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The Influence of Operating Parameters on the Biodelignification of *Eucalyptus globulus* Kraft Pulps in a Laccase–Violuric Acid System

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Abstract The potential of a laccase mediator system on the delignification of *Eucalyptus globulus* kraft pulp was investigated under diverse operating conditions. A series of enzymatic treatments were performed to determine how biodelignification is influenced by pulp consistency, oxygen pressure, reaction time, dose of laccase, and dose of mediator. The mediator used was violuric acid. The results revealed that the extent of biodelignification remains practically constant between 1% and 2.5% of pulp consistency then decreases. However, its effect is less pronounced than the mediator or laccase charge. In fact, an increase in the mediator charge from 1% to 4% leads to an increase in the extent of delignification (fixed laccase charge=20 IU/g and reaction time 120 min) from 28.4% to 52.2%. On the other hand, at a mediator charge of 4%, the increase of laccase charge from 10 to 40 IU/g has a similar impact on delignification. As for oxygen pressure, the extent of delignification increases with oxygen pressure, from 38.9% to 48.6%, when air and pure oxygen at 4 bar gauge are used. From the standpoint of reaction time, delignification is characterized by a very fast phase followed by a much slower one, leading to a plateau.

Keywords Pulp consistency · *Eucalyptus globulus* · Kraft pulp · Laccase · Oxygen pressure · Violuric acid

Introduction

Eucalyptus globulus is the most widely used raw material in the Portuguese pulp and paper industry. Kraft pulping is a dominant and very well-established process for brownstock production. For its part, bleaching faces a continuous challenge because of its threat to the environment.

In fact, biotechnology has provided alternative approaches to the challenges faced by the pulp and paper industry [1, 2]. For example, xylanases have been used to enhance the

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bleachable properties of pulp by instigating the spread of entrapped lignin from the cell wall [3, 4]. Furthermore, treatments with ligninolytic enzymes may provide milder and cleaner processes for biodelignification of kraft pulp, without damaging the cellulose [5, 6]. In addition, the new pulping and bleaching technologies will reduce the capital requirements of the pulp mill of the future, and biotechnology also seems attractive in this respect. The mild reaction conditions, which generally characterize enzymatic systems, coupled with their catalytic properties, i.e., selective action, suggest that enzyme-based bleaching systems would have low capital requirements and low operating costs and therefore would be useful for bleaching sequences in the future.

It has been proven that the use of laccase, in combination with a chemical mediator, is very effective in delignifying kraft pulps. Studies from several authors [7–9] have demonstrated that the laccase–mediator biobleaching system is very selective for lignin removal. Consequently, the use of a laccase–mediator system (LMS) seems a very attractive technology for biodelignifying hardwood and softwood kraft pulps. Several studies have shown the potential of laccase–mediator stages in an environmentally friendly full bleaching sequence [10–12].

In order to implement LMS at an industrial scale, the effect of operating factors (such as reaction time, laccase and mediator charges, pH, and pulp consistency) should be carefully studied. The consistency of the pulp and mixing conditions are key parameters when two or more phases are present in the reaction medium. Consistency determines the amount of extra water which, according to the intensity of mixing, then determines the thickness of the water film surrounding each fiber. This presents a mass transfer resistance to diffusion. In addition, consistency affects the rheological behavior of the pulp suspension, which determines the rate and the mix quality of the reagents, liquids or gases such as oxygen, as well as the specific interfacial gas–liquid and liquid–solid areas. Obviously, consistency also determines the concentration of the reagents and products in the reaction medium, which affect the reaction rates.

At a very low consistency, the fiber surface is almost completely accessible, and mass transfer resistance (outside the fibers) is basically non-existent; however, the concentration of reagent is low at a given chemical charge. Accordingly, the rate of chemical reaction can be relatively low. On the other hand, the thermal energy efficiency of the process is extremely low due to the considerable amount of water that must be heated. When the consistency increases, the initial reagent concentrations also increase, and the global reaction rate can rise, in spite of the expected increase in mass transfer resistance. On an industrial scale, the working consistency of a bleaching process is moderate [8% to 12% oven-dry basis (o.d.)] if one uses medium-consistency pumps and mixers. This technology enables the pulp suspension to fluidify and so mixes liquid and gaseous reagents with the pulp very efficiently. For laccase–mediator systems, the effect of consistency has received very little attention.

In the present study, we shall attempt to investigate the response of *E. globulus* kraft pulp, (with an initial kappa number of 15.5) to laccase–violuric acid treatment. This investigation will describe the influence of operating factors (such as pulp consistency, oxygen pressure, and charges of laccase and mediator) on biodelignification of *E. globulus* kraft pulps.

Materials and Methods

Pulp Production

Hardwood chips from 8-year-old *E. globulus* clone trees were used in this study to prepare unbleached conventional kraft pulp. Screened wood chips with an average thickness of

4 mm were pulped under the conditions of reaction summarized in Table 1. Experiments were carried out with 1,000 g of wood (oven-dry basis) in a forced circulation digester. The cooked chips were disintegrated, washed, and screened on an L&W screen with a slot 0.3 mm wide.

Chemicals

All the chemicals were of analytical grade, purchased from Aldrich, Sigma-Aldrich, Fluka, and Merck and used as received. The enzyme was obtained from *Trametes versicolor* laccase, has a redox potential of 780 mV [13], and was in the form of spray-dried powder (after removing the polysaccharide fraction by a freezing and thawing process) from Bioscreen e. K., Germany.

Laccase Activity

Laccase activity was measured by monitoring the rate of oxidation of syringaldazine. The substrate oxidation under controlled conditions was measured following extinction and observed to change at 525 nm every 15 s for at least 1 min, at room temperature.

The activity of the enzyme was 20 IU/mg of solid, expressed in International Units (IU); 1 IU is defined as 1 μmol oxidation of syringaldazine per minute per milligram of solid enzyme. The laccase was kept frozen at −20°C before use.

Laccase–Mediator Treatment

All LMS experiments were carried out using a 3.8-l capacity Parr reactor equipped with a pressure gauge, stirrer, and temperature controller. The pulp (25 g) was suspended at the required consistency [mass pulp/(mass pulp+mass water)] and placed in the reactor. In brief, the pulp slurry was heated to 45°C, and the appropriate amount of mediator was added by mixing over 5 min. Violuric acid was used as the mediator; with a dose range between 1% and 6% (on o.d. pulp). The pulp slurry was then adjusted to pH 4.5 with either glacial acetic acid or a saturated sodium bicarbonate solution. Afterwards, the appropriate amount of laccase was introduced into the well-stirred mixture. The following doses were studied: 10, 20, 40, and 60 IU/g of pulp (o.d.). The reactor was sealed and pressurized with oxygen. Upon completion of the reaction, the pulp was removed from the reactor and washed in a Büchner funnel with distilled water.

Table 1 Conventional kraft cooking conditions of *E. globulus* and pulp yields.

Cooking conditions	*E. globulus*
Active alkali charge on wood (%)	19.0
Sulfidity (%)	30.0
Liquor/wood ratio (l/kg)	4:1
Cooking temperature (°C)	160
Time to temperature (min)	90
Time at temperature (min)	60
Pulp yield on wood (%)	55.1
Rejects on wood (%)	0.5

Alkaline Extraction Stage

Following the laccase–mediator system treatment and washing the pulp, an alkaline extraction stage (E) was performed in a 4-mm thick heat-resistant plastic bag at 10% consistency, 70°C, for 1 h, using a 2% charge of NaOH. After treatment, the pulp was removed from the bag, filtered, and thoroughly washed with distilled water until the filtrate was colorless and pH neutral.

Pulp Characterization

The lignin content of the unbleached pulps, as well as that of the laccase-treated pulps, was determined by $KMnO_4$ oxidation of the pulp in accordance with ISO 302 standard and expressed as kappa number. Each reported kappa number represents the average of three laccase–mediator treatments followed by alkaline extraction LMS(E) experiments.

Results and Discussion

The goal of this study was to investigate the influence of the main operating parameters on the biodelignification of *E. globulus* kraft pulp. The operating variables investigated were pulp consistency, oxygen pressure, reaction time, and laccase and mediator charges. Delignification was evaluated using kappa numbers. Although hexenuronic acids can represent over 30% of the $KMnO_4$ consumption in kappa numbers for unbleached pulps, it was shown in a previous paper that, with the same kind of pulp [6], the remotion of HexA in LMS(E) treatment is negligible. At most it represents half one unit of kappa number. In addition, the remotion is not significantly dependent on reaction conditions.

Consistency

A comparative study was carried out to determine if the biodelignification response of laccase–mediator system is influenced by the consistency of the pulp slurry (1%, 2.5%, 4%, and 10% consistency). The experiments were performed in accordance with the general procedure described in the experimental section, and the following parameters have remained fixed: amount of pulp=25 g o.d.; laccase dose=20 IU/g; mediator=4%; temperature=45°C; time=120 min; O_2 pressure=6 bar; pH=4.5. Only the total reactional volume was changed as a consequence of pulp consistency.

Figure 1 shows the evolution of kappa number reduction with the pulp consistency, and a consistent decrease in delignification is regularly observed, particularly at 10% consistency. The extent of delignification decreases from 47.7%, at 1% consistency, to 41.9%, at 10% consistency. Although the lowest initial concentration of both laccase and mediator occurs during the 1% consistency experiments, the extent of delignification is the highest. When the consistency is increased from 1% to 2.5%, delignification remains practically at the same level, although the initial concentration of laccase and mediator is more than twice as high. Our previous experience with the Parr reactor and its mixer indicates that its performance is good at least until 3% consistency. Consequently, taking into account the increase in initial concentration, we would expect that the biodelignification rate and perhaps the extent of biodelignification would be higher at 2.5% than at 1%, which is not the case. In former experiments performed by other

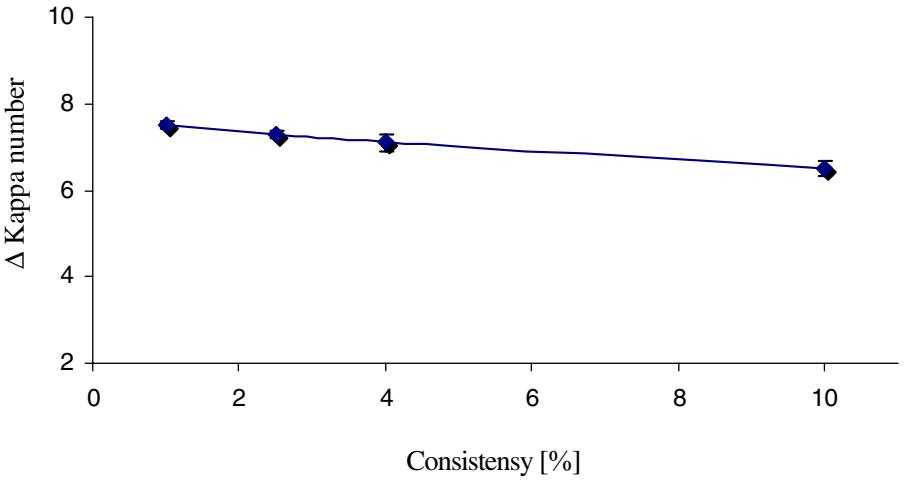

Fig. 1 Kappa number reduction vs. pulp consistency, in biodelignification of *E. globulus* kraft pulp

groups, the performance normally increased by raising the consistency, but no mixing was carried out during the treatment [5]. As the consistency increases to 10%, the extent of delignification decreases, in spite of having the highest initial concentration of both laccase and mediator, which is probably a consequence of poor mixing and/or nonactivation of the enzyme.

All the remaining laccase–mediator system treatments in this paper take into account these results of consistency testing and were carried out at 2.5% consistency.

Oxygen Pressure

Increasing the oxygen pressure will enhance the concentration of dissolved oxygen in its aqueous phase. The solubility of oxygen in water increases from 39 to 296 mg/l, when the pressure of oxygen increases from 1 to 8 bar. The amount of dissolved oxygen also depends on the temperature, pH, reaction medium, etc. Additionally, it has been noticed that oxygen pressure significantly affects redox potential [14]. The effect of oxygen pressure was investigated at 2.5% consistency and under the same reaction conditions as were used for the consistency study, except for oxygen pressure, which took on numbers of 1, 4, 6, and 10 bar. It was also tested with air from the atmosphere. Delignification increases from 38.9% with air to 47.9% with oxygen at 11 bar.

The data plotted in Fig. 2 show that there is a significantly different effect of oxygen under pressure from the atmospheric air environment until we reach 5 bar (4 bar gauge); above 5 bar, the effect seems to be negligible. Nelson et al. [9] also reported decreasing effectiveness when oxygen at 780 kPa was replaced by air. At 390 kPa, this study also observed lower performance, which is not the case in this work. It should be noted, however, that we operate at 2.5% of consistency and that the reactor was equipped with a mechanical mixer specially designed for this kind of reactions, while Nelson et al. [9] operated at 10% consistency and with a rotating vessel. Therefore, the oxygen transfer rate in these experiments is certainly better, and the same oxygen concentration in the liquid phase is probably attained with a lower oxygen pressure.

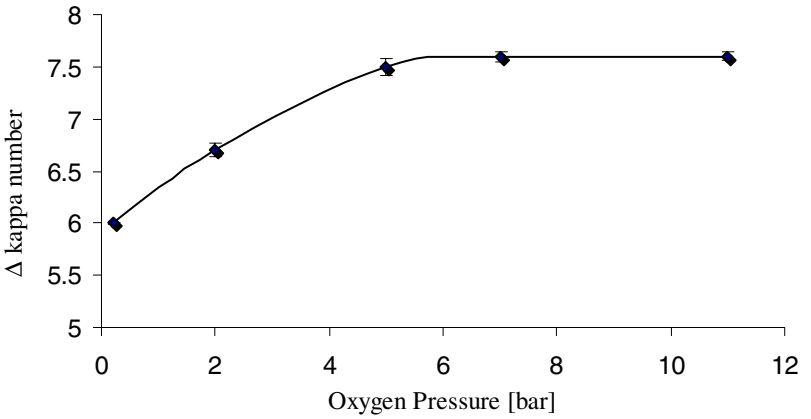

Fig. 2 Effect of oxygen pressure on kappa number reduction (average of three experiments), for $LMS_{VA}(E)$ treatments (fixed conditions: laccase dose=20 IU/g; mediator dose=4%; temperature=45°C; time=2 h; pH=4.5)

Reaction Time

The choice of reaction time for the bleaching stage on the industrial scale is a compromise between the kinetics of delignification and degradation of carbohydrates. Traditional reaction times are of the order of hours, although several kinetic studies at low and ultra-low consistency found that in most cases a few minutes are enough. The reason behind the usually long reaction times is probably a function of the absence of proper mixing inside the bleaching reactor (bleaching tower) located after the mixer, where the chemicals are mixed with the pulp suspension. Global kinetics depends on the chemical reaction but also on mass transfer resistances [15, 16]. According to the reaction conditions, global kinetics can be controlled by the transfer and diffusion of the species in the medium or by the chemical reactions, or both.

Fixing consistency at 2.5%, laccase dose at 20 IU/g of pulp, mediator dose at 4%; temperature at 45°C, O_2 pressure at 6 bar, and pH=4.5, we investigated the following reaction times: 30, 60, 120, and 240 min. Figure 3 illustrates the change in kappa number reduction with reaction time.

In accordance with the results reported for other bleaching stages [15, 16], Fig. 3 allows us to distinguish two reaction phases. A rapid phase, during the first minutes of the reaction, followed by a slow phase. At least two hypotheses have been advanced to justify the existence of the two: one related to lignin reactivity and another related to the accessibility of lignin. According to the chemical hypothesis, the first stage marks the rapid degradation of the reactive and easily oxidizable lignin [17], while the second stage is due to the less reactive lignin reactions. According to the accessibility theory, the different reaction rates are explained by the more or less rapid accessibility of the lignin in the pulp [18, 19]. Although the effect of time on delignification is significant, most of the lignin is removed after a 30-min treatment, thus highlighting the efficiency of the system. A further increase in reaction time does not yield much higher levels of biodelignification. Essentially, the data seems to suggest that delignification approaches a plateau at reaction times greater than 1 h. In the next section, we shall see that the extent of delignification at this plateau depends on the mediator and laccase charges.

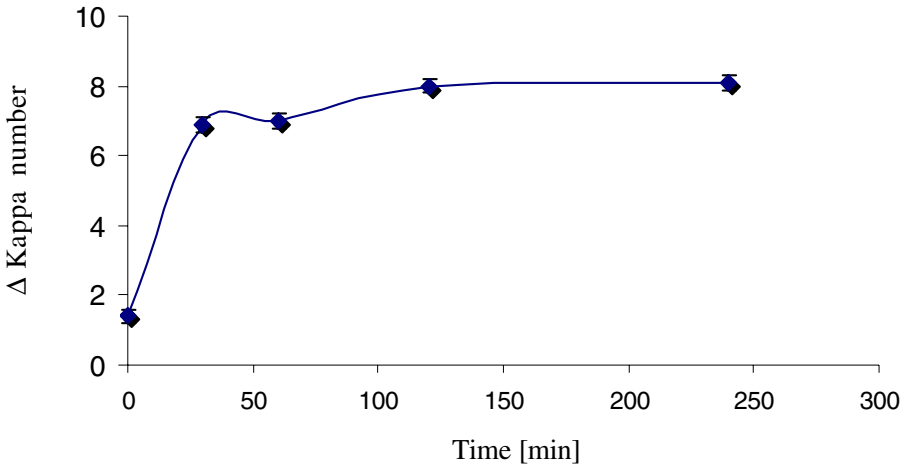

Fig. 3 Δ kappa number vs. reaction time

Mediator Dose

The dose of mediator also has an important effect on biodelignification. Figure 4 represents the biodelignification response at the various doses of violuric acid (VA) in the presence of fixed dose of laccase (20 IU/g). The drop in kappa number substantially increased as the mediator dose was increased from 1% to 6%. In addition, the rate of delignification in the initial phase is also highly dependent on the violuric acid charge.

At a dose of laccase of 20 IU/g of pulp, a charge of 4% of mediator seems to be recommended. These data agree substantially with those reported by Barreca et al. [20].

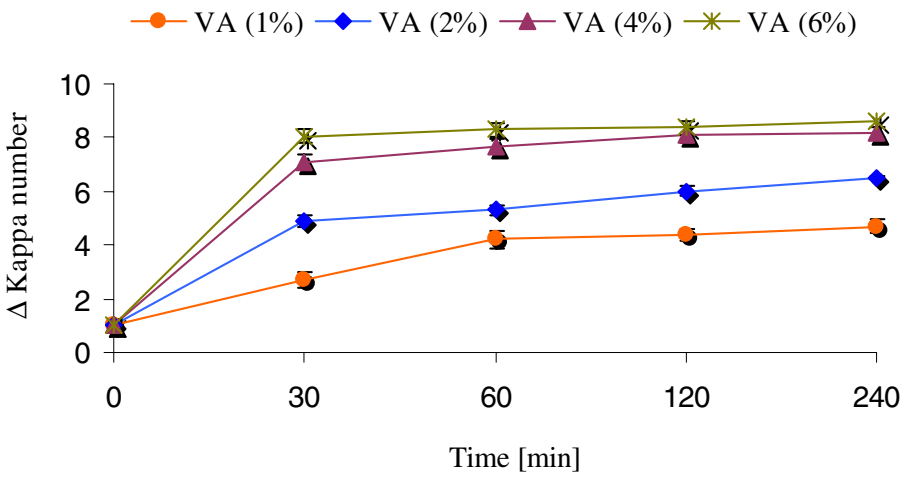

Fig. 4 Δ kappa number reductions vs. time, for different violuric acid doses, at a laccase dose of 20 IU/g of pulp

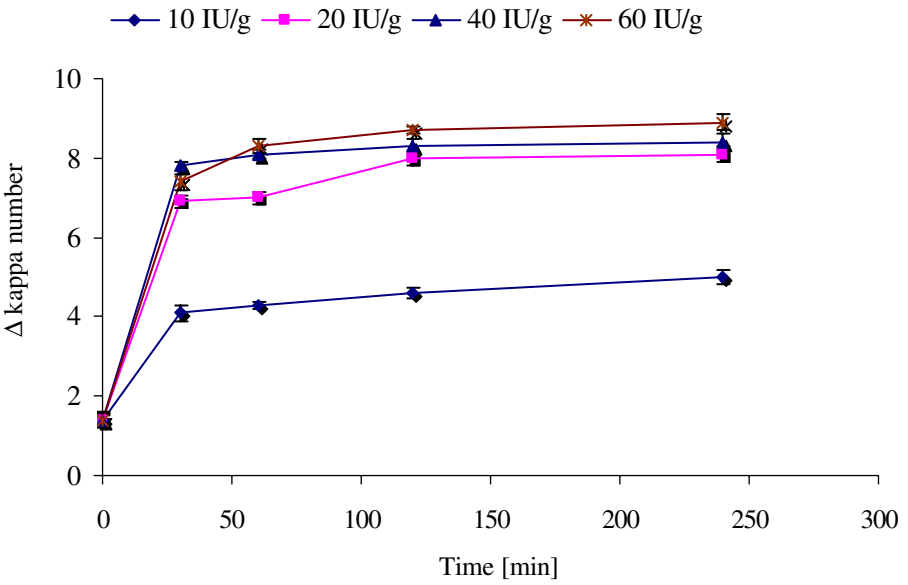

Fig. 5 Δ kappa number reduction vs. time, at 4% of mediator and different laccase doses

Laccase Dose

Figure 5 shows that biodelignification was drastically enhanced as the enzyme dose was increased from 10 to 20 IU/g. However, when the laccase dose was further doubled to 40 IU/g, efficiency in biodelignification did not increase significantly. If the dose of laccase was increased further, the delignification rate and its extent practically leveled off. This

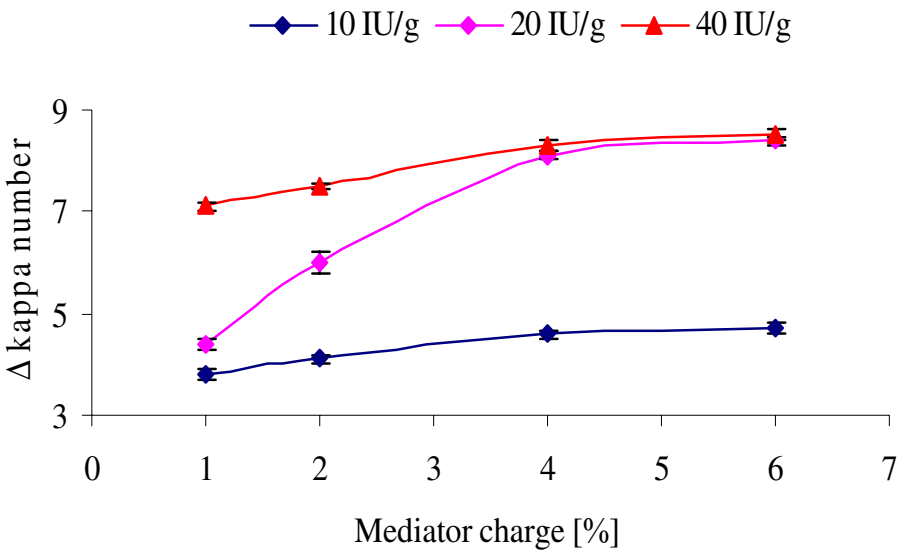

Fig. 6 Effect of laccase and violuric acid–mediator on biodelignification

means that at a laccase dose higher than 40 IU/g, the system is saturated, making any further addition of enzyme ineffective for improving biodelignification.

Interaction Between the Mediator and Laccase

The interaction between the violuric acid and laccase doses and biodelignification can be seen in Fig. 6, for a reaction time of 120 min. Using this reaction time, we are in the plateau zone, where delignification remains practically constant, and therefore, we are evaluating the effect of laccase and mediator charges on maximum delignification. The extent of biodelignification increased drastically as the dose of mediator was increased for an intermediate dose of laccase (20 IU/g). For the lowest (10 IU/g) and the highest (40 IU/g) laccase doses, the effect of the mediator is exposed, but it is significantly lower than for an intermediate laccase level. These results seem to indicate that at 10 IU/g, the laccase oxidation/reduction cycle is the limiting factor on the global process, involving laccase and mediator redox cycles and the reduction of oxygen to water on one hand and oxidation of lignin on the other hand. At 40 IU/g, the extent of biodelignification is so high that the improvement in kappa number is small. These results can hypothetically be explained by assuming that the mediator redox cycle is very effective if the laccase cycle drives it, but other mechanisms, like enzyme nonactivation or lignin inaccessibility, could be present.

Conclusions

The data presented in this manuscript have confirmed the suitability of treatment with laccase combined with VA as a mediator to biodelignify *E. globulus* kraft pulps. The operating variables such as pulp consistency, oxygen pressure, time, dose of laccase, and dose of violuric acid have an effect on biodelignification. Delignification efficiency decreased at a laboratory scale when the pulp consistency was increased from 1% to 10%, but this can certainly be overcome at an industrial scale with the high-intensity mixer currently used.

An increase in the dose of VA from 1% to 6% (on o.d. pulp) yielded a substantial increase in the extent of biodelignification from 28.4% to 54.2%, at a laccase charge of 20 IU/g and a reaction time of 120 min. Most of the lignin was removed within the first 30 min, but small increases in biodelignification were observed for each increment in reaction time. A very positive effect on biodelignification was also noticed as the dose of laccase was increased from 10 to 20 IU/g. Further increases in the dose of laccase led to only small improvements. Interactions between laccase and mediator doses were also observed. In the future, additional mechanistic studies will be performed to investigate these interactions, using the initial rate approach.

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