Testing the Influence of both Surfactant Addition and Refining Treatment on the Efficacy of the Xylanase Enzyme for Xylan removal in Bamboo Pulp

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Abstract

Methods for reducing chemical consumption associated with the removal of hemicellulose from lignocellulosic materials, and pulp brightening, for the production of dissolving pulps are explored. The use of the enzyme xylanase enhanced with the addition of surfactants has been shown to reduce hemicellulose in plant materials by breaking down xylan, a component of hemicellulose [10]. The addition of the enzyme xylanase has also been shown to aid in the bleaching process by reducing the amount of bleaching agents required [2]. But because refining pulp has been determined to release xylan through mechanical action, which is naturally trapped in the pores of the cell wall [4], this study aims to find the effects of the addition of Tween 80 (a non-ionic surfactant) and Bio-Terge AS-40 (an anionic surfactant) with xylanase (DP 407) when added to both refined and unrefined pulps. This study finds that refining the pulp had the greatest outcome for xylan removal and the addition of Tween 80 had no effect for additional xylan removal. Bio-Terge AS-40 had a negative impact on xylan removal for both refined and unrefined pulp. Refined pulp with the addition of xylanase and 1% (by weight of oven dried pulp) of Tween 80 had the greatest effect for pulp brightening. The brightness was increased from 39.95% ISO to 42.76% ISO. This same sample also showed the greatest improvement for whiteness, increasing from 79.65% to 81.13%. The addition of Tween 80 to the treated refined pulp also saw the greatest reduction in redness and yellowness.

Key words: Dissolving Pulps, Xylanase, Tween 80, Bio-Terge AS-40, Refining, Pulp Brightening

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Introduction

Dissolving pulps are used to create a wide variety of products from rayon to photographic film to cellophane and more [1]. Dissolving pulps have unique and special properties such as high brightness, even molecular weight distribution, and extremely low hemicellulose, lignin and resin content [1]. Removal of hemicellulose is critical for commercially produced dissolving pulps, but this removal is a chemical intensive process requiring a prehydrolysis sulphate process and/or an alkaline purification treatment [1]. As such, alternative pre-treatment methods for removing hemicellulose are being investigated so as to reduce the amount of chemicals needed to reach the final product. It is thought that using alternative methods could reduce the amount of effluents released by pulp and paper mills, thereby creating a more environmentally friendly method [1].

The addition of the enzyme xylanase has been confirmed in several studies to help break down hemicellulose in lignocellulosic materials [5]. It has been suggested that xylanase can help by synergistically enhancing the activity of cellulase [5].

Further research has suggested that the addition of additives such as non-ionic surfactants can enhance the performance of xylanase. There are three main thoughts as to why this may be so [5]:

a) The surfactant is acting as a lubricant for the enzyme, preventing it from binding to xylan which allows for more efficient enzyme recycling.
b) The surfactant contributes to cell wall collapse which exposes more surface area for the xylanase to work on.
c) The surfactant creates a protective barrier around the xylanase, guarding it from unfavorable temperatures and pH levels, essentially stabilizing the enzyme.

Additionally, the addition of anionic surfactants has been shown to increase the activity levels of other enzymes including cellulase, phytase, amylase and ligninase [17].

Mechanical refining of lignocellulosic materials has also been confirmed to release xylan that had been trapped in the pores of cell walls [4]. During mechanical refining, pulp fibers are forced between two metal plates: one rotor and one stator plate, each plate exhibiting a series of grooves and gaps. Through this intense shearing action, fibers experience a fibrillation of their surface which increases the surface area [8]. Mechanical refining also increases the amount of fines (fibers<0.2mm) in a pulp sample [8].

When xylanase is added to pulp before or after O_2 delignification it can help improve brightness and reduce the amount of chemicals needed for beaching [2]. Studies [2] have indicated that xylanase itself does not have a bleaching effect, but rather improves the bleach-ability of pulp by either:

a) Removing xylan from cell walls which would have later re-precipitated into the pores of cell walls or
b) By partially extracting chromophore groups attached to remaining xylan.

Tolan et al. [15] found this to be the case during a long term industrial trial. They found that after treating unrefined pulp with xylanase in their digesters and then bleaching, their pulp was brighter and showed fewer impurities.

Due to these findings, this study will test the effects of adding a non-ionic surfactant and an anionic surfactant each to refined and unrefined bamboo pulp samples in varying concentrations. Bamboo pulp was chosen due to its high xylan content [13]. The non-ionic surfactant that will be used is Tween 80 (polysorbate 80) which has been shown to improve xylanase activity [17]. The anionic surfactant we will be using is Bio-Terge AS-40 which does not appear to have ever been used for this purpose. For this experiment, the color space of the pulp will also be tested to see if xylanase can improve this quality. CEI L*a*b* color testing will be performed which plots the color of pulp somewhere on a sphere of color [11]. The north and south ends of the sphere on the L* axis are white and black respectively. The a* axis represents green and red where green is a negative value and red is positive. The b* axis represents blue and yellow where negative values show blue and positive values represent the color yellow. All figures are reported in degrees [11]. This study aims to gain a better understanding of the effectiveness of the use of surfactant with xylanase in conjunction with refining in terms of xylan removal from lignocellulosic materials and its brightening and color changing effects.

Method

Enzyme Activation

The bamboo pulp that was used had gone through O_2 delignification but, not pre-hydrolysis. The original consistency of the pulp was 64.76% of dry weight. Half the pulp was mechanically refined using a PFI Mill. The pulp was refined at a consistency of 10% dry weight at 9000 revolutions and at a gap of 2 millimeters. The remaining pulp was left unrefined.

A total of 14 samples were created at a target consistency of 5% dry weight and at pH 5. Seven of the samples contained unrefined pulp and the other seven contained refined pulp. Each of the 14 samples contained 5 grams of oven dry pulp. All samples were treated with a dosage of xylanase (DP 407) enzyme of 1500 mL/tonne of dry weight pulp as per the recommendation from the manufacturer. Six of the samples (three refined and three unrefined) received a dosage of Tween 80, a non-ionic surfactant, and six of the samples (three refined and three unrefined) received a dosage of Bio-Terge AS-40, an anionic surfactant. The remaining two samples (one refined and one unrefined) did not receive any surfactant. See table 1 for the experimental setup.

Sample ID	U-NO	U-T 0.5	U-T 1.0	U-T 2.0	R-NO	R-T 0.5	R-T 1.0	R-T 2.0
Xylanase Enzyme (mL/tonne of o.d. pulp wt.)	1500	1500	1500	1500	1500	1500	1500	1500
Tween 80 (% of o.d. pulp wt.)	0.0	0.5	1.0	2.0	0.0	0.5	1.0	2.0
Sample ID	**-**	**U-B 0.5**	**U-B 1.0**	**U-B 2.0**	**-**	**R-B 0.5**	**R-B 1.0**	**R-B 2.0**
Xylanase Enzyme (mL/tonne of o.d. pulp w.t.)	-	1500	1500	1500	-	1500	1500	1500
Bio-Terge AS-40 (% of o.d. pulp wt.)	-	0.5	1.0	2.0	-	0.5	1.0	2.0

Table 1 Experimental setup for enzyme reaction. Samples were diluted with 0.1 M sodium acetate – citric acid buffer (pH5), sealed in plastic bags and placed in a hot water bath at 70°C for 3 hours.

The samples were then diluted to a consistency of 5% dry weight using 0.1 M sodium acetate – citric acid buffer at pH 5. The bags were sealed and massaged thoroughly for proper mixing and then submerged in a hot water bath at 70°C for three hours. The samples were massaged vigorously every 30 minutes to ensure proper mixing. After three hours, the samples were immediately transferred to the deep-freeze to stop the reaction.

Collecting the filtrate

The frozen samples were transferred to a cold water bath (15°C) to thaw. The cold water bath was used in lieu of a hot water bath to avoid furthering the enzyme activity. The samples were then vacuum filtered through Grade 1 Whatman filter papers. The filtrate of each sample was collected in 50 mL falcon tubes and put into the freezer to preserve.

Monomer/oligomer analysis sample preparation

Two 19.303 mL samples were drawn from each filtrate sample and dispensed into septa bottles. Each sample then received 0.697 mL 72% sulfuric acid. The final volume of each treated sample was 20 mL. The bottles were then crimp sealed with a rubber stopper and a metal lid for an air tight seal.

A sugar solution was also prepared as a standard solution. The purpose of the sugar solution is to measure the degradation of sugars during the autoclaving process. The solution contained a mixture of the sugars arabinose, glucose, galactose, xylose, mannose and deionized water. From this stock solution, five standard solutions were created of varying sugar concentrations by mixing in different proportions of deionized water. All samples were made in 20 mL quantities and all samples contained 0.697 mL of 72% sulfuric acid. These samples were mixed in septa bottles, crimp sealed, and added to the tray of prepared filtrate samples.

All 33 samples (filtrate samples and standard solutions combined) were then autoclaved at 121°C for one hour. The samples were allowed to cool overnight.

High Performance Liquid Chromatography

From each autoclaved filtrate sample a total of two samples were created of two different concentrations. Of the two per sample, one contained 100 µL of the autoclaved filtrate solution and the second contained 50 µL of the autoclaved filtrate solution. Every sample was given a 50 µL dose of a

fucose solution (4mg/mL). Enough nanopure water was added to each sample to bring the volume of each sample to 1000µL. The five standard sugar solutions were then used to create seven standard solutions of varying concentrations. Each standard also received a dose of 50 µL of the fucose solution and the appropriate amount of nanopure water to bring the total volume of each of the seven standards to 1000 µL. At this point there were a total of 56 filtrate samples (four samples per filtrate sample) and seven standard samples. A 'blank' sample was included which contained only 1000 µL distilled water.

All 64 samples were thoroughly mixed using a Spin-Genie and then filtered through a 0.45 µm syringe filter before being placed into the High Performance Liquid Chromatography (HPLC). From the HPLC, the xylose concentration of each sample is found which will be used as a means to understand the effects of both refining and the addition of surfactants on xylan removal.

Brightness Testing

Bleaching

No pulp samples were bleached for this experiment in order to gain a better understanding of the role of xylanase in the brightening process.

Handsheets

A series of handsheets were made in conjunction with TAPPI standard T205 sp-2. Note that due to the limited pulp used in each enzyme treated sample, a total of two handsheets per sample was the maximum achievable. Therefore, slight changes to the TAPPI standard had to be made in order to compensate for the small amount of pulp available. The procedure used is explained below.

4 grams from each sample was taken and each diluted to 1333.33 grams using water that had been purified through a reverse osmosis filter in order to reach a consistency of 0.3% dry weight. Each individual sample was then disintegrated for 15000 revolutions (approx. 5 minutes). The previously mentioned steps were the only steps that had been modified as the TAPPI standard suggests disintegrating 24 grams at a consistency of 1.2% and diluting the slurry to 0.3% after disintegration. However, I only had 4 grams of treated pulp for each sample left to work with.

From this pulp slurry, 400 mL was used for each handsheet resulting in handsheets which had a grammage of 60 g/m². The handsheets were produced in a Lab-Tech Semi-Automatic Handsheet Maker. The handsheets were then stacked and pressed between blotting papers and metal plates at 50 psi for 5 minutes in the complimenting handsheet press. The blotting papers were then exchanged for fresh ones, the handsheets re-stacked and they were then pressed for an additional 2 minutes at 50 psi.

The handsheets were then transferred to the Controlled Temperature and Humidity (CTH) room (23°C ± 2°C, 50% RH ± 2%) and stretched on rings for 24 hrs.

Brightness Testing

The brightness and color space of the handsheets were tested using the Technidyne Color Touch system using the C(ISO) Pad test.

Results

Xylan Removal

As can be seen in table 2, the addition of Tween 80 only shows an effect for pulp that was left unrefined. The unrefined pulp samples saw an increase in xylose concentration as the amount of Tween 80 increased. For unrefined pulp, the sample with the greatest amount of xylan removal was that treated with 2% Tween 80. A peak for optimal Tween 80 addition was not found for unrefined pulp. As for the refined pulp, the addition of Tween 80 made very little difference no matter the amount added. Every refined sample showed higher concentrations of xylose than any unrefined sample.

As for the addition of the anionic surfactant, table 3 shows that for both refined and unrefined pulp, the addition of Bio-Terge AS-40 reduces xylan removal. The refined pulp samples showed a greater removal of xylan at every point when compared with any data point from the unrefined samples.

In both cases, the act of refining the pulp yielded the highest concentration of xylose, from which we can assume the most xylan removal. Xylose concentration appears to vary linearly with surfactant addition.

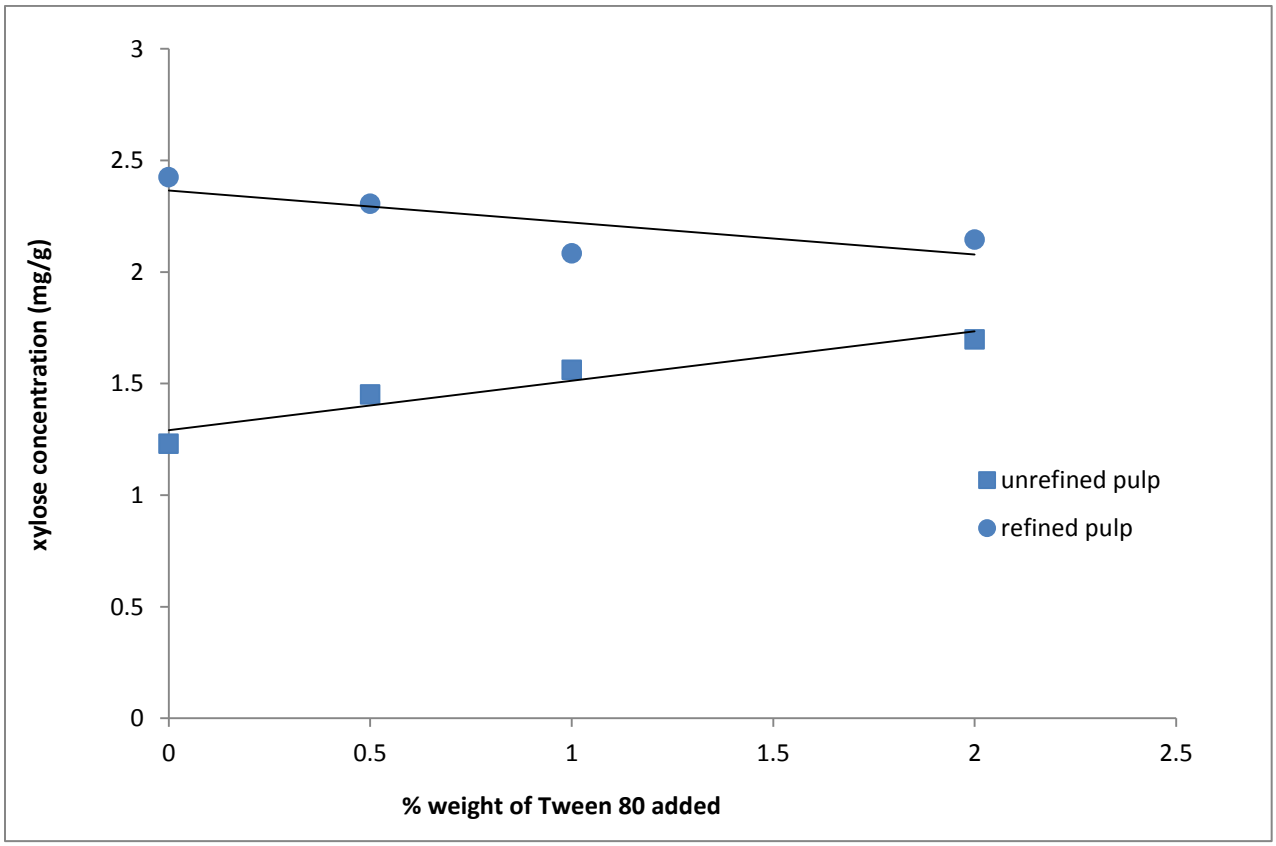

Table 2 Xylose concentration found in the filtrate of both refined and unrefined bamboo pulps which had been treated with xylanase enzyme (DP 407) varying amounts of Tween 80.

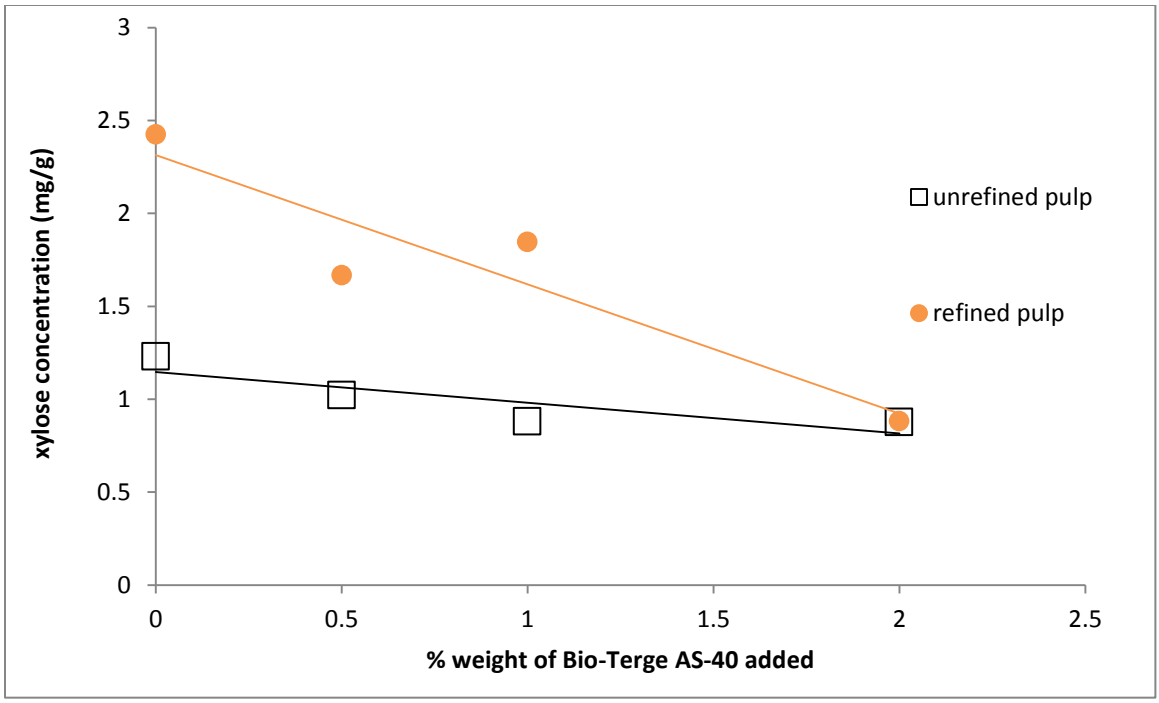

Table 3 Xylose concentration found in the filtrate of both refined and unrefined bamboo pulps which had been treated with xylanase enzyme (DP 407) varying amounts of Bio-Terge AS-40

Tables 2 and 3 were calculated as follows:

$$\left[\frac{\left(\frac{nC * min_{xyl}}{nC * min_{fuc}} - b_{ssc}\right)}{m_{ssc}}\right] * \left[\frac{\mu L_{af} + \mu L_{H^2O} + \mu L_{fuc}}{\mu L_{fuc}}\right] = xylose\ concentration\ (mg/g)$$

Where:

$nC * min_{xyl} = nanocurrents\ per\ minute: xylose$

$nC * min_{fuc} = nanocurrents\ per\ miute: fucose$

$b_{ssc} = y\ intercept\ of\ the\ standardized\ sugar\ curve$

$m_{ssc} = slope\ of\ the\ standardized\ sugar\ curve$

$\mu L_{af} = microliters\ of\ autoclaved\ filtrate\ sample\ used\ in\ the\ hplc\ solution$

$\mu L_{H^2O} = microliters\ of\ water\ used\ in\ the\ hplc\ solution$

$\mu L_{fuc} = microliters\ of\ fucose\ used\ in\ the\ hplc\ solution\ (50\ \mu L)$

Brightness and Color Space

Pulps that were left unrefined and pulps that were treated with the surfactant Bio-Terge AS-40 showed no improvement in brightness. However, the pulp that was refined and treated with 1% Tween 80 has shown increased brightness from 39.95% ISO to 42.76% ISO. This improvement has been realized without the addition of any bleaching agents.

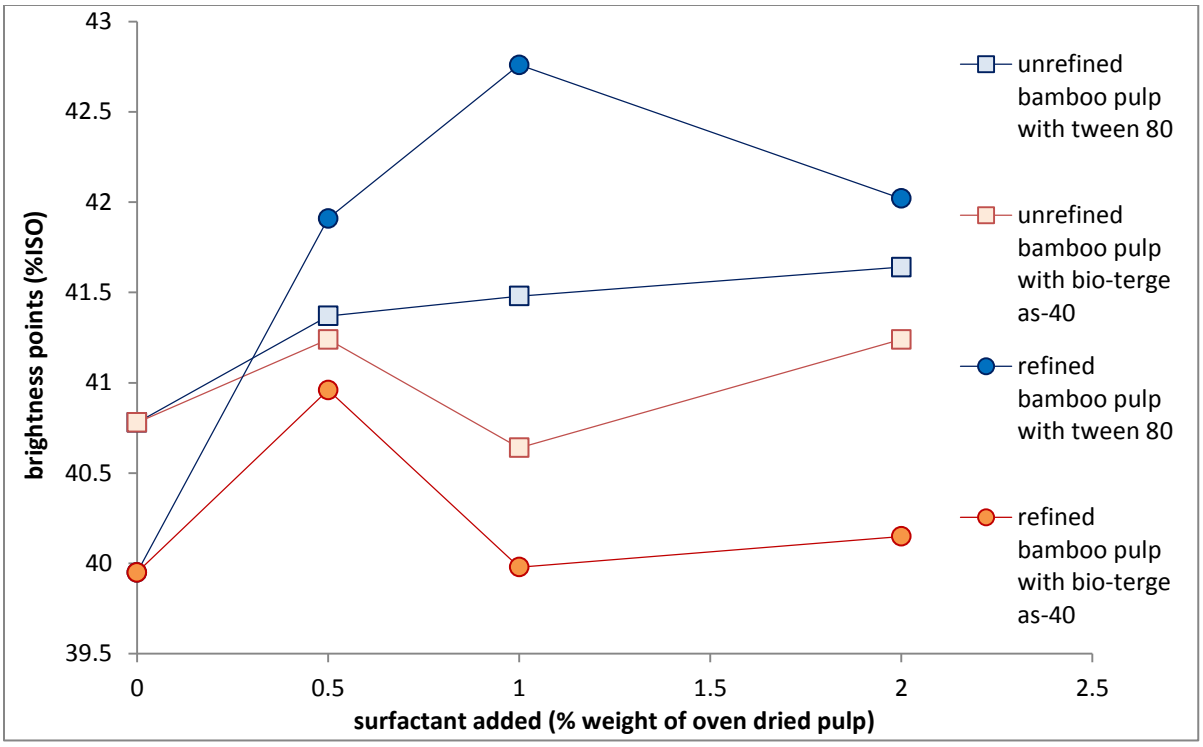

Table 4 Pulp brightness measured on the Technidyne Color Touch, C(ISO)

The greatest improvement in whiteness can be found in the samples that were both refined and treated with Tween 80 (Table 5). This is especially so when treated with Tween 80 in the amount of 1% of the weight of oven dried pulp: the whiteness improves from 79.65° to 81.13°.

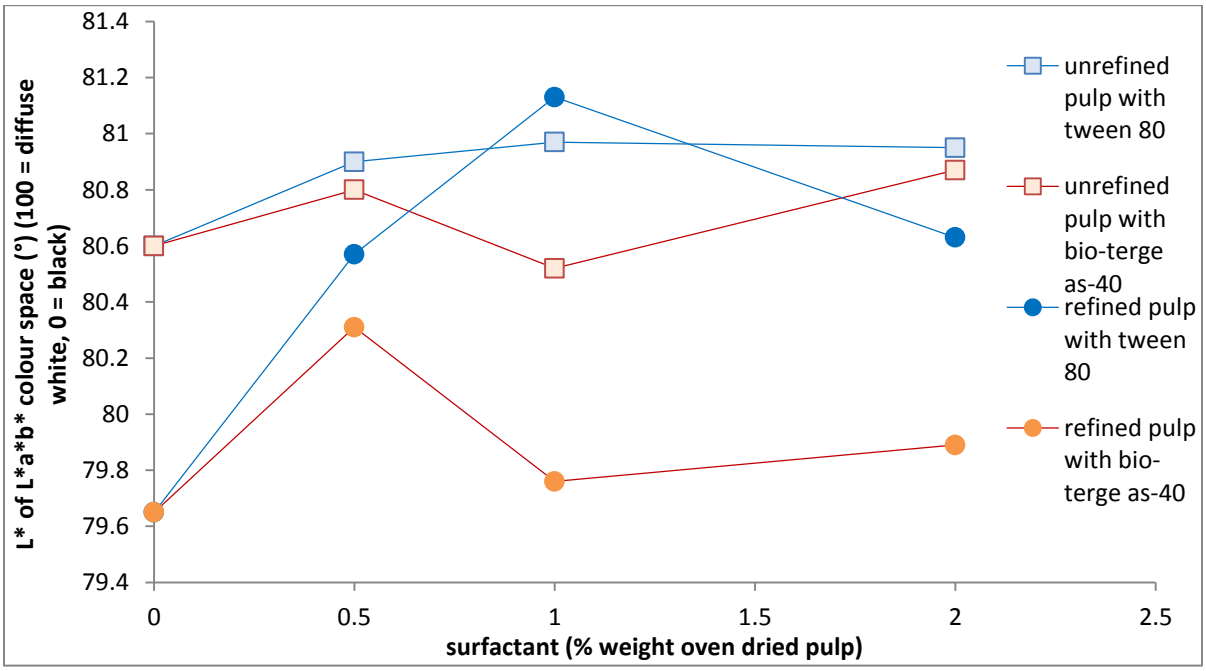

Table 5 L* of CEI L*a*b* color space (measured in degrees) where 100° equals a diffuse white and a value of 0° equals black

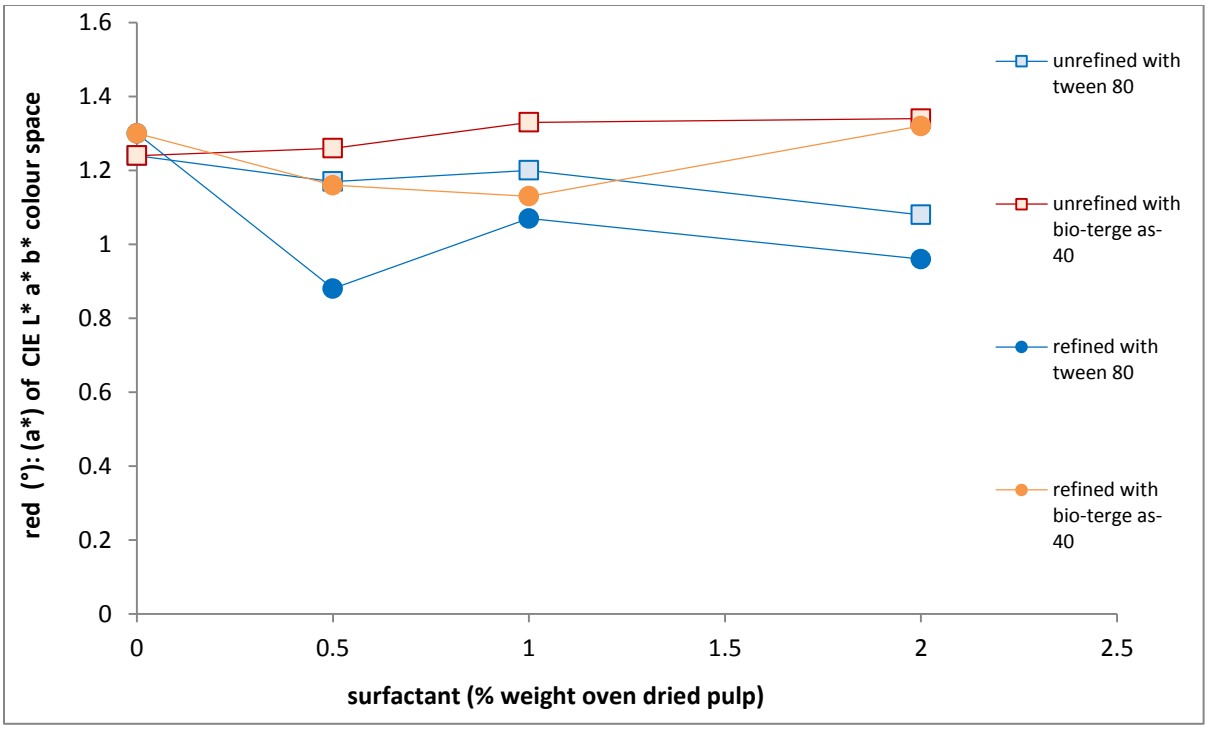

Table 6 a* of CEI L*a*b* color space. a* depicts the redness of pulp and is reported in degrees.

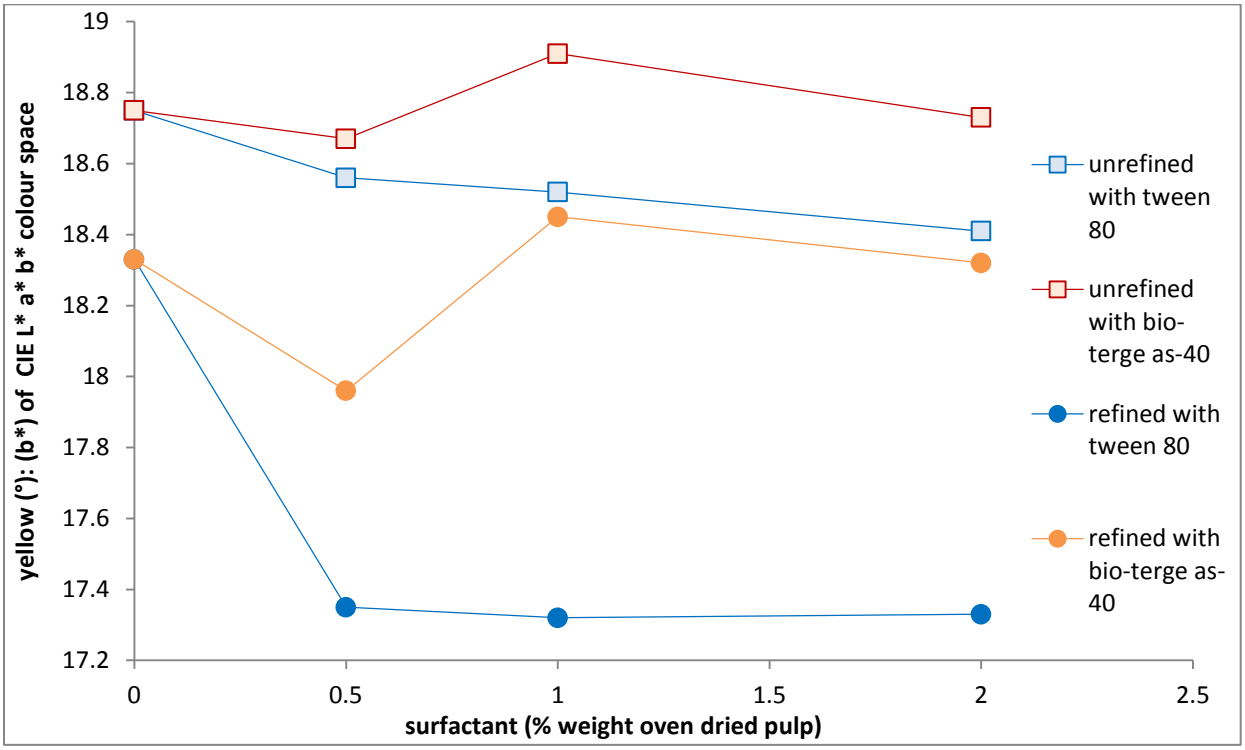

Table 7 b* of CEI L*a*b* color space. b* depicts the yellowness of pulp and is reported in degrees.

Minimal improvement is made in terms of reducing the redness of the pulp (see table 6). However, once again, the greatest improvement for redness reduction is made by the pulp that had been refined and treated with Tween 80 (Table 6). The redness was reduced by 0.42° for the sample which was refined and untreated with Tween 80 to the sample which had been refined and treated with 0.5% Tween 80.

A small improvement is seen in terms of reducing yellowness of the pulp for the sample that had been refined and treated with Tween 80 (see table 7). The yellowness was improved by 1.01° from the refined sample containing no Tween 80 to the sample which contained 1% Tween 80.

Discussion

Xylan Removal

Tween 80

According to the results for this experiment, refining and adding xylanase has the greatest effect for xylan removal and the addition of any surfactant is unnecessary for further xylan removal from refined pulp. Perhaps the mechanical release of xylan trapped in the pores of cell walls, enhanced with the addition of xylanase is a more effective method of xylan removal than adding a surfactant. It could be that the mechanical action on the fibers allows the xylan to be more accessible to xylanase. Alternatively, with the unrefined pulp, the surfactant Tween 80 may have allowed the xylanase enzyme to better recycle itself, but similar results to the refined samples were not realized because the xylan groups remained trapped in the pores of the cell walls rendering them unavailable to the xylanase enzyme.

Bio-Terge AS-40

As for the pulp treated with Bio-Terge AS-40, there was a definite decrease in xylanase activity with surfactant addition. It is possible that although anionic surfactants have been shown to improve activity for such enzymes as cellulase, phytase, amylase and linginase [17], that anionic surfactants, or Bio-Terge AS-40 in particular, hinder the effectiveness of the enzyme xylanase.

This finding could also show support for reason ‘c’ as previously mentioned: that the surfactant, in this case Bio-Terge AS-40, is creating a protective barrier around the enzyme rather than binding to the fiber itself. Wood fibers are known to have many anionic chemical groups attached to the surface such as carboxyl groups and hydroxyl groups [9]. Furthermore, if the fibers have gone through oxygen delignification, which this pulp has, then this would further the negative charge as many of the chemical groups on the surface would have gone through a reduction from being oxidized [9]. If the Bio-Terge AS-40 happened to coat the xylanase enzyme as previously suggested, then the xylanase would carry a negative charge as well and would have had a more difficult time reaching the reaction site due to the two negative charges repelling each other.

The refined pulps fared better as far as xylan removal and this could be because the increased surface area due to fibrillation allowed for greater access to xylan for xylanase.

Brightness and Color Space Improvement

The brightness, yellowness, whiteness and redness of pulp that was refined and treated with Tween 80, the non-ionic surfactant, improved. Xylanase itself is not a bleaching agent. It seems most plausible that the Tween 80 helped to improve the optical qualities of the pulp by washing away impurities in the pulp.

These findings could help to support the two theories as for why xylanase improves the bleach-ability of pulp. The xylanase helped with removing xylan from the cell walls which could have later re-precipitated into the pores of the cell walls. And the xylanase could have also helped to improve the optical qualities by partially removing chromophore groups from xylan which remained attached to the cell walls. Perhaps the addition of the surfactant Tween 80 further improved the optical qualities by helping to wash away, or prevent, the removed xylan and/or chromophore groups from reattaching to the cell walls.

It is possible that the Bio-Terge AS-40 did not show a marked improvement for the optical qualities of the paper because the negative charge played a role in preventing the xylanase from reaching the reaction sites on the cell walls. The xylanase was unable to do as much work as when paired with Tween 80. And perhaps the Bio-Terge AS-40 did a poorer job of washing away impurities due to its anionic properties. Chromophore groups and auxochrome groups are responsible for the color of pulp and both can be found attached to xylan [11]. Chromophore groups are chemical groups containing a π electron, for example an unsaturated bond (e.g. >C=CC=O, >C=NH, -N=N-). An auxochrome is responsible for enhancing the color, making it appear more saturated. These groups have isolated electron pairs (e.g. –OH, COOH, and –OR) [11]. So, it is perhaps because the Bio-Terge AS-40 carries with it a negative charge that it actually repelled some of the released negatively charged auxochrome groups allowing them to re-precipitate into the pores of the cell walls and thus did not wash them away as efficiently as Tween 80, the non-ionic surfactant.

Conclusion

Xylan removal from lignocellulosic materials is best achieved with the act of refining and the addition of xylanase. The addition of surfactant to refined pulp proved unnecessary. Tween 80 can help to remove xylan from unrefined pulp and Bio-Terge AS-40 shows a negative effect for xylan removal.

The addition of the non-ionic surfactant Tween 80 to refined pulp treated with xylanase did show an improvement in optical qualities. Most likely, the Tween 80 helped to wash away removed xylan groups, chromophore groups, auxochrome groups and impurities in the pulp.

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