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Treball Final de Grau

Decolourization of recycled paper by biotechnological methods
Decoloració del paper reciclat per mètodes biotecnològics

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A la Doctora Cristina Valls Vidal, tutora del treball de fi de grau, per la seva exigència, el constant suport, l'orientació i la confiança d'acompanyar-me durant la trajectòria del projecte. A tots aquells altres professors del Grup de recerca en Enginyeria Paperera (Celbiotech)" que han col·laborat tot aportant el seu gra de sorra en el meu projecte, amb suggeriments i plantejament de qüestions que m'han inquietat i han donat impuls a la meva constància. A les meves amistats, les meves companyes del laboratori del "Grup de recerca en Enginyeria Paperera (Celbiotech)"; també vull esmentar el gran ajut d'Álvaro González i Helena Marques pels seus assessoraments lingüístic i visió crítica externa. I com no, la família: els meus pares Josep Manel i Anna amb el seu estímul, recolzament i estima inqüestionable. A la meva parella Nora Caviedes per no rendir-se mai amb mi i ajudar-me en tot moment.

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1. SUMMARY

This project is done in collaboration with the "Grup de recerca en Enginyeria Paperera (Celbiotech)" of the Universitat Politècnica de Catalunya (UPC), which investigates new methods for deinking recycled fibres, using biotechnological methods and ozone.

The main objective is to increase the use of fibres recycled from coloured paper. One of the main problems of this raw material is the presence of contaminants, such as colourants, which hinder its potential and causes the obtained product to loss part of its value. A big inconvenience of using recycled fibres from the disintegration of coloured paper as raw material is that the type of colourant used is unknown. Therefore, parameters based on measuring the optical properties of the paper sheets where used to determine the efficiency of the colour elimination sequences. The first part consisted in applying different enzymatic stages (laccase-mediator system) and a bleaching chemical agent to the red/black recycled fibres for determining the efficiency of eliminating colour in the dough.

Afterwards, different biotechnological agents (enzymes) and chemical treatments will be applied to the red recycled fibres using a combination of mediators and doubling the dose of mediator used in the red fibres in order to determine which treatment gives the best results. Later on, we will apply a selected colour elimination sequence to the red and black recycled fibres, with the aim of determining the best biochemical sequence. At the same time, studies with another oxidizing agent (ozone) will be done in order to determine if the enzymatic stages can substitute the ozone stage.

Last part of the project consists in doing physical test to determine if the paper has lost any of its properties after the treatments. The enzymatic activity of the effluents will be calculated as well in order to determine which mediator performed better and the possibility of recirculating these effluents will be studied.

Keywords: Decolourization, recycled paper, biotechnological methods.

2. RESUM

El present treball s'emmarca dins del Grup de Recerca en Enginyeria Paperera (Celbiotech) de la Universitat Politècnica de Catalunya (UPC), encaminada a la recerca de nous mètodes de decolorar fibres reciclades, mitjançant mètodes biotecnològics i ozó.

L'objectiu d'aquest treball és potenciar l'ús de fibres reciclades que provenen dels papers acolorits en la fabricació de paper d'alt valor afegit. Un dels principals problemes d'aquesta matèria prima és la presència de contaminants, com els colorants, fent que no sigui tant atractiu i determina la seva utilització com a paper de menys valor afegit. Un gran inconvenient d'utilitzar com a matèria prima fibres reciclades procedents d'una desintegració del paper acolorit, és que es desconeixen els tipus de colorants utilitzats i, per aquest motiu, s'han utilitzat per determinar l'eficàcia de les seqüencies d'eliminació del color paràmetres basats en la mesura de les propietats òptiques de les fulles de paper.

En una primera etapa de la investigació s'apliquen diverses etapes enzimàtiques (sistema lacasa-mediador) i un agent químic blanquejant a les fibres reciclades vermelles/negre per determinar l'eficàcia de l'eliminació del color de la pasta. Posteriorment s'aplicaran agents biotecnològics (enzims) i tractaments químics a les fibres reciclades vermelles utilitzant combinació de mediadors i duplicant la dosis de mediador utilitzat en les fibres vermelles per determinar el millor tractament. Seguidament s'aplicarà una seqüencia d'eliminació de color seleccionada a les fibres reciclades de color vermell i negre, amb l'objectiu de determinar la millor seqüencia bioquímica d'eliminació del color. També es realitzaran estudis amb un altre agent oxidant (Ozó) per determinar si les etapes enzimàtiques poden substituir l'etapa d'ozó.

Per últim, es realitzaran proves físiques per determinar si els papers han perdut propietats després dels tractaments i es mesurarà l'activitat enzimàtica residual.

Paraules clau: Decoloració, paper reciclat, mètodes biotecnològics.

3. INTRODUCTION

3.1. HISTORY OF PAPER

The first paper production is from 105 B.C. in China. The paper is recycled since it began to be produced in masse in the 14th century. The improvement of the production techniques allowed that fibres like Kozo (bark from paper mulberry) and bamboo could be used (McGinnis & Shafizadeh, 1981). The production and the use of paper spread out, but for many centuries it was luxury for few people. Finally, in the 19th century steam paper machines made it possible to produce paper with wood pulp fibre. From that moment, the industrial production of paper had two consequences: the universal access to paper (literacy, newspapers, letters, etc.) and greater logging (Andreu Terren, 2013).

However, one can't talk about a real movement in favour of paper recycling until well into the 20th century. And it was not until 1993 that for the first time more tons of paper are recycled than are thrown away.

From that moment paper recycling makes a great progress to the present. Nowadays in Spain 70 % of the paper consumed is recycled. ("Desde cuando se recicla el papel", 2012)

3.2. PULP AND PAPER PRODUCTION

The manufacturing of paper pulp consists in the separation of cellulose fibres which are joined by lignin. The final properties of the paper depend on the plant species used (raw material) and on the process for getting the pulp (Garcia, 2007). To produce paper pulp, it is necessary to obtain a suspension of cellulosic fibres with specific characteristics in relation to fibre size, distribution of the sizes, composition, flexibility, resistance, etc. The processes of fibre separation and resulting pulps can be mechanics, chemicals or a combination of both (Blain, 1993).

3.3. RECYCLING PROCESS AND RECUPERATION OF RECYCLED FIBRES

The recycling process consists of different Stages. The objective is to eliminate the contaminants that can be found in the recovered fibre, to avoid problems in subsequent stages and to obtain a recovered fibre of a suitable quality for the type of paper to be made (López Calvo, 2004).

The development of new technologies more environmentally friendly and more effective in the elimination of contaminants makes it possible a greater use of this raw material (CEPI, 2005) (CEPI, 2003)

In the recycling plant takes place those Stages whose final objective is to eliminate the contaminants and get recovered fibres of an adequate quality to make paper. Each process eliminates one or more contaminants.

- Disintegration: The objective is fibre individualization and ink removal (Biremann, 1996) (Schwarz, 2000); at this time the high density materials are also eliminated (Biremann, 1996).
- Depuration system: They separate paper fibres from unwanted elements (Biremann, 1996).
- Deinking: Process aiming at eliminating the ink particles present in the fibre suspension (Smook, 1990). It also causes the elimination of the "stickies" and other contaminants present on the paper (Muguet, 1996) (Schwarz, 2000).
- Dispersion: Process for improving the aspect of recovered paper by the dispersal and the fragmentation of contaminants at high temperature. This process eliminates compounds like humidity resistant agents, stickers, etc. (Schwarz, 2000).
- Bleaching: In this stage, oxidizing (P) and reducing (Y/F) chemical agents are used to eliminate those contaminants that give colour, like inks and dyes (Muguet, 1996) (Biermann, 1996).

There are two alternatives to recycling. The problem is that they are not sustainable and they contaminate much more than recycling.

- Incineration: The paper can be burn to obtain energy. This alternative dumps are avoided and as a consequence there is a lower methane production. On the other

hand, to obtain virgin fibres to make paper requires more energy than using recycled fibres (Finnveden,1998) and CO2 emissions are lower (Counsel, 2006).

- Disposal of solid waste: This should be the last option since products like methane are produced and they have higher greenhouse effect than CO2 (ASPAPEL, 2003).

3.4. COMPOUNDS THAT PROVIDE OPTICAL PROPERTIES TO PAPER

3.4.1. Organic colourant

Colourants are a real solution and they are one of the most common contaminants in recycled paper. These compounds are added to give optical properties to paper.

Willam Henry Perkin discovered accidentally the first commercial synthetic colourant in 1856 (Griffiths, 1990). From that moment ten thousand new synthetic colourants have been manufactured (Wesenberg, 2003).

The main problem when studying colourants is their complexity. The colour always appears as a consequence of the joint action of two different atomic groups: chromophore and auxochrome. The chromophore group is functional group such as -C = C-, -N = N- (azo compound) and aromatic rings with many electrons in n-orbitals and/or π-orbitals that give the colour that is observed. The chromophore is responsible for the colour by itself. On the other hand, the auxochrome is the part of the structure responsible for fixation of the colourant to the fibre and can be ammonia groups ($-NH_2$), hydroxyl groups (-OH), sulphonic groups (HSO_3) and carboxylic groups (-COOH).

Depending on the use, there are different criteria for choosing a colourant:

- Fastness: Homogeneity of the colour on the dyed surface.
- Bleeding resistance: Colour stability in contact with water.
- Light resistance: Durability of the colour exposed to light.
- Others: Specific properties according to the use given to paper.

Some representative colourants are showed in Figure 1:

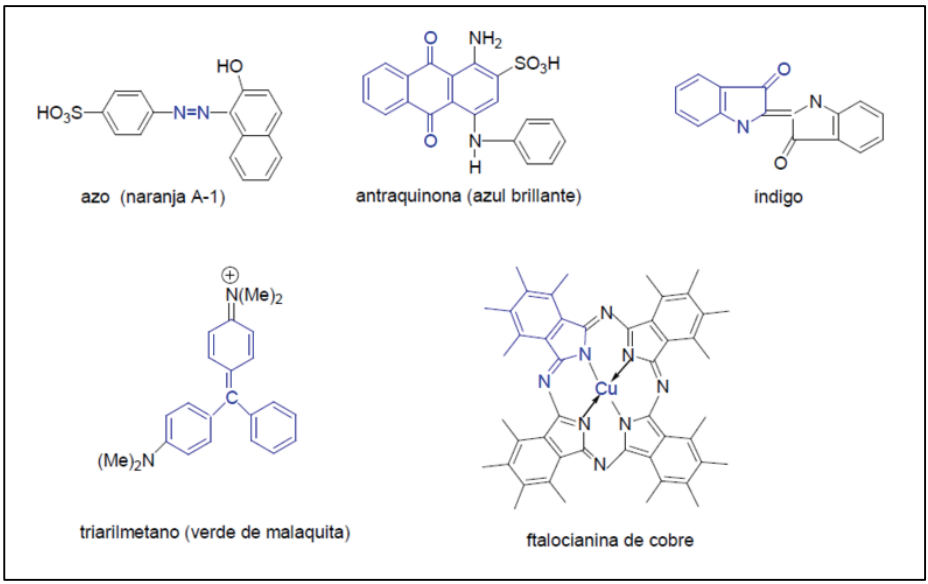


Figure 1 : Different organic colourants (Sanz Tejedor, 2015)

The fibres of cellulose, cotton, linen and rayon don't have acidic or basic groups and they can't form ionic bonds. However, they have a lot of hydroxide groups and can be dyed with molecules that form hydrogen bonds with them. The colourants used for this purpose are called "direct colourants" (they are the ones that dye directly the fibre). Structurally, these colourants have to be linear, flat and long, with several binding sites (like cellulose). These sites bind hydroxide groups from the fibre and they have to have minimum water solubility. The main limitation of these colourants comes from weakness of hydrogen bonds, and for this reason pieces of cotton cloth lose colour with successive washings. These colourants useful to paint paper (Sanz Tejedor, 2015).

3.4.2. Pigments

Pigments are particles of colloidal size. There are two types of pigments, the carbon black and organic pigments.

3.4.2.1 Carbon black

Carbon black is produced by the partial combustion or thermal decomposition of hydrocarbons. Several methods are used, including the furnace black, thermal black, lamp black and acetylene.

The furnace black process is the most common. In this process, natural gas (or another fuel) is burned to form a hot gas stream that is directed into a tunnel. Aromatic oil is sprayed in and the black forms as the gas moves down the tunnel. The reaction is stopped with the addition of water, and the product is collected as a low density powder (fluffy black) or is further processed into millimetre sized pellets.

Carbon black aggregates consist of primary particles that are fused together to form fractal like structures. The aggregates associate with each other because of van der Waals forces to form agglomerates and can be formed into millimetre sized pellets during manufacturing. The primary particles are typically 10 – 75 nm in diameter and the aggregates are typically 50-300 nm in diameter. The surface area of carbon blacks is largely determined by the size of the primary particles and is usually in the range of 30 –200 m^2/g, but there are some outside these ranges with some exceeding 500 m^2/g.

The primary particles of the carbon black are made up of amorphous carbon and small graphitic crystallites roughly 15-25A in size. Research has shown that the crystallites near the surface are parallel to it and the ones in the centre are randomly arranged (Cabot Corporation, 2009).

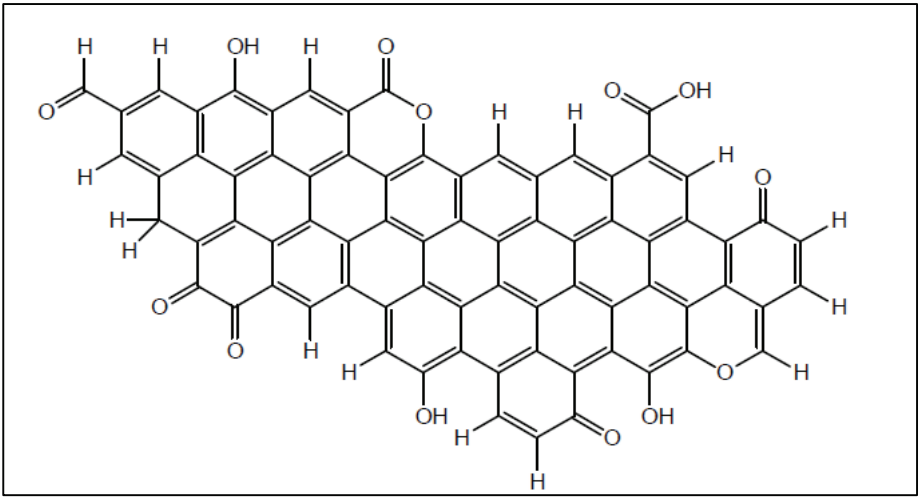


Figure 2 : Functional groups on the surface of carbon black (Cabot Corporation, 2009)

3.4.2.2 Organic pigments

It is well known that a robust composite colour match can be built from a white, a black and from two colourants (A and B) which have hue angles correspondingly smaller and larger than the required colours. The closer the hues of A and B are to the required colour, the better the match.

The most widely used set of pigments for inkjet (IJ) are cyan (C), magenta (M), yellow (Y) and black (K). This set is commonly called as CMYK. All composite colours are built by superposition of these four primaries.

There are significant differences between organic pigments and carbon black that affect the ability to bond to their surfaces. First, organic pigments are molecular crystals. The molecules of the organic pigments are bound together with relatively weak bonds compared to the covalent bonds in carbon black particles. Consequently, the individual pigments have specific, well defined compositions. Further, most organic pigments are not good electron donors, as is carbon black.

There are several methods for modifying the surface of organic pigments. Some are effective for a limited range of pigments, while others are more general. (Cabot Corporation, 2009)

3.5. BIOTECHNOLOGY IN DEINKING

There are several operations in the paper industry in which energy and/or chemical components can be saved if the conventional procedure is substituted by a procedure which uses an enzyme. Several examples can be found: using xylanases (Valls, 2010), the use of amylases and xylanases in deinking (Zollner, 1998), or the use of oxidative enzymes (laccases and peroxidases) in the elimination of chlorine from recycled fibres (Franks, 2001) (Knutson et al. 2004) (Li, 2000) (Arjona, 2008).

In past works the elimination of a flexographic ink (hydrophobic ink) using flotation as the main unitary operation has been shown. This system is known to be efficient for the deinking of offset printing paper (Nyman, 2011) (Fillat, 2012). Another method for the elimination of flexographic ink is using the laccase-mediator system using synthetic and natural mediators. These studies have been carried out combining inks and paper separately.

The pigments, on the other hand, are hydrophilic, and consequently, it is not possible to apply the method of flotation. Arjona (2008) proposed the elimination of a colourant from a commercial coloured paper using the laccase-mediator system with the enzyme *Trametes versicolour* and synthetic mediator HBT.

The present work carries out the same work as Arjona (2008) but using the laccase-mediator system with the enzyme *MyceliopthoraTthermophila* and different natural mediators.

In the next pages the structure, the mechanisms of laccase and the different mediators will be explained.

3.5.1. Enzymes and mediators

3.5.1.1 Laccases

Laccase has the capacity of oxidizing different aromatic compounds with the consequence of reducing the oxygen to water (Riva 2006). This enzyme is very antic from a phylogenetic point of view, since it can be found in several organisms: plants, insects, fungus and bacteria. (Mayer et al 2002).

Its investigation, from an industrial point of view, has focused in the laccase from fungus, since they are the easier to obtain, being the amount of contaminants lower than in other organisms. (Mayer et al 2002).

Laccase is a protein with a molecular weight between 50 and 100 KDa. Its primary structure is very different from the secondary structure. The tertiary structure consists of a globular protein with three domains from the β-sandwich cupredoxin type, similar to the ones found at the ceruloplasmin and ascrobate oxidase. (Durán, 2002).

The active centre of laccase is a group of 4 copper atoms with a constant formulation. The different copper atoms have different properties and different names.

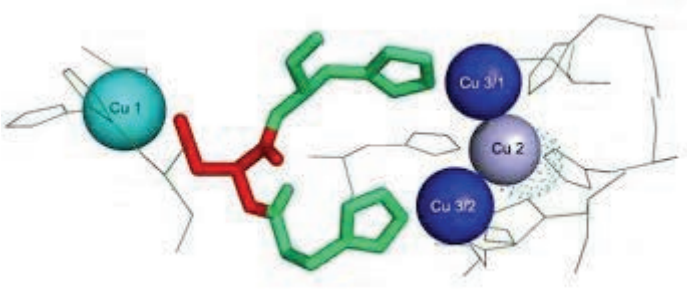


Figure 3: Structure of the catalytic centre of laccase from Coriolopsis gallica UAMH 8260 Source: http://www.rcsb.org/pdb/explore/explore .do?structureId=4A2H

- Type 1 copper: The importance of the redox potential of type 1 copper is that it oxides the substrate and transfers the electron to the type 2 copper (Call, 1997) (Shleev, 2005). The potential of type 1 copper determines in last instance the substrate, which can be oxidized by the laccase. Depending on the redox potential of type 1 copper, there can be differentiated: enzymes with a low redox potential (340-490 mV), mid redox potential (470-710 mV); and high redox potential (730-780 mV) (Shleev, 2005).
- Type 2 copper: It can be found near the type 3 copper and they form a trinuclear group (Call et al. 1997). It combines with two histidines and a molecule of water as bond. This copper ion it's implied in the capture and transference of the electron. (Bajpai, 1999).
- Type 3 copper: They form a grid of diamagnetic copper atoms. Each atom is coupled together and coordinates with three histidines and an atom of oxygen. This trinuclear group of the 2/3 type is the spot of the active centre where the association of molecular

oxygen and its reduction to water is produced. The type 1 copper does not interfere in the process (Call, 1997) (Claus, 2004) (Durán, 2002).

The catalysis mechanism of laccase consists of a monoelectronic oxidation of a molecule of colourant which produces a radical and the reduction of a molecule of oxygen to water. In this reaction the 4 atoms of copper participate actively. Sometimes, the colourant cannot be oxidized directly by the laccase, that's why mediators which are oxidized by the laccase are used. Due to having a lower molecular weight, the oxidant agent can enter the inside of the fibre more easily and attack the colourant. And for this reason it allows to increase the amount of compounds which intervene with laccase forming radicals that can interact with the colourants (Call, 1997) (Riva, 2006).

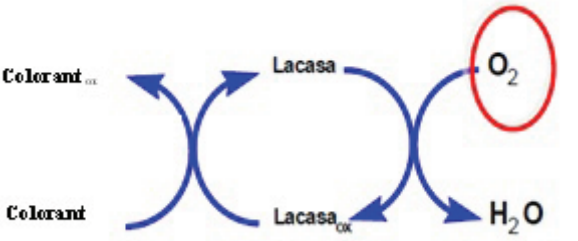


Figure 4: Action of laccase alone (Image from Valls 2015 modified by the author)

This union between laccase and mediator is called laccase-mediator system (L). Due to the characteristics of the enzyme, this system needs an alkali pH in order to increase the efficiency of the treatment.

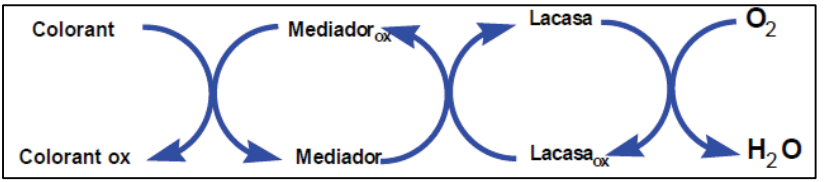


Figure 5: Action of the laccase-mediator system (Image from Valls 2015 modified by the author)

Redox potential of oxygen is around 700-800mV. As seen above, laccases have potential between 340-780mV; therefore, oxygen can oxidise laccase. On the other hand, the potential of some synthetic mediators is around 600mV, and for this reason, there are laccases that can't oxidise the mediator. However, the redox potential of natural mediators is between 300-400mV and thus laccases can oxidise natural mediator and he latter oxidise the colourant.

3.5.1.2 Mediators

There are two types of mediators:

- Synthetic: the ones that contain an N-OH functional group. They are the most effective mediators of laccase used for the bleaching of the dough. The first mediator used capable of bleaching the dough was the ABTS, but more have appeared since then. Thanks to the radical formed they are capable of degrading the colourant of the dough.

Figure 6: Lacasse inactivation problem (Valls 2015).

- The problem of synthetic mediators is its expensive price and its effluents are very toxic. For this reason, the use of natural mediators is being investigated (Valls, 2015).
- Natural: They are directly involved in the natural degradation of the lignin by the fungus of the white moisture. They are phenolic compounds derived from lignin and can be obtained from the black bleach obtained during Kraft cooking. The natural mediators more frequently used are shown in Figure 7.

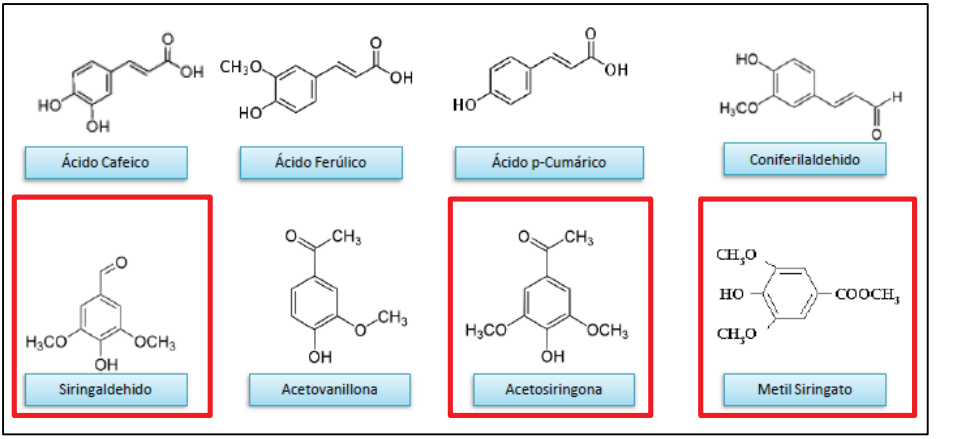


Figure 7: Different natural mediators (Valls, 2015).

The advantages of the use of natural mediators are less toxicity and that they don't inactivate the enzyme as much as synthetic mediators.

The application of the first natural laccase-mediator system in deinking dates from 2007. It has been seen that natural mediators are less effective than the synthetic ones in deinking (Valls, 2010) (Fillat, 2012). In the present work natural mediators are applied for deinking coloured fibre. The natural mediators used are: acetosyringone (AS), syringaldehyde (SA) and methyl syringate (MeS).

3.5.2. Laccase-mediator system biodeinking (Stage L)

Bleaching is a process which the objective of increasing the whiteness in the fibres by removing lignin and other components avoiding the degradation of carbohydrates (Reeve, 1996).

Laccase is an enzyme which, with or without mediator, is capable of destroying the colourant present in effluents (Torres, 2005). There are some colourants that laccase cannot destroy without the assist of the mediator.

The mediators are smaller molecules which can interact with lignin where laccase cannot (Archibal, 1995) (Balakshin, 2001).

3.5.3. Chemicals agents in bleaching

3.5.3.1 Treatment with hydrogen peroxide (Stage P)

Hydrogen peroxide is one of the oxidizing agents used in the elimination of colour in the recycled fibres and in the bleaching of the dough. During the bleaching it is dissociated in different products and it is considered that the perhydroxile anion (HOO^-) is the component responsible of the bleaching (Presley, 1996).

This treatment will be done in an alkali environment since the hydroxyl groups react with the hydrogen peroxide resulting in the perhydroxxile anion (Ackermann, 2000). The presence of metallic ions in the environment produces undesired secondary reactions which reduce the efficiency of the treatment. By adding a chelating compound, such as DTPA, it is possible to increase this efficiency (Presley, 1996). In an alkali environment and high temperature, a peeling reaction also takes place (degradation of the superficial layer of the fibre) (McGinnis, 1990). By adding magnesium sulphate this can be avoided, since the Mg^{2+} acts as a protector (McDonough, 1996).

$$H_2O_2 \leftrightarrow HOO^- + H^+$$

$$H_2O_2 \rightarrow HO^- + HO^+$$

$$H_2O_2 \rightarrow HOO^- + H^- \qquad HO^- \uparrow\downarrow H^+$$

$$H_2O_2 \rightarrow 2HO^- \text{ (Radical Hydroxyl)}$$

$$O_2 \text{ (Anion radical superoxide)} \rightarrow HOO^- + H^+$$

$$H_2O_2 + HO^- \rightarrow HOO^- + H_2O$$

3.5.3.2 Treatment with formamidine sulphinic acid (Stage F)

It's a reducing agent capable of reducing the cromophore groups present in colourants (Figure 8) and it is advisable to use them in the final Stage of the bleaching elimination (Ackerman, 2000).

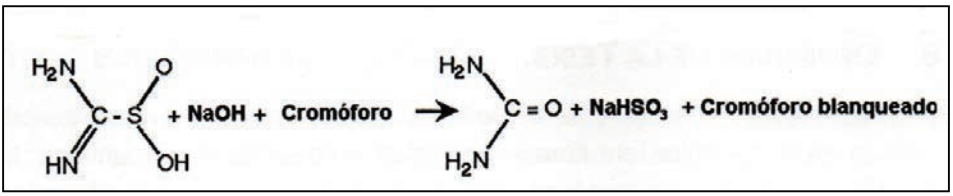


Figure 8: Actuating mechanism F (Ackerman 2000).

3.5.3.3 Treatment with ozone (Stage Z)

Ozone is a strong candidate to replace traditional bleaching agents derived from chlorine (Byrd, 1992), due to its high oxidizing potential and its capability to remove lignin and bleaching cellulosic paste. Despite this, its implementation at an industrial scale has been hindered due to its high cost and low selectivity (Van Heiningen, 1997) (Broli, 1993).

Ozone, or O_3 has a molecular weight of 48 g/mol. It's a gas with a high oxidizing potential, only matched by fluorine and atomic oxygen. Is a gas highly electronegative and very instable, which is produced in the presence of oxygen every time an electric charge is produced. It's a colourless gas, almost black in liquid state and purple-blue when solid. It's more soluble in water than oxygen, especially at low temperatures. Despite this is an instable gas which decomposes in aqueous environment. The main parameters that affect its stability of the solution are temperature, pH and the presence of metallic ions and hydrogen peroxide. The decomposition of ozone generates hydroxyl radicals (-OH), which can accelerate this decomposition even more. The decomposition reactions of ozone are shown below (Roncero, 2011).

$$O_3 + OH^- \rightarrow O_2^{o} + HO_2^{\circ}$$
$$HO_2^{\circ} \leftrightarrow O_2^{-} + H^+ \quad pK_a = 4.8$$
$$O_2^{\circ -} + O_3 \rightarrow O_3^{o-} + O_2$$
$$O_3^{\circ} + H^+ \rightarrow {}^{o}OH + O_2$$
$$O_3 + HO_2^{\circ} \rightarrow 2\,O_2 + {}^{\circ}OH$$
$$O_3 + {}^{\circ}OH \rightarrow 0_2 + HO_2^{\circ}$$
$$HO_2^{\circ} + {}^{\circ}OH \rightarrow O_2 + H_2O$$

Ozone reacts with most of the chemical groups present in the waste colourants, unlike oxygen and hydrogen peroxide. But it also tends to react with carbohydrates, which are the main cause of the decrease of the viscosity of the dough and, therefore, the selectivity of the process. This selectivity issue, added to the bleaching with ozone tends to hinder its application in the bleaching of TCF (Colodette, 1993).

In the image below a flux diagram of the installation used is shown.

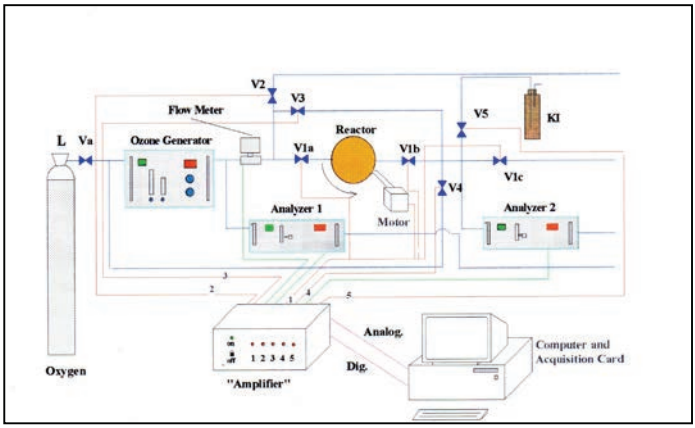


Figure 9: Flux diagram of the installation (Serra, 1996)

4. OBJECTIVES

This project will be done in collaboration with the "Grup de Recerca en Enginyeria Paperera de la Universitat Politècnica de Catalunya (UPC), which focuses on the research of new methods for decolouring cycled fibres using biological methods and ozone.

Colourants are typical contaminants in the recycled papers. These compounds are added to papers in order to produce the desired optical properties. In the traditional recycling process, huge quantities of chemical products are used in order to remove these colourants. Therefore, the process is expensive and detrimental for the environment. Moreover, the reduced optical properties of recycled fibres obtained restrict their use in high added value papers. In this context, the use of biotechnological products (enzymes) could reduce the consumption of chemical products, reducing both, price and the environmental impact. Laccase alone is not effective and needs the use of mediators to increase their oxidative action (laccase-mediator system). Mediators may be natural or synthetic. In this work natural mediators will be applied (alone or combined) in order to evaluate the most effective combination. The oxidant power of laccase-mediator system will be compared with ozone. Ozone is a powerful oxidizing agent. To evaluate decolourization, paper sheets of treated samples will be done. Both, optical and mechanical properties will be evaluated.

Therefore, the main objective of this project is:

Developing a sequence for eliminating the colour of recycled coloured fibres combining the application of one or several enzymatic Stage (laccase-mediator system) and different conventional bleaching agents and/or ozone.

To fulfil this objective, different secondary objectives were considered.

- Studying the influence of the structure of different mediators in the oxidative capacity of the laccase-mediator system.
- Determine the relevance of the mediator used during the enzymatic Stage.

- Evaluating bleaching sequences completely chlorine-free, including the treatment with the enzymatic laccase-mediator, hydrogen peroxide (P), fomamidine sulphinic acid (F) and ozone(Z).
- Obtaining paper with excellent optic properties
- Studying the physical properties
- Evaluating the possibility of studying the economic feasibility doing the study of the enzymatic activity of the effluents.
- Evaluating different natural mediators for bleaching; both for red paper and black carbon.
- Studying the best sequence combining different mediators to see the synergy between them.
- Comparing the effectivity between the laccase-mediator system and ozone.

5. MATERIALS AND METHODS

All the experimental can be found in annexed 1

5.1. RAW MATERIAL

The study which takes places in this project focuses in the deinking of recycled paper. The obtainment of the paper, of different brands like Motif and Liderpapel, will be task of the Cellbioth group.

The answer of a raw material in a decolouration process depends of several factors, one of them being the origin of the paper (which we don't know).

5.2. LACCASE-MEDIATOR SYSTEM BIODEINKING (STAGE L)

For the experiments done at the laboratory, the working conditions are previously determined:

- Laccase used origins from fungus *Myceliopthora Thermophila (of low redox potential)* provided by Nouozyme.
- pH meter in the process. For laccase the convenient pH is 7. For this reason, a sodic phosphate tampon of 200 mM, which needs to be prepared, will be placed in the process.
- When working at the laboratory a consistency of 5% is used. This percentage refers to 100 total grams for each 5 grams of dry dough (gr odp). At an industrial level a consistency of 10% is used.
- Individualization of the fibres. This facilitates de bleaching since the contact layer is hols between the enzyme and the dough.
- For knowing the amount of enzyme that needs to be added calculations with the enzymatic activity and a proportion of 20 units of enzyme per gram of dry dough will be used.
- The dose of mediator will be between 1.5 and 3 grams of mediator for each 100 gr odp.
- The density of all the solids and liquids is considered to be of 1000 Kg/L during all the experiments.

- The residence time for the treatment is of 4h and the temperature of 50ºC. Conditions stablished according to previous studies (Valls 2015) (Nyman 2011) (Fillat 2012).
- All the compounds mentioned above are supplied by Sigma-Aldrich.

These conditions are the optimum conditions according to the statistic plans for bleaching paper dough.

Figure 10 Reactor Easydye

5.3. CHEMICAL AGENTS IN BLEACHING

5.3.1. Treatment with hydrogen peroxide (Stage P)

The experiments are done under determined conditions. These conditions are:

- A pH meter will be placed in the process. In the treatment with hydrogen peroxide the most convenient pH is a basic pH. For this reason, sodium hydroxide (1.5%) will be introduced in the process.
- When working at the laboratory a consistency of 5% is used. This percentage refers to 100 total grams for each 5 grams of dry dough (gr odp). At an industrial level a consistency of 10% is used.
- Individualization of the fibres. This facilitates de bleaching since the contact layer is hols between the enzyme and the dough.
- For knowing that the amount of hydrogen peroxide is of 3 grams of H_2O_2 per each 100 gr odp will be added.
- The density of all the solids and liquids is considered to be of 1000 Kg/L during all the experiment.

- The residence time is of 2h and the temperature of 90ºC. Conditions stablished according to previous studies (Valls 2015) (Nyman 2011) (Fillat 2012). For avoiding the peeling, magnesium sulphate is introduced (0.2%).
- For removing problems caused by heavy metals DTPA 1% in weight is introduced.

These conditions are the optimum conditions according to the statistic plans for bleaching paper dough.

Figure 11 Reactor Easydye

5.3.2. Treatment with Formamidine Sulphinic Acid (Stage F)

The experiments will be done in absence of oxygen, since this decreases its efficiency. The conditions where a bigger effect can be observed are with a pH between 7 and 9, a temperature of 40 to 90ºC and the recommended dose is of around 0.5% of acid (Ackerman, 2000).

5.3.3. Treatment with ozone (Stage Z)

The working conditions are: low consistency (0.5%), ozone dose of 0.8 and acid pH (addition of sulfuric acid until pH reaches 2.5) since it improves the ozone reaction. The ozone dose can vary between 0.4 and 1% but it can't never be bigger than 1%, since in previous studies is shown that above 1% the quantity of dough obtained decreased significantly. Another important parameter is the consistency of the treatment. In our case it's of 0.5% (low

consistency). This is for improving the efficiency of the treatment, since in the other experiments (done at higher consistency) the formation of lumps was reported, meaning that only the superficial layer of the dough was whitened. There are two reasons why the peroxide and ozone systems are not combined: the pH of the environment (ozone → acid, H_2O_2 → basic) and the formation of free radicals that can destroy the fibres.

Figure 12 Ozone equipment

5.4. MEASUREMENT OF THE OPTIC PROPERTIES.

5.4.1. Parameters of the optic properties.

Colour is not a property of the object, but an interaction between the object and the incoming light. The sensation of colour is determined by several factors: the observer, the source of the light and the object.

- Source of the light: There are different sources and models of obtaining the light. It can be obtained by applying a filter to a halogen lightbulb of tungsten of xenon. The source of light used in this investigation is the D_{65}. This source of light has a spectral distribution of

"plankian radiator" with a temperature of 6500K. It's the equivalent to a daylight and it's used for measuring the colour of the dough (Hunt, 1998a) (Pospon, 1996).

- Illumination: It's a spectral distribution of the wavelength. It's not a physical source. There are different standard illuminations. The one use during this investigation is the D_{65}. The illumination has the objective of normalizing the data obtained through the reflectance obtained and eliminating the degradation that that the source of light can have due to its constant use or to normalize the data obtained after using a determined source of light.
- Observer: Each person has a different perception of the colour. Because of this reason the standard observer parameters are normalized. The ones used are the 2nd and 10th.
    - 2nd observer is the one that a normal person observes when looking through a whole of the size of ¼ of a dollar when a series of beams of a determined wavelength are projected into a wall.
    - 10th observer is the one that a normal person observes when looking through a whole of the size of a baseball ball when a series of beams of a determined wavelength are projected into a wall.

When the measure is done the source of light need to be specified as well as the illumination and the observer (Popson, 1996) (Pauler, 1990).

Figure 13 : Remission spectrophotometer

5.4.2. Parameters based on the reflectance

When the beams of light affect a paper sheet, different phenomes take place: part of the radiation is absorbed, part is reflected and another part is transmitted. The sighting of an object is because of the reflection of the light from the object into the eye (Holmberg, 2000) (Lips, 1991).

5.4.3. Reflectance curves

The intrinsic reflectance factor (R_∞) it's the relation in percentage of the reflected radiation of the group of sheets combined, opaque enough to not letting the light and the reflected radiation by a perfect reflective diffusion. Due to working with paper made of recycled coloured fibres, we measure R_∞ using a D_{65} source of light and an illumination/observer of $D_{65}/10^{th}$ since the obtained data can be approached to what a person can see. The colour of a specific material comes determined by a R_∞ with a visible wavelength; when representing this data, the reflectance curve or spectrophotometry can be drawing and it has a different shape depending on the colour (Biermann, 1996) (Popson,1996) (Pauler, 1990).

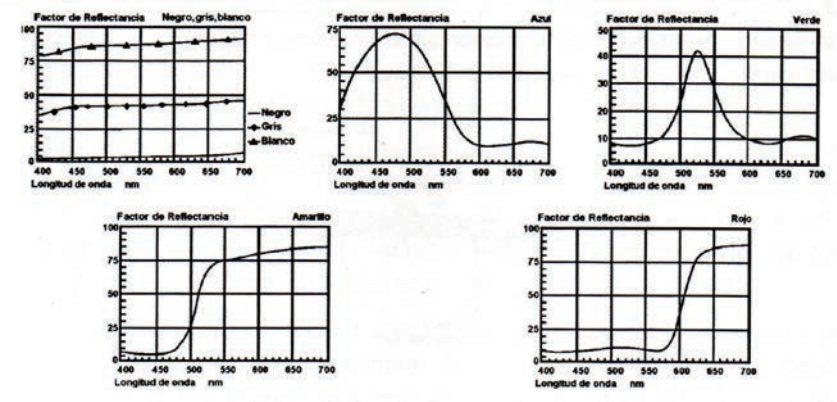


Figure 14: Spectrophotometric curves (Pauler, 1990).

5.4.4. Brightness measures

The brightness is the factor of the fuzzy reflectance measured at an effective wavelength of 457 nm. This parameter is specific for measuring the efficiency of a bleaching sequence (Biermann, 1996) (Popson, 1996) (Pauler, 1990).

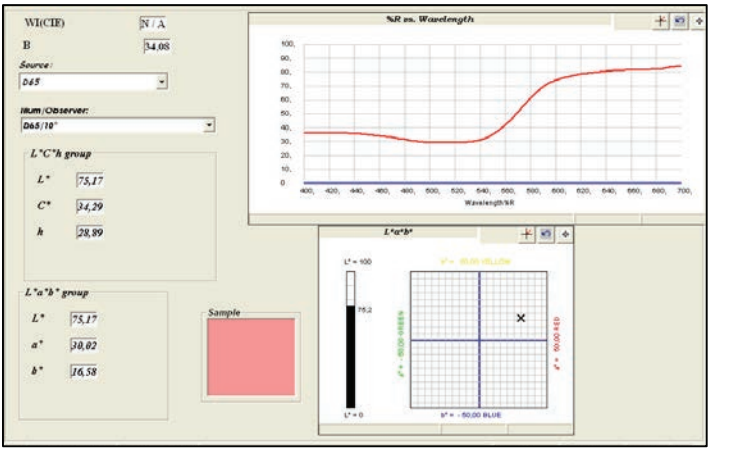


Figure 15: Determining coordinates CIEL * a * b *

5.4.5. k/s index

The paper fibres have elements that absorb the light. These elements can be remains of lignin or colourants if the paper comes from a coloured recycled paper. Using the theory of Kubleka-Munk the next formula can be obtained:

$$k/_S = \frac{(1 - R_\infty)^2}{2R_\infty}$$

Equation 1: Equation k/s index.

K: specific coefficient of the absorbance of light. It's defined as the limit value of light energy absorbed per unit of weight when the weight tends to zero (Hunt, 1998b) (Loras, 1991) (Pauler, 1990).

S: specific coefficient of the light dispersion. It's defined as the limit value of light energy spread per unit of weight when the wright tends to zero (Hunt, 1998b) (Loras, 1991) (Pauler, 1990).

5.4.6. Chromatic coordinates CIEL*a*b*.

It's defined as the three-dimensional space where each one of the points it's associated to a colour. The wavelength of the a*b* axis goes from 100 to -100 and the L* axis from 100 to 0. All colours can be characterized by its chromatic coordinates.

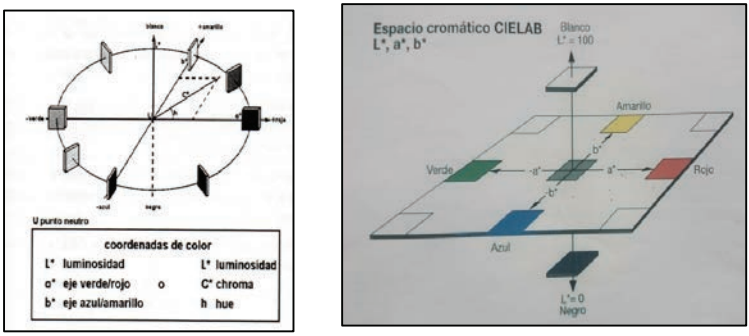


Figure 16: Dimensional space CIEL * a * b *.

The coordinates L*a*b* are obtained when transforming to colour units the values of the three-stimulus X (red), Y (green) and Z (blue) of a sample. These value can be used to determine the answer of the human eye when capturing determined colours. For calculating the difference of colour (AE*) we use the chromatic system L*a*b* using the following equation:

$$AE^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Equation 2: Colour difference

5.4.7. Chroma

Parameter used for determining if a colour is dark or light. It can be calculated doing the module of a* and b* and its value oscillates between 0 and 60. The equation can be seen below.

$$C^*(Chroma) = \sqrt{(a^*)^2 + (b^*)^2}$$

Equation 3: Chroma

5.4.8. Dye Removal Index (DRI)

The DRI represents the decrease of the observed module after applying a sequence of colour elimination to the dough in comparison with the maximum module of the vector, defined by the chromatic coordinates of the initial dough. The equation used for calculating the DRI is:

$$DRI = \left(\frac{\Delta R^2}{R_1^2}\right) x\ 100$$

Equation 4: Dye Removal Index

Where ΔR^2 and R_1^2 are:

- (L_1^*, a_1^*, b_1^*) chromatic coordinates of the initial dough.
- (L_2^*, a_2^*, b_2^*) chromatic coordinates of the final dough.

5.5. PHYSICAL AND MECHANICAL PROPERTIES.

To determine if the treatment applied to the paper has suffered any anomalies to the physical of mechanical properties, the next techniques are applied: weight, thickness, traction resistance and scaling resistance.

For obtaining representative results, the samples of paper need to be previously conditioned in an environment of 23ºC and 50% of relative humidity, as specified in the ISO 187.

- **Weight:** It's determined by weighting a known area of paper as specified in ISO 536. In Figure 17 the apparatus used to determine this parameter can be seen.

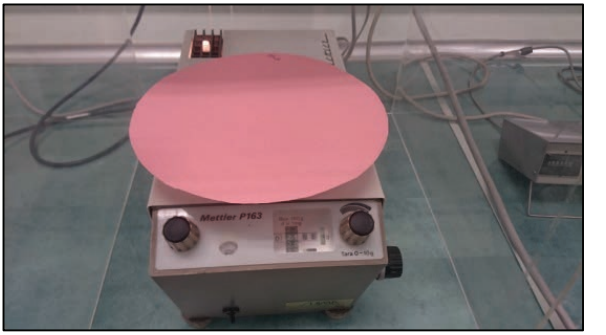


Figure 17: Balance

- **Thickness:** It can be measured using a micrometre with an area and compression pressure on the determined sheet according to ISO 638. In Figure 18 the apparatus used to determine this parameter can be seen.

Figure 18: Micrometre

- **Traction resistance:** The strength that is needed for breaking a piece of paper in a narrow stripe when the length of the paper and the velocity of the charge applied are perfectly specified; it also determined the extension of the paper. The procedure followed can be found at ISO 1924-2. In Figure 19 the apparatus used to determine this parameter can be seen.

Figure 19: Tensile test

- **Scaling resistance:** It can be determined by placing the sample of paper on a rubber diaphragm, to which a defined pressure and a velocity are applied, measuring the value of the pressure when the sample broke. The specified procedure can be found at ISO 1974. In Figure 20 the apparatus used to determine this parameter can be seen.

Figure 20: Bursting test

5.6. RESIDUAL ENZYME ACTIVITY

The activity of the enzyme will be determined using a spectrophotometer. Using the initial value of the initial activity we calculate the percentage of inactivity of the enzyme.

For calculating the percentage of inactivity we use the following formula:

$$Inactivity\ percentage = \frac{Initial\ Activity - Final\ activity}{Initial\ Activity} * 100$$

Calculating the initial activity:

$$12\ g\ odp * \frac{100\ total\ grams}{5\ gps} = 240\ total\ grams$$

$$240\ gr\ totals - 12\ g\ odp = 228\ grams = 228\ ml$$

Assuming density equal to water

$$20\ \frac{U}{g\ odp} * 12\ g\ odp = \frac{240\ U}{228\ ml} = 1.05\ \frac{U}{ml}$$

And applying the formula we calculate the final activity:

$$Activitat\left(\frac{U}{ml}\right) = \frac{\Delta_{Abs} * \Delta_{SE} * D_C * 1000}{29300}$$

Where Δ_{Abs} is the value of the absorvence obtained with the spectophotometre. Δ_{SE} is 1 because is direclty obtained without diluting the enzime and Dc presents different values depending of the concentration of the effluent. The table below shows how the solutons are prepared for studying the absorbance of the effluents.

In our case the value of D_C is of 20, since we have the effluent with low concentration.

Deionized water ([µL]	ABTS [µL]	Enzyme [µL]	Final volume	Flask dilution [D_C]
490	500	10	1000	100
475	500	25	1000	40
450	500	50	1000	20

Figure 21 : Table effluent concentration

6. RESULTS

The main objective of the project is to determine if using the laccase mediator can deink a recycled paper already coloured. Another objective is determining which colourant compound used is in the paper.

6.1. DETERMINATION OF THE COLOURANT COMPOUNDS

For determining if it's a colourant or a pigment we will take the effluents of the paper after being one day in water. Using the microscope, we obtained the following results.

In Figure 22 we can see the red effluent at different magnifications. We cannot be sure if it's a pigment or a colourant. The problem is that we couldn't concentrate the effluents since the first one was impossible to differentiate. For concentrating we used the rotavapour at a temperature of 70ºC and a pressure of 31.2 Kpa approximately. But when increasing the temperature, we cannot determine if they are colourant particles polymerized and conglomerated or the pigment itself which conglomerated. The only thing we can determine is that the particles give the red colour to the dough.

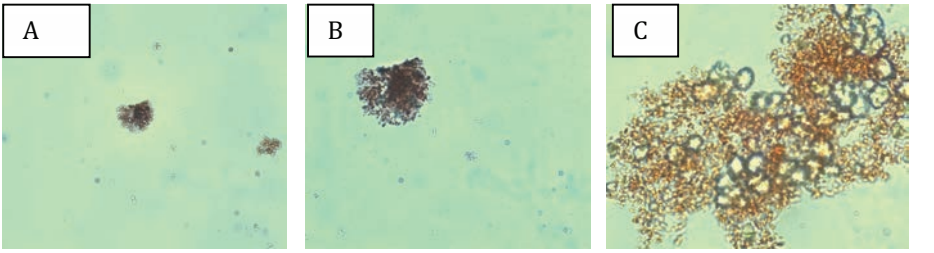


Figure 22: Image A at 200 magnifications, image B at 400 magnifications and C a 1000 magnifications.

In Figure 23 we can see the black smoke at different magnification measures. We can confirm that it is a pigment since the small particles of colloidal length with a "Brownian movement" due to the electrostatic repulsion of the particles.

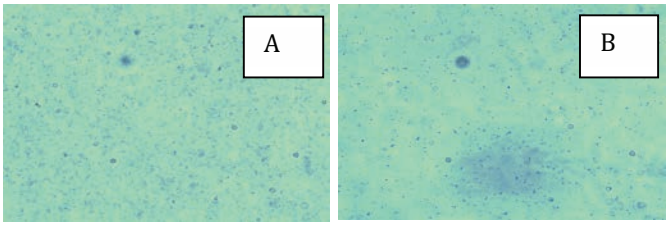


Figure 23 : Image A at 400x or image B a 1000x

6.2. EVALUATION OF THE BEST LACCASE-MEDIATOR ON RED PAPER.

Evaluation according to its optical properties. After each treatment the optic properties CIEL*a*b are measured; absorbance (k/s index); reflectance and dye removal index.

The Figure 24 shows the initial chromatic coordinates.

	Chromaticity Coordinates					
	L*	a*	b*	C*	h*	B
Red Initial Pulp	57.29	48.95	31.98	58.47	33.16	9.88

Figure 24 : Initial chromatic coordinates

Our objective is to determine if the laccase-mediator system is more effective than the laccase alone and try to deinking the paper until we reach the same optical properties than those of bleached eucalyptus pulp, which is considered the final objective of the experiments. For this reason, a control stage (enzyme without mediator), will be done and the difference of action between the mediators will be tested. In Figure 25 the different treatments of this section are shown.

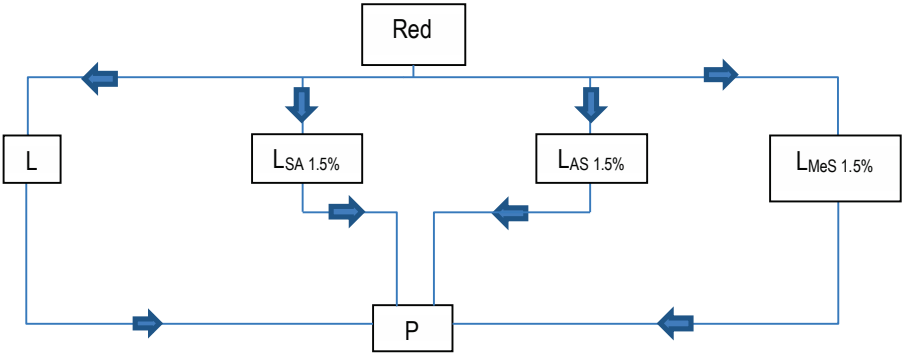


Figure 25: Treatments with different mediators and control stage.

In Figure 26 a higher absorbance peak can be seen, which corresponds to the initial paper. This paper has an absorbance between 400 and 600 nm. It is possible to determine that the next peak is the one from the control stage, where it can be seen that the laccase alone cannot oxidize the colourant. This supports the fact that a laccase-mediator system needs to be used for oxidizing more colourant. We can observe that the mediator that performs the worst is the SA and the two best ones are the AS and the MeS. The high values of k/s indicate that that area absorbs the light. Low values of k/s indicate that reflects the light in that zone of the spectre. In the red colour it can be seen that between 0 and 600 nm of wavelength are the values with a considerable peak. On the other hand, the values of 600 to 700 nm of wavelength are low, and therefore reflect the light.

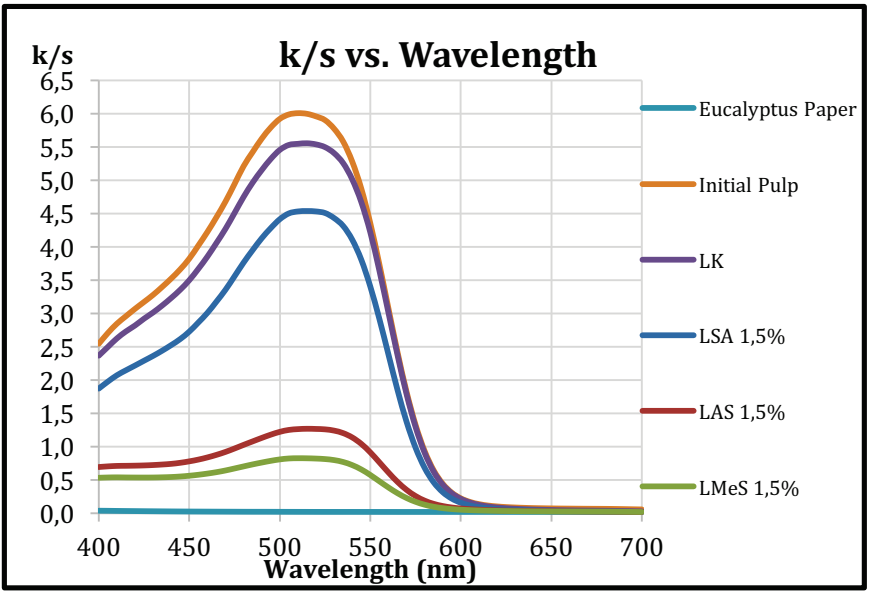


Figure 26 : Graphic of the treatments using different mediators and control stage.

In Figure 27 we can see that the treatments which improve the elimination of colour are the ones that have lower a* and b*. A* ranges from red to green and b* from yellow to blue. It can

be seen that our sample has high a* (red) and also b* (yellow). AS and MeS decrease a* and b* being more efficient MeS. For this reason, we can say that the best treatment is the MeS.

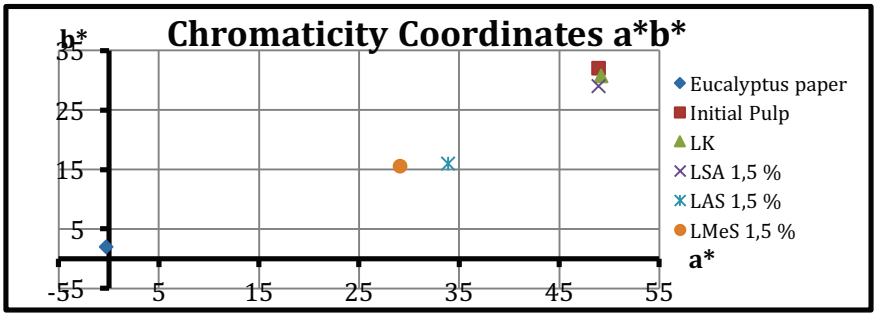


Figure 27 : Graphic coordinates CIEL * a * b *

The next step of the experiment is trying checking if the oxidizing agent used, like hydrogen peroxide, causes a great change in the loss of colour. In Figure 28 the final loss of colour compared to the initial amount can be seen. According to the DRI we can see that the treatment with laccase alone and the treatment with the mediator SA are the ones that remove the smallest amount of colour. However, with AS and MeS mediator we can have observed the same tendency than after the L stage.

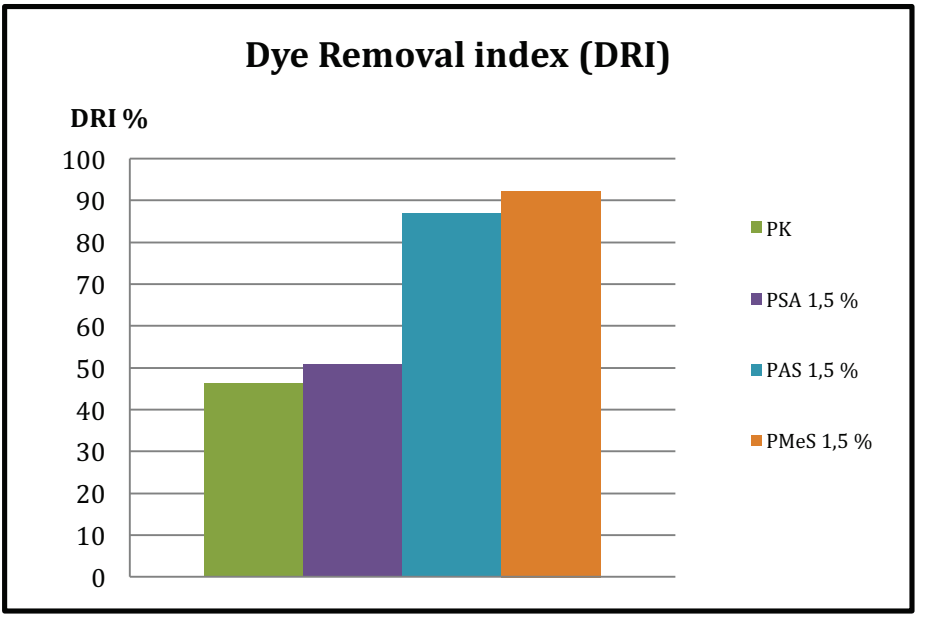


Figure 28 : DRI of P stage of the different treatments.

Summarizing, we can see that MeS is the most effective treatment for removing colour, followed by the AS and SA, which cause very little effect. After stage P and the DRI, we can see that the loss of colour is of approximately 90%, but in Figure 29, we can see that the reflectance curve of the eucalyptus paper, used as reference, has not been reached yet.

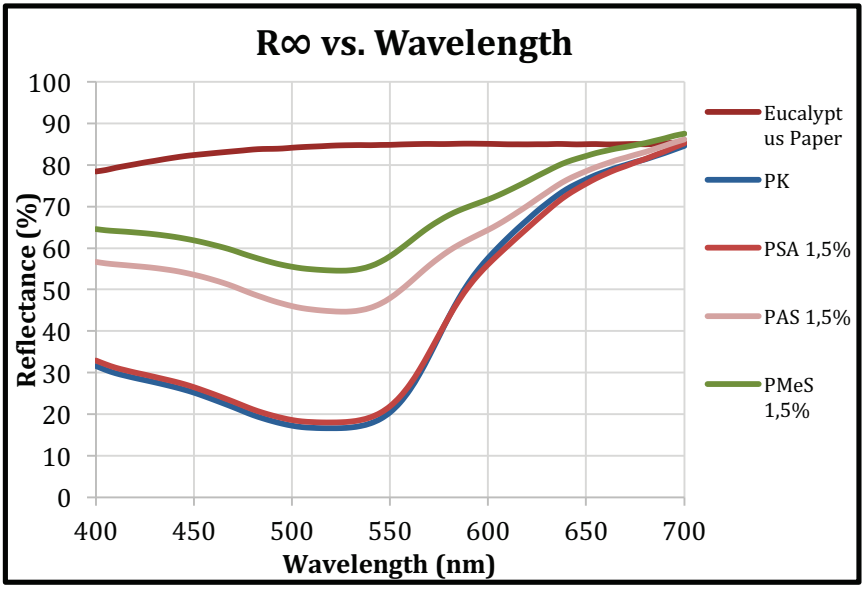


Figure 29 : Reflectance graph of stage P with control stage and the different mediators.

In Figure 30 we can see the loss of colour. Next figure shows the different papers treated, being the lower ones the treated with stage L and the top ones the treated with stage P.

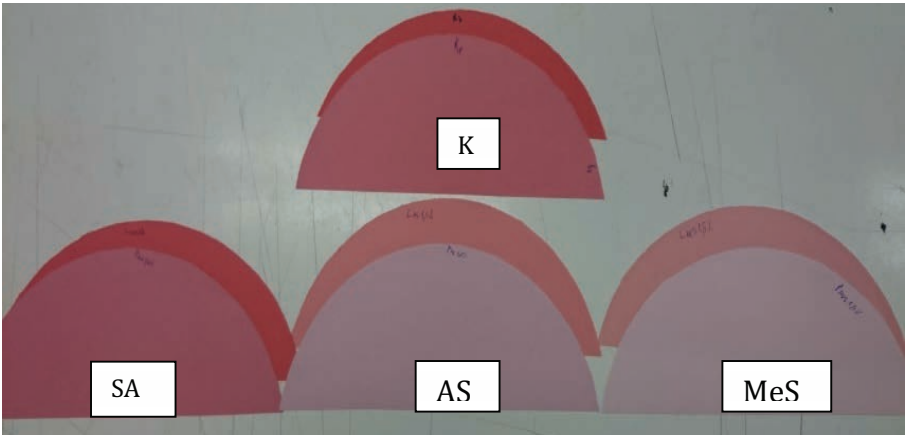


Figure 30 : Different treatments used.

6.3. STUDYING THE MEDIATOR COMBINATIONS

The next step is to combine different mediators to see if they have a synergic effect. Since we add 1.5 of each mediator, treatment at 3% will be compared. At Figure 31 the path that we will follow can be seen:

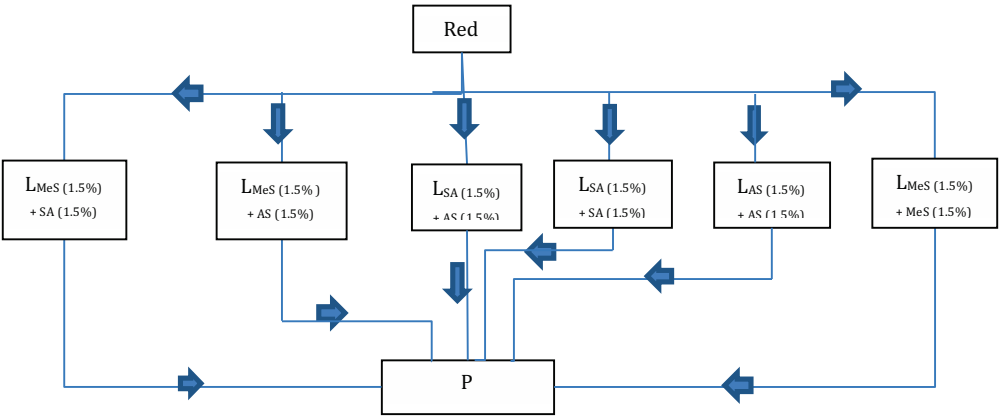


Figure 31 : Scheme of the combination of mediators.

In Figure 32 we can see how in stage L the combination of mediators gives negative results, since the laccase has to oxide both mediators and, therefore, a loss in effectivity occurs. The SA and AS make the effect of MeS worst.

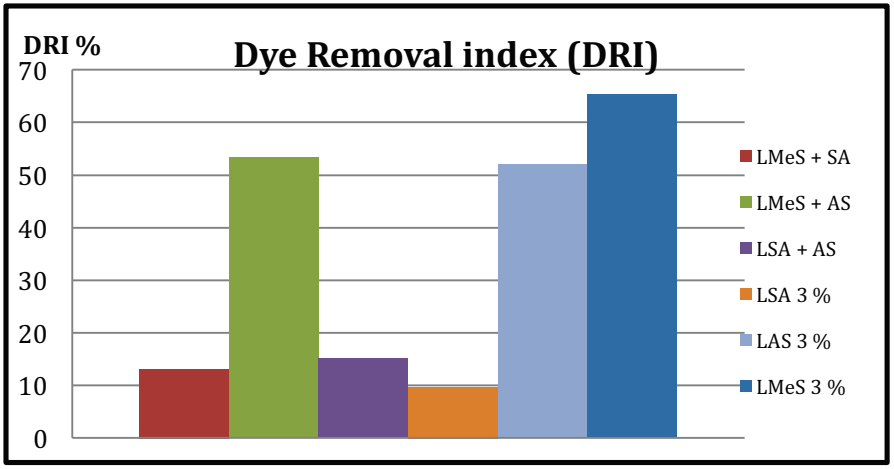


Figure 32 : Dye removal index with combination of mediators.

In Figure 33 we can see L(luminosity) vs Chrome that when increasing the dose of mediator, the elimination of colour is not affected, therefore, is better the ones that uses 1.5% of mediator. We can also confirm that the best one is the MeS with 1.5%. Eucalyptus has an L of 95 and Chroma of 0. The most efficient treatment will be the closest to this value. It can be observed that the most efficient is MeS 1.5%.

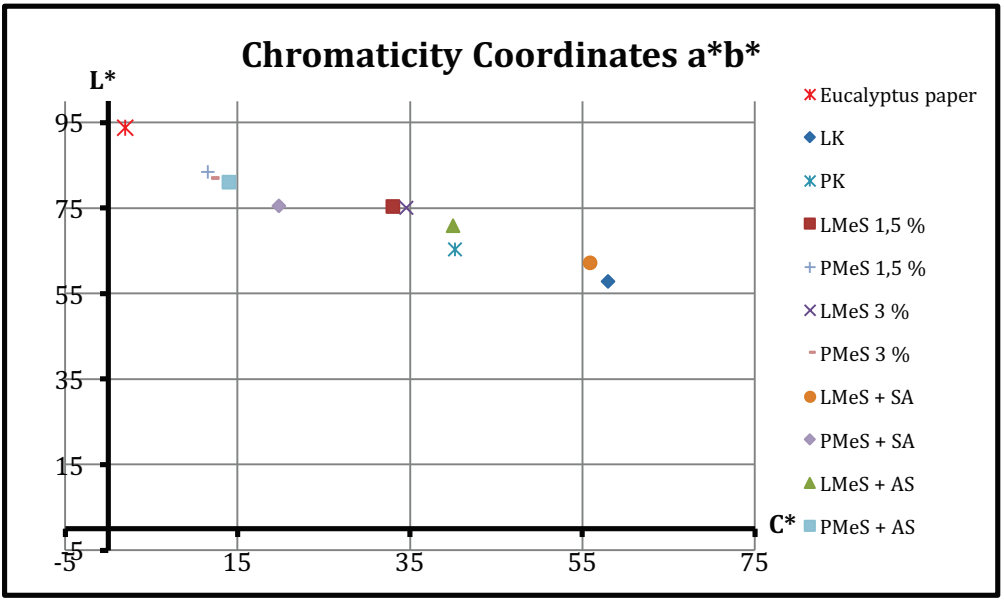


Figure 33 : Graph C* vs L*

In Figure 34 we can also appreciate the loss of colour. In the figure below different samples of treated paper can be seen, where the lower ones are the ones treated with stage L and the top ones the treated with stage P.

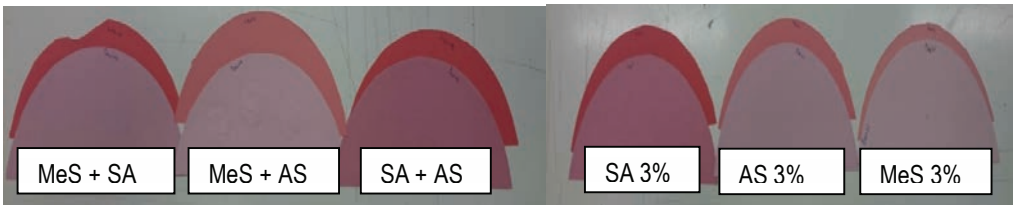


Figure 34 : Loss of colour of the different treatments used.

6.4. COMPLETE BIODEINKING SEQUENCE

As shown above, MeS 1.5% is the most efficient followed by AS. We are going to see if by making a decolouration sequence we can approach to our final objective. It has been demonstrated that adding the two mediators together the effect is worse than using them separately. After each treatment the dough will be washed in order to see if there is more effect. As we have mentioned before, when decolourising the recycled fibres, we use the ozone treatment. For comparing the effectiveness of the different sequences used we will do a sequence where the first treatment will be the ozone one.

The following sequences will be used: L_{MeS} L_{AS} P P F, L_{MeS} P L_{AS} P F, L_{AS} P L_{MeS} P, L_{MeS} P L_{MeS}P, Z P L_{MeS} P. The figure bellow shows the procedure to follow.

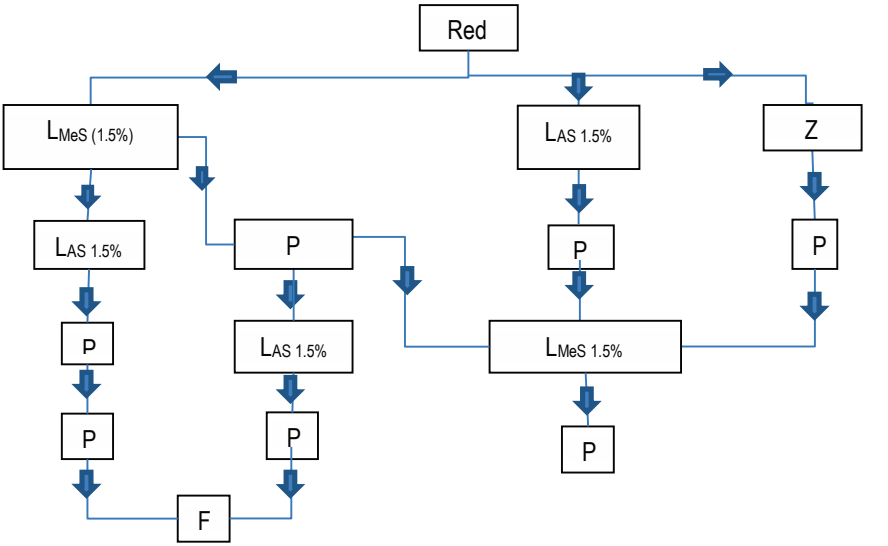


Figure 35 : Sequence of elimination of colour.

In Figures 36, 37, 38, 39 and 40 the colour loss of each colour elimination sequence is shown.

In Figure 36 it can be observed that applying L_{AS} after L_{MeS} has more effect if the dough is washed between each stage.

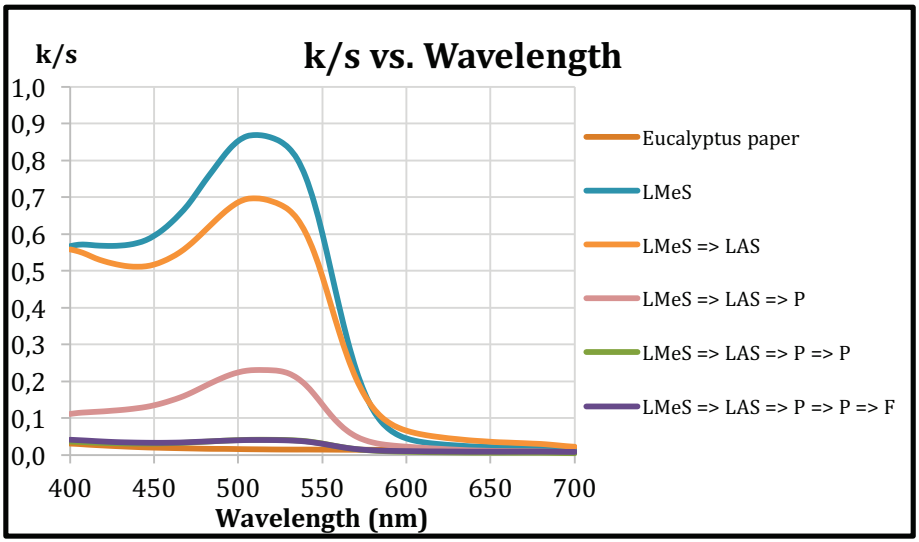


Figure 36 : Sequence LMeS LAS P P F

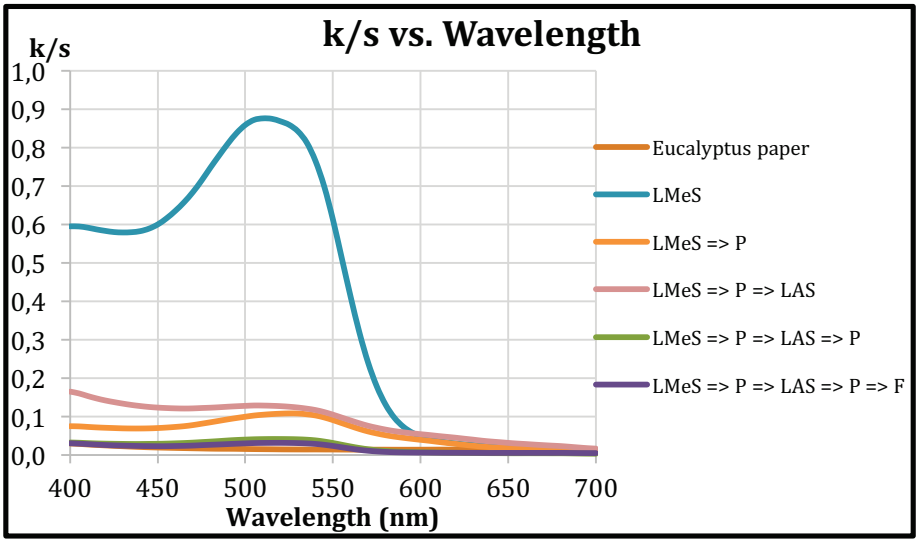


Figure 37 : Sequence LMeS P LAS P F

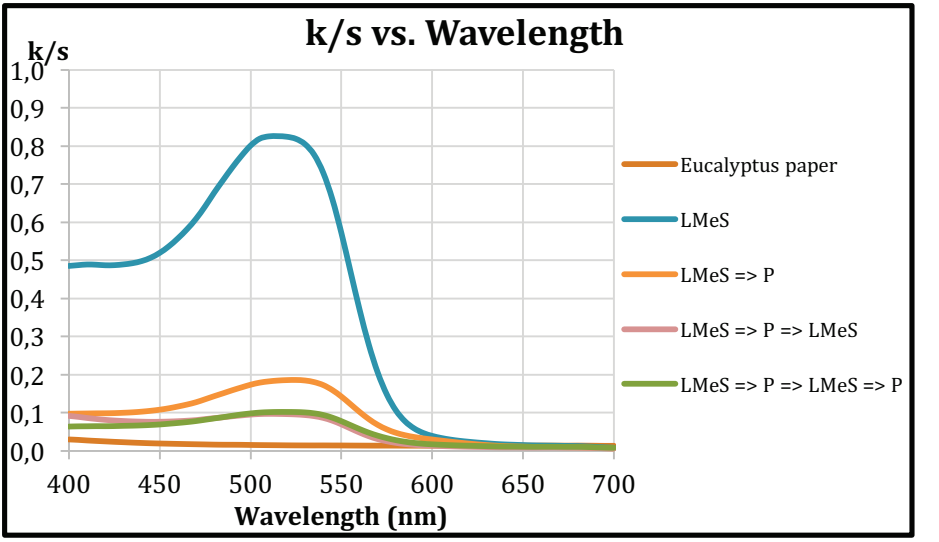


Figure 38 : Sequence LMeS P LMeSP

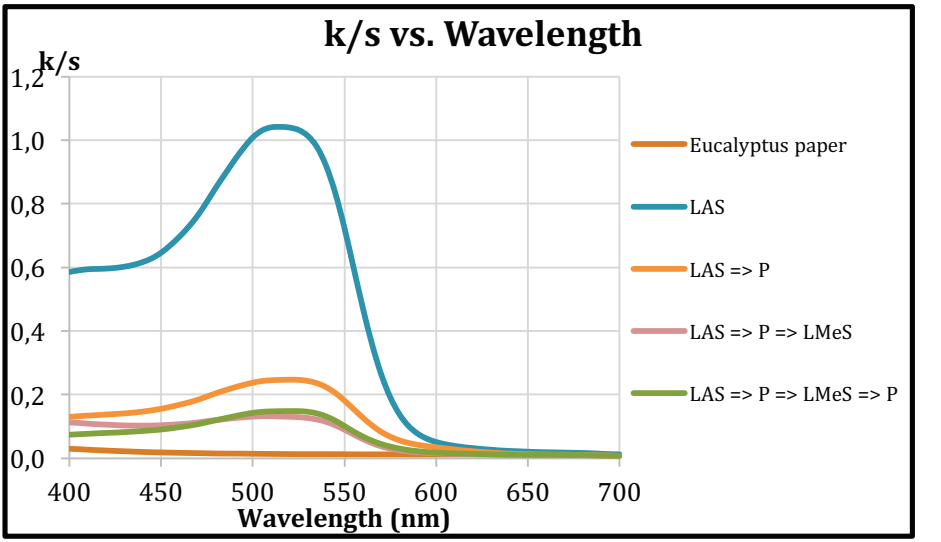


Figure 39 : Sequence LAS P LMeS P

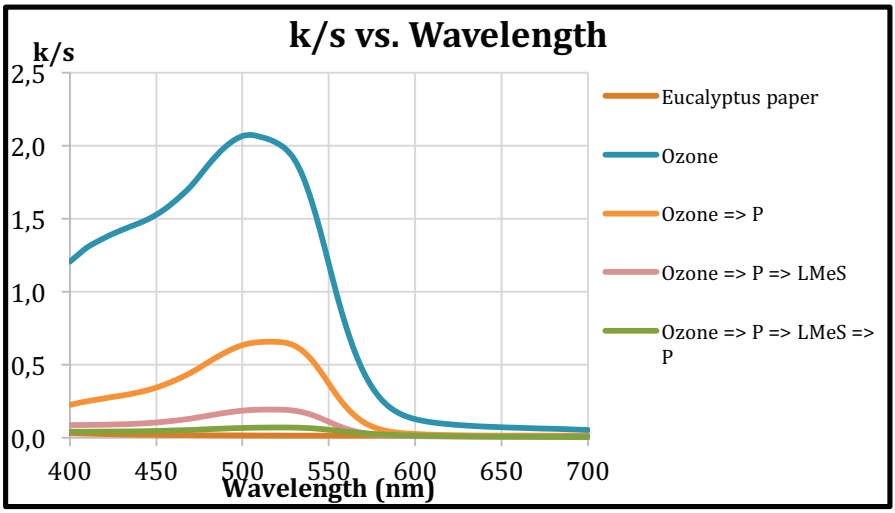


Figure 40 : Sequence Z P LMeS P

In Figures 36, 37, 38, 39 and 40 it is shown that as we move forward in the sequence the colour removed and in some cases be reach spectrum similar to that of the eucalyptus.

In order to do a good comparison, the DRI of all the stages in the last P stage.

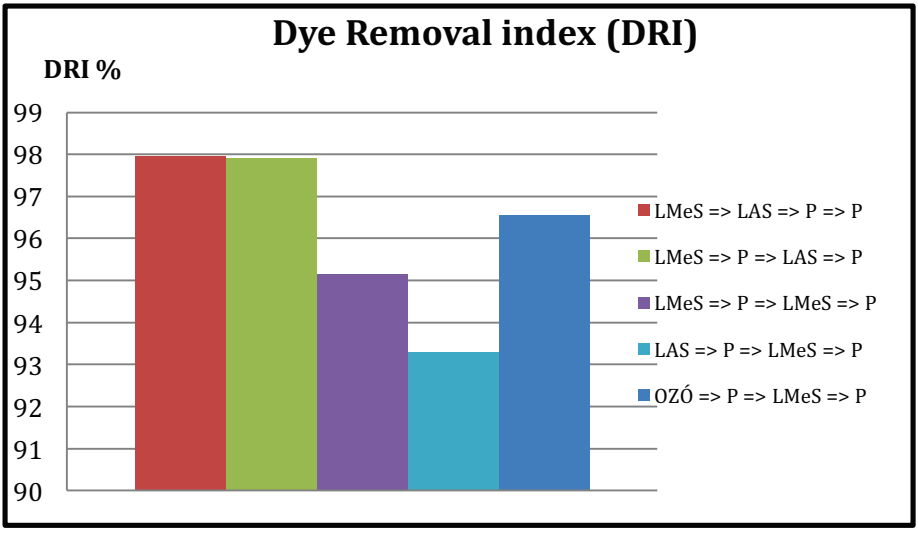


Figure 41 : Comparison of the loss of colour after the different sequences of elimination of colour.

In Figure 42 you can see a graph with the last stage P of the sequences removal colour. You can see the two best are those combining AS and MeS.

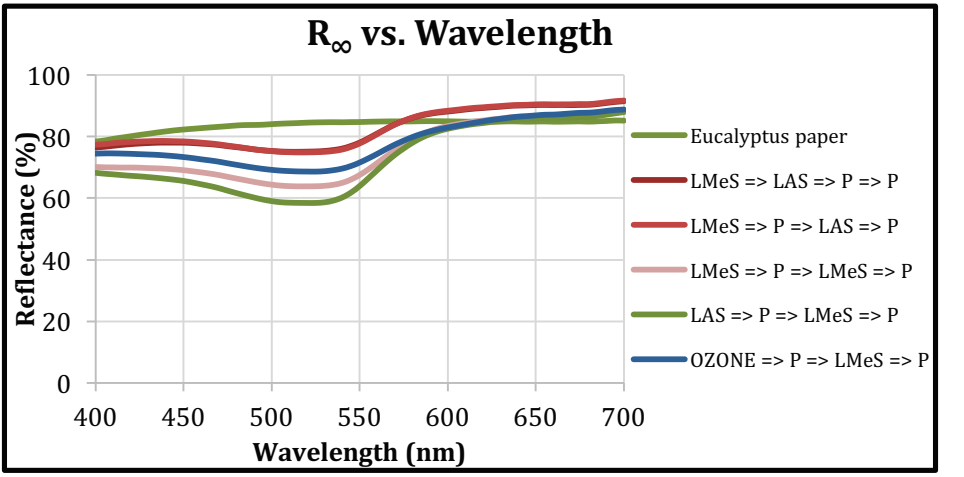


Figure 42 : Different sequence of elimination of colour.

Summarizing, the best sequence of decolourising is the ones that combines first the MeS and then the AS. For this reason, the stage F is applied, which is a stage that uses a reductor to try to achieve the final objective. We can see that the order of the treatments affects, since if we use the MeS first and the AS after the efficiency is better than if we change the sequence. We can also see that the treatment using ozone is not as effective as expected. On the other side, applying the MeS twice is not more effective than the combination of MeS and AS. In Figure 43 we can see the difference between treatments. In this case the differences are not so easy to appreciate.

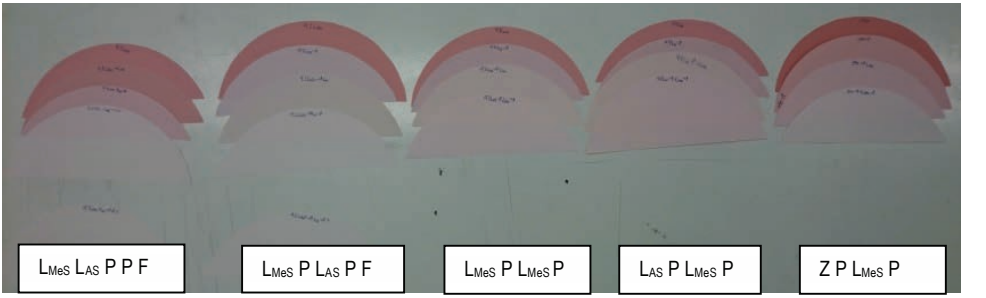


Figure 43 : Different treatments used

Finally in Figure 44 shows the colour difference (ΔE). You can see that the samples that show the greatest ΔE are those that have a greater loss of colour and therefore are more efficient. The best is MeS 1.5% and the worst is SA 1.5%.

Treatments	ΔE*	Treatments	ΔE*
L_K	1.40	P_K	22.59
L_{SA} 1.5 %	4.38	P_{SA} 1.5 %	25.32
L_{AS} 1.5 %	26.03	P_{AS} 1.5 %	50.54
L_{MeS} 1.5 %	31.46	P_{MeS} 1.5 %	55.00
L_{SA} 3 %	4.40	P_{SA} 3 %	34.51
L_{AS} 3 %	23.15	P_{AS} 3 %	50.50
L_{MeS} 3 %	29.90	P_{MeS} 3 %	53.96
$L_{MeS + SA}$	6.38	$P_{MeS + SA}$	46.55
$L_{MeS + AS}$	23.64	$P_{MeS + AS}$	52.03
$L_{SA + AS}$	6.75	$P_{SA + AS}$	35.51
		Z	18.36
		Z => P	36.23

Figure 44 : Colour difference

6.5. EVALUATION OF THE BEST LACCASE-MEDIATOR ON BLACK PAPER.

At this moment, there is no bibliography that studies the Black carbon oxidation in the paper.

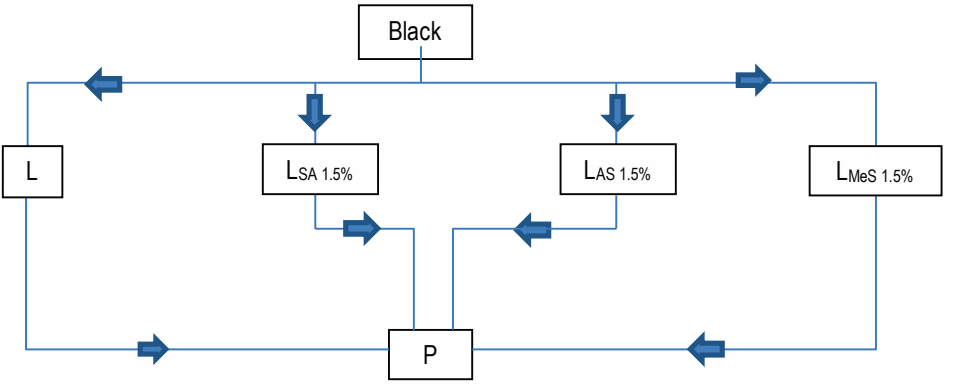
The Figure 45 shows the initial chromatic coordinates.

	Chromaticity Coordinates					
	L*	a*	b*	C*	h*	B
Initial Pulp	35.65	-0.94	-1.63	1.89	240.12	9.33

Figure 45 : Initial chromatic coordinates

From previous observations, we can say that the best treatment for recycling the red paper is the laccase-mediator system, using the natural mediator methyl syringate (MeS). We consider doing a repetition of the experiments using the different mediators but using the black paper as a starting point. As we said before, the black carbon is a pigment. In the previous experiments our objective is to turn this recycled paper in a paper completely free of colourants. For this reason, we will try to achieve a decolourized paper similar to the eucalyptus paper, which will be our final objective.

The next figure shows the different experiments done.



In Figure 46 and 47 the reflectance curves and the wavelength are show. We can see that the black carbon does not reflect the light, but absorbs it. That's why the curves are almost a straight lane. We can also see that the fact that the curves are a straight lane means that there are no hidden colours behind the black carbon. We can also see that the laccase-mediator system doesn't cause a big effect in the black carbon. There is little difference between the treatment and the stage of control. But we can appreciate that in stage P (Figure 47) a loss of colour is produced when using different treatments. The best one is the MeS. The same trend is observed than in red paper with the different mediators.

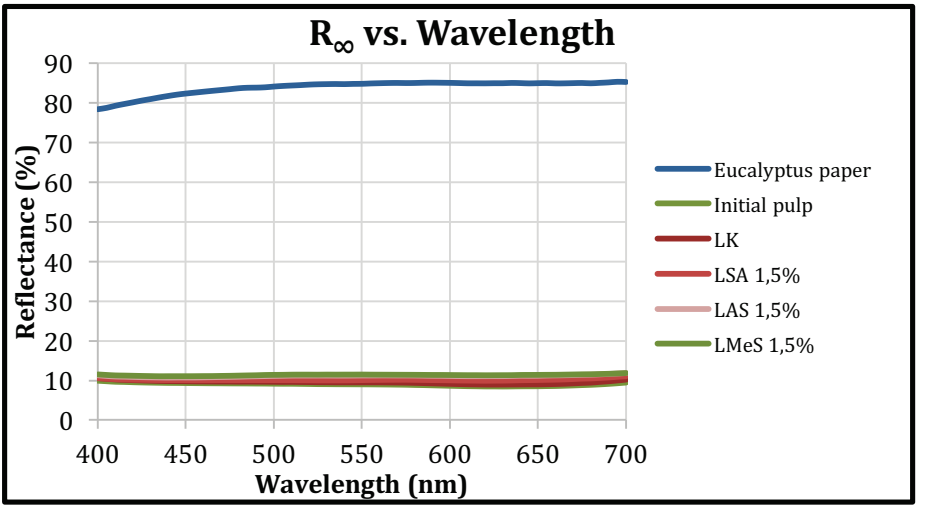


Figure 46 : Valorisation of the treatments with the lacasse-mediator system with black carbon paper.

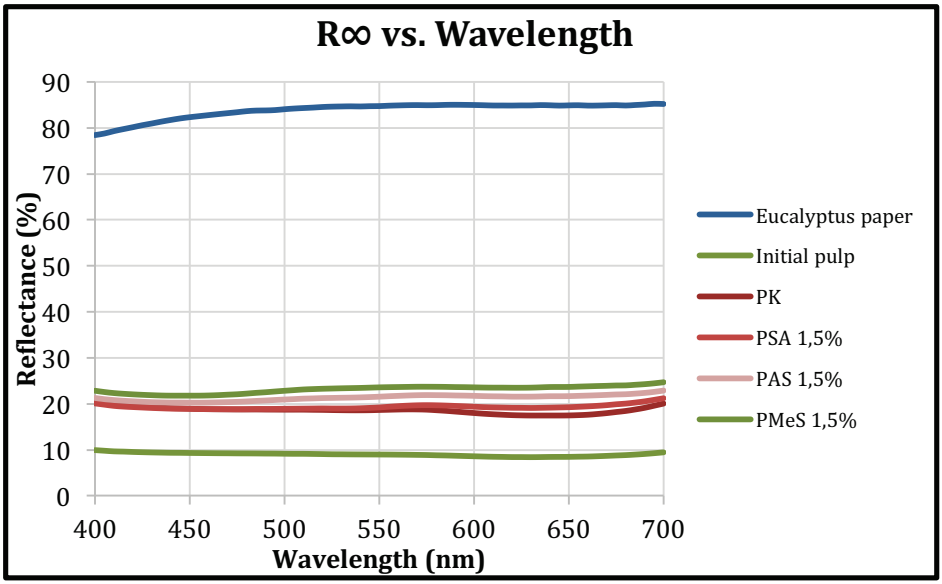


Figure 47 : Reflectance graph of stage P with control stage and the different mediators.

In order to confirm the last statements, we do a graph to compare L* and C*. We cannot assure it completely since the difference between them is not so big but in Figure 48 we can see that MeS is better and that the L stages don't affect the initial. We can also assure that the using the laccase-mediator system in comparison with the control stage don't show significant differences. On the other hand, between stage L and P significant differences can be appreciated. For this reason, a decolouration system will be applied.

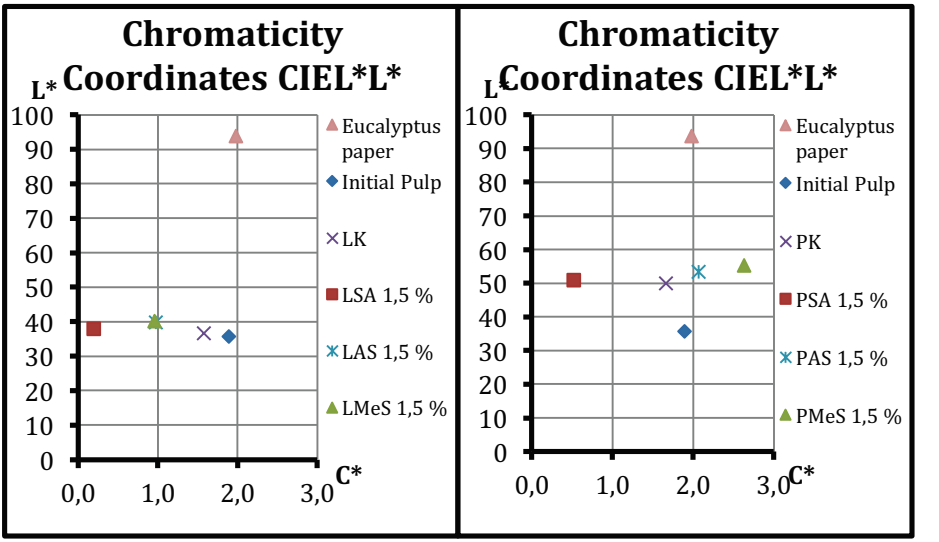


Figure 48 : Graph L* vs C*

As mentioned before, we cannot appreciate the difference between using different mediators (Figure 49), but we can see the difference between the two stages. The ones from behind are the ones treated with stage L and the ones in front where treated with stage P.

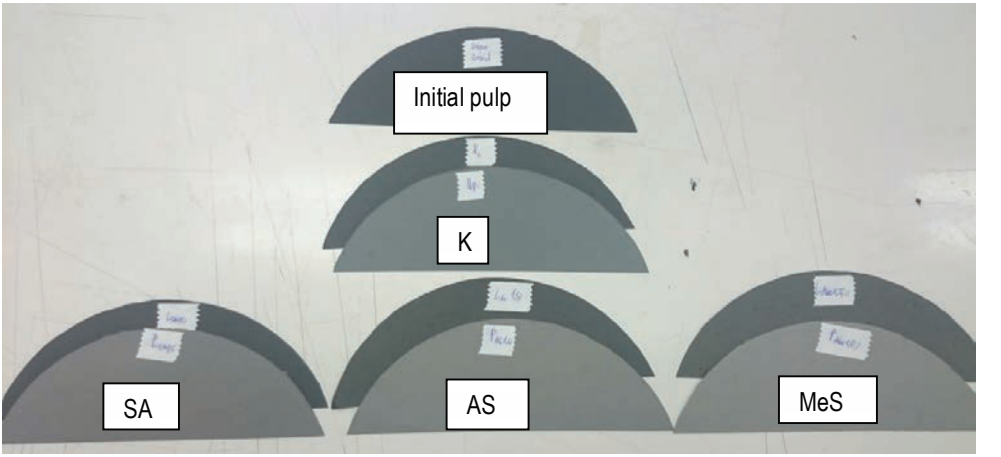


Figure 49 : Difference between the different treatments used.

6.6. COMPLETE BIODEINKING SEQUENCE

Seeing the low effect that the treatments have we will also do a decolouration sequence. After each stage the dough will be washed with water; this also produces a loss of colour. The sequences choose are the ones that gave better results when treating the red colour: L_{MeS} P L_{AS} P, L_{MeS} P L_{MeS} P, Z P L_{MeS} P.

The figure below shows the different sequences choose.

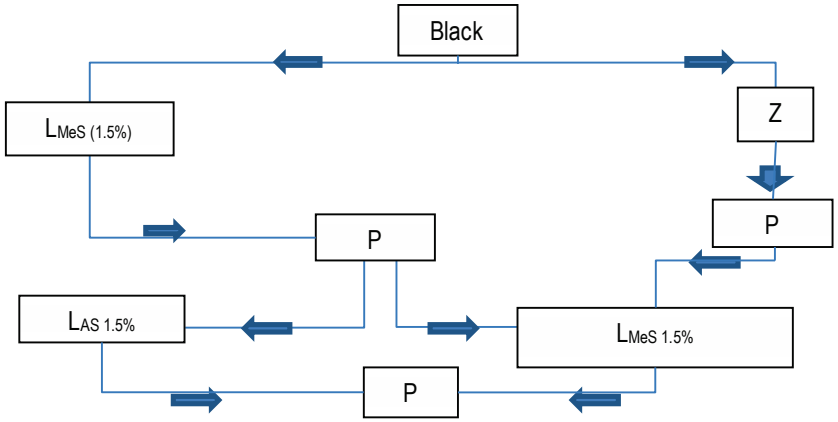


Figure 50 : Sequence of elimination of colour.

The next figures show the different stages used in each treatment. We can see that using the stages in series, alternating between the washed, increases the efficiency of the experiment. We can also see that each time that we do a treatment, the L* increases. In Figure 51 and 52 we can see that the measure that we change in the sequences of eliminating colour, the C* also increases gradually. On the other hand, in Figure 53, where ozone was used, we cannot see any increase or decrease of C* but an increase of L* is showed. The Chroma goes down in the L stage and later on grows in the next stages. The black pulp has a low a*, b* and C*.

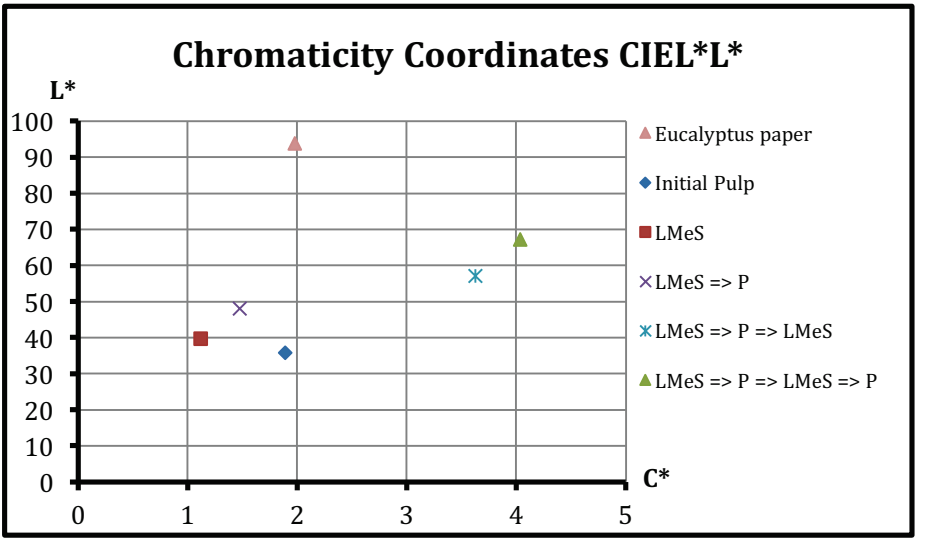


Figure 51 : Sequence of elimination of colour LMeS P LMeSP

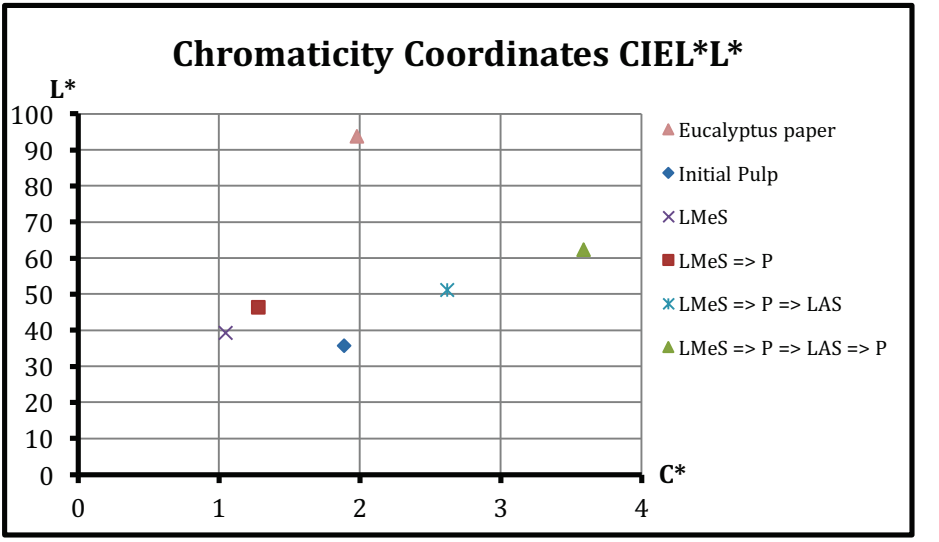


Figure 52 : Sequence of elimination of colour LMeS P LAS P

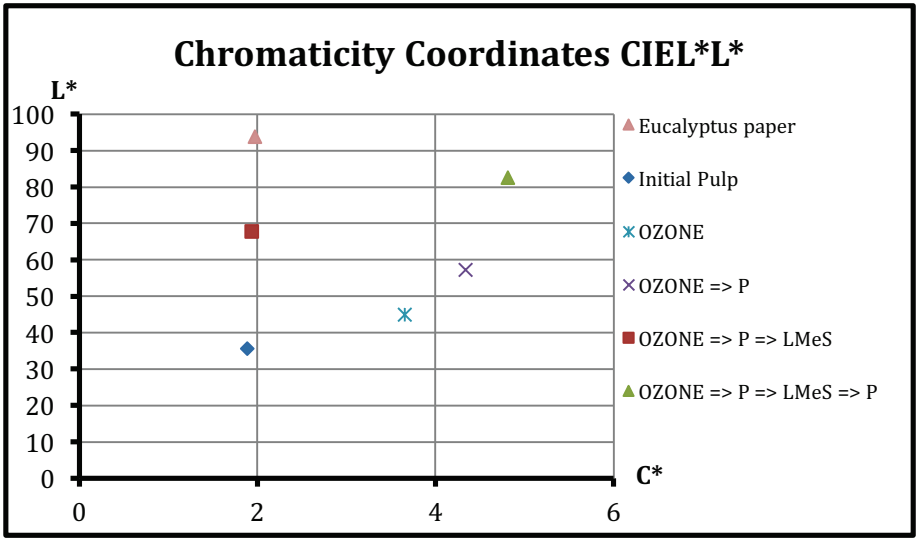


Figure 53 : Sequence of elimination sequence of elimination of colour Z P LMeSP

In order to do a proper comparision, we will use the DRI of the different sequencies in the last stage of the decolouration. In Figure 54 we can see that the best sequence is the one that uses ozone and the worst one the one using MeS followed by AS (contrary to that obtained with red paper).

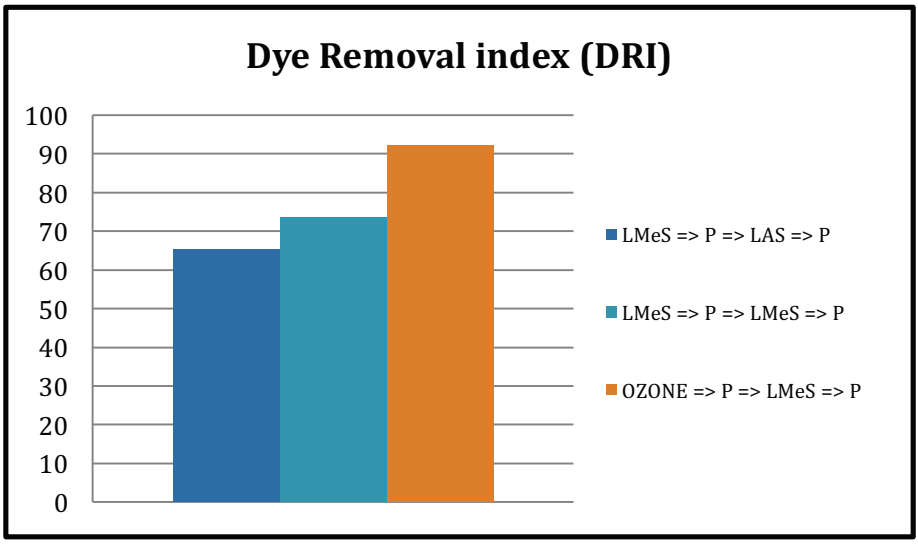


Figure 54 : Dye removal index

In Figure 55 the you can see a graph with the last stage P of the sequences removal colour. You can see that the two best are those combining ozone and MeS.

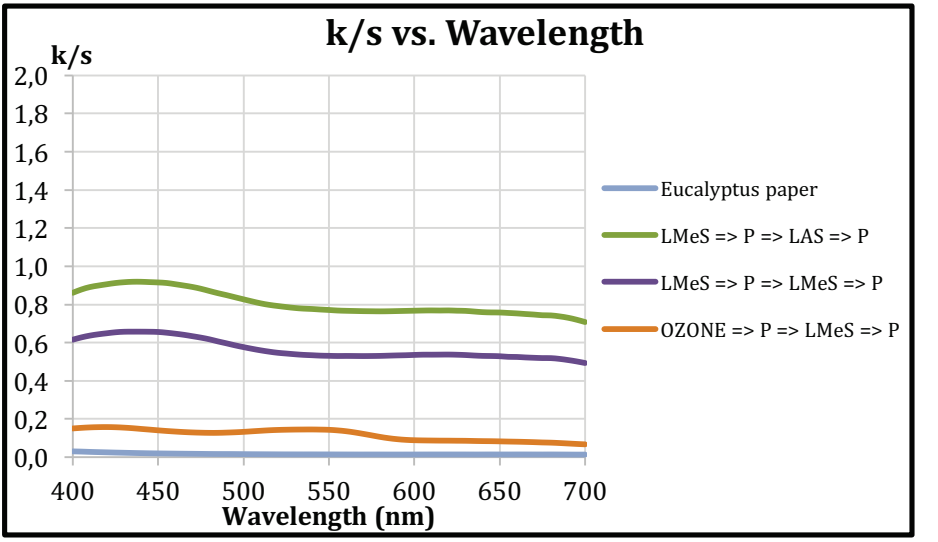


Figure 55 : Different sequence of elimination of colour.

In the figure below we can see the loss of colour in each of the sequences

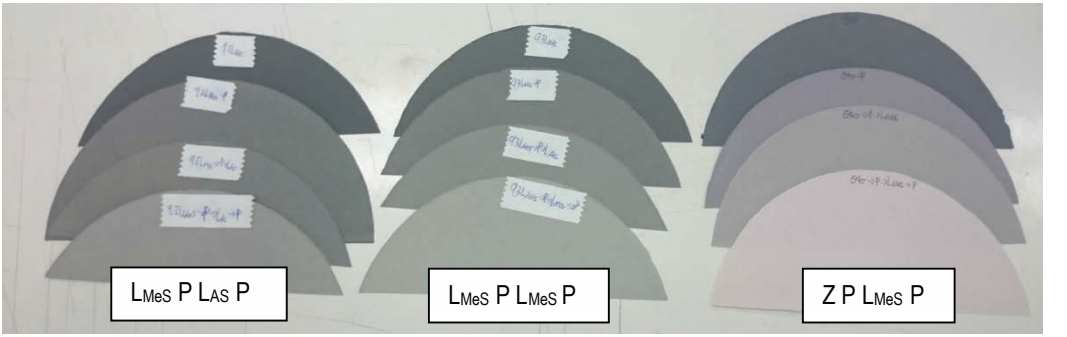


Figure 56 : In the figure below we can see the loss of colour in each of the sequences.

Finally Figure 57 shows the colour difference (ΔE). You can see that the ones that have a greater ΔE* are those who have a greater loss of colour and therefore are more efficient. The best is MeS 1.5% and the worst is SA 1.5%.

Treatments	ΔE*	Treatments	ΔE*
L_K	1.05	K_P	14.23
L_{SA} 1.5 %	2.77	P_{SA} 1.5 %	15.37
L_{AS} 1.5 %	4.89	P_{AS} 1.5 %	18.07
L_{MeS} 1.5 %	5.13	P_{MeS} 1.5 %	20.08

Figure 57 : Colour difference

6.7. COMPARISON OF THE ELIMINATION OF COLOUR BETWEEN RED AND BLACK

The final part is to compare the loss of colour between red and black. For comparing it we will use the DRI, since it has into consideration the initial colour. We can see in Figure 58 how in stage L the laccase alone is more effective with black than with red; on the other hand, when using the mediators is more effective in red than in black. The effect is more evident for AS and MeS. We can also see that stage P is more effective in red than in black.

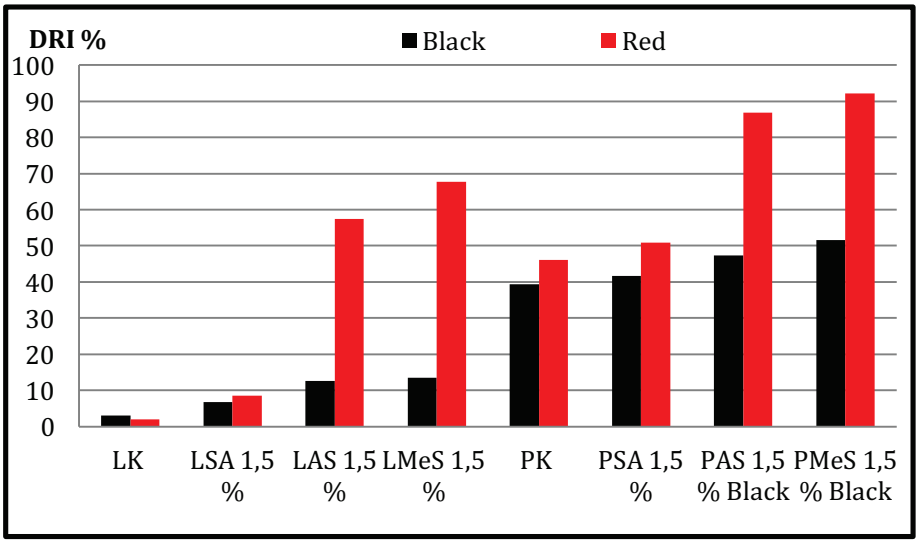


Figure 58 : Comparison between black and red.

The table below shows the difference between treatments L and P according to the DRI. We can see how in the treatment with laccase alone and the mediator SA there is a bigger difference between stages L and P in the red paper than in the black paper. On the other hand, when using the MeS and AS mediators the difference is bigger in the black paper than in the red one.

	Difference between red and black		
K	36.22	Black	Red major
	44.16	Red	
1.5 SA	34.86	Black	
	42.32	Red	
1.5 AS	34.76	Black	Black major
	29.46	Red	
1.5 MeS	38.12	Black	
	24.60	Red	
Ozone	27.96	Black	Equal
	28.64	Red	

Figure 59 Difference between red and black

6.8. MECHANICAL PROPERTIES

In Figure 60 we can see how the MeS in the red is the one that gives fewer strength to the traction, and SA the biggest. On the other hand, in the black paper what gives better results is the MeS and the worst is AS. The AS in the black paper causes a loss of mechanical properties when compared with the initial state. All the others have caused an increase in the maximum strength. The lowest value is obtained with ozone.

RED				
Treatment	Weight	Grams	Thikness	Max strenght
Units	gr		µm	N
Initial pulp	2,17	68,95	102 ± 1.00	63,36 ± 1.44
L_K	2,31	73,44	117,2 ± 1.10	33,945 ± 4,43
$L_{SA\ 1.5\%}$	2,21	70,32	124,4 ± 2.40	36,63 ± 0.09
$L_{AS\ 1.5\%}$	2,26	71,91	120,8 ± 1.80	38,655 ± 1.49
$L_{MeS\ 1.5\%}$	2,36	75,10	121,4 ± 3.98	35,245 ± 1.32
Ozone in red	2,28	72,58	130,4 ± 2.60	32,355 ± 0.87
BLACK				
Treatment	Weight	Grams	Thikness	Max strenght
Units	gr		µm	N
Initial pulp	2,27	72,23	124,2 ± 4.90	36,64 ± 0.05
L_K	2,28	72,64	120,2 ± 1.50	38,68 ± 1.45
$L_{SA\ 1.5\%}$	2,25	71,53	124,6 ± 13.10	34,94 ± 5.05
$L_{AS\ 1.5\%}$	2,28	72,45	117,8 ± 1.30	31,82 ± 0.45
$L_{MeS\ 1.5\%}$	2,28	72,52	117 ± 1.70	38,83 ± 2.21
Ozone in black	2,25	71,66	120,4 ± 2.25	30,355 ± 0.90

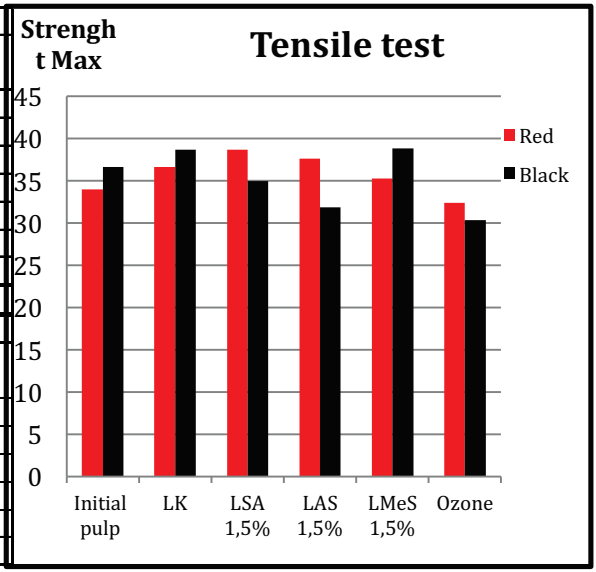


Figure 60 : Tensile test

The next figure shows the resistance to the escalation. In Figure 61 we can see that in both red and black, the one that gives better resistance to the escalation is MeS. Between the different mediators there are no big difference in red and black. With laccase alone red loses a bigger amount of resistance to the escalation.

Red		Black	
Initial pulp	1.41 ± 0.01	Initial pulp	1.227 ± 0.09
L_K	1.25 ± 0.01	L_K	1.5 ± 0.12
$L_{SA\ 1.5\%}$	1.487 ± 0.06	$L_{SA\ 1.5\%}$	1.597 ± 0.04
$L_{AS\ 1.5\%}$	1.477 ± 0.04	$L_{AS\ 1.5\%}$	1.487 ± 0.08
$L_{MeS\ 1.5\%}$	1.497 ± 0.04	$L_{MeS\ 1.5\%}$	1.657 ± 0.07
Ozone	1.467 ± 0.14	Ozone	1.387 ± 0.18

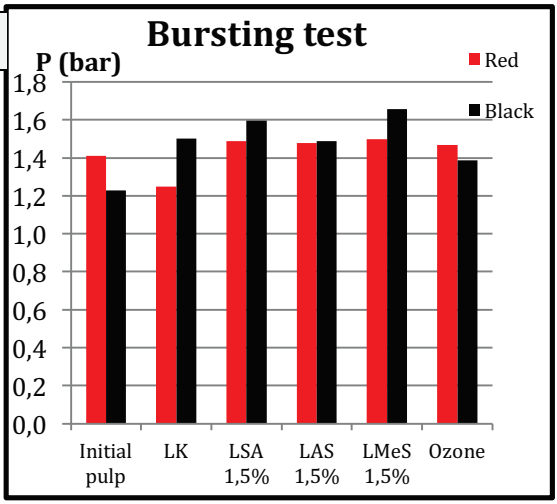


Figure 61 : Bursting test

6.9. RESIDUAL ENZYME ACTIVITY

Residual enzyme activity of the effluents of paper treated using different mediators and papers red or black showed differences between each one of the treatments used (Figure 62 and 63) :

Initial residual enzyme activity is 1.05U/ml

All the calculations of the experiments have been done according to the corresponding rules (see attached in Materials and Methods)

Red Pulp			
Treatment	Δ_{Abs}	**Activity (U/ml)**	**Percentage of inactivity (%)**
L_K	0.317	0.216	79.346 ± 0.10
	0.32	0.218	
$L_{SA1.5\ \%}$	0.379	0.259	76.622 ± 1.20
	0.342	0.233	
$L_{AS1.5\ \%}$	0.01	0.007	99.351 ± 0.00
	0.01	0.007	
$L_{MES1.5\ \%}$	0.008	0.005	99.546 ± 0.06
	0.006	0.004	
$L_{SA\ 3\%}$	0.422	0.288	71.629 ± 1.01
	0.453	0.309	
$L_{AS\ 3\%}$	0.018	0.012	98.767 ± 0.06
	0.02	0.014	
$L_{MeS\ 3\%}$	0.004	0.003	99.675 ± 0.06
	0.006	0.004	
L_{MeS+SA}	0.012	0.008	99.286 ± 0.06
	0.01	0.007	
L_{MeS+AS}	0.012	0.008	99.546 ± 0.32
	0.002	0.001	
L_{SA+AS}	0.043	0.029	97.244 ± 0.03
	0.042	0.029	

Figure 62: Absorbance of the red effluents.

Black pulp			
Treatment	Δ_{Abs}	**Activity (U/ml)**	**Percentage of inactivity (%)**
L_K	0.302	0.206	80.546 ± 0.13
	0.298	0.203	
$L_{SA\ 1.5\%}$	0.470	0.321	70.430 ± 0.91
	0.442	0.302	
$L_{AS\ 1.5\%}$	0.050	0.034	95.784 ± 0.97
	0.080	0.055	
$L_{MeS\ 1.5\%}$	0.010	0.007	99.481 ± 0.13
	0.006	0.004	

Figure 63 : Absorbance of the black effluents.

As we can see, the treatment which consumes more enzyme, and therefore has a bigger percentage of inactivity is the treatment using MeS. It is also the treatment which gives better results when whitening paper dough. The isolated laccase (LK) already losses enough activity within 4h in the conditions of the treatment, but adding mediators it increases even more. It indicates that we can't reuse the enzyme.

7. CONCLUSIONS

The reflectance curves and the k/s curves show the optic properties of the paper used, showing that they are composed of only of one colourant. When comparing the obtained information about the chromatic coordinates with the k/s curves we can see a relation between both parameters: the lower the L* is the bigger the values beneath the curve k/s are.

The oxidizing capability of the colourant with the laccase-mediator system is better than the system using laccase alone. The laccase-mediator system which oxides the colourant best is the one that uses natural Methyl Syringate (MeS) both in red and black papers.

The best combination of mediator with a bigger oxidation capability is the one that combines MeS and AS in the red colour. When increasing the concentration of mediator, the efficiency of the treatment does not increase.

The combination of MeS with other mediators combined in one treatment results worse than when we use them separately. When comparing all the treatments where natural MeS is used as mediator we can see that the best of all is the MeS by itself with a concentration of 1.5%.

In the whitening sequence of the red paper, the two best sequences where those where MeS was used first followed by AS (L_{MeS} L_{AS} P P F or L_{MeS} P L_{AS} P F). On the other hand, for black paper the best sequence is that where first we used a treatment with ozone followed by the laccase-mediator system with 1.5% of MeS (Z P L_{MeS} P).

Stage F doesn't cause a big change in the whitening of paper.

When comparing the loss of colour in red and black paper we can see that the laccase-mediator system is more efficient in red paper than in black paper. The treatments with the laccase-mediator system showed small improvements on the mechanical properties. The characteristics of the effluent show that the best treatment of the laccase-mediator system is the ones that has bigger enzymatic inactivity, therefore the MeS mediator.

8. REFERENCES AND NOTES

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9. ACRONYMS

a* X axis CIEL * a * b * system
ABTS 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
AS **A**cetosyringone mediator
b* Y axis CIEL * a * b * system
C* Chroma; degree of colour saturation
D_{65} Light source and lighting
DRI Dye removal index
DTPA Diethylenetriaminepentaacetic acid
F Colour removal stage with formamidine sulfonic acid
HBT *N,N'*-Bis-(1*H*-tetrazol-5-yl)-hydrazine
K Control **c**olour removal stage
k/s Kubelka-munk coefficient
L Removal stage with the laccase-mediator system
L* Brightness, Z axis CIEL * a * b * system
MeS Methyl syringate mediator
P Colour removal stage with hydrogen peroxide
odp Oven dried pulp
R_∞ Intrinsic reflectance factor
SA Syringaldehyde mediator
TCF Totally chlorine-free paper
wp Wet pulp
Y Colour removal stage with sodium hydrosulphite
Z Colour removal stage with ozone

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APPENDICES

11. APPENDIX 1: EXPERIMENTAL METHOD

(Same steps will be applied at each experiment)
Declaration of paper recycled using biotechnological methods.
In all the experiments the density will be considered, both for solids and liquids, equal to the density of water (ρ= 1000 Kg/L).

Preparation of the paper to be treated at the reactor

The tutor of the projects gives us a brief update of the methods we are about to use. The methods consist on using different mediators such as: MeS (Methyl syringate), SA (syringaldehyde) and AS (acetosyringone) with a 1, 5% in weight for every mediator and a 3% in weight for every mediator.

Image 1

A mix of mediator (1,5% MeS +1,5% SA, 1,5% MeS +1,5% AS, 1,5% SA +1,5% SA) will be prepared as well to compare its effectiveness when compared to the treatment using ozone. An experiment without using mediator (K_L), only with lacasa will be done to determine the necessity of using mediators.
First of all, we take the red wet paper and introduce it in a plastic flask of 5L of capacity filled with deionized water for turning the paper into a homogenous dough (Image 1), leaving it to rest for one day.

Glass	Red paper (gr)	Decalcified water (gr)
1	30	1035,5
2	30	1011,5
3	30	1011,5
4	30	1020,5
5	30	1011,5

Then we prepare the tampon solution of sodium phosphate 200 mM.
Sodium phosphate monobasic (A)

$$500\ ml * \frac{0{,}2\ mol}{1000\ ml} * \frac{137{,}99\ gr}{1\ mol}$$
$$= 13{,}799\ gr\ sodium\ phosphate\ monobasic$$

Sodium phosphate dibasic (B)

$$500\ ml * \frac{0{,}2\ mol}{1000\ ml} * \frac{177{,}99\ gr}{1\ mol}$$
$$= 17{,}799\ gr\ Sodium\ phosphate\ dibasic$$

We weight the two types of phosphates in a flask of 200mL. Once weighted, we dissolve the, in deionized water using a magnetic agitator. Then we introduce them in a volumetric flask of 500mL. Once the two solutions are prepared, we take 156 mL of A, which has a pH of 4.67, and add B to it until the solution reaches a pH of 7. This process will be done using a pH meter (Image 2). This will take approximately 244 of B to take place. All this values are established for these procedures.

Image 2

Once the solution has the desired pH, we store it in a glass flask inside a fridge.

We take the paper samples which were previously introduced in water and place each of them separately in the normalized disintegrator (See specification sheet in Annex 2) (Image 3). This is a device typically used in the paper industry.

The number of spins that need to be done for producing a chemical dough with a content greater than 20% in weight of dried material is of around 30.000 spins.

- Sample number 2 was in a slightly broken flask and during the time that the paper was left to rest, some of the water has leaked out of the flask. The amount leaked is small enough to assume that it won't affect the results obtained.

Image 3

Once the mixture of paper and water has turned into dough, we filter it (Image 4) (See specification sheet in Annex 3), since we are interested in obtaining dough with the lowest amount of water possible. After filtration, we crumble the solid in small pieces and place them into a bag (Image 5). In order to know the amount that we need to put in every bag (gr odp) we need to calculate its humidity (gr wt). The humidity will be obtained using a humidity analyser which works using infrared rays and doing a gravimetric balance (Image 6) (See specification sheet in Annex 4).

Image 6

Image 5

Image 4

The dough has 27,48g of dried material (gr odp) for each 100g of humid dough (gr wp). Therefore, the amount of humid dough will be calculated as follows:
With mediators:

$$12\ gr\ odp * \frac{100\ gr\ wp}{27{,}48\ gr\ odp} = 43{,}66\ gr\ wp$$
$$\rightarrow 12\ gr\ odp + 31{,}66\ gr\ Water$$

With ozone:

$$15\ gr\ odp * \frac{100\ gr\ wp}{27{,}48\ gr\ odp} = 54.58\ gr\ wp$$
$$\rightarrow 15\ gr\ odp + 39.58\ gr\ Water$$

Therefore, 10 bags of 12 gr odp and 1 of 15 gr odp will be needed.

Treatment with the lacasa-mediator system (L)

Experiment 1

We take 4 bags of 12 gr odp and place them in the reactor. We know that our experiment has a consistency of 5%. With this we can calculate the amount of gr that we will need to ensure this consistency. Supposing that the density is equal to 1000 Kg/L:

$$12\ gr\ odp * \frac{100\ gr\ totals}{5\ gr\ odp} = 240\ gr\ total = 240\ ml\ total$$

We calculate the amount of enzyme (lacasa: Myceliopthora thermophila) that we will need knowing that it has 20 units/gr odp and an activity of 855, 33 unities per mL:

$$12\ gr\ odp * \frac{20\ U}{1\ gr\ odp} * \frac{1\ ml}{855{,}33\ U} = 0{,}28\ ml = 280\mu l$$

We calculate the amount of mediator needed as well, knowing that the dose is of 1,5% sps.

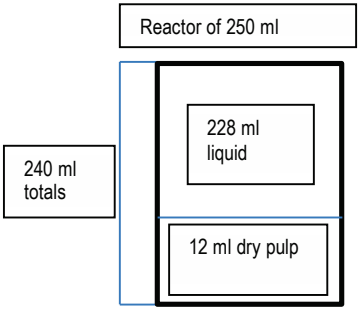
$$12\ gr\ odp * \frac{1{,}5\ mediator}{100\ \ gr\ odp} = 0{,}18\ gr\ of\ mediator$$
$$= 0{,}18\ ml\ of\ mediator$$

With the total quantity of grams, we calculate the amount of liquid needed.

$$240\ gr\ totals - 12\ \ gr\ odp = 228\ gr\ of\ liquid$$
$$= 228\ ml\ of\ liquid$$

We know from previous experiments that the amount of tampon is 4 times lower than the amount of liquid that we will need.

$$\frac{228\ ml\ of\ liquid}{4} = 57\ ml\ of\ tampon$$



228 ml of liquid are formed of:

- Tampon → 57 ml
- Enzyme → 0,28 ml
- Water from the dough → 31,66 ml
- Mediator → 0,18 ml
- Deionized water →? ml

$$228\ ml\ of\ liquid - 57\ ml\ of\ tampon - 0{,}28\ ml\ of\ enzime - 31{,}66\ ml\ of\ water\ from\ the\ dough - 0{,}18\ ml\ of\ mediator = 138{,}88\ ml\ deionzied\ water.$$

K_L has to be prepared as well. It will be the same than the others but without the mediator. Therefore more deionized water will be needed:

$$228\ ml\ of\ liquid - 57\ ml\ of\ tampon - 0{,}28\ ml\ of\ enzime - 31{,}66\ ml\ water\ from\ the\ dough = 139.06\ ml\ of\ deionized\ water$$

We will dissolve the mediator*, using a magnetic agitator and introducing the water (138, 38 mL)

- *We can see that the mediators have low solubility. We will leave them to rest for approximately 45 minutes to try to dissolve the maximum amount possible.

Once the mediator has partially dissolved, we preparer de reactor with 250 mL of capacity. We introduced 43,66 gr wp in the EASYDYE reactors (Image 7) (See specification sheet in Annex 5). Then we introduce the mediator and the deionized water used to dissolve it. We take the 57 mL tampon and place it in the

Image 7

Image 8

same flask that we used for dissolving the mediator in order to recover the maximum amount of mediator possible. With a pipette of 10 µl – 300 µl, we pick the Lacasa (280 µl) and place it in the flask. We close the reactor and put it in the EASYDYE Reactor. It's an apparatus widely used in the ink industry which works at atmospheric pressure and temperature. In our case, the working temperature is of 50ºC. The experiment will take place in a period of 4h.

Once the reaction time has finished, we filter the mixture with a Buchner filter in order to separate the dough from the remaining liquid. We pass it through the filtration system (image 8) and clean it 3 times with decalcified water and one with deionized water. After that, we crumble the solid in small pieces and put them in a bag (Image 4). We calculate the effectiveness of each of the drying methods. With the remaining water we calculate the pH of each one.

	Dryness
K_L	25,27 gr odp/100 gr wp
L_{AS} at 1,5 % in weight	21,53 gr odp/100 gr wp
L_{SA} at 1,5 % in weight	27,66 gr odp/100 gr wp
L_{MeS} at 1,5 % in weight	23,12 gr odp/100 gr wp

	pH	**Temperature (ºC)**
KL	7,32	20
L_{AS} at 1,5 % in weight	7,42	20
L_{SA} at 1,5 % in weight	7,33	20,8
L_{MeS} at 1,5 % in weight	7,26	20,8

Once we finished cleaning, we clean the filter with a chromic mixture prepared by the teacher. A company of from the Generalitat de Catalunya, hired by the UPC of Terrassa takes care of the wastes.

Image 9

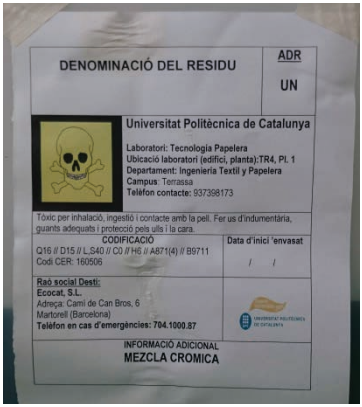


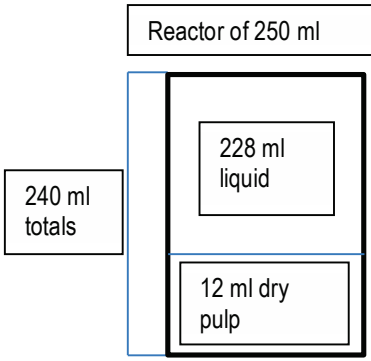
Image 10

Experiment 2

Same procedure as in experiment 1 but instead of using 1,5% of mediator we use 3%. Therefore the amount of mediator is the described below:

$$12\ \ gr\ odps * \frac{3\ gr\ mediator}{100\ \ gr\ odp} = 0{,}36\ gr\ of\ mediator$$

The proportion of enzyme will be the same (0, 28 mL) and the total dough is 240 mL.



228 mL of liquid are formed of:

- Tampon → 57 ml
- Enzyme → 0,28 ml
- Water from the dough → 31,66 ml
- Mediator → 0,36 ml
- Deionized water → ? ml

$$228\ ml\ of\ liquid - 57\ ml\ of\ tampon - 0{,}28\ ml\ of\ enzime - 31{,}66\ ml\ of\ water\ from\ the\ dough - 0{,}36\ ml\ of\ mediator = 138{,}7\ ml\ deionzied\ water.$$

The K_L, since there is no mediator, will remain unchanged. That's why we won't do it this time.

We will introduce the reactor in the EASYDYE*

Once the reaction time has passed, we filter with a Buchner filter (image 11) and separate the dough from the bleach (remaining liquid). We pass it through a filtration system and wash it 3 times with decalcified water and one with deionized water. After that,

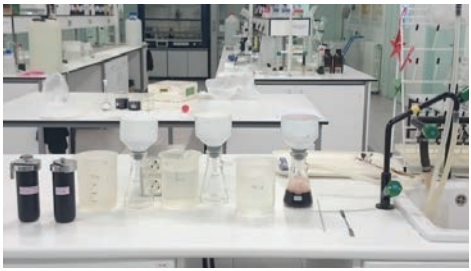


Image 11

we crumble the solid in small pieces and put them in a bag. We calculate the effectiveness of each of the drying methods. With the remaining water we calculate the pH of each one.

*In the EASYDYE we put the reactor, which has a temperature sensor with deionized water to control it. In theory, the control reactor has to have the same capacity that the ones that we are using. In my case, the first experiment was done with a reactor of 125 mL due to logistic problems. For this reason the same reactor was used in the following experiments to reduce the error.

	Dryness
L_{AS} at 3 % in weight	22,84 gr odp/100 gr wp
L_{SA} at 3 % in weight	20,61 gr odp/100 gr wp
L_{MeS} at 3 % in weight	21,36 gr odp/100 gr wp

	pH	**Temperature (ºC)**
L_{AS} at 3 % in weight	7,19	20,2
L_{SA} at 3 % in weight	6,97	20,9
L_{MeS} at 3 % in weight	7,10	21,1

Experiment 3

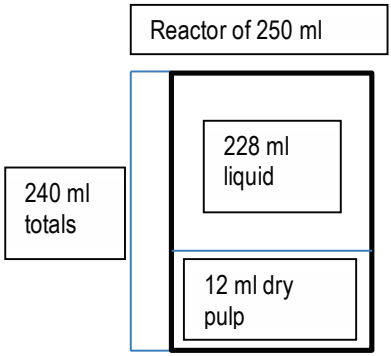
Combination of the different mediators in a proportion of 1,5% in weight of each one.

$$12\ gr\ odp * \frac{1{,}5\ gr\ mediator}{100\ \ gr\ odp} = 0{,}18\ gr\ for\ each\ mediator$$

Combinations:

- MeS (0,18 gr) + AS (0,18 gr)
- MeS (0,18 gr) + SA (0,18 gr)
- SA (0,18 gr) + AS (0,18 gr)

The experimental procedure will be the same than in experiment 2. The proportion of enzyme will be the same (0, 28 mL) and the total dough is 240 mL.



228 mL of liquid are formed of:

- Tampon → 57 ml
- Enzyme → 0,28 ml
- Water from the dough → 31,66 ml
- Mediator → 0,36 ml
- Deionized water → ? ml

$$228\ ml\ of\ liquid - 57\ ml\ of\ tampon - 0{,}28\ ml\ of\ enzime - 31{,}66\ ml\ of\ water\ from\ the\ dough - 0{,}36\ ml\ of\ mediator = 138{,}7\ ml\ deionzied\ water.$$

The K_L, since there is no mediator, will remain unchanged. That's why we won't do it this time.

We will introduce the reactor in the EASYDYE*

Once the reaction time has passed, we filter with a Buchner filter (image 11) and separate the dough from the bleach (remaining liquid). We pass it through a filtration system and wash it 3 times with decalcified water and one with deionized water. After that, we crumble the solid in small pieces and put them in a bag. We calculate the effectiveness of each of the drying methods. With the remaining water we calculate the pH of each one.

	Dryness*
$L_{MeS + AS}$	29.08 gr odp/100 gr wp
$L_{MeS + SA}$	32,17 gr odp/100 gr wp
$L_{SA + AS}$	29.39 gr odp/100 gr wp

*The results obtained are higher since after the filtration we squeezed the sample manually increasing the degree of drying.

	pH	Temperature (ºC)
$L_{MeS + AS}$	7,42	21,5
$L_{MeS + SA}$	7,29	21,7
$L_{SA + AS}$	7,33	21,5

Treatment with Hydrogen peroxide (P)

All the experiments are done following the same methodology.

We want to treat 6 gr odp with a consistency of 5%. With these two considerations we calculate the total volume needed for the experiments.

$$6\ gr\ odp * \frac{100\ g\ totals}{5\ gr\ odp} = 120\ g\ totals = 120\ ml\ totals$$

For doing the whitening experiments 3% de H_2O_2, 1,5% de NaOH, 1% de DTPA, 0,2% de $MgSO_4$ need to be added to each reactor. Therefore, we calculate the amount that we need to add for 6 gr odp:

H_2O_2

$$6\ gr\ odp * \frac{3\ g\ H_2O_2}{100\ gr\ odp} * \frac{1000\ ml}{29.1\ gr\ H_2O_2} = 6{,}18\ ml\ of\ H_2O_2$$

NaOH

$$6\ gr\ odp * \frac{1{,}5\ g\ NaOH}{100\ gr\ odp} * \frac{1000\ ml}{40\ gr\ NaOH} = 2{,}25\ ml\ of\ NaOH$$

$MgSO_4$

$$6\ gr\ odp * \frac{0{,}2\ g\ MgSO_4}{100\ gr\ odp} * \frac{1000\ ml}{20\ gr\ MgSO_4} = 0{,}6\ ml\ of\ MgSO_4$$

We calculate the amount of DTPA. The problem is that the DTPA is in weight %, therefore we calculate the grams of solution that we need to add.

DTPA

$$6\ gr\ odp * \frac{1\ g\ DTPA}{100\ \ gr\ odp} * \frac{100\ gr\ of\ solution\ of\ DTPA}{0{,}4\ gr\ DTPA}$$
$$= 15\ gr\ of\ solution\ of\ DTPA$$
$$= 15\ ml\ of\ solution\ of\ DTPA$$

Since each dough has a different degree of dryness, we need to calculate it for each dough in order to know the amount of wet dough that we will need. This means that for each experiment different amounts of deionized water will be needed. For calculating this amount we follow the next:

We know that the mL of H_2O_2, NaOH, $MgSO_4$ and DTPA are the totals are the same for each experiment. For simplifying the calculations we use the constant A:

$$A = ml\ total - ml\ \ H_2O_2 - ml\ NaOH - ml\ MgSO_4$$
$$- ml\ of\ solution\ of\ DTPA = 95{,}97\ ml$$
$$ml\ of\ deionized\ water = A - gr\ wp = 95{,}97\ ml - gr\ wp$$

Experiment 1

K_p

$$6\ \ gr\ odp * \frac{100\ gr\ wp}{25{,}27\ \ gr\ odp} = 23{,}74\ gr\ wp$$
$$= 23{,}74\ ml\ of\ wet\ dough.$$
$$ml\ of\ deionized\ water = 95{,}97\ ml - 23{,}74\ ml$$
$$= 72{,}23\ ml\ of\ deionized\ water$$

$P_{SA\ 1{,}5\ \%}$

$$6\ \ gr\ odp * \frac{100\ gr\ wp}{27{,}66\ gr\ odp} = 21{,}69\ gr\ wp$$
$$= 21{,}69\ mlof\ wet\ dough.$$
$$ml\ of\ deionized\ water\ \ = 95{,}97\ ml - 21{,}69\ ml$$
$$= 74.27\ ml\ of\ deionized\ water$$

$P_{AS\ 1{,}5\ \%}$

$$6\ \ gr\ odp * \frac{100\ gr\ wp}{21{,}53\ \ gr\ odp} = 27{,}86\ gr\ wp$$
$$= 27{,}86\ ml\ of\ wet\ dough.$$
$$ml\ of\ deionized\ water\ \ = 95{,}97\ ml - 27{,}86\ ml$$
$$= 68{,}10\ ml\ of\ deionized\ water$$

$P_{MeS\ 1{,}5\ \%}$

$$6\ \ gr\ odp * \frac{100\ gr\ wp}{23{,}12\ \ gr\ odp} = 25{,}95\ gr\ wp$$
$$= 25{,}95\ ml\ of\ wet\ dough.$$

$$ml\ of\ deionized\ water\ = 95{,}97\ ml - 25{,}95\ ml$$
$$= 70{,}01\ ml\ of\ deionized\ water$$

Experiment 2

$P_{SA\ 3\ \%}$

$$6\ \ gr\ odp * \frac{100\ gr\ wp}{20{,}61\ \ gr\ odp} = 29.11\ gr\ wp$$
$$= 29.11\ ml\ of\ wet\ dough.$$
$$ml\ of\ deionized\ water = 95{,}97\ ml - 29{,}11\ ml$$
$$= 66{,}85\ ml\ of\ deionized\ water$$

$P_{AS\ 3\ \%}$

$$6\ \ gr\ odp * \frac{100\ gr\ wp}{22{,}84\ \ gr\ odp} = 26{,}26\ gr\ wp$$
$$= 26{,}26\ ml\ of\ wet\ dough.$$
$$ml\ of\ deionized\ water\ = 95{,}97\ ml - 26{,}26\ ml$$
$$= 69{,}70\ ml\ of\ deionized\ water$$

$P_{MeS\ 3\ \%}$

$$6\ \ gr\ odp * \frac{100\ gr\ wp}{21{,}36\ gr\ odp} = 28{,}08\ gr\ wp$$
$$= 28{,}08\ ml\ of\ wet\ dough.$$
$$ml\ of\ deionized\ water\ = 95{,}97\ ml - 28{,}08\ ml$$
$$= 67{,}88ml\ of\ deionized\ water$$

Experiment 3

$P_{MeS + SA}$

$$6\ \ gr\ odp * \frac{100\ gr\ wp}{32{,}17\ \ gr\ odp} = 18{,}65\ gr\ wp$$
$$= 18{,}65\ ml\ of\ wet\ dough.$$

$$ml\ of\ deionized\ water = 95{,}97\ ml - 18{,}65\ ml$$
$$= 77{,}31\ ml\ of\ deionized\ water$$

$P_{MeS + AS}$

$$6\ gr\ odp * \frac{100\ gr\ wp}{29{,}08\ gr\ odp} = 20{,}63\ gr\ wp$$
$$= 20{,}63\ ml\ of\ wet\ dough.$$

$$ml\ of\ deionized\ water\ = 95{,}97\ ml - 20{,}63\ ml$$
$$= 75{,}33\ ml\ of\ deionized\ water$$

$P_{SA + AS}$

$$6\ gr\ odp * \frac{100\ gr\ wp}{29{,}39\ gr\ odp} = 20{,}41\ gr\ wp$$
$$= 20{,}41\ ml\ of\ wet\ dough.$$
$$ml\ of\ deionized\ water\ = 95{,}97\ ml - 20{,}41\ ml$$
$$= 75{,}55\ ml\ of\ deionized\ water$$

Once added in the reactor, we place it in the EASYDYE (Image 12) at a temperature of 90ºC during 120 minutes.

Image 12

Once the reaction time has passed, we filter with a Buchner filter (image 11) and separate the dough from the bleach (remaining liquid). We pass it through a filtration system and wash it 3 times with decalcified water and one with deionized water. After that, we crumble the solid in small pieces and put them in a bag. We calculate the effectiveness of each of the drying methods. With the remaining water we calculate the pH of each one.

	pH	Temperature (ºC)
K_p	9,59	22,0
$P_{SA\ 1,5\ \%}$	9,61	21,6
$P_{AS\ 1,5\ \%}$	9,61	21,6
$P_{MeS\ 1,5\ \%}$	9,55	21,7
$P_{SA\ 3\ \%}$	9,43	21,7
$P_{AS\ 3\ \%}$	9,09	21,7
$P_{MeS\ 3\ \%}$	8,95	21,6
$P_{MeS\ +\ SA}$	9,34	21,5
$P_{MeS\ +\ AS}$	9,36	21,6
$P_{SA\ +\ AS}$	9,07	21,5

	Dryness
K_p	29,51 gr odp/100 gr wp
$P_{SA\ 1,5\ \%}$	30,26 gr odp/100 gr wp
$P_{AS\ 1,5\ \%}$	29,48 gr odp/100 gr wp
$P_{MeS\ 1,5\ \%}$	28,25 gr odp/100 gr wp
$P_{SA\ 3\ \%}$	28,73 gr odp/100 gr wp
$P_{AS\ 3\ \%}$	26,54 gr odp/100 gr wp
$P_{MeS\ 3\ \%}$	28,35 gr odp/100 gr wp
$P_{MeS\ +\ SA}$	31,25 gr odp/100 gr wp
$P_{MeS\ +\ AS}$	33,04 gr odp/100 gr wp
$P_{SA\ +\ AS}$	41,05 gr odp/100 gr wp

We can see that with the combination of mediators the filtration process is slower since the sample is more refined and causes the formation of hydrogen bonds between the fibres of paper making the filtration process more difficult..

Experiment 4: Treatment with ozone

For the treatment with ozone we need to disintegrate 5 gr odp in 1L of distilled water. Knowing the dryness of the initial dough we can find that for treating 5 gr odp we need 18,19 gr wp. In order to conduct the experiment we need to increase the acidity of the mixtures water-paper with sulfuric acid at 98% until we reach a pH of 2,5

For doing the treatment with ozone we will use the equipment shown in image 13 (see specification sheet in Annex 5). The conditions which we will work with are the next:

- Low consistency (0,5%)
- Ozone dose of 0,8%

In image 14 we can see the effect of the treatment with ozone. The container on the right has been treated with ozone and the one on the left has not.

Once the treatment has finished, we wash the dough 3 times with decalcified water and one with deionized water and calculate the dryness.

Dryness = 30,54 gr odp /100 gr wp
In order to compare the different treatments we need to do the stage P. We will also do a stage F with 6 gr odp.

Image 13

Image 14

Reduction treatment (Stage F)
In a stage in which the main component is the formamidine sulfinic acid (FAS). The conditions of the treatment will be of 1% of FAS, 0,5% of NaOH and 5% of consistency. This treatment will be done in polyethylene bags, in a bath at 60ºC during 120 min. Every 10 minutes we agitate the sample manually in order to facilitate the reaction.
For the ozone:
FAS

$$6\ gr\ odp * \frac{1\ gr\ FAS}{100\ gr\ odp} = 0{,}06\ gr\ of\ FAS$$

NaOH

$$6\ gr\ odp * \frac{0{,}5\ gr\ NaOH}{100\ gr\ odp} * \frac{1000\ ml\ of\ solution}{40\ gr\ NaOH} = 0{,}75\ ml\ of\ solution\ of\ NaOH$$

Consistency

$$6\ gr\ odp * \frac{100\ gr\ total}{5\ gr\ odp} = 120\ gr\ total$$

Deionized water

$ml\ H_2O = 120 - 19{,}76 - 0{,}06 - 0{,}75 = 99{,}44\ ml\ of\ H_2O$

Experiment 5

Combination of mediators

We will follow the next schemes:

5.1 L_{MeS} => L_{AS} => Stage P => Stage P => Stage F

- L_{MeS}: treating 20 gr odp in two samples of 10 gr odp each. Mixing the samples once the treatment has finished.
- L_{AS}: Treating 14 gr odp in two samples of 7 gr odp each. Mixing the samples once the treatment has finished.
- Stage P: Treating 9 gr odp
- Stage P: Treating 5 gr odp
- Stage P: Treating 2 gr odp

5.2 L_{MeS} => Stage P => L_{AS} => Stage P => Stage F

- L_{MeS}: Treating 20 gr odp in two samples of 10 gr odp each. Mixing the samples once the treatment has finished.
- Stage P: Treating 14 gr odp in two samples of 7 gr odp each. Mixing the samples once the treatment has finished.
- L_{AS}: Treating 10 gr odp
- Stage P: Treating 6 gr odp
- Stage F: Treating 3 gr odp

5.3 L_{MeS} => Stage P => L_{MeS} => Stage P => Stage F

- L_{MeS}: Treating 20 gr odp in two samples of 10 gr odp each. Mixing the samples once the treatment has finished.
- Stage P: Treating 14 gr odp in two samples of 7 gr odp each. Mixing the samples once the treatment has finished.
- L_{AS}: Treating 10 gr odp
- Stage P: Treating 6 gr odp.
- Stage F: Treating 3 gr odp

5.4 L_{AS} => Stage P => L_{MeS} => Stage P => Stage F

- L_{MeS}: Treating 20 gr odp in two samples of 10 gr odp each. Mixing the samples once the treatment has finished.
- Stage P: Treating 14 gr odp in two samples of 7 gr odp each. Mixing the samples once the treatment has finished.
- L_{AS}: Treating 10 gr odp
- Stage P: Treating 6 gr odp.
- Stage F: Treating 3 gr odp

Treatments	5.1 L_{MeS}	5.1 L_{MeS} => L_{AS}	5.1 L_{MeS} => L_{AS} => P	5.1 L_{MeS} => L_{AS} => P => P	5.1 L_{MeS} => L_{AS} => P => P => F	5.2 L_{MeS}	5.2 L_{MeS} => P	5.2 L_{MeS} => P => L_{AS}	5.2 L_{MeS} => P => L_{AS} => P	5.2 L_{MeS} => P => L_{AS} => P => F
Grams of dry pulp	10	7	9	5	2	10	7	10	6	3
Dryness before treatment (%)	29,75	29,80	27,23	29,02	30,70	29,75	31,10	28,98	30,57	29,92
Gr wp	33,61	23,48	33,05	17,22	6,51	33,61	22,50	34.50	19,62	10,02
Consistency (%)	5	5	5	5	5	5	5	5	5	5
Total grams	200	140	180	100	40	200	140	200	120	60
Reactor	Big	Big	Big	Little	----	Big	Big	Big	Little	----
Apparatus	EASYDYE	EASYDYE	EASYDYE	EASYDYE	Water bath	EASYDYE	EASYDYE	EASYDYE	EASYDYE	Water bath
Grams of mediator	0,15	0,105	----	----	----	0,15	----	0,15	----	----
Tampon (ml)	47,5	33,25	----	----	----	47,5	----	47,5	----	----
ml of enzyme	0,23	0,163	----	----	----	0,23	----	0,23	----	----
Hydrogen peroxide (ml)	----	----	9,27	5,15	----	----	7,21	----	6,18	----
Sodium hydroxide (ml)	----	----	3,37	1,87	0,25	----	2,62	----	2,25	0,375
Magnesium sulphate (ml)	----	----	0,9	0,5	----	----	0,7	----	0,6	----
DTPA (ml)	----	----	22,5	12,5	----	----	17,5	----	15	----
FAS (ml)	----	----	----	----	0,02	----	----	----	----	0,03
Deionized water (ml)	118,51	83,02	110,91	67,76	33,22	118,51	89,47	118,51	82,35	49,57
pH	7,39	8,27	9,96	11,36	----	7,44	9,69	7,42	11,22	----
T (ºC)	19,2	20,2	23,7	24		19,2	29,1	19	23,5	
Dryness after treatment (%)	29,80	27,23	29,02	30,70	----*	31,10	28,98	30,57	29,92	30,6

Treatments	5.3 L_{MeS}	5.3 L_{MeS} => P	5.3 L_{MeS} => P => L_{MeS}	5.3 L_{MeS} => P => L_{MeS} => P	5.3 L_{MeS} => P => L_{MeS} => P => F	5.4 L_{AS}	5.4 L_{AS} => P	5.4 L_{AS} => P => L_{MeS}	5.4 L_{AS} => P => L_{MeS} => P	5.4 L_{AS} => P => L_{MeS} => P => F
Grams of dry pulp	10	7	10	6		10	7	10	6	
Dryness before treatment (%)	29,75	28,71	25,54	27,07		29,75	27,21	27,57	29,26	
Gr wp	33,61	24.38	39,15	22,16		33,61	25,72	36,27	20,50	
Consistency (%)	5	5	5	5		5	5	5	5	
Total grams	200	140	200	120		200	140	200	120	
Reactor	Big	Big	Big	Little	----	Big	Big	Big	Little	----
Apparatus	EASYDYE	EASYDYE	EASYDYE	EASYDYE	Water bath	EASYDYE	EASYDYE	EASYDYE	EASYDYE	Water bath
Grams of mediator	0,15	----	0,15	----	----	0,15	----	0,15	----	----
Tampon (ml)	47,5		47,5	----	----	47,5	----	47,5	----	----
ml of enzyme	0,308	----	0,308	----	----	0,308	----	0,308	----	----
Hydrogen peroxide (ml)	----	7,66	----	6,56	----	----	7,66	----	6,56	----
Sodium hydroxide (ml)	----	2,62	----	2,25		----	2,62	----	2,25	
Magnesium sulphate (ml)	----	0,7	----	0,6	----	----	0,7	----	0,6	----
DTPA (ml)	----	17,5	----	15	----	----	17,5	----	15	----
FAS (ml)	----	----	----	----		----	----	----	----	
Deionized water (ml)	118,43	87,14	112,88	73,43		118,43	85,80	115,77	75,09	
pH	7,15	9,77	7,70	9,28	----	7,05	9,80	7,95	9,27	----
T (ºC)	8.5	21,6	21,4	20,7		10,5	21,5	21,2	20,9	
Dryness after treatment (%)	28,71	25,54	27,07	30,50		27,21	27,57	29,26	30,58	

We don't need to know the dryness since this time we will place all the dough we have because we have prepared 2 gr odp and for making a sheet we will need 2,3 gr odp. For this reason, we won't do the mechanic properties in this experiment.

Experiment 6

Black pulp

The experimental procedure will be the same than in experiment 2.

	pH	Temperature (ºC)
K_L	7,34	17,4
K_P	9,70	21,5
$L_{SA\ 1.5\%}$	7,13	18,1
$P_{SA\ 1.5\%}$	9,57	21,5
$L_{AS\ 1.5\%}$	7,19	18
$P_{AS1.5\%}$	9,58	21,5
$L_{MeS\ 1.5\%}$	7,31	18,5
$P_{MeS\ 1.5\%}$	9,48	21,5

	Dryness
K_L	30,15
K_P	27,64
$L_{SA\ 1.5\%}$	30,96
$P_{SA\ 1.5\%}$	26,72
$L_{AS\ 1.5\%}$	30,41
$P_{AS1.5\%}$	28,95
$L_{MeS\ 1.5\%}$	28,88
$P_{MeS\ 1.5\%}$	26,27

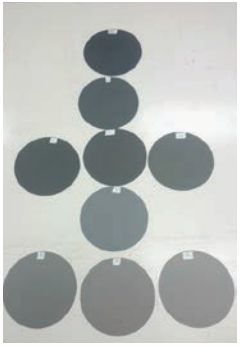


Image 16

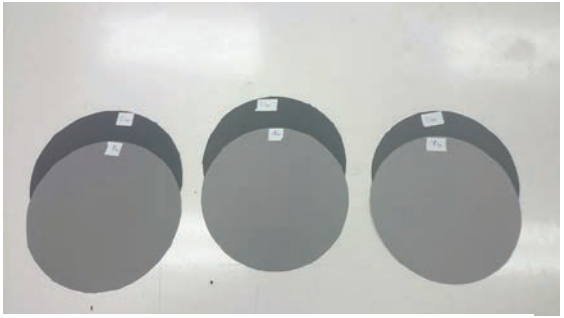


Image 15

Experiment 7 Black pulp

Treatments	7.2 L_{MeS}	7.2 L_{MeS} => P	7.2 L_{MeS} => P => L_{AS}	7.2 L_{MeS} => P => L_{AS} => P	7.3 L_{MeS}	7.3 L_{MeS} => P	7.3 L_{MeS} => P => L_{MeS}	7.3 L_{MeS} => P => L_{MeS} => P
Grams of dry pulp	10	7	10	6	10	5,7	8,29	5,4
Dryness before treatment (%)	30,11	28,32	28,02	26,53	30,11	25,81	27,55	22,12
Gr wp	33,21	24,71	35,68	22,53	33,21	22,38	30,1	24,60
Consistency (%)	5	5	5	5	5	5	5	5
Total grams	200	140	200	120	200	114	165,8	108
Reactor	Big	Big	Big	Little	Big	Big	Big	Little
Apparatus	EASYDYE	EASYDYE	EASYDYE	EASYDYE	EASYDYE	EASYDYE	EASYDYE	EASYDYE
Grams of mediator	0,15	----	0,15	----	0,15	----	0,12	----
Tampon (ml)	47,5		47,5	----	47,5	----	47,5	----
ml of enzyme	0,308	----	0,308	----	0,308	----	0,225	----
Hydrogen peroxide (ml)	----	7,66	----	6,56	----	6,24	----	5,91
Sodium hydroxide (ml)	----	2,62	----	2,25	----	2,13	----	2,025
Magnesium sulphate (ml)	----	0,7	----	0,6	----	0,57	----	0,54
DTPA (ml)	----	17,5	----	15	----	14,25	----	13,5
FAS (ml)	----	----	----	----	----	----	----	----
Deionized water (ml)	118,83	86,81	116,36	73,05	118,83	65,43	87,82	61,43
pH	7,11	9,43	6,92	8,92	7,10	9,41	7,01	8,86
T (ºC)	20.9	21	21,1	21,7	20,7	20,9	21,1	21,5
Dryness after treatment (%)	28,32	28,02	26,63	27,88	25,81	27,55	22,12	27,59

Treatments	7.5 Ozone => P	7.5 Ozone => P => L_{MeS}	7.5 Ozone => P => L_{MeS} => P
Grams of dry pulp	5	10	6
Dryness before treatment (%)	31,85	27,59	24,84
Gr wp	15,69	36,24	24,15
Consistency (%)	5	5	5
Total grams	140	200	120
Reactor	Big	Big	Little
Apparatus	EASYDYE	EASYDYE	EASYDYE
Grams of mediator	----	0,15	----
Tampon (ml)		47,5	----
ml of enzyme	----	0,308	----
Hydrogen peroxide (ml)	5,70	----	6,56
Sodium hydroxide (ml)	1,875	----	2,25
Magnesium sulphate (ml)	0,5	----	0,6
DTPA (ml)	12,5	----	15
FAS (ml)	----	----	----
Deionized water (ml)	63,72	115,79	71,17
pH	8,75	7,09	9,15
T (ºC)	23,7	23,6	23,7
Dryness after treatment (%)	27,59	24,84	28,51

Preparation of the sheet.

In order to evaluate the discoloration that has been caused for the different treatments, we will make paper sheets following the rule ISO 4269-2 (Rapid-Köthen method) (See specification sheet in Annex 4). We weight 2,3 gr odp in 2 L of deionized water. In order to prepare 2,3 gr odp we need to use de dryness of each past to calculate the equivalent gr wp.

$$2{,}30\ gps * Dryness(\frac{gph}{gps}) = gph$$

RED			
Treatments	Dryness (gr odp/100 gr wp)	**Treatments**	**gr wp**
K_L	25,27	**K_L**	9,01
L_{AS} at 1,5 % in weight	21,53	**L_{AS} at 1,5 % in weight**	8,31
L_{SA} at 1,5 % in weight	27,66	**L_{SA} at 1,5 % in weight**	10,68
L_{MeS} at 1,5 % in weight	23,12	**L_{MeS} at 1,5 % in weight**	9,94
L_{AS} at 3 % in weight	22,84	**L_{AS} at 3 % in weight**	11,15
L_{SA} at 3 % in weight	20,61	**L_{SA} at 3 % in weight**	10,07
L_{MeS} at 3 % in weight	21,36	**L_{MeS} at 3 % in weight**	10,76
$L_{MeS + AS}$	29,08	**$L_{MeS + AS}$**	7,14
$L_{MeS + SA}$	32,17	**$L_{MeS + SA}$**	7,90
$L_{SA + AS}$	29,39	**$L_{SA + AS}$**	7,82

RED			
Treatments	Dryness (gr odp/100 gr wp)	**Treatments**	**gr wp**
K_p	29,51	**K_p**	7,79
$P_{SA\ 1,5\ \%}$	30,26	**$P_{SA\ 1,5\ \%}$**	7,60
$P_{AS\ 1,5\ \%}$	29,48	**$P_{AS\ 1,5\ \%}$**	7,80
$P_{MeS\ 1,5\ \%}$	28,25	**$P_{MeS\ 1,5\ \%}$**	8,14
$P_{SA\ 3\ \%}$	28,73	**$P_{SA\ 3\ \%}$**	8,00
$P_{AS\ 3\ \%}$	26,54	**$P_{AS\ 3\ \%}$**	8,66

$P_{MeS\ 3\ \%}$	28,35	**$P_{MeS\ 3\ \%}$**	8,11
$P_{MeS\ +\ SA}$	31,25	**$P_{MeS\ +\ SA}$**	7,36
$P_{MeS\ +\ AS}$	33,04	**$P_{MeS\ +\ AS}$**	6,96
$P_{SA\ +\ AS}$	41,05	**$P_{SA\ +\ AS}$**	5,60

RED			
Treatments	**Dryness (gr odp/10(gr wp)**	**Treatments**	**gr wp**
OZONE	30,54	**OZONE**	7,53
OZONE => P	30,36	**OZONE => P**	7,57
5.1 L_{MeS}	29,80	**5.1 L_{MeS}**	7,71
5.1 L_{MeS} => L_{AS}	27,23	**5.1 L_{MeS} => L_{AS}**	8,44
5.1 L_{MeS} => L_{AS} => P	29,02	**5.1 L_{MeS} => L_{AS} => P**	7,92
5.1 L_{MeS} => L_{AS} => P => P	30,70	**5.1 L_{MeS} => L_{AS} => P => P**	7,49
5.1 L_{MeS} => L_{AS} => P => P => F	*	**5.1 L_{MeS} => L_{AS} => P => P => F**	*
5.2 L_{MeS}	31,10	**5.2 L_{MeS}**	7,39
5.2 L_{MeS} => P	28,98	**5.2 L_{MeS} => P**	7,93
5.2 L_{MeS} => P => L_{AS}	30,57	**5.2 L_{MeS} => P => L_{AS}**	7,52
5.2 L_{MeS} => P => L_{AS} => P	29,92	**5.2 L_{MeS} => P => L_{AS} => P**	7,68
5.2 L_{MeS} => P => L_{AS} => P => F	30,60	**5.2 L_{MeS} => P => L_{AS} => P => F**	7.51

RED			
Treatments	**Dryness (gr odp/100 gr wp)**	**Treatments**	**gr wp**
5.3 L_{MeS}	28,71	**5.3 L_{MeS}**	8,01
5.3 L_{MeS} => P => L_{AS}	25,54	**5.3 L_{MeS} => P => L_{AS}**	9,00
5.3 L_{MeS} => P => L_{MeS} => P	27,07	**5.3 L_{MeS} => P => L_{AS} => P**	8,49
5.3 L_{MeS} => P => L_{MeS} => P	30,50	**5.3 L_{MeS} => P => L_{AS} => P**	7,5

5.4 L_{AS}	27,21	**5.4 L_{AS}**	8,45
5.4 L_{AS} => P	27,57	**5.4 L_{AS} => P**	8,34
5.4 L_{AS} => P => L_{MeS}	29,26	**5.4 L_{AS} => P => L_{MeS}**	7,86
5.4 L_{AS} => P => L_{MeS} => P	30,58	**5.4 L_{AS} => P => L_{MeS} => P**	7,52
5.5 OZONE	27,80	**5.5 OZONE**	8,27
5.5 OZONE => P	29,69	**5.5 OZONE => P**	7,74
5.5 OZONE => P => L_{MeS}	32,74	**5.5 OZONE => P => L_{MeS}**	7,02
5.5 OZONE => P => L_{MeS} => P	31,72	**5.5 OZONE => P => L_{MeS} => P**	7,24

BLACK

Treatments	Dryness (gr odp/100 gr wp)	Treatments	gr wp
K_L	30,61	**K_L**	7,51
K_P	28,48	**K_P**	8,07
$L_{SA\ 1.5\%}$	31,08	**$L_{SA\ 1.5\%}$**	7,40
$P_{SA\ 1.5\%}$	27,13	**$P_{SA\ 1.5\%}$**	8.47
$L_{AS\ 1.5\%}$	31,29	**$L_{AS\ 1.5\%}$**	7,35
$P_{AS1.5\%}$	29,46	**$P_{AS1.5\%}$**	7,80
$L_{MeS\ 1.5\%}$	29,18	**$L_{MeS\ 1.5\%}$**	7,88
$P_{MeS\ 1.5\%}$	26,67	**$P_{MeS\ 1.5\%}$**	8,62

BLACK

Treatments	Dryness (gr odp/100 gr wp)	Treatments	gr wp
7.2 L_{MeS}	28,32	**7.2 L_{MeS}**	8,12
7.2 L_{MeS} => P	28,02	**7.2 L_{MeS} => P**	8,20
7.2 L_{MeS} => P => L_{AS}	26,63	**7.2 L_{MeS} => P => L_{AS}**	8,63
7.2 L_{MeS} => P => L_{AS} => P	27,88	**7.2 L_{MeS} => P => L_{AS} => P**	8,24
7.3 L_{MeS}	25,81	**7.3 L_{MeS}**	8,91

7.3 L_{MeS} => P => L_{AS}	27,55	**7.3 L_{MeS} => P => L_{AS}**	8,34
7.3 L_{MeS} => P => L_{AS} => P	22,12	**7.3 L_{MeS} => P => L_{AS} => P**	10,39
7.3 L_{MeS} => P => L_{AS} => P	27,59	**7.3 L_{MeS} => P => L_{AS} => P**	8,33
7.5 OZONE	31,85	**7.5 OZONE**	7,22
7.5 OZONE => P	27,59	**7.5 OZONE => P**	8,33
7.5 OZONE => P => L_{MeS}	24,84	**7.5 OZONE => P => L_{MeS}**	9,25
7.5 OZONE => P => L_{MeS} => P	28,51	**7.5 OZONE => P => L_{MeS} => P**	8,06

*Nor the dryness or the gr wp are needed since the sample will only be treated with 2 gr odp, therefore we will use all the dough for making the paper sheet.
Once we have weighted the necessary amount for each experiment, we place them separately in 2L of deionized water and passed them through the disintegrator. Once disintegrated, we prepare sheets with the equipment shown in Image 17 (See specification sheet in Annex 6).

Image 17

First of all we pass it through the filter for eliminating the remaining water (Image 18), after that we place a filtration paper and press it for extracting all the remaining water (Image 19). Then we put it in the stove to dry it (Image 20). These stoves have a vacuum system which facilitates the water leaving the paper. These procedures will be done for every treatment (L, P, ozone and F).

Image 18

Image 19

Image 20

- Once filtered, the percentage of water remaining in the solid is of around 90%. After pressing it the percentage is reduced to 70%.
- In this equipment the mater and heat transference unit operation known as drying takes place. The red plate is the hot focus and the other the cold focus. With the transferred temperature we cause the liquid water in the paper sheet to evaporate. This vapour water, using a small vacuum, is taken out of the system through the cold focus. With this system we achieve a sheet with a humidity of 6—8%, which is the amount of humidity that commercial paper has.

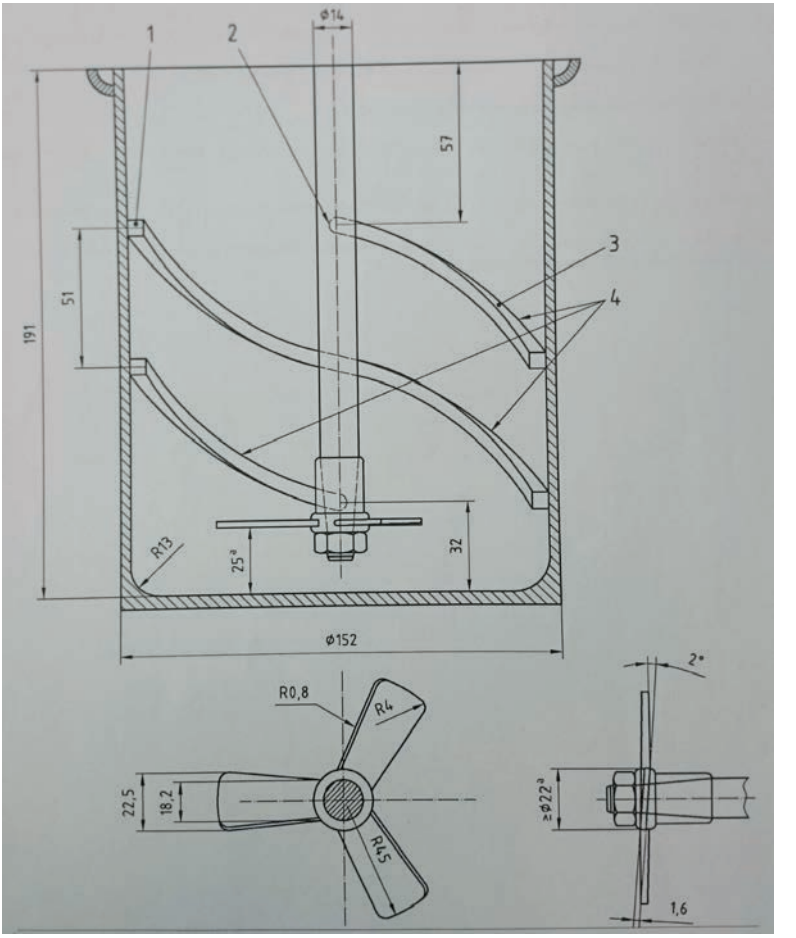
After this we take a look at the optic and mechanic properties in order to compare the treatments.

12. APPENDIX 2: SPECIFICATION SHEET NORMALIZED DISINTEGRATOR

Full de especificació del desintegrador normalitzat				
Part		Dimensió	Valor específic	Tolerància
Recipient		Altura interna	191 mm	± 2 mm
		Diàmetre intern	151 mm	± 2 mm
		Radi farcit	13 mm	± 2 mm
Deflectors		Secció quadrada	6,5 mm	± 1 mm
		Altura de la base del recipient	32 mm	± 1 mm
		Distancia del vora	57 mm	± 1 mm
		Extrem arrodonides	3 mm	± 0,5 mm
		Bores arrodonides	0,4 mm	± 0,1 mm
		Especiat (centres)	51 mm	± 1 mm
Agitador		Diàmetre del cercle de arrossegament en el extrem de la paleta	90 mm	± 0,5 mm
		Diàmetre del boixa	≥ 22 mm	-
		Distancia entre la paleta i la base del recipient (punt mes baix)	25 mm	± 2 mm
Paletes del agitador		Amplada en el boixa	18,2 mm	± 0,5 mm
		Amplada màxima	22,5 mm	± 0,5 mm
		Espessor	1,6 mm	± 0,5 mm
		Bores arrodonides	0,8 mm	± 0,2 mm
		Extrem arrodonides	4 mm	± 1 mm
		Passos	2º	± 15
Eix de agitador	Diàmetre	≤ 20 mm		-
	Extrem cònic	Per muntar qualsevol boixa de agitació		
	Velocitat de rotació	3150 rpm		
	Freqüència de rotació	49 s^{-1}		± 1,5

Llegenda

1. Secció 6,5mm x 6,5 mm
2. Extrem R3
3. Bores arrodonides R 0,4
4. Quatre reflectors, cada un a mitja volta del recipient (es mostren tres)

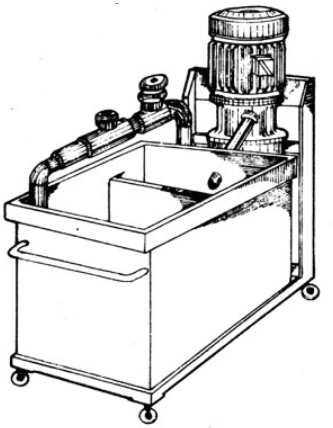


Unitats en mm

13. APPENDIX 3 SPECIFICATION SHEET FILTER EQUIPMENT LABORATORY

Full de especificació d'equip de filtrat de laboratori

TECNIUM



Portàtil

Anticorrosiu

Alt buit

Elevat rendiment

Us universal en

- Filtració
- Evaporació
- Cristal·lització
- Separació
- Etc

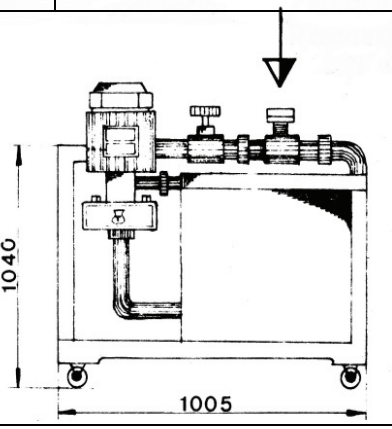
Útil per a gasos, vapors i líquids

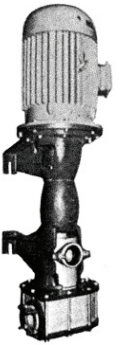
Es subministra tot es equip complert

Bomba BVS -15/424	s. full tècnic 1554
Ejecto TBT 1	s. full tècnic 2200

El grau de buit màxim: La següent taula expressa el buit obtingut, que dependrà del caudal de fluid aspirat.

Grau de buit en m.m.Hg.	Caudal de fluid aspirat en L/h	
	Aigua	Aire
730	150	150
700	250	300
600	350	450
500	500	700
350	650	1000

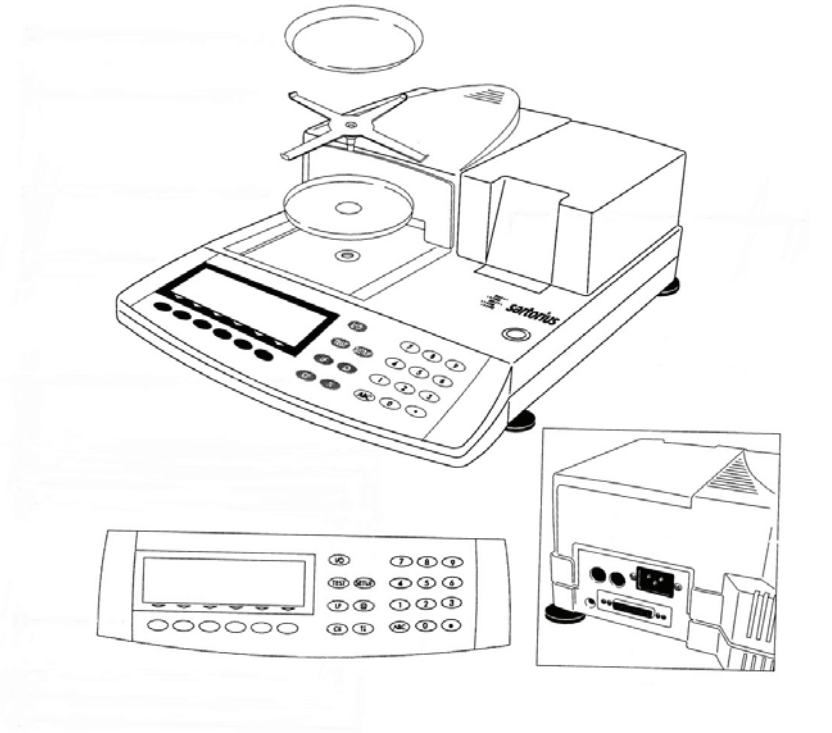


Funcionant la bomba a 15ºC i aspirant aigua o aire, respectivament. Al aspirar vapors condensats, el caudal augmenta	Característiques orientatives
Especificacions de la bomba	
	BVS model For out-dorr tank mounting Flow rates: 1-1000 m^3/h • Materials: • PP-PVC-PE • PVDF-PTFE • CTFE • Titanium • Stainless Steel • Hastelloy`s • Uranus
I.A. 1.8.6 Ed. 10-75	CASALS CARDONA IND. S.A. MANRESA - BARCELONA	Fig: 6500

14. APPENDIX 4 SPECIFICATION SHEET MOISTURE ANALYZER SARTORIUS

Full de especificació de la Moisture Analyzer Sartorius (Analitzador de humitat electrònic)	
Model	MA100C-0CE230V1, MA100H-0CE230V1, MA100Q-0CE230V1
Funció de secat	
Font de calefacció	Radiador ceràmic quars de superfície o radiador rodo de halògens
Rang de temperatura	30 - 300ºC
Escalo de temperatura	Ajustable en escalons de 1 ºC
Ajust de temperatura	Amb joc de ajust de temperatura YTM03MA
Funció de mesurament	
Capacitat de pesat màx.	100 g
Escalo real	1 mg, 0.01%/0.001% humitat
Rang de tara	g < 100% de la capacitat de pesat màx.
Escalo de verificació	0.001 g
Alçada Min.	0.1 g
Rang de temperatura de l'entorn	+15 ºC .... +25 ºC
Diàmetre del platets	Ø 90 mm
Aparell (hardware)	
Dimensions (A x P x A)	350 x 453 x 156 mm
Pes net, aprox.	8 kg
Tensió de la xarxa	230 V o 115 V commutable al canvi d'unitats calefactora, -15% ... +10%

Freqüència de xarxa	48 – 60 Hz
Fusibles de xarxa	2 (conductors zero/fase), 6.3 AT, 5 x 20 mm
Rang de temperatura que empleat	+10 ... +30 ºC
Consum elèctric	Màxim 700 VA



15. APPENDIX 5 SPECIFICATION SHEET EASY DYE DATACOLOR AHIBA IR

Full Specification Easy Dye Datacolor Ahiba IR	
Applications	
Fibres:	All
Substrates:	Piece, Skein, Loose, Tops
Minimum Liquor Ratio:	1:5 natural fibers
	1:3 syntheyic fibers
Heating / Cooling	
Heating system:	Infra-Red
Max. Heating Power:	3 KW
Temperature Range:	20º - 140ºC
Cooling:	Forced Air
Dyeing Capacities	
Beaker Sizes & Maximum:	150cc x 20
Number of Dyeing Positions:	300cc x 15
	500 cc x 8
	1000cc x 8
	5 Liter Drum x 1
Beaker Sizes & Maximum:	50cc x 10
Ideal Sampie Size:	5 grams for 150cc beaker
	10 grams for 300cc beaker
	25 grams for 500cc beaker
	50 grams for 1000cc beaker
	250 grams for 1 Liter Drum
Dosing:	Manual Dosing injector

Controller	
Controller Type:	Icon/Button driven
Program Capacity:	99 dyeing programs
Program Name:	User selectable number
Programmable Steps:	15 Steps
Language:	N/A
Display Size:	114mm X 64mm
Controller Parameters	
Time:	In minutes
Temperature:	0.1ºC or ºF
Temp. Gradient:	0.1ºC or ºF/min.
Reversing:	Automatic every 1 minute
Program Halt:	Selectable On/Off for each step
Physical / Electrical	
Dimensions H/W/D:	864 x 597 x 648 mm
	34 x 23.5 x 25.5 in.
Weight:	73 kg
	161 lbs.
Voltage:	230V AC 50/60 HZ Single Stage
Insulation:	Fiberglass

16. APPENDIX 6 ISP SHEET FORMER

FORMADOR DE HOJAS ISP

(Tipo Rapid-Köthen)

Está diseñado mediante la **Norma UNE (57042/2),** que regula el modo para la determinación de los análisis que hacen referencia a la preparación de hojas para ensayos fisicos que consiste en preparar por succión hojas circulares de **200 mm. / diámetro** a partir de una suspensión de pasta sobre un tamiz .

La hoja así preparada se transfiere a un secador que por medio de vacío y temperatura evita la contracción de la misma.

Consta de :

COLUMNA DE FORMACION

SISTEMA DE SECADO

SISTEMA DE MANDO Y DISTRIBUCION

MESA

I.S.P.

Investigación Sistemas Papeleros, S.L.

COLUMNA DE FORMACION

Consta de dos bridas superpuestas, unidas entre sí por una bisagra, todo en fundición en bronce . La brida superior tiene dos filas de orificios de **1,5 mm/diámetro** con una distancia de 7 mm. entre ellas ; la fila inferior con orificios horizontales de salida al centro de la columna , mientras que la superior presenta los orificios con un ángulo de **30°**, hacia arriba .

La brida inferior va soldada a una columna de inox. dividida en dos depósitos distintos, el primero **(superior)** está destinado como receptor del agua filtrada después de la **1ª succión (vacío 1°)** , el segundo sirve como depósito auxiliar de captación de agua para la bomba y su posterior lanzamiento hacia la torre de formación.

Esta brida inferior aloja al soporte de la tela de formación que va con deflectores biselados a un ángulo de **75°** en sus dos bordes superiores que están en contacto con la tela y su misión consiste en la orientación de la fibra.

La brida superior lleva un tubo de metacrilato con una capacidad de **12 lts.** para la solución de la muestra.

Una base en bronce con corrección de nivel y como soporte del conjunto de la torre de formación.

SISTEMA DE SECADO

En el equipo estándar consta de dos planchas de secado y un equipo termostático , con su propia cubeta en inox, sistema de programación de la temperatura y protección de la resistencia . Cada plancha lleva en su parte superior un vacuostato manual para eliminar el vacio producido , una membrana de silicona por moldeo y una brida de sujeción para la membrana.

La parte inferior de la plancha está formada por la base de asentamiento para la parte superior que, a su vez, sirve como soporte del vaso de recogida de condensados y alojamiento del calderín de agua fría . Entre la parte superior e inferior está la base de reposo de la hoja húmeda con tres mallas superpuestas y perfectamente estiradas para el secado de la muestra.

La conexión de vacio está tomada en el centro del vaso de condensados, en el punto más bajo del conjunto de la plancha, para facilitar su salida y disminuir el tiempo de secado.

Un vacuómetro para cada plancha con apertura de señal por electroválvula directamente mandada.

Un reloj-avisador por cada plancha de **0-30 min.**

Una bomba de recirculación de agua caliente con toma de la cubeta del equipo termostático y salida hacia entrada de la(s) plancha(s) con retorno a la cubeta en un sistema cerrado y una regulación de caudal por potenciómetro al motor de la bomba.

SISTEMA DE MANDO Y DISTRIBUCION

Panel de mandos en la mesa de fácil ejecución, con interruptor de tensión general , marcha y paro de la bomba del sistema, conmutador de resistencia de recirculación , marcha de proceso , selector , programador (autómata).

SELECTOR . (OFF - AUT. - MAN. - REC.MAN. - REC.AUT.)

OFF. (Sin función)
AUT. (Funcionamiento automático pulsando marcha proceso)
MAN.(" manual " " "

1ª pulsación .- LLENADO
2ª " .- AGITACION
3ª " .- CALMADO
4ª " .- VACIO 1º
5ª " .- VACIO 2º (se ilumina)
6ª " .- PARO

REC.MAN. (Recirculación manual pulsando marcha proceso)

1ª pulsación.- LLENADO AGUA LIMPIA
2ª " .- LLENADO AGUA RECIRC.
3ª " .- AGITACION
4ª " .- CALMADO
5ª " .- VACIO 1º
6ª " .- VACIO 2º
7ª " .- PARO

Recirculación manual y recirculación automática con selección en el programador .

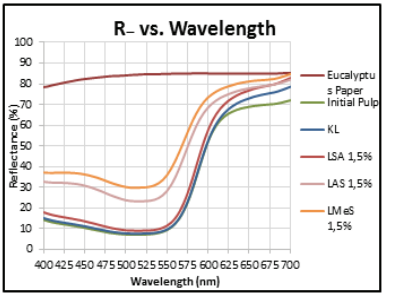
RECIRCULACION MANUAL .-

Señalizando el selector hacia REC.MAN. y pulsando el botón de marcha proceso tendremos el 1º pulso agua limpia ,2º pulso agua de recirculación, 3º pulso entrada del torbellino de aire, 4º pulso calmado, 5º pulso 1º vacio, 6º pulso 2º vacio, 7º pulso paro .

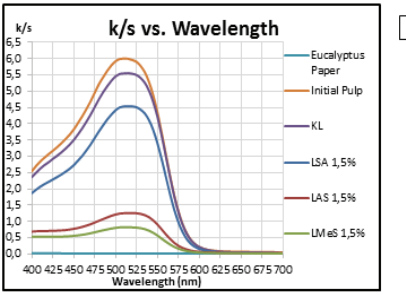
17. APPENDIX 7 : GRAPHICS

Evaluation of the best laccase-mediator on red paper.

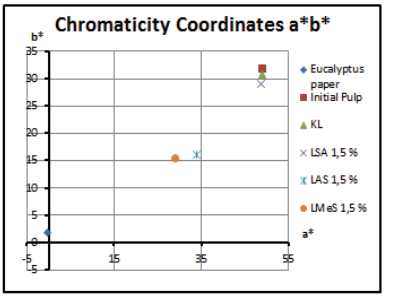
A



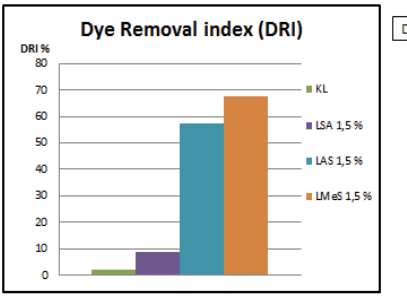
B



C



D



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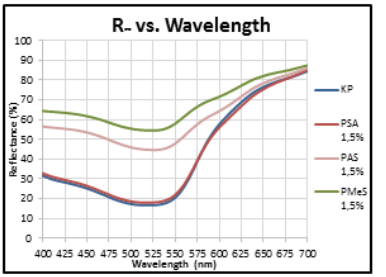

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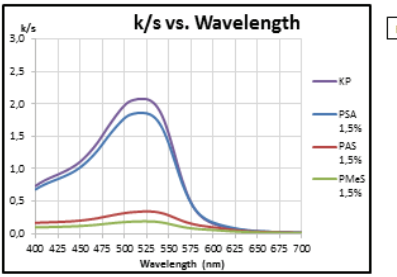

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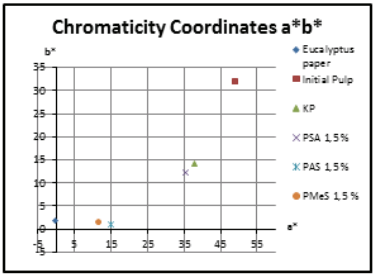

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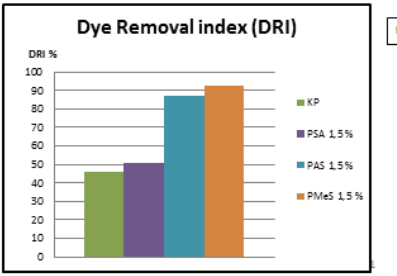
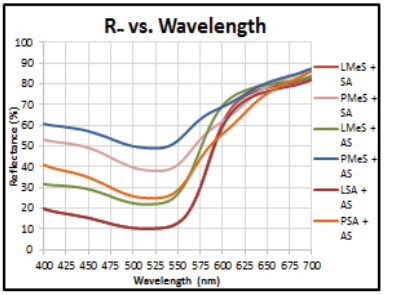


Studying the mediator combinations

A



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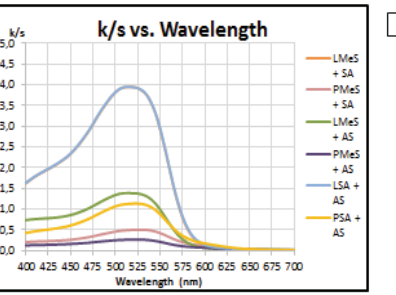

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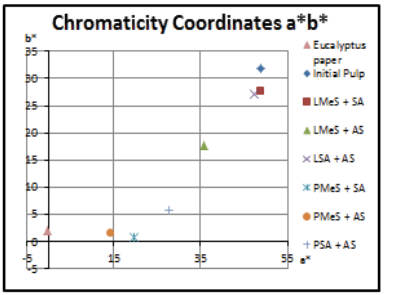

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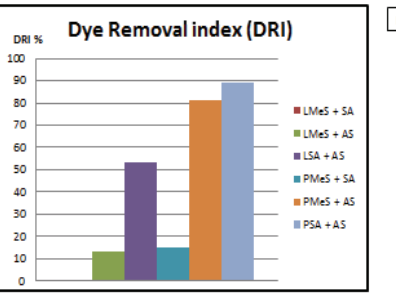

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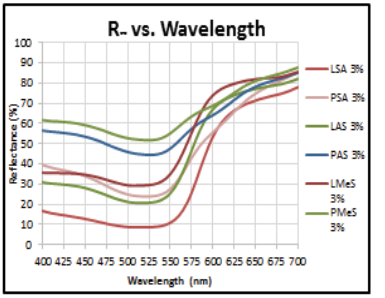

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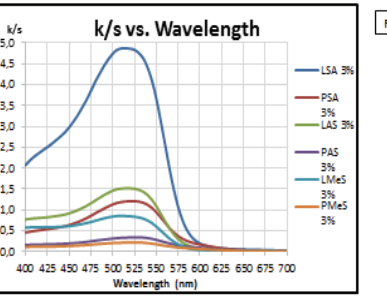

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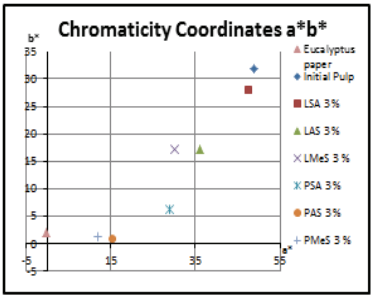

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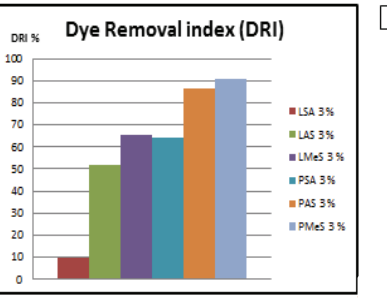
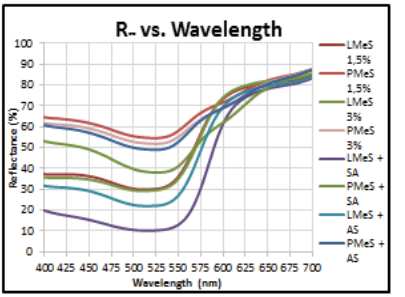


I



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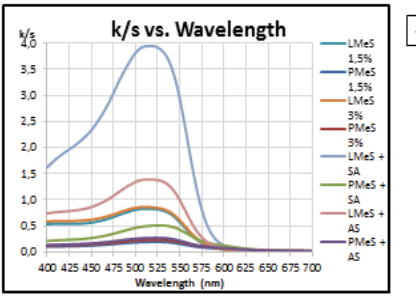
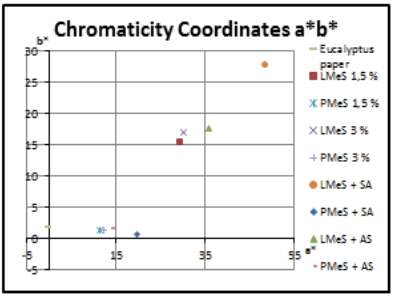
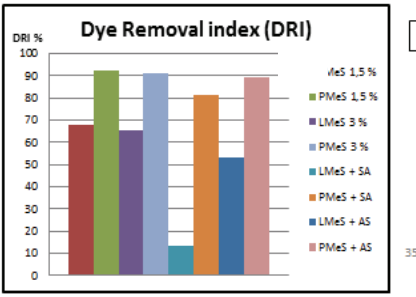


K



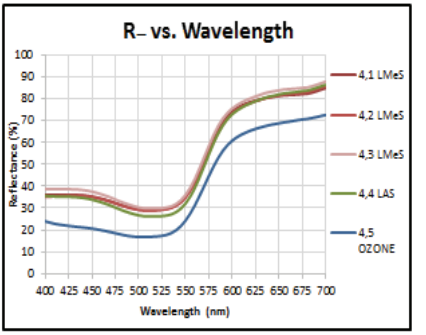
L



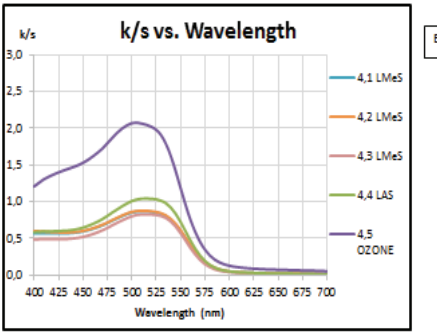
35

Complete biodeinking sequence

A



B



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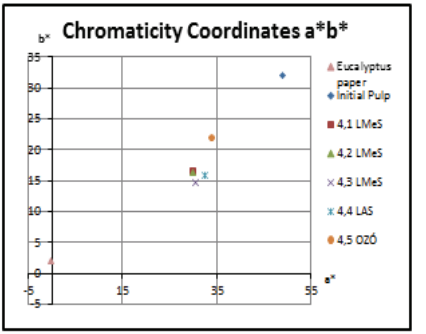

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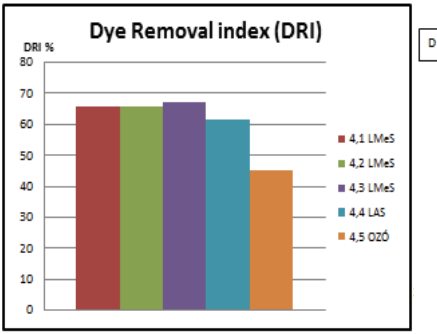

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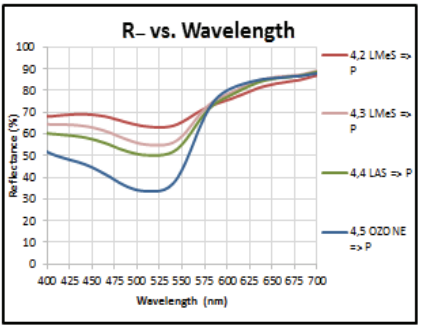
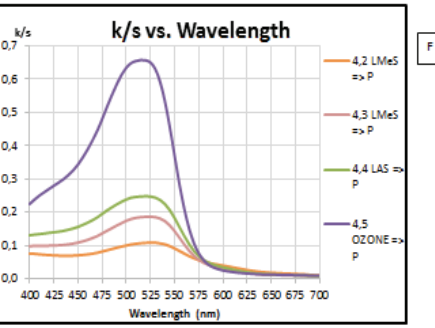
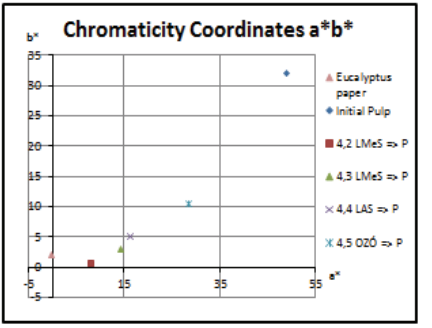

R– vs. Wavelength
Reflectance (%)
Wavelength (nm)
4,2 LMeS => P
4,3 LMeS => P
4,4 LAS => P
4,5 OZONE => P

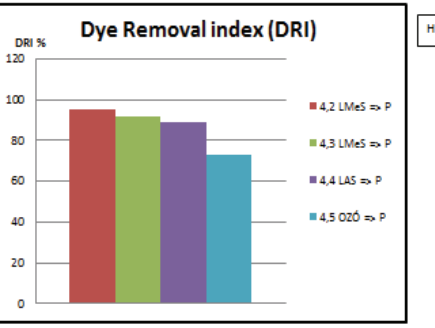
F


k/s vs. Wavelength
k/s
Wavelength (nm)
4,2 LMeS => P
4,3 LMeS => P
4,4 LAS => P
4,5 OZONE => P

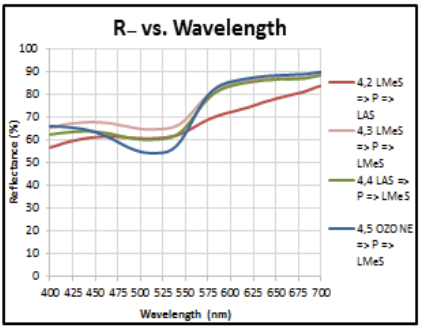
G


Chromaticity Coordinates a*b*
b*
a*
Eucalyptus paper
Initial Pulp
4,2 LMeS => P
4,3 LMeS => P
4,4 LAS => P
4,5 OZÓ => P

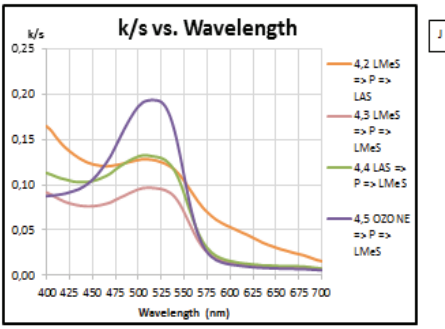
H


Dye Removal index (DRI)
DRI %
4,2 LMeS => P
4,3 LMeS => P
4,4 LAS => P
4,5 OZÓ => P

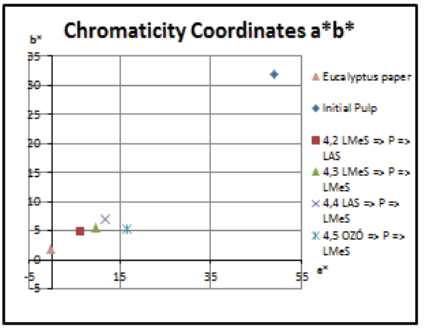
I


R– vs. Wavelength
Reflectance (%)
Wavelength (nm)
4,2 LMeS => P => LAS
4,3 LMeS => P => LMeS
4,4 LAS => P => LMeS
4,5 OZONE => P => LMeS

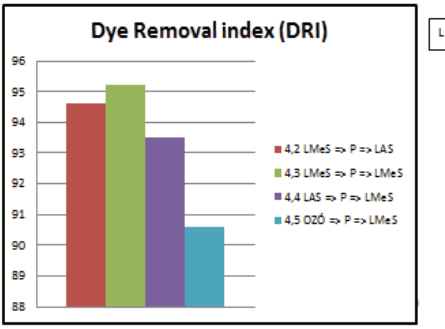
J


k/s vs. Wavelength
k/s
Wavelength (nm)
4,2 LMeS => P => LAS
4,3 LMeS => P => LMeS
4,4 LAS => P => LMeS
4,5 OZONE => P => LMeS

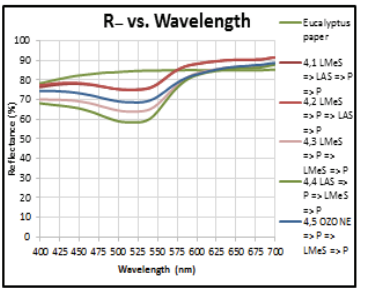
K


Chromaticity Coordinates a*b*
b*
a*
Eucalyptus paper
Initial Pulp
4,2 LMeS => P => LAS
4,3 LMeS => P => LMeS
4,4 LAS => P => LMeS
4,5 OZÓ => P => LMeS

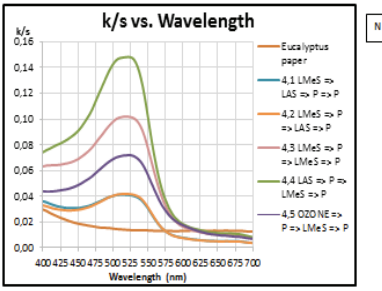
L


Dye Removal index (DRI)
4,2 LMeS => P => LAS
4,3 LMeS => P => LMeS
4,4 LAS => P => LMeS
4,5 OZÓ => P => LMeS

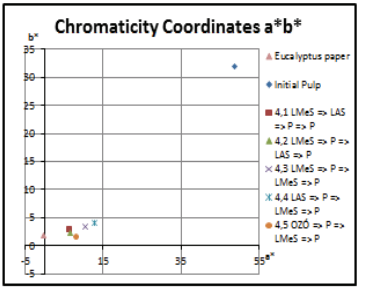
M


R– vs. Wavelength
Reflectance (%)
Wavelength (nm)
Eucalyptus paper
4,1 LMeS => LAS => P => P
4,2 LMeS => P => LAS => P
4,3 LMeS => P => LMeS => P
4,4 LAS => P => LMeS => P
4,5 OZO NE => P => LMeS => P

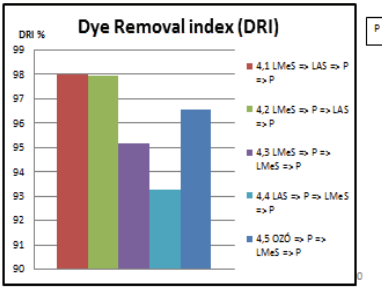
N


k/s vs. Wavelength
k/s
Wavelength (nm)
Eucalyptus paper
4,1 LMeS => LAS => P => P
4,2 LMeS => P => LAS => P
4,3 LMeS => P => LMeS => P
4,4 LAS => P => LMeS => P
4,5 OZO NE => P => LMeS => P

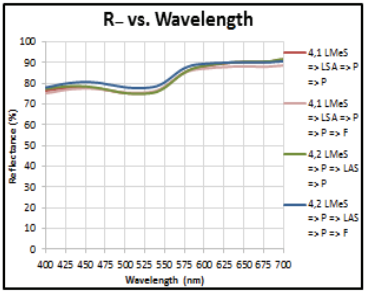
O


Chromaticity Coordinates a*b*
b*
a*
Eucalyptus paper
Initial Pulp
4,1 LMeS => LAS => P => P
4,2 LMeS => P => LAS => P
4,3 LMeS => P => LMeS => P
4,4 LAS => P => LMeS => P
4,5 OZÓ => P => LMeS => P

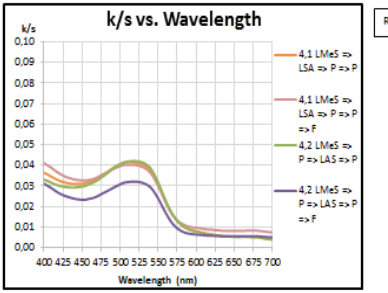
P


Dye Removal index (DRI)
DRI %
4,1 LMeS => LAS => P => P
4,2 LMeS => P => LAS => P
4,3 LMeS => P => LMeS => P
4,4 LAS => P => LMeS => P
4,5 OZÓ => P => LMeS => P

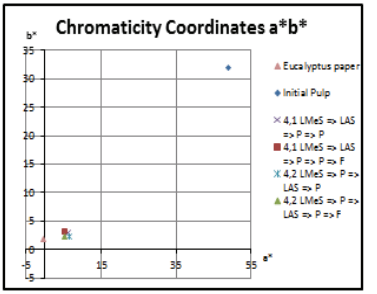
Q


R– vs. Wavelength
Reflectance (%)
Wavelength (nm)
4,1 LMeS => LSA => P => P
4,1 LMeS => LSA => P => P => F
4,2 LMeS => P => LAS => P
4,2 LMeS => P => LAS => P => F

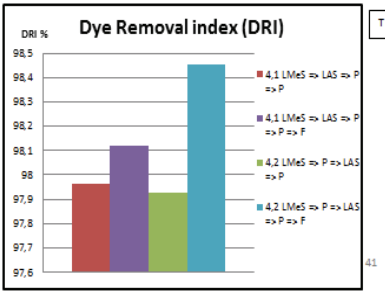
R


k/s vs. Wavelength
k/s
Wavelength (nm)
4,1 LMeS => LSA => P => P
4,1 LMeS => LSA => P => P => F
4,2 LMeS => P => LAS => P
4,2 LMeS => P => LAS => P => F

S

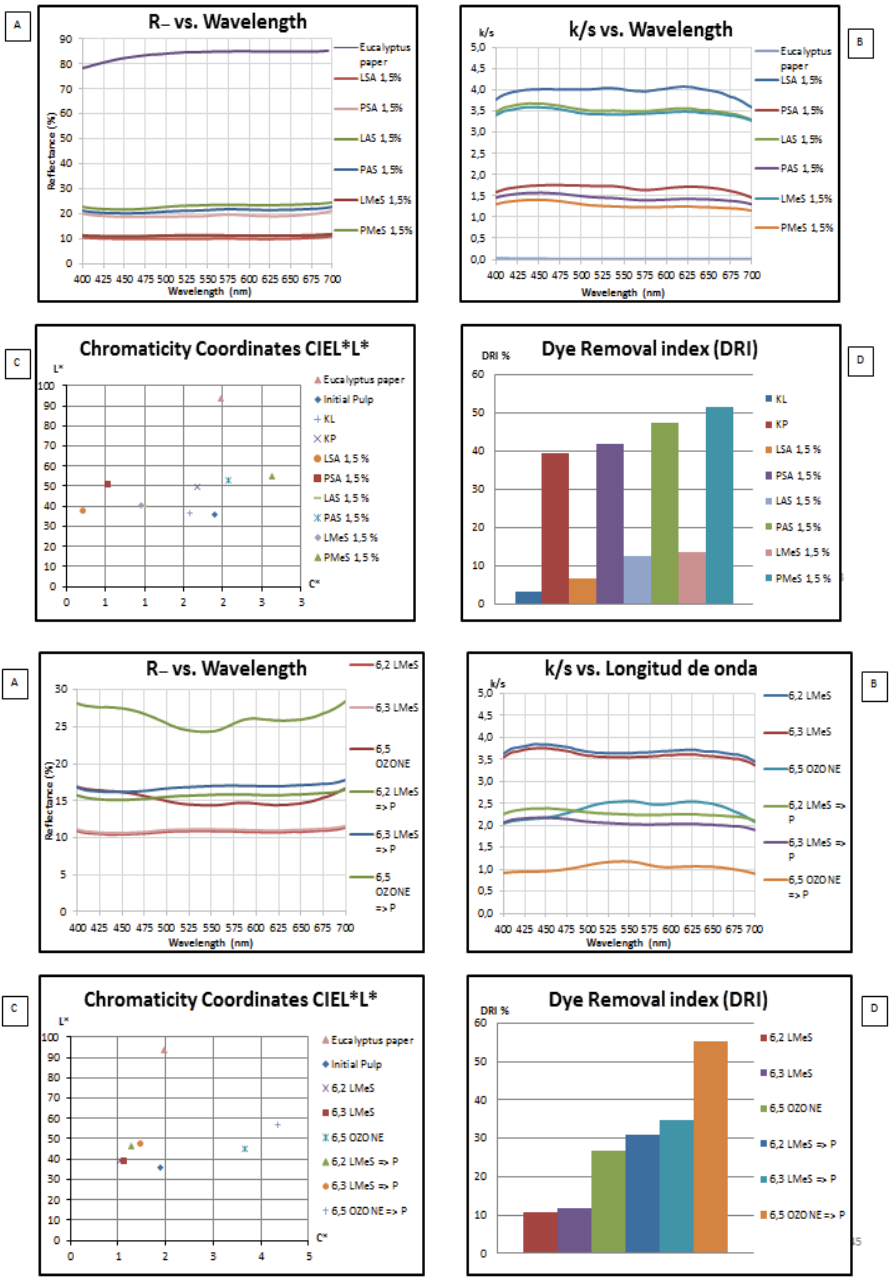

Chromaticity Coordinates a*b*
b*
a*
Eucalyptus paper
Initial Pulp
4,1 LMeS => LAS => P => P
4,1 LMeS => LAS => P => P => F
4,2 LMeS => P => LAS => P
4,2 LMeS => P => LAS => P => F

T


Dye Removal index (DRI)
DRI %
4,1 LMeS => LAS => P => P
4,1 LMeS => LAS => P => P => F
4,2 LMeS => P => LAS => P
4,2 LMeS => P => LAS => P => F

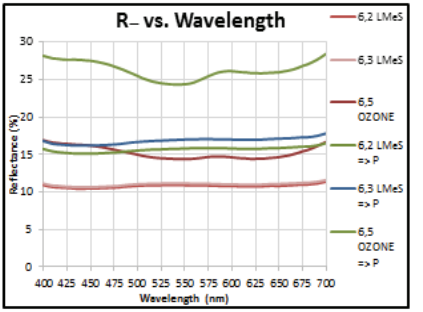
Black paper

Evaluation of the best laccase-mediator on black paper.

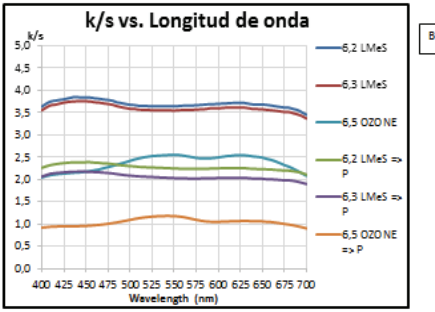


Complete biodeinking sequence

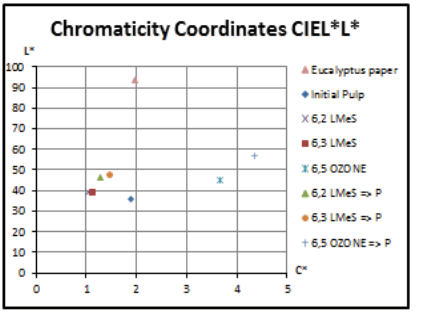
A



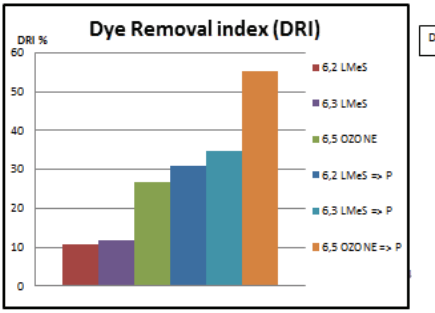
B



C



D



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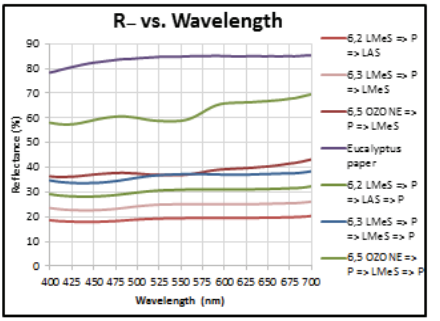
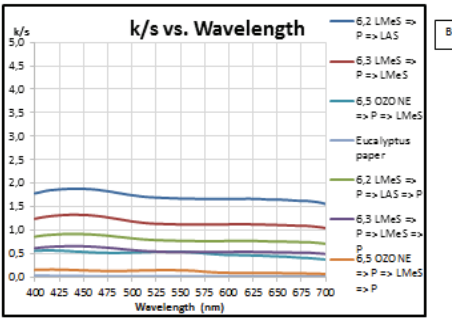


B



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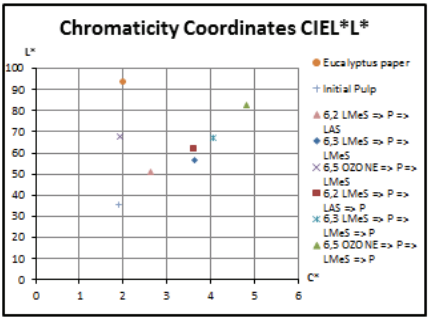

D