INSTITUTO SUPERIOR DE ENGENHARIA DO PORTO
MESTRADO EM ENGENHARIA QUÍMICA




Valorização do licor negro da pasta do papel para a indústria de curtumes

MAGDALENA MARTA TOMASZEWSKA
Julho de 2017

POLITÉCNICO
DO PORTO

INSTITUTO SUPERIOR DE ENGENHARIA DO PORTO
MESTRADO EM ENGENHARIA QUÍMICA

VALORIZATION OF BLACK LIQUOR FOR LEATHER TREATMENT

MAGDALENA MARTA TOMASZEWSKA
Julho de 2017

POLITÉCNICO
DO PORTO

2017

Erasmus project

VALORIZATION OF BLACK LIQUOR FOR LEATHER TREATMENT

MAGDALENA TOMASZEWSKA

Table of contents

Figure Index

Table Index

Abstract

The project was connected with the valorization of black liquor from paper pulp for leather treatment process.

The main goal of the project was using the black liquor to prepare a compact product for leather retanning process and finding its environmental impact on leather treatment process in comparison with standard process.

There were made 10 leather retanning trials. One of them was a standard retanning process, to which compact retanning processes were compared. Compact processes varied through amount of added compact product, kind of compact product, time of dye action (75 – 180min), temperature of retanning process (50 – 60ºC) and time of formic acid action (30 – 90min). The compact retanning process was distinguished by the fact that compact product was added instead the various products used in the standard retanning process and the quantity of water used was less . Leather and wastewater from the different processes were evaluated.

Results showed that CP4 process represents the lowest values of COD (chemical oxygen demand): 9,37 mg/g of leather, TS (total solids): 86,04 mg/g of leather, SS (suspended solids): 3,14 mg/g of leather and the best values of mechanical resistance in comparison with standard process and remaining compact processes.

Having these results, it can be concluded that connection of the lower amount of compact product, the lower temperature of retanning process, the shorter time of dye action and the longer time of formic acid action, allows to obtain a lower negative impact on environment and the highest tear and cracking resistance of leather.

1 - Introduction

1.1 – Framework

The project was connected with the valorization of black liquor from paper pulp for leather treatment.

Researches were carried out according to Project Erasmus in Center of Innovation in Engineering and Industrial Technology, which is located at ISEP – Higher Institute of Engineering in Porto.

1.2 – Objectives and activities

The aim of the project was to use the black liquor from pulp paper to prepare a compact product for leather retanning and find its environmental impact on leather treatment process in comparison with standard process.

Research consisted of three main activities.

First of them was making a characterization of black liquor that was used in the next stage of the project: leather treatment. The characterization of black liquor consisted of few parts: lignin detection, tannin detection, lignosulphonate detection, evaluation of moisture content, evaluation of mineral matter content, evaluation of organic matter content, evaluation of total solids content and evaluation of glucose content.

In the second activity two compact products were prepared with the black liquor and used in leather retanning.

The third activity consisted in wastewater and leather evaluationfor the processes used. For leather distension and strength of grain and tear load were evaluated. For wastewater TS (total solids) content, SS (suspended solids) content and COD (chemical oxygen demand)were evaluated.

1.3 – Report presentation

For a better understanding of the work, this report is divided into five chapters.

Chapter 1 – Introduction: presents the framework and the main objectives of this work.

Chapter 2 – State of Art: enhances the black liquor and the leather industry.

Chapter 3- Experimental Work: the experimental description is made, in which all the procedures used in characterization of the black liquor, leather treatment and evaluation of wastewater are mentioned.

Chapter 4 – Results and Discussion: is dedicated to the main results obtained experimentally and its discussion.

Chapter 5 – Conclusions: presents the conclusions drawn from the study.

2 - State of art

2.1 - Black liquor

Black liquor is a thick and dark liquid that is a byproduct of the process that transforms wood into pulp, which is then dried to make paper (Restolho, et al. 2009). One of the main ingredients in black liquor is lignin, which is the material in trees that binds wood fibers together and makes them rigid, and which must be removed from wood fibers to create paper (Pereira, et al. 2017).

The black liquor is an aqueous solution of lignin residues, hemicellulose, and the inorganic chemicals used in the process. The black liquor comprises 15% solids by weight of which 10% are organic chemicals and 5% are inorganic chemicals (Hamaguchi, Cardoso e Vakkilainen 2012).

Most kraft pulp mills use recovery boilers to recover and burn much of the black liquor they produce, generating steam and recovering chemicals (sodium hydroxide and sodium sulfide) (Hamaguchi, Cardoso e Vakkilainen 2012).

Lignin, and therefore black liquor, contains the bulk of the energy content of wood.

Black liquor is used as fuel at papermaking facilities to generate electricity as well as the heat needed to remove the water from pulp to make paper, thus making pulp mills possible to be nearly energy self-sufficient by producing approximately 66 percent of their energy needs onsite (Hamaguchi, Cardoso e Vakkilainen 2012).

Black liquor is not a hazardous waste, using it for fuel is good for environment. It avoids fossil fuel use and is highly efficient. Moreover black liquor is a carbon-neutral biomass-based fuel that results in no increase of carbon dioxide in the atmosphere.

2.2 – Leather

Leather is a durable and flexible material created by the tanning of animal rawhide and skin. It was used from the primitive times and is widely used today. Despite of the fact that leather is not a textile material, it acquires significant use in textiles (Kesarwani, Jahan e Kesarwani 2015).

The types of the skins often used by manufactures are from cattle, sheep and pigs (Kesarwani, Jahan e Kesarwani 2015).

Processing of leather consists of three main phases – preparation, tanning and post-tanning.

1. Preparation phase

In Beamhouse, the skins are preserved in sodium chloride salt, stored in controlled cool rooms and presorted for quality and weight. The skin is soaked in water to remove blood, dirt and salt. Then the liming is done to remove the hairs, nails and soluble proteins. Skins are treated with acid and salt in preparation for tanning (EPA 1997).

2. Tanning phase

During tanning the skin fibers absorb the tanning agents. That's when the skin becomes leather. There can be distinguished vegetable tanning (done with natural or synthetic tannin), chrome tanning (done by using chromium sulphate and other salts of chromium) aldehyde tanning (done with glutaraldehyde) and synthetic tanning (tanned by using aromatic polymers), depending on a substance used for process (EPA 1997).

3. Post-tanning phase

During the samming process water is removed. The leather is reduced of substances in order to achieve a specified thickness. Then in skiving irregularities are removed and the acid resulting from the tanning process is neutralized. Leather is dyed and treated with reactive oils to achieve correct softness. Then leather needs to be dried.
Two methods are used to drying. The vacuum process during which moisture is removed by suction and the oven process, when leather is fixed on a frame and dried in the oven. In the end leather is given to its final surface treatment such as coating with pigments or dyes (EPA 1997).

3 - Experimental work

The experimental work consisted of the characterization of black liquor, leather retanning trials, and wastewater and leather evaluation.

3.1 - Characterization of black liquor

Before starting the experimental part, it is important to know the characteristics of the raw material involved in the research work. So the first step is to characterize the black liquor according to the next determinations:

- Lignin detection
- Tannin detection
- Evaluation of moisture content
- Evaluation of mineral matter content
- Evaluation of organic matter content
- Evaluation of total solids content
- Evaluation of glucose content
- Lignosulphonate detection

3.1.1 - Lignin detection

The lignin was determined by the standard Tappi T222: 2006. To detect lignin in black liquor there were prepared specimens of dried black liquor and cold 72% solution of H_2SO_4 – in this way the carbohydrates in black liquor were hydrolyzed and solubilized by sulphuric acid. Then acid-insoluble lignin was filtered off, dried and weighted.

3.1.2 - Tannin detection

There were used two methods to detect tannin in black liquor.

The first one was Folin - Ciocalteu method (Tambe e Bhambar 2014). In this method the sample containing black liquor solution, Folin- Ciocalteu phenol reagent, 35% Na_2CO_3 solution and water was compared with calibration curve of gallic acid (Appendix A1). The absorbance was measured at 725 nm with UV-Vis spectrophotometer (Shimadzu UV 160-A).

The second method was tyrosine method (Appendix A2) from the spectrophotometer DR/2000 procedure. The tannin content was detected in a sample that contained TanniVer 3 Tannin-Lignin Reagent, Na2CO3 solution and black liquor solution. This test measures all hydroxylated aromatic compounds, including tannin, lignin, phenol and cresol. The results were reported as total tannin and lignin and expressed as mg/L tannic acid. The measurement wavelength is 700 nm for spectrophotometers.

3.1.3 - Moisture, mineral, organic and total solids content

The moisture content and total solids (Appendix A3) were evaluated with the dryness of black liquor in the oven at 103ºC until constant weight. Then the samples were ignited in a furnace at 550ºC for 3 hours, which was needed to estimate mineral and organic matter (Appendix A4). All the procedures were based in the standard methods.

3.1.4 - Glucose content evaluation

The glucose content (Appendix A5) was evaluated by the colorimetric method using 3,5-Dinitrosalicylic acid (DNS reagent) in a UV-Vis spectrophotometer (Shimadzu UV 160-A) at 540nm.

3.1.5 - Lignosulphonate content

Lignosulphonate content (Appendix A6) was determined by UV-Vis spectrophotometer 160 A (Shimadzu) at 273nm. The absorbance of black liquor solution was compared with calibration curve of sodium lignosulphonate.

3.2 - Leather treatment

This stage of project consisted of making 10 trials of leather retanning.

One of them was the standard process, to which there were compared compact processes by evaluation of wastewater impact and mechanical tests of leather.

The main goal was to obtain compact processes, more sustainable concerning the environment, energy and productivity.

For the compact processes there was prepared a compact product described in Table 3. 1, below.

Table 3. 1 - Preparation of compact products R1 and R2

	Compact R1	Compact R2
Black liquor	120 g	100 g
Fortan A40	25 g	25 g
Fortan SML	25 g	25 g
Corilene HLG	10 g	10 g
Mimosa extract	-	20 g
Water	20 g	20 g

In processes from CP1 to CP7 there was used compact product R1 and in CP8 and CP9 there was used compact product R2. The difference was adding another amount of black liquor and extra adding Mimosa in R2.

Processes varied among themselves– each of them was made in different conditions. The table shows conditions in which standard process and every compact process were leaded.

Table 3. 2 – Conditions of Processes.

		SP	CP1	CP2	CP3	CP4	CP5	CP6	CP7	CP8	CP9
Amount of compact product R1	9,6 g		X	X	X	X		X			
	14,4 g						X		X		
Amount of compact product R2	9,6 g									X	
	14,4 g										X
Time of dye action	75 min	X	X			X	X	X	X	X	X
	120 min			X							
	180 min				X						
Temperature of retanning part of process	50 °C	X	X	X	X	X	X				
	60 °C							X	X	X	X
Time of formic acid action	30 min	X	X	X	X						
	90 min					X	X	X	X	X	X
Not added compact product		X									

As it is shown in Table 3. 2, processes varied through amount of added compact product, kind of compact product, time of dye action (75 – 180min) , temperature of retanning process (50 – 60ºC) and time of formic acid action (30 – 90min).

Despite of fact that compact processes were made in different conditions and varied between themselves, there were some connections between them. In the scheme below (Figure 3. 1) are presented dependencies between all compact processes.

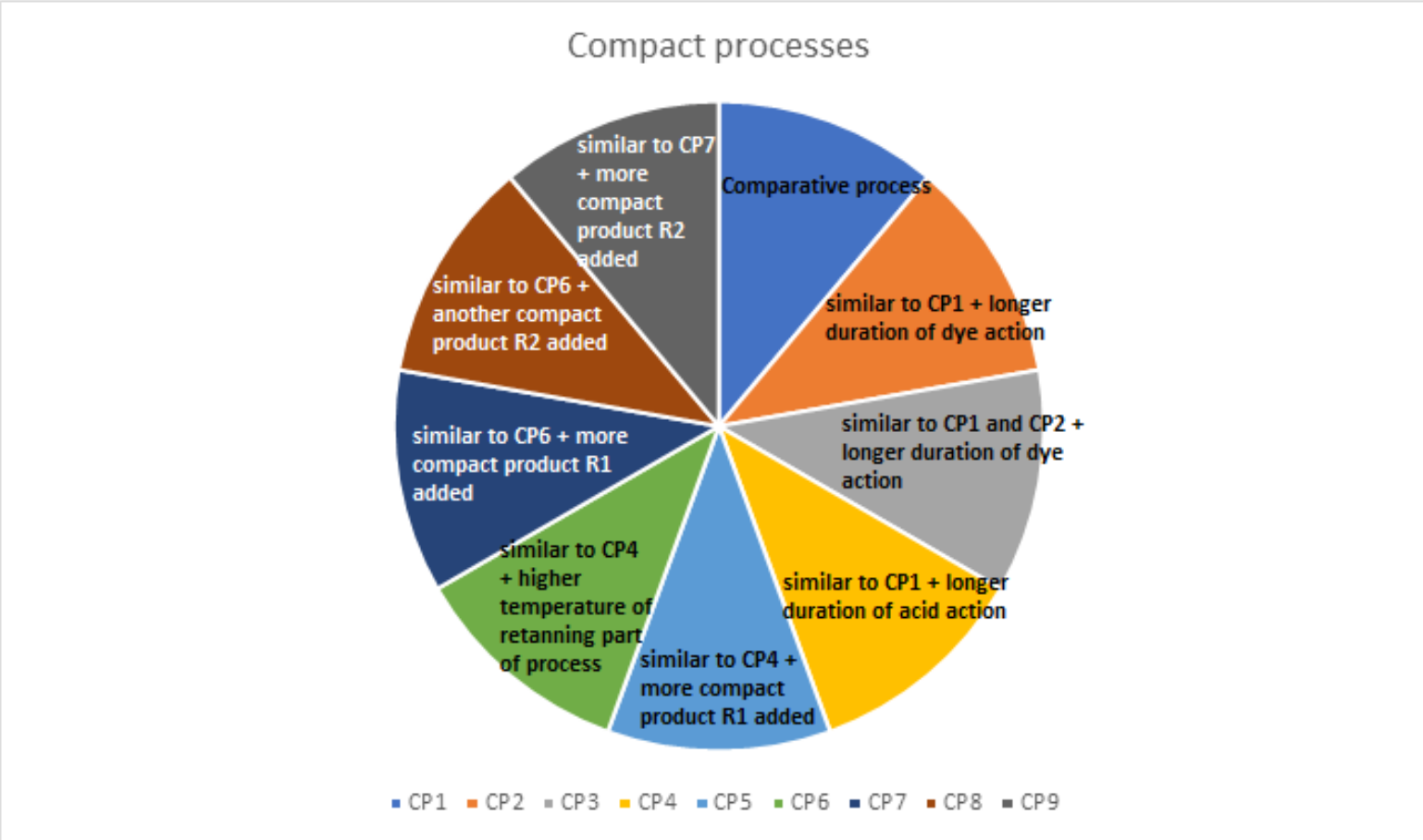
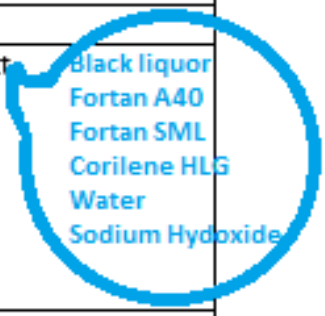


Figure 3. 1 – Comparison of Compact processes.

In following Table 3.3, there is presented a comparison of standard and compact process.

Table 3. 3 - Comparison of standard and compact process

	Standard process	Compact process
Wash	Water	Water
Neutralize	Water Sodium Formiate Sodium Bicarbonate	Water Sodium Formiate Sodium Bicarbonate
Wash	Water	-
Retan	Water Fortan A40 Indinol EAF Indinol RS Inditan VOC Mimosa Corante	Compact Product Corante
Fix	Formic Acid	Formic Acid
Fatliquoring	Water Indinol BE Indinol HS Indinol EAF Indinol LOX	Water Indinol BE Indinol HS Indinol EAF Indinol LOX
Fix	Formic Acid	Formic Acid
Wash	Water	Water



From Table 3. 3, it is possible to observe that the only difference between compact process CP1 and standard process SP is the compact product addition and the longer retanning time of SP.

3.3 - Leather evaluation

After making all of leather treatment processes, there was made leather evaluation. Leathers have been sent to CTIC (Technological Center of Leather Industry) to check their mechanical resistance.

There was determined the distension and strength of grain by Lastometer method (ISO 3379:1976 – IULTCS/IUP 9:1976) and tear load – double edge tear (ISO 3377-2:2002 – IULTCS/IUP 8:2002).

3.4 - Evaluation of wastewater

After each compact process, wastewater was collected and evaluated in total solids content, total suspended solids content and COD - chemical oxygen demand.

3.4.1 - Total solids content

To estimate total solids content, 5 ml of wastewater from each process were dried to a constant weight in a oven (WTB Binder) at 103±0.2ºC (Appendix A1).

3.4.2 - Total suspended solids content

To estimate the total suspended solids (Appendix A7) it was made a filtration with glass fiber filters of samples of wastewater. After process of filtration, filters were situated inside the oven (WTB Binder) at 103±0.2ºC and after finishing changes in weight, they were balanced.

3.4.3 - Chemical oxygen demand

In the determination of oxygen demand (Appendix A8), the sample of wastewater reacts with standard potassium dichromate solution for 2 hours. After this time, samples were evaluated by the spectrophotometer DR/2000. The result is expressed in mass of oxygen consumed over volume of solution which in SI units was milligrams per liter (mg/L).

4 – Results and Discussion

In this chapter the results obtained are presented and discussed for all the experimental work done.

4.1 - Characterization of black liquor

In the Table 4.1 there are presented the results obtained for the black liquor characterization.

Table 4. 1 – Results of black liquor characterization.

Lignin content	30, 08 %
Tannin content ➔ Folin-Ciocalteu method ➔ Tyrosine method	 11, 38 g/l 115 g/l tannic acid
Mineral matter content	17, 44%
Organic matter content	82, 57 %
Moisture content	51, 25 %
Total solids content	48, 75 %
Glucose content	10, 35 g/l
Lignosulphonate content	18, 63 g/l

Table 4.1 shows that black liquor contains big amount of tannin and lignin. These results were useful during leather retanning processes, because of the fact that the more tannic acid skin absorbs, the more resistant and durable it is.

4.2 – Leather evaluation

In Table 4.2, below, there are presented obtained results of leather mechanical resistance. There can be observed that compact process CP4 has higher mechanical resistance in comparison with remaining compact processes and standard process. All of them were above minimum values needed for footwear.

Table 4. 2 – Results of mechanical resistance tests of leather.

	SP	CP1	CP2	CP3	CP4	CP5	CP6	CP7	CP8	CP9
Strength of grain [N] (min = 300 N)	496,5	488,5	487,7	491,0	564,7	458,1	352,2	428,7	402,6	305,4
Distension of grain [mm] (min = 7 mm)	11,2	10,6	10,3	11,8	9,7	9,2	8,3	8,3	9,1	7,4
Tear Load [N] (min = 120 N)	197	155	143	135	210	148	212	122	177	192

4.3 - Evaluation of wastewater

In the following Table 4.3, there are presented obtained results of wastewater evaluation.

Table 4. 3 – Results of wastewater evaluation.

	SP	CP1	CP2	CP3	CP4	CP5	CP6	CP7	CP8	CP9
Total solids [mg total solids/g of leather]	98,16	96,37	166,13	133,46	86,04	104,63	93,87	102,55	85,15	106,13
Total suspended solids [mg suspended solids/g of leather]	4,46	3,18	4,81	4,46	3,14	6,18	4,25	5,37	3,96	4,78
COD [mg/g of leather]	34,20	32,83	37,22	47,13	9,37	34,18	49,87	71,88	52,93	63,64

Obtained results presented in Table 4.3 shows that wastewater from compact processes CP1, CP4, CP6 and CP8 presents lower values of total solids and total suspended solids in comparison with standard process SP. It can be also observed that in case of chemical oxygen demand, lower values are presented by compact processes CP1, CP4 and CP5.

5 - Conclusions

To sum up the project, there can be draw some conclusions based on obtained results.

For total and suspended solids:

- The longer the action of the acid, the better the process
- The longer the dye works, the worse the process
- The more added compact product, the worse the process

For COD:

- Connection of shorter duration of dye action and lower temperature of retanning part of process, makes process better

Taking into account all of obtained results, it can be concluded that compact process CP4 is the best of processes, that have been done.

Wastewater of CP4 process represents very low value of COD and total and suspended solids in comparison to another compact processes and standard process.

The result of total solids of CP4 is 12% lower than result of SP, in case of total suspended solids it is 2% lower and in case of COD , it is 250 % lower in comparison with SP.

Moreover, leather that was subjected to this process, had the greatest Distension and strength of grain and tear load.

Having these results, it can be concluded that connection of low amount if compact product R1, low temperature of retanning part of process with short duration of dye action and long duration of formic acid action make it possible to obtain process which has low negative impact on environment and which makes the leather resistant.

Bibliography

EPA. “Leather tanning - Final report.” Emission Factor Documentation for AP-2 Section 9.15, 1997.

Hamaguchi, Marcelo, Marcelo Cardoso, e Esa Vakkilainen. “Alternative technologies for biofuels production in kraft pulp mills - potential and prospects.” *Energies* 5 (2012): 2238 - 2309.

Kesarwani, Priyanka, Shahnaz Jahan, e Kirti Kesarwani. “A review on leather processing.” *International Journal of applied research* 1, nº 9 (2015): 977-982.

Pereira, Susana R., Diogo J. Portugal-Nunes, Dmitry V. Evtuguin, Luisa S. Serafim, e Ana M.R.B. Xavier. “Advances in ethanol production from hardwood spent sulphite liquors.” *Process Biochemistry* 48, nº 2 (2017): 272 - 282.

Restolho, Jose Augusto, Antonio Prates, Maria Norberta Pinho, e Maria Diná Afonso. “Sugars and lignosulphonates recovery from eucalyptus spent sulphite liquor by membrane processes.” *Biomass and Bioenergy Journal* 33, nº 11 (2009): 1558-1566.

Tambe, V.D., e R.S. Bhambar. “Estimation of total phenol, tannin, alkaloid and flavonoid in Hibiscus Tiliaceus Linn. wood extracts.” *Research and Review: Journal of Pharmacognosy and Phytochemistry* 2, nº 4 (2014): 41 - 47.

Appendices

Appendix A: Characterization

Appendix A1: Tannin detection – Folin Ciocalteu method

A1.1 Principle

The tannin detection was determined by a colorimetric method based on the measurement of blue color formed by the reduction of phosphomolybdiate acid by tannin in aqueous alkali.

A1.2 Apparatus and materials

- Analytical balance
- UV/Vis Spectrophotometer

A1.3 Procedure

- A minimum of 3 determinations is needed to get a good average result.
- 7,5mL of distilled water and 0,5mL of Folin-ciocalteuphenol reagent and 1 mL of 35% Na_2CO_3 solution were added to a volumetric flask of 10mL.
- To the same volumetric flask 0,1mL of the sample diluted 100x was added and the volume completed with distilled water.
- The mixture was shaken and kept 30min at room temperature.
- After the 30min, the absorbance was measured against the blank at 725nm in the spectrophotometer.
- The blank was prepared in the same manner as the sample but with addition of distilled water instead of the sample.
- The Calibration curve was prepared with a set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml).

A1.4 Calibration Curve

First step was to prepare gallic acid solution, needed to do calibration curve.

Mass of acid gallic = 100 mg, completed until 1 l in a volumetric balloon.

C= 100 mg/ 1000 ml = 0,1 mg/ ml = 100 ug/ ml

Table A1. 1 – Calibration curve of gallic acid.

Gallic acid solution	water	Final volume	Concentration
20 ml	80 ml	100 ml	20 ug/ml
40 ml	60 ml	100 ml	40 ug/ml
60 ml	40 ml	100 ml	60 ug/ml
80 ml	20 ml	100 ml	80 ug/ml
100 ml	0 ml	100 ml	100 ug/ml

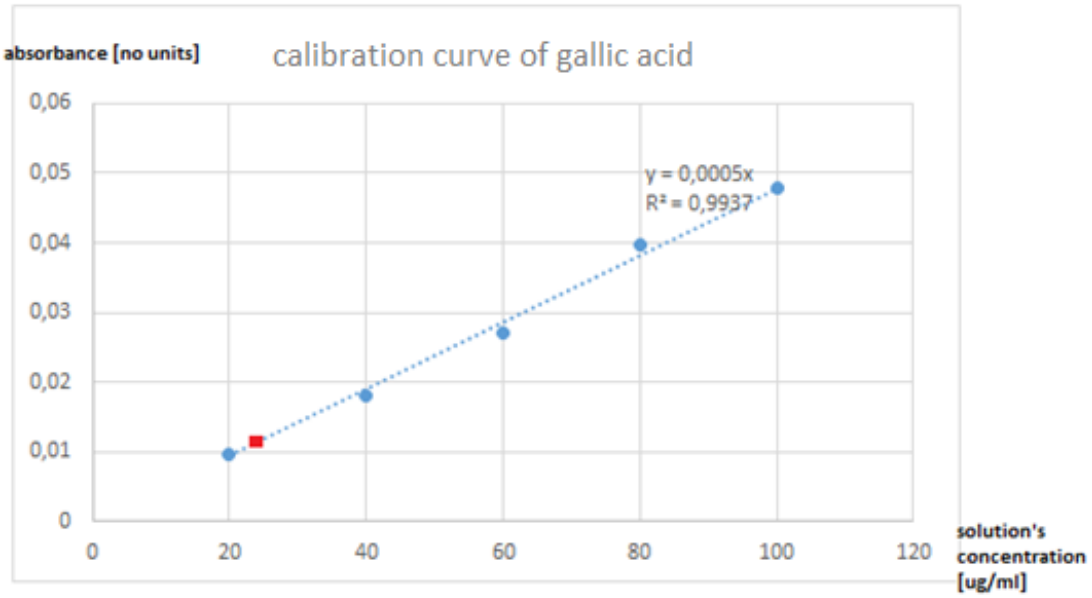


Figure A1. 1 – Calibration curve of gallic acid.

Appendix A2: Tannin detection – Tyrosine method

A2.1 Principle

The tannin detection by the colorimetric tyrosine method based on the measurement of blue color formed by the presence of all hydroxylated aromatic compounds, including tannin, lignin, phenol and cresol.

A2.2 Apparatus and materials

- Dr2000 Spectrophotometer

A2.3 Procedure

- Preparation of black liquor solution – 1 g of black liquor, then filled to 500 ml by distilled water in volumetric flask and it was taken 1 ml of black liquor solution and filled in 100 ml by distilled water in volumetric flask.

Procedure of detection tannin:

- A minimum of 3 determinations is needed to get a good average result.
- Fill a sample cell (the blank) with 25 ml of deionized water
- Pipet 0,5 ml of TanniVer 3 Tannin-Lignin Reagent into cell
- Swirl to mix
- Pipet 5,0 ml of Sodium Carbonate Solution into cell, swirl to mix
- Place the blank into cell holder, close light shield
- Press SHIFT TIMER – 25-minute reaction period will begin
- After the timer beeps, the display showed 0,0 mg/l TANNIC ACID
- Remove the blank from the cell holder
- Place the prepared sample into cell holder, close the light shield
- Press SHIFT TIMER – 25- minute reaction periods will begin
- After this period the display showed the results of absorbance measured against the blank at 700nm in the spectrophotometer
- The blank was prepared in the same manner as the sample but with addition of distilled water instead of the sample

Appendix A3: Moisture content and Total Solids

A3.1 Principle

The analysis sample was dried at a temperature of 105 ± 2°C and the percentage moisture was calculated from the loss in mass of the test sample.

A3.2 Apparatus and materials

- Drying oven (WTB Binder)
- 3 crucibles
- Analytical balance
- Desiccator

A3.3 Preparation

- The crucibles were cleaned for 1 hour at 900°C in a furnace.
- The crucibles were allowed to cool down in the furnace until 100°C.
- The crucibles were removed from the furnace, and placed on a heat resistant plate for 5-10 minutes and then transferred to a desiccator and allowed to cool until room temperature.

A3.4 Procedure

- 3 determinations were done to get a good average.
- The empty crucible was weighed.
- 5g of black liquor was placed in the crucible.
- The uncovered crucible was heated with the sample at 105°C until constant weight.
- The crucible and its content were transferred to a desiccator. It was allowed to cool to room temperature.
- The moisture content in the sample was calculated.

A3.5 Calculations

The moisture content was calculated using the following formula:

$$Moisture(\%) = \frac{(m_2 - m_3)}{(m_2 - m_1)} x100$$

Or

$$Moisture(\%) = \frac{m_{moisture}}{m_{sample}} x100$$

Where:

M_{ad} is the % moisture content of the test sample used for determination.

m_1 is the mass in grams of the empty crucible

m_2 is the mass in grams of the crucible plus sample before drying.

m_3 is the mass in grams of the crucible plus sample after drying.

Appendix A4: Organic and Mineral content

A4.1 Principle

The organic and mineral content was determined by calculation from the mass of the residue remaining after the sample was ignited in air under 550 ± 5 °C, for 3 hours.

A4.2 Apparatus and materials

- 3 heat crucibles
- Furnace
- Analytical balance
- Desiccator

A4.3 Preparation of Crucibles

- The empty crucibles were cleaned in a furnace at 900°C for 1 hour.
- The dishes were cooled down in the furnace until 100°C.
- The dishes were removed from the furnace. The dishes were placed on a heat resistant plate for 5-10 minutes and transferred to a desiccator to cool to room temperature.

A4.4 Procedure

- A minimum of 3 determinations is needed to get a good average result.
- The dried sample was weighed in each crucible.
- The crucibles were placed in the furnace.
- The temperature of the furnace was raised to 550°C and the samples were ignited for 3 hours.
- The crucibles were cooled down in the furnace until 100°C.
- The crucibles were removed from the furnace, and they were placed on a desiccator and allowed to cool until room temperature.
- The organic and mineral content in the sample was calculated

A4.5 Calculations

The mineral content of the sample (A_d), expressed as a percentage by mass on a dry basis, was calculated using the following formula:

$$\%\,\mathrm{min}\,eral = \frac{(m_3 - m_1)}{(m_2 - m_1)} x100$$

Where:

m_1 is the mass, in g, of the empty crucible.

m_2 is the mass, in g, of the crucible plus the test sample.

m_3 is the mass, in g, of the crucible plus ash.

$$\%organic = 100 - \%\min eral$$

Appendix A5: Glucose content

The sugar content was determined by using the 3,5-Dinitrosalicylic acid (DNS) method.

A5.1 Principle

The sugar content was determined by using the 3,5-Dinitrosalicylic acid (DNS) method. This method is based on the oxidation of a reducing sugar by the 3,5-Dinitrosalicylic acid, with the formation of 3-amino-5-nitrossalicilic acid and an oxidized sugar. The absorbance of the solutions is measured at a wavelength of 540 nm in a UV-VIS spectrophotometer (Shimadzu UV-160A).

A5.2 Apparatus and materials

- Analytical balance
- Ultrasonic bath
- Centrifuge
- Filtration system
- Paper filters MACHEREY-NAGEL 4-12 µm/ϕ 47 mm
- UV-VIS spectroscopy equipment UV-160A (SHIMAZU).

A5.3 Procedure

- Preparation of glucose solution
  Mass of glucose = 100 mg, then filled by the water in 100 ml volumetric flask
  Concentration = 0,1 %
- Three determinations were done to get a good average.
- For the blank 1 mL of distilled water and 1 mL of DNS were measured to a glass tube.

- For the sample, in 6 tubes the following quantities of the sample, water and DNS were added.

Table A5. 1 – Preparation of samples trials..

Trial	Sample (mL)	Water (mL)	DNS (mL)
S1	0,1	0,9	1
S2	0,1	0,9	1
S3	0,2	0,8	1
S4	0,2	0,8	1
S5	0,5	0,5	1
S6	0,5	0,5	1

- All the tubes were closed and mixed with a shaker.
- The tubes were put in boiling water for 5min (this is to let the sugars react with the DNS).
- After the 5 minutes the tubes were cooled down.
- After cooling down 8 mL of distilled water was added.
- The content of the tubes were transferred to the UV-VIS spectrometry cells.
- The absorbance at 540 nm was measured against the blank.
- The concentration of the sugars in both samples were calculated.
- The sugar content was expressed as a concentration in g Glucose/100 g sample.

A5.3 Calibration curve

Table A5. 2 - Calibration curve of glucose

trial	glucose solution(ml)	water(ml)	concentration(ug/ml)	DNS (ml)
P1	0,1	0,9	**10**	1
P2	0,2	0,8	**20**	1
P3	0,4	0,6	**40**	1
P4	0,5	0,5	**50**	1
P5	0,7	0,3	**70**	1
P6	0,8	0,2	**80**	1
P7	1	0	**100**	1

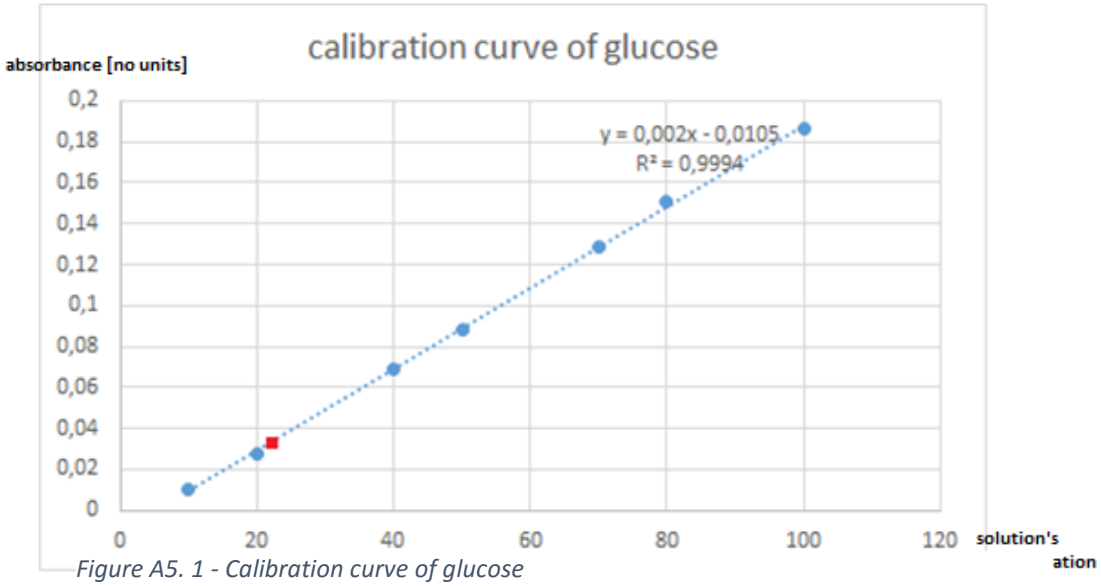


Figure A5. 1 - Calibration curve of glucose

Appendix A6: Lignosulphonate content

The lignosulphonate content was determined by colorimetric method in the UV-Vis spectrophotometer (Shimadzu UV 160-A).

A6.1 Apparatus and materials

- Analytical balance
- UV-VIS spectroscopy equipment UV-160A (SHIMAZU).

A6.2 Procedure

- Preparation of solution of sodium lignosulphonate for the calibration curve

Mass of sodium lignosulphunate = 100 mg, then filled by the distilled water in 100 ml volumetric flask

Concentration = 0,1 %

Then it was done solutions in concentrations: 10, 20, 40, 60, 80, 100 ug/ml

- Preparation of solution of black liquor in concentration 0,2%. Then it was diluted one more time to have proper measures in spectrophotometer.
  5 ml of black liquor solution was diluted in 100 ml of water→ concentration of black liquor solution, that will be taken into account is now 0, 166%.

- Three determinations were done to get a good average.

- The absorbance at 273 nm was measured against the blank (distilled water.

A6.3 Calibration curve

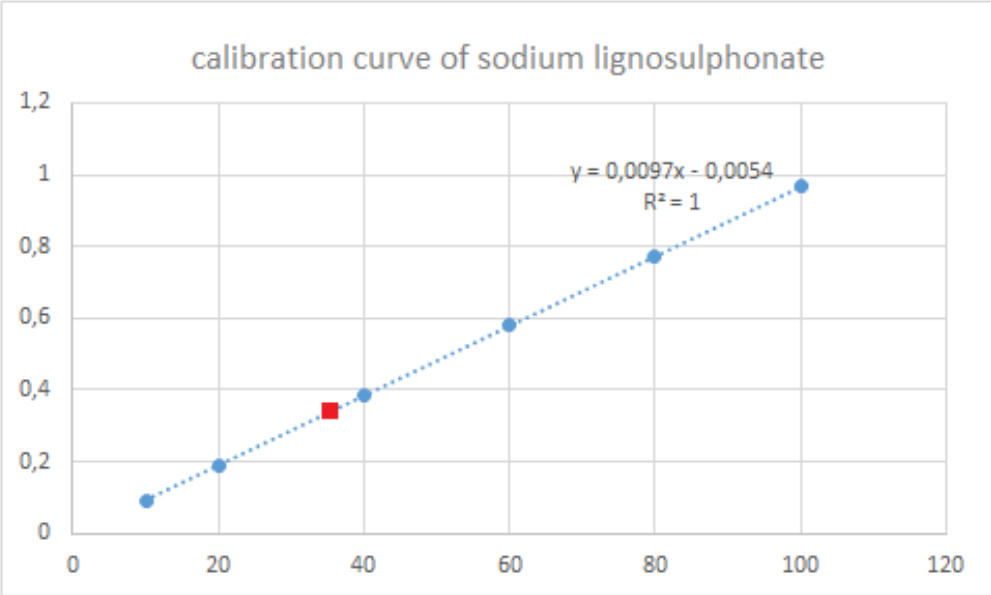


Figure A6. 1 – Calibration curve of lignosulphonate.

Appendix A7: Total Suspended Solids content

A7.1 Principle

Total suspended solids are the solids that remain in the filter paper after the filtration of a certain amount of sample.

A7.2 Apparatus and materials

- Analytical balance
- Filtration system
- Glass fiber filters 1,2 µm/ϕ 47 mm
- UV-VIS spectroscopy equipment UV-160A (SHIMAZU).

A7.3 Procedure

- In the first step the filters were prepared by applying three successive amounts of 20mL of distilled water.
- The filters were dried in the oven ate 103ºC for 1 hour and then cool down in desiccator and measured the weight.
- For the samples, three determinations were done to get a good average.

- With continuous stirring it was measured 20mL of wastewater for the filtration system.
- After the end of filtration, the filters were dried in the oven at 103ºC until the constant weight.
- The filters were cooled down in the desiccator and weighted.

$$TSS\ (g/L) = \frac{m(dried\ residue(105 \circ C))(g)}{V(sample)\ (ml)} X\ 1000ml/L$$

Appendix A8: Chemical Oxygen Demand content

A8.1 Principle

Chemical oxygen demand determines the amount of oxygen that is required for chemical oxidation of organic matter using a strong chemical oxidant.

A8.2 Apparatus and materials

- Analytical balance
- Eco 16 Thermoreactor (Velp Scientifica)
- Spectrophotometer DR 2000 .

A8.3 Procedure

- Heat previously the reactor at 150ºC.
- With continuous stirring of the sample 2,5mL was measured for a glass tube and mixed with 1,5mL of digester reagent and 3,5mL of catalysed reagent.
- Then the tubes react for 2 hours at 150ºC in the reactor.
- After cooling down the COD was measured in the spectrophotometer against the blank at 620nm.
- Three determinations were done to get a good average.
- The blank was prepared the same manner as the sample but with distilled water instead of wastewater.