J Mater Sci (2008) 43:1311–1317
DOI 10.1007/s10853-007-2284-4

Quantification of dislocations in hemp fibers using acid hydrolysis and fiber segment length distributions

Lisbeth G. Thygesen

Received: 25 July 2007 / Accepted: 31 October 2007 / Published online: 12 December 2007

Abstract Natural fibers such as flax or hemp may be used in composite materials. However, their use for this purpose is hampered by the large natural variation in tensile strength and other quality parameters. The first step in managing these variations is to develop methods for fast and reliable determination of relevant parameters. One quality parameter of the fibers is the amount of structural distortions known as dislocations or kink bands. Here, a method developed for the quantification of dislocations in pulp fibers was adapted and tested successfully for hemp yarn segments. The method is based on acid hydrolysis and subsequent determination of the fiber segment length distribution. The premise of the method is that acid hydrolysis causes fibers to break in the dislocations rather than in other places. By use of polarized light microscopy and image analysis it was found that the premise was correct, and furthermore results showed that fibers broke more often in large dislocations than in small ones. However, it was also found that the hemp fiber segments did not break in all dislocations, and strict standardization of the procedure for acid hydrolysis is therefore necessary if results from different batches of fibers are to be compared.

Introduction

Natural fibers may be used in composite materials, but the large variation in the quality of these fibers poses a problem. If relevant quality parameters could be measured fast and reliable it could help manage this difficulty. In this article a way of measuring the amount of structural distortions within the cell walls is tested on hemp fibers. The method was originally developed for pulp fibers.

Plant fiber walls contain cellulose microfibrils that in bast fibers normally run nearly parallel to the longitudinal direction of the fiber. However, at some locations the angle between the longitudinal direction of the fiber and that of the microfibrils differ from the angle found in the bulk fiber wall. These local misalignments are known as dislocations, kink bands, microcompressions or slip planes. The origin, occurrence, and effects of these defects were reviewed by Nyholm et al. [1]. The exact structural characteristics of these regions remain unknown. In accordance with earlier publications on natural fibers, the word 'dislocations' is here used as a broad term for regions with structural deviations from the bulk cell wall, even though the word 'dislocations' has a more narrow definition within metals and ceramics. Dislocations are found both in the living plant and in processed fibers, and it is known that compression stress applied in the longitudinal direction of the fibers increases the amount of dislocations [2]. The relationship between fiber performance and the occurrence of dislocations has been studied, both for fibers relevant for fiber composites [3–8] and for pulp and paper [1, 2, 9], but until recently such studies were hampered by the lack of reliable methods for the quantification of the amount of dislocations. However, Ander and co-workers have developed a method for the quantification of dislocations in pulp fibers [10–13]. The method is based on acid hydrolysis of the fibers and subsequent mild stirring, a treatment that causes the fibers to break into shorter segments. Afterwards the length distribution of the segments is found automatically using either the FiberMaster instrument [14] or the Kajaani instrument. Both these

L. G. Thygesen (✉)
Forest & landscape, University of Copenhagen, Rolighedsvej 23, DK-1958, Frederiksberg C, Denmark
e-mail: lgt@life.ku.dk

instruments are used routinely within the pulp and paper industry in Scandinavia. The premise of the method is that the acid hydrolysis will weaken the fibers in the dislocations, so that they will brake primarily at these locations during the subsequent stirring. This implies that a strong fiber shortening should correspond to a high occurrence of dislocations. Ander and Daniel [10, 12] and Ander et al. [13] presented micrographs showing fibers breaking in the dislocations, but strictly speaking no quantitative data is presented that unequivocally proofs that fiber breakage takes place in the dislocations more often than in other places.

In the present study it is demonstrated that the method of Ander et al. [13] may be applied also to hemp fibers, and, perhaps more importantly, the premise of the method is checked by use of polarized light microscopy and image analysis according to the method of Thygesen and Ander [15].

Materials and methods

Hemp yarn

As the purpose of this study merely was to adapt, demonstrate, and test the method of Ander et al. [13] on hemp, it was decided to use hemp yarn as an easy accessible source of single hemp fibers instead of carefully isolating fibers from hemp stems by hand using precision tweezers. The product name of the yarn was "Imperial" and it was purchased from Linificio e Canapificio Nazionale (Italy) by Bo Madsen, Risø National Laboratory, Roskilde, Denmark.

Since the FiberMaster instrument used could only handle fiber segments up to 7 mm length, the hemp yarn was cut into 5 mm segments prior to the acid hydrolysis to insure that this would be the maximum length occurring in the samples. This implies that the samples that are here denoted reference samples do not correspond to virgin hemp fibers with regard to length distribution, but to fiber segments that were up to 5 mm long.

Acid hydrolysis

For each sample 150 mg of 5-mm long hemp yarn segments were put in demineralized water for 24 h at room temperature. The water was drained off using a Munktell 1002 filter, the fiber segments were transferred to 40 mL 1 N HCl in a 100 mL container, and the container was placed at 80°C for 24 h. The mixture was finally stirred at 500 rpm for 1 h at room temperature, after which the acid was washed off using a Munktell 1002 filter and demineralized water. Reference samples were produced in the same way, except that demineralized water was used instead of HCl. A total of five samples of each kind were included in this study.

The procedure used here is more severe than the standard method developed for spruce kraft pulp fibers [13].

FiberMaster analysis

The fiber segment lengths were determined for two HCl-treated samples and two reference samples at STFI Packforsk, Stockholm, Sweden by use of a FiberMaster instrument [14]. This instrument takes micrographs of fiber segments in a suspension and uses image analysis to calculate the length and width of each fiber segment. With the FiberMaster standard software only objects of lengths more than four times the width are accepted as fiber segments and included in the output data. A so-called form-factor is also calculated, a factor which describes how straight or bend the fiber segment is [14]. The form factor is calculated from the length of the fiber and from the smallest possible rectangle that can be drawn around the fiber in the captured image. The form factor equals the diagonal of this rectangle divided by the length of the fiber. Only fiber segments with acceptable form factors are included.

In this article results from the FiberMaster are presented as pooled results from the two HCL-treated samples and pooled results from the two reference samples. Each sample is divided into two subsamples during analysis. Measurements for one subsample from one of the reference samples were unsuccessful, i.e., the results from the reference fiber segments are based on three subsamples, not four. The FiberMaster instrument used here can measure fiber segment up to 7 mm length, i.e., measurements on virgin hemp fibers would not have been possible.

Kajaani analysis

The fiber segment lengths were determined for two HCl-treated samples and two reference samples at M-Real, Örnsköldsvik, Sweden using a Kajaani FS-200 instrument. In this instrument fiber segments are led through a narrow, transparent cylinder, and the length of a segment passing through is calculated from the flow rate and the time it blocks out a light beam. Results from the Kajaani instrument are here presented as mean values that for each sample type (reference and HCl treatment) and are based on two samples. The principle of measurement used by the Kajaani instrument does not imply an upper limit to the measurable fiber segment length, i.e., measurements on virgin hemp fibers are also possible.

Polarized light microscopy and image analysis

A Leitz light microscope (orthoplan, 30.5.16.8 FSA/GW-/402a LILOM) equipped with two polarization plates, a 40x objective for light field microscopy, a rotating stage and a variable 100 W Hg light source was used. The camera was a Nikon DN100 digital net camera. Images were captured as 1,280 × 960 RGB bitmap files. From an image of an object of known size (a 1/100 mm scale) it was derived that one pixel corresponded to approximately 0.27 × 0.27 μm.

All image processing was carried out using Gimp (version 1.2) for Linux. Matlab version 7.0 with the Image Processing Toolbox was used for image analysis according to the method of Thygesen and Ander [15]. This method implies that two images are taken per fiber segment under crossed polars: one image in which the fiber edges are made to light up, and one in which only the dislocations are seen. From each of these two images a mask is found representing the fiber and the dislocations, respectively. From these two masks a number of values characterizing the amount, the sizes, and the distribution of the dislocations may be calculated (for details please refer to [15]).

The method of Thygesen and Ander [15] was applied to study the dislocations still present in the segments after the reference treatment or the acid hydrolysis, i.e., the lengths of the fiber segments were not used in this test. The Thygesen and Ander method [15] is not applicable if results for individual fiber segments are the objective, however, for groups of 20–30 fiber segments, the mean values are not significantly different from the correct means [15]. In the present study the method was applied to two sets of fiber segments and for each set approximately 100 fiber segments were assessed. The two sets of fiber segments were prepared in the same way as the fiber suspensions used for the FiberMaster and the Kajaani analysis.

Results and discussion

Polarized light microscopy and image analysis

Polarized light microscopy and image analysis were used to test the Ander method's premise that fiber segments predominantly break in the dislocations during the acid hydrolysis and/or the subsequent stirring. Figure 1 shows the distributions of the sizes of the relative dislocation areas for the two sets of fiber segments. For a given fiber segment the relative dislocation area is calculated as the area of the dislocations relative to the total fiber areas as seen in a microscope using transmitted light and crossed polars. It is thus a parameter reflecting a two-dimensional representation of a three-dimensional reality. Figure 1 shows that the relative dislocation area was lower for the

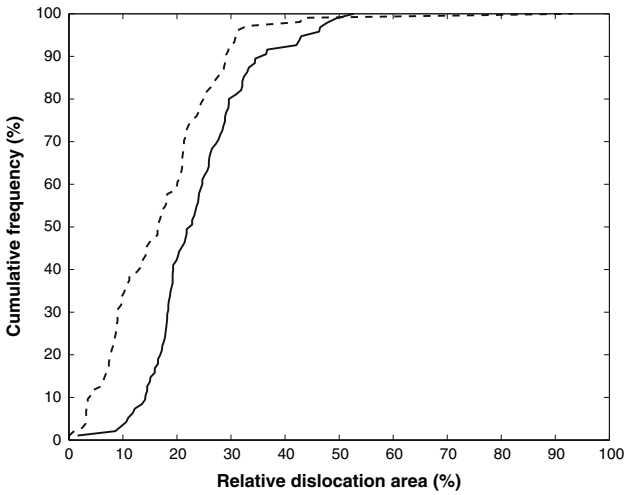

Fig. 1 Cumulative frequency of the relative dislocation areas for 97 fiber segments from the reference treatment (solid line) and 107 fiber segments from the HCl treatment (dashed line). The relative dislocation area is for each fiber segment the total area of the dislocations in per cent of the total fiber segment area as seen using polarized light microscopy. HCl treatment reduces the mean relative dislocation area

fiber segments subjected to acid hydrolysis. A one-sided t-test showed that the mean relative dislocation area was significantly lower for these fiber segments (at the 1 ‰ level). This means that acid hydrolysis *does* reduce the amount of dislocations in the fiber segments, which in turn implies that fiber breakage predominantly takes place in dislocations, and that (part of) a cleaved dislocation is lost during this process. If the dislocations were simply cut with no loss of material the relative dislocation area would in theory remain unaffected. Ander et al. [13] found that the HCl treatment released sugars from spruce fiber segments. The xylose release was 1.9% and 1.7% from mill and laboratory kraft pulp spruce fibers, respectively. Arabinose, galactose, glucose, and mannose were also released, but to a smaller extent. Even though this result suggests that hemicellulose is degraded more heavily than cellulose, their finding supports the present result that dislocations that are cleaved are reduced or disappear from the fiber segments. Another possible explanation for the disappearing dislocations could be that the remaining bits of misaligned microfibrils straighten out when the dislocation is cleaved due to a release of stress when the microfibrils become free to move at one end.

Figure 1 also shows that even though acid hydrolysis causes the fibers to break in dislocations, they do not break in all dislocations as the HCl-treated fibers still contain dislocations. Since breakage in all dislocations is not achieved, standardization of the Ander method is very important in order to allow comparison between different batches of fibers.

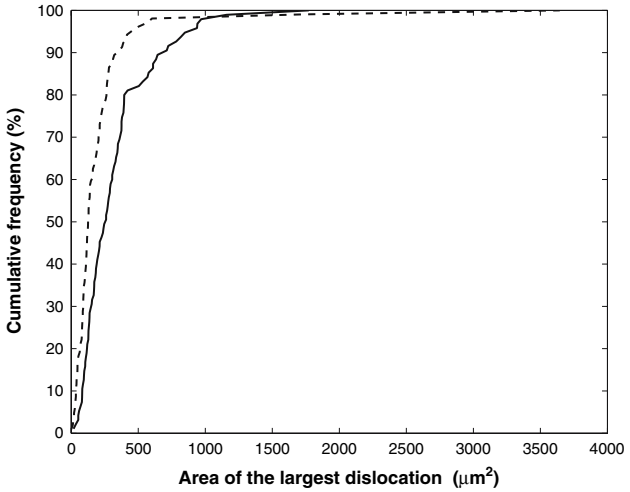

Fig. 2 Cumulative frequency of the absolute sizes (areas) of the largest dislocations found for each fiber segment using polarized light microscopy. The figure includes the largest area found for each of 97 fiber segments from the reference treatment (solid line) and for each of 107 fiber segments from the HCl treatment (dashed line). HCl treatment reduces the mean size of the largest dislocation found in each fiber segment

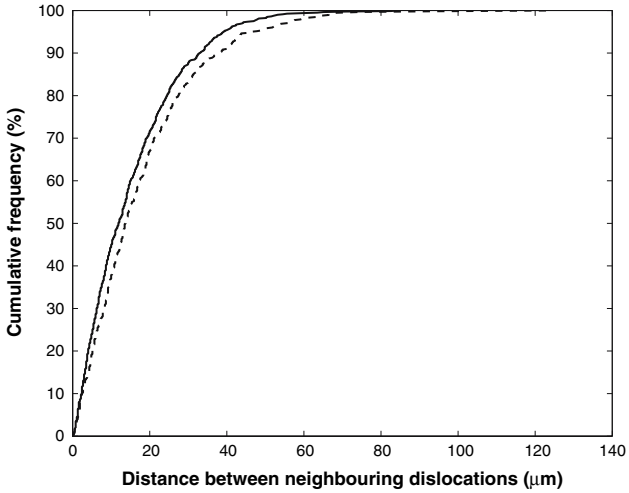

Fig. 3 Cumulative frequency of the approximate distances between neighboring dislocations found in 97 fiber segments from the reference treatment (798 distances, solid line) and in 107 fiber segments from the HCl treatment (576 distances, dashed line). HCl treatment increases the mean distance between neighboring dislocations

Figure 2 concerns the absolute size of the largest dislocation found in each fiber segment. A significant difference was found (at the 5% level, one-sided t-test) between the two sample types also for this parameter. The largest dislocations in the reference fiber segments were larger than the largest dislocations found in the HCl-treated fiber segments. This implies that fiber breakage more often takes place in large dislocations than in small dislocations.

Figure 3 shows for the two sets of fiber segments the approximate distances between dislocations in the longitudinal direction. The distances are only approximate as they are found from a projection of the dislocations on to the lower edge of the images, i.e., this procedure presumes that the longitudinal direction of the fiber is parallel to the lower image edge. Although this orientation of the fibers was aimed for, it was not achieved in all images and this implies that some of the distances included in the figure are too short. Only distances between neighboring dislocations seen in the images are included, i.e., distances from a dislocation to the left or right image edge are excluded. The mean distance between neighboring dislocations was found to be significantly longer for the HCl-treated fiber segments (at the 1 ‰ level, one-sided t-test).

The results of the test show that the premise of the Ander method holds. The fiber segments *do* tend to break in dislocations, and furthermore, breakage does more often take place in large dislocations than in small ones. However, even though the mean distance between neighboring dislocations is longer after acid hydrolysis and stirring, HCl-treated fiber segments still contain many dislocations. This implies that strict standardization of the method is critical if comparable results are to be obtained.

Fiber segment length distributions from the Kajaani instrument and from the FiberMaster

The results from the Kajaani instrument are given in Fig. 4(a), which shows the length weighted fiber segment length distribution for the reference fibers and for fibers subjected to acid hydrolysis. A clear difference is seen between the two treatments, HCl treatment results in strong fiber shortening. The figure shows that quantification of dislocations is possible for hemp fibers using acid hydrolysis and the Kajaani instrument.

The mean length weighted fiber segment length was 0.53 and 0.49 mm for the two HCl-treated samples, 3.57 and 3.58 mm for the two reference samples. The corresponding mean lengths according to an arithmetic mean were 0.30 and 0.29 mm, respectively 1.35 and 1.43 mm. These results show that both sample types contain more short segments than long segments, but that the reference samples contain more long segments relative to the amount of short segments than the HCl-treated samples do.

The reference samples show a dominant peak around a fiber segment length of 5 mm, corresponding to the length of the hemp yarn segments used for this study. Fibers isolated from hemp stems would have a different length distribution, most likely with a peak between 10 and 20 mm, provided that no fiber shortening took place during the defibration step.

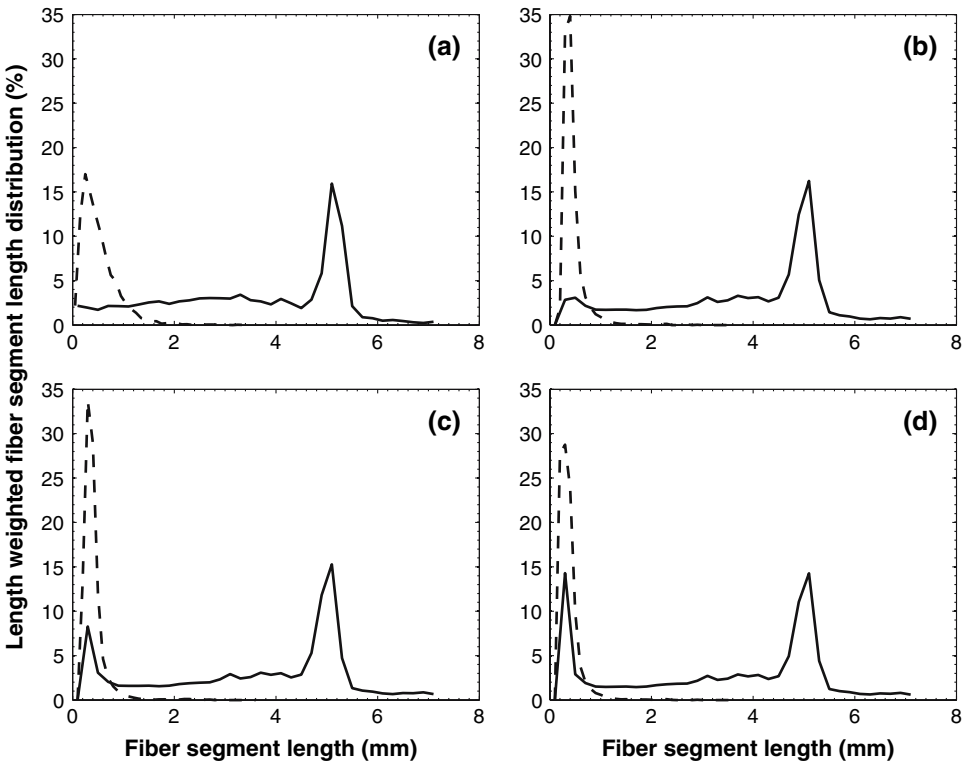

Fig. 4 Length weighted fiber segment length distribution according to the Kajaani instrument (**a**) and the FiberMaster instrument (**b**, **c,** and **d**). FiberMaster results are given in three versions: (**b**) is the standard fiber data from the instrument, (**c**) is for all objects (fibers and particles) with a length which is at least twice the width, and (**d**) is based on all objects. Solid lines correspond to reference samples, dashed lines correspond to HCl-treated samples. For reference samples length classes 0–0.2, 0.2–0.4, …, 7.0–7.2 mm were used, for HCl-treated samples classes 0–0.1, 0.1–0.2, …, 3.5–3.6 mm were used. Acid hydrolysis is seen to cause strong fiber shortening

Contrary to the Kajaani instrument, the FiberMaster sorts registered objects into fibers and particles and discards data from particles. Fibers are defined as objects at least 80 μm long (Elisabeth Björk, STFI-Packforsk, personal communication 2003), and at least four times as long as they are wide [14]. The form factor of a fiber candidate is also calculated [14], and if this calculation results in a physically impossible form, the data for the object is discarded. Registered objects may be either parts of fibers or other objects present in the suspension. Table 1 shows the number of objects registered in the hemp samples analyzed, sorted into fibers and particles, and into length classes. The table illustrates the power of the FiberMaster when it comes to obtaining representative data for natural fibers: this FiberMaster study includes data for not less than 240.146 objects totally, of which 51.046 objects were accepted as fiber segments.

It was presumed that some fiber segments risked being discarded as particles in the present study where fibers were broken into short segments prior to the FiberMaster analysis. Figure 5 shows micrographs of a reference sample and of a HCl-treated sample, and it shows that fiber segments shorter than 80 μm occur for the HCl-treated sample. Segments may also be seen that appear to have a length to width ratio lower than 4:1. For the reference sample small fiber segments (<80 μm) may be found. These segments do not appear to be fibers cleaved in dislocations, but rather they seem to be irregular fragments torn from the fibers. Segments longer than 80 μm and of a length to width ratio lower than 4:1 do not appear to be present in the reference sample. Figure 5 indicates that fragments are wrongly discarded by the automatic FiberMaster fiber/particle criteria. If compared to the data in Table 1 it may be seen that for the reference samples 86% of the objects were discarded because they were shorter than 80 μm, while the corresponding number for the HCl-treated samples was 73%. This means that relatively more

Table 1 Overview of the number of objects registered by the FiberMaster, sorted into classes by the FiberMaster standard software

	Object length	Reference		HCl treated	
		No.	%	No.	%
Fibers	0.08–0.5 mm	7455	7	35432	27
	0.5–1.5 mm	2105	2	1035	1
	1.5–3.0 mm	1555	1	30	0
	3.0–4.5 mm	1309	1	1	0
	>4.5 mm	2124	2	0	0
	All fibers	**14548**	**14**	**36498**	**27**
Particles	<20 μm	17669	16	10525	8
	20–50 μm	57361	53	51198	39
	50–80 μm	16797	16	31086	23
	Others	956	1	3508	3
	All particles	**92783**	**86**	**96317**	**73**

Results have been pooled for each sample type

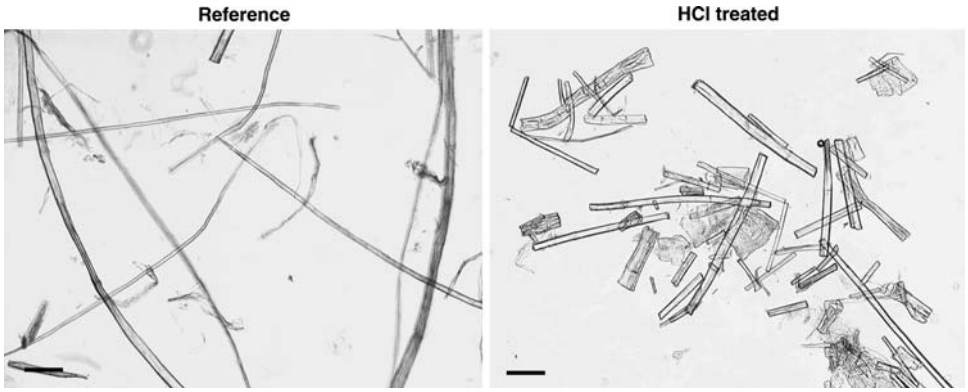

Fig. 5 Micrographs of reference fiber segments and HCL-treated fiber segments. The length of the scale bar is 100 μm in both images

objects were discarded from the reference samples due to the length criterion than from the HCl-treated samples. According to micrographs such as those in Fig. 5, however, the characteristics of the discarded objects differ between the two sample types: for the HCl-treated samples the discarded objects include fiber segments cleaved in dislocations, this do not appear to be the case for the reference samples.

In order to investigate the possible effect of the wrongly discarded fiber segments, The FiberMaster standard data were compared to values based on raw output data. The weighted fiber segment length distributions were calculated for three different subsets:

Set I: Objects identified as fibers by the FiberMaster standard criteria, i.e., objects longer than 80 μm (longest dimension), at least four times as long as they are wide, and with an acceptable from factor. The weighted length distribution for this set is shown in Fig. 4(b).

Set II: Objects that are at least twice as long as they are wide. No minimum length was applied and no form factor calculated. The weighted length distribution for this set is shown in Fig. 4(c).

Set III: All objects. The weighted length distribution for this set is shown in Fig. 4(d).

Figure 4(b) shows that the results for Set I agree qualitatively with the Kajaani results given in Figure 4(a): HCl treatment gives strong fiber shortening. However, some differences are seen when comparing Fig. 4(a and b) more in detail. For Set I the distributions for both sample types descend to zero for fiber lengths below 50–100 μm, this is simply the effect of the 80 μm limit. The peak around 0.5 mm for the HCl-treated samples is higher and more narrow than the corresponding peak for the Kajaani data. The reason for this difference is not clear, but could possibly be a result of less precise fiber length measurements from the Kajaani instrument.

Figure 4(c and d) show that it is only the length distribution for the reference samples that is affected markedly if the fiber/particle criteria are altered. This is the opposite of what was expected from images like those in Fig. 5. When the criteria are relaxed a new peak emerges for the reference fibers for the same segment length as the single peak of the HCl-treated fibers. On one hand it is plausible that if short segments are due to fiber cleavage at weak points, the stirring that the reference samples were subjected to could have resulted in breakage into segments of about the same length as those resulting from the more severe HCl treatment, albeit to a smaller extent. On the other hand, micrographs like those in Fig. 5 did not indicate that segments similar to those from the HCl treatment were created by the reference treatment.

In order to better understand the FiberMaster results in Fig. 4(b–d) the raw data were sorted both according to length and according to length:width ratio. This is shown in Fig. 6 according to which both sample types contain a

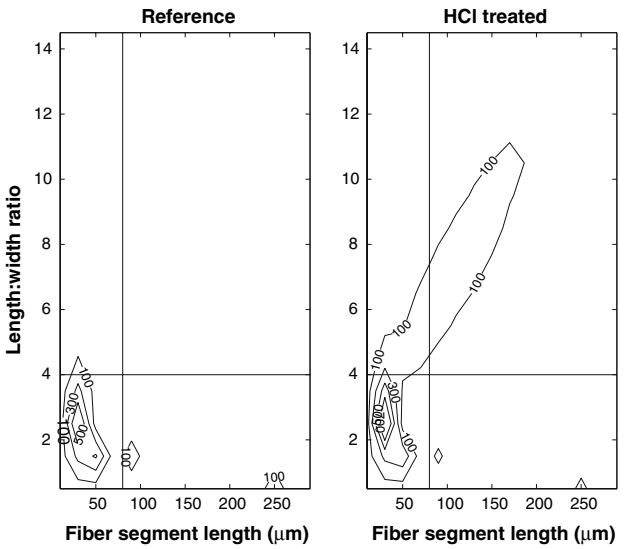

Fig. 6 Contour plots based on raw FiberMaster data. The plots show data from the two sample types sorted into classes according to both fiber segment lengths (0–20 μm, 20–40 μm, etc.) and length:width ratios (<1, 1–2, 2–3, etc.). The frequency is given as the total segment length in mm, i.e., it is length weighted but not normalized. The vertical lines show the 80 μm length limit and the horizontal lines show the length:width ratio limit of 4:1

population of short particles (<80 μm) with low length:width ratios (<4). The two peaks are so alike that the most plausible explanation is that they both reflect the same phenomenon, possibly air bubbles. However, each sample is de-aerated at start up of the FiberMaster analysis (Elisabeth Björk, STFI-Packforsk, personal communication 2005), which ought to remove or at least reduce the amount of air bubbles present during analysis. Figure 6 also shows that only the HCl samples contain fiber segments just above both the two limits (<80 μm and 4:1), as also indicated by Table 1. The effect of this difference is that inclusion of the small objects into the data significantly alters the weighted length distribution of the reference samples, while for the HCl-treated samples the population of small objects are absorbed into the already existing peak in the length distribution. Thus, by including fiber segments shorter than 80 μm and with length:width ratios lower than 4, unwanted objects affect the weighted length distribution of the reference samples negatively, while the desired short fiber segments of the HCl-treated samples do not markedly alter the weighted length distribution of these samples. This means that the FiberMaster fiber/particle criteria appear to be adequate also for this type of analysis, a result that was unexpected given micrographs such as those in Fig. 5.

The present study concerned hemp fibers, which both in size and in composition are different from pulp fibers. Nevertheless, it was possible to adapt the Ander method to these fibers, and it is therefore likely that the method could be adapted to other types of natural fibers also, for example flax and nettle.

Conclusions

The method of Ander et al. [13] was tested for quantification of the amount of dislocations in hemp fiber segments. It was found that the method also applies to hemp, given a few modifications in the preparation of samples. The premise of the method is that acid hydrolysis causes fiber breakage in dislocations, and this premise was proven to be correct. If the purpose of the analysis is to compare the length distribution after acid hydrolysis with the length distribution of virgin hemp fibers, the Kajaani instrument is a better choice than the FiberMaster as the measuring principle of the Kajaani instrument does not impose an upper limit on the fiber length.

Acknowledgements The author wishes to thank Paul Ander for helpful discussions regarding his method and Elisabeth Björk for access to the raw data output from the FiberMaster in addition to the standard result files. Ann Marklund is thanked for the Kajaani measurements. Ulla Gjøl Jacobsen's laboratory assistance and Bo Madsen's donation of the hemp yarn is much appreciated. This study was initiated within the project 'High performance hemp fibers and improved fiber network for composites' financed by the Danish Research Council. Most of the work reported here was carried out at the Department of Civil Engineering, Technical University of Denmark.

References

1. Nyholm K, Ander P, Bardage S, Daniel G (2001) Nord Pulp Pap Res J 4:374
2. Terziev N, Daniel G, Marklund A (2005) Holzforschung 59:163
3. Davies GC, Bruce DM (1998) Tex Res J 68:623
4. Hughes M, Sèbe G, Hague J, Hill C, Spear M, Mott L (2000) Compos Interface 7:13
5. Bos HL, Van Den Oever MJA, Peters OCJJ (2002) J Mater Sci 37:1683. doi:10.1023/A:1014925621252
6. Lamy B, Pomel C (2002) J Mater Sci Lett 21:1211
7. Baley C (2004) J Mater Sci 39:331. doi:10.1023/B:JMSC.0000007768.63055.ae
8. Thygesen LG, Eder M, Burgert I (2007) J Mater Sci 42:558. doi:10.1007/s10853-006-1113-5
9. Ander P, Marklund A, WURC report no. 4 (2003) ISBN 91-576-6647-4, available from P. Ander, Department of Wood Sciences, Swedish University of Agricultural Sciences, P.O. Box 7008, SE-75007 Uppsala, Sweden
10. Ander P, Daniel G (2004) In: Stanzl-Tschegg SE, Gindl M, Sinn G (eds) Proceedings of the 2nd international symposium on wood machining, Vienna, July 2004, BOKU, Vienna, p 247
11. Ander P, Daniel G (2004) In: Proceedings of the 8th European workshop on lignocellulosis and pulp, Riga, August 2004, Latvian Institute of Wood Chemistry, p 49
12. Ander P, Daniel G (2004) In: Schmitt U, Ander P, Barnett J, Emons AM, Jeronimidis G, Saranpää P, Tschegg S (eds) Wood fiber cell walls: methods to study their formation, structure and properties, SLU, Uppsala, p 203
13. Ander P, Daniel G, Garcia-Lindgren C, Marklund A (2005) Nord Pulp Pap Res J 20:84
14. Karlsson H, Franson P-I, Mohlin U-B (1999) In: Proceedings from the 6th International conference on new available technologies, Stockholm, 1999, SPCI, Stockholm, p 367
15. Thygesen LG, Ander P (2005) Nord Pulp Pap Res J 20:64