MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology, Leather and Fur

QUALIFICATION THESIS

on the topic **Enzymatic separation and purification of saccharides from wheat straw machine pulp black liquor**
First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group BEBT-20
Tian JIALIANG

Scientific supervisor
Iryna VOLOSHYNA, Ph.D., As. prof.

Reviewer
Liubov ZELENA, Ph.D., As. prof.

Kyiv 2024

KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN

Faculty: Chemical and Biopharmaceutical Technologies
Department: Biotechnology, Leather and Fur
First (Bachelor's) level of higher education
Specialty: 162 Biotechnology and Bioengineering
Educational and professional program Biotechnology

APPROVE
Head of Department of Biotechnology,
Leather and Fur, Professor,
Doctor of Technical Science
Olena MOKROUSOVA
_____
«___»_____2024

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Tian Jialiang

1. Thesis topic **Enzymatic separation and purification of saccharides from wheat straw machine pulp black liquor**
Scientific supervisor Iryna Voloshyna, Ph.D., As. prof.

approved by the order of KNUTD "_____" _____2024, №_____

2. Initial data for work: assignments for qualification thesis, scientific literature on the topic of qualification thesis, materials of Pre-graduation practice

3. Content of the thesis (list of questions to be developed): literature review; object, purpose, and methods of the study; experimental part; conclusions

4. Date of issuance of the assignments_____

EXECUTION SCHEDULE

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	From 01 April 2024 to 11 April 2024	
2	Chapter 1. Literature review	From 06 April 2024 to 20 April 2024	
3	Chapter 2. Object, purpose, and methods of the study	From 21 April 2024 to 30 April 2024	
4	Chapter 3. Experimental part	From 01 May 2024 to 10 May 2024	
5	Conclusions	From 07 May 2024 to 12 May 2024	
6	Draw up a bachelor's thesis (final version)	From 20 May 2024 to 25 May 2024	
7	Submission of qualification work to the supervisor for feedback	From 25 May 2024 to 27 May 2024	
8	Submission of bachelor's thesis to the department for review (14 days before the defense)	27 May 2024	
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)	31 May 2024	
10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)	03 June 2024	

I am familiar with the task:

Student _____ Tian JIALIANG

Scientific supervisor _____ Iryna VOLOSHYNA

SUMMARY

Tian Jialiang. Enzymatic separation and purification of saccharides from wheat straw machine pulp black liquor. – Manuscript.

Qualification thesis on the specialty 162 «Biotechnology and Bioengineering». – Kyiv National University of Technologies and Design, Kyiv, 2024.

In recent years, the concept of biomass refining has opened up a new way for the efficient utilization of biomass resources, and the application prospect of biomass conversion into high value-added products is very broad. With the development of biomass refining technology for pulping and paper making, the high value utilization of hemicellulose in black liquor has attracted extensive attention. In the process of using hemicellulose in black liquor to produce high value-added products, lignin and furfural in black liquor will hinder its utilization. In order to realize the separation, purification and high value utilization of sugar substances in black liquor, this paper takes wheat straw machine pulping black liquor as the research object, using calcium hydroxide, resin, activated carbon and laccase to purify the black liquor, while removing lignin and other impurities, as far as possible to reduce the loss of xylose. Then, xylanase was used for hydrolysis and directed degradation of black liquor to produce xylo-oligosaccharide, in order to provide a certain theoretical basis and technical support for the high-value utilization of black liquor.

Firstly, calcium hydroxide combined with ion exchange resin was used to treat wheat straw machine pulp black liquor to remove impurities such as lignin and furfural. The effects of calcium hydroxide and resin dosage on each component of black liquor were investigated. The results showed that calcium hydroxide and resin treatment could effectively remove impurities such as lignin, furfural and 5-HMF in black liquor. The optimal dosage of calcium hydroxide was 0.6 wt%, and the removal rates of lignin, furfural and 5-HMF in black liquor were 27.53%, 40.23% and 31.56%, respectively, and the loss rate of xylose was 7.26%. On the basis of D301 resin treatment, 67.85% of the lignin was removed.

Then laccase combined with xylanase was used to treat the purified black liquor to improve the removal rate of lignin and prepare xylo-oligosaccharide by enzymolysis. Finally, the black liquor was further purified by activated carbon adsorption. When the laccase dosage was 2 U/g, the xylanase dosage was 2 U/g, the temperature was 50℃, the pH5.5 and the time was 4 h, the lignin removal rate was increased by 19.62%, and the xylooligosaccharide content was increased by 52.25%. The optimal condition was 0.4wt % activated carbon, and the lignin removal rate was 20.07% and the loss rate of xylose was 6.45%.

Keywords: Wheat straw machine pulp black liquor; Carbohydrate substance; Biological enzymes; Separation and purification; Xylo-oligosaccharide

TABLE OF CONTENTS

INTRODUCTION

China has the longest history of making pulp and paper from non-wood fibers in the world. As early as more than a thousand years ago, China used straw, wheatgrass and bamboo as raw materials for handmade paper. China uses the most varieties of non-wood fiber raw materials in the world, and has used no less than 30 varieties of non-wood fiber raw materials, including crop residues such as straw, wheatgrass, cotton stalk, corn stalk, bagasse, etc. These agricultural wastes are typical renewable fiber material resources [1]. Black liquor of pulping and papermaking is the waste water produced in the process of chemical pulping cooking or chemical mechanical pulping chemical pretreatment, and is the main pollution source of pulping and papermaking industry [2]. However, the solid black liquor contains a large number of organic substances such as hemicellulose, cellulose and lignin. From the perspective of resources, pulping black liquor is also a very unique biomass resource. It is of great significance to separate and extract sugars and aromatic substances from it for the production of functional materials, chemicals and fuels, which is the strategy of China's green, low-carbon and high-quality development

in this paper, using wheat straw machine pulp black liquor as raw material, firstly design a purification process of xylose/xylose in black liquor, selectively separate the impurities such as lignin and furfural in black liquor, and then enzymolize the purified black liquor to prepare and extract xylose oligosaccharides.

The tasks of the study The sugars are separated from the black liquor

The gaol of the study The sugars are separated from the black liquor

The relevance of the topic is black liquor

The purpose of the study is the Isolated sugar

The objectives of the study black liquor

The subject of the study black liquor

Research methods filtration

The scientific novelty enzyme treatment, Black liquor treatment

The practical significance of the results obtained is xylooligosaccharide

Approbation. (Appendix).

CHAPTER 1

LITRATURE REVVIEW

1.1 Status of paper black liquor pollution

In recent years, the rapid development of papermaking industry has made an important contribution to the growth of China's national economy, but it has also brought serious environmental problems, especially the pollution of water environment. In the process of papermaking, a large number of wastewater and waste pulp are discharged into the water body, containing pollutants such as organic matter and heavy metals[4], which have a serious impact on aquatic organisms and ecosystems. The alkaline papermaking process produces a large amount of black liquor, which is a kind of organic wastewater that is extremely difficult to degrade and is the main source of pollution in the pulping and papermaking industry. In China, the pollution degree of papermaking black liquor ranks third[5] after petrochemical industry and metal smelting. Black liquor contains a large number of organic matter and inorganic matter, organic matter mainly includes pulping raw materials in the cooking process of dissolved matter and degradation products, such as cellulose, hemicellulose, lignin, starch, resin, pigment and organic acid, among which lignin accounts for more than 30% of the solid matter of black liquor, the largest contribution to the COD and chroma of black liquor. Black liquor COD is generally up to tens of thousands of mg/L, if the direct discharge of waste water, not only will cause serious pollution to the water body, and the black liquor caused by environmental problems and treatment costs also greatly limit the development of pulp and paper industry, agricultural production and people's health have a serious infringement[6].

1.2 Status of paper black liquor treatment

The current black liquor treatment technology mainly includes alkali recovery, acid evolution, flocculation precipitation, biological gas production and so on. Although these methods have achieved better comprehensive utilization and pollution control effect, there are still some shortcomings [7] For example, in wheatgrass pulping black liquor, due to the high content of silicon and oligosaccharides in hemicellulose degradation products, the viscosity of the black

liquor is large, the solid calorific value and the isothermal expansion volume are low, which makes the alkali recovery of wheatgrass black liquor face the problems[8]. of low extraction rate of black liquor and high evaporation steam consumption. From the perspective of ecological engineering, the recycling project of straw pulp black liquor is to burn black liquor to indirectly obtain the cooking alkali required by the paper mill, which not only leads to high-cost alkali recovery, but also torch natural organic resources such as lignin and oligosaccharides with sustainable utilization and high added value to nothing, resulting in a serious waste[9]. of biomass resources in the black liquor.

The layered multistage comprehensive utilization of black liquor in papermaking is an innovative comprehensive treatment technology. It achieves the goal of pollution control by recycling resources, transforming the single utilization of raw materials into the comprehensive utilization technology of renewable biological resources such as cellulose, lignin and glycans. This method has certain economic benefits, social benefits and environmental benefits. Through continuous research and practice, people have found a variety of effective recycling ways of black liquid alkali and lignin extraction, and achieved good comprehensive utilization and pollution control effects.[10].

In the process of using sugar in black liquor to produce high value-added products, lignin and furfural in black liquor will hinder its utilization. For example, when using xylan to produce functional xylo-oligosaccharide products, lignin, furfural, etc., reduce the biological activity of xylanase, making the rate low. Therefore, it is necessary to separate and purify the black liquor first, and reduce the loss of sugar substances as much as possible while removing impurities. In recent years, researchers have proposed a number of methods to remove lignin and furfural from black liquor, including physical methods, chemical methods and biological methods[11]..

1.2.1 Physical law

The main physical methods for treating black liquor are activated carbon method, resin method [12]. and membrane method. As a porous material with a huge specific surface area, activated carbon has strong adsorption capacity, can

effectively adsorb organic substances and colored substances in black liquor, widely used in a variety of fields, especially in the field of water treatment, and low price, wide range of sources, non-toxic, recyclable, renewable, is an excellent adsorption material[13]. Resin is a kind of polymer compound, according to the active group can be divided into cationic and anion exchange resin, according to the pore size can be divided into gel type and large pore type ion exchange resin[14] Resin treatment of black liquor refers to the method of using ion exchange resin to remove lignin and other substances in black liquor through cation and ion exchange or adsorption, so as to achieve the separation of target substances. The membrane method uses specific membrane materials, such as ultrafiltration membrane, nanofiltration membrane or reverse osmosis membrane, to separate organic substances, colored substances and other impurities in the black liquor from the black liquor through the separation effect of the membrane, so as to achieve the treatment and purification of the black liquor. Membrane method, as an efficient black liquor treatment method, has a remarkable effect, but because of the high cost of membrane, it may bear a heavy burden[15]for small and medium-sized enterprises.

1.2.2 Chemical method

Chemical treatment mainly includes flocculation method and alkali method. [16] The flocculation method is mainly by adding polymer flocculants (such as polyaluminum chloride, kaolin, etc.) to the black liquor, forming flocculants with the lignin groups in the black liquor, and then filtering and separating, so as to achieve the separation and purification of the components in the black liquor. Alkali method, also known as overneutralization method, usually uses calcium oxide or calcium hydroxide to neutralize acid degrading substances in black liquor, and has a better removal effect on furfural. Although the flocculation method and the alkali method can effectively remove impurities such as lignin in the black liquor, it will also cause a high degree of sugar loss, so it is necessary to control the amount of addition, and reduce the loss[17] of sugar substances while removing impurities.

1.2.3 Biological law

Biological treatment of black liquor means that under the action of biological enzymes, the complex chemical chain of pollutants in the black liquor is

decomposed into small molecular organic matter, water, carbon dioxide and other inorganic[18] matter, and then the organic matter in the polluted substance is decomposed into free groups, and then through chemical polymerization reaction to generate polymer compound precipitate that can be directly filtered and removed, so as to achieve harmless treatment of black liquor. In the process of treating purified black liquor, the biological enzymes that can be used mainly include lignin peroxidase, manganese peroxidase and laccase, among which laccase[19] is the most commonly used.

Laccase is a copper containing polyphenol oxidase, which can oxidize phenol terminal groups to form relatively stable free radicals, and then couple the free radicals to form covalent bonds, so that small molecular lignin can be condensed to form large molecular lignin, and then removed[20]. Laccase has a wide range of sources, and more than 100 kinds of laccase have been isolated from nature. And laccase treatment reaction conditions are relatively mild, low energy consumption, is an environmentally friendly treatment. Laccase polymerization to promote the separation of lignin in black liquor can not only improve the separation efficiency of each component in black liquor, but also contribute to the subsequent processing and utilization[21] of carbohydrates.

1.3 Preparation of xylo-oligosaccharides

Xylo-oligosaccharide is a kind of functional oligosaccharide which is connected by the xylose molecule through the β-1,4 glucoside bond. It has good physical and chemical properties, and has the effects[22] of lowering cholesterol, preventing hypertension and arteriosclerosis, anti-aging and inhibiting tumor. It is widely used in food, medicine, chemical industry and other fields. For example, it can be used as food additives, drug sustained-release agents, and raw materials for bioenergy production[23].

1.3.1 Acid hydrolysis method

Acid hydrolysis method refers to the hydrolysis of xylan in raw material under the action of acid at high temperature to obtain xy[24]lo-oligosaccharides with low polymerization degree, which has the advantages of high yield and rapid reaction. However, acid hydrolysis is to randomly cut the glycosidic bond of xylan, resulting

in a high yield of xylose, and high temperature will also lead to the generation of furfural and other by-products. The black liquor after acid hydrolysis also needs a large amount of alkali to neutralize before it can be discharged, [25] and the cost is high.

1.3.2 Enzymatic hydrolysis method

The preparation of xylo-oligosaccharides by enzymatic hydrolysis is mainly based on the selective cutting of the glycosidic bonds between the xy[26]lo-oligosaccharides by endo-xylanase, so as to break them and degrade them into xylo-oligosaccharides. Xylan endonuclease is an enzyme that randomly acts on the β-1, 4-glucoside bond in xylan chain to produce short-chain xylan, xylo-oligosaccharide and xy[27]lose. Xylanase is widely used in the fields of pulp and paper industry and biomass refining for the deconstruction and biotransformation of hemicellulose components. Due to the high selectivity of xylanase, the xylose oligosaccharide yield is higher and the xylose yield is lower[28]

Enzymatic method is an environmentally friendly preparation process with strong specificity, mild reaction conditions, green environmental protection, and high yield and purity of xylo-oligosaccharide[29].

1.4 Main contents of the paper

In this paper, a separation and purification process of oligoxylan in wheat straw machine pulp black liquor was designed. Firstly, impurities such as lignin and furfural in the black liquor were selectively separated, and then the purified black liquor was enzymolized to prepare xylo-oligosaccharides. The main research contents are as follows:

(1) Calcium hydroxide combined with ion exchange resin was used to treat wheat straw machine pulp black liquor to remove impurities such as lignin and furfural in the black liquor. The effects of calcium hydroxide and resin dosage on each component of black liquor were investigated.

(2) laccase combined with xylanase was used to treat the purified black liquor, polymerize small molecule lignin, improve the removal rate of lignin, and prepare xylo-oligosaccharide by enzymatic hydrolysis. Finally, the black liquor was further purified by activated carbon adsorption, and the influence of different factors on the

content of xylooligosaccharide during laccase and xylanase treatment was discussed.

Conclusions to chapter 1

This paper briefly analyzes the research progress at home and abroad and the relevant methods and discusses the experimental process

CHAPTER 2

OBJECT, PURPOSE, AND METHODS OF THE STUDY

2.1 Preparation for experiment

2.1.1 Experimental materials

The wheat straw chemical mechanical pulping black liquor used in this experiment was provided by Shandong Century Sunshine Paper Group Co., LTD.

2.1.2 Experimental drugs

Experimental drugs include calcium hydroxide, laccase and xylanase, etc. The detailed specifications are shown in Table 2.1.

Table 2.1 Main experimental reagents

Experimental reagents	Specifications	Manufacturer
Calcium hydroxide	Analytically pure	Tianjin Dingsheng Xin Chemical Co., LTD
D301 Resin	Analytically pure	Zhengzhou Hexheng New Material Technology Co., LTD
Laccase	0.5 U/mg	Shanghai McLean Biochemical Technology Co., LTD
xylanase	$5\times10_4$ U/ml	Shandong Longcott Enzyme Preparation Co., LTD
Potassium bromide	Spectral pure	Tianjin Kemiou Reagent Co., LTD
Activated Carbon	Phosphoric acid activated, wood, over 200 mesh	Guangdong Haiyan Activated Carbon Co., LTD

2.1.3 Experimental instruments

Experimental instruments include ion chromatograph, high performance liquid chromatograph, ultraviolet spectrophotometer, Fourier infrared spectroscopy, oscillation

incubator, etc. See Table 2.2 for detailed specifications.

Table 2.2 Main experimental instruments

Names of instruments	Model number	Manufacturer
Ion chromatograph	ICS-5000+	Thermo Fisher Technologies USA
High performance liquid chromatograph	1260 Infinity II	Agilent Technologies Inc
Ultraviolet spectrophotometer	SHIMADZU UV-2600	Spectral Mass Analysis Detection Technology Co., LTD
Fourier infrared spectroscopy	Bruker, Germany	
Oscillating incubator	ZQZY-78AE	Shanghai Zhichu Instrument Co., LTD
Magnetic Stirrer	DF-101S	Gongyi Yuhua Instrument Co., LTD
Freeze dryer	LGJ-12N	Beijing Yaxing Yike Technology Development Co., LTD
Centrifugal Separator	H2050R	Hunan Xiangyi Laboratory Instrument Development Co., LTD

2.2 Experimental Treatment

2.2.1 Calcium hydroxide treatment

At room temperature, add 30 g each of the original black liquor to 50 mL beakers and add 0.2 wt%, 0.6 wt%, 0.8 wt%, 1.0 wt%, 1.5 wt%, 3.0 wt% calcium hydroxide powder, respectively. Then, the beakers were placed on a magnetic stirrer, stirred at 250 rpm for 90 min and moved to a centrifuge, centrifuged at 10000 rpm for 15 min. The centrifuged solids were collected, and the supernatant was collected and stored and recorded as primary treatment liquid (L1).

2.2.2. Resin treatment

2.2.2.1 Resin pretreatment

Industrial grade ion exchange resin contains a small amount of oligomers, organic solvents and harmful metal ions. These impurities may eluate together with elution components during the separation experiment, which will pollute the eluent and pollute the liquid chromatographic column. Therefore, pretreatment should be carried out before use to remove the impurities on the surface and inside of the resin. The formation of precipitate should be avoided during pretreatment.Pretreatment of cationic resin: inject water into the chromatographic column, load the resin into the chromatographic column, keep the liquid level higher than the resin level, soak for one day and night. Use 4% hydrochloric acid, twice the volume of the resin, to pass through the resin layer at a certain flow rate, and soak for 2 hours. Rinse the resin layer with deionized water at a certain flow rate until the effluent pH 4 to 5. Use 2% sodium hydroxide, 4 times the volume of the resin, to pass through the resin layer at a certain speed and soak for 2 hours. Rinse the resin layer with deionized water at a certain flow rate until the effluent pH 8 to 10. Pretreatment of anionic resin: water is injected into the chromatographic column, the resin is loaded into the chromatographic column, the liquid level is kept higher than the resin level, and soaked for one day and night. Use 2% sodium hydroxide, 4 times the volume of the resin, to pass through the resin layer at a certain speed, and soak for 2 hours. Rinse the resin layer with deionized water at a certain flow rate until the effluent pH 8 to 10. Use 4% hydrochloric acid, twice the volume of the resin, to pass through the resin layer at a certain flow rate and soak for 2 hours. Rinse the resin layer with deionized water at a certain flow rate until the effluent pH 4 to 5.

2.2.2.2 Resin screening

The same amount of the primary treatment liquid (L1) was accurately weighed and put into three triangle bottles respectively, and 5wt % of the pre-treated D301, D392 and D20 resin were added respectively. The resin was shaken in a constant temperature shaking table at 40℃for 5 h, and centrifuged after the reaction, and the content changes of each component in the supernatant were measured.

2.3 Optimization of D301 resin treatment conditions

Add 30 mL of primary treatment solution (L1) to the 50 mL triangular bottle, and add 2wt%, 4 wt%, 5 wt%, 8 wt% and 10 wt% D301 resin respectively. The triangular bottle is placed in a constant temperature shaker and shaken at 200 rpm. To explore the effects of treatment time (1, 2, 5, 8, 10 h), treatment temperature (30, 40, 50, 60, 80℃) and pH value (2, 4, 6, 8, 10)on the lignin and glycan content in L1. After the reaction, centrifuge was centrifuged at 8000rpm for 10 min, and the supernatant was collected and stored and recorded as the secondary treatment solution (L2).

2.4. Laccase treatment

Add 20 g of secondary treatment solution (L2) to each 50 mL triangular bottle, adjust the pH value to 4.5, add 0.5 U/g, 1 U/g, 2 U/g, 5 U/g laccase solution, place the triangular bottle in a constant temperature shaking table, shake at 45℃ at 200 rpm for treatment. To explore the effect of treatment time (1, 2, 3, 5 h) on the lignin content in L2. After the reaction, L2 was laced in a water bath at 100℃ for 15 min to inactivate laccase, and then centrifuge at 8000rpm for 10 min. The supernatant was collected and stored and recorded as laccase treatment solution.

2.5. Xylanase treatment

Xylanase activity was determined in accordance with GB/T 23874-2009. Unit of enzyme activity: The amount of xylanase required to release 1 mol of reducing sugar from xylanan solution with a concentration of 5 mg/mL per minute at 37℃ and pH value of 5.5. Add 20 g of secondary treatment solution (L2) into each 50 mL triangular bottle, adjust the pH value to 5.5, and add 1 U/g, 2 U/g, 3 U/g, 5 U/g, 10 U/g of xylanase solution, respectively.The triangular bottle is placed in a constant temperature shaking table, and shaken at 55℃ at200 rpm. To explore the effect of treatment time (1, 2, 4, 8, 12h) on the xylooligosacchar 聚合度2~6ide content in L2. After the reaction, L2 was placed in a water bath at 100℃ for 15min to inactivate xylanase, and then centrifuge at 8000 rpm for 10 min. The supernatantwas collected and stored and recorded as xylanase treatment solution.

2.6. Laccase combined with xylanase treatment

Add 20 g secondary treatment solution (L2) into each 50 mL triangular bottle, add 2 U/g laccase solution and 2 U/g xylanase solution respectively, place the triangular bottle in a constant temperature shaking table, shake at 200 rpm for 4 h. To explore the effects of pH value (3.5, 4, 4.5, 5.5, 7) and treatment temperature (35, 45, 50, 55, 60℃) on the content of lignin and xy 聚合度 2~6lo-oligosaccharide in L2. After the reaction, L2 was placed in a water bath at 100℃ for 15 min to inactivate the xylanase, and then centrifuged at 8000 rpm for 10 min. The supernatant was collected and stored and recorded as the tertiary treatment solution (L3).

2.7. Activated carbon treatment

At room temperature, add 30 g of each tertiary treatment solution to 50 mL beakers, and add 0.2 wt%, 0.4 wt%, 0.6 wt%, 1.0 wt%, 1.5 wt%, 3.0 wt% activated carbon powder, respectively. Then the beakers were placed on a magnetic stirrer, stirred at 300 rpm for 15 min,and moved to the centrifuge, centrifuged at 10000 rpm for 10 min, and recorded as the fourstage treatment liquid (L4).

2.7.1 Determination of solid matter content in black liquor

Weigh the original black liquor of a certain quality, put it into a glass petri dish that hasbeen dried in an oven at 105℃ to a constant weight, and then put it in an oven at 105℃ to a constant weight. After taking it out, put it in a dryer to cool and then weigh it. As shown in formula 2.1

$$\text{Solid content (mg/L)} = m_1 - m_2 \quad \text{... (2.1)}$$

In the formula m_1 is the quality of glass petri dish and absolute dry black liquor, mg; m_2 is the mass of glass petri dish, mg.

2.7.2 Determination of ash content in black liquor

Weigh a certain quality of the original black liquor, put it into a porcelain crucible that has been burned to constant weight, and then put it in a Muffle furnace, and burn it to constant weight at 600℃. As shown in formula 2.2

$$\text{Ash content (\%)} = (M - {}_2m_1) / m_3 \quad \text{.... (2.2)}$$

Where m_1 is the quality of porcelain crucible, g; m2 is the quality of porcelain crucible and ash, g; m3 for the mass of black liquor, g.

2.7.3 Determination of main sugar components in black liquor

Accurately measure 5 mL of the original black liquor into a pressure resistant bottle, add 174 μL of concentrated sulfuric acid with mass fraction of 72%, mix well, seal the pressure resistant bottle into an oil bath, and react at 121℃ for 60 min. All hemicellulose oligosaccharides could be decomposed into monosaccharides by acidolysis. The supernatant was diluted at an appropriate ratio. ICS-5000 ion chromatography was performed with CarboPacPA20 (3 mm×150 mm) as the analytical column and CarboPacPA20 (3 mm×30 mm) as the protection column. EC detector (Au electrode for working electrode, Ag/AgCl electrode for reference electrode); The sample size was 25 μL; Column temperature was 30℃; The mobile phase was 250 mm/L sodium hydroxide and distilled water gradient leaching, and the

flow rate was 0.4 mL/min. The content of monosaccharides and total sugars in the supernatant was determined. The concentration of monosaccharides before and after acid hydrolysis of black liquor can be measured by the above method, and the content of oligosaccharides can be calculated by the increase of monosaccharides after acid hydrolysis.

2.7.4 Determination of acetic acid, furfural and 5-HMF content in black liquor

1 mL of the original black liquor was diluted 30 times by HPLC-1260 Infinity II high performance liquid chromatography with Bio-Rad Aminex HPX-87H (300× 7.8mm) as the analytical column. The detection wavelength was 210 nm; The sample size was 25 μL; Column temperature was 50℃; The mobile phase was 5 mmol/L sulfuric acid and the flow rate was 0.6 mL/min. The contents of acetic acid, furfural and 5-HMF in black liquor were determined.

2.7.5 Quantitative determination of lignin content

The determination of lignin content includes the determination of acid insoluble lignin and acid soluble lignin. The acid insoluble lignin was determined by gravimetry. 50 mL of the sample was accurately measured and added to 1740μL 72% concentrated sulfuric acid solution for acid hydrolysis at 121℃ for 60 min. Then centrifuge, freeze dry the obtained precipitated part, weigh its mass m, calculate the

concentration of acid insoluble lignin according to the volume and mass. The acid-soluble lignin is detected by ultraviolet spectrophotometer, the detection wavelength is 205 nm, the filtrate obtained after the acid-hydrolysis sample is filtered into the colorimetric dish, and the absorption value is measured by ultraviolet spectrophotometer with 3% sulfuric acid as the reference solution. If the absorbance value is too large, the 3% sulfuric acid solution is diluted in the volumetric bottle, so that the final absorbance is between 0.2-0.7.The method of calculating the acid soluble lignin content (B) in the sample is: B ()g/L =A110× D

Where A is the absorbance value of the diluted supernatant sample at 205 nm; D is the dilution multiple of the supernatant sample; 110 is the absorption coefficient, L/(g·cm-1).7.6 Infrared spectrum analysis.Take 1 mg of centrifugally dried solid sample, grind with 100 mg of dried KBr with agate mortar and press tablet. FT-IR testing of lignin is performed using Bruker seriesspectrophotometers, scanning the lignin sample in the wave number range of 4000 and 400 cm-1 at a resolution of 4 cm-1

Conclusions to chapter 2

The experimental instruments, experimental methods, experimental means and the separation and purification methods of sugars were analyzed.

CHAPTER 3
EXPERIMENTAL PART

3.1 Analysis of chemical components of wheat straw machine pulp black liquor

As can be seen from Table 3.1 and Figure 3.1, the content of monosaccharides, glycans and other components in this batch of wheat straw chemical mechanical pulping black liquor is relatively low, especially the content of monosaccharides is extremely low, and the total content is only 17 mg/L. The main glycans in the black liquor were polyarabinose, polygalactose, polyglucose, xylose and polymannose, among which xylose was the main one, accounting for 51.8% of the total glycans; Polymannose content was the least, accounting for 0.8% of the total glycan. Other products mainly included acid-soluble lignin, acetic acid, furfural and 5-HMF, in which acid-soluble lignin and acetic acid were the main products. The total soluble solid content in black liquor was 31590 mg/L, and the proportion of ash was 1.04%. The chemical composition of wheat straw chemical mechanical pulping black liquor used in this experiment is similar to the chemical composition of black liquor reported by predecessors, so the research method and experimental results of this experiment can provide reference for other related research.

Table 3.1 Component analysis of wheatgrass chemical-mechanical pulping black liquor

Monosaccharides (mg/L)	Glycans (g/L)	Other substances (g/L)					
Arabinose	5	Arabinose	0.912	Acid soluble lignin	2.204	Solid matter	31590 mg/L
Galactose	2	Galactose	0.421	Acetic acid	3.353		
Glucose	2	Glucose	0.304	Furfural	0.156	Ash	1.04%

Xylose	8	Xylose	1.788	5-HMF	0.404		
mannose	0	Mannose	0.028				

Total content	17	Total content	3.453	Total content	6.117

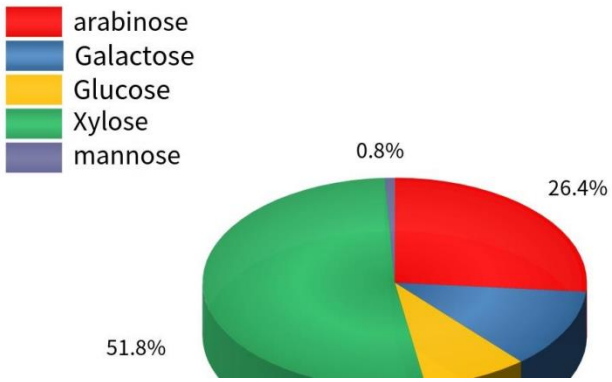
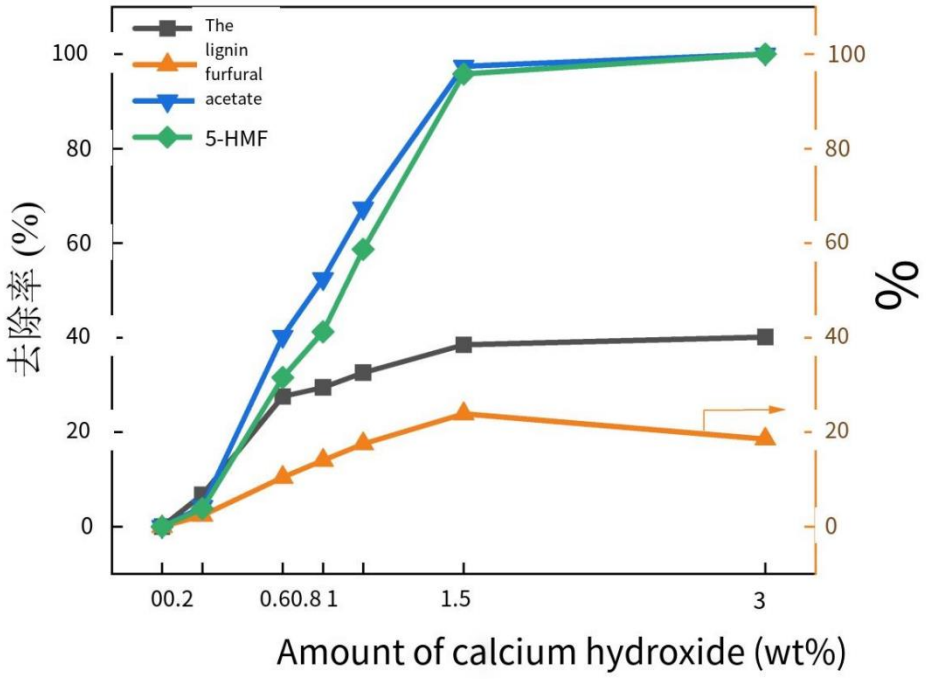


Figure 3.1 Composition of carbohydrate substance (glycan) in black liquor

3.2 Effects of calcium hydroxide treatment on components in black liquor

As can be seen from Fig. 3.2, with the increasing of calcium hydroxide dosage, the lignin removal rate in black liquor gradually increased, and when the calcium hydroxide dosage increased to 1.5wt %, the lignin removal rate was 38.46%. The reasons for lignin removal can be attributed to two points: the free Ca^{2+} combined with the ionic groups of dissolved lignin to form a complex precipitation; Undissolved calcium hydroxide particles can adsorb soluble lignin to some extent. With the increase of calcium hydroxide dosage, the lignin removal rate did not change significantly, indicating that calcium hydroxide could only remove part of lignin in black liquor through complexation and adsorption. The removal rates of furfural and 5-HMF in black liquor increased rapidly with the increase of calcium hydroxide dosage. When the dosage of calcium hydroxide was 1.0 wt %, the removal rates of furfural and 5-HMF were 67.35% and 58.66%, respectively. When the calcium hydroxide dosage was 1.5wt %, it was almost completely removed, because furfural and 5-HMF could degrade and self-condensation [29] under alkaline conditions.



With the increasing of calcium hydroxide dosage, the acetic acid content in black liquor showed a trend of gradual increase. This is because under the alkaline condition, the acetyl group on the hemicellulose side chain falls off to form acetic acid, and the carbohydrate compounds also degrade to form acetic acid under the alkaline condition, thus increasing[30] the acetic acid content in the black liquor. The shedding of the acetyl group makes the main chainof the xylose more regular, which is conducive to improving the accessibility of the subsequentenzymatic hydrolysis reaction process. The acetic acid formed can also be recovered byextraction to meet the needs [31] of industrial production. When the amount of calcium hydroxideincreased from 0 to 1.5wt %, the acetic acid content in black liquor increased by 23.86%. When the amount of calcium hydroxide was further increased to 3.0wt %, the acetic acid content decreased slightly, which may be caused by the adsorption of acetic acid by undissolved calcium hydroxide particles.

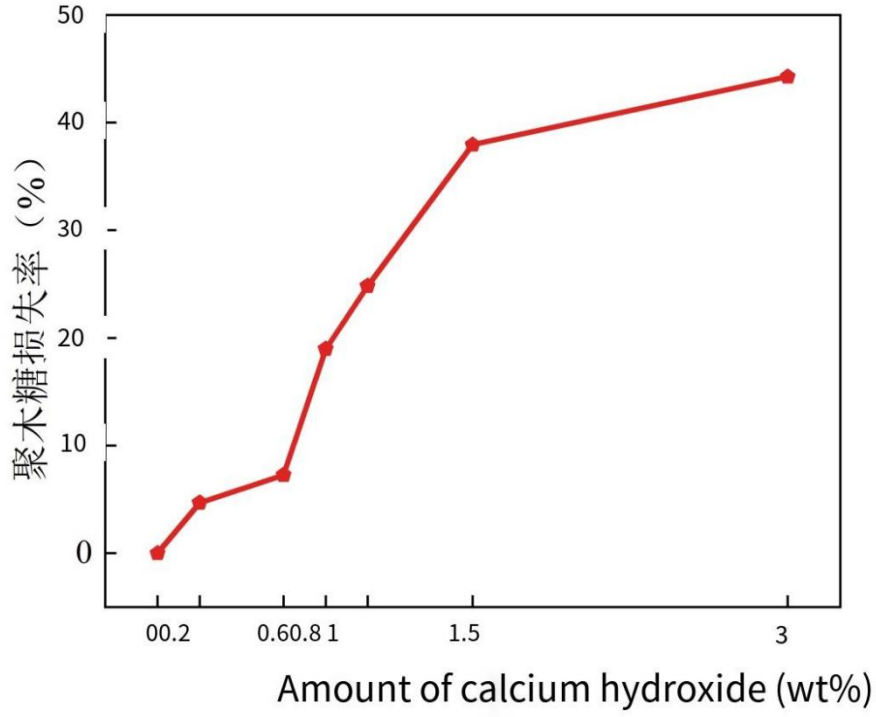


FIG. 3.3 Effect of calcium hydroxide dosage on loss rate of xylose in black liquor

As can be seen from FIG. 3.3, the loss rate of xylose in black liquor showed a gradual increasing trend with the increase of calcium hydroxide dosage. This is mainly due to the degradation reaction of carbohydrate substances in alkaline environment, and the reactionbecomes more intense[32] with the increase of calcium hydroxide dosage. At the same time, a small amount of carbohydrates are absorbed by undissolved calcium hydroxide particles,resulting in a large loss of xylose. For example, when the amount of calcium hydroxideincreased from 0 to 3.0wt %, the content of xylose in black liquor decreased from 1.788 g/L to 0.997 g/L, and the loss rate was 44.24%.

The inhibition of lignin, furfural and 5-HMF in black liquor can be effectively removed by the flocculation and adsorption of calcium hydroxide, so as to reduce the inhibition of the activity of subsequent enzymatic hydrolysis. However, higher calcium hydroxide dosage would lead to a large amount of loss of xylose. The removal rate of impurities such as lignin and loss rate of xylose were taken as evaluation indexes, and 0.6wt % of calcium hydroxide was selected as the optimal

condition. The removal rates of lignin, furfural and 5-HMF in black liquor were 27.53%, 40.23% and 31.56%, respectively, and the loss rate of xylose was 7.26%。

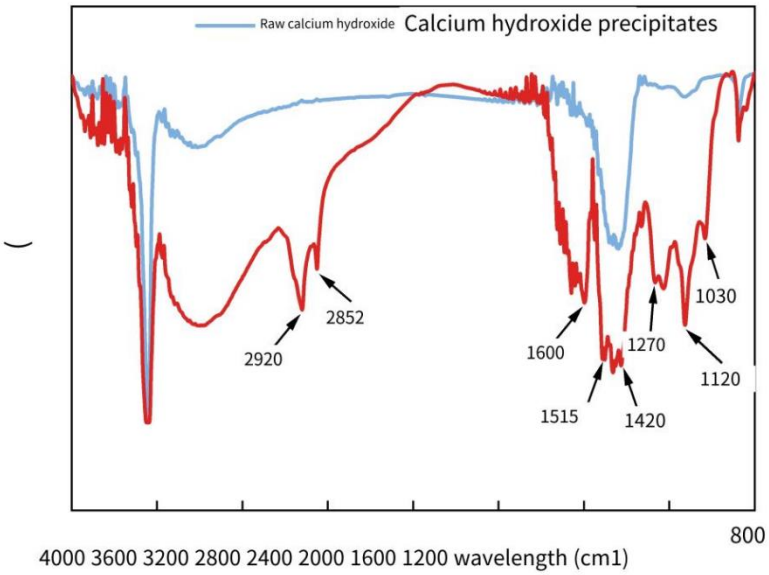


Fig. 3.4 Infrared spectrum of calcium hydroxide before and after black liquor treatment

Fig. 3.4 shows the infrared spectrum of calcium hydroxide before and after black liquor treatment. It can be clearly seen that, compared with the original calcium hydroxide, a new characteristic peak appeared on the infrared spectrum after treating the black liquor. C-H stretching vibration absorption peaks of methyl and methylene were observed at 2920 cm-1 and 2852 cm-1. The absorption peaks at 1600, 1515, 1460 and 1420 cm-1 were associated with C-H deformation[33] associated [34]with binding aromatic ring vibration and aromatic skeleton vibration. The absorption peak of the guaiac-based unit is 1270 cm- 1, and the stretching of CC binding C = O occurs at 1220 cm -1. The characteristic absorption peak at 1120 cm-1 was caused by the C-H vibration of the lilac - based benzene ring. The in-plane deformation vibration[35] of aromatic C-H was detected at 1030 cm- 1. The above results showed that some lignin and lignin derivatives were adsorbed by calcium hydroxide during the adsorption of black.

3.3 Influence of resin treatment on components in black liquor

3.3.1 Screening of resin

Different types of resins differ in their ability to adsorb the components in black liquor.Table 3.2 shows the effect of black liquor treated by 3 different types of resins under the same conditions. It can be seen that with the same amount added, the lignin removal rate of D392resin is the highest, reaching 70.15%, the xylose loss rate of D201 resin is the highest, reaching20.16%, and the lignin removal rate of D301 resin is 64.12%, which is not much different from that of D392 resin, and the xylose loss rate is low. Overall consideration, in the follow-up treatment process, the D301 resin is selected to treat the calcium hydroxide treatment solution(L1)

Table 3.2 Treatment effect of 3 kinds of resins

	Lignin Removal rate /%	Furfural Removal rate /%	5-HMF Removal rate /%	Polyxylose Loss rate /%
D301	64.12	75.46	84.21	7.89
D392	70.15	72.54	83.11	13.81
D201	55.86	35.87	45.68	20.16

3.3.2 Optimization of resin treatment conditions

In the experiment, the lignin removal rate and xylose loss rate were evaluated, and the influence of different temperatures on the resin treatment effect was investigated when the resin dosage was 5 wt%, the initial pH7, and the treatment time was 5 h. Temperature is one of the factors affecting the treatment effect of resin. As can be seen from FIG. 3.5 (A), temperature has a great influence on the treatment effect of resin. As the temperature increases, the viscosity of the black liquor system decreases, thus speeding up the diffusion of lignin molecules, which is conducive to the adsorption of lignin molecules by resin and gradually increasing the removal rate of lignin. When the temperature rises to 60℃, the lignin removal rate decreases on the other hand, possibly because the adsorbed molecules are desorbed because the temperature is too high. The loss rate of xylose increased with

the increase of temperature. The optimum temperature for treating L1 with resin D301 at 50℃ was considered comprehensively.

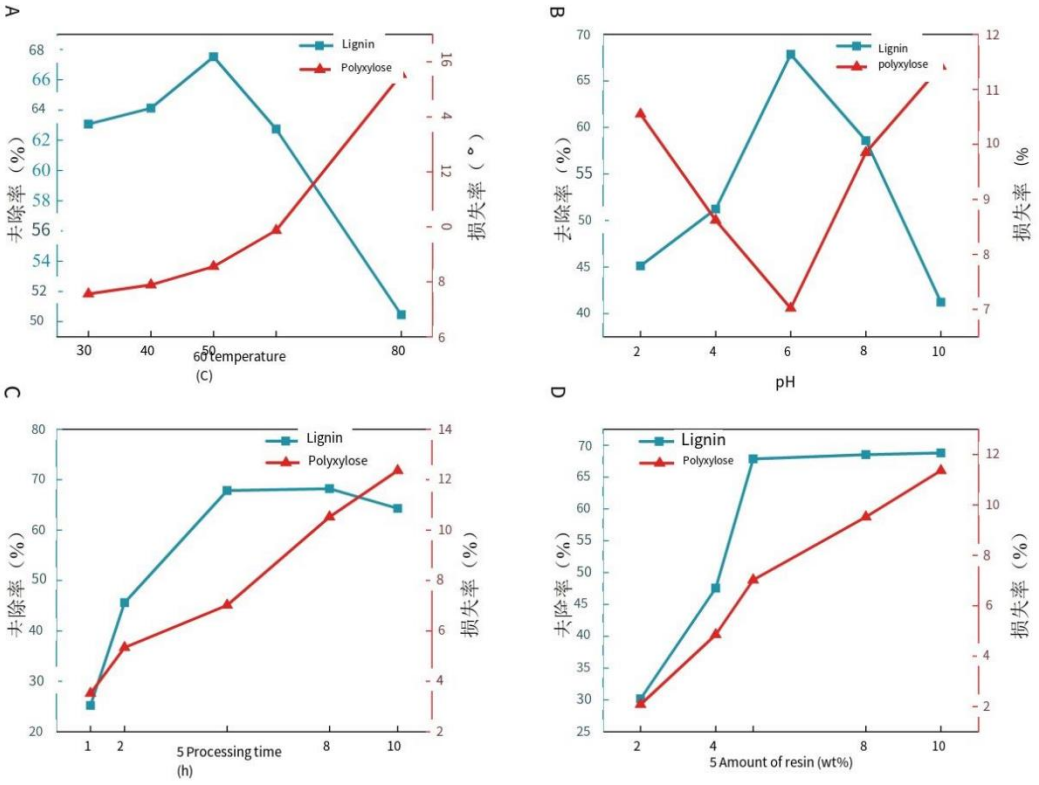


Fig. 3.5 Influence of different treatment factors on resin treatment effect

Processing temperature.

(2) Initial pH

In the experiment, the lignin removal rate and xylose loss rate were used as evaluation.indexes. When the resin dosage was 5 wt%, the treatment time was 5 h, and the treatment temperature was 50℃, the influence of different initial pH on the resin treatment effect was explored respectively.

As can be seen from FIG. 3.5 (B), either too low or too high pH value is detrimental to the removal of lignin and the retention of xylose. In a pH value of 5-8, the loss rate of xylose can be controlled at about 8%. When pH is 6, the lignin removal rate is the highest, reaching 67.85%, and the xylose loss rate is the lowest, 7.02%. The optimal initial pH for L1 treatment with pH6 was considered comprehensively.

Treatment time

In the experiment, the lignin removal rate and xylose loss rate were used as evaluation indexes, and the influence of different treatment time on the resin treatment effect was respectively explored when the resin addition amount was 5 wt%, the initial pH6 and the treatment temperature was 50℃

As can be seen from FIG. 3.5 (B), either too low or too high pH value is detrimental to the removal of lignin and the retention of xylose. In a pH value of 5-8, the loss rate of xylose can be controlled at about 8%. When pH is 6, the lignin removal rate is the highest, reaching 67.85%, and the xylose loss rate is the lowest, 7.02%. The optimal initial pH for L1 treatment with pH6 was considered comprehensively.

the amount of resin

In the experiment, the lignin removal rate and xylose loss rate were used as evaluation indexes. When the treatment time was 5 h, the initial pH6 and the treatment temperature was 50℃, the influence of different resin dosage on the resin treatment effect was explored respectively. As can be seen from FIG. 3.5 (D), with the gradual increase of the amount of resin, the lignin removal rate showed an upward trend. When the amount of resin was 0-5 wt%, the lignin removal rate increased rapidly, and the xylose rate also showed an upward trend with the increase of the amount of resin. After comprehensive consideration, 5 wt% was selected as the suitable amount for resin treatment of L1. In summary, D301 resin has a good treatment effect on L1, and the optimal treatment conditions are treatment temperature 50℃, initial pH6, resin dosage 5 wt%, treatment time 5 h. Under these conditions, the lignin removal rate is 67.85%, and the xylose rate is 7.02%.

3.4 Laccase treatment

As can be seen from Figure 3.6, when the dosage of laccase is 0.5 U/g, 1 U/g, 2 U/g, and the treatment time is less than 3 h, the laccase removal rate in the laccase treatment solution increases rapidly with the extension of the treatment time, and reaches the highest at 3 h. When the treatment time was further extended to 5 h, the lignin removal rate did not change significantly. At the treatment time of 3 h, when the laccase dosage was increased to 5 U/g, the lignin removal rate was decreased, which may be due to the two effects of laccase on lignin polymerization and

depolymerization. Increasing the laccase dosage would depolymerize part of the lignin after polymerization, so the lignin removal rate of lignin was decreased. After comprehensive consideration, the optimal laccase dosage was 2 U/g and the treatment time was 3 h.

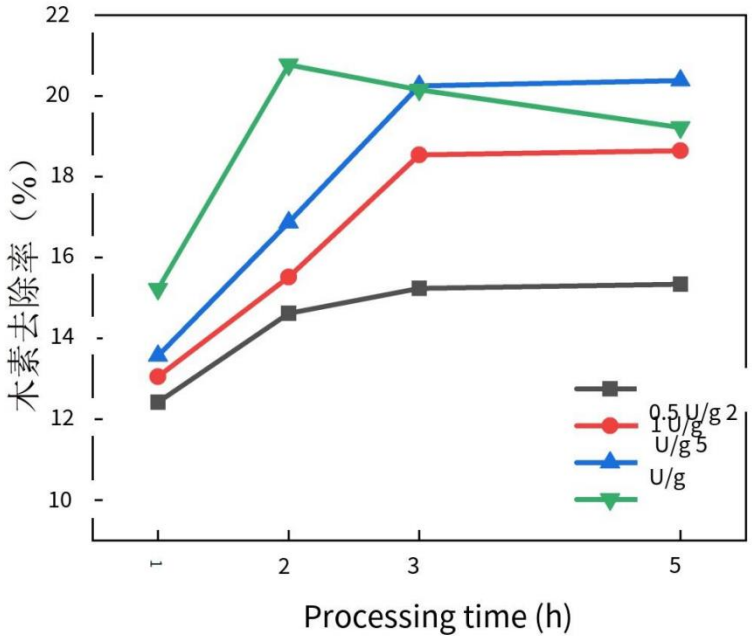


Figure 3.6 Effect of laccase treatment on lignin removal rate in black liquor

3.5. Xylanase treatment

As can be seen from Figure 3.7, when the dosage of xylanase is 1 U/g, 2 U/g and 3 U/g, with the extension of the treatment time, the xylo-oligosaccharide content in the xylanase treatment solution increases rapidly at first and then tends to be stable, and reaches a large value when the treatment time is 4 h. When the enzyme dosage was 5 U/g and 10 U/g, the xylooligosaccharide content in the xylanase treatment solution rapidly increased at first and then rapidly decreased with the extension of the treatment time, and the xylo-oligosaccharide content reached the maximum value at 2 h. The reason is that when the enzyme dosage is high, the number of enzyme molecules in the xylanase treatment solution is large, and the probability of the enzyme molecules binding with the reaction substrate increases. Therefore, the enzyme molecules can be fully combined with the reaction substrate in a short time, and the rate of xy-looligos accharide preparation by xylanase

hydrolysis is effectively improved. The higher the amount of xylanase, the remaining enzyme molecules will be more, will further cut the xy-looligosaccharide bond to form xylo-oligosaccharide, thereby reducing the content of xy-looligosaccharide. Therefore, the optimal xylanase

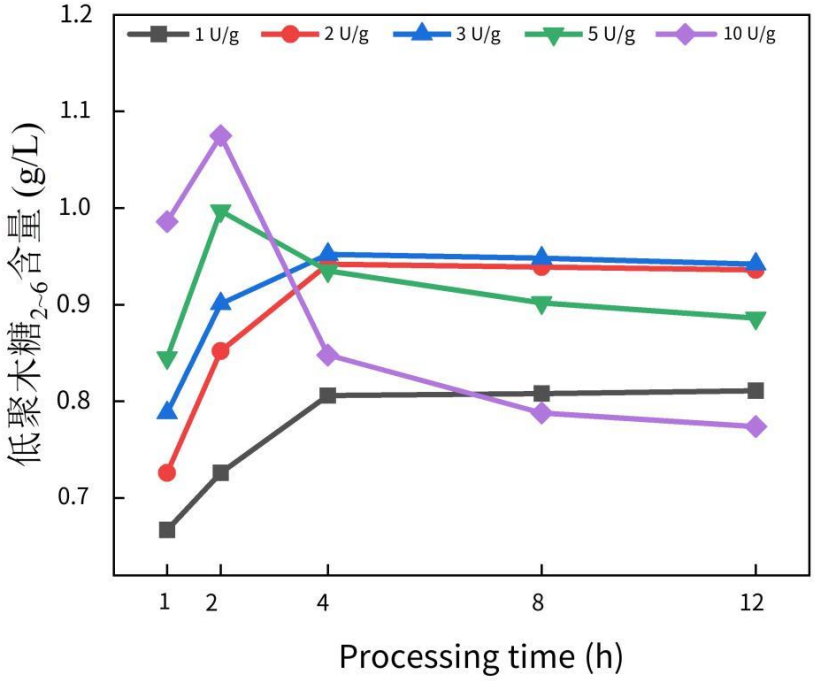


Figure 3.7 Effect of xylanase treatment on xylo-oligosaccharide content in black liquor dosage is 2 U/g and the treatment time is 4 h.

3.6. Laccase combined with xylanase treatment

As can be seen from FIG. 3.8 (A), with the gradual increase of pH value, both the lignin removal rate and xylo-oligosaccharide content in L2 showed a trend of first increasing and then decreasing. When pH value is 4.5, the highest lignin removal rate is 20.02%, and when pH continues to rise, the lignin removal rate decreases. When pH value is 5.5, the highest xylo-oligosaccharide content is 0.945 g/L, and the continuous increase of pH results in the decrease of xylo-oligosaccharide content. Overall consideration, the optimal pH value should be 5.5. As can be seen from Figure 3.8 (B), with the increase of treatment temperature, the lignin removal rate and xylo-oligosaccharide content also showed a trend of first increasing and then decreasing, and reached the maximum value at 45℃ and 50℃,

respectively, with the lignin removal rate and xylo-oligosaccharide content being 20.18% and 0.947 g/L, respectively. Taking into consideration, the optimal treatment temperature should be 50℃. Laccase-combined xylanase treatment can effectively remove lignin from black liquor and significantly increase the content of xylo-oligosaccharide. After laccase-combined xylanase treatment, the removal rate of lignin is increased by 19.62%, and the content of xylo-oligosaccharide is increased by 52.25%. Therefore, xylanase treatment is an effective method to prepare xylo-oligosaccharide in black liquor.

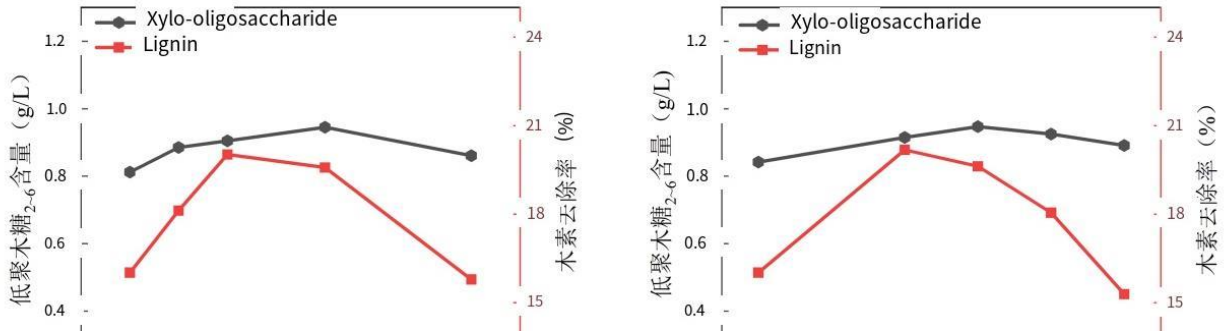


Figure 3.8 Effects of pH value and treatment temperature on lignin removal rate and xylo-oligosaccharide content in L2

3.7. Activated carbon treatment

After laccase combined with xylanase, some lignin still existed in the black liquor. As a kind of porous material with huge specific surface area, activated carbon has good physical and chemical adsorption properties, and is widely used in many fields, especially in the field of water treatment, and low price, wide source, non-toxic, recyclable, renewable, is an excellent adsorption material. The effects of different amounts of activated carbon on the content of each component in L3 were studied experimentally. It can be seen from FIG. 3.9 that with the increase of the amount of activated carbon, the removal rates of lignin, furfural and 5-HMF in L3 showed a gradual increasing trend, while the acetic acid content in L3 did not change significantly. Studies have shown that activated carbon adsorption is an effective method to remove impurities such as lignin, furfural and 5-HMF from black liquor, but the adsorption effect on acetic acid is not good. When the amount

of activated carbon is lower than 0.4wt %, with the increase of activated carbon, the content of xylose in L3 does not change significantly, which is caused[36] by the adsorption selectivity of activated carbon. When the amount of activated carbon is low, there are fewer adsorbable sites on the surface of activated carbon. [37]Since the hydrophobicity of lignin and furfural is higher than that of carbohydrates, lignin and furfural have higher affinity with the surface of activated carbon and are easier to be adsorbed by activated carbon and removed[38]. At the same time, because lignin occupies more active sites of activated carbon, the adsorption effect of activated carbon on xylose is not obvious, and the loss rate is low. However, when the input of activated carbon continues to increase, because most of the lignin and furfural and other impurities have been adsorbed by activated carbon, the adsorption sites on the surface of activated carbon become more and more, the adsorption capacity of sugar increases, and the adsorption selectivity of lignin decreases, so that the loss rate of polysaccharide in black liquor increases[39]rapidly. Based on the loss rate of xylose and the removal rate of lignin and other impurities as evaluation ndexes, the optimal condition was selected as the dosage of activated carbon of 0.4 wt%. At this time, the removal rates of lignin, acetic acid, furfural and 5-HMF in L3 were 20.07%, 5.34%, 42.26% and 40.27%, respectively, and the loss rate of xylose was 6.45%.

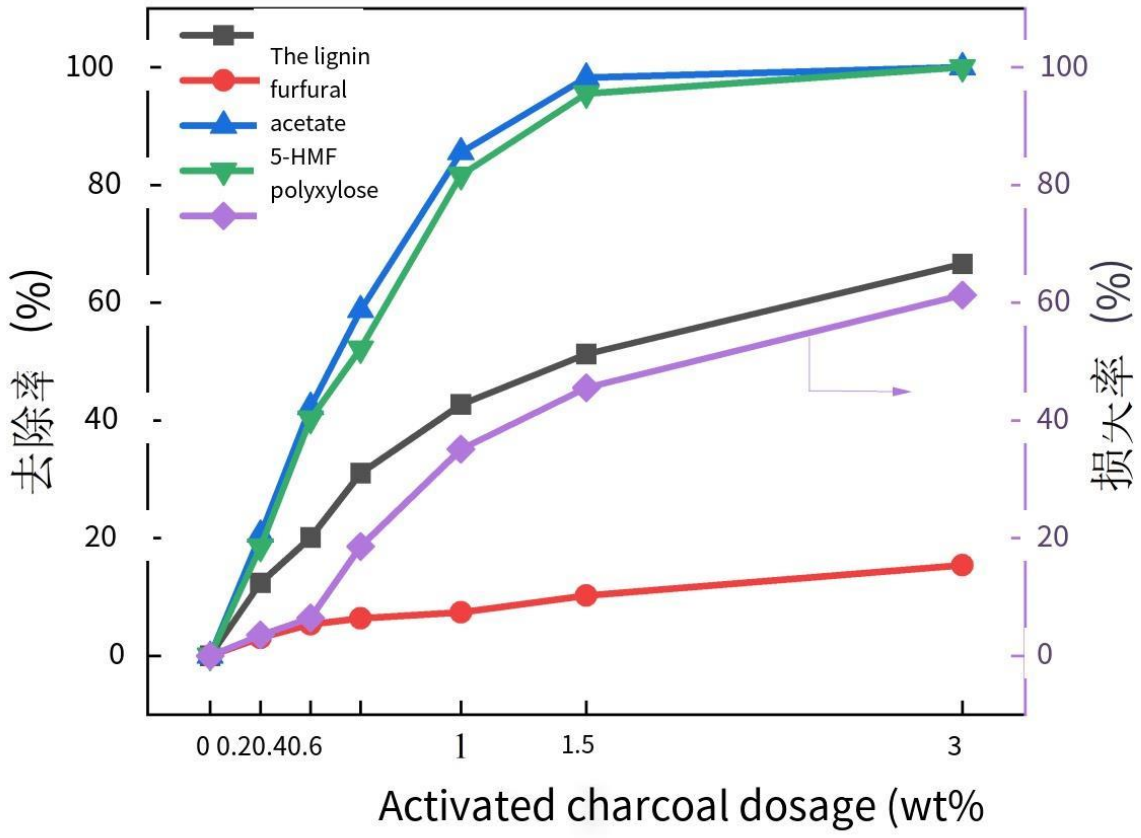


Figure 3.9 Influence of activated carbon dosage on the content of each component in L2

Conclusions to chapter 3

The experimental results and the trend of the process are analyzed

CONCLUSIONS

This paper takes wheat straw machine pulp black liquor as the research object, focusing on the separation and purification of sugar substances. Using calcium hydroxide, resin,activated carbon, laccase and xylanase to separate and purify the sugars in the black liquor and prepare xylo-oligosaccharides, and optimize the processing conditions. The research results not only alleviated the problems of heavy pollution load, difficult separation and purification, low utilization rate of hemicellulose, but also provided a reference for the industrial treatment of wheat straw chemical mechanical pulping black liquor. The main conclusions are as follows:

(1) Calcium hydroxide can effectively remove lignin, furfural, 5-HMF and other inhibitors in black liquor, so as to reduce the inhibition of subsequent enzymatic reaction activity, but more calcium hydroxide will lead to higher loss of xylose. Taking the removal rate of lignin and other impurities and the loss rate of xylose as evaluation indexes, 0.6 wt% of calcium hydroxide was selected as the optimal condition. At this time, the removal rates of lignin, furfural and 5-HMF in black liquor were 27.53%, 40.23% and 31.56%, respectively, and the loss rate of xylose was 7.26%.

(2) The optimal conditions for the treatment of black liquor by D301 resin were as follows: treatment temperature 50℃, initial pH6, resin dosage 5 wt%, treatment time 5 h. Under these conditions, the lignin removal rate was 67.85%, and the xylose rate was 7.02%.

(3) Laccase combined with xylanase treatment can effectively remove lignin from black liquor and significantly increase xylo-oligosaccharide content. When laccase dosage is 2 U/g, xylanase dosage is 2 U/g, temperature is 50℃, pH5.5, and time is 4 h, the lignin removal rate in black liquor is increased by 19.62%, and xylo-oligosaccharide content is increased by 52.25%.

(4) Under the optimal condition of 0.4wt % activated carbon, the removal rates of lignin, acetic acid, furfural and 5-HMF in black liquor were 20.07%, 5.34%, 42.26% and 40.27%, respectively, and the loss rate of xylose was 6.45%.

Prospects

In the laboratory, the substance in the black liquor for enzyme treatment is generally to be pretreated by physical method so as to keep the activity of the enzyme stable, and in recent years, the discovery of a variety of different enzymes, it may not carry out complex physical treatment can also be carried out enzyme treatment. Experimental data show that in this experiment, the black liquor after enzyme treatment contains more xylo-oligosaccharides. Due to the time problem of this experiment, the physical method has not been optimized too much. More physical methods should be explored for the treatment of fermentation inhibitors in the black liquor, so as to further explore its impact on the enzymolysis black liquor and optimize the efficiency of the enzymolysis black liquor. In addition, xylanase and laccase from different sources can also be used to treat different substances in the related black liquor respectively, in order to propose a huge xylooligosaccharide yield.

LIST REFERENCES

[1]. Cao X Y & Yang L. (2021). Application of non-wood fiber raw materials in papermaking industry. Guangdong Chemical Industry (02),272-273+275.

[2]. Li W & Li K W. (2020). Discussion on the present situation and prospect of scientific control of pulp and paper pollution. East China Paper (05),29-32.

[3]. Liu M. (2020). Treatment and resource utilization of black liquor and secondary black liquor in papermaking.(in Chinese) Energy Conservation and Environmental Protection (05),38-39.

[4]. JIANG Y T, WANG X Y, WU Z, et al. Purification of xylo-oligosaccharides from bamboo with non-organic solvent to prepare food grade functional sugars[J]. Results in Chemistry, 2021, 3: 100153.

[5]. Zhinan Feng, Raimo Alen, Jarmo Lou he lainen. Characteriza-tion of black liquors from soda -AQ pulping of wheat straw. Appita Journal, 2001, 52 (2):234~238

[6]. Xu Y H, Liu G G. (2003). Treatment and recovery of wheat straw pulp black liquor. Environmental Protection (04),25-27.

[7]. Fu D. (2015). Research progress of paper black liquor treatment technology. Liaoning chemical (4), 385-386 + 391. Doi: 10.14029 / j.carol carroll nki issn1004-0935.2015.04.060.

[8]. Abha Gupta Rajeev M. Mathur Arvind G.Kulkarni. Non-process elements in non-wood black liquors: their effect on tho-peration and energy efficiency of chemical recovery systems-Appita Journal，2001，54 (6): 518~522.

[9]. SARAWAN C, SUINYUY T N, SEWSYNKER-SUKAIY, et al. Optimized activated charcoal detoxification of acid-pretreated lignocellulosic substrate and assessment for bioethanol production[J]. Bioresource technology, 2019,286: 121403

[10]. Yang R C, Zhou S T. (1994). Study on the catalytic effect of sulfuric acid on black liquor of straw pulp making. Environmental Science (05),46-48+94.

[11]. JANUSZG, PA WLIK A, sW IDERSKA-BUREK U , et al. Laccase properties , physiological function sand evolution[J]. International Joumal of Molecular Sciences, 2020,21 (3): 966

[12]. hang Quanxing, Zhang Zhengpu, Li Aimin, et al. Review and prospect of the
development of ion exchange and adsorption resins in China [J]. Acta Polymerica Sinica, 2018(7):814-828. DOI: 10.11777/ j.ssn1000-3304.2018.17317.

[13]. HUANG Q L, ZHANG H R, XIONG L, et al. Controllable synthesis of styrene-divinylbenzene adsorption resins and the effect of textural properties on removal performance of fermentation inhibitors from rice straw hydrolysate[J]. Industrial & engineering chemistry research,2018, 57(14): 5119-5127. DOI: 10.1021/acs.iecr.8b00545

[14]. Wang Yu, Zhang Jianmin, Zhang Qingmei. [14] Wang Y, Zhang J M, Zhang Q M, et al. Study on biological treatment of papermaking black liquor [J]. Journal of Xi 'an Institute of Technology and Technology, 2003, 17 (1) : 88-90.

[15]. Huang Yanqiu, Xie Zhiru, Piao Shenghua, et al. Application of macroporous adsorbent resin in the purification of traditional Chinese medicine [J]. Guangdong Chemical Industry, 2018, 45(8): 130-132. (in Chinese) DOI: 10.3969/j.issn.1007-1865.2018.08.059.

[16]. WU Le, WU Jianqun, Yu Dunxi, et al. Effect of O2/CO2 combustion on interaction between Ca and Fe in Shenhua coal [J]. Acta Chemologica Sinica, 201566(2) : 753-758.

[17]. Zhang Bin, Zhang Chengci, Ji Wenhui. Treatment and resource utilization of
papermaking black liquor [C]// Environmental Engineering Branch of Chinese Society of Environmental Sciences. Proceedings of the 2019 Annual Science and Technology Conference of Chinese Society of Environmental Sciences -- Sub-Forum on Innovation and Application of Environmental Engineering Technology (1). Shanghai Kaixin Separation Technology Co., LTD.; , 2019:3. DOI: 10.26914 / Arthur c. nkihy. 2019.071072.

[18]. JANUSZG, PA WLIK A,sW IDERSKA-BUREK U, et al. Laccaseproperties, physiological functions, and evolution[J]. International Joumal of Molecular Sciences, 20,21 (3) : 966.

[19]. Yang D,Chang Y, Wu X, et al. Modification of sulfomethylated alkali lignin catalyzed by horseradish peroxidase[J].RSC Advances, 2014, 4(96) : 53855-53863

[20]. WEI Huali, Shu LAN, PEI Jicheng et al. [J]. Chinese Journal of Papermaking, 2003,18(2):56-59. (in Chinese

[21]. Liu J. Dual function of laccase/xylanase prehydrolysate conversion of furfural [D].Tianjin university of science and technology, 2022. DOI: 10.27359 /, dc nki. Gtqgu. 2022.000755.

[22]. Samanta A K, Jayapal N, Jayaram C, et al. Xylooligosaccharides as prebiotics from agricultural byproducts: Production and applications[J]. Bioactive Carbohydrates and Dietary Fibre, 2015, 5(1): 62-71.

[23]. Vazquez M J, Alonso J L, Dom I nguez H, et al. Xylooligo saccharides: manufacture and applications[J].Trends in Food Science & Technology, 2000,11(11): 387-393. (in Chinese

[24]. Kumar P, Barrett D M, Delwiche M J, et al. Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production[J]. Industrial & Engineering ChemistryResearch, 2009, 48(8): 3713-3729

[25]. Warrand J, Janssen HG. Controlled production of oligosaccharides from amylose by acid-hydrolysis under microwave treatment: Comparison with conventional heating[J]. Carbohydrate Polymers, 2007, 69(2): 353-362

[26]. LIAN Z N,WANG Y,LUO J,et al. An integrated process to produce prebiotic xylooligo saccharides by autohydnoly sis, nanofilration and endo-xylanase from alkali-extracted xylan [J/OL].Bioresource 'Technology, 2019,3 14: 123685 [2022-12-10]. HTPS: / l doi ore / 10. 1016 / j.b iortech. 2020.123685.

[27]. Yang Dou, Zhang Weibo, Zhao Qianyun. Study on preparation of Xylo-oligosaccharide[J]. Food and Food Industry,2017,24(1): 53-56. (in Chinese

[28]. Wang Y S, Zhu X, Jin L Q, et al. Post-hydrolysis of the prehydrolysate from eucalyptus pulping with xylanase. Journal of Cleaner Production, 2017, 142:2865-2871.

[29]. Chi Z, Rover M,Jun E, et al. Overliming detoxification of pyrolytic sugar syrup for direct fermentation of levoglucosan to ethanol[J]. Bioresource Technology, 2013, 150(12): 220-227

[30]. Mosier N S, Richard H, Mark B, et al. Industrial scale-up of p H-controlled liquid hot water pretreatment of corn fiber for fuel ethanol production[J]. Applied Biochemistry & Biotechnology, 2005,125(2): 77-97.

[31]. Yang G, Jahan M S, Ahsan L, et al. Influence of the diluent on the extraction of acetic acid from the prehydrolysis liquor of kraft based dissolving pulp production process by tertiary amine[J]. Separation& Purification Technology, 2013, 120(120): 341-345.

[32]. Wang X, Zhuang J, Fu Y, et al. Separation of hemicellulose-derived saccharides from woodhydrolysate by lime and ion exchange resin[J]. Bioresource Technology, 2016, 206: 225-230.

[33]. Zhang Y, Feng X X, Chang S Y, et al. Ultrasonic - of preparation of low poly xylose by enzymatic hydrolysis of wheat bran [J]. Journal of Shanghai chemical industry, 2021 46-48 (05) : 23-26. DOI: 10.16759 / j.carol carroll nki. Issn 1004-017 - x. 2021.05.010.

[34]. Konduri M K, Kong F, Fatehi P. Production of carboxymethylated lignin and its application as a dispersant[J]. European Polymer Journal, 2015, 70:371-383

[35]. Liu Z, Fatehi P,Jahan MS,et al. Separation of lignocellulosic materials by combined processes of pre-hydrolysis and ethanol extraction[J]. Bioresource Technology, 2011, 102(2): 1264-1269

[36]. Kubo S, Kadla J F. Hydrogen Bonding in Lignin: A Fourier Transform Infrared Model Compound Study[J]. Biomacromolecules, 2005, 6(5): 2815

[37]. Fatehi P, Ryan J, Ni Y. Adsorption of lignocelluloses of model pre-hydrolysis liquor on activated carbon[J]. Bioresource Technology, 2013, 131:308-314

[38]. Fatehi P, Chen J. Extraction of Technical Lignins from Pulping Spent Liquors, Challenges and Opportunities[J], 2016: 35-54

[39]. Shen J, Kaur I, Baktash M M, et al. A combined process of activated carbon adsorption, ion exchange resin treatment and membrane concentration for recovery of dissolved organics in pre-hydrolysis liquor of the kraft-based dissolving pulp production process[J]. Bioresource Technology, 2013, 127: 59-65