UNIVERSIDADE FEDERAL DE LAVRAS

ALISSON FARLEY SOARES DURÃES

EVALUATION OF DISLOCATIONS IN CELLULOSE PULPS SUBMITTED TO PRE-TREATMENTS

LAVRAS-MG

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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência e Tecnologia da Madeira, para a obtenção do título de Mestre

Dr. Gustavo Henrique Denzin Tonoli

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APROVADA em 30 de Julho de 2018.
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2018

“O que eu faço é uma gota no meio de um oceano. Mas sem ela, o oceano não seria o mesmo.”

Madre Teresa de Calcutá

Dedico a Deus por renovar minhas forças todos os dias,
à minha família que mesmo de longe sempre me dá motivos para continuar
e aos amigos que compartilhou de perto o meu crescimento, à vocês
Dedico.

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RESUMO

Dislocations são designações dadas as deformações permanentes da parede celular da fibra celulósica. As fibras da madeira podem ter a resistência comprometida com as *dislocations* na parede celular. A aplicação de tratamentos químicos, enzimáticos e a desfibrilação mecânica podem contribuir com a redução da resistência da fibra, atuando no aumento das *dislocations* e como facilitador para a desconstrução da parede celular da fibra vegetal. Nesse sentindo o trabalho avaliou o efeito de pré-tratamentos químicos e enzimáticos em fibras celulósicas branqueadas e não branqueadas na formação de *dislocations* na parede celular das fibras, além de avaliar as *dislocations* em fibras após pré-tratamento enzimático e desfibriladas mecanicamente. A polpa foi submetida ao pre-tratamento químico com hidróxido de sódio nas concentrações de 5% por 2 h e 10% por 1 h e 2 h e com peróxido de hidrogênio com solução alcalina. Para o pré-tratamento enzimático foram utilizadas as enzimas A, B e C do tipo endoglucanase e a enzima D correspondente a mistura da endoglucanase com exoglucanase. Para as fibras que foram desfibriladas mecanicamente, foi utilizado o pré-tratamento com enzima B e analisadas as *dislocations* antes (0 passagens) e após a desfibrilação mecânica (5, 10, 15, 20, 25 e 30 passagens). As análises de caraterização das *dislocations* nas paredes celulares das fibras foram realizadas por meio de microscopia ótica com luz polarizada, analisando-se o índice de *dislocations*, ângulo das *dislocations* e curvatura das fibras. Os resultados mostraram aumento do índice de *dislocations* para as fibras branqueadas e não branqueadas após os pré-tratamentos químicos, além de redução no ângulo das *dislocations*. A retilineidade das fibras após os pré-tratamentos foi alterada em relação ao tratamento controle, gerando fibras mais encurvadas. Para o pré-tratamento enzimático em fibras branqueadas, o índice de *dislocations* apresentou variação em função da enzima utilizada, gerando redução de *dislocations* para enzima C e aumento de *dislocations* para a enzima B, enquanto que para as fibras não branqueadas a ação enzimática na parede celular das fibras gerou baixo índice de *dislocations*, bem como, pouca diferença no ângulo das *dislocations* observadas. Para as fibras branqueadas pré-tratadas com enzimas e sem pré-tratamento processadas mecanicamente, a enzima B não causou redução no comprimento das fibras, mas permitiu aumento significativo das *dislocations* com o aumento das passagens pelo desfibrilador. O aumento das *dislocations* ocorreu até 20 passagens no desfibrilador mecânico, quando a fibra atingiu o índice máximo de *dislocations* em relação a todas as condições e passagens. O ângulo das *dislocations* foi reduzido com a ação enzimática (B), sendo estatisticamente diferente do controle para todas as passagens analisadas, assim como o índice de curvatura das fibras, que em função da quebra das fibras durante as passagens, reduziu a ponto de não se observar curvatura.
Os resultados mostram que a aplicação de pré-tratamentos químico, enzimático e mecânico nas fibras podem afetar a integridade da fibra, criando dislocations, além de gerar modificações nos ângulos das dislocations.

Palavras-chave: *Slip plane*. Desfibrilação. Celulose microfibrilada. Nanofibras celulósicas.

ABSTRACT

Dislocations are designations given to the permanent deformations of cell wall cellulosic fiber. Wood fibers may have its resistance compromised with dislocations on the cell wall. The application of chemical and enzymatic treatments and mechanical defibrillation can contribute to the reduction of fiber resistance, acting in the increase of dislocations and as a facilitator for the deconstruction of the cellular wall of the vegetal fiber. In this sense the work evaluated the effect of chemical and enzymatic pre-treatments on bleached and unbleached cellulosic fibers in the formation of dislocations in the cell wall of the fibers, besides evaluating the dislocations in fibers after enzymatic pre-treatment and mechanically defibrillated. The pulp was subjected to chemical pre-treatment with sodium hydroxide at concentrations of 5% for 2 h and 10% for 1 h and 2 h and with hydrogen peroxide with alkaline solution. For the enzymatic pre-treatment, the enzymes A, B and C of the endoglucanase type and the enzyme D corresponding to the mixture of endoglucanase with exoglucanase were used. For fibers that were mechanically defibrillated, a pre-treatment with enzyme B was used and the dislocations were analyzed before (0 passages) and after mechanical defibrillation (5, 10, 15, 20, 25 and 30 passages). Characterization analyzes of the dislocations on the cell walls of the fibers were carried out by optical microscopy with polarized light, analyzing the index of dislocations, angle of dislocations and the curvature of the fibers. The results showed an increase on the dislocations index for bleached and unbleached fibers after chemical pre-treatments, as well as a reduction in the angle of dislocations. The straightness of the fibers after the pre-treatments was altered in relation to the control treatment, generating more curved fibers. For the enzymatic pre-treatment in bleached fibers, the index of dislocations presented variation as a function of the enzyme used, generating reduction of dislocations for enzyme C and an increase of dislocations for the enzyme B, whereas for the unbleached fibers the enzymatic action on the wall cellular fibers generated a low index of dislocations, as well as, little difference in the angle of observed dislocations. For bleached fibers pre-treated with enzyme and without pre-treatment mechanically processed , enzyme B did not cause reduction in fiber length but allowed a significant increase in dislocations with an increase in the defibrillator passages. The increase of the dislocations occurred up to 20 passages in the mechanical defibrillator, when the fiber reached the maximum index of dislocations in relation to all conditions and passages. The angle of the dislocations was reduced with the enzymatic action (B), being statistically different from the control for all passages analyzed, as well as the fiber curvature index, which, due to the fiber breaking during the passages, reduced to the point of not observing curvature . The results show that the application of chemical, enzymatic and mechanical pre-treatments in the fibers can affect the integrity of the fiber, creating dislocations, besides generating modifications in the angles of the dislocations.

Keywords: Slip plane. Defibrillation. Microfibrillated cellulose. Cellulosic nanofibres.

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LISTA DE ABREVIATURAS

BF	Bleached fibers
CRD	Completely Randomized Design
HD	Height of the *dislocations*
ID	Index of *dislocations*
LF	Length of the fiber
mm	Milimetro
ND	Número de *dislocations*
Rpm	Rotação por minuto
Ton	Tonelada
UF	Unbleached Fibers

SUMÁRIO

PRIMEIRA PARTE

1. INTRODUÇÃO

As fibras vegetais são materiais renováveis, podendo ser aplicadas em diversos setores industriais. As fibras são consideradas lignocelulósicas e constituem a formação da madeira sendo composta por celulose, lignina e hemicelulose. As fibras são responsáveis por conferir rigidez à madeira.

A parede celular das fibras pode ser comprometida quando a madeira é submetida a esforços de compressão paralela às fibras ocasionados pela interferência do homem, como a derrubada da árvore e processamento da madeira ou por processos naturais gerados por fatores ambientais e pelas características da própria árvore. Essas modificações provocam deformações na parede celular da fibra celulósica, e são denomindas *dislocations,* também conhecidas como *slip planes* ou *kinks* (NYHOLM, 2001).

As *dislocations* na extensão da fibra são consideradas pontos de enfraquecimento (HIDAYAT et al, 2012) e influenciam diretamente na resistência mecânica da fibra. Nesse sentido, o processo de desfibrilação mecânica, que é utilizado para desconstruir a parede celular das fibras e obter as micro/nanofibrilas, pode ocasionar as *dislocations* em função dos impactos das fibras no desfirilador, ou podem romper as fibras nas *dislocations* já presentes na parede celular das fibras. Assim a desfibrilação pode ser favorecida quando houver *dislocations* na sua constituição, ou seja, as *dislocations* podem atuar como facilitador para a quebra da fibra vegetal durante o processo de produção de nanofibrilas.

Existem diversos métodos de produção de nanofibrilas, porém as práticas mais promissoras são aquelas que fazem uso de processo mecânico que gera o cisalhamento da polpa celulósica. Entretanto, em virtude da inviabilidade econômica proporcionada pelo gasto energético, a sua aplicação em larga escala ainda não é muito usual. Assim, encontrar soluções que proporcionem redução desse custo de desfibrilação é fundamental para alavancar pesquisas sobre geração de novos produtos a partir de nanofibrilas.

Pré-tratamentos anteriores à desfibrilação mecânica podem ser feitos com o intuito de otimizar o processo e minimizar os gastos energéticos. Dentre os pré-tratamentos tem-se aqueles realizados mecanicamente, ou então fazendo uso de alternativas químicas ou enzimáticas (ABRAHAM et al., 2011). Os pré-tratamentos químicos e enzimáticos promovem modificações na estrutura das fibras celulósicas gerando quebra e formação de novas ligações. As regiões fragmentadas de *dislocations* iniciadas após o pré-tratamento podem contribuir com a otimização do processo de desconstrução da fibra durante a desfibrilação no tratamento mecânico.

A relação das *dislocations* com a fragmentação da fibra vegetal ainda é pouco conhecida e explorada, principalmente quando aplicados pré-tratamentos. As dislocations podem ser a chave para o entendimento e controle do processo de desfibrilação e em função disso torna-se importante a pesquisa sobre a implicação de diferentes pré-tratamentos nas características das *dislocations* e suas consequências na evolução da desfibrilação mecânica. Portanto esse trabalho busca avaliar o efeito de pré-tratamentos químicos e enzimáticos na formação de *dislocations* em fibras branqueadas e não branqueadas de polpa celulósica de *Eucalyptus* sp. e também avaliar o impacto do pré-tratamento enzimático sobre as *dislocations* de fibras branqueadas ao serem submetidas a vários ciclos de desfibrilação mecânica.

1.1 Conteúdo da Dissertação

A dissertação está subdividida em três partes. A primeira parte é composta de 3 seções, contemplando (i) introdução, (ii) revisão de literatura, contendo os principais tópicos abordados neste trabalho, contribuindo para o melhor entendimento do artigo que será apresentado na segunda parte deste trabalho e (iii) considerações finais sobre a revisão de literatura, em que serão expostos os principais pontos observados na mesma. A segunda parte contém o artigo e a terceira parte contém as conclusões desta dissertação.

O artigo aborda as análises de *dislocations* na parede celular das fibras de polpa branqueada e não branqueada de *Eucalyptus* sp, após serem submetidas a pré-tratamentos químicos com hidróxido de sódio e também com peróxido de hidrogênio em concentração e tempo de contato diferentes e tratamento enzimático com três enzimas do tipo endoglucanase e outra enzima do tipo endoglucanase com exoglucanase. Também foi avaliado *dislocations* em fibras de polpa branqueada de *Eucalyptus* sp., submetida a pré-tratamento enzimático e sem tratamento após serem submetidas a diferentes passagens no processador mecânico.

Este trabalho buscou contribuir com informações sobre a influência de pré-tratamentos em polpas celulósicas com ênfase nos seus impactos sobre as *dislocations* presentes na parede celular das fibras. A abordagem da integridade da fibra através da avaliação microscópica das *dislocations* permite estabelecer uma etapa de conhecimento importante sobre o efeito de pré-tratamentos químicos e enzimáticos nas características morfológicas/anatômicas das fibras de polpas kraft.

2. REFERENCIAL TEÓRICO

Os tópicos seguintes constituem os principais temas abordados para execução e entendimento do presente trabalho.

2.1 Fibras vegetais

As fibras vegetais apresentam como função principal a sustentação mecânica da árvore (METCALFE; CHALK, 1989). Elas são constituídas basicamente de material lignocelulósico e morfologicamente esbeltas, com extremidades afiladas e pontiagudas (BURGUER; RICHTER, 1991). As fibras vegetais possuem como componente majoritário a celulose, estas por sua vez são encontradas na parede celular dos vegetais atuando como agente estrutural, fornecendo rigidez as plantas (MOON et al., 2011), podem ser encontradas em madeira, algodão, juta, sisal, algas e seres tunicados (HENRIKSSON et al., 2007; EICHHORN et al., 2010; MOON et al., 2011).

A celulose, principal constituinte das fibras, é um polissacarídeo formado por unidades de glicose. A organização das ligações existentes na sua constituição confere regiões organizadas denominadas cristalinas as quais são intermediadas por regiões menos organizadas chamadas de amorfas, sendo orientadas em diferentes camadas, formando ângulos que conferem características como elasticidade, dureza e resistência às fibras (TIENNE et al., 2009). Em virtude das regiões amorfas serem menos organizadas, apresentam maior acessibilidade à sua estrutura, sendo mais susceptíveis ao ataque de enzimas e reagentes químicos.

Fibras de celulose podem ser modificadas em diferentes derivados com propriedades físicas unicas (DE CAMPOS et al., 2013; GARCÍA et al., 2016), podendo ser aplicadas em diversos segmentos industriais como têxteis, na indústria automotiva

(JOHN; THOMAS, 2008), como reforço na construção civil (SILVA, 2015) e na criação de dispositivos, como catalizador hibrido (ARANTES et al., 2017). Além desse uso da celulose como matéria-prima, tem-se também a exploração das nanofibrilas de celulose que compõe as fibras. Essas nanofibrilas que podem ser aplicadas na composição de diversos produtos, apresentando características únicas (KAUSHIK; SINGH, 2011; CHEN et al., 2016) tais como: elevada área de superfície, boas propriedades mecânicas, térmicas e elétricas (DEEPA et al., 2011). Assim as nanofibrilas podem ser empregada em reforços de matrizes cimentícias (FONSECA et al., 2016), em produtos biodegradáveis, indústria de cosméticos (IOELOVICH, 2008), na medicina (FÉLIX et al., 2017) e para a indústria de papel tanto na fabricação de nanofilmes de celulose (POTULSKI et al., 2016) como na melhoria das propriedades ópticas e físicas do papel (POTULSKI et al., 2018).

2.2 Nanofibrilas de celulose e métodos de obtenção

O termo nanofibrilas de celulose (CNFs) também pode ser encontrada na literatura, com outros sinônimos, como nanofibras de celulose e microfibrilas (IWAMOTO; NAKAGAITO; YANO, 2007; BANDERA et al., 2014), todos são aplicados para designar o produto gerado a partir da celulose microfibrilada (ABDUL KHALIL et al., 2014). A celulose microfibrilada remete a celulose que foi submetida a um tratamento que possibilitou a sua desintegração em nanofibrilas (SIRÓ; PLACKETT, 2010). As CNFs são unidades de fibras compostas pela combinação linear de cadeias de celulose com regiões amorfas e cristalinas (TONOLI et al., 2016). As regiões cristalinas são originadas por meio da biopolimerização e cristalização da celulose, sendo áreas da celulose altamente organizadas, enquanto a região amorfa é uma região que não possui organização tridimensional das cadeias de celulose (HABIBI et al., 2007; PENG et al., 2012).

As nanofibrilas de celulose podem ser obtidas por diversos caminhos englobando métodos biológicos, enzimáticos, químicos, mecânicos e/ou a combinação entre eles (HUBBE et al., 2008; KANMANI et al., 2017). Dentre os métodos biológicos, pode-se destacar a celulose de tunicados (RUPPERT, FOX, BARNES, 1996) e as nanofibrilas bacterianas (KLEMM et. al., 2011), nos métodos enzimáticos destacam-se aqueles que utilizam enzimas para gerar a degradação da celulose, como observado nos trabalhos de Henriksson et al. (2007), Pääkkö et al. (2007), Janardhnan e Sain (2011). Dentre os métodos químicos, os processos usuais são aqueles que utilizam a modificação de pH do meio como um pré-tratamento para facilitar a redução das fibras a escala nanométrica, como os trabalhos de Qua e Hornsby (2011), que utilizaram o ácido sulfúrico, e também os trabalhos de Zheng et al. (2002), Beltrami, Scienza e Zattera (2014), que utilizaram hidróxido de sódio como pré-tratamento do processo. Dentre os métodos de tratamentos mecânicos de polpa celulósica para obtenção de nanofibrilas podem ser destacados: cryocrushing, homogeneização de alta pressão, microfluidização e cisalhamento mecânico.

Dentre os novos métodos e equipamentos para processamento mecânico pode-se destacar aqueles desfibriladores que utilizam discos cerâmicos paralelos, que desfibrilam a parede celular das fibras, gerando as nanofibrilas (KLEMM et al., 2011). Como exemplo pode-se destacar o "grinder" que é um moinho que gera o cisalhamento mecânico. Este equipamento possui dois discos de alumina (Al_2O_3) com diâmetro de 150 mm, sendo um disco giratório e o outro fixo, com uma abertura ajustável entre eles, por onde passa o material que será desfibrilado em escala nanométrica por força de cisalhamento gerada pela rotação dos discos, (SIRÓ; PLACKETT, 2010). Tem-se como produto final um gel constituído de nanofibrilas.

Diversos trabalhos apresentados na literatura relatam a produção de nanofibrilas, como: Iwamoto et al. (2007), Wang, Li e Zhang (2013), Bufalino et al. (2014), Guimarães Jr. et al. (2015), Mirmehdi et al. (2018), Guimarães Jr., Teixeira e Tonoli (2018), os quais apresentam em comum a utilização do desfibrilador mecânico, que possui alta eficiência no processo de produção. Entretanto, como desvantagem associada, é um procedimento que gera consumo elevado de energia devido ao aumento do número de passagens das fibras pelo desfibrilador mecânico para obtenção de nanofibrilas (BUFALINO et al., 2015). Esse gasto energético é um dos maiores entraves para potencializar a produção de nanofibrilas em escala industrial (SPENCE et al., 2011).

Em busca da otimização do tempo e menor gasto energético no processo, pode-se observar o desenvolvimento de pesquisas que abordam a inclusão de pré-tratamentos para promover modificações nas estruturas das fibras, como os processos químicos e os enzimáticos (SIRÓ; PLACKETT, 2010; CHINGA-CARRASCO, 2011).

2.3 Pré- tratamentos

Os materiais lignocelulósicos são compostos de longas cadeias de celulose envolvidas por hemiceluloses e lignina, o que agrega maior dificuldade de separação das fibrilas, no processo de obtenção das nanofibrilas, por tornar esses materiais estruturalmente resistentes à hidrólise e pouco reativos (LEHNINGER; NELSON; COX, 2002). Com isso para eliminar as barreiras que inviabilizam a eficiência do processo de produção de nanofibrilas é fundamental aplicar pré-tratamentos nas fibras. Os pré-tratamentos são considerados etapas onerosas, entretanto essenciais para permitir otimização da desfibrilação (MISHRA; SABU; TIWARI, 2018). Segundo Fassanella (2008), muitos métodos têm sido pesquisados e verifica-se a necessidade em desenvolver opções ambientalmente seguras e economicamente viáveis. Esses pré-tratamentos agem nas fibras gerando modificações na sua estrutura, podendo atuar sobre áreas sensíveis da parede celular ou até mesmo comprometendo outras áreas com a criação de pontos de enfraquecimento, como as *dislocations*. Estudos contemplando a relação de pré-tratamentos associados com a criação das *dislocations*, são pouco conhecidas. Para preencher essa lacuna existente na literatura esse trabalho está sendo desenvolvido para buscar compreender essa relação.

2.3.1 Pré-tratamento químico

O pré-tratamento alcalino pode ser realizado utilizando hidróxido de sódio (NaOH), o qual é capaz de promover a “limpeza” da fibra, removendo lignina, hemicelulose e ceras, deixando a área cristalina mais exposta, além de clivar as regiões amorfas. No pré-tratamento com NaOH, a concentração e o tempo de reação são fundamentais para garantir bons resultados (LI et al., 2007; FASSANELLA 2008; SILVA, 2009; FARUK et al., 2012).

Após a adição do NaOH, as ligações de hidrogênio se rompem, assim os íons Na^+ formam novas ligações com os sítios livres, gerando modificações em suas propriedades de adsorção, facilitando o intumescimento/turgescência da fibra (GURGEL, 2007). O rompimento de ligações nesses processos gera modificações estruturais na parede celular das fibras, deixando a superfície da fibra mais rugosa e também são disponibilizados grupos que antes não existiam para que novas ligações possam ser realizadas

O peróxido de hidrogênio tem capacidade de modificar a superfície das fibras por meio da remoção dos constituintes não-celulósicos (remoção de lignina residual e agentes cromóforos por meio de oxidação), tornando a fibra mais branca e brilhante. A sua aplicação se estende para branqueamento de fibras nos setores têxtil (NABI SAHEB; JOG, 1999; IMTIAZUDDIN; TIKI, 2015), e de papel e celulose (HE; NI, 2008), e na produção de compósitos com aplicação de fibras lignocelulósicas (ENRIQUEZ; MOHANTY; MISRA, 2016). A utilização do peróxido de hidrogênio junto com hidróxido de sódio é chamada peróxido-alcalino. Segundo Hendriks e Zeeman (2009), a solução peroxido-alcalino é capaz de branquear e deixar a celulose mais acessível.

2.3.2 Pré-tratamento enzimático

O pré-tratamento enzimático pode ser considerado um processo ambientalmente viável por não envolver solventes químicos ou reagentes tóxicos (SIQUEIRA et al. 2010a; SAELEE et al, 2016). É um pré-tratamento promissor por apresentar melhorias na relação custo-benefício (SUKUMARAN et al., 2009; NIE et at., 2018).

Neste pré-tratamento as enzimas atuam nas regiões amorfas, quebrando as ligações glicosídicas β-1,4 localizadas entre as moléculas de glicose que compõem a celulose (HUBBE et al., 2008). Para a eficiência do processo, as condições de reação devem ser conhecidas e controladas afim de garantir apenas clivagem nas regiões amorfas (ZHU et al., 2011), fornecendo assim bons resultados em seu processo de produção. Os parâmetros que devem ser avaliados são tempo de hidrólise (h), concentração de substrato e concentração de enzima (MEYABADI; DADASHIAN, 2012).

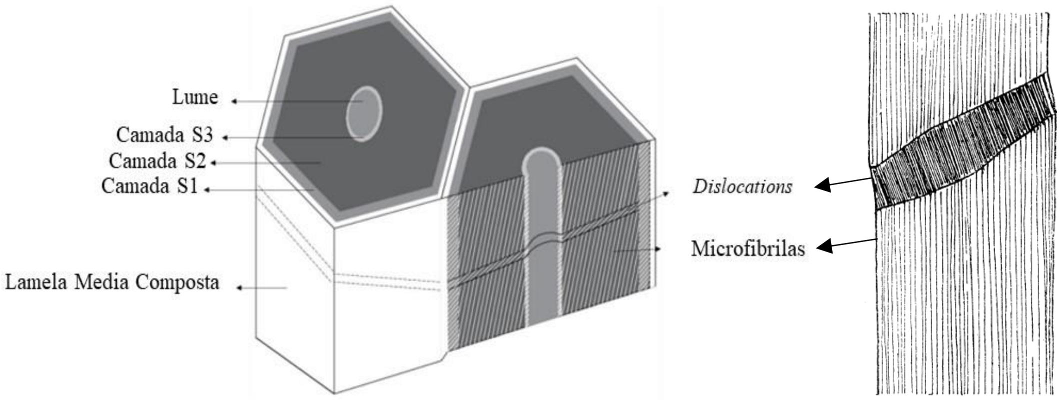
De acordo com Durán et al. (2011), a produção de nanofibrilas a partir do emprego das enzimas pode ser realizado por meio de processos combinados com agitação mecânica ou homogeneização de alta pressão. O emprego das enzimas como facilitador do processo de desfibrilação com posterior obtenção de nanofibrilas de celulose, foi utilizado por Pääkko et al. (2007), onde foi demonstrada a eficiência da enzima endoglucanase na hidrólise da celulose. Nobuta et al. (2015) e Tian et al. (2017) também utilizaram enzimas como pré-tratamento no processo de obtenção de nanofibrilas.

Outros trabalhos estudaram a ação enzimática sobre as fibras, como Suchy et al. (2009), que relataram o impacto da celobiohidrolase sobre a resistência das fibras e sua morfologia, onde foi observado aumento de áreas fragilizadas (*dislocations)* na parede celular e para concentrações maiores dessa enzima surgiram interrupções na forma de rachaduras. Thygesen et al. (2011), ao usar enzima do tipo endoglucanase verificaram que a sua atuação é seletiva em regiões de *dislocations* durante o início da hidrólise.

2.4 *Dislocations*

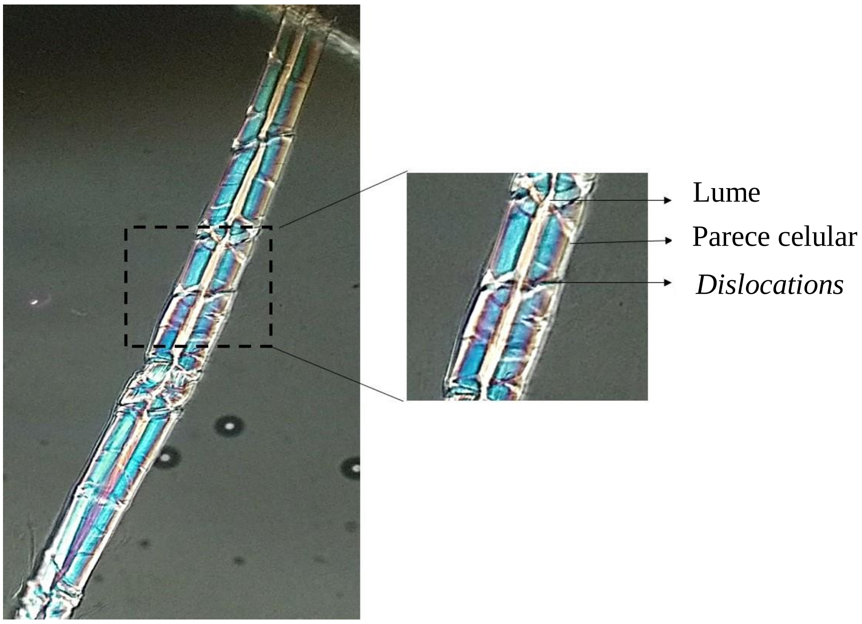
Dislocations, slip planes e *kinks,* são designações dadas as deformações na parede celular da fibra celulósica observadas pela modificação do ângulo das microfibrilas (FIGURAS 1 e 2). Nos estudos iniciais sobre as *dislocations,* Robinson (1920) afirmou que essas deformações na parede celular das fibras geram o enfraquecimento da madeira e que são causadas por forças compressivas aplicadas paralelamente às fibras. Essas alterações afetam a estrutura da fibra da madeira, promovendo a redução das propriedades mecânicas da madeira (LIMA, 1999), ou seja, a ruptura da fibra com *dislocations* ocorre com esforços inferiores ao que ela suportaria. Fibras com *dislocations* são indesejáveis na produção de papel e compósitos pois diminui a resistência das fibras, como observado por Nyholm et al. (2001) e Hughes (2012), respectivamente.

Figura 1. Ilustração referente a *dislocation* em fibras.



Fonte: Moulin; Lima (2017) Fonte: Robinson (1920)

Figura 2. Fibra branqueada de *Eucaliptus sp*. com presença de *dislocations* em sua extensão.



O desenvolvimento de *dislocations* pode ser ocasionado de forma natural, por fatores ambientais, como flexão do caule gerado pelo vento ou redução na disponibilidade hídrica para o desenvolvimento das plantas (THYGESEN; ASGHARIPOUR, 2008) ou pelas características da própria árvore, como tensões de crescimento. As *dislocations* também podem ser ocasionadas de forma induzida, como os esforços gerados pela interferência do homem como a derrubada da árvore, transporte e processamento da madeira (DINWOODIE, 1970). Além desses fatores, Bienfait (1926), destaca a retração da madeira como forma de surgimento de *dislocations*.

A ocorrência de *dislocations* se inicia por uma perturbação que ocorre nos ângulos das microfibrilas, em que a camada de microfibrilas da parede celular desliza em relação a outra (FIGURA 1), gerando uma zona de reorientação (HOFFMEYER et al., 1993). Essa reorientação propicia o aparecimento de *dislocations*.

As modificações na morfologia das fibras na geração da dislocation acontecem de forma sequencial, surgindo primeiramente a separação da fibra com a lamela média (intercelular), posteriormente a geração da falha por toda a parede secundária (intracelular) e finalizando com a ruptura transversal à toda a parede da fibra, configurando a *dislocation* (COTÉ; HANNA, 1983).

A literatura disponível sobre *dislocations* nas fibras da madeira contempla como principais objetivos a análise dos fatores que são precussores dessa deformação. Escassez de informações é observada sob quantificação das *dislocations* e sobre as suas consequências na utilização da madeira e suas fibras.

Um dos trabalhos que utilizou a quantificação das *dislocations* foi realizado por Thygesen, Eder e Burgert (2007), que por meio de análises das fibras individualizadas com as *dislocations* no microscópio de luz polarizada, realizou-se a quantificação das *dislocations* considerando a relação da área ocupada pelas *dislocations* com a área da fibra. Outros trabalhos foram realizados por Ander, Hildén e Daniela (2008) e Thygesen (2008) que utilizaram a hidrólise ácida e por Thygesen et al. (2014) que utilizaram a hidrólise enzimática para quebrar a fibra nos locais com as presenças de *dislocations* e assim quantificá-los.

3. CONSIDERAÇÕES FINAIS DO REFERENCIAL TEÓRICO

Esta revisão bibliográfica apresentou informações essenciais para compreensão da abordagem sobre as *dislocations* em fibras e o uso de pré-tratamentos para facilitar sua desconstrução. A partir da leitura de vários artigos científicos, foi possível lapidar o enfoque desse trabalho bem como definir os experimentos adotados nessa pesquisa. Diante dos tópicos abordados notou-se a necessidade de encontrar informações sobre a influência de pré-tratamentos sobre as fibras e entender o impacto gerado na parede celular. Foram encontradas poucas publicações nos últimos anos com enfoque sobre *dislocations,* como também conteúdos que abordam aplicação de pré-tratamentos em fibras visando alterações na morfológia das fibras. A influência da presença das *dislocations* nas fibras em sua desfibrilação com as várias passagens no desfibrilador também não foi reportada na literatura.

Apartir das lacunas observadas nessa revisão, pretende-se com essa pesquisa contribuir com informações acerca da avaliação da integridade das fibras de polpas celulósicas, por meio da caracterização de suas *dislocations* antes e após pré-tratamentos químicos, enzimáticos e sua evolução durante a desfibrilação mecânica.

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SEGUNDA PARTE

ARTIGO: Optimization of the nanofiber production process through the evaluation of dislocations in cellulose pulps submitted to chemical and enzymatic pre-treatments.

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ABSTRACT

The cell wall of plant fibers may have irregular regions called dislocations, which are weak areas of the cell wall. This work evaluated, the effect of the chemical and enzymatic pre-treatment in the generation of dislocations in unbleached and bleached fibers of eucalyptus, as well as the influence of enzyme pre-treatment on fiber dislocations during mechanical defibrillation. The bleached and unbleached pulps were subjected to chemical pre-treatments with NaOH and H_2O_2+NaOH. For the enzymatic treatments the activity of 4 enzymes was evaluated, being three of them of the endoglucanase type and one as a mixture of endoglucanase and exoglucanase. For the mechanical defibrillation the untreated and enzyme treated fibers were collected before and after 0, 5, 10, 15, 20, 25, and 30 passages in the mechanical defibrillator. The dislocations were evaluated through a polarized light microscope for the calculation of the index of dislocations and angle of dislocations. The chemical pre-treatments with NaOH and H_2O_2+NaOH resulted in the increase of the index of dislocations for bleached and unbleached fibers. There was the reduction of the index of dislocation with enzyme C and increase the index of dislocation in enzyme B for the bleached fibers, while for the unbleached fibers the index of dislocation may have been protected by the presence of lignin in their constitution. There was greater reduction of dislocation angles for the chemical pre-treatment, as well changes in the curl of bleached and unbleached fibers for chemical pre-treatments. For the mechanically defibrillated fibers, the impact of the enzyme on the fibers did not cause a reduction in the length of the fibers, but allowed a significant increase of the dislocations. The increase of the dislocations occurred up to 20 passes for the fibers treated with enzymes and without pre-treatment, which was the point whose the fiber reached the maximum dislocation index. The angle of the dislocations was reduced with the enzymatic action, as well as the index of curvature of the fibers. The results show that the action of chemical and enzymatic pre-treatments can affect the integrity and recalcitrance of the fibers by creating dislocations.

KEYWORDS: slip plane, defibrillation, mechanical process, nanofibrils, nanofibers, microfibrillated cellulose (MFC)

1 INTRODUCTION

The vegetal fibers are renewable and biodegradable materials. They have been used for the development of products using micro/nanofibril, such as paper [1, 2, 3], paints, coatings [4, 5], biomedical and pharmaceutical products [6, 7], fiber-cements [8] and electronic/magnetic devices [9, 10, 11]. The term “micro/nanofibrils” refers to long, flexible micro and nanofibers consisting of alternating crystalline and amorphous domains [12]. Such micro/nanofibrils have been obtained by mechanical defibrillation procedures [13, 14, 15].

The disintegration of the fibers to get this nanocomponds can be favored by the presence of structurally weakened regions in the cell wall [16], which are known as dislocations, also widely called slip planes and/ kinks [17, 18, 19].

From the structural point of view, the dislocations are constituted of regions with modified microfibril angles [20]; which generates disorganizations in the ultrastructure of the cell wall [21]. Thus when the cell wall presents several dislocations, they may act reducing the mechanical properties of fibers [16].

The dislocations are deformations caused by compressive stress [17]. Dislocations can be generated in a natural or induced way. Among them, it can be distinguished those generated by the intensity of the growth stress, whose the bending originated in the stem caused by the action of the wind induces the longitudinal compression of the cell wall [20]. The compressive load exerted on the wood by the mass of the trees canopy [22], and the low availability of water for the plant growth, can also generate dislocations [23]. After thinning, the origin of the dislocations can occur in the transport of wood, wood processing [21], mechanical shocks and variations in the drying processes of the wood.

In literature, just very few studies were reported evaluating the impact of pre-treatments on the fiber dislocations. Among them it can be reported the studies with application of acid hydrolysis [24] and some enzymes [25] in order to break the fibers in the regions with the presence of dislocations. The use of mechanical shearing for defibrillation of the fibers contributes to generate dislocations and reduction in fiber size by breaking these regions (dislocations) during the friction of the fibers in the mechanical defibrillator [26].

Based on the characteristics of the dislocations produced by different pre-treatments, it may be possible to understand and optimize the production of nanofibrils with mechanical shearing of pulp fibers. As a consequence, the objective of this work was to verify the impact of chemical and enzymatic pre-treatments in bleached and unbleached fibers on their dislocation characteristics, as well as the influence of enzymatic pre-treatments on their mechanical defibrillation.

2 EXPERIMENTAL

2.1 Materials

Bleached and unbleached kraft pulps of *Eucalyptus* sp. used in this study were provided by Klabin S.A. and the enzymes A, B, C e D were supplied by Novozymes Latin America LTDA.

Hydrogen peroxide (H_2O_2) and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich.

2.2 Chemical and enzymatic pre-treatments applied to bleached and unbleached celullose

Cellulose pulp fibers were submitted to the different pre-treatments described below:

(i) NaOH treatment under the influence of the reagent concentration and the reaction time: 20 g of cellulose was immersed in aqueous solution of 1 L of 5% NaOH for 120 min under bath of 80ºC. Also, other samples were submitted to pre-treatment with 10% NaOH at 80ºC for 60 min and other for 120 min. All pre-treatments remained under constant mechanical agitation (~ 355 rpm). Subsequently, it was washed with water to remove excess reagent and oven-dried. This procedure was applied for the bleached and unbleached cellulose.

(ii) Treatment with hydrogen peroxide (H_2O_2) and NaOH: 20 g of unbleached cellulose was used in 1 L of 5% NaOH aqueous solution with 16% H_2O_2 in a 60°C heated bath with stirring at 700 rpm for 90 min. Subsequently, it was washed with water to remove excess reagent and dried in an oven.

(iii) Enzymatic pre-treatment: 30 g of cellulose immersed in 1 L of distilled water was used, with heating at 50°C and pH ~ 6.5. Under these conditions, 100 g of enzyme / ton of pulp were added under constant stirring at 750 rpm for 120 min. At the end of the process, the material was washed with distilled water at

90°C for denaturation of the enzymes. This procedure was performed with endoglucanase type enzymes (A, B and C), and with the mixture of endoglucanase and exoglucanase (enzyme D). Subsequently, it was washed with water to remove excess reagent and oven-dried.

2.2 Chemical characterization of bleached and unbleached pulps

The chemical characterization of the fibers was performed before and after the chemical and enzymatic pre-treatments. An ADionex ICS 5000 ion chromatography system was used. For the soluble lignin content, the Tappi UM 250 (1976) standard was used and the monosaccharides were determined according to Wallis; Wearne and Wright [27].

2.3 Mechanical defibrillation of the enzymatic-treated fibers

Mechanical defibrillation of the bleached fibers was performed using untreated pulp (i) and pulp pretreated with enzyme (ii), as shown below:

(i) The untreated *Eucalyptus* sp. pulp fibers (120 g) were saturated in 6 L of distilled water for 72 h in order to cause their intumescence and subsequently subjected to 15 min of mechanical stirring for maximum individualization of the pulp fibers.

(ii) The enzymatic pre-treated *Eucalyptus* sp. pulp fibers (120 g) were used in 4 L of distilled water at 50°C and pH corrected to 6.5. The enzyme B (endoglucanase activity) was added in the suspension in a ratio of 100 g of enzyme / ton of pulp, under constant stirring at 750 rpm for 120 min. At the end of the process the material was washed with distilled water at 90°C for denaturation of the enzymes and then oven-dried at 40ºC. Subsequently the enzyme-treated pulp was saturated in 6 L of distilled water for 72 h and subjected to 15 min of mechanical stirring as done for the untreated fibers.

After the preparation of the two fiber conditions (untreated and enzyme-treated), they were processed in the mechanical defibrillator (SuperMassColloider Masuko Sangyo Co., LTD.), using 1500 rpm, around 4-6 A electric current, and carried out by means of 30 passages as performed by Guimarães Junior et al. [14], Mirmehdi et al. [2], Guimarães Junior, Teixeira and Tonoli [28]. Pulp samples were collected before the defibrillation process (0 passages), and after every five passages, i.e. 5, 10, 15, 20, 25 and 30 passages. Then, the samples from all theses condition were analyzed under microscopy.

2.4 Microscopic characterization of the dislocations

The analyzes were conducted under a light polarized microscope, so it was possible to visualize the dislocations in the fibers, which were placed on temporary slides and analyzed under a microscope equipped with a turntable (from 0 to 360º). The fibers were aligned vertically with the assistance of the Image Pros Plus image analyzer software associated with the microscope (Olympus BX-51). Then, it was obtained the height of the dislocations (HD), the angle of dislocation, the fiber width (FW) and length of the fiber (LF). The index of dislocations (ID) was calculated using Eqs. 1 and 2 [29]. Twenty fibers were analyzed for each treatment for angle of dislocations (Figure 1) and for fiber width and length.

$$\mathrm{HD} = \mathrm{FW} \text{ x tang } \theta \qquad (1)$$

For the determination of the ID, the sum of all HDs values present in the fiber is necessary (ΣHD), divided by the length of the fiber (LF), as applied in Eq. (2).

$$\mathrm{ID}\ (\%) = \left(\frac{\Sigma HD}{\mathrm{LF}}\right) \text{ x } 100 \qquad (2)$$

For the determination of the angle of the dislocations, the angle corresponding to the longitudinal direction of the fiber was used (ɵ), as shown in Figure 1, and the angles were applied in Eq. (3).

$$\text{Angle of } dislocations = 90° - \theta \qquad (3)$$

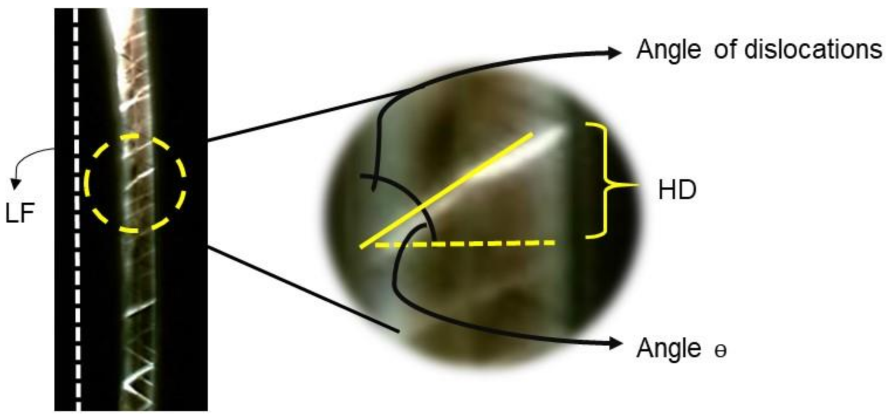
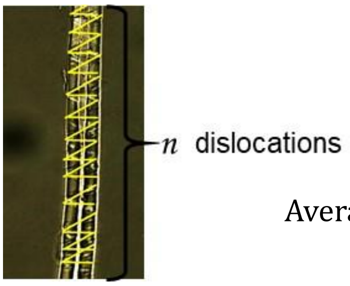


Figure 1. Schematic representation of the fiber for the characterization of the fiber dislocations. LF means fiber length; FW means fiber width; HD means fiber height.

The mean value of the angle of dislocations was obtained by summing all angles of the dislocations, divided by the number of dislocations present in the fiber (Figure 2). Were applied in Eq. (4).



$$\text{Average angle of dislocations} = \frac{\sum(90° - \theta) + \cdots (90° - \theta)}{n\ dislocations} \quad (4)$$

Figure 2. Schematic representation of the fiber for determination of the mean of the dislocations angle. *n* means several.

2.5 Fiber curl index

To determine fiber curl, the distance between the two ends of the fiber was measured and the distance between the average of that length and the fiber curvature (arrow) was measured, and the values were applied in Eq. (5) (Figure 3).

$$\text{Index curl} = \frac{\text{Arrow}}{\text{Length}} \quad (5)$$

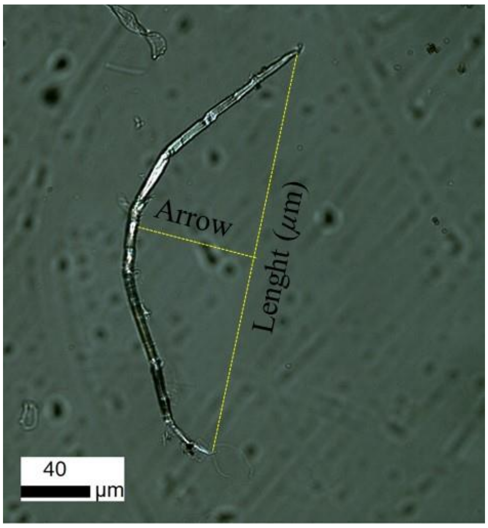


Figure 3. Schematic representation of the fiber for determination of curl.

2.6 Statistical analysis of dislocations

The analysis of the characteristics of the dislocations was performed in 20 fibers per pre-treatment and for each passage through the mechanical defibrillator. For the analyzes in the fibers with chemical and enzymatic pre-treatments, the scott-knott test was applied to 5% of significance. For the mechanically defibrillated fibers, a completely randomized experimental design (DRC) was used and the regression was applied, with significance of 5%.

3 RESULTS AND DISCUSSION

3.1 Effect of chemical pre-treatments on dislocations of unbleached and bleached fibers

The dislocations in the unbleached and bleached fibers of *Eucalyptus sp*., after chemical pre-treatments are present in Table 1. The chemical pre-treatment increased ID in the unbleached and bleached fibers. The action of NaOH on the fibers is effective to remove polyses and lignin, in addition to depolymerizing the amorphous region of the cellulose. In the pre-treatment, the swelling of the fibers ocurred, which distracts the cellulose chains, causing hydrogen bonds to rupture [30, 31]. The hydroxyls from NaOH also favor the breaking of the bonds forming new associations with the cellulose chain, which changed the roughness of the surface, besides leaving them with greater contact surface [26, 32]. The use of NaOH increased the ID of the samples, independent of sample concentration.

Table 1. Average and standard deviation values of index of dislocation (ID) and angle of dislocation of unbleached and bleached fibers after different chemical pre-treatments.

	Unbleached Fibers						
	Control	**Control ***	**Control ****	**NaOH 5%****	**NaOH 10%***	**NaOH 10% ****	**H_2O_2**
ID	58 ± 4 b	55 ± 7 b	57 ± 8 b	63 ± 6 a	65 ± 10 a	65 ± 6 a	63 ± 9 a
	(7)	(13)	(14)	(10)	(16)	(10)	(14)
Angle (º)	72 ± 1 a	70 ± 1 b	71 ± 1 b	71 ± 2 b	71 ± 1 b	71 ± 1 b	71 ± 1 b
	(2)	(2)	(2)	(2)	(2)	(1)	(1)
	Bleached Fibers						
	Control			**NaOH 5%****	**NaOH 10%***	**NaOH 10% ****	
ID	24 ± 3 b	-	-	29 ± 6 a	34 ± 3 a	30 ± 5 a	-
	(14)	-	-	(22)	(10)	(17)	-
Angle (º)	76 ± 1a	-	-	61 ± 5 c	70 ± 1 b	67 ± 3 b	-
	(2)	-	-	(8)	(2)	(4)	-

* Heating for 1 h at 80°C; ** heating for 2 h at 80°C; UF: unbleached fibers. Value in brackets corresponds to coefficient of variation (%). Means followed by the same letter in the row do not differ by the scott-knott test at the 5% level of significance.

The heat not altered the ID in the unbleached fibers. The effect of H_2O_2+NaOH pre-treatment was similar to the NaOH. The increase of NaOH concentration (10%) did not add more dislocations in unbleached fibers. The dislocations generated by chemical pre-treatments showed lower angle in relation to the fibers without chemical pre-treatment. The reduction of the angle occurs due to the degradation of the most amorphous chains. Images of unbleached fibers with chemical pre-treatment are shown in Figure 4. Figures 4b and 4d show the high number of index of dislocation in the chemical pre-treatment.

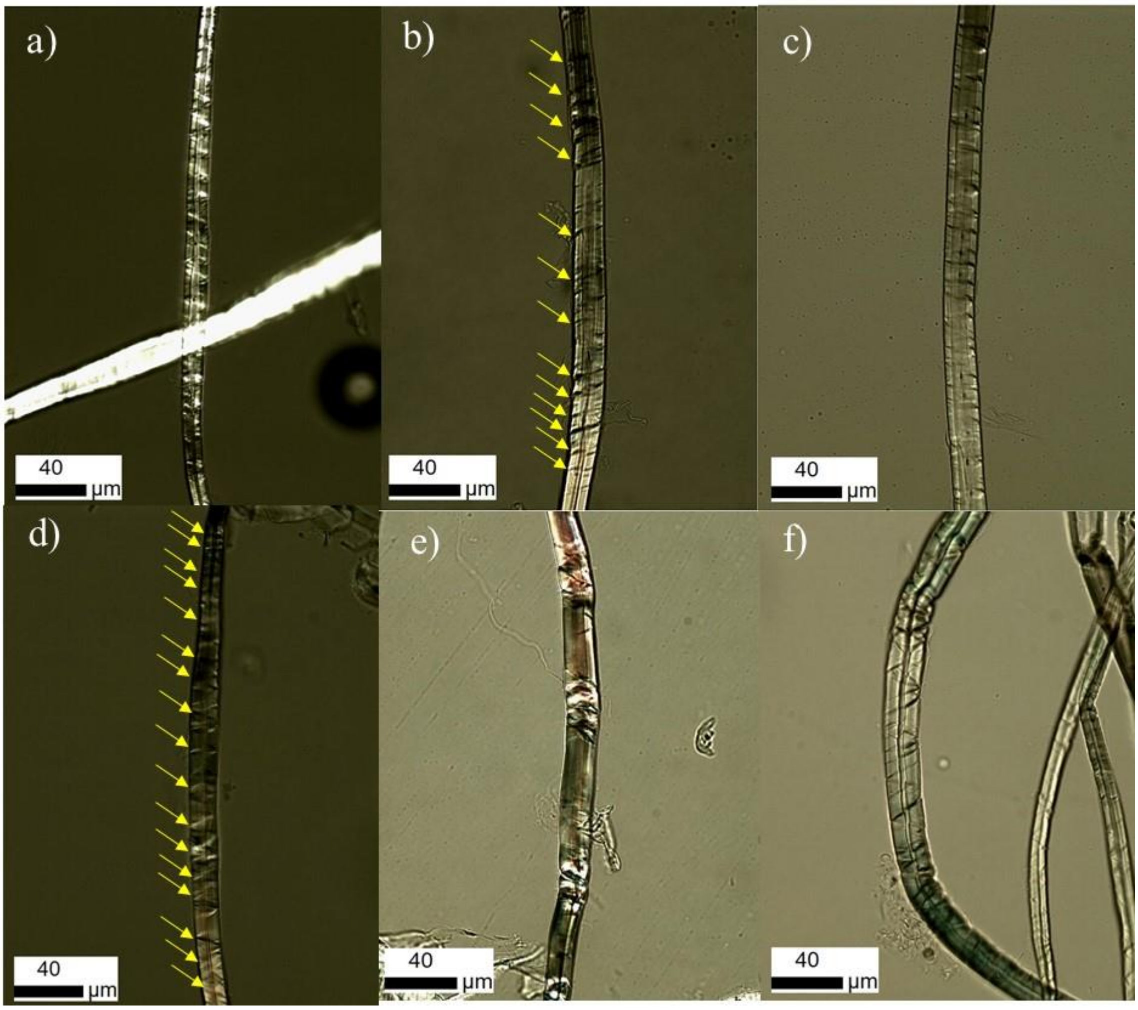


Figure 4. Typical ligh-pollarized microscopic images of individualized pulp unbleached fibers of *Eucalyptus sp*. showing the dislocations in samples with chemical pre-treatment: a) heating for 1 h; b) heating for 2 h showing the presence of dislocations (arrows for the index of dislocations ~ 57%); c) NaOH 5% for 2 h; d) NaOH 10% for 1 h (with 65% of index of dislocations); e) NaOH 10% for 2 h; and f) H_2O_2/ NaOH.

The effect of pre-treatment in the bleached fiber was similar to the unbleached fiber, just NaOH increased ID, independent of its concentration, however the NaOH 5% generated dislocations with smaller angles, whit 15º lower than the dislocations of the control bleached fibers. The larger angle formed in the fiber indicates that the dislocations occupy more space in the fibers, besides indicating greater deformation. Images of bleached fibers before and after chemical pre-treatments are depicted in Figure 5. Figures 5a and 5d showed the dislocations (arrows) and change of ID with the chemical pre-treatment.

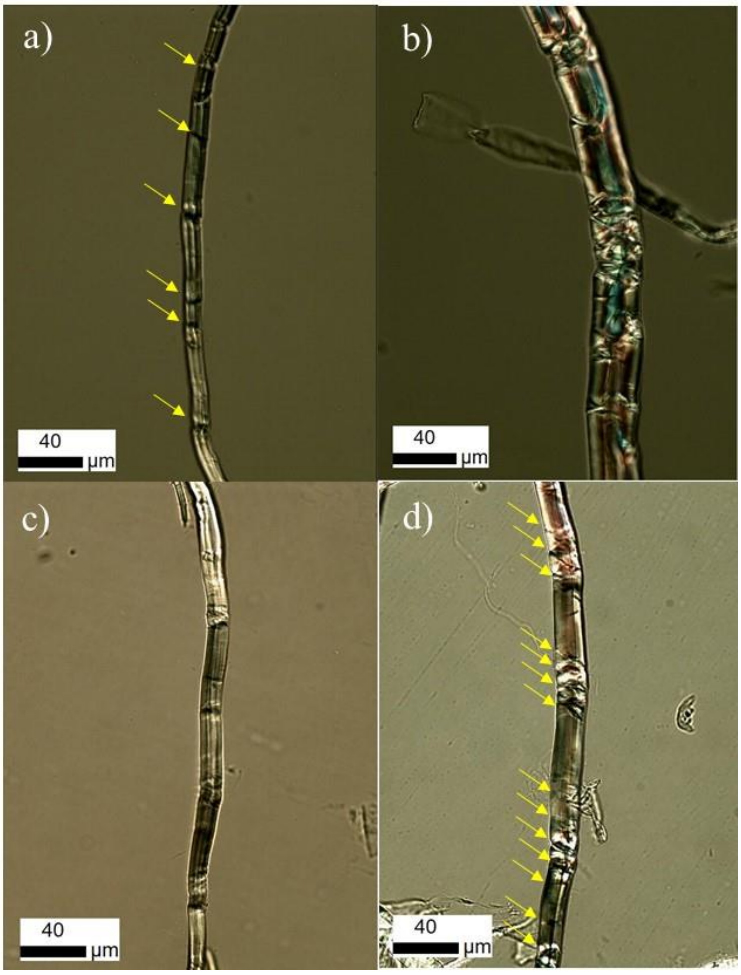


Figure 5. Typical ligh-pollarized microscopic images of individualized pulp bleached fibers of *Eucalyptus sp*. showing the dislocations in samples with chemical pre-treatments: a) control without heating with 24% of index of dislocations; b) NaOH 5% for 2 h; c) NaOH 10% for 1 h; and d) NaOH 10% for 2 h (with 30% of index of dislocations).

The action of temperature on the unbleached fibers did not statistically changed the ID when compared to the control without heating, while for the bleached fibers heating may have contributed to the appearance of the dislocations. The presence of lignin in unbleached fibers may have acted as a barrier to reach the cellulose chains [33], preventing the appearance of dislocations in the fibers. Table 2 shows that soluble (3%) and insoluble (16%) lignin content is higher in the unbleached fibers compared to bleached fibers, which presented only soluble lignin (0.14%).

Table 2. Chemical composition of bleached and unbleached pulps before and after chemical and enzymatic pre-treatments.

	Unbleached Fibers							Bleached Fibers			
	Gluc	Arab	Galac	Xyl	Man	Insol. lig.	Sol. lig.	Gluc	Xyl	Insol. Lig.	Sol. Lig.
	(% in mass)										
Control	64	0.08	0.24	14	0.24	16	3	76	15	ND	0.14
NaOH 5% 2h	69	0.06	0.15	7	ND	15	3	83	9	ND	0.16
NaOH10% 1h	74	0.06	0.08	5	0.27	16	3	92	3	ND	0.10
NaOH10% 2h	72	0.06	0.13	5	0.26	16	3	94	2	ND	0.12
$H_2O_2$16%+NaOH 5%	64	ND	0.20	7	0.61	12	3	-	-	-	-
Control	59	ND	0.31	10	0.30	17	3	75	12	ND	ND
Enzyme A	54	0.35	0.58	9	ND	17	3	75	13	ND	ND
Enzyme B	60	ND	0.60	10	ND	18	3	76	13	ND	ND
Enzyme C	55	0.31	0.58	9	ND	15	3	77	13	ND	ND
Enzyme D	54	0.35	0.58	9	ND	18	3	76	12	ND	ND

Gluc means glucose; arab means arabinose; galac means galactose; xyl means xylose; man means manose; insol. lig. means insoluble lignin; sol. lig. means soluble lignin; ND means not detected.

3.2 Effect of enzymatic pre-treatments on dislocations of unbleached and bleached fibers

The evaluation of the dislocations made in the unbleached and bleached fibers of *Eucalyptus* sp., after enzymatic pre-treatments were presented in Table 3. Heating and stirring of the unbleached and bleached fibers at 50°C caused the increase of ID in comparison to the control without heating, which corroborates the results of Zeng et al [26]. Fiber drying can generate dislocations [34], therefore fibers treated under heating were subjected to one additional drying that could have caused shrinkage of the fibers and appearance of higher ID in comparison to the control without heating and stirring.

Table 3. Average and standard deviation values of index of dislocation (ID) and angle of the dislocations of unbleached and bleached fibers from different enzyme pre-treatments.

	Unbleached Fibers					
	Control	**Control heating***	**Enzyme A**	**Enzyme B**	**Enzyme C**	**Enzyme D**
ID	58 ± 4 b (7)	64 ± 8 a (12)	62 ± 7 b (12)	61 ± 9 b (13)	66 ± 7 a (13)	64 ± 8 a (10)
Angle (º)	72 ± 1 a (2)	72 ± 1 a (2)	71 ± 2 a (2)	71 ± 1 a (2)	71 ± 1 a (1)	72 ± 1 a (1)
	Bleached Fibers					
	Control	**Control heating***	**Enzyme A**	**Enzyme B**	**Enzyme C**	**Enzyme D**
ID	24 ± 3 d (14)	39 ± 5 b (14)	39 ± 3 b (7)	49 ± 9 a (19)	17 ± 4 e (26)	34 ± 7 c (22)
Angle (º)	76 ± 1 a (2)	72 ± 3 b (3)	71 ± 2 b (2)	72 ± 1 b (1)	71 ± 2 b (2)	70 ± 1 c (2)

* Heating for 2 h at 50°C. Value in brackets corresponds to coefficient of variation (%). Means followed by the same letter in the row do not differ by the scott-knott test at the 5% level of significance.

In the unbleached fiber the enzymes C and D were statistically similar to the control with heating, whereas the enzymes A and B were statistically imilar to the control, that is, the enzymatic action may not have contributed effectively to the ocurrence of dislocations in unbleached fibers, indicating that dislocations were generated only by heating the fibers. Images of unbleached fibers before and after the enzymatic pre-treatment are presented in Figure 6. Figures 6b and 6c showed the dislocations (arrows) in the enzyme- treated fibers.

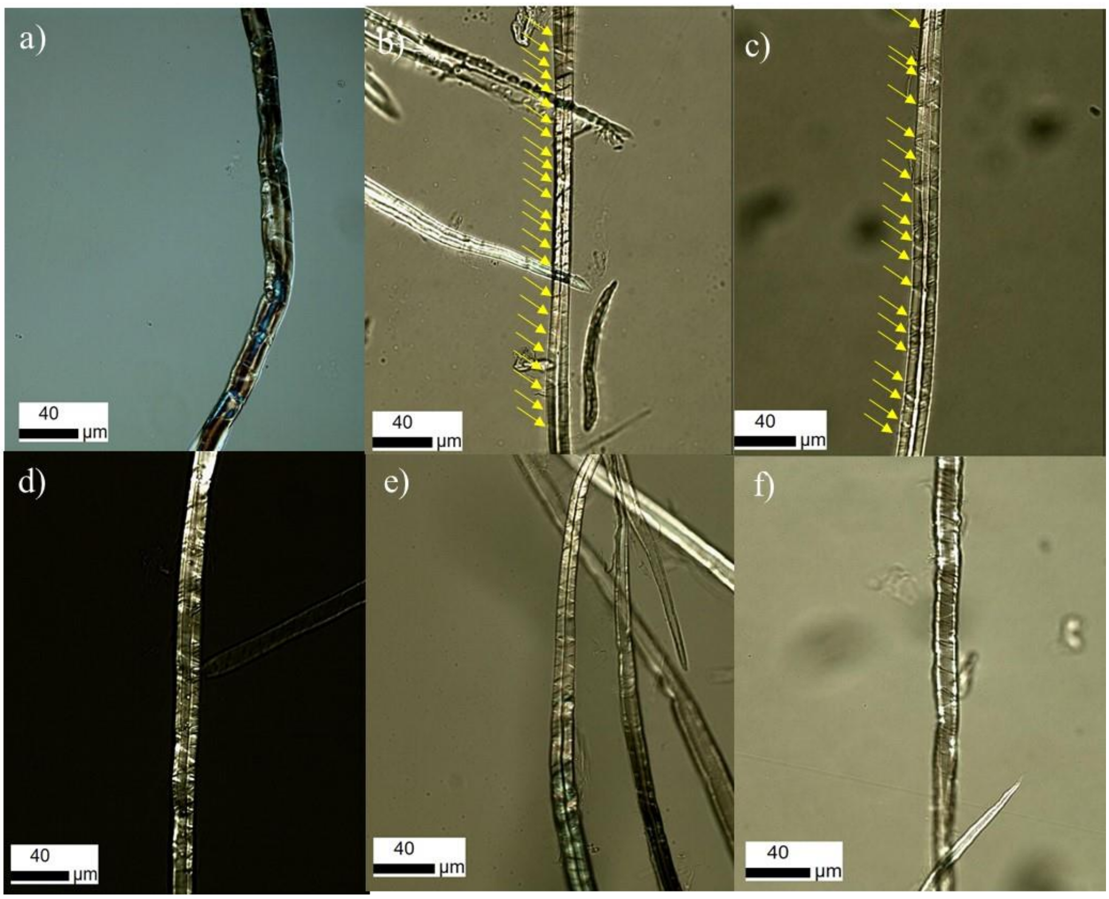


Figure 6. Typical ligh-pollarized microscopic images of unbleached fibers of *Eucalyptus sp*., showing the dislocations in samples with enzymatic pre-treatment: a) control; b) control fibers with heating at 50°C (arrows indicate presence of dislocations, ID ~ 64%); c) enzyme A (with ID ~ 62%); d) enzyme B; e) enzyme C; and f) enzyme D.

The application of enzymes did not caused the appearance of dislocations in the cell wall of unbleached fibers. Variation in the amount of enzyme as well as the time of action of the enzymes were not tested in the present work, but are important factors to be considered in further studies. As already discussed, the unbleached fibers contains high levels of lignin (17%) (Table 2), which is a hydrophobic molecule that prevents water or enzymes in aqueous solution to penetrate the cell wall of the fibers, acting as a physical barrier, reducing accessibility to the cellulose chains [35], as well as the hemicelluloses, which coat the microfibrils giving protection to the cellulose chains, and can be considered barriers for the enzymatic attack [36]. According to Hidayat et al. [18], the dislocations happen more easily after bleaching or delignification.

The angle of the dislocations for unbleached fibers remained unchanged, but in the bleached fibers there was the significant decrease of the angle (Table 3). The organization of the microfibrils that constitute the dislocations have been modified by the enzymatic action, which in addition to the heating may have favored modifications in the structural arrangement of the bonds, causing displacement in the angle of the dislocations.

In the bleached fibers, the use of the enzymes caused increase of the ID in relation to the control, with the exception of the enzyme C (Table 3). The enzyme B caused higher ID, similarly to the observed with enzymatic pre-treatment by Suchye et al. [37] with the increase of dislocations in the cell wall of the fiber as a function of the enzymatic concentration. According to those authors and in agreement with Gurnagul et al. [38], the emergence of the dislocations was caused by irregular structures already present in the fiber, which was intensified with the enzymatic action, exposing the S2 layer of the fiber wall. Wallace [39] and Thygesen et al. [40] observed the breakage of the fiber at the spots/sites of dislocations in the fibers after enzymatic treatment, due to the greater accessibility and fragility of these regions to the enzymatic attack. However, there is the possible increase of dislocation by mechanical stirring during hydrolysis, as reported by Thygesen et al. [40] that showed the dislocations caused by mixing of the fiber during enzymatic hydrolysis process. This effect was also documented by other authors [18].

For samples treated with the enzymes C and D, there was a decreased amount of dislocations in the fibers in relation to the control samples with heating, proposing possible breaking of the fibers in the regions of dislocations. This reduction of the dislocations caused by by enzymatic hydrolyses also was reported by Wallace [39] and Rao [41]. According to Ander et al. [16] the dislocations are regions with less ordered cellulose structures and this may facilitate enzymatic action. Images of the bleached fibers before and after enzymatic pre-treatment are presented in Figure 7.

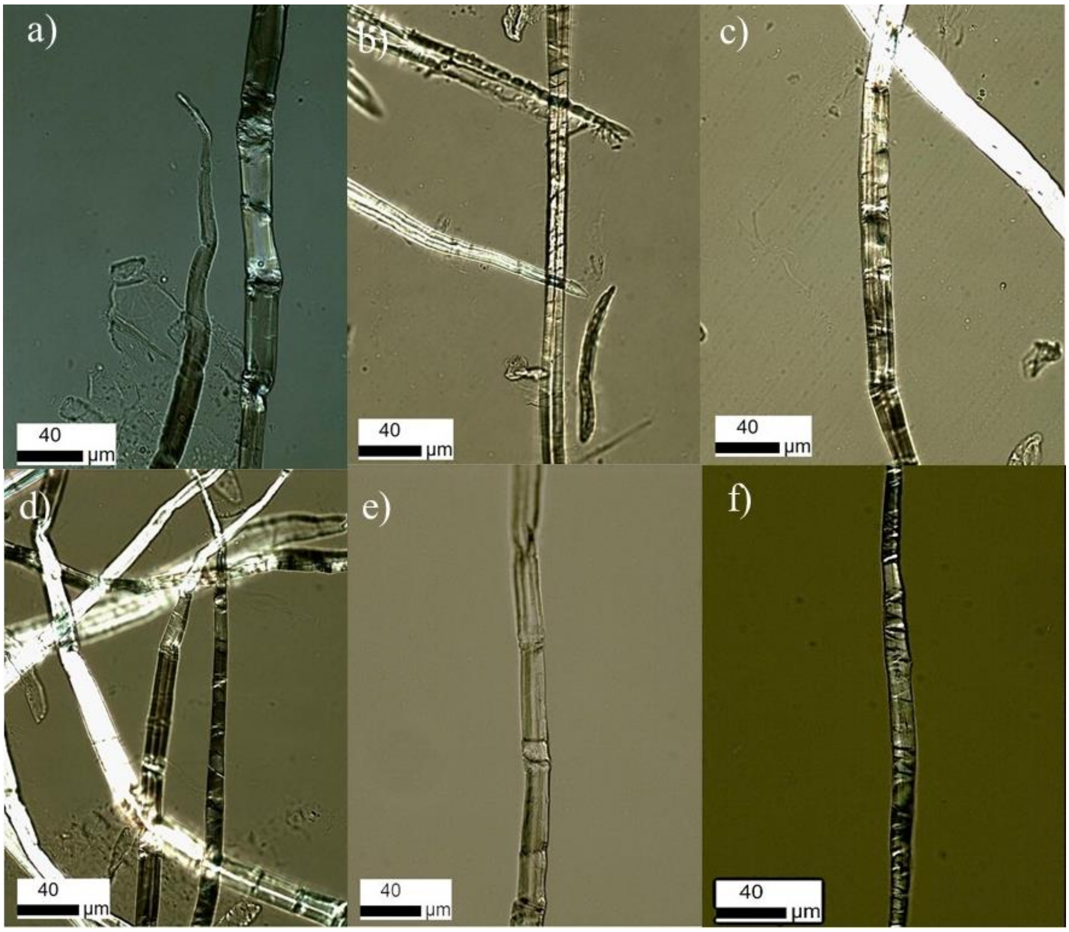


Figure 7. Typical ligh-pollarized microscopic images of *Eucalyptus sp*. bleached fibers, showing the dislocations in samples with enzymatic pre-treatments: a) control; b) control fibers with heating at 50°C; c) enzyme A; d) enzyme B; e) enzyme C; and f) enzyme D.

3.3 Influence of pre-treatments on curl index of the fibers

According to Page et al. [42] the tendency of the fibers to curl may be related to the origin of the fibers and the way fibers were pulped. Finding fibers without curvature is difficult because it is influenced by the mechanical process and the chemical pre-treatments during pulping [43]. The average and standard deviation values of the curl index for pre-treated unbleached and bleached fibers are presented in Figure 8.

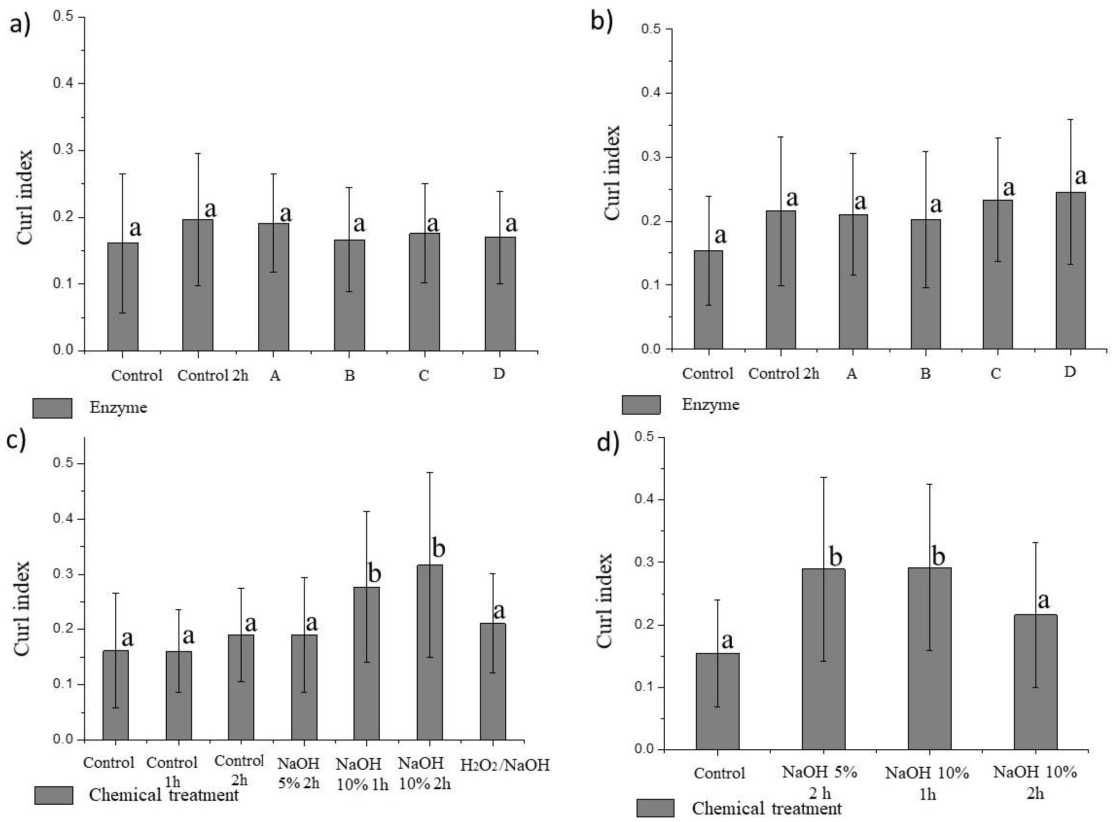


Figure 8. Average and standard deviation values of curl index for: a) unbleached fibers with enzymatic pre-treatment; b) bleached fibers with enzymatic pre-treatment; c) unbleached fibers with chemical pre-treatment; and d) bleached fibers with chemical pre-treatment. The letters on the bars correspond to scott-knott's statistic at 5%.

The increase of the fiber curl may be related to higher flexibility of the fibers, as a function of the increased dislocations. The dislocations tend to favor fiber twisting at these points, increasing the curl as observed by Kibblewhite [44]. Bending of the fiber interferes with the properties of the paper, affecting the load capacity, stress distribution and paper strength [45], and can be corrected by means of the refining processes [42].

The heating is a factor that contributed to increase the curvature of the fiber. The influence of temperature was observed by Page et al. [42] that reported the influence of heating time on the curl of the fibers. Zeng et al. [26], when applying different mechanical treatments in association with fiber heating evidenced an increase of the curved fibers.

Figure 9 shows some curved fibers after application of chemical and enzymatic pre-treatments. For unbleached fibers, the pre-treatments with NaOH in the higher concentrations (10%) were more influential in curl.

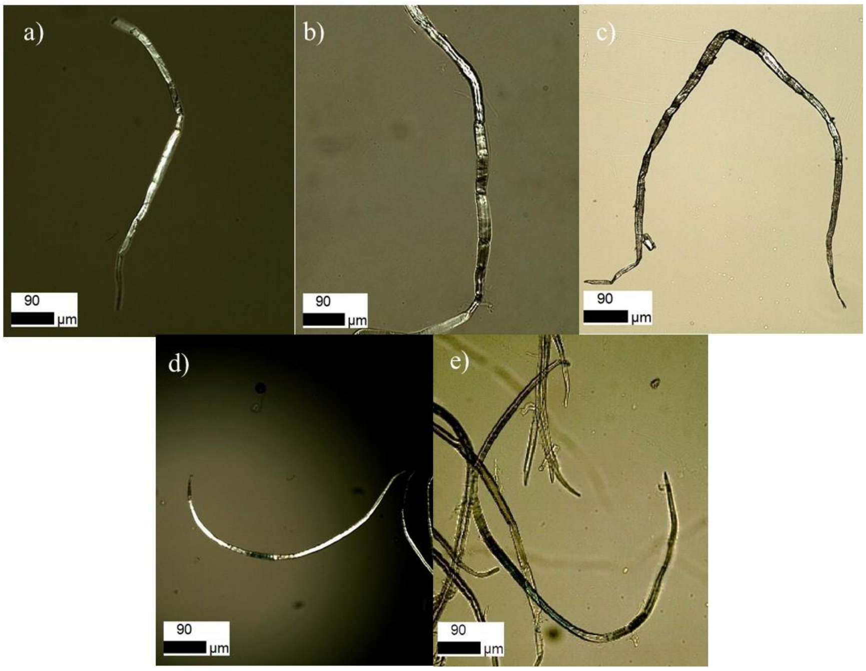


Figure 9. Typical light-polarized microscopic images of Eucalyptus sp. fibers, showing curl on pre-treated samples: a) bleached fiber pre-treatment with enzyme B; b) unbleached fiber pre-treatment wich enzyme A; c) unbleached fiber pre-treatment with NaOH 10% 2h; d) unbleached fiber pre-treatment with NaOH 10% 1h; and e) bleached fiber pre-treatment with NaOH 10% 1h.

3.4 Evolution of dislocations with the mechanical defibrillation

Figure 10 shows the evolution of the morphological properties of control bleached fibers (untreated and without heating) and of fibers treated with enzyme B along the increase of the mechanical defibrillation passages. ID increased with the increase of the number of defibrillation passages through the grinder. According to Siró and Plackett [46], the defibrillation cycles promote the breaking of hydrogen bond in the cell wall structure by shearing forces and individualization of the micro/nanofibrils. It was observed that the ID of the samples increased more rapidly for enzyme-treated fibers in the initial passages (first 15 passages) than for untreated samples (Figure 10a).

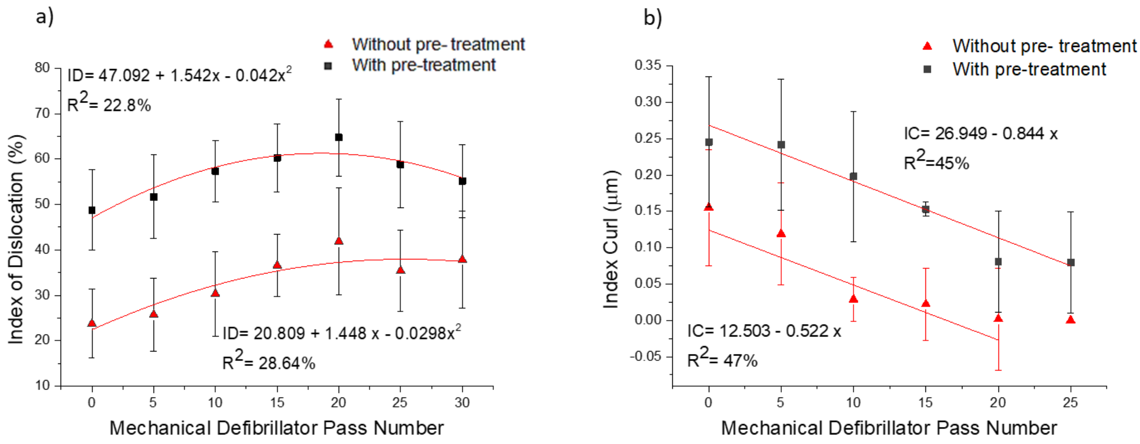


Figure 10. Evolution of the morphological properties of control bleached fibers (untreated and without heating) and of fibers treated with enzyme B along the increase of the mechanical defibrillation passages: a) index of dislocation (ID) of the fibers and b) fiber curl index. The points observed are the average results of 20 fibers analyzed.

The initial ID of the starting bleached pulp fibers (untreated and no passages through the defibrillator) was 24±3% (Figure 11a). This high ID value is associated to the mechanical stresses during the wood milling and kraft pulping processes that generate microcompression and modifications in the cell wall of the fibers [21, 26, 42, 47, 48].

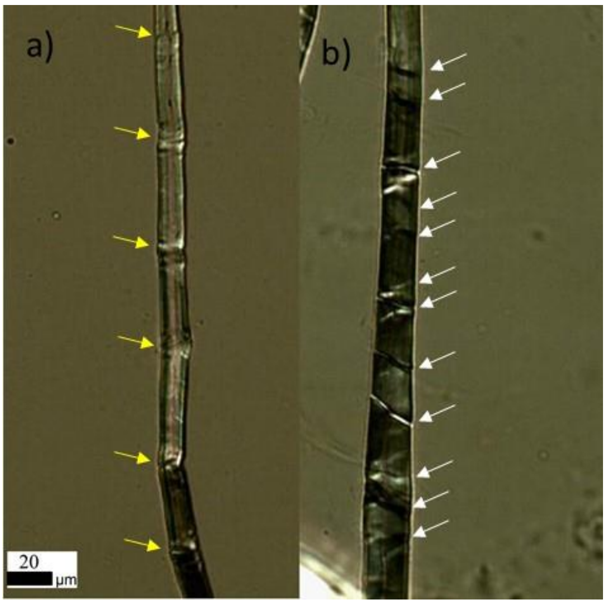


Figure 11. Typical ligh-pollarized microscopic images of *Eucalyptus* sp. fibers showing the dislocations (arrows) in samples with no passages through the mechanical

defibrillation: a) control (untreated); and b) with enzymatic pre-treatment using enzyme B.

Figure 12 shows control (untreated) fibers processed in different passages in the mechanical defibrillator. The ID values of the control fibers found their maximum with around 20 passages through the defibrillator, presenting values of around 42±12% (Figure 10a). The flexibility of the fibers may increase with the fiber bonds in the dislocations [21, 49], and consequence can increase the stretch and tearing resistance of pulp and paper sheets [50], also enhancing the qualities of softness and smooth [39].

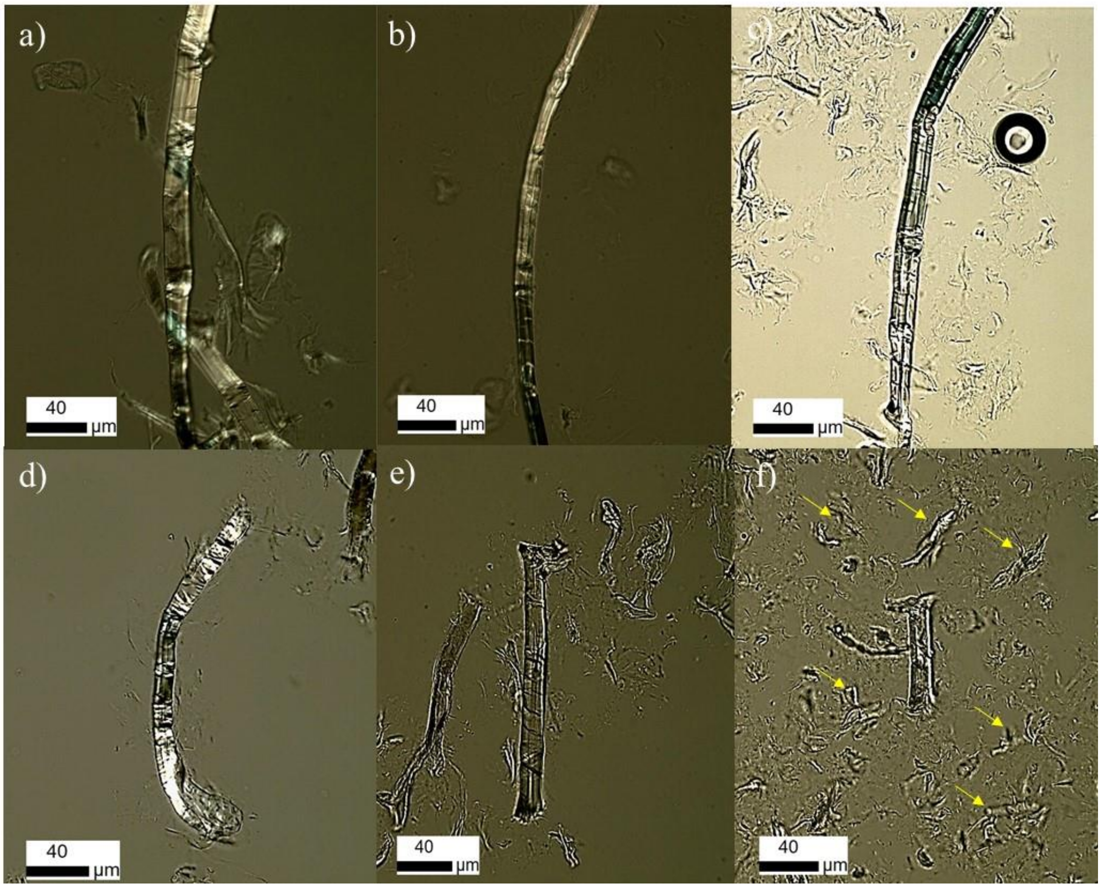


Figure 12. Typical light-polarized microscopic images of *Eucalyptus* sp. bleached fibers, showing dislocations on control (untreated) sample after the passages through the defibrillator: a) 5 passages; b) 10 passages; c) 15 passages; d) 20 passages; e) 25 passages; and f) 30 passages (arrows indicate the presence of fiber debris).

The ID for enzymatic treated fibers before defibrillation (no passage through the defibrillator, passage 0) was around 49±9% (Figure 11b), showing the increase of initial dislocations with the action of the enzymes, as aleady reported in the previous sections and also observed by Suchy et al. [37], who noticed that the higher concentration of enzymes in the fibers, caused an increase of the dislocations and appearance of cracks in the fibers. Regions with misorganized fibrils such as at dislocations and ponctuations in the fibre wall are more susceptible to enzymatic action and production of britle areas [38]. When the S1 layer already has the presence of irregular regions before enzymatic attack, this place are initial points for attack of the enzymes [16], promoting the breakdown of the local chemical bonds, reduction of recalcitrance and resulting in the appearance of new dislocations [18]. Enzymes (endoglucanase) acting within the cell wall are very specific and are able to disrupt cellulose chains in the fibrils and allow water to come in and break further hydrogen bonds, leading the enzymes to go deeper into the cell walls and contributing to forming new dislocations [51].

The ID of the samples with enzymatic pre-treatment varied from 49% (0 passage) to 65% (30 passages) during mechanical defibrillation (Figure 10a) and also reaches their maximum values at around 20 passages through the defibrillator. The typical light microscopy images of the fibers with dislocations for each number of passages is presented in Figure 13.

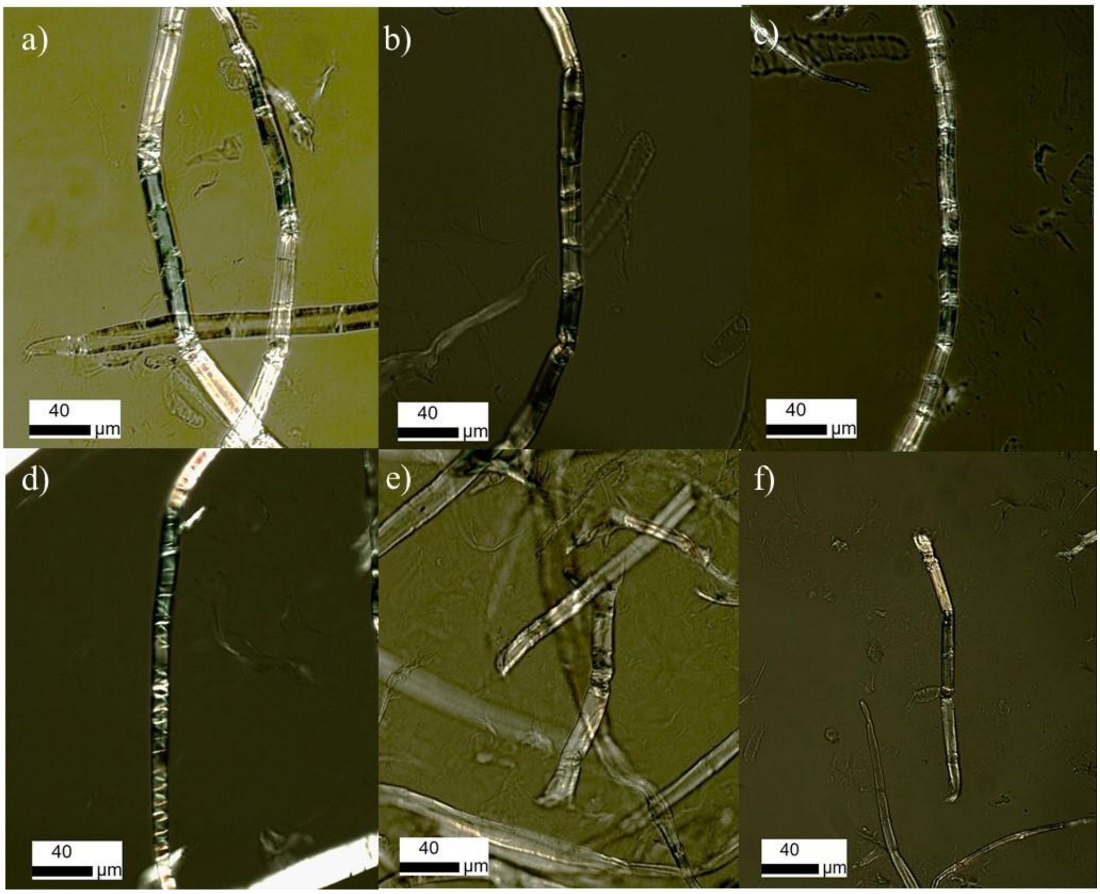


Figure 13. Typical light-polarized microscopic images of *Eucalyptus* sp. bleached fibers, showing dislocations in samples treated with enzyme B after the increasing number of passages through the defibrillator: a) 5 passages; b) 10 passages; c) 15 passages; d) 20 passages; e) 25 passages; and f) 30 passes (with small presence of fiber debris).

The increase of the passages in the mechanical defibrillator caused the reduction of fiber length. The passages in the mechanical defibrillator caused the fracture of the fiber and consequently its reduction in length, which may be justified by the rupture of the already britle regions in the dislocations [26]. The small bundles of fibrils were better dissociated for enzyme-treated fibers, as observed in Fig. 13f. Siddiqui et al. [52] analyzed the action of enzymes in long fibers, prior to the mechanical process and concluded that the enzyme treatment had little effect on the length of the fibers.

The curl of the enzyme-treated and control fibers also decreased with the evolution of the passages through the defibrillator, as shown in Figure 10b. The same behavior was observed by Rao [41] after the application of enzymes in the fibers and tracheids for a period of 10 h.

The angle of dislocations in the fibers during the defibrillation cycles for untreated and enzyme-treated fibers did not varied significantly along the mechanical defibrillation passages, whose the mean value was around 77±1° for untread fiber and 70±1° for enzyme-treated fibers. However, there were significant reduction of the angle of the dislocations in the enzyme-treated fibers in relation to the fibers without treatment (Figure 14).

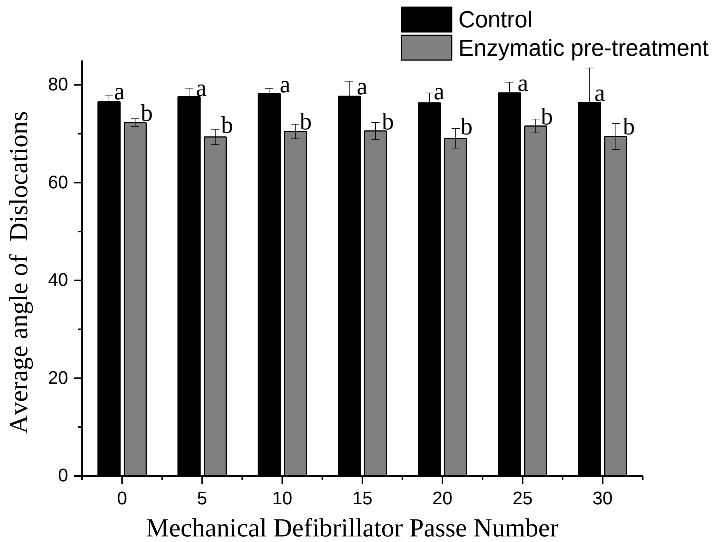


Figure 14. Variation of the angle of the dislocations of the bleached fibers, without treatment (control with no heating) and pre-treated with enzyme B, with the increase of the mechanical defibrillation passages. The letters for each bar correspond to the scott-knott test applied to 5% of significance.

The study on the angle of dislocations in several species was carried out by Dinwoodie et al. [34] that verified the angle of dislocations was associated to the angle of the micro/nanofibrils of the secondary wall, and also to their modulus of elasticity, being an inherent characteristic for each evaluated species. In the present study it was possible to observe that the enzymes generated dislocations with lower angles.

4 CONCLUSIONS

Chemical and enzymatic pre-treatments influence the index of dislocations. Chemical pre-treatments were able to increase the initial amount of index of dislocations (ID) for the unbleached and bleached fibers. The higher concentration of NaOH (10%) did not cause additional changes to ID relative to the lower NaOH concentration (5%). The angles of the dislocations were higher for the bleached fibers in comparison to the unbleached fibers. For the bleached fibers, the ID values were higher with the enzyme B and lower for enzyme C. Heating during the fiber treatment may have been a contributing factor to the increase of ID. The pre-treatment of the unbleached fibers did not promote increase of ID of the fibers, probably because of the high lignin content of the fibers. The angle of the dislocations was lower for the pre-treated bleached fibers. The chemical pre-treatments led to more curved fibers, which is directly related to the chemical changes (removing of hemicelluloses) and to the increase of the number of dislocations in the fibers. The enzymatic pre-treatment did not caused significant changes of curl of the fibers. These results suggest that the impact of the pre-treatments is directly related to the starting characteristics of the fibers, and may contribute to decrease of fiber recalcitrance, with the increase of fiber fractures and reduction of their stiffness and mechanical strentgh. The enzymatic pre-treatment led to high ID in relation to the control (untreated) fibers before the mechanical treatment. In the control and enzyme-treated fibers the ID increased until around 20 passages through the defibrillator. Dislocations generated by the enzymes were characterized by smaller angles, and may be an important parameter to identify the dislocations generated by the enzymatic treatments.

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TERCEIRA PARTE

CONCLUSÕES DESTA DISSERTAÇÃO

O estudo da morfologia da fibra, por meio da avaliação de *dislocations* ainda é pouco reportado na literatura, principalmente quando se relaciona a pré-tratamentos que antecedem a produção de nanofibrilas. Com isso o artigo produzido nessa pesquisa buscou complementar as lacunas existentes sobre o assunto a fim de permitir uma explicação mais detalhada sobre a parede celular da fibra (*dislocation*) e como ela se comporta ao ser submetida aos pré-tratamentos químicos e enzimáticos, e como isso pode influenciar no processo de desfibrilação.

O artigo apresentou dados referentes a relação das *dislocations* com aplicação de tratamentos químicos e enzimáticos sobre fibras branqueadas e não branqueadas de *Eucaliptus* sp e também a aplicação de pré-tratamento enzimático em fibras branqueadas combinado com o processo mecânico de desfibrilação. Os pré-tratamentos químicos e enzimáticos aplicados nas fibras não branqueadas possibilitaram aumento do índice de *dislocation*. Porém nas fibras branqueadas os pré-tratamentos químicos (NaOH e H_2O_2+NaOH) provocaram notório aumento deste índice de *dislocation* enquanto que no enzimático parece haver a redução ou surgimento de novas regiões de *dislocations* nas fibras, dependendo dos tipos de celulases utilizadas.

Os pré-tratamentos químicos e enzimáticos também geraram mudanças nos ângulos das *dislocations,* na constituição química e no índice de encurvamento das fibras. Em relação aos ângulos das *dislocations*, modificações maiores foram observadas para os pré-tratamentos químicos em fibras branqueadas enquanto que nas fibras não branqueadas a modificação dos ângulos foram mais brandas. Os pré-tratamentos acarretaram modificações no teor de glicose, dos açúcares constituintes das hemiceluloses e da lignina solúvel e insolúvel. Em relação ao índice de encurvamento, alterações significativas foram observadas para as fibras tratatadas quimicamente, enquanto que as fibras tratadas com enzimas não diferiram entre si.

Pré-tratamento enzimático do tipo endoglucanase também foi apresentado como uma possibilidade de otimização do processo de desfibrilação, por meio da geração de *dislocations* nas fibras. A desfibrilação mecânica realizada com as fibras sem tratamento causou aumento do índice de *dislocation*, como também a redução gradual do tamanho das fibras em cada passagem pelo desfibrilador. Para as fibras com pré-tratamento enzimático a redução do comprimento inicial das fibras não foi observada, entretanto a enzima aplicada nas fibras aumentou o índice de *dislocations* em comparação com as fibras sem tratamento, além de reduzir os ângulos das *dislocations*. A desfibrilação mecânica para ambas as polpas resultou em maior incidência de *dislocations* na 20ª passagem pelo desfibrilador.

Como sugestão para pesquisas futuras sobre o tema em questão, poderiam ser realizadas análises de *dislocations* em fibras submetidas a diferentes escalas de aquecimento para entender sua influência na parede celular das fibras. Poderia ser verificada a influência do processo de agitação das polpas em diferentes rotações, e a sua implicação no aparecimento das *dislocations*. Outro caminho que pode ser explorado é a confecção de papel a partir de fibras com diferentes intensidades de *dislocations* e realizar sua caracterização mecânica.