate cellulose and the fibers are shortened which is a property that stands in contrast to nanofibril production.

Sample 74C: Softwood sulfite has whether optimal nor suboptimal properties as a raw-material for producing nanofibrils when it is dissolved in DMAc/LiCl (9%) as can be see above.

Zimmermann et al. (2010) wrote that the homogeneity of the NFC material seems to be more important for the reinforcement potential than the DP. According to their research a proper pre-treatment and choice of starting cellulose materials can reduce energy consumption.

Summarizing all results of different cellulose pulps which have been treated in the dissolution and "in-situ"-precipitation experiments shows that the purified cotton linters sample yielded in the second best GS value. It also showed the far best TS value result which underlines the hypothesis from Zimmermann et al. (2010) that the grade of homogeneity of the nano fibers is high and leads to a very satisfying level of GS.
Zimmermann et al. say that it is generally found that strengths properties decrease with decreasing DP of the cellulose.
During refining treatment Zimmermann et al. (2010) described that the DP also correlates strongly with the aspect ratio of the fibrils. As a conclusion one can say that the shorter the fibrils are the lower is their DP.
Combining these facts and the results from my thesis it can be said that cotton linters (sample 67) obviously shows the best properties from all used cellulose samples.

The second conclusion would be that ultrasonic treatment as performed is effective in some way but does not show that long duration times defining the TS value.
According to this "in-situ" precipitation seems to be more intense in producing nano characteristic fibers.

Disadvantages are in this manner the longer preparation steps in order to produce the cellulose solution with 9% DMAc/LiCl used as a dissolution solvent and the circumstance that the procedure needed to be done very accurate.
In comparison ultrasonic treatment procedure is simpler and quicker but seems to be less effective.

4 SUMMARY AND PROSPECT

Aim of this thesis was to observe and test the influences of different additives and pre-treatment steps on the result of fibrillation. Cotton linters was used as standard cellulose sample. Besides, the experiments done to test the regeneration ability of dissolved cellulose in water were also done with 4 additional cellulose types to compare them.

1) Pre-tests had shown that efficiency depends widely on the concentration of cellulose in water during milling procedure. This concentration was defined as 0.25%. Also the refiner-gap showed best milling efficiency and influence on temperature at a gap distance of 0.159 mm.
2) As another point the influence of oxidative damage of the fiber was tested. It could be observed that oxidation treatment with 0.1 mol/L sodium metaperiodate solution led to an increased fibrillation. Comparing the absolute results of the alternative oxidation, the TEMPO-mediated oxidation, which was also done, the achieved fibrillation results by 0.1 mol/L sodium metaperiodate were more satisfying which can also be seen in figures 11 and 12. The very low GS and the much higher WRV show that periodate oxidation treatment led to very good results despite of a poor chemical input and a simpler pre-treatment procedure.
3) Irradiation treatment with high dosages (200 kGy) gave results which are not very appreciated in producing nanofibers. The higher dosages were used the more cellulose fibrils were shortened until the average lengths of cellulose fibrils ended up at approximately 0.2 µm when a dosage of 200 kGy was applied (figure 21) whereas lower dosages led to satisfying results of GS and TS values.
4) The addition of DMSO and NaOH as swelling additives was also investigated. As a first diagnose one can see that a 1:1 mixture of DMSO and water did not result in a very high grade of fibrillation but worked better than milling of cellulose in pure DMSO.

   NaOH swelling tests were done at two concentrations of sodium hydroxide (10% and 18%) and showed that despite of occurring mercerization (Klemm et al. 2002) almost no fibrillation was caused according to photos made by optical microscope. The observed morphology of cotton linters fibers fits to the measured GS and TS values. As a consequence a possible treatment of cotton linters cellulose with sodium hydroxide solutions does not lead to the aim in order to yield high concentrations of nano fibrils.
5) Enzymatic treatment did not resulted in higher fibrillation grades. There appeared a certain fibrillation but not at a satisfying level. Although in one of the tests related to

enzymatic treatment procedures better GS values were found, the yield is not high. The suggestion that the used enzyme would be inactivated during ultrasonic treatment did not occur.

6) Alkenyl succinic anhydride (ASA) and carboxymethyl cellulose (CMC) additives that should influence the fibrillation did show some effects but were not very effective. ASA as a widely used sizing agent resulted in a slightly better fibrillation grade considering all measured test values compared to CMC. Carboxymethyl cellulose treated cotton linters sample presented in figure 28 shows almost no fibrillation. As a conclusion applying CMC and ASA in order to produce nano fibrils are not advisable, moreover especially ASA is not very convenient to use and needs intense post-washing and purification procedures to concentrate the treated clean cellulose fibers.
7) An astonishing result was achieved by using a simple ultrasonic bath because it was an experiment without any chemical additives. The GS, TS and WRV values were on very good levels and also SEM/ESEM-micrographs confirm an effective influence on cotton linters cellulose. It can be said that using ultrasonic to break up the cellulose in loose fibers and MK/MKO mill as an additional treatment yielded in a relatively high concentration of nano fibrillated fibers. As already mentioned this procedure required no chemicals and as consequence no purification steps are necessary.
8) The method of pre-swelling cotton linters samples in deionized water was done to compare also a very minimalistic pre-treatment with other treatments. The results are that the major parameters which are the indicator for fibrillation grade are good. They are comparable to those resulted by ultrasonic treatment. Greatest disadvantage is the long time period for swelling before milling. Ultrasonic treatment shortens this time up from 72h swelling time in pure water up to 1 h in the used ultrasonic bath. Moreover, one has to mention that the used ultrasonic device had not much power (80W according to manual).
9) One of the most auspicious experiments which were done with DMAc/LiCl used as a 9% solution. The fibrillation which occurred was astonishing. The cellulose which is regenerated by putting the cellulose solution into a defined volume of deionized water showed a jellylike behavior like cellulose fibrillated in a high pressure homogenizer. The advantage is the enormous yield of fibrillated or rather regenerated cellulose structures (figure 39). The complex dissolution procedure and the post-purification steps including centrifuge treatment are costly in terms of time and are negative aspects. But overall the very good GS, TS and furthermore the satisfying WRV values which indicate high fibrillation grade show the big potential of this method. This method was also done with several other cellulose types as described in 3.7. but treatment of the standard sample

cotton linters and Rosenthal kraft softwood (sulfate pulping) showed the most satisfying results.

So the prospects for further researches could be an improvement of ultrasonic treatment and especially putting a bigger focus on “in-situ”-precipitation of cellulose in water out of a solution in different cellulose solvent systems like DMAc/LiCl or ionic liquids, as well as the in-situ regeneration of cellulose derivatives in high shear fields. Furthermore a possible recycling of DMAc/LiCl could be investigated. Besides long-time pre-swelling of cellulose in deionized water these two mentioned methods bring along some interesting production procedures for nano fibrillated cellulose.

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