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Master's in Chemical Engineering
MASTER'S THESIS

Investigation of an Environmentally Method for the Oxidation of Hemicellulose Model Compounds

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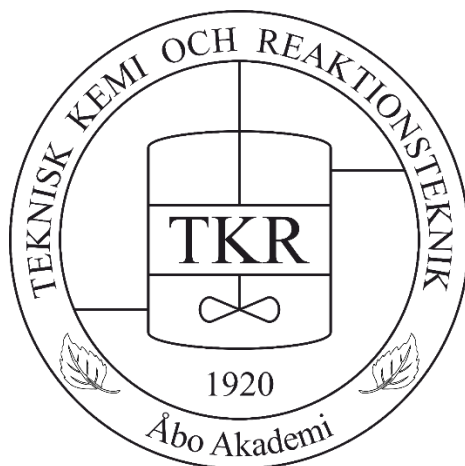Laboratory of Industrial Chemistry and Reaction Engineering

Åbo Akademi University

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Investigation of an Environmentally Method for the Oxidation of Hemicellulose Model Compounds

Master's Thesis

Álvaro Asensio Ricor

TÍTULO: Investigation of an Environmentally Method of Hemicellulose Model Compounds Oxidation

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PREFACE

I think that the preface is always the most difficult part of the thesis. There is not enough space for describing on one page my experience of this journey. Let me start from the beginning.

Two years ago…I didn't know how my life would change during the following years. I was in Valladolid doing my master degree studies, when I decided to stay in Spain, get my diploma and start to work. However…COVID,COVID!! I was without job, without a subject and the master thesis still undone… I think that in the first moment that I applied for doing my thesis in Finland, all things change.

Of course my application for going of Erasmus was accepted, I had 9 months for preparing all my suitcases and go to Finland. In that moment, I found also job for 9 months, that would be perfect! Erasmus and my first experience working in a multinational environment. But that is not all, I had the opportunity to meet an excellent team, excellent people that today are in contact with me.

I don't want to follow telling you my experience in Spain, we are going to focus on Finland. First day in Finland, what am I doing in this country? Maybe Finland is a cold country, but you will have the opportunity to meet incredible and warm people. I suppose that I must express my infinite gratitude to many people. Sorry Pasi, in this case I need to start for my colleague of master, my "unofficial tutor" German Araujo, thanks and very thanks you made my Erasmus easier, the adaptation had not been the same without you. Now yes, you have been my supervisor, you have helped me with this demanding research and without you this research would have obtained these good results. Thanks to all my workmates Tomasso, Ole, Giuseppe, Christophe, Marie, Mouad, Ana and all other numerous friends because I have really good moments with you.

Regarding my Erasmus I think that my experience would not have been complete without my friend of laboratory Alberto. I don't have any doubt that my best memories from this experience are with you, scare you in the laboratory, built our hall of fame or visit incredible countries and places. But when I said Alberto, I need to complete it with Iraide because we were only together 5 months - What months!!. Thanks and many thanks for so many good moments. GUAU GUAU para que nos entendamos.

During my project I had hard challenges, for this reason I would like to express my gratitude to the Ms Annika Smeds, for her enormous help conducting GC-MS analyses for the identification of products, and to Dr Robert Lassfolk for NMR investigation of products.

In addition, I would like to express my gratitude to my master's coordinator, Professor Juan García Serna, which made it possible for me to pursue my master's degree at the University of Valladolid. Also, I would like to thank my supervisor at Åbo Akademi University, Professor Tapio Salmi, for the opportunity to work with him.

Of course, thanks to my parents, because without you this would not be possible. Your support during all my degree has been the main motor for finish all. Last but not least, the most important person, you and only you: Patricia Derqui, the best nights from my Erasmus were when I spoke with you, it is incredible but each day which I spent far from you I love you a bit more. Thanks for your support - I love you so much. GRACIAS A TODOS, GRAZIE A TUTTI, THANKS TO EVERYONE!

ABSTRACT

Keywords: galactose, oxidation, hydrogen peroxide, FePcS, catalysis, reaction.

This research work was carried out at the Laboratory of Industrial Chemistry and Reaction Engineering (TKR) at Åbo Akademi University (Turku/Åbo, Finland) in collaboration with the University of Valladolid (Valladolid, Spain) under the supervision of Tapio Salmi (Professor of the Academy of Finland) and Juan García Serna (Full Professor at the University of Valladolid) as part of the Erasmus Plus exchange program.

Large amounts of hemicellulose are burnt in paper industry as a waste treatment. In our research, we have studied the possibility of use this hemicellulose for obtaining useful by-products such as acids which can be used in another kind of industries. All our studies start with a model compound of hemicellulose: galactose.

The research effort of this thesis was focused on development a method of oxidation which was carried out with a more environmentally friendly oxidant, hydrogen peroxide (H_2O_2), and a special metal complex catalyst (FePcS), of which only a small amount is needed.

The work comprised batch and semibatch studies, determination of low molecular by-products and determination of optimal conditions for obtaining these products. This resulted in a waste-free oxidation method, which only produces water and oxygen as side products.

The galactose oxidation was studied in both semibatch and batch modes in respective to the oxidant (H_2O_2) addition. Semibatch mode obtained better results because keeping the concentration of the oxidant constant during the reaction. To identified the compounds, HPLC, GC-MS and NMR analyses were used during this research.

REFERAT

Undersökning av en miljövänlig metod för oxidation av modellkomponenter från hemicellulosa

Nyckelord: galaktos, oxidation, väteproxid, FePcS, katalys, reaktionskinetik

Detta forskningsarbete genomfördes vid Laboratoriet för teknisk kemi och reaktionsteknik (TKR) vid Åbo Akademi (Åbo, Finland) i samarbete med universitetet i Valladolid (Valladolid, Spanien) under ledning av akademiprofessor Tapio Salmi (Finlands Akademi) och Juan García Serna (professor vid universitetet i Valladolid) som en del av utbytesprogrammet Erasmus Plus.

Stora mängder hemicellulosa förbränns i pappersindustrin som avfallsbehandling. I vår forskning har vi studerat möjligheten att använda denna hemicellulosa för att få fram användbara biprodukter såsom syror som kan användas vid andra industrier. Alla våra studier börjades med en modellförening av hemicellulosa: galaktos.

Forskningsinsatsen i denna avhandling var fokuserad på att utveckla en oxidationsmetod som genomfördes med en mer miljövänlig oxidant, väteperoxid (H_2O_2), och en speciell metallkomplexkatalysator (FePcS), av vilken endast en liten mängd behövs.

Arbetet omfattade satsvisa och halvkontinuerliga experiment, identifiering av lågmolekylära biprodukter och bestämning av optimala betingelser för att erhålla dessa produkter. Detta resulterade i en avfallsfri oxidationsmetod, som endast producerar vatten och syre som biprodukter.

Galaktosoxidationen studerades i både satsvisa och halvkontinuerliga system i avseende på tillsatsen av oxidant (H_2O_2). Halvkontinuerlig drift gav bättre resultat, eftersom koncentrationen av oxidationsmedlet hölls konstant under reaktionen. För att identifiera föreningarna användes HPLC-, GC-MS- och NMR-analyser i denna forskning.

RESUMEN

Palabras claves: galactosa, oxidación, peróxido de hidrogeno, FePcS, catálisis, reacción.

El presente trabajo fue realizado en el laboratorio de Química Industrial e Ingeniería de la Reacción en Åbo Akademi University (Turku/Åbo, Finlandia) en colaboración con la Universidad de Valladolid (Valladolid, España) bajo la supervisión de Tapio Salmi (Profesor de la Academia de Finlandia) y Juan García Serna (Catedrático de la Universidad de Valladolid) en el marco del programa de intercambio Erasmus Plus.

Hoy en día, la industria de papel quema grandes cantidades de hemicelulosa como posible tratamiento de residuos. En este trabajo, se estudió la posibilidad de aprovechar dicha hemicelulosa para obtener co-productos útiles como ácidos que pueden ser aprovechados en otro tipo de industrias. En el proyecto se tomó como punto de partida para todos los experimentos un compuesto modelo de la hemicelulosa: la galactosa.

El objetivo de esta investigación se centró en el desarrollo de un método de oxidación utilizando un agente oxidante que afecte lo menos posible al medio ambiente, el peróxido de hidrógeno (H_2O_2) y un complejo metálico (FePcS) como catalizador, el cual solo se utilizó en pequeñas cantidades.

El trabajo abarca: estudios en modo discontinuo y semicontinuos, identificación de los co-productos de bajo peso molecular de la reacción y la determinación de las condiciones óptimas para obtener dichos productos. Este proyecto finalizó con la obtención de un método de oxidación que solo produce agua y oxígeno como productos secundarios.

La oxidación de la galactosa fue estudiada en modo semicontinuo y discontinuo con respecto a la adición del oxidante. El modo semidiscontinuo obtuvo mejores resultados ya que mantuvo constante la concentración del oxidante durante la reacción. Para identificar los compuestos, se utilizaron las técnicas (cromatografía líquida de alta eficacia) HPLC, cromatografía de gases/espectrometría de masas (GC-MS) y resonancia magnética nuclear (RMN) durante esta investigación.

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1 INTRODUCTION

According to new research, the population of Earth reached of 7.8 billion in August 2020 and this huge amount will be projected until 10 billion in 2057. The high annual population growth contributes severe environmental problems and food security, which affect the Sustainable Development Goals (SDGs) (Leong et al., 2021). Specifically, environmental concerns such as pollutions, climate change, global warming, and reduction of natural resources; these global problems need an urgent solution to decrease the negative impact.

European Union (EU) is carrying out plans such as "The zero pollution" which is a cross-cutting goal that contributes to the United Nations 2030 Agenda for Sustainable Development and complementing the 2050 climate-neutrality target in synergy with the clean and circular economy and restored biodiversity goals. The major goal of this action plan is to create a roadmap for incorporating pollution prevention into all relevant EU policies, maximizing synergies in an effective and appropriate way(European Comission, 2021).

Figure 1. Sustainable Goals

1.1. **Concept of biorefinery**

Softwood presents an enormous lignocellulosic biomass resource, which can be used as a valuable and renewable feedstock for the production of chemicals and biofuels. The major components of lignocellulosic biomass are cellulose, hemicelluloses and lignin. However, despite many decades of effort, commercial development of biomass-to-biofuel conversion processes is still not an economically very viable proposition.

In biochemical biomass-to-biofuel conversion processes, lignin is typically produced as a waste stream. Also, industries such as pulp production use chemically aggressive and environmentally hazardous reagents. The employment of solid catalysts, organic solvents, and green reagents is related with some research trends in the development of new environmentally friendly high-tech processes of lignocellulosic biomass conversion into valuable compounds. (Kuznetsov et al., 2021). "Development of selective and robust catalytic processes specifically designed for lignin conversion must be a core effort in a biorefinery program" as US Department of Energy told in one of their reviews.(Ma et al., 2015).

The so-called second generation biorefinery is focused on using lignocellulosic biomass produced by the agriculture, forestry, and food industries, producing chemicals from residues. Outstanding benefits of this strategy include the accessibility of lignocellulosic material, which account for 75% of renewable biomass and the lack of competition for cultivable soil. However, the chemical diversity of its elements, including cellulose, hemicellulose, lignin and extractives, makes the transformation processes difficult.(Pinales-Márquez et al., 2021)

A 40-50 w/w% of lignocellulosic biomass is made up of cellulose, a glucose-based polymer linked by 1,4-glycosidic bonds, 16-33 w/w% of hemicelluloses, heteropolymers containing different monomers of different sugars, including arabinose, galactose, glucose, mannose, and xylose. Therefore, in order to extract the components of fuels and chemicals from these materials, thermal, chemical, catalytic or biological processes must be performed.(Mckendry, 2001.)

The goal of the green chemical industry is to decrease or eliminate the use and generation of hazardous compounds. In this field, hydrogen peroxide (H_2O_2) is a great oxidant which is widely used in chemical industry, especially in hydrocarbon nitridation and oxidation processes. With the recent increase of the green chemical sector, the demand of H_2O_2 has expanded significantly. China consumed more than 3.2 x 106 t of H_2O_2, accounting for more than half of worldwideH_2O_2 consumption in 2018 and it is still expanding at a rate of more than 5% per year (Shi et al., 2022).
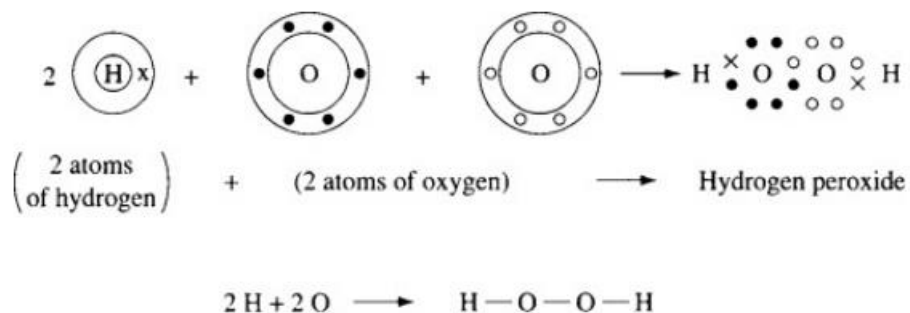
1. Introduction

Figure 2. Structure of hydrogen peroxide (Shi et al., 2022).

Hydrogen peroxide is an oxidizing agent that is similar to oxygen in effect but is significantly stronger. The oxidizing activity of hydrogen peroxide results from the presence of the extra oxygen atom compared with the structure of water. This extra oxygen atom is described as a "peroxidic oxygen" and is otherwise known as "active oxygen". Hydrogen peroxide has the ability to oxidize some compounds directly such as alkenes. The peroxidic oxygen adds to the double bond producing a hydroperoxide (Woodard & Curran, 2006).

1.2. **Hemicellulose and galactose in the industry**

Hemicellulose can be defined as cell wall polysaccharides, which binds strongly to cellulose microfibrils by hydrogen bonds and Van der Waals force. The main plant-derived polysaccharides found in hemicellulose were D-xylose, D-mannose, D-galactose, L-arabinose and 4-O-methyl-D-glucuronic acid. Hemicellulose is used to make alcohol by fermentation and sorbitol by reduction, which have significant uses in food, toothpaste, cosmetics, the production of explosives and papermaking. More significantly, because of their distinct physical, chemical, and physiological features, xylooligosaccharides, one of the degradation products of hemicellulose, are widely used in the functional food and pharmaceutical industries. Hemicellulose, whose structure and molecule varies, is derived from various plant sources and plant locales. For instance, the chemical structures of the hemicellulose from various biomass species, such as gramineous plants, softwoods and hardwoods, differ.(Huang et al., 2021)
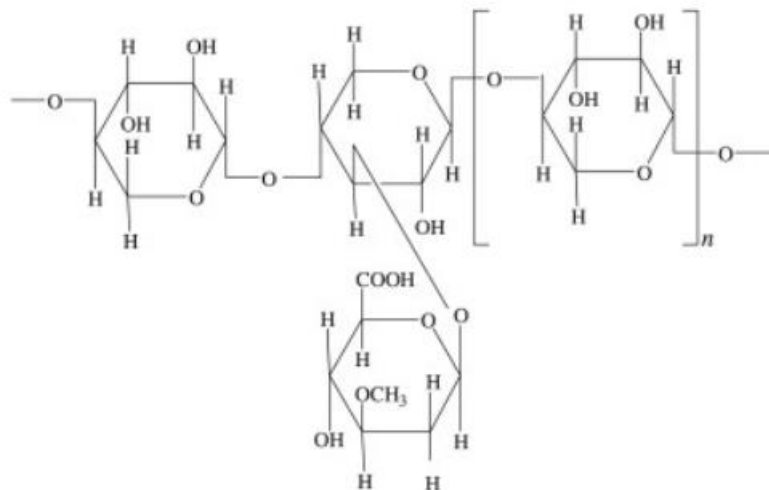

Figure 3. Structure of hemicellulose

Since hemicellulose is tightly attached to cellulose and lignin in plant cell walls, the pretreatment of lignocellulosic biomass is often carried out before the modification. Hemicellulose can be extracted using chemical-physical, and biological pretreatments. After separation and purification, hemicellulose with a higher purity can be achieved increasing its reactivity and use effectiveness. Hemicelluloses that are directly isolated from lignocellulosic biomass typically feature rigid molecular chains and strong hydrogen bonds between molecules. To achieve the best application performance of hemicellulose, a number of processes including as plasticization, mixing chemical reaction, are beneficial. Hemicellulose contains a large number of hydroxyl groups, which enable it to be altered by etherification,

esterification, and other processes in accordance with various bonding modes between hydroxyl groups and substituent groups.

Hydrothermal extraction, steam explosion, acidic and alkaline pretreatments, solvent extraction, ultrasonic-assisted and microwave-assisted techniques are some of the methods used to separate and extract hemicellulose from lignocellulosic biomass. By severing the chemical and hydrogen connections between hemicellulose and lignin as well as between hemicellulose and cellulose, the components of the cell wall are separated. Hemicellulose typically undergoes partial or total degradation during the pretreatment steps. Additionally, nearly no method can completely extract hemicellulose without also dissolving the other components. (Lu et al., 2021)
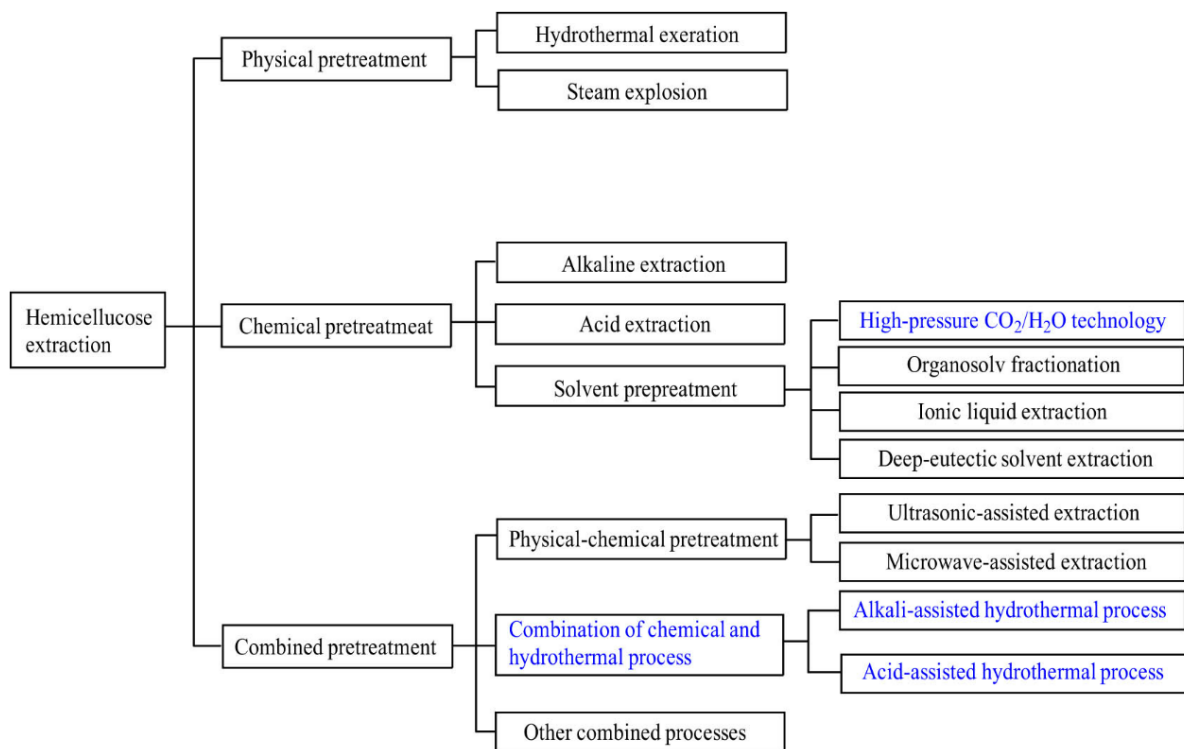

Figure 4. Hemicellulose extraction methods. (Lu et al., 2021)

1.2.1 Galactose

As a starting point, galactose is chosen as reactive in this research. Once results are obtained, the number of sugars will increase until the final research where we should use hemicellulose from wood biomass. Taking as a reference other studies where the galactose was the first reactive used to start with the investigation. (Kusema & Murzin, 2013)
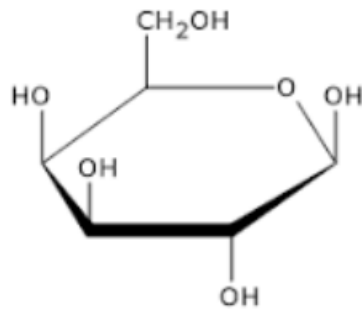
Figure 5. Structure of D-Galactose

The chemical formula of galactose, a monosaccharide, is $C_6H_{12}O_6$, the same as glucose. The only structural difference between it and glucose id the placement of one hydroxyl group. Galactose, however, has different molecular and metabolic properties from glucose as a result of this distinction.

Figure 6. Lactose structure

Lactose, a disaccharide created by combining one molecule of glucose with one of galactose, is the main dietary source of the sugar. (Williams, 1993)

1.3. **Use of metal phtalocyanines**

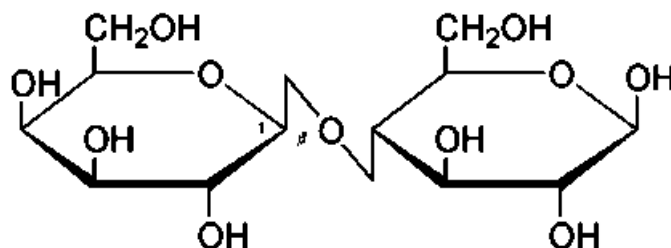The present interest in using renewables as raw materials has generated a lot of research for environmentally friendly and cost-effective methods for converting them into useful products. Polysaccharides are plentiful non-toxic, biodegradable, natural polymers with a high degree of functionalization. Their complex polymeric nature is advantageous for a variety of applications (Sorokin, 2013).

Traditionally, some polysaccharide oxidation processes such as starch oxidation has been performed by using agents such as hypochlorite or iodate and hydrogen peroxide in the presence of significant amounts of catalyst such as $FeSO_4$. Large amounts of inorganic waste are produced in these processes, for that raison more environmentally friendly catalyst with oxidation agents has been studied (Tolvanen et al., 2009). To tackle this challenging goal some authors have suggested that tetrasulfonatophthalocyanine metal complexes (MPcS) could be useful for oxidation processes (Kachkarova-Sorokina et al., 2004a).

The FePcS $-$ H_2O_2 system was used for clean modification of starch, cellulose and its derivatives such as guar gum and inuline. This friendly oxidant system was used because of their significant advantages such as:

1. Use of low concentrations for efficient oxidation.
2. Good solubility in water.
3. The absence of complexation products. (Tolvanen et al., 2011)

For clean one-step modification of polysaccharides without the creation of waste or side product, only modest volumes of water, H_2O_2, and an inexpensive and widely available on an industrial scale catalyst are used. The process can be carried out under homogeneous circumstances or with a polysaccharide suspension in an aqueous catalyst solution.
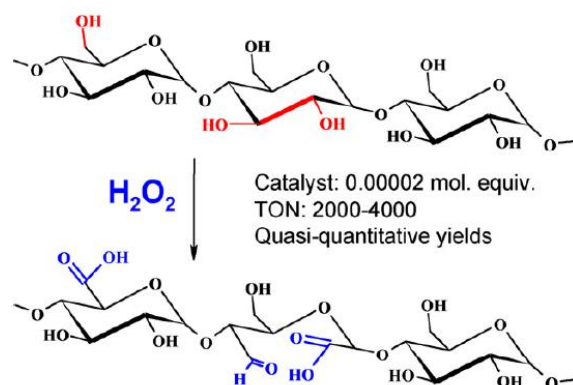
Figure 7. Schematic representation of oxidation of starch (Sorokin & Kudrik, 2011).

A homogeneous dispersion of the catalyst in the solid substrate can be achieved by mixing solid polysaccharides, such as starch, with a tiny amount of an aqueous solution of the catalyst. Catalyst oxidation starts as H_2O_2 is added. The efficiency of using H_2O_2 to produce carbonyl and carboxyl groups was found to be around 80%. During the reaction the catalyst is bleached and colourless. Due to the extremely low catalyst charge, a trace amount of residual iron can be eliminated by washing with cold water or even left in the material, depending on the application of the modified polysaccharide.

Products which can be obtained with this method do not have any impurities and they have been successfully tested for the surface coating of paper, latex and paint preparation and also in applications where the purity of product is an important issue, e.g. in cosmetic formulations. (Sorokin & Kudrik, 2011)

This oxidation method meets all criteria of green chemistry and therefore in this work the metal sulfophthalocyanine (MPcS) complex were chosen as the catalyst in the present work.
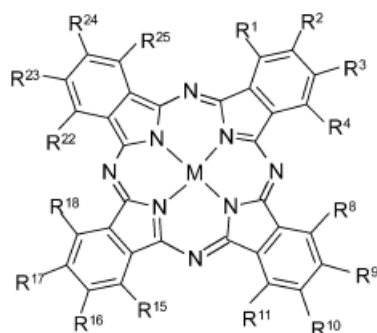

Figure 8. General structure of substituted phthalocyanine complexes.

1.4. Hydrogen peroxide as a green oxidant

Hydrogen peroxide (H_2O_2) is a safe and biodegradable oxidant which is commonly used for pulp and paper bleaching, wastewater treatment, substrate oxidation and in fuel cells. H_2O_2 is currently produced mostly using the indirect anthraquinone method, in which a palladium catalyst reduces an anthraquinone (such as 2-ethylanthraquinone or the 2-amyl derivative) . The high expenses of the anthraquinone process, which are caused by the reuse of extraction solvents, the hydrogenation catalyst, and the costly quinone has prevented H_2O_2 from becoming a widely used green oxidant and fuel. An alternate pathway for on-site preparation has been found in the direct synthesis of H_2O_2 from a combination of H_2 and O_2. The production of H_2O_2 by photocatalysis is the most sustainable process, because water and oxygen are earth-abundant and environmentally benign source materials and solar light is used as the energy to drive the reaction between them preparation. (Fukuzumi et al., 2021)

As a peroxygen, H_2O_2 has a lot of environmental applications, one of them is working as a green oxidant as reported by (Klein-Koerkamp et al., 2009a). Conventional oxidation of polysaccharides is efficient but generate huge amounts of waste, for instance in the case of NaOCl as the oxidant are obtained toxic by-products. Therefore, H_2O_2 might become the solution as a green oxidant for avoiding the production of harmful and contamination of final products as well as limiting the purifaction procedures (Kachkarova-Sorokina et al., 2004). However, hydrogen peroxide is not active without proper activation. Some authors such as Dr. P.Tolvanen reported the use of this oxidant with homogeneous catalysts such as FePcS. (Tolvanen et al., 2011)
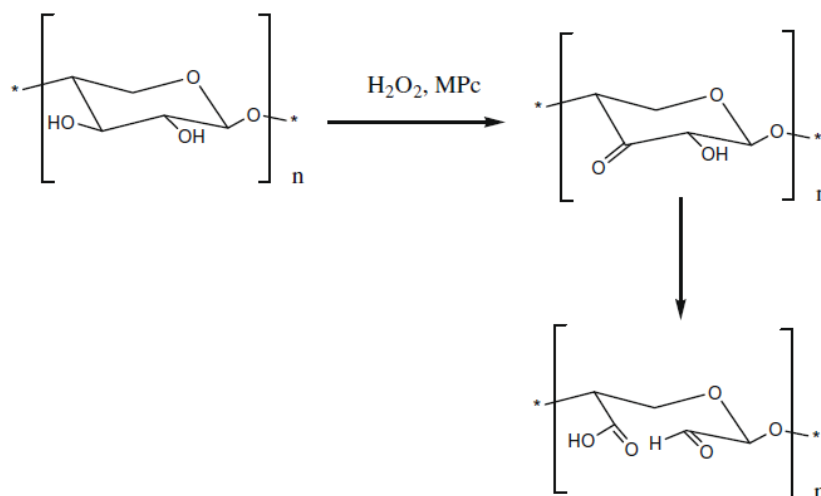
Figure 9. General scheme of polysaccharide oxidation.

1. Introduction

Oxidation of other polysaccharides with a higher molecular weights have been performed with the same system FePcS/H_2O_2/H_2O with good carboxyl and carbonyl yields (Klein-Koerkamp et al., 2009). Therefore, this research has been conducted with the same approach to carry out the oxidation.

1.5. Product: Formic acid, tartronic acid and glycolic acid

1.4.1 Formic acid

In this demanding research, three main products were obtained in the oxidation experiments: formic acid, tartronic acid and glycolic acid. The industrial importance of these products is described in this chapter.

Formic acid manufacturing has traditionally started with biomass-based precursors. Careful examination of previous procedures has already resulted in new production concepts and it is believed that this approach will continue to provide new improvements. The figure 6 display the approaches based on historical methodology.
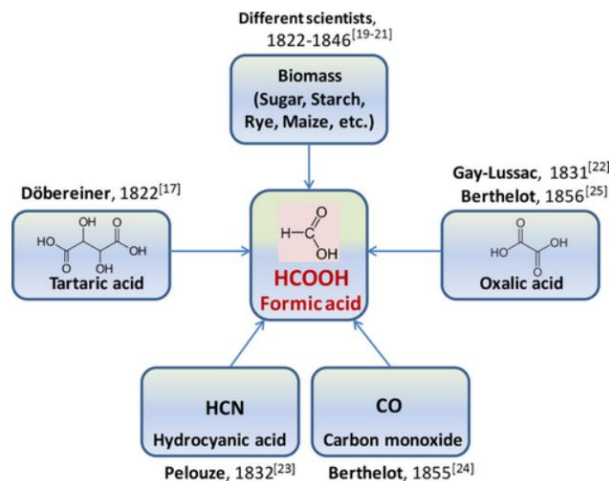

Figure 10. Historical methodology to obtain Formic acid (Bulushev & Ross, 2018a).

Regarding this research, formic acid can be obtained from biomass cellulose as you can see in the figure 7:
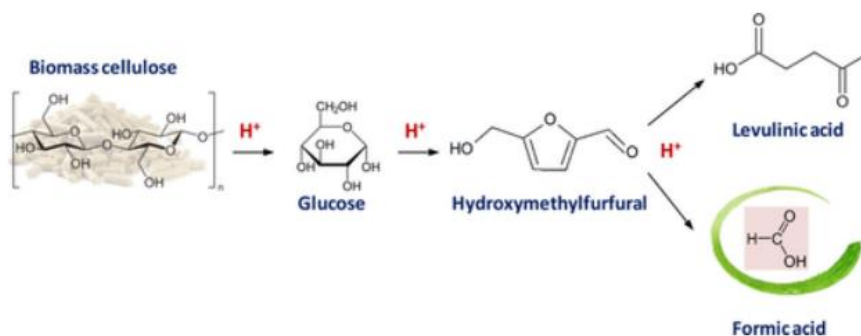

Figure 11. Oxidation process to obtain Formic acid (Bulushev & Ross, 2018a).

1. Introduction

The chemical, agricultural, textile, leather, pharmaceutical and rubber sectors use formic acid as a commodity. In chemical processes, formic acid can replace various inorganic acids and is less corrosive than many of them. It also does not cause nitrate, phosphate or sulfathe loadings in wastewater. Formic acid and formate ion are easily biodegradable, according to the data of European Chemical Agency. Furthermore, in seawater and sweetwater, formic acid and formate ion are easily biodegradable.

The global formic acid production is projected to be up to 950,000 tons per year worldwide. This high demand is due to its generally harmless and noncorrosive qualities, which make it easy to handle. The market for this chemical is expected to grow at a rate of 5.6 percent each year through 2019 (Bulushev & Ross, 2018b).

In addition, due to its intrinsic features (it is non-toxic, liquid at a room temperature, suited for easy transportation, handling and safe storage) formic acid has recently emerged as one of the most promising compounds for chemical storage of hydrogen.
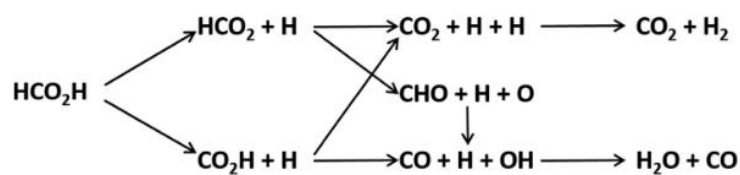

Figure 12. Proposed formic acid decomposition routes

It is also an important chemical intermediate, by-product, and final product, as well as a major product in biomass processing (Navlani-García et al., 2019).

1.4.2   Tartronic acid

In industry, tartronic acid is considered as an important compound for the synthesis of fine chemicals and new polymers and in fine chemical industry this acid is used as a reactant in the catalytic oxidation to produce mesoxalic acid which is another potential compound used as an intermediate chemical in some reactions. (Coelho et al., 2018)

In addition, this compound is mainly used in alimentary industry due to its capacity of inhibiting the transformation of sugars to fats, preventing the accumulation of fat in the body and therefore reducing the weight (Li et al., 2022).

Tartronic acid is a high value-added chemical which is commonly used as a pharmaceutical and anti-corrosive protective agent, for this raison scientists are searching for a cost-efficient and environmentally friendly method for obtaining this acid.
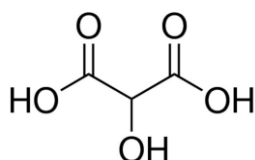
Figure 13. Tartronic acid structure.

The main process for obtaining tatronic acid is by means of glyceric acids. Although the glyceric acid is the common process for obtaining this acid, the tartronic acid yield of this reaction is, some authors explain that this is due to the difficulty in further oxidation of glyceric acid and the occurrence of C-C cleavage or decarboxylation. (Cai et al., 2014) Therefore, it is important to find paths for obtaining a better yield of this acid.

1.4.3   Glycolic acid

Glycolic acid is a small two-carbon α-hydroxy acid with both alcohol and acid groups. Glycolic acid is used as a dyeing and tanning agent in textile business, as a flavour and preservative in alimentary industry and as a skin care agent in the pharmaceutical sector. It is also used in cleaning agents and adhesives for industrial and home use, and it is frequently incorporated in emulsion polymers, solvents, as well as for ink and paint to improve the flow characteristics and gloss. Glycolic acid can be transformed to a biodegradable polymer (PGA) with good mechanical properties, and it is utilized to make a co-polymer with lactic acid for different medical purposes.
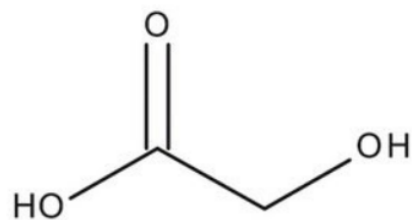
Figure 14. Molecular structure of Glycolic acid

In 2015, the glycolic acid market was worth US$ 159.6 million and it has been constantly growing due to increased use of glycolic acid in cosmetics and household cleaning solutions. By 2024, the market is estimated to be worth $415 million. Glycolic acid is created naturally by some chemolithotrophic iron- and sulfur-oxidizing bacteria or by hydrolyzing glycolonitrile with alcaligenes. In addition, glycolic acid is produced by a variety of yeast and acetic acid bacteria from ethylene glycol oxidation.(Salusjärvi et al., 2019)

The research of new oxidation processes to obtain glycolic acid has been an increasing trend recent years. Some authors have invented these kind of processes with the addition of metal catalysts for maximizing the amount of glycolic acid (Ruiz et al., 2021).
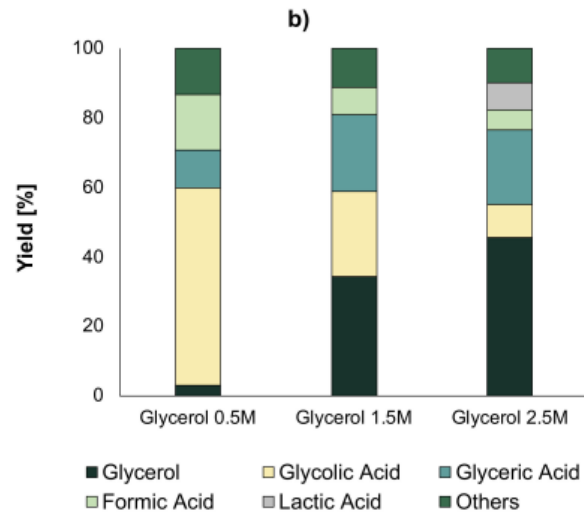
Figure 15. Results of conversion to obtain glycolic acid (Ruiz et.,2021)

In the light of the above discussion, it can beconcluded that all products are very useful nowadays in the industry and therefore the research and goal of this project became to obtain a reaction for getting these products.

1.6. **Aim and scope of the research**

In this work we developed further an environmentally friendly option with galactose for obtaining useful products, using an iron tetrasulfophthalocyanine complex (FePcS) as the catalyst and hydrogen peroxide as an oxidant, which forms only water as stoichiometric co-product. To achieve this objective, the following tasks were performed:

- Preliminary study for a product identification of the interaction of galactose, hydrogen peroxide and iron catalyst during the oxidation process.
- Investigation of kinetics using two different reactor types (batch and semibatch mode).
- Product identification from HPLC with different identification techniques such as GC-MS and NMR analyses
- Optimization of the reaction conditions for improving the galactose conversion and product yield, by changing reaction parameters such as temperature, pH, type of reactor configuration and types of catalysts.

2 MATERIAL AND METHODS – EXPERIMENTAL SECTION

2.1. Equipment

2.1.1   SemiBatch reactor

In the first 9 experiments, a batch mode reactor system was used. All reactants, including H_2O_2 were added immediately in the beginning of the experiment. From experiment 10 and forward, a semibatch reactor system was used (see next section 2.1.2). The configuration consisted of a reactor, a heat exchanger, a pH control system and a reflux condenser.

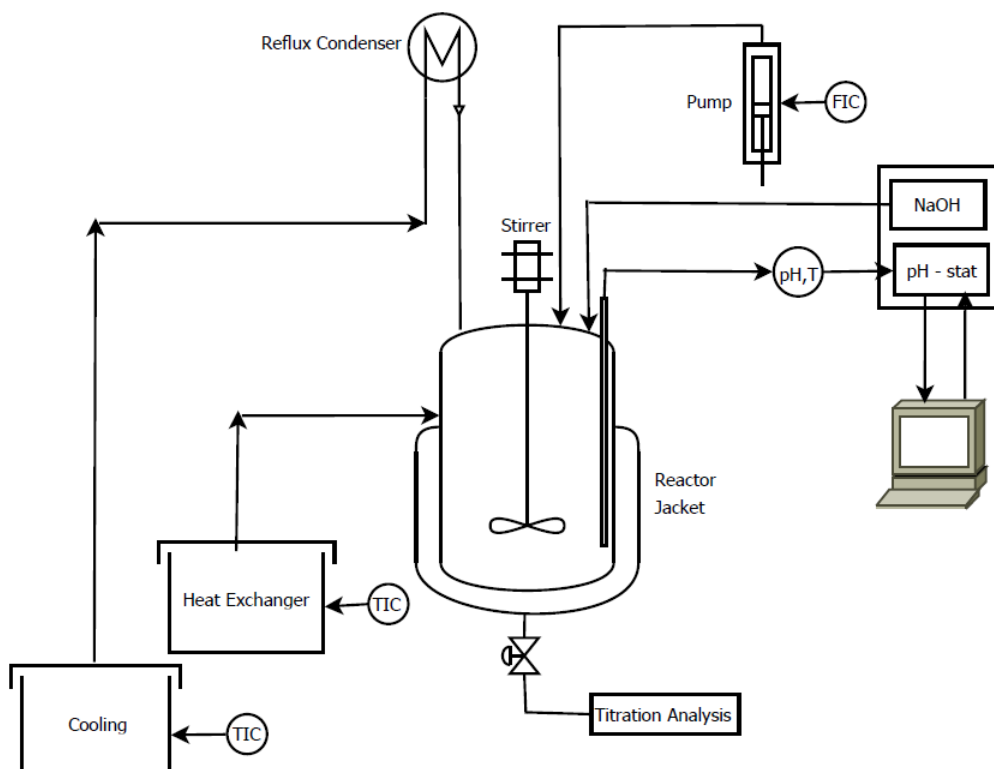The system is displayed in Figure 12:

Figure 16. Flow diagram flow of the batch reactor system

2.1.2 Semibatch reactor

The reactor setup was designed to work as a semi-batch system. This system comprised a glass reactor used to study the kinetics the oxidation of selected sugars such as galactose, in the presence of a catalyst; a heat exchanger which was incorporated in order to provide an energy source. The reactor was surrounded by a heating jacket using circulating water, in order to maintain the desired temperature. The reactor was equipped with a stirrer with a propeller head made from stainless steel, under which the SpinChem rotating basket was located.

A precision pump installed with a syringe (Alaris, Asena), was used to control the dosage of the oxidant, hydrogen peroxide. Typically in the case of semibatch experiment, the flow velocity was set to 2.7 mL/h, and the total dosing time thus 8 hours. It was important to select a slow enough H_2O_2 feed rate, since the preliminary experiments indicated that the catalyst may be degraded in the presence of a too high H_2O_2 concentration. Since the reactor was operating at an elevated temperature (typically 60°), a reflux condenser was incorporated in the system to avoid the evaporation.

The temperature and pH of the experiment were measured with electrodes installed in-situ, and followed with a computer. The pH was controlled with a Titrando pH-stat device (Metrohm AG. This device added NaOH to the solution to reach the desired pH and to keep it constant. The molar strength of the NaOH was in all experiments 0.10 M.

Vigorous stirring was ensured by using a mechanical impeller, which was equipped with a propeller-type head, made from stainless steel. In all experiments, a stirring velocity of 500 rpm was used.

A constant precaution was carried out when the reactor was turned on. Therefore, as a security measure, the reactor was installed in a fumehood for avoiding any kind of escape of liquids or gases to the laboratory space during experiments.

The schematic configuration of the reactor system is presented in Figure 13:
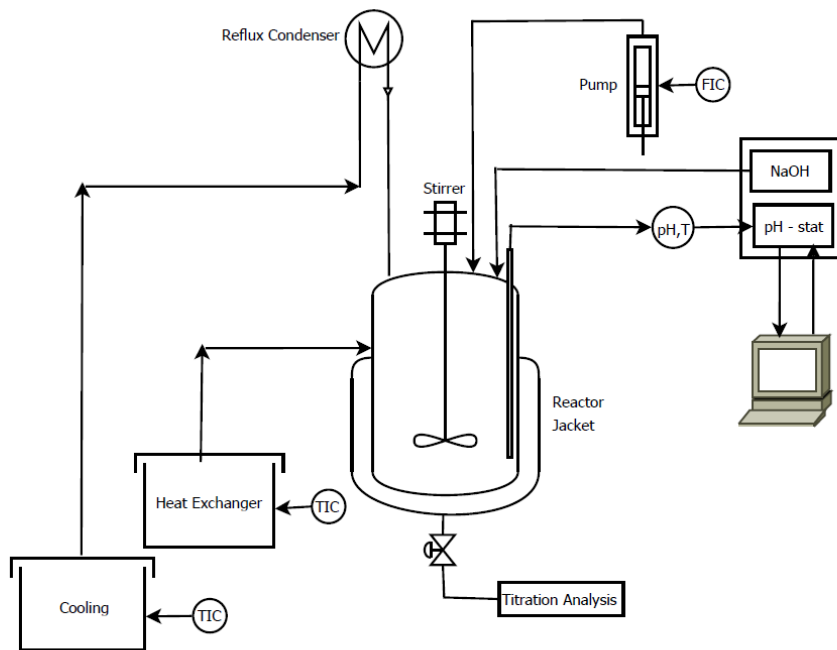
Figure 17. Flow diagram of the semibatch reactor system.

2.2. **Raw Materials**

During this project, main raw materials were:

- The Galactose reagent (99,9%): for the oxidation experiments was purchased from Sigma – Aldrich.
- Hydrogen peroxide (30 wt.%): It was utilized as an oxidant, in several experiments which was diluted with deionized water.
- Water-soluble metal tetrasulfophthalocyanine complex was brought from "Institut de Recherches sur la Catalyse et l'Environnement de Lyon". (Sorokin & Kudrik, 2011b)
- Sodium hydroxide solution (0,1M): to maintain the pH level in the reactor during the experiment.

The hydrogen peroxide titration content in the samples was determined by iodometric titration (KI, Sigma-Aldrich, 10 wt. % H_2SO_4 for acidification, 0,05 M $Na_2S_2O_3$).

Several homogeneous catalyst of MPcS were tested in this work. Most experiments used iron as the active metal (FePcS), but also copper (CuPcS) and cobalt (CoPcS) were investigated. The following figure illustrated the iron structure catalyst:

Figure 18. Iron tetrasulfophthalocyanine catalyst (FePcS)

It can be pointed out, that the MPcS catalysts are forming a very strong color (typically dark blue) when dissolved in water. It can be possible, that a change (disappearance) of the strong color during a reaction (which we have observed during this work) can indicate that the catalyst is defragmenting and loosing its catalytic power. In Figure 15 the initial color is illustrated in a sample collected from experiment 17 (catalyst=CuPcS):

Figure 19. Sample taken from experiment 17

2.3. **Standard galactose oxidation procedure**

Experiments were performed in the batch and semi batch glass reactor of 1000 mL with raw materials mentioned previously. The reactor is showed in Figure 16.

Figure 20. Batch and semi-batch reactor.

All experiments were done to match an initial galactose concentration of 2.0 wt% i.e 5.36g of galactose was diluted in 250 mL of deionized water at room temperature, subsequently added into the reactor. Afterwards, the reactor had been heated to the desired temperature (these temperatures varied between 50-60 °C) the experiment was started. By means of the pH control, the pH was adjusted the solution to the desired pH value.

In the first set of experiments (1-9), hydrogen peroxide was added instantaneously to the batch reactor. However, this amount of oxidant destroyed the catalyst (the intense color disappeared rapidly) and therefore the conversion of galactose into products was very low (less than 10%). In the second set of experiment (10-28), hydrogen peroxide was added continuously by means of a pump to the semi batch reactor, this design extends the catalyst lifetime. 0.16M was the concentration of

hydrogen peroxide in several experiments due to a higher concentration destroyed the catalyst of the reaction. For revealing the decomposition process of hydrogen peroxide, experiments with catalyst, galactose and H_2O_2 were performed at three different temperatures.

Figure 21. Reactor with homogeneous catalyst

Before the experiment, a specified amount of catalyst was dissolved in 20 mL of deionized water at room temperature, typically the amount of catalyst was 37 mg, corresponding to a catalyst-to-galactose molar ratio of 1:143. In some experiments (18 and 28), different amounts were used in order to investigate the catalyst amount on kinetics. Subsequently, it was added to the reactor. At the beginning of the experiment, pH 7 and batch reactor were selected based on previous work derivated to the oxidation of starch by means of hydrogen peroxide (Tolvanen et al., 2013). It was observed that when the reaction started, the colour of the catalyst solution disappeared 3-4 hours later. The strong color in the semibatch mode experiments indicated that the catalyst is more stable at these conditions, since the hydrogen peroxide concentration remains lower during reaction. It can therefore be postulated that semibatch mode allows a longer catalytic experiment, and it was thus decided to perform the remaining experiments in semibatch.

2.4. Procedure for hydrogen peroxide and catalyst concentration experiments

All the experiments were conducted in a 1L reactor with recirculating water inside the reactor jacket to keep the temperature. The reactor was a stirred tank, which ensured homogeneous mixing during the experiment. Hydrogen peroxide was added in two different ways:

- At the beginning of the experiments with a batch reactor, it was added instantaneously.
- With a semi-batch reactor, hydrogen peroxide was added continuously during 7-8 hours with a pumping speed of 2.7 mL/h.

In the batch reactor experiments, a specific amount of galactose (5.5 g, 2% wt.) was diluted in 250 ml of deionized water at room temperature, and the solution was heated up until the temperature and the pH were adjusted to the desired level. Regarding the hydrogen peroxide concentration, all the experiments were carried out with an amount of 0.6M. Concerning the catalyst concentration, experiments were performed with the same amount (35 mg) but changing the catalyst (FePcS, CuPcS, CoPcS).

In the semi-batch reactor, the same amount of galactose and water were heated up to the desired temperature and the pH was adjusted to the desired level. However, hydrogen peroxide was added continuously and the concentration was changed to 0.1-0.16M. In order to reach this concentration, hydrogen peroxide (30% wt in water) was used and diluted with deionized water to reach a concentration of 0.16M. Subsequently, this solution was added to the experiment by means of a springe pump. Concerning to catalyst concentration, experiments were done with different amounts of the catalyst for studying the relationship between the catalyst and the final products.

Table 1. Experiments with different amounts of catalyst and H_2O_2 concentrations.

H_2O_2 Concentration	Batch Reactor	Semibatch Reactor
0.6M	x	x
0.16M	x	x
0.1M		x
Mass of catalyst	**Batch Reactor**	**Semibatch Reactor**
35 mg	x	x
70 mg		x
105 mg		x

2.5. Product analysis

2.5.1 Hydrogen peroxide titration

During these experiments, two methods were used for analysing the hydrogen peroxide. First, laboratory documentation (Tolvanen, 2021) was used to apply the first method. To perform the analysis, several samples of 0.2 gr were withdrawn and placed in a 250 mL Erlenmeyer flask. To avoid decomposition of hydrogen peroxide, samples were analysed within 30 minutes after they had been withdrawn. Subsequently, 10 mL of sulphuric acid was added. After that, 1 g of potassium iodide was added to the mixture, in that point the mixture changes the colour; depending the amount of hydrogen peroxide it can be yellow or orange, in this last case would be the highest concentration. Three droplets of $(NH_4)_2MoO_4$ was added and finally was titrated with 0.1 N sodium thiosulfate, the colour of the solution changes from orange to transparent-blue. The following reactions occurred:

$$H_2O_2 + 2I^- + 2H^+ \rightarrow I_2 + 2H_2O$$

$$2S_2O_3^{2-} + I_2 \rightarrow S_4O_6^{2-} + 2I^{2-}$$
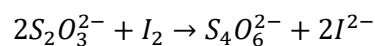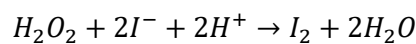
The hydrogen peroxide concentration was calculated with the following equation:

$$M_{H_2O_2}\left(\frac{mol}{L}\right) = 5 \cdot 10^{-5} \cdot \frac{V_{Na_2SO_3(mL)}}{m_{sample\,(gr)}}$$

Where $M_{H_2O_2}$ is the concentration of hydrogen peroxide, $V_{Na_2SO_3}$ is the volume of sodium thiosulfate (in mL) and m_{sample} is the mass of the sample in grams (typical amount of sample was 0.10-0.20 g).

The second method was based on Greenspan and MacKellar method (Greenspan & Mackellar, 1948). The reaction stoichiometry is,
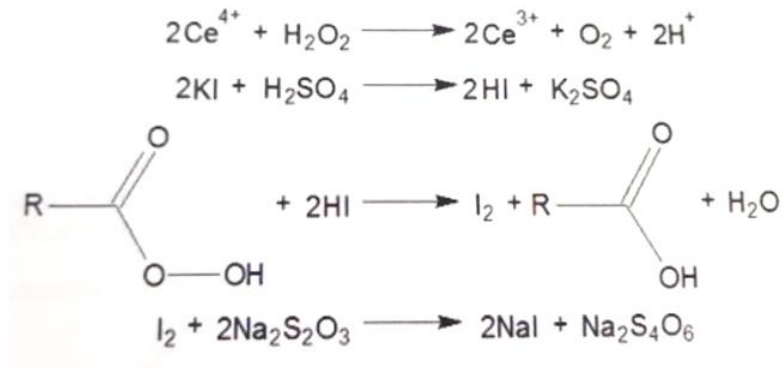
Figure 22. Greenspan and Mackellar analysis mechanism.

In this method 0.1 gr of samples were taken and placed in a 250 mL Erlenmeyer flask. 50 mL of 10% sulfuric acid solution was added, a piece of ice was put into the flask to decrease the temperature to 10°C or below. After that, three droplets of ferroin indicator were added and the solution was titrated with ammonium cerium sulfate from orange colour to light blue. The expression for calculating the percentage of hydrogen peroxide is:

$$wt\%H_2O_2 = \frac{M_{cerium} \cdot V_{cerium} \cdot MM_{H_2O_2} \cdot 100}{2 \cdot m_{sample}}$$

2.5.2 Gas chromatographic analysis (GC)

For a qualitative analysis of the samples in order to identify formed products, gas chromatography was used by Wood and Paper Laboratory at Åbo Akademi. Before the analysis, the samples were freezed and dried in a freeze drying. After that, 40 μL of pyridine, 160 μL BSTFA and 40 μL TMCS were added. In addition, samples were kept a few seconds in an ultrasonic bath and then in the oven at 70°C for 30 minutes. Finally, the samples were shooked few seconds and pipetted into test tubes.

For the definite analysis, 5 mL of acetone was added to the samples and then 1 μL was injected in the column. The size of HP-1 column was 30 m x 0.2 mm x 0.11 um. The column was heated as follows: from 60° C, increased to 320° C at 6° C/min.

2.5.3   High performance liquid chromatography (HPLC)

The analysis of galactose and products were performed by high performance liquid chromatography (HPLC, VWR Hitachi Chromaster) equipped with a 300x7.8 mm Bio-Rad Aminex HPX-87C column and a refractor index (RI) detector (VWR Hitachi Chromaster 5450). The operating temperature of the column was $80°$ and a flow rate 0.6 ml/min using 1.2 mM $CaSO_4$ as the eluent, resulting in a column backpressure raging between 80-85 bar. The injection volume of the sample was set to $10 \ \mu L$.

The products from galactose oxidation were done by comparing the retention times with available standards. The amount of product concentrations obtained in the experiments were calculated by calibration of sample series with known concentrations. The slope from the linear plot of the concentration as a function of the peak area was used to determine quantitatively the concentrations. The remaining data concerning calibration are available in Appendix.

3 RESULTS AND DISCUSSION

3.1 Experiment matrix

To follow the discussion of results in a smooth way, an experiment matrix was created (Table 2), which lists all performed kinetic experiments in a chronological orderand highlighting the different variables (or conditions) that were alternated. The different variables were: the type of reactant (galactose or arabinose), the regulated pH value (non =not regulated, and 3,4,5,6,7,8), type of catalyst (Fe-Pcs, Cu-PcS, Co-PcS) the reactor configuration (H_2O_2 in batch or semibatch), and the total added amount of H_2O_2 (0.10, 0.16, 0.60 mol/L) recalculated to molarity.

Table 2. Experiment Matrix

Galactose Oxidation	Reactant		ph							Active metal in MPcS cat.			Reactor config.		Conc. H_2O_2 mol/L		
Experiments	Galactose	Arabinose	non	3	4	5	6	7	8	Iron	Copper	Cobalt	Batch	Semi-Batch	0.6	0.16	0.1
Experiment 1								x		x			x		x		
Experiment 2									x	x					x		
Experiment 3	x							x		x			x		x		
Experiment 4	x								x	x			x		x		
Experiment 5	x								x			x	x		x		
Experiment 6	x							x		x			x		x		
Experiment 7	x							x		x				x	x		
Experiment 8	x							x						x	x		
Experiment 9	x							x		x			x		x		
Experiment 10	x							x		x				x			x
Experiment 11	x		x							x				x			x
Experiment 12	x		x									x		x		x	
Experiment 13	x		x								x			x		x	
Experiment 14	x					x					x			x		x	
Experiment 15		x				x				x				x		x	
Experiment 16	x					x					x			x		x	
Experiment 17	x					x					x			x		x	
Experiment 18	x					x				3x				x		x	
Experiment 19	x					x								x		x	
Experiment 20	x						x			x				x		x	
Experiment 21	x				x					x				x		x	
Experiment 22	x			x						x				x		x	
Experiment 23	x		x							x				x		x	
Experiment 24				x						x				x		x	
Experiment 25	x					x				x				x		x	
Experiment 26	x			x						x (Het.)				x		x	
Experiment 27	x			x						x			x			x	
Experiment 28	0.5x			x						2x				x		x	

At the beginning of these studies blank experiments were done in order to see how affect the temperature to the hydrogen peroxide (Table 2 entry 1-2). After this, the first experiments with a sugar reactant (entries 3-6,9) were carried out as batch mode adding all reactants and catalyst immediately at the start of the experiment. Subsequently, it was found that huge amounts of hydrogen peroxide somehow affected the catalyst and therefore the sugar oxidation reaction was retarded for some reason. Several observations were indicated this phenomena, such as the rapid discoloration of the reactant solution and low conversion of the reactant when analyzed with GC-MS- Thus, some actions

actions such as the decreasing of concentration of hydrogen peroxide and the change of mode from batch to semibatch reactor were performed.

Experiments from entries 10 to 26 where subsequently performed in semibatch mode (adding H_2O_2 slowly) and using three different MPcS catalysts (active metal Fe, Cu or Co), and additionally varying the concentrations of the catalyst or the amount of H_2O_2 .At the middle of our research (entries from 14 to 28), HPLC analyses were performed to see the products which were obtained.

3.2   Batch experiments

3.2.1   Effect of temperature on the H_2O_2 decomposition

The effect of temperature was investigated in batch mode(experiments 1,3) by using two different temperatures. The temperature was controlled by a reactor jacket filled with distillated water.

Based on the previous studies of doctor P.Tolvanen (Tolvanen et al., 2011), these experiments were carried out under similar conditions: reaction temperatures 50° and 60°C, a stirring rate of 500 rpm, a pH of 7 and a concentration of hydrogen peroxide of 0.6M. Iron tetrasulfophthalocyanine (FePcS) was the only catalyst used in the study of temperature effect. It can additionally pointed out, that in Tolvanen's work the reactant was solid starch particles, and therefore their study was restricted to temperatures below the gelationation temperature of starch (60°C). Moreover, at higher temperatures than 60°, H_2O_2 starts to rapidly decompose which may not be desired in our current study.

The consumption of hydrogen peroxide during the experiment is shown in Figure 19. The kinetic curves of H_2O_2 were different in each experiment.
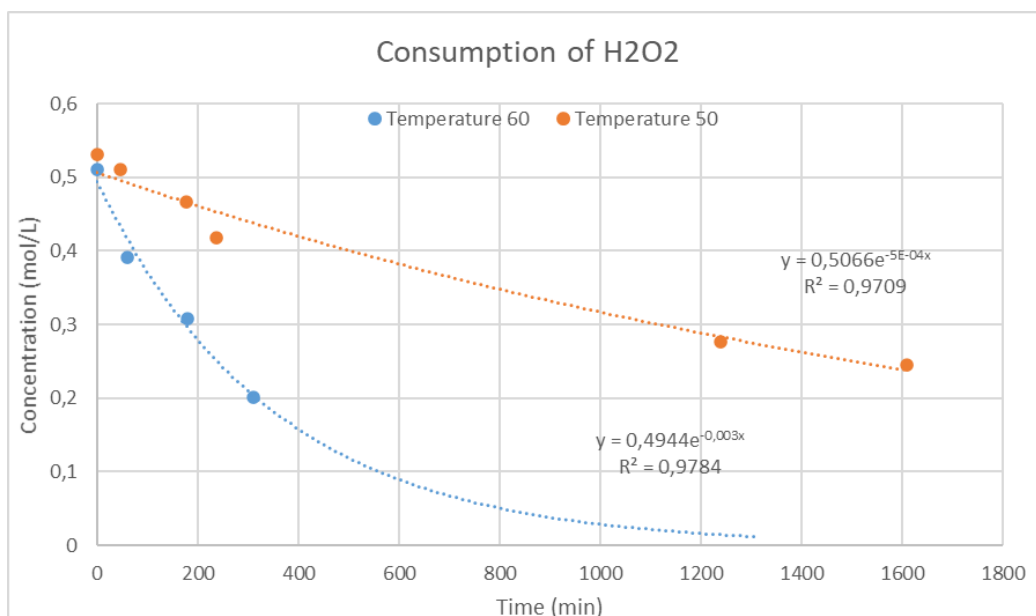

Figure 23. The concentration of hydrogen peroxide in the absence of sugars as a function of time, at temperatures 50 and 60°C and in the absence of sugar reactant. The dots represent experimental values, the dashed lines represent an exponential fit of the dots.

3. Results and discussion

In these experiments, the concentration of hydrogen peroxide can be roughly said to exponentially decrease vs reaction time. Considering these results, it can be concluded that the kinetic curves are influenced by the temperature, if it is increased 60 degrees, the consumption of hydrogen peroxide is clearly faster than at 50 degrees. Increasing more the temperature might damage the catalyst due to the high temperatures and hydrogen peroxide would decompose.

3.2.2 Effect of pH

The effect of pH on the oxidation of galactose in batch reactor was investigated (experiments 1,3) by conducting experiments at two different pH values 7 and 8. The influence of pH in these experiments on the H_2O_2 is illustrated in Figure 20. During these early experiments, the kinetics of product formation was not yet developed. The pH was controlled and kept constant with a pH-Stat device (790 Titrando, Metrohm AG) This device controlled the pH adding NaOH (strength 0.1M) to maintain the desired pH. Note that in figure 20, the results are normalized in order to get a better understanding of them.
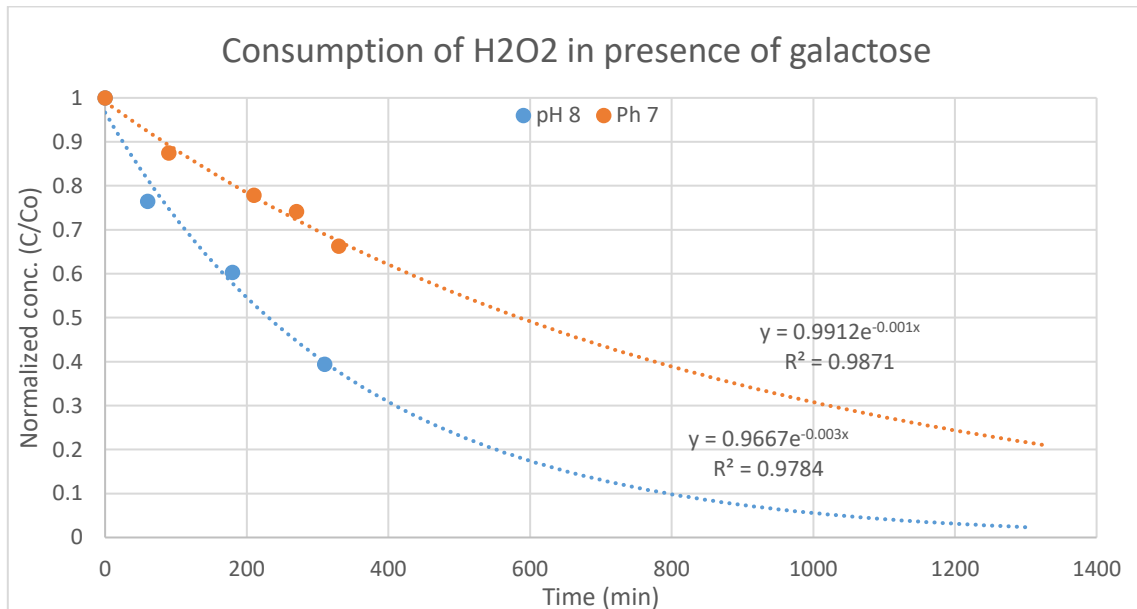

Figure 24. Behaviour of the H_2O_2 at different pH values.

The results display that the consumption of hydrogen peroxide during the experiment was faster at basic pH. However, operating at more basic pH can affect to the hydrogen peroxide stability and therefore the instability of this compound during the process is revealed as it has been mentioned by other authors, as well (Sorokin et al., 2004).

3.2.3   Effect of type of reactor operation: batch and semibatch

In this project one of the most difficult challenges was to obtain a good conversion of galactose and a good yield in new products. During these experiments, it was very important to consider the amount of hydrogen peroxide and catalyst. If the concentration of hydrogen peroxide was too high, the catalyst could be deactived or even destroyed, i.e. by defragmentation (Genuino et al., 2021)

Iron tetrasulfophthalocyanine catalyst has a specific colour, dark blue, when it is dissolved in water. During the experiment, the colour became gradually more and more light until the solution turned transparent or yellow, if the volume of sodium hydroxide was considerable (due to the control of pH). Therefore, a good qualitative way in order to know the concentration of remaining active catalyst during the course of reaction is the colour of the solution.

The colour of the solution is due to the metal phtalocyanines which have a characteristic colour (dark blue) when are dissolved in water. When hydrogen peroxide reacts with the catalyst forming products, the catalyst concentration decreases and therefore the colour of the solution becomes more transparent.
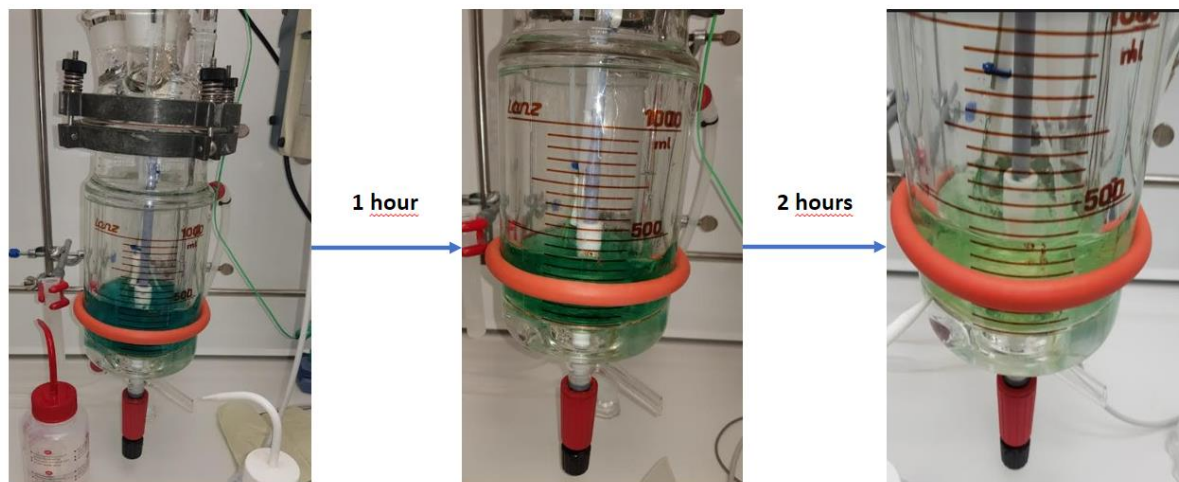

Figure 25. Different colours during the experiment 7.

For this reason, one of the backbones of this project was to maintain the colour of the catalyst solution during the experiment. The catalyst life time depends on the hydrogen peroxide concentration; if the amount of hydrogen peroxide was high the change of colour was faster. Therefore it was decided to decrease the concentration of H_2O_2 and to use the semibatch configuration, in order to maintain relatively low concentrations during the entire experiments.

3. Results and discussion

In addition to H_2O_2 conversion, HPLC analyse were made to check if the conversion of galactose might have increased in the semi batch reactor. Next figures show the difference between semibatch a batch reactor. The kinetics of semibatch experiments will be explained more in detail later (Section 3.3.2).
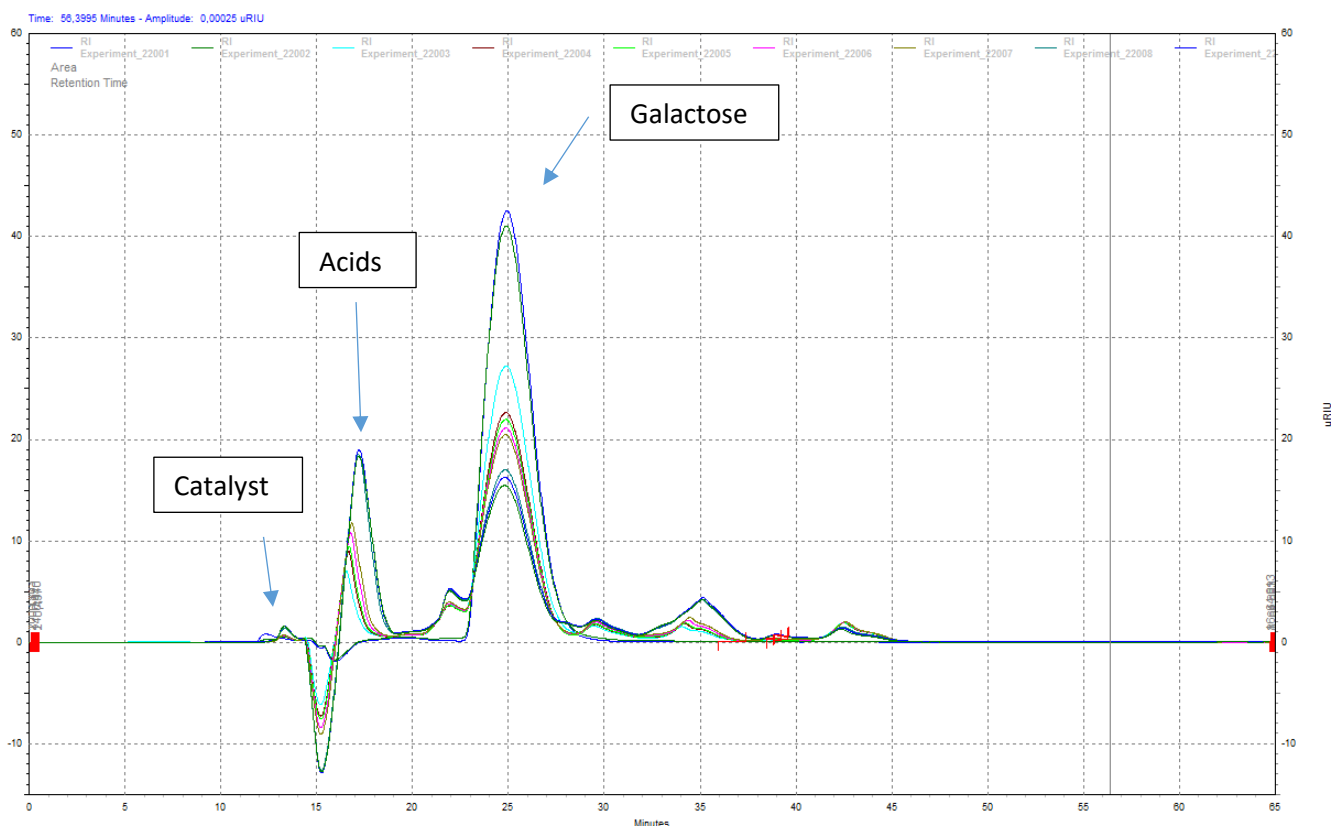

Figure 26. Kinetic visualization of the formed products in experiment 15. The blue line is the zero sample. X-Axis is the retention time, y-axis is the peak height.

HPLC chromatograms show the consumption of galactose and the formation of products. As figure 22 shows, the first peak, represents a mixture of several acids. The third peak represents galactose in the experiment. The calibration curve of this area corresponds to a galactose concentration of 0.162 M. Concerning to the acids, the graphic shows an area of 21.258.004 which means a significant concentration if it is compared with the concentration of galactose.
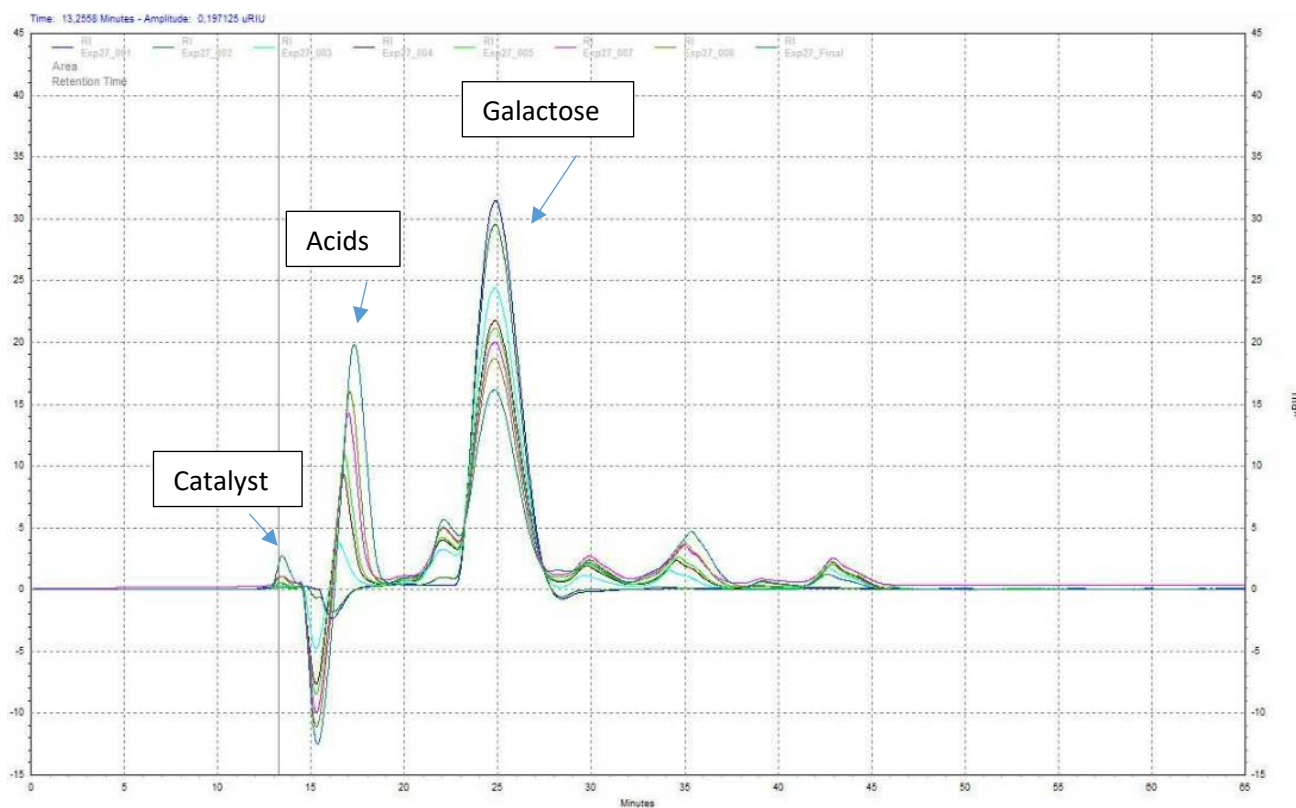
3. Results and discussion

Figure 27. HPLC analysis of samples from batch reactor

This graphic in Figure 23 could indicate that the initial concentration of galactose is lower (0.12M) than the Figure 22, even though the same amount of galactose was added in both experiments. An explanation might be that when hydrogen peroxide was added, a small amount of galactose was destroyed due to the high concentration of H_2O_2. Furthermore, the area of acids is over 11.5 million a.u which was two times less than in the semi batch reactor. Another interesting observation is that the catalyst concentration in the solution is higher than semi batch reactor, thus, it might be that the reaction has a lower yield compared to semi batch mode. Based on this experience, it was decided to use the semibatch reactor in the subsequent experiments.

3. Results and discussion

3.3 SEMIBATCH EXPERIMENTS

3.3.1 Catalyst concentration

As explained in a previous article (Genuino et al., 2021), it is necessary to control the ratio of H_2O_2 and catalyst concentration. For this reason, several experiments at pH 5 (19,18,25) were carried out in order to find the optimal concentration for obtaining acid products. Generally the mass of catalyst (FePcS) was 37,5 mg and the concentration of hydrogen peroxide was 0.16M. The results are displayed in Figure 24 and 25.
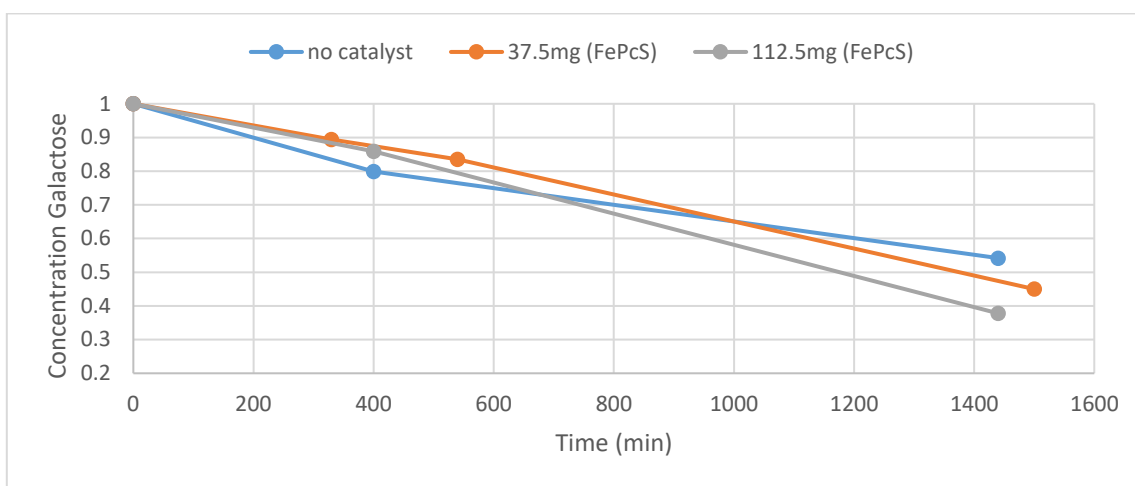
Figure 28. Concentration of galactose in experiments 18,24,28 with different amount of catalyst.

In light of these results, it can be assumed that a high catalyst concentration improves the conversion of galactose.
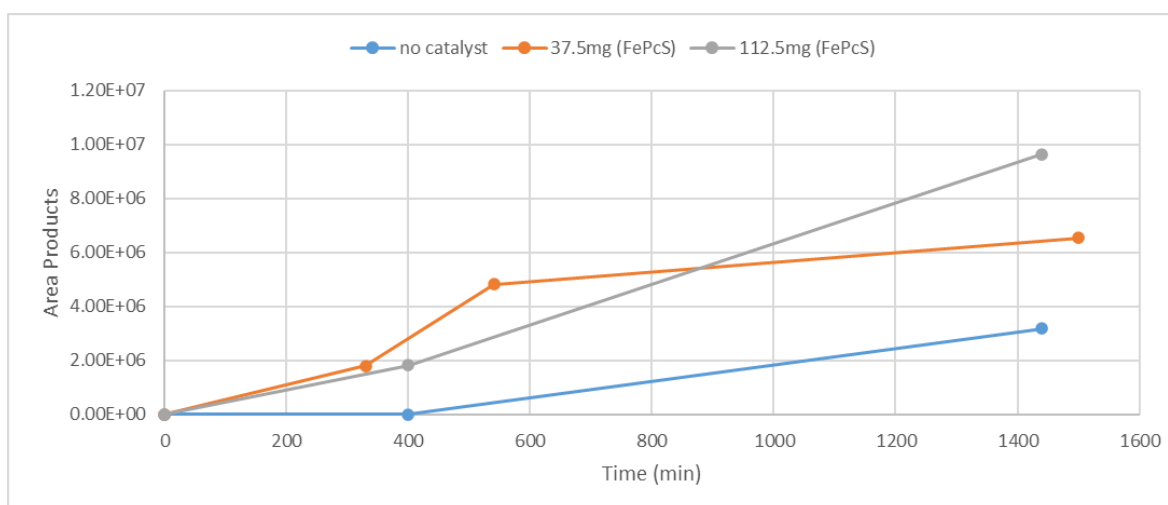

Figure 29. Yield of products in several experiments with different amount of catalyst.

35

3. Results and discussion

The kinetic product curves of each experiment with different mass of catalyst are displayed in Figure 25. The results show that if the mass of catalyst is high, the product formation will be higher than in those experiments having a lower catalyst concentrations.
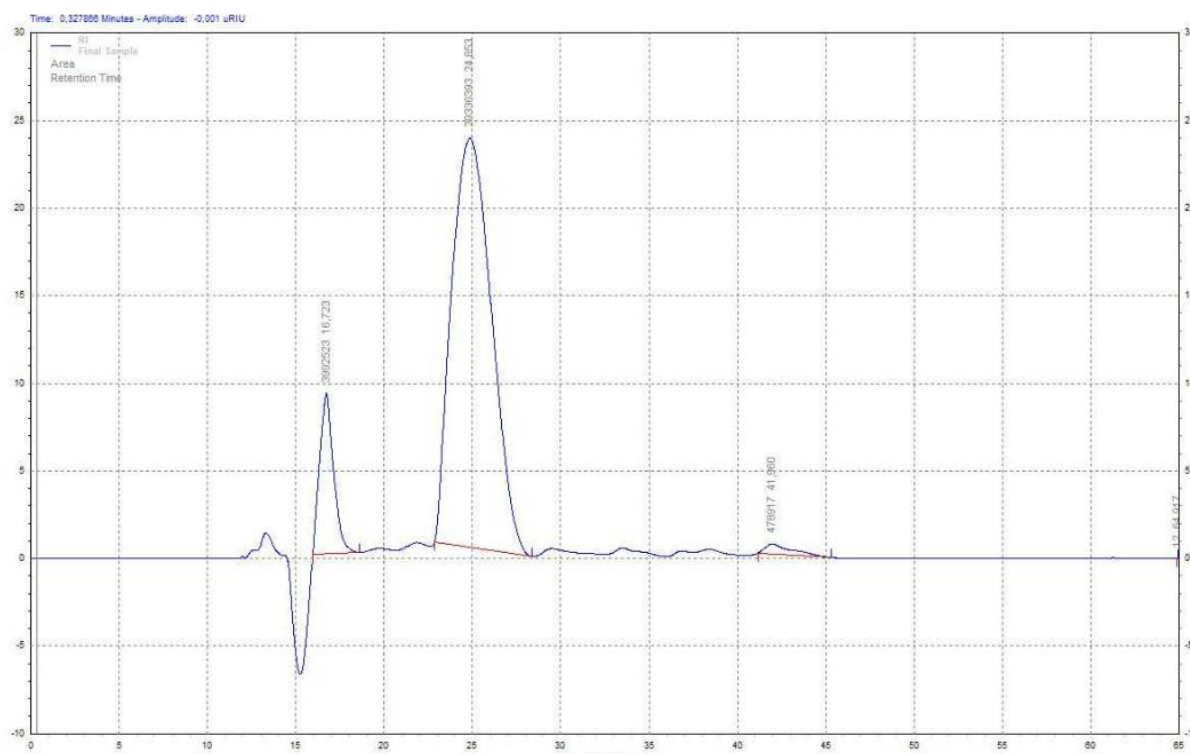

Figure 30. Experiment without catalyst

The HPLC results from experiment 19 are shown in Figure 26. This experiment was conducted without catalyst. Three peaks appear in the chromatography; the first one is assigned to acids formed in the experiment, the second is unreacted galactose (RT = 25 min) and finally a third peak (RT = 42 min) which was not identified. It can be concluded that the concentrations of acids increase with an increasing catalyst concentration.
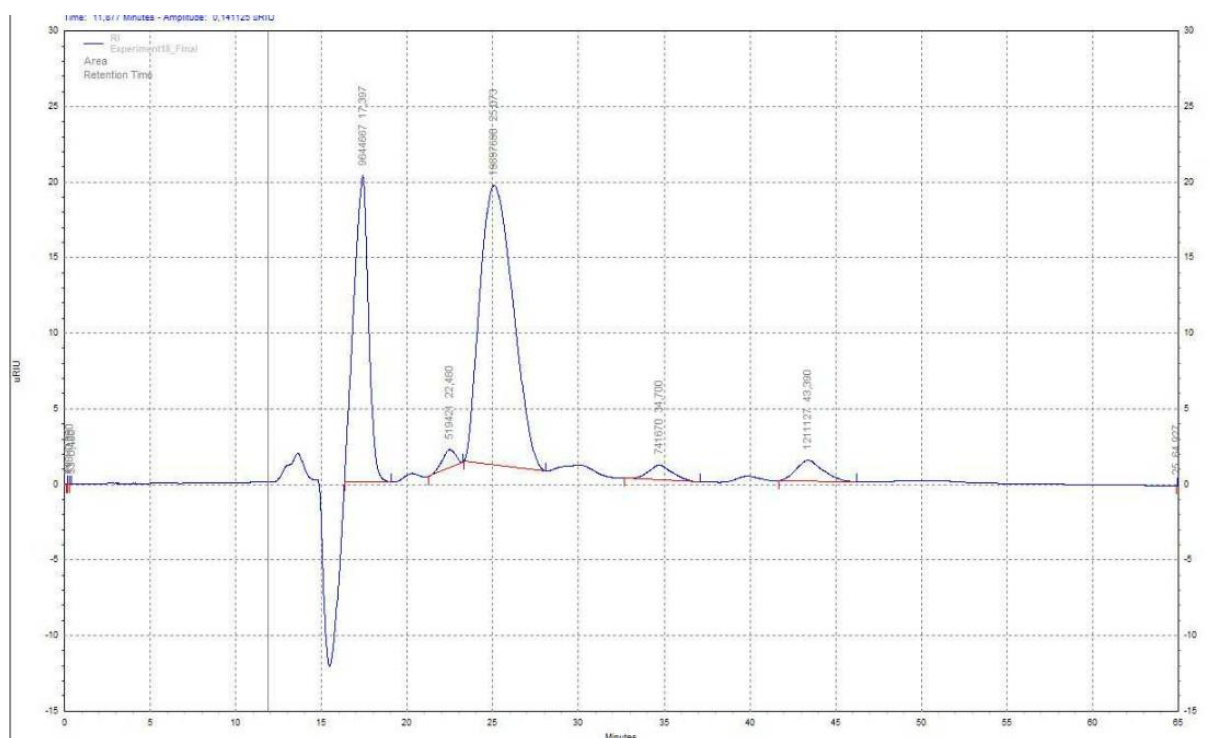
3. Results and discussion

Figure 31. Experiment with three times the mass of catalyst.

The increase of acid peak is due to the increase of catalyst in the experiment is illustrated in Figure 27. Another significant peaks appeared but these compounds were not identified.
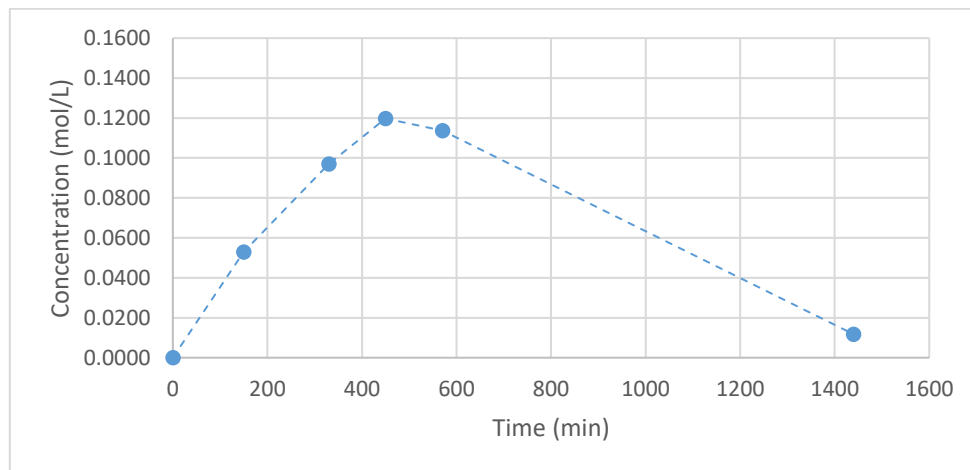

Figure 32. Conversion of H_2O_2 in semibatch experiment 18

Regarding the concentration profile of hydrogen peroxide, Figure 28 shows an increase of the catalyst mass enhances the consumption velocity of this. In this experiment, the concentration of H_2O_2 reached at highest point the value 0,12M, whereas the theoretical maximum value was 0.16M; it is possible that one part of H_2O_2 was reacting, decomposing during the experiment. On the other hand, the final concentration was around 0.01M, therefore almost all H_2O_2 was consumed in the experiment.
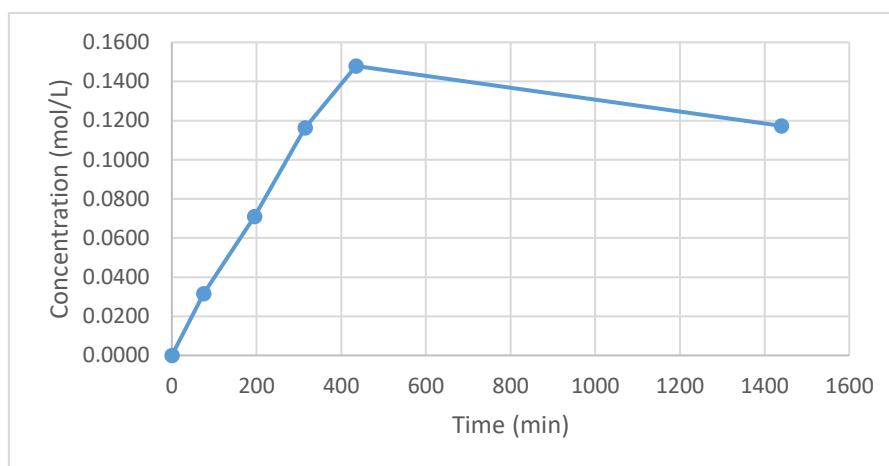
3. Results and discussion

Figure 33. Concentration profile of hydrogen peroxide in semibatch experiment 19

Figure 29 illustrated the concentration profile of H_2O_2 in experiment 19. Compared with other experiments the consumption was slower, and this was because the experiment was carried out without catalyst. As you can see the initial concentration was similar, reaching almost 0.16M. However, the final concentration was 0.12M, so it was assumed that the amount of catalyst improved the reaction between H_2O_2 and galactose.

3. Results and discussion

3.3.2   Effect of pH

The study of pH the effect was made to optimize the oxidation conditions during the experiment, keeping the same temperature as in batch reactor,60°C. Figure 30 shows experiments 20,21,22 and 25 which were conducted at different pH values similar concentrations of galactose and hydrogen peroxide.
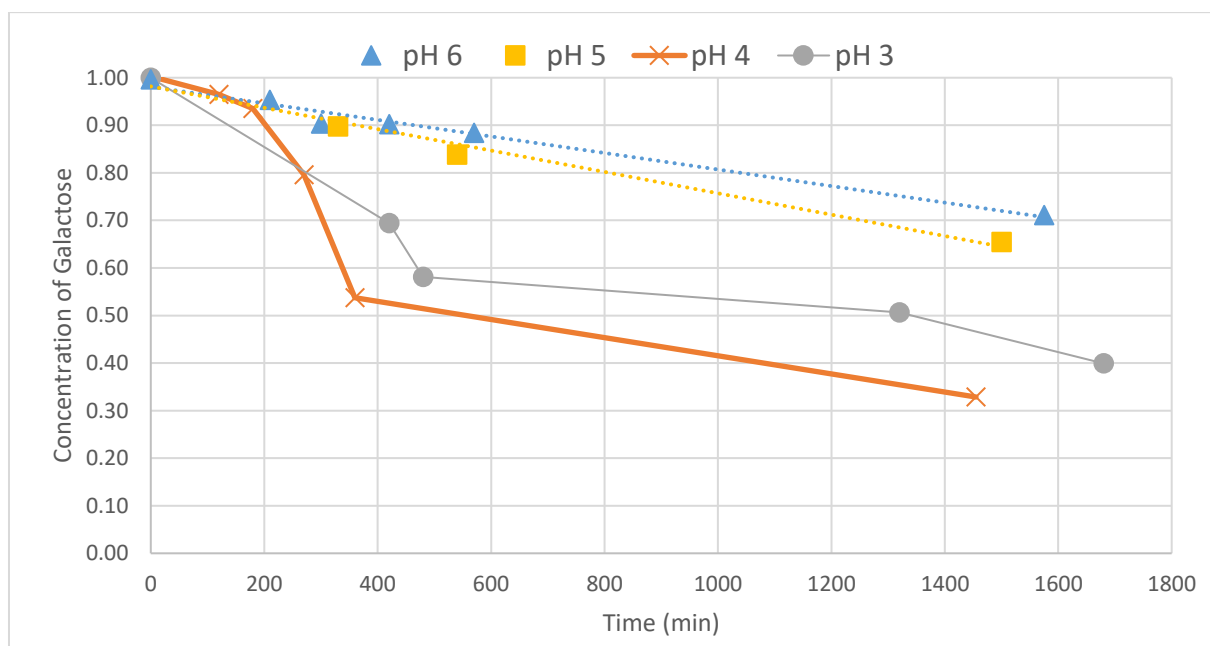

Figure 34. Concentration of galactose at different pH values.

Figure 30 displayed that the maximum conversion of galactose appears at pH 4. Figure 31 helps to understand that the best pH is the most acids one. The product yields are shown in Figure 31:
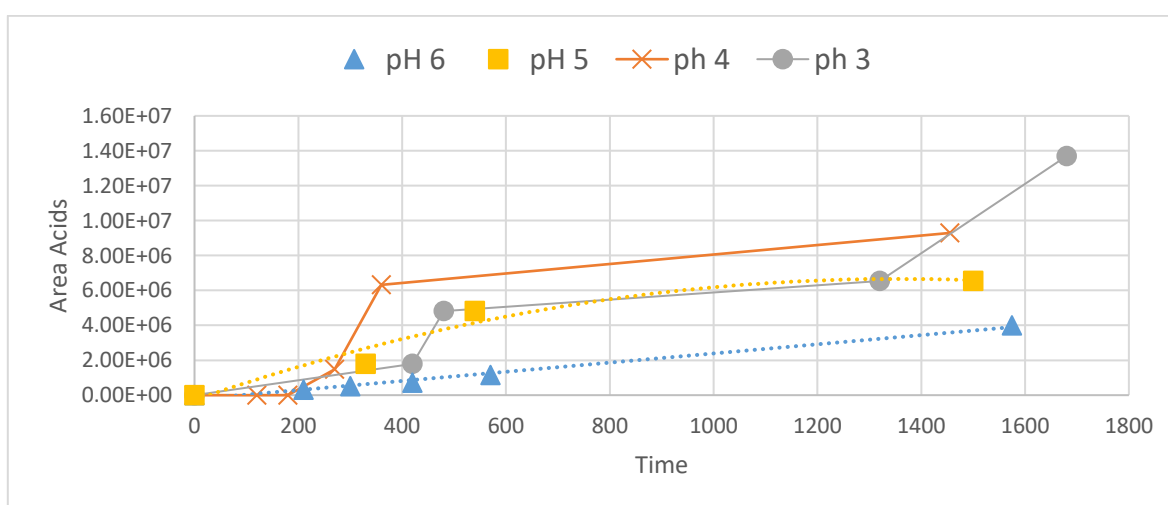

Figure 35. Area of acids at different pH values

The highest yields of acid products were obtained when using a reaction pH level of 3.

3. Results and discussion

3.3.3 Chemical analysis of samples

3.3.3.1 HPLC analysis

During all the experiments, one of the most difficult challenges was the compound identification. For this raison, two kinds of analysis were used GC-MS and HPLC. Regarding HPLC, two columns were used; the first one was showed previously for revealing parameters such as conversion and yield. However, there were many difficulties for finding which peak corresponded to each acid, therefore, a second specific column for acids was applied.

Table 3. Retention times of plausible compounds

Compound	RT (min)
Glucuronic Acid	9,409
Galactonic Acid	11,405
Gluconic Acid	10,687
Acetic Acid	10,767
Formic Acid	17,149
Levulonic Acid	20,678
Lactic Acid	15,622
Ethyl - Glicol	19,826
Sorbitol	12,393

For performing the analysis, several pure compounds were used in order to reveal which retention time they had in the column. As retention times were obtained, these were compared with final samples from other experiments.
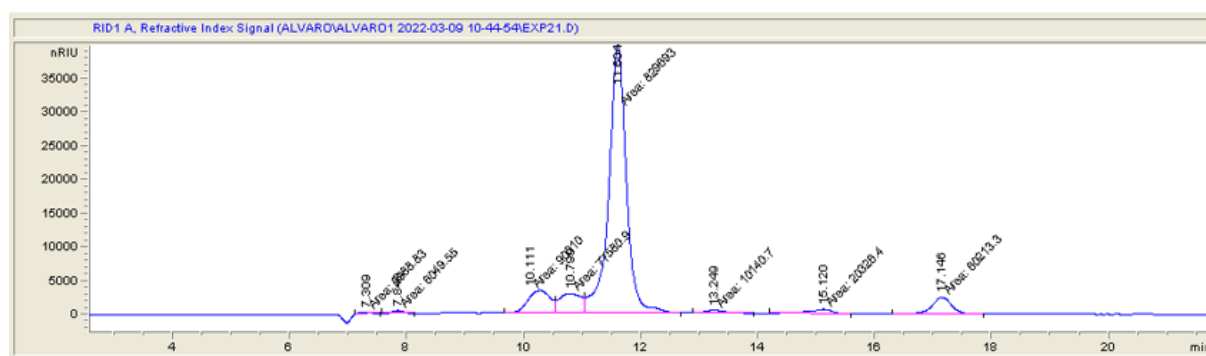

Figure 36. HPLC analysis, experiment 21

The HPLC analysis to the final sample of experiment 21 is displayed in Figure 32. The parameters in this experiment was a pH=4, hydrogen peroxide concentration 0.16M and 37 mg of FePcS.
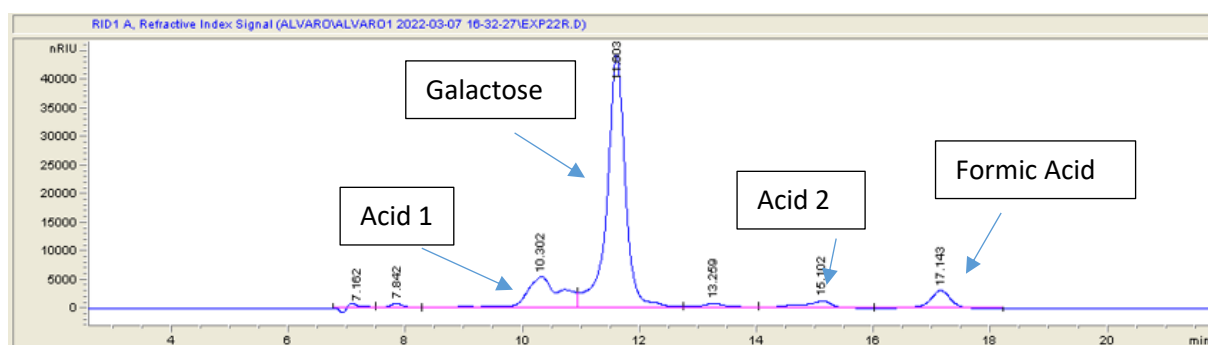
3. Results and discussion

Figure 37. HPLC analysis, experiment 22

The HPLC analysis at the final sample of experiment 22 is shown in the figure 33. The pH at this experiment was 3 (as previously mentioned, it is better to work with low pH) the others parameters were identical with these in experiment 21.

Formic acid with a retention time of 17.143 minutes was confirmed, rest of compound were not identified until NMR and GC-Ms analysis. However, the yield of formic acid was higher with a low pH; while at pH=4 the yield was 5.26%, at pH=3 the yield was 9,79%.

However, the amount of unreacted galactose was high. Therefore, the next step was to increase the amount of catalyst and decrease the concentration of galactose, to 2 wt% (experiment 28).
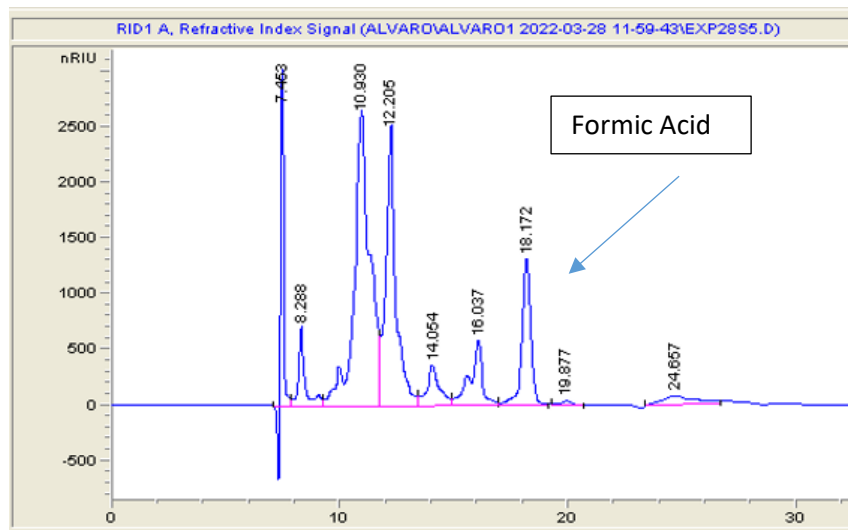

Figure 38. HPLC analysis of experiment 28

The final experiment 28 gave really good results, as you can see in the figure 34. The conversion of galactose was almost completed, at least there was no peak at 11.6, but instead a peak at 10.9 and at 12.2 had appeared. The presence of formic acid was confirmed in this experiment.

The reason behind the success of these results was the increase of Catalyst –to-galactose mass ratio. In most of the experiments the ratio was:

3. Results and discussion

$$Mass\ ratio = \frac{Mass\ catalyst}{Mass\ Galactose} = \frac{37mg}{5320\ mg} = 0.007\frac{mg\ catalyst}{mg\ galactose}$$

After these experiments, it was decided to increase this ratio in an exponential way in order to see if the amount of galactose was too high. Keeping the same pH=3 at 60°C, the concentration of galactose was decreased from 2 wt% to 0.5wt%. In addition, the amount of catalyst was increased from 35 mg to 70 mg.

$$Mass\ ratio = \frac{Mass\ catalyst}{Mass\ Galactose} = \frac{70\ mg}{1330\ mg} = 0.052\frac{mg\ catalyst}{mg\ galactose}$$

Next step was to discover which compounds could be peaks which were watched in the HPLC analysis. Two types of analysis were made, GC – MS and NMR.

3.3.3.2 GC-MS analysis

In order to discover which kind of compounds were present in the samples withdrawn from the reactor GC-MS analysis was carried out. Characteristics of these analyses were described in chapter 2 of this thesis.

Several experiments were analysed with this technique and similar results as HPLC analysis were obtained.
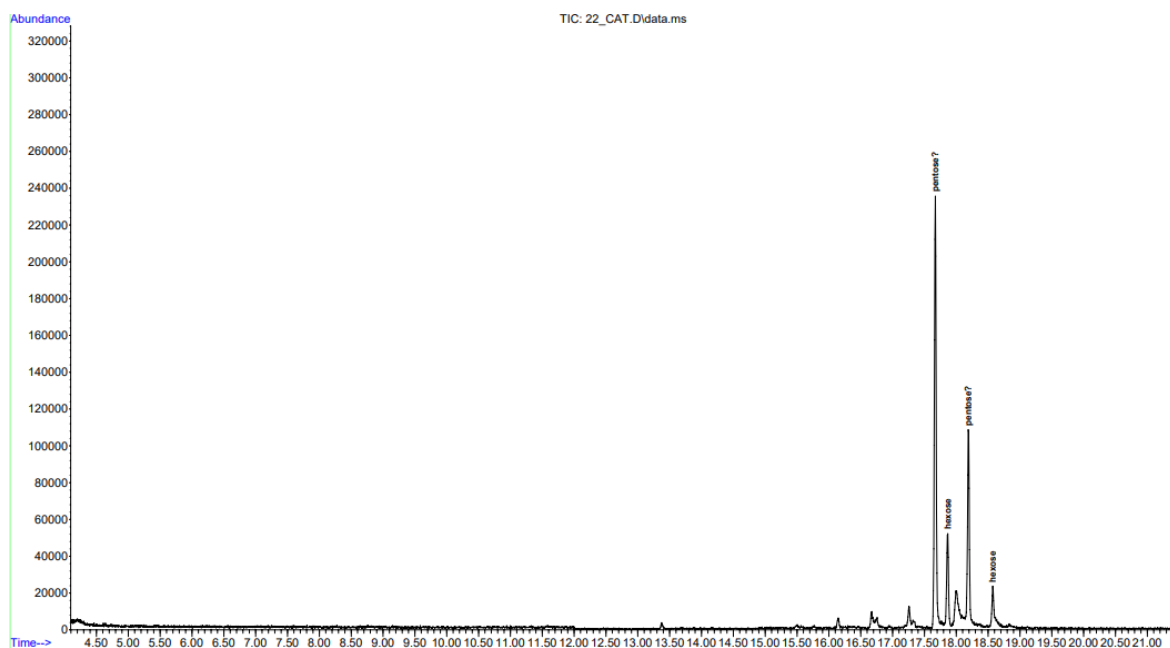

Figure 39. GC-Ms analysis of experiment 22

3. Results and discussion

The results from the GC-MS analysis were in agreement with the HPLC results. Two peaks appeared which had exactly similar mass spectra as silylated xylose (or some other pentoses), but they were eluted among the hexoses, which should not be possible. They can not be hexonic acids because such compounds were available in the MS database, and the mass spectra were not identical to any of those.

The analysis was carried out to the experiment 28 for discover which kind of compounds were in the sample.
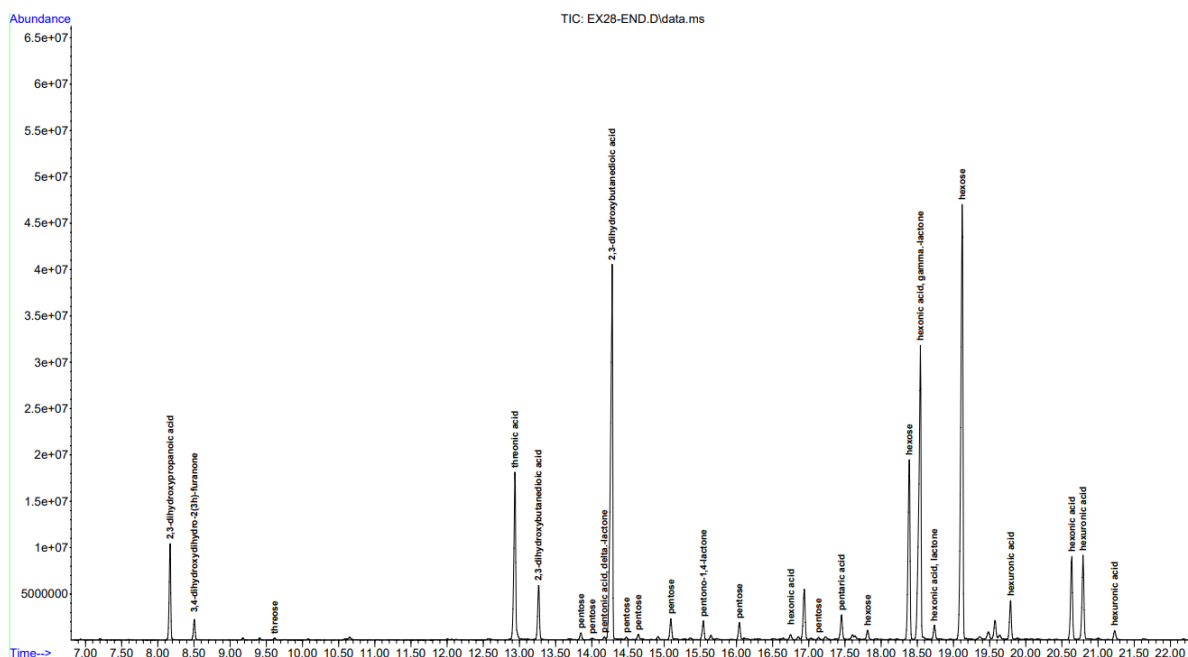

Figure 40. GC-MS analysis of experiment 28

Several compounds were recognized in the GC-MS analysis. The following compounds which were identified are following Table 4 to give a better perception.

Table 4. Main compounds discovered in experiment 28

Compounds
2,3 - dihydroxypropanoic acid or Glyceric Acid
Threonic Acid
2,3-dihydroxybutanedioic acid or Tartaric Acid
Hexonic acid, gamma-lactone
Hexuronic Acid

On the other hand, two samples were withdrawn at the end of the experiment 28 and as the addition of hydrogen peroxide in the semibatch reactor was completed at 400 minutes, a kinetic curve was constructed using the semiquantitative data obtained from GC-MS. This experiment was performed with a pH=5, 2 wt% of galactose and 35 mg of catalyst.

3. Results and discussion

Table 5. GC-Ms from experiment 14

Experiment 14 - Galactose, Fe cat, ph 5			
Compound	400min Conc. %	1200min Conc. %	#
Hexonic acid	0.64	1.59	1
Galacturonic acid	0.16	0.26	2
Threonic acid	0.00	0.10	3
Tetronic acid	0.00	0.62	4
2,3 Dihidroxybutanodioic	0.00	0.11	5
2-Keto-t-hexonic acid	0.00	0.58	6
Galactonic acid	0.00	2.18	7

Table 5 shows that Hexonic acid and for Galactonic acid are main products obtained during this experiment. The behaviour is similar with all the products, the reaction starts when hydrogen peroxide feed is completed, with the exception of hexonic acid which appeared in considerable amount already in the first sample. In addition to this, with the assistance of the department of Organic Chemistry, a series of NMR analyses was conducted. In this way, NMR analysis guaranteed the identification of the compounds.

3.3.3.3 NMR Analysis

During experiment 28, several samples withdrawn at different times for the correct identification of the compounds. Three compounds were identified which could give a explanation on which reaction were taking place in the reactor.

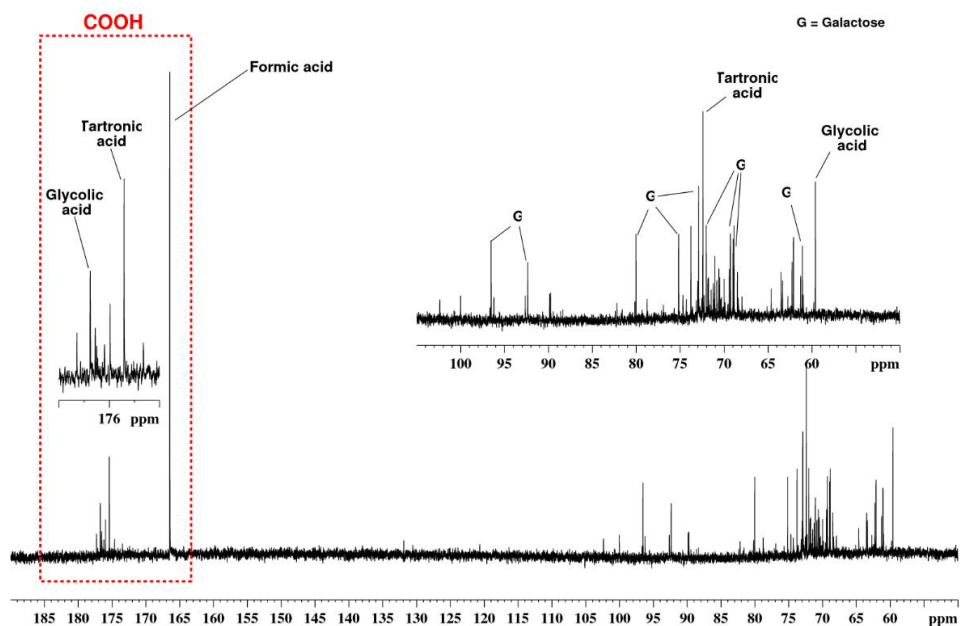
Figure 41. NMR analysis from experiment 28.

One of these compounds was formic acid as confirmed us HPLC analysis previously. This acid reached of an almost 70 weight-% in the sample at low pH. On the other hand, tartronic acid and glycolic acid were identified at the beginning of the experiment and their concentration remained constant after the feeding of hydrogen peroxide had been finished.
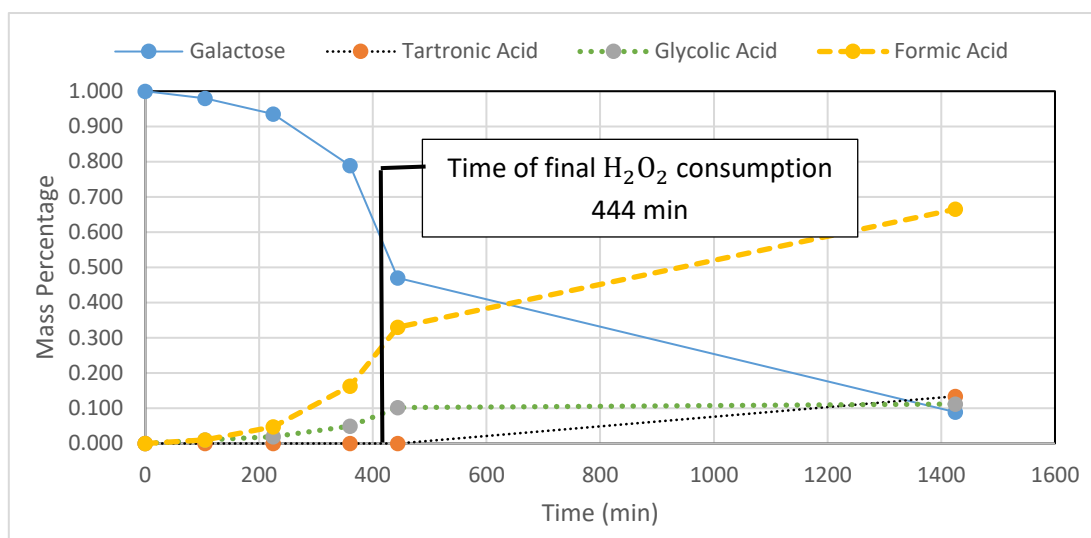
3. Results and discussion

Figure 42. Kinetic Curve from NMR analysis

At the end of the experiment the formic acid concentration reached a 66 wt% and the concentration of tartronic acid and glycolic acid were 13 wt% and 11 wt%, respectively. The concentration of glycolic acid increases at the same time that formic acid as the Figure 38 illustrates:
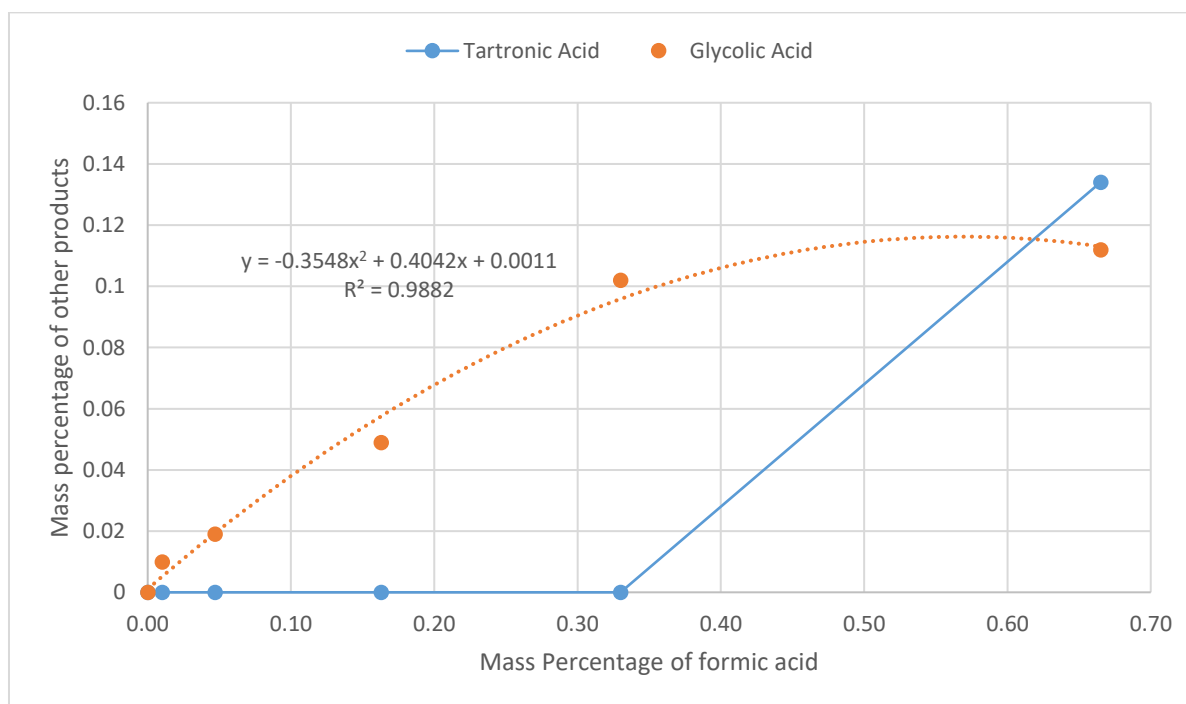
Figure 43. Product comparative which was obtained

The galactose conversion is a 91%. Therefore, with this information, it is possible to identify the reaction sequence which could be a hydrolysis process and subsequently an oxidation process.

$$C_6H_{12}O_6 + H_2O_2 \rightarrow C_3H_4O_5 + C_2H_4O_3 + CH_2O_2 + H_2O$$
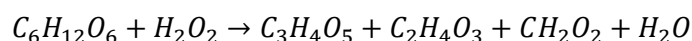
3. Results and discussion

In addition to this, the catalyst change was studied during this project with NMAR analysis, however, unfortunately the concentration of catalyst was too low to be seen properly.

4 CONCLUSIONS AND FUTURE PERSPECTIVES

The aim of this work was to develop a new environmentally friendly method for homogeneously catalyzed oxidation of model compounds from hemicellulose and kinetic data have been recorded from batch and semibatch reactor in order to receive information regarding the products which were generated during the reaction, as well as the conversion of galactose. Numerous batch and semibatch experiments were carried out to receive important data regarding the decomposition of H_2O_2. The effect of catalyst during the reaction has been studied, as well.

Batch and semibatch experiments were performed. In batch experiments were appreciated that the degradation of the catalyst was really fast due to the high concentration of hydrogen peroxide, therefore several improvements in batch mode were carried out such as the decreasing of hydrogen peroxide concentration from 0.2M to 0.16M and the change to semibatch mode.

When semibatch experiments were performed, some improvements such as the optimization of amount of catalyst were conducted. The catalyst ratio is increased from 145:1 to 700:1 (catalyst: galactose). To analyse the results of each experiment different samples were withdrawn and used to perform HPLC, GC-MS analyses.

Regarding the pH, working with an acid pH improved the product yield. As a consequence, several experiments are performed with pH = 3 - 4 in order to analyse what products improved their yield and how affected the change of another parameter (catalyst concentration or hydrogen peroxide concentration).

At the end of our research, last experiment showed a high conversion of galactose in order to reach a 66 w/w% of acetone and and concentration of tartronic acid and glycolic acid of 13 wt% and 11 wt%, respectively. Next step in this investigation would be to improve the yields of glycolic and tartronic acids, because as it was showed previously (section 1.4) these two compounds have several uses in the industry.

Many research opportunities can be derived from the results of this work, such as the improvement of reaction yield and galactose conversion, the optimization of the reaction with a more complex model compound from hemicellulose or with the hemicellulose and the scaling up of the semibatch reactor and hemicellulose oxidation procedures.

4. Conclusions and future perspectives

From the viewpoint of industrial scaling up, the next step could be the use of the prepared catalyst reaction in a semibatch mode and a reactor that allows taking advantage of the high mass and heat transfer rates, and low resistance to the diffusion associated with this kind of catalyst. Furthermore, the oxidation of other compounds containing carbonyl groups can be screened, whether they are individual sugars of high commercial importance such as D-xylose or D-arabinose to produce glycolaldehydes or compounds from the oxidation of lignocellulosic materials such as sugar mixtures or levulinic acid.

5 REFERENCES

Bulushev, D. A., & Ross, J. R. H. (2018a). Towards Sustainable Production of Formic Acid. In *ChemSusChem* (Vol. 11, Issue 5, pp. 821–836). Wiley-VCH Verlag. https://doi.org/10.1002/cssc.201702075

Bulushev, D. A., & Ross, J. R. H. (2018b). Towards Sustainable Production of Formic Acid. In *ChemSusChem* (Vol. 11, Issue 5, pp. 821–836). Wiley-VCH Verlag. https://doi.org/10.1002/cssc.201702075

Cai, J., Ma, H., Zhang, J., Du, Z., Huang, Y., Gao, J., & Xu, J. (2014). Catalytic oxidation of glycerol to tartronic acid over Au/HY catalyst under mild conditions. *Cuihua Xuebao/Chinese Journal of Catalysis*, *35*(10), 1653–1660. https://doi.org/10.1016/S1872-2067(14)60132-7

Coelho, L. C. D., Filho, N. M. L., Faria, R. P. V., Ferreira, A. F. P., Ribeiro, A. M., & Rodrigues, A. E. (2018). Separation of tartronic and glyceric acids by simulated moving bed chromatography. *Journal of Chromatography A*, *1563*, 62–70. https://doi.org/10.1016/j.chroma.2018.05.052

European Comission. (2021). *Towards Zero Pollution for Air, Water and Soil*.

Fukuzumi, S., Lee, Y. M., & Nam, W. (2021). Recent progress in production and usage of hydrogen peroxide. In *Chinese Journal of Catalysis* (Vol. 42, Issue 8, pp. 1241–1252). Science Press. https://doi.org/10.1016/S1872-2067(20)63767-6

Genuino, H. C., Meinds, T. G., Broekman, J. O. P., Staal, M., Brinksma, J., Wielema, T., Picchioni, F., Browne, W. R., Deuss, P. J., & Heeres, H. J. (2021). Iron Tetrasulfonatophthalocyanine-Catalyzed Starch Oxidation Using H2O2: Interplay between Catalyst Activity, Selectivity, and Stability. *ACS Omega*, *6*(21), 13847–13857. https://doi.org/10.1021/acsomega.1c01407

Greenspan, F. P., & Mackellar, D. G. (1948). Analysis of aliphatic per acids. In *Ind. Eng. Chem., Anal. Ed* (Vol. 20, Issue 1). Smith and Wilcox. https://pubs.acs.org/sharingguidelines

Huang, L. Z., Ma, M. G., Ji, X. X., Choi, S. E., & Si, C. (2021). Recent Developments and Applications of Hemicellulose From Wheat Straw: A Review. In *Frontiers in Bioengineering and Biotechnology* (Vol. 9). Frontiers Media S.A. https://doi.org/10.3389/fbioe.2021.690773

Kachkarova-Sorokina, S. L., Gallezot, P., & Sorokin, A. B. (2004a). A novel clean catalytic method for waste-free modification of polysaccharides by oxidation. *Chemical Communications*, *24*, 2844–2845. https://doi.org/10.1039/b411694a

Kachkarova-Sorokina, S. L., Gallezot, P., & Sorokin, A. B. (2004b). A novel clean catalytic method for waste-free modification of polysaccharides by oxidation. *Chemical Communications*, *24*, 2844–2845. https://doi.org/10.1039/b411694a

Klein-Koerkamp, C., Granet, R., Zerrouki, R., Villandier, N., Jérôme, F., Barrault, J., & Krausz, P. (2009a). Efficient oxidative modification of polysaccharides in water using H2O2 activated by

5. References

iron sulfophthalocyanine. *Carbohydrate Polymers*, *78*(4), 938–944. https://doi.org/10.1016/j.carbpol.2009.07.021

Klein-Koerkamp, C., Granet, R., Zerrouki, R., Villandier, N., Jérôme, F., Barrault, J., & Krausz, P. (2009b). Efficient oxidative modification of polysaccharides in water using H2O2 activated by iron sulfophthalocyanine. *Carbohydrate Polymers*, *78*(4), 938–944. https://doi.org/10.1016/j.carbpol.2009.07.021

Kusema, B. T., & Murzin, D. Y. (2013). Catalytic oxidation of rare sugars over gold catalysts. In *Catalysis Science and Technology* (Vol. 3, Issue 2, pp. 297–307). Royal Society of Chemistry. https://doi.org/10.1039/c2cy20379k

Kuznetsov, B. N., Sudakova, I. G., Garyntseva, N. v., Tarabanko, V. E., Yatsenkova, O. v., Djakovitch, L., & Rataboul, F. (2021). Processes of catalytic oxidation for the production of chemicals from softwood biomass. *Catalysis Today*, *375*, 132–144. https://doi.org/10.1016/j.cattod.2020.05.044

Leong, H. Y., Chang, C. K., Khoo, K. S., Chew, K. W., Chia, S. R., Lim, J. W., Chang, J. S., & Show, P. L. (2021). Waste biorefinery towards a sustainable circular bioeconomy: a solution to global issues. In *Biotechnology for Biofuels* (Vol. 14, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s13068-021-01939-5

Li, Y., Ramaswamy, H. S., Li, J., Gao, Y., Yang, C., Zhang, X., Irshad, A., & Ren, Y. (2022). Nutrient evaluation of the seed, pulp, flesh, and peel of spaghetti squash. *Food Science and Technology (Brazil)*, *42*. https://doi.org/10.1590/fst.70920

Lu, Y., He, Q., Fan, G., Cheng, Q., & Song, G. (2021). Extraction and modification of hemicellulose from lignocellulosic biomass: A review. In *Green Processing and Synthesis* (Vol. 10, Issue 1, pp. 779–804). De Gruyter Open Ltd. https://doi.org/10.1515/gps-2021-0065

Ma, R., Xu, Y., & Zhang, X. (2015). Catalytic oxidation of biorefinery lignin to value-added chemicals to support sustainable biofuel production. In *ChemSusChem* (Vol. 8, Issue 1, pp. 24–51). Wiley-VCH Verlag. https://doi.org/10.1002/cssc.201402503

Mckendry, P. (n.d.). *Energy production from biomass (part 1): overview of biomass*.

Navlani-García, M., Salinas-Torres, D., Mori, K., Kuwahara, Y., & Yamashita, H. (2019). Enhanced formic acid dehydrogenation by the synergistic alloying effect of PdCo catalysts supported on graphitic carbon nitride. *International Journal of Hydrogen Energy*, *44*(53), 28483–28493. https://doi.org/10.1016/j.ijhydene.2018.11.057

Pinales-Márquez, C. D., Rodríguez-Jasso, R. M., Araújo, R. G., Loredo-Treviño, A., Nabarlatz, D., Gullón, B., & Ruiz, H. A. (2021). Circular bioeconomy and integrated biorefinery in the production of xylooligosaccharides from lignocellulosic biomass: A review. In *Industrial Crops and Products* (Vol. 162). Elsevier B.V. https://doi.org/10.1016/j.indcrop.2021.113274

Ruiz, C. P. T., Dumeignil, F., & Capron, M. (2021). Catalytic production of glycolic acid from glycerol oxidation: An optimization using response surface methodology†. *Catalysts*, *11*(2), 1–20. https://doi.org/10.3390/catal11020257

5. References

Salusjärvi, L., Havukainen, S., Koivistoinen, O., & Toivari, M. (2019). Biotechnological production of glycolic acid and ethylene glycol: current state and perspectives. In *Applied Microbiology and Biotechnology* (Vol. 103, Issue 6, pp. 2525–2535). Springer Verlag. https://doi.org/10.1007/s00253-019-09640-2

Shi, Y., Xia, Y., Xu, G., Wen, L., Gao, G., & Zong, B. (2022). Hydrogen peroxide and applications in green hydrocarbon nitridation and oxidation. In *Chinese Journal of Chemical Engineering* (Vol. 41, pp. 145–161). Materials China. https://doi.org/10.1016/j.cjche.2021.09.030

Sorokin, A. B. (2013). Phthalocyanine metal complexes in catalysis. In *Chemical Reviews* (Vol. 113, Issue 10, pp. 8152–8191). American Chemical Society. https://doi.org/10.1021/cr4000072

Sorokin, A. B., Kachkarova-Sorokina, S. L., Ce´cile, C., Donze´, D., Pinel, C., & Gallezot, P. (n.d.). *From native starch to hydrophilic and hydrophobic products: a catalytic approach*.

Sorokin, A. B., & Kudrik, E. v. (2011a). Phthalocyanine metal complexes: Versatile catalysts for selective oxidation and bleaching. *Catalysis Today*, *159*(1), 37–46. https://doi.org/10.1016/j.cattod.2010.06.020

Sorokin, A. B., & Kudrik, E. v. (2011b). Phthalocyanine metal complexes: Versatile catalysts for selective oxidation and bleaching. *Catalysis Today*, *159*(1), 37–46. https://doi.org/10.1016/j.cattod.2010.06.020

Tolvanen, P. (2021). *Exothermal Reaction in an Adiabatic Cascade reactor*.

Tolvanen, P., Mäki-Arvela, P., Sorokin, A. B., Salmi, T., & Murzin, D. Y. (2009). Kinetics of starch oxidation using hydrogen peroxide as an environmentally friendly oxidant and an iron complex as a catalyst. *Chemical Engineering Journal*, *154*(1–3), 52–59. https://doi.org/10.1016/j.cej.2009.02.001

Tolvanen, P., Sorokin, A., Mäki-Arvela, P., Leveneur, S., Murzin, D. Y., & Salmi, T. (2011a). Batch and semibatch partial oxidation of starch by hydrogen peroxide in the presence of an iron tetrasulfophthalocyanine catalyst: The effect of ultrasound and the catalyst addition policy. *Industrial and Engineering Chemistry Research*, *50*(2), 749–757. https://doi.org/10.1021/ie100868k

Tolvanen, P., Sorokin, A., Mäki-Arvela, P., Leveneur, S., Murzin, D. Y., & Salmi, T. (2011b). Batch and semibatch partial oxidation of starch by hydrogen peroxide in the presence of an iron tetrasulfophthalocyanine catalyst: The effect of ultrasound and the catalyst addition policy. *Industrial and Engineering Chemistry Research*, *50*(2), 749–757. https://doi.org/10.1021/ie100868k

Tolvanen, P., Sorokin, A., Mäki-Arvela, P., Leveneur, S., Murzin, D. Y., & Salmi, T. (2011c). Batch and semibatch partial oxidation of starch by hydrogen peroxide in the presence of an iron tetrasulfophthalocyanine catalyst: The effect of ultrasound and the catalyst addition policy. *Industrial and Engineering Chemistry Research*, *50*(2), 749–757. https://doi.org/10.1021/ie100868k

5. References

Tolvanen, P., Sorokin, A., Mäki-Arvela, P., Murzin, D. Y., & Salmi, T. (2013). Oxidation of starch by H2O2 in the presence of iron tetrasulfophthalocyanine catalyst: The Effect of catalyst concentration, pH, solid-liquid ratio, and origin of starch. *Industrial and Engineering Chemistry Research*, *52*(27), 9351–9358. https://doi.org/10.1021/ie4003969

Williams, C. A. (1993). *Encyclopaedia of Food Science, Food Tecnology and Nutrition*.

Woodard & Curran. (2006). *7 Methods for Treating Wastewaters from Industry General*.