LENZINGER BERICHTE

Scientific Reports on Wood, Pulp and Regenerated Cellulose

"Der Fortgang der wissenschaftlichen Entwicklung
ist im Endeffekt eine ständige Flucht vor dem Staunen."

Albert Einstein

Dedicated to Professor Dr. Herbert Sixta on the occasion of his retirement at Aalto University, Department of Bioproducts and Biosystems, School of Chemical Engineering in March 2022

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Lenzinger Berichte 96 (2021)

Editorial

DEDICATED TO PROF. DR. HERBERT SIXTA ON THE OCCASION OF HIS RETIREMENT

In regards to Prof. Dr. Herbert Sixta's official retirement in March 2022 it goes without saying that this latest issue had to be dedicated to him and his work. Without a doubt, the journal would no longer exist without the support and involvement of Herbert during his time at Lenzing AG.
In the first article of this issue, DDr. Haio Harms describes Herbert's career in more detail, furthermore we would like to let people have their say in this editorial who have accompanied Herbert along his career path, worked with him, collaborated with him and learned from him:

Dr. Andrea Borgards, Vice-President Operations and Technology Pulp Lenzing AG/Austria:
Difficult to imagine – Herbert as pensioner! He has been so active and successful in research and science for so many decades. Over 400 publications in scientific journals and patents are an impressive footprint.
Herbert has also played a very important role model in the early days of my career, for which I have finally now the opportunity to express my gratitude. I met Herbert in 1993 as we were both giving presentations at TAPPI Pulp conference. Me as a rather shy student giving a presentation on the results of my diploma thesis, while Herbert was giving some insights on the real world of dissolving wood pulp production. A few years later he called and I started at Lenzing. Not only me but a full generation of Lenzing researchers benefitted from his endurance, structured approach, and the knowledge that profound improvements in our processes also require a profound understanding of the chemistry and physics involved. Our joint business trips to our Brazilian plant were remarkable not only for the extent of working hours, the achieved product and process improvements and last but not least the caipirinhas involved. Herbert was also ahead of his time in diversity aspects, allowing e.g part-time for high level scientists, which also gave me the opportunity to follow his footsteps when he decided for his purely scientific career in Finland.
Dear Herbert, I am convinced that you will not be a classical pensioner. I wish you lots of curiosity also in this part of your life. Thank you and all the best!

Herwig Schottenberger's Happ-Appraisal (Univ.-Prof. Dr. Herwig Schottenberger, University Innsbruck/Austria):
Needless to enumerate, the scientific achievements of Professor Herbert Sixta in Biopolymer Chemistry are impressive and of lasting impact.

Howsoever, I was lucky enough to get to know eager Herbert from the first semester of our chemistry studies at Innsbruck University as a very entertaining and friendly person in private life as well.
Admittedly, Herbert was always a step ahead of me in the disciplines of studies, but also personal affairs, and thus he settled into a smart "work-wife balance" early on by founding a charming family and becoming a proud and happy father, and later on a doting grandfather. However, it wasn't always easy for him though, as things didn't always go so smooth in this respect. In our first weeks as freshmen, when we went to Innsbruck's (in)famous basement disco called Scotch Club in order to soak in the urban nightlife (amongst other, more mundane intentions), the bouncer denied poor Herbert entry on account of having to be at least 18 years of age. The bouncer also did not accept our confirmations that "Happ" belonged to our study cohort.
Even after going back to his apartment to get his passport, the door attendant still wouldn't let him in, claiming that the ID was obviously faked. While I felt really sorry for him in this moment, this feeling was soon to turn into the opposite, and I always was a bit envious of his eternal young and fresh appearance. He looked like an angel (see picture), and he drove the bike like hell; in this regard, a veritable member candidate of the notorious motorcycle gang Hells Angels.
While Herbert rarely needed electrolytes for hangover recovery, he considered Ionic Liquids, among many other favorite subjects, a high-potential research field of his industrial and academic objectives. Fortunately, this interest of Professor Sixta brought us together once again, forming the scientific part of our joint life journey for which I am honored and extremely grateful.
https://www.researchgate.net/publication/228666834_Ionic_liquids_Current_developments_potential_and_drawbacks_for_industrial_applications
Dear Herbert, it's great to hear that you are willing to continue sharing your expertise by being part of the Ioncell startup. All the best to you, and stay a grandmaster, grandfather, as well as a curious child at heart!

Prof. Dr. Thomas Heinze, Friedrich Schiller University Jena/Germany:

I have been knowing Herbert Sixta since many years; I met him at Lenzing AG discussing cellulose solvents for chemistry and shaping in particular fiber spinning and learning about fundamentals from a company-based point of view. I was inspired by his enthusiasm about the bright future of cellulose on many areas of applications. As an innovative researcher, he became Professor of Chemical Pulping Technology at Helsinki University of Technology and contributed to the field of special ionic liquids as an alternative to the existing man-made cellulose fibers making processes from the basics up to the commercial scale. He is a well-known and leading expert in the fields of pulp, cellulose textile and fiber chemistry. Based on the two-volume book "Handbook of Pulp", which was published in 2006 by the prestigious publisher WILEY-VCH, he taught students and researchers in these fields. Herbert contributes with his extraordinarily vast knowledge to the discussions of the experts group of the "Cellulose and Cellulose Derivatives" a section of the Pulp and Paper Chemists and Engineers Association in Germany (ZELLCHEMING). As the chair of this expert group, I learned that he was always open taking responsibility for various aspects of our work. He was chairperson for the round robin test about the evaluation of methods to determine molar mass and molar mass distribution of cellulose. Dear Herbert, all the best to you and your family.

Dr. Frank Meister, Head of Department Native Polymers and Chemical research, TITK Rudolstadt/Germany:

When I started my professional career as a young man of 34 at the Thuringian Institute of Textile and Plastics Research (TITK) at Rudolstadt, Herbert Sixta was already well known as a highly respected expert in cellulose pulp sciences and regenerated fibre manufacturing. At this time we didn't have any opportunity to discuss my passionate questions on dissolving pulp, its processing and application in direct dissolution and Lyocell fibre spinning procedures. A couple of years later I've got numerous opportunities to learn from his highly rated scientific publications and lectures at prestigious conferences and symposia. I was deeply impressed by his excellent studies and epoch-making findings in pulp science and technology. Another couple of years, when Herbert Sixta changed from Lenzing Research to Aalto University and became a Professor of Chemical Pulping Technology, we met in the experts group of the "Cellulose and Cellulose Derivatives" a section of the Pulp and Paper Chemists and Engineers Association in Germany (ZELLCHEMING). And finally, in 2015, Herbert Sixta invited my group and me to visit him at Aalto University and to report on our R&D activities in physical modification of Lyocell fibres by long lasting incorporation of different kind of additives in fibre cross sections. Again, I was surprised by his huge extent of very different scientific themes in his academic research, but also by his valued estimation of all kind of R&D in cellulose science.

PD Dr. Hedda Katrin Weber, Green Swanlings e.U. Bioeconomy Consulting & Leadership Coaching in R&D, Graz/Austria, former co-worker:

During my time in Lenzing AG, it was an exciting journey to work with Herbert Sixta. I learned amazing facts about pulping processes and related topics since Herbert never hesitated to share his vast knowledge. He had a good idea of which research and development were needed. Since R&D usually has another time horizon than the immediate production needs, his ideas were not always met with overwhelming enthusiasm. Nonetheless, he imperturbably focused on reaching his research goals and the results often came in handy at a later time.

After moving to Finland he started in a rather modest environment and I am deeply impressed with his achievements at Aalto University to date. I am also grateful for his encouragement and support for my habilitation.

In short, it has been a privilege to work with Herbert and I wish him a fulfilling time as Emeritus Professor.

Rosi´s and Antje´s memories of the start of a long and productive collaborative journey (Prof. Dr. DDr.h.c. Thomas Rosenau, Prof. Dr. Antje Potthast, both BOKU Vienna/Austria):

In the mid 90ies we spent a year of our PhD time at North Carolina State University in Raleigh, being frequent visitors to Joe Gratzl´s legendary garden parties. One night, the BBQ was joined by a young fellow from Austria, named Herbert, apparently a PhD student like us, as we thought at that time. He looked about our age, liked beer a lot, was quiet and modest, yet at the same time extremely well-versed when it came to topics of pulping, bleaching or fiber making. When talking about our PhD work with Herbert, we naturally wanted to know also about his research topic - and of course we were a bit shocked when we learned that we had actually been talking all the time to one on the research bosses of Lenzing AG. This was the starting point for an almost live-long collaboration with Herbert, and it indirectly also influenced our professional path that a few years later lead us to Austria and BOKU University. Together with Joe who had always remained in close contact with Lenzing AG as professor at NC State, the idea was born to found a Christian-Doppler-Lab in the field of pulp and cellulose science. Herbert and Haio Harms propelled this idea from Lenzing´s side, and soon an Austrian institution was found to host the lab, BOKU University Vienna, a lab director, Paul Kosma at BOKU, and two postdocs (namely us), to make that idea come true.

Funnily, the scheduled start in May 1999 was even surpassed, and we had to come to BOKU already in November 1998. The Christian Doppler laboratory for "Pulp reactivity" had taken up its duty, and the next seven years were filled with mutual research and close cooperation with Lenzing AG. Herbert had always been not only our direct contact at the company, but also our mentor and fatherly friend during these days (despite of continuing to always look incredibly young as in the day we first met). Eventually more than 40 SCI articles and 50 conference papers resulted from that time, and the close links between the BOKU research and Lenzing AG as one of the oldest industrial cooperation partners were forged.
Every CD lab has a seven-year life span, and also our CD lab ended and our ways parted. Herbert went to accept a professorship at Aalto University, we stayed at BOKU University. However, the scientific topics once set by the collaboration with Lenzing and this first CD-lab have continued to accompany us all the time. A second Christian-Doppler laboratory, this time entitled "Advanced cellulose chemistry and analytics" was founded in 2008, and in 2013 a new "Institute of Chemistry and Renewable Resources" was founded, located at the BOKU Tulln site. In this way the research topics that caught us roughly 15 years ago were quasi institutionalized and made permanent. Also later, there was constant scientific exchange, mutual discussion, publications, exchange of ideas and students with Herbert and his institute at Aalto.
Without exaggeration, we can state that Herbert was one of the figures who have centrally influenced and determined our scientific career – both with regard to research topics and research location. It has always been a pleasure and privilege to work with him, and to have known his family.
Dear Herbert, we would like to convey a big thank you that our ways crossed fruitfully so many times, for the inspiration and friendship. We wish you all the best. We are sure that there is no real retirement for you, you will always remain active and productive. For these activities and for the time with your family we wish you all the best.
Rosi & Antje

Dr. Petra Wollboldt, Head of R&D AustroCel Hallein GmbH/Austria and former co-worker:
I still remember the telephone call from Herbert when he offered me a post-doc position in his research group, my first steps into professional life. In his second sentence he switched from "Sie" to "Du", and this is a good description how Herbert Sixta swiftly comes to the core of things without many diversions. I had the chance to work with him for about 10 years and his enthusiasm and dedication to topics related to cellulose and wood chemistry inspired me and are the reason I stayed in this field. I know Herbert as a person with neverending energy driving forward and ignoring obstacles, and he is undoubtedly one of the key influencers in my professional life. Thank you Herbert for your inspiration!

Dr. Marina Crnoja-Cosic, Director New Business Development and Member of Management Team, Kelheim Fibers GmbH/Germany and former co-worker:
It was almost 25 years ago, just when I decided to start my professional career in the man-made fiber industry, that I met Herbert for the first time. He was also my very first boss during my 20 years Lenzing time.
He gave me the possibility to enter the world of pulp, to discover the processing behind and how it is linked to fiber solutions as well as the products made out of them. He also supported me in my decision to specialize in textile processing and application development. I was always impressed by his enthusiasms concerning research and by his visionary thinking regarding new processes in pulp and novel spinning technologies.
I had also great respect for Herbert's decision to continue his research work at Aalto University. I'm deeply impressed by what he achieved during his time in Finland and how his great research work and visions now become reality by being transferred to commercialization.
Many thanks, Herbert, for supporting me in my professional work. But my biggest thanks to you for always being a person who respects and appreciates others.

Prof. Dr. Michael Hummel, Professor of Biopolymer Chemistry and Engineering, Aalto University/Finland, and former coworker:
Herbert moved to Finland in 2007 when he accepted a professorship at Aalto University (at that time Technical University of Helsinki). As I learned later from various representatives of the Finnish pulp and paper industry, key industrial players have pursued the faculty to explicitly recruit Herbert, whose reputation preceded him. When I joined his team in 2009, he had already established a large group covering several research areas. We started to work on the Ioncell® process, not knowing yet how far this work would take us. In my more than ten years of research with Herbert, I always appreciated his clear vision and determination. When others often only saw the problem, he strove to find a solution. I also enjoyed our many casual conversations during a beer at a conference or when having a pizza in our local "Spelunke". Herbert was a strong promotor of fundamental research but always had an eye on applicability and scalability. It was this holistic research thinking and his perseverance in the Ioncell® process that has recently been acknowledged with the Marcus Wallenberg Prize.

During his time at Aalto University, Herbert shaped the Department of Forest Products Technology (later Bioproducts and Biosystems) both as professor and as department head. He renewed and expanded the analytical equipment, set up state-of-the-art research infrastructure, and – driven by the momentum of Ioncell® – led the establishment of sustainable textile chemistry and engineering as entirely new research field at the department. This was an important milestone to maintain high-level education in textile technology at Finnish universities. And although Herbert's time as professor at Aalto University might have ended, I am sure his passion for science and research will keep him active for many years to come.

By a few of Herbert's PhD graduates:

How did we get to know Herbert? Some of us had classes with him during the master's studies, while others got external recommendations to pursue a doctoral degree with him owing to his renowned reputation. During his lectures, one could immediately see his leading character that is full of charisma, and his incredible presentation skills. We once overheard someone at a conference calling Herbert 'the pope of cellulose', while we were still learning about his achievements in the field. Despite Herbert's position as the Head of the Department of Bioproducts and Biosystems in Aalto University, he was always open to our thoughts and suggestions.

As a supervisor, Herbert was always easily approachable, and happily available for a quick chat. Especially at the beginning, many PhD students can feel lost at times or detached from the big picture of their research topic. This was never the case with Herbert, since right from the start, he'd fully engage his students into the topic. From his close guidance and expertise, we could learn a lot about regenerated cellulose fibers and pulping. When students came to choose a professor for their dissertation, they would ask among their peers, 'How's Herbert as a supervisor?' 'Quite demanding', most people would say. Often, throughout the way, you'd find Herbert challenging us with more scientific questions, which might not have been very understood at start, and we'd often ask ourselves 'Isn't it already enough that we have all these sets of data?' However, over time we too developed a scientist mind-set and realized that we will never stop asking questions and challenge our knowledge since we can never know enough.

Herbert always acknowledged his students on their work, especially, in front of external partners. Never have we once heard Herbert talking as 'I did this' or 'I instructed that', although he could, instead he would always use 'we'. Whenever there was an appraisal for his work, he would openly refer to his students' efforts. We remember him once saying, before he was asked to present our group's work in an international event, 'I am only the one to give the presentation, but it's your work and your achievement!'. Now, as we look back, we clearly see that learning from Herbert has influenced each one of us. Today, we are all in respectable positions in- and outside of Finland, and we will not forget our journey with you Herbert. Thank you for pushing us to finish and we genuinely wish you happiness in your future.

Dr. Thomas Röder, Senior R&D Expert Lenzing AG and Editor-in-chief Lenzinger Berichte:

In my first 10 years at Lenzing AG I had the privilege of working with Herbert Sixta. It was an intense learning phase. Herbert's irrepressible curiosity to elicit the secrets of nature and to use these findings beneficially has always left its mark on him. Almost legendary were Herbert's emails that began with "I respectfully request/Ich bitte höflichst um...". When you received such an email, it was clear that the task had to be done immediately.

He often did not take the sensitivities of the production managers into account. The complicated chemical and physical processes in pulp and cellulose fiber production cannot be described with simple numbers or models just because a manager wants it that way.

With his visions and their realisation, he played a major role in ensuring that Lenzing AG coped well with many a crisis and is where it is now. Prof. Dr. Hans-Peter Fink from the Fraunhofer Institute in Potsdam/Golm once said to me: "You in Lenzing do more basic research than we do at Fraunhofer institute!" Anyway, time has proven Herbert right. It is precisely this basic research that we still benefit from today.

Dear Herbert, thank you very much and stay the way you are! I wish you and your family all the best and hope that you will continue your contributions to science.

A Brief Personal Review of Academic and Corporate R&D for the Chemical Process Industry

Haio Harms
former CEO Kelheim Fibres GmbH/Germany

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Abstract

Lecture held on the occasion of the retirement of Professor Dr. Herbert Sixta at the Scientific Biorefineries Seminar, Department of Bioproducts and Biosystems, School of Chemical Engineering, Aalto University, Espoo, Finland, 30 March 2022

Dear colleagues, ladies and gentlemen!

Last year Michael Hummel and Herbert Sixta asked me to give a presentation at the annual Biorefineries Seminar, which Herbert organised for 15 years in December for the senior students of the "Department of Bioproducts and Biosystems" and for the interested industrial and academic community. Unfortunately, the seminar had to be cancelled because of Corona / SarsCov19. But with half a year's delay I'm grateful to give now a presentation about research and development

- in the fields of **Pulping chemistry and technology** with special emphasis on dissolving pulps,
- of **Cellulose chemistry** and the chemistry of the fractionation of biomass, in particular lignocellulosic biomass,
- about the chemical and mechanical **purification of pulps**, the oxygen delignification of unbleached pulps, about TCF- and ECF- bleaching techniques and about Organosolv fractionation methods with particular emphasis on GVL/water pulping of hardwood.
- This will bring me to the isolation and characterization of **lignin** and its valorization to monoaromatic structures, to bio-oil and as co-polymer in composite material with cellulose and to the valorization of **hemicelluloses** as furanic compounds.
- I will especially focus on the **valorization of cellulose** to regenerated cellulose fibers and to cellulose derivatives on **dry-jet wet spinning** of cellulose solutions and last but not least I will also cover **Cellulose textile chemistry**, novel dying technologies for cellulose-based textiles, and novel pathways of functionalization of cellulose-based textiles.

If someone in this room should ask what this comprehensive set of chemistry topics has to do with "a review of R&D for the chemical process industry", and how I will be able to do this in just the next 45 minutes, I have a very good reply:

I'm going to do it by talking about Professor Herbert Sixta!

Because the list of these topics is what he represents! It is the list of his academic and private interests, the list of topics of his publications and projects and – which is likely the reasons for his success – also the description of his hobbies: It cannot be but a hobby, which – without any external pressure – keeps you busy almost every day, until late hours, during weekends, and even during holidays spent around visiting other research institutions ...

Dear Herbert!

You told me that a "laudation" should not be part of this programme. You thought that nobody would be interested in what made you the person we like and admire, the person who managed to master both worlds of industrial and academic research and to bring this department to its present impressive condition.

You also told me that you don't really enjoy to be the centre of admiration! You asked me to talk about something that could be interesting for the young academics invited to this symposium.

I'm convinced that everybody here, who had the privilege to learn from you and to work or cooperate with you, is interested to understand how in a long and extraordinary career in Company-, and University-research you could acquire all the abilities needed to generate such impressive results. Therefore I will concentrate on the way how we jointly tried to manage an important section of **Research and Development in Lenzing AG** and on some of the conclusions we drew from it.

Professor Dr. Herbert Sixta

University

Herbert studied Chemistry at the University of Innsbruck. Coming from a background of medical doctors the legend tells, that his family for 1 year thought that he was studying Medicine.

He got his Diploma based on a paper on "Infrared reflection and electron energy-loss spectroscopy for the investigation of solid surfaces" and continued with a PhD-thesis in physical chemistry on "The interaction of CO, H2, O2 and simple saturated Hydrocarbons with Pt-, Ru-, Cu and Ru/Cu- supported Catalysts". Since I did my PhD in physical chemistry as well, this of course was a very good recommendation. But it also proved to be a very good recommendation for his wife Eva so he could start his family at that time.

Here I want to add a short remark on **studying chemistry** and the subjects of its curriculum. It is still a surprise to me that in the course of my professional life there was no chemical discipline, which I didn't need at one or the other occasion – maybe with the exception of statistical thermodynamics and some theoretical chemistry, but definitely including the basic understanding from having practised the H_2S cation separation procedures. For a technical position in the chemical process industry a broad chemistry knowledge is indispensable. Chemical processes are full of surprises and the ability to find out what a certain problem could be needs a general chemical "feeling". Early specialisation is not helpful.

Dr. Sixta receiving his PhD and with his wife, Innsbruck 1980

Environmental protection and biotechnological reserach

In 1982 Herbert and his family moved to Upper Austria and he started to work for Lenzing AG in the department for **Environmental Protection**. In fact, very soon he was practically in charge of the department because its formal head was a member of the Austrian Parliament who was extremely busy in politics.

In view of the dramatic growth of synthetic fibres at that time Lenzing Management had one more of its periodical panic attacks that Viscose fibre would soon be a dead product. In line with the glittering topics of industry success stories in the media they thought that Lenzing could survive as a "Biotechnological Company" and as a "High Performance Fibre Company".

Lenzing AG's production site in Upper Austria

As for a commercial person "Environment" is dealing with "Biotechnology" Herbert was asked to develop concepts for new "biotechnological products" based on the enzymatic and bacterial conversion of Lenzing process byproducts. Already during second world war Lenzing had a production of alcohol from lignocellulosic substrates and used the proteins of bacteria grown on Hemicelluloses to produce sausages. Later on there was a project to use Hemicellulose as a dietary substitute for wheat flour. So, in 1987 Herbert became head of a new Department for **Biotechnological Research** and did the best thing he could do: He started to concentrate on the **Lenzing pulping processes**.

Parallel to his Biotechnology assignment I was asked to work on "High performance polymers" after having joined Lenzing almost at the same time. Based on the Acrylic dry spinning technology owned by Lenzing I started to run a demonstration plant for "P84" high temperature-, and flame resistant Polyimide fibres. However, after 2 years I was asked to switch to Viscose technology, I moved to Indonesia and started to work for South Pacific Viscose, a Viscose fibre producing subsidiary of Lenzing. It was there, in about 1986, when I first professionally met Herbert. I was in charge of Production and Technology, and Herbert came to help me with the mass balances of sulphur containing gas streams in the Viscose fibre process.

Network on lignocellulosic substrates

It is a major problem that commercial people and most managers don't understand that successful **R&D needs special competences**. Driven by the latest Business Consultants Philosophy declaring that innovation just consists of "doing things differently" the Lenzing management in 1991 thought to add value to the business by reorganising R&D: Since operational managers know how "to do things", let them also manage innovation! No customer is willing to pay for the "intellectual satisfaction of R&D people" who want to understand things. The results of time-consuming discussions amongst scientists and literature studies can be saved. And – of course – if R&D then comes up with surprising innovation proposals with costly capex, things start to become really difficult. The central R&D group was dissolved and distributed to the operational units. Only a small central "Innovation scouting group" was kept. Part of Herbert's group went back to Environmental treatment and the Biotechnological activities were stopped.

Herbert's pulp related activities however could continue. They were kept as a small personal project directly under Dr. Zauner, Lenzing's Technical director at that time, who was guided by his dear friend, **Joe Gratzl**, an Austrian professor at the North Carolina State University in Raleigh, USA and one of the leading specialists in Pulp and Paper Science of that time. This turned out to be a fortunate constellation. In his regular consultations of Lenzing a long-lasting friendship between Joe and Herbert was established and Joe became a mentor for Herbert. Regularly, after a whole day of discussions about pulping, bleaching and wastewater treatment they continued with informal late hours sessions in the hotel. The ideas developed in these sessions became the base for a series of pioneering new pulping and bleaching technologies

Prof. Gratzl introduced Herbert also to **Prof. Rudolf Patt** from Hamburg University in Germany, who in 1987 developed the ASAM Pulping process. This resulted in the development of a close cooperation. They both recommended several excellent young scientists who started to work in Lenzing or in University cooperations. They jointly published several high impact papers. Herbert, by the way, was one of the first experts

in the German speaking pulp and paper community who published in English and in international journals. He started to build up scientific contacts with institutes, universities and companies first in Austria and Germany, finally all over the world.

Herbert started to work not only on **sulfite pulping**, but also on **soda**, **Kraft** and **ASAM pulping**. With the background of dissolving pulps for Viscose fibres he studied beech **wood** as applied in Lenzing and eucalypt as used by Saiccor. After Voest Alpine of Austria shut down their alkaline pulping engineering business Herbert took the chance to take over some of their project managers to keep the knowhow. In the years around 1993 they started to work on the development of the new **Continuous Batch Cooking** (CBC) process being a substantial improvement of Kraft displacement batch cooking, combining the advantages of batch operation with the continuous preparation of cooking liquors in the tank farm. The CBC Process for paper pulp was the base for the development of the VisCBC process. Herberts knowledge on eucalypt alkaline pulping and TCF bleaching was the scientific base to establish this technology in about 1995 in the Bacel plant, a new mill for very high quality dissolving pulp in Brazil, at that time a Lenzing joint venture which exceeded all expectations.

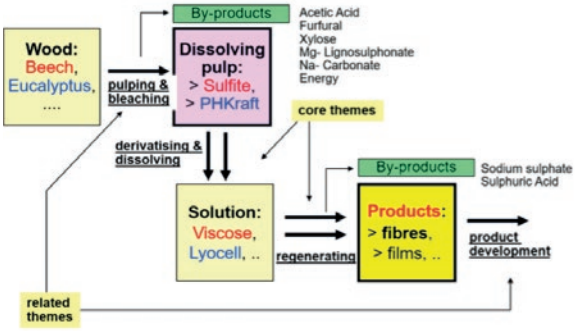
Dr. Sixta with Prof. Joe Gratzl (left) and with Prof. Rudolf Patt (right) with on the occasion of his habilitation 1995

Prof. Gratzl and Dr Zauner introduced Herbert also to Prof. Helmut Starck from the Institute for Pulp- and Paper- Technology of the Technical University of Graz. The high appreciation of Herbert's work resulted in the proposal of his habilitation by Prof. Starck. In 1995 an excellent paper on "Chemical Pulp Production considering environmentally friendly Cooking- and Bleaching processes" was rewarded by his habilitation at the Faculty for mechanical engineering at the Technical University of Graz.

In the years 2000 a Xylane Cold caustic extraction (CCE) process was patented, as a purification step for pulp and also as a means to transfer Xylane to other pulp qualities or to isolate it as a powder.

Industrial research and development supplemented by science

Some years after I came back from Indonesia, the Lenzing management realised that the 1991 reorganisation of R&D was maybe not such a good decision, as they had wished it to be. Day to day routine of operational business managers was not the environment to stimulate new ideas, to develop and implement new concepts. Innovations did not come just by themselves. In 1995 they asked me to re-establish a strong R&D function for the Lenzing group.



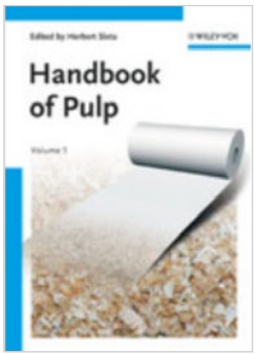
Overview about Lenzing core business around 1995

My concept for the new R&D-division deviated from the traditional perspective of Lenzing as a pure textile fibres manufacturer. Besides "Viscose R&D" and "Lyocell R&D" it included also "Pulp R&D" as an essential department focussed on dissolving pulp as a "product". So far the pulping processes were mainly dealt with as standard upstream technology. Since the quality of dissolving pulp is defined by its ability to give good solutions for the fibre spinning processes, the responsibility for Viscose dope manufacturing was added to the portfolio of this group, which was headed by Herbert. A smaller R&D services groups took care for IPR, literature and cooperations-administration.

This was the beginning of 13 years of successful cooperation with Herbert and of a growing, personally very rewarding relationship. We continued to be in close contact also after 2008 when I changed as CEO to Kelheim Fibres.

Herbert was not satisfied in limiting himself to practical development work. He realized the largely empirical state of knowledge about the Lenzing processes and their underlying chemistry. He understood that apart from small optimisation steps no substantial progress was possible on this base.

He started to add a scientific focus to the different practical projects he supervised and started to build up a strong **analytical group** specialised on lignocellulosic substrates. He introduced or developed several analytical methods for pulp and fibre characterisation. Amongst many other methods he adapted the DMAc/LiCl method to measure the DP-distribution of pulp, which was standardised in a round robin and is now the industry standard. The ISEC (Inversed size exclusion chromatography) method for pore size distribution was introduced and the CCOA and FDAM methods for cellulose carbonylic- and carboxylic groups were developed. The installation of new automatized and IT controlled small scale **pulping and bleaching pilot plants** turned out to be the key for the development of new and optimised pulping and bleaching processes. The rather obsolete old **Treiber Viscose equipment** was brought up to state of the art and together with a small Davenport viscose fibre spinning apparatus proved to be a reproducible testing tool for dissolving pulp quality.



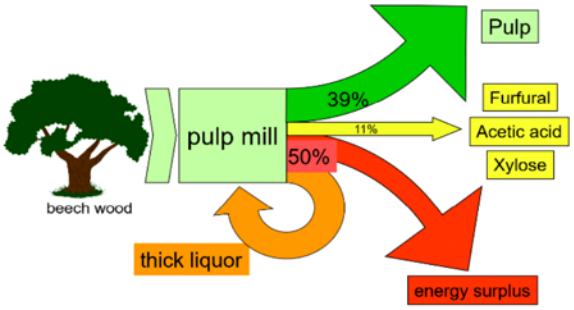
After a couple of years in this new Corporate Research constellation Herbert started to work intensively in his "leasure time" on his most important publication, the "Handbook of Pulp", which was released in 2006.

In this two volume set he brought together a team of authors to produce the first comprehensive handbook on the market. The aim of the book is initially to provide a short, general survey on pulping processes, followed by a comprehensive review in certain specialized areas of pulping chemistry and technology. It describes all traditional and modern processes for the pulping of Chemical Pulp and Mechanical Pulp, for Recovered Paper and Recycled Fibers as well as for waste liquor treatment, pulp bleaching and environmental aspects, while also covering the analytical Characterization of Pulps.

Wood biorefinery

Herbert's experience with the effluent treatment plant just degrading potentially valuable biomaterial at high costs triggered a big portion of his work in the next decades. He realised that this was one of the fundamental business relevant topics for Lenzing and as it turned out was key for its overall competitiveness and a major factor in its survival as a fibre producer in Europe's high cost environment. Several new technologies, processes and patents have been the consequence of Herbert's approach to innovation.

This type of fundamental topics to me is typical for companies in the chemical process industry producing commodities or semi commodities with usually very similar cost structures and very limited price elasticity. They are often neglected since these topics are only indirectly linked to the final product and since there is usually only an empirical state of understanding them. But they are key to improving the competitiveness by reducing the net costs of production: this is what innovation in process chemistry is about!



WOOD biorefinery in Lenzing [H. Harms, Lenzinger Berichte, 86 (2006) 1-8, figure 10]

Already in the 1970's Lenzing was forced by environmental regulations to restrict itself to the use of beech wood because of the lower BOD load to the effluent. In the beginning the ban of spruce was a severe blow for the company. However, in the end it turned out to be an advantage since the processes developed in the next decades – many of them masterminded by Herbert – ended up in the conversion of the Lenzing pulp mill into a true "Wood Biorefinery". The acetylated beechwood pentosanes are converted into food-grade acetic acid, furfural and xylose, increasing the recovery of dry wood matter from 39% to about 50% and substantially contributing to the income of the company. The combustion of the lignin containing fractions covers the energy of the pulp mill and most of the energy needed by the viscose fibre production. Innovative processes increasing the Xylose concentration in the thick liquor further increased the income.

Another topic intensively linked to government regulations and environmental trends was the development of the **Totally Chlorine Free** (TCF) dissolving pulp production process with new Ozone- and new EOP-Bleaching processes patented by Lenzing in about 1990. It was of special importance because of the public awareness related to Chlorine in hygiene products. This process however was economically not yet feasible since the ozone bleaching was developed at high consistency and at lab scale only. The breakthrough came in 1992 with the patented new medium consistency Ozone bleaching technology based on a new mixing technology implemented in Lenzing for the first time in the world. This TCF-pulp now met the environmental regulations imposed on Lenzing. Later the technology was also implemented in the Bahia and in the Paskow mill.

A purely inorganic problem had to be solved by Herbert's group in the context of the poor performance and excessive Sulfate formation in the **Magnesium-Monosulfite splitting** (MSS) plant of the tertiary SO_2 recovery in the pulp mill.

The key property of dissolving pulp, its reactivity, and the potential effects of an activating pretreatment have been of special interest to Herbert. They resulted in studies on pulp grinding, electron beam irradiation, enzymatic activation and tribological treatement. A **Chemical Slurry Ageing** (CSA) process for the Viscose technology was patented in 1998. It is an alternative process for Cellulose pre-ageing in an ageing drum and reduces the Hemicellulose load in the Viscose by removing the Hemis at an early stage and by reducing the Hemi formation. **Nanofiltration** was another process developed for the removal of Hemicellulose from the press-lye.

Lenzing did an intensive evaluation of several **pulp dissolving technologies** in the early 80's before making the decision for NMMO. But after this decision new approaches to dissolve cellulose had to be evaluated. An Australian formic acid process failed because the remaining acidic groups started to degrade the regenerated fibre. Professor Robin Rogers gave an impressive demonstration of a cellulose instantly dissolving in a beaker with hot ionic liquid, but he had not yet considered a depolymerisation reaction. Herbert's follow-up of the concept of **Ionic Liquids** with Professor Herwig Schottenberger brought first positive results and generated the initial knowledge which later on was used for the development of the Ioncell technology in Aalto.

The innovation system in the chemical process industries

From the beginning Herbert and I had very similar views about the principles of Corporate R&D in industries like Lenzing. In many cases these were quite different from what business consultants taught at that time. We both were convinced that product development in the Chemical Process Industries usually cannot be done without **process development**. Since the products are usually commodities or semi-finished goods which basically can be produced by everybody, success is intensively linked to being better in production. Like Viscose fibres or Wood pulp, both more than 100 years old, this type of products usually have very long **lifecycles**. They are typically produced using complex closed loop processes and very **capex intensive** plants and equipment. Improving the plant utilisation and innovative process steps to reduce the costs of production are always a key objective. Innovative technological concepts however are preferably limited to using the actually installed equipment. As a consequence the flexibility, and quality of new equipment define the long-term development potential of the company. Product innovation besides quality improvements is usually targeted at property modifications to generate additional applications or niche markets. Since the production facilities usually cannot be used for development work **appropriate facilities** like pilot plants and analytical methods are required and of course persons with **specialised experience**, who are not readily available on the market. It needs a lot of **time** to build up a versatile competence and continuity to keep it.

Business consultants employed by chemical process industries usually claim that it is wrong for a company to do **Basic Research**. In a competitive environment it is considered too costly. However the limited and predominantly empirical state of knowledge about many of the technological and chemical business aspects needs to be overcome to achieve a sustainable flow of innovation. Development work of corporate R&D needs to be supplemented by knowledge-based research / basic research: It needs to be done – by whomsoever! But of course it needs to be organised in a way compatible with industry: being precompetitive by nature public research institutions can be involved and the public interest justifies a public (co)funding.

Our analysis of the state of knowledge about the core processes and the key technologies in Lenzing showed an urgent need for a better understanding. Observations in many cases had no explanations. However,

we could not think of establishing all the fields of competences and the methods required by dramatically increasing our own R&D resources. We therefore started a search for competent research institutions. We tried to present and offer ourselves as a convincing "innovation company", as a competent partner to public research and research funding institutions, industry associations and individual industrial parties of the relevant research community.

A lot of efforts went into continuing **"Lenzinger Berichte"**, an internationally renowned and intensively quoted reviewed Journal referenced in Chemical Abstracts. Since 1953 it has been one of the most important publication platforms in the field of man-made cellulosic fibres and – in this context – of Wood and Cellulose.

Public accessibility to the generated knowledge in most cases is a virtual problem only: As long as such basic research is done in an active cooperation with a leading industry in its own field, this industry will be first in profiting from the results. "Cooperation", however, means that there is at least one competent person in the company responsible for the **transfer** of the knowledge. In some industries a **technology monitoring/ scouting** function is established which, however, will only work if a minimum of company activities in the relevant fields of interest and persons with the specific competence are available.

All these activities resulted in several longterm "Private Public research Partnerships" mostly managed by Lenzing Pulp R&D.

The **"Christian Doppler Laboratory for pulp reactivity"** was a cooperative project started in 1998 with Professor Paul Kosma from the Institute of organic chemistry of the University of Natural Resources and Life Sciences in Vienna (BoKu) as the academic partner. The Austrian CD-Research society explicitly focusses its funding to Industry/University cooperations doing 7 years of basic research and with annual budgets of presently up to 750.000 EUR. The research programme drafted by Herbert was about aspects from the isolation of wood cellulose, to the derivatization procedures and the regeneration of fibres with special emphasis on the physico-chemical structure, morphology and reactivity of cellulosic materials. It had more than 20 scientific staff, amongst them Thomas Rosenau and Antje Potthast, now themselves Professors at the BoKu University. The Lab in turn had several collaborations, amongst others with Joe Gratzl at NC State University, Professor Otto Glatter at the Institute for Physical Chemistry of the University of Graz, with Dr. Buchner at the University of Regensburg and Dr. Binder at the technical University in Vienna. The level of activities and the output was amazing. 134 publications and 38 conference contributions are listed in the Web.

In 2000 another academic collaboration, the **WOOD Kplus Competence Center**, was established with BoKu, the University of Linz and several industry partners from various wood processing chains. The area "Wood- and Cellulose-Chemistry" was located at the Lenzing site and started with Herbert as the responsible key researcher. The Area invested an "Analytic Center" in Lenzing which worked for the research programme but also sold analytical services to third parties. The partnership was continued until 2017.

Lenzing in these decades established several other CD-Labs besides Pulp also dealing with topics in the fields of Viscose and Lyocell fibre properties, manu-

CD-Lab Meeting 2000 in Lenzing: From left: Haio Harms, Paul Kosma, Thomas Röder, Thomas Rosenau, Herbert Sixta, Antje Potthast, Otto Glatter.

The WOOD Kplus crew stationed in Lenzing (2008): from the left in the Backline: Moritz Leschinsky, Gerhard Zuckerstädter, Mario Buchberger, Philipp Schröder, Benjamin Neuhaus, Andreas Stockinger, Herbert Sixta; Frontline: Sandra Schlader, Hedda Weber, Petra Wollboldt, Gabriele Schild.

facturing, processing and applications: these were CD-Labs on the "Chemistry of Cellulosic Fibres and Textiles" of Prof. Bechtold in Dornbirn, Prof. Stana in Maribor and Prof. Phillips in Manchester, on "Applied Thermofluiddynamics" of Prof. Brandstätter in Leoben, on "Advanced Cellulose Chemistry and Analytics" of Prof. Rosenau and Prof. Potthast in Tulln, on "Paper strength" of Prof. Schennach and on "Fibre swelling" of Prof. Hirn in Graz.

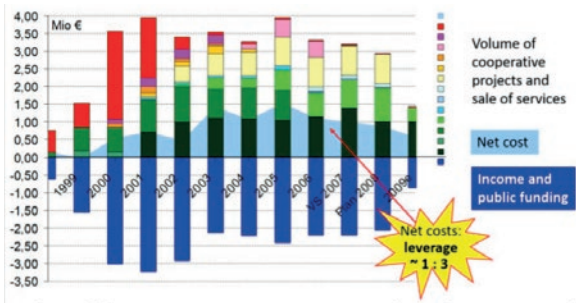
Apart from the scientific results and the improved understanding of Lenzing's chemistry and technologies, these activities produced a **network** of contacts and a multitude of medium and shortterm cooperative projects. They helped the participation in several EU projects, the "EU-carbohydrates centre of excellence" and the "forest-based products technology platform". Lenzing R&D also had substantial income from selling **research services** to third parties especially in industries active in the fields of cellulose films, casings and sponges and pulp manufacturing.

All this helped to reach the required **critical mass** of activities. The value of the cooperative activities directly managed by Lenzing Research in the years 1999 until 2008 was in the range of annually 3,5 to 4 Mio €. The majority was basic research. The corresponding financial coverage by Government- or EU-subsidies or payments by industry partners was between 2 and 2,5 Mio. €. The net costs to Lenzing therefore had a leverage of about 1:3.

The total activity level was substantially increased and even more important: work on themes of relevance for the Lenzing technology, which had virtually disappeared has sustainably been **reintroduced** at Universities in the region.

Herbert at the same time received widespread recognition in the international community of academic and industrial specialists. In 2005 he was one of the few scientists who like Hermann Staudinger (1951), Burkart Philipp (1982) and Joe Gratzl (1992) was awarded by **Zellcheming**, the platform of pulp- and paper-scientists in Germany, the **Alexander Mitscherlich medal** for outstanding work in the field of "Cellulose research and Cellulose chemistry".

Marketing of R&D Services at the exhibition of the International Cellulose Chemists Conference of Cellcheming in Wiesbaden 2001. From left to right: Haio Harms, Marina Crnoja-Cosic, Susanne Möderl, Tanja Kosch, Herbert Sixta.



Cost structure of Lenzing R&D projects in relation to sale of R&D services and public funding

Alexander Mitscherlich medal for outstanding work in the field of Cellulose research and Cellulose chemistry. Zellcheming, Wiesbaden 2005 (Robert Hock, Herbert Sixta)

Yet another R&D reorganisation

After about 10 years of almost undisturbed and very successful R&D, with the implementation of many fundamental innovations, making Lenzing the true "leader in Manmade Cellulosic Fibre technologies", a new group of inexperienced MBA business consultants was brought into Lenzing by shareholders who wanted to prepare the company for a sale to competitors.

Not willing to understand anything about relations management, knowledge generation and the complex activity leverage system, they identified an "enormous cost savings potential" by cutting the cooperative research activities and – as in 1991 – Management, with almost the same arguments decided "to add value to the business" by cutting R&D budgets, splitting up R&D and distributing it to different operational business units. It was obviously too difficult to care for R&D and for converting its results into profitable business at the same time.

I was made responsible for Lenzing group corporate services with subsidiary management, internal revision, legal services, IPR management and a small R&D scouting group. In 2008 I changed as CEO to Kelheim Fibres, a Speciality Viscose Fibre producer in Germany where I spent the most rewarding years of my professional career. At the same time Herbert accepted a call of Aalto University in Finland as a Professor for Bioproducts and Biosystems. He continued for some time offering his expertise to Lenzing but finally moved to Finland completely. About his activities and achievements here at Aalto University I need not talk: You know them better than I do! His group is amazing! The results of his work here are breathtaking!

Whoever is interested in the details of Herbert's research output in Aalto, the many doctoral theses he supervised, and the projects he managed, should look at his personal profile on the Aalto homepage:

https://research.aalto.fi/en/persons/herbert-sixta

Herbert, I'm grateful for the many years of very fruitful collaboration and for your friendship. I'm sure we will see many more fruits that have grown from your "hobbies" in the field of lignocellulosic substrates.

The group of the department for Bioproducts and Biosystems, 2022

Postscriptum in June 2022

It is only a couple of days when I received the first example of these fruits. It was the invitation from "The Marcus Wallenberg Foundation" requesting my attendance at "The Marcus Wallenberg Prize Ceremony in the presence of Their Majesties the King and Queen of Sweden" in honour of Herbert Sixta as one of the MWP 2022 Laureates. The price was awarded to him and Ilkka Kilpeläinen "for the development and use of novel ionic liquids to process wood biomass into high performance textile fibres."

Herbert: Congratulations again, and all the best for your future!

Last, but not least I want to thank Herbert's former colleagues in Lenzing Gabriele Schild, Thomas Röder, Gregor Kraft and Geri Meister who share my admiration for Herbert and helped mew to put my memory in order and to fill its holes.

Herbert Sixta and Ilkka Kilpeläinen

Outlook on Global Fiber Demand and Supply 2030

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Abstract

The Covid-19-induced decline in global fiber demand has already recovered by 2021. This article tries to explore the outlook for the global fiber market until 2030, both in terms of top-line demand as well as in terms of the composition of supply from different fiber types. Demand is expected to reach 142 million tons, based on underlying population and per-capita consumption growth. Supply growth is expected to come almost exclusively from man-made fibers, given natural fibers are supply-constrained. Among man-made fibers, wood-based cellulosic fibers will benefit from the increasing trend towards sustainability, which synthetic fibers cannot cater to.

Historical fiber market growth

Fibers are the basis of many products we use every day, not just in clothing applications, but also in less apparent end uses such as household wipes, filters, automotive interiors, or even battery separators. With the rise of many of those end uses, the global fiber market grew at a compound annual growth rate of 2.8% over the last five decades, from roughly 27 million tons[1] in 1970 to 113 million tons in 2021 [1].

Over the full period, this can be broken down to 1.5% annual population growth [2] and another 1.3% annual growth in per-capita fiber consumption. In the first half of that period (1970-1995), 73% of total growth was attributable to population growth alone. That changed in the following years: since 1995, the majority (73%) of growth came from an increase in per-capita fiber consumption (see Figure 1), which reached 14.4 kg, almost double the 7.3 kg in 1970. This coincided with disposable income in many developing countries reaching levels where more people could afford to buy larger amounts of textile and nonwoven products.

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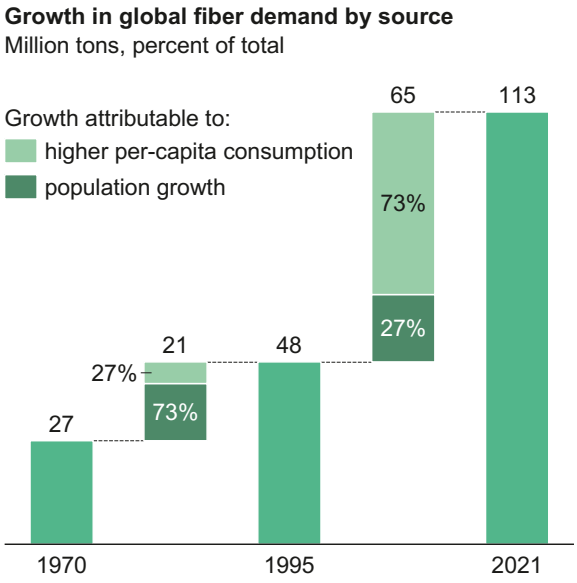


Figure 1: *Growth in global fiber demand by source, 1970-2021*

[1] Note that all fiber market volumes in this article refer to the sum of staple fibers and filaments, but do not include spunmelt nonwovens, since the process does not involve fibers

The global fiber market in the Covid-19 pandemic

In the Covid-19 pandemic, global fiber demand – similar to many other discretionary expenditures – experienced a major decline based on a weakness of end markets. In most countries, strict measures to fight the pandemic led to consumers unable or unwilling to shop. A weighted average calculated from statistics of apparel retail sales of 42 countries, published by statistical offices and national banks, suggests a -19% nominal decline in global apparel demand 2020 compared to 2019 [3]. At the same time, the high demand for medical and hygiene products caused a significant increase in demand for nonwoven fibers [4]. 2021 marked the first year of recovery from the pandemic. Global consumer demand for apparel exceeded pre-pandemic levels starting May 2021 and ended the full year roughly on the same level as 2019 (see Figure 2).

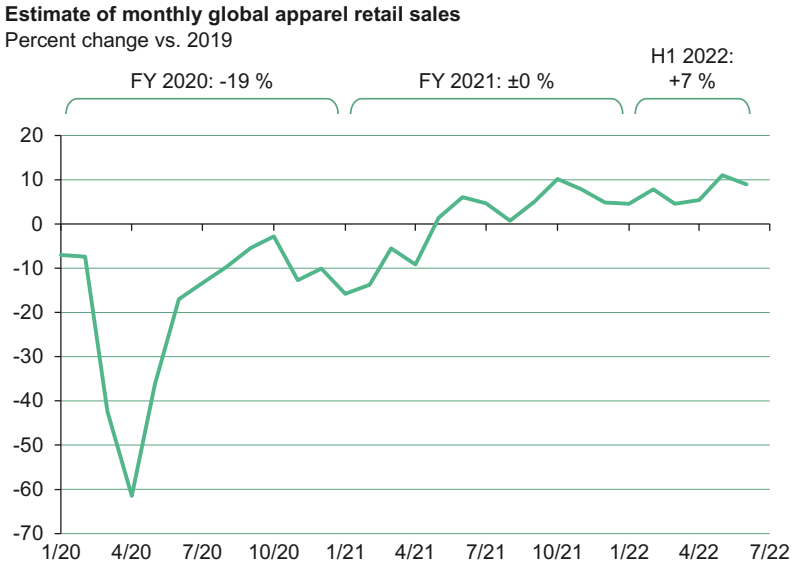


Figure 2: *Estimate of weighted average global apparel retail sales*

Corresponding demand for fibers declined from approximately 113 million tons in 2019 to below 99 million tons in 2020 (a -12% YoY decline) and increased again to more than 113 million tons in 2021 – roughly +1% above the level of 2019 (see Figure 3) [5]. It is appropriate to say the global fiber market lost two years of growth.

This article tries to explore the outlook for the global fiber market until 2030, both in terms of top-line demand as well as in terms of the composition of supply from different fiber types.

Overall fiber demand growth until 2030

Similar to historical fiber market growth, we can dissect future growth into population growth and per-capita fiber consumption. We can also assume fiber demand to continue to closely follow volume growth in the underlying apparel, footwear, home textiles, technical textiles, and hygiene markets.

In terms of population growth, the UN [6] expects 0.9% annual growth from 2021 to 2030. Given its high latency, we take this rate as granted as a structural growth driver.

Euromonitor [7] expects main fiber end markets to grow at 2.5-3.0% p.a. at constant prices (a good proxy for volumes) from 2021 to 2026. According to the source, the apparel and footwear market is set to grow at 2.9% p.a., home textiles at 2.7% p.a., and retail hygiene (wipes, sanitary protection, adult incontinence, and diapers) at 2.8%. Since no forecast is available until 2030, we extrapolate the available forecast for 2026 further into the future.

Many global trends support a further growth in per-capita fiber consumption. The number of middle class households (defined by their disposable income of USD 15,000–45,000) which constitute the bulk of consumer spending in most countries, is expected to grow by 60% from 2020 to 2040, “accounting for more than one in three households globally” [8]. Similarly, through optimization of value chains and a

move towards low-cost manufacturing locations, textile products have become much more affordable. While clothing accounted for 11.5% of US household expenditure in 1950 [9] it was only 3.0% in 2019 (even 2.3% in 2020) [10]. In hygiene applications, certain pandemic-induced behaviors are expected to remain sticky – particularly increased hygiene awareness –, but that effect is expected to wane over time. Particularly the adult incontinence market is expected to experience support by an ageing population, with the worldwide absolute population >65 years more than doubling from 728 million in 2020 to 1,549 million in 2050 [11], as well as products slowly shedding their associated stigma.

On the other hand, certain trends, which might lead to a slowdown in per-capita fiber consumption, are on the rise. For example, trends such as frugality, degrowth, conscious spending, and keeping long-life products start to gain popularity, but so far remain somewhat limited to certain population groups in most mature markets. Hence, we do not expect them to be a major inhibitor to growth in per-capita consumption.

Large regional differences in growth exist. Textile and nonwovens end markets in North America and most of Europe are mature and relatively saturated. They exhibit limited (and partly even negative) population growth and already high per-capita consumption. On the other hand, in many Asian, Middle Eastern, and African countries, both population and per-capita GDP (historically good proxies for rising per-capita fiber consumption) are forecasted to grow above world average [12]. Therefore, we can expect much of the growth in fiber demand to come from those regions.

Overall, we expect global fiber demand to continue annual growth at a rate of 2-3% through 2030, broken down to roughly 1% population growth and 1-2% growth in per-capita consumption. Such a growth would lead to total fiber demand of close to 142 million tons in 2030 (see Figure 3). This growth rate is in a similar range as other recently published estimates, such as 3.0% by Textile Exchange for 2020-30 [13], 2.4% by Hawkins Wright for 2019-26 [14], and 2.2% by Tecnon OrbiChem for 2020-30 [15].

Of course, unforeseeable "black swan events" hold the power to derail consumer spending and, as a consequence, also fiber demand. Examples include the Global Financial Crisis of 2007-08, as well as the Covid-19 pandemic starting 2020, with the latter costing the global fiber market two years of growth. The Russia-Ukraine conflict is likely another such event, fueling inflation and leading to low consumer confidence in European markets and potentially limited appetite for discretionary purchases such as apparel. In addition, the conflict holds the potential to influence fiber supply. Disrupted grain exports might lead to acreage being diverted away from cotton towards food crops in other parts of the world. Rising energy prices and fertilizer costs make growing cotton less economical.

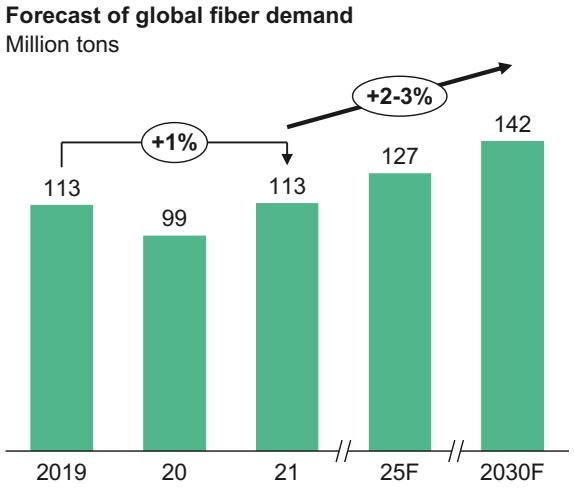


Figure 3: *Forecast of global fiber demand, 2019-2030*

Composition of future fiber supply

The aforementioned 142 million tons of fiber demand in 2030 correspond to an incremental demand of almost 30 million tons per year compared to 2021. We will take a look at which fiber types are most likely to capture this growth (see Figure 4), based on consumers' demand for comfortable and aesthetic, yet affordable clothes. When doing so, two different perspectives are of particular relevance: first, which fiber types are able to scale their output that rapidly, and second, which fiber types satisfy the increasing demand from consumers for higher sustainability standards.

There have been multiple efforts in the past to forecast the fiber mix in terms of volume, including by Hämmerle [16] and Eichinger [17]. Some of those earlier assumptions for future fiber supply remain valid. As outlined by Eichinger, "Future fiber demand … can only be met by man-made fibers in the light of the stagnant or shrinking production of natural fibers."

Production of the most prevalent natural fiber, cotton, has been relatively stagnant for almost 20 years – of course with seasonal fluctuations [18]. Cotton harvest

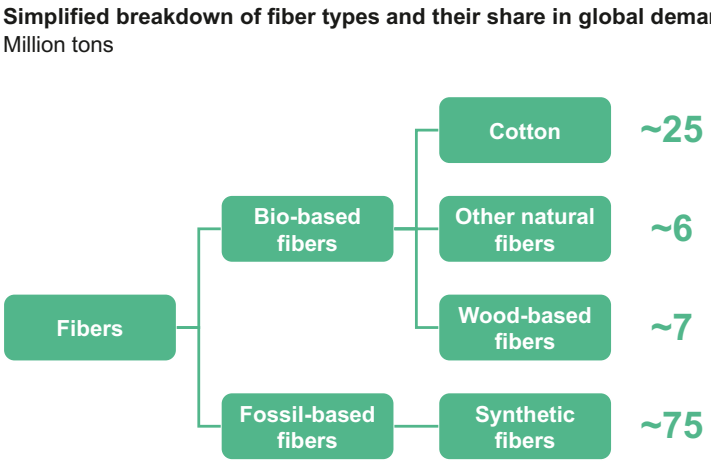


Figure 4: *Simplified breakdown of fiber types*

can be analyzed as a function of planted area and yield. Historically, since the 1950s the global cotton planted area was relatively stable in the range of 30 to 36 million hectares. Global average cotton yield grew gradually over time with improving agricultural practices, then jumping in the 2000s to ~800 kg/ha with the widespread adoption of genetically modified (GMO) cotton (see Figure 5). Combined, this resulted in recent seasonal cotton harvests in the 25 to 28 million tons range.

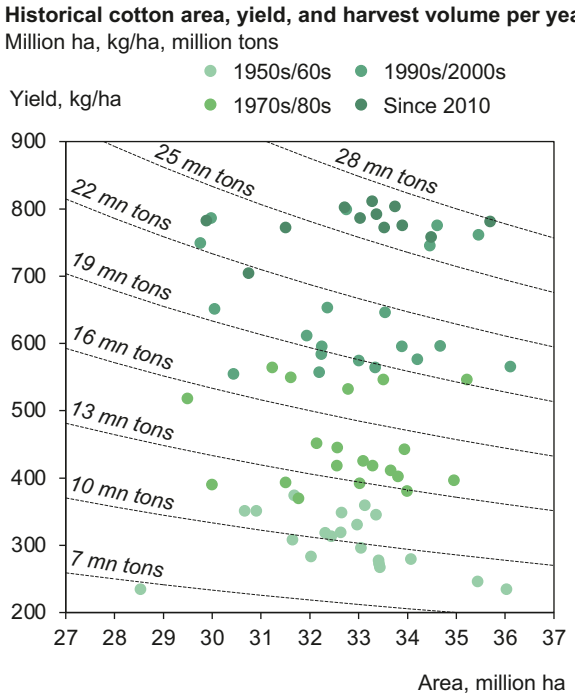


Figure 5: *Historical cotton area, yield, and harvest volume 1950-2021*

Cotton cultivation in several world regions is criticized for high water and pesticide consumption, as well as labor issues, e.g., in China's Xinjiang province. Only a fraction of the global cotton harvest is produced to higher standards with the market share of the most sustainable option organic cotton equaling less than 1% [19].

Going forward, cotton harvest can be expected to remain in the 25-30 million tons range. In its latest publication, the International Cotton Advisory Committee (ICAC) expects global cotton production to reach 27.9 million tons by the 2026/27 season [20] while the latest OECD-FAO outlook expects 30.1 million tons by 2030 [21]. Further improvements in farming practices and an ever-increasing share of GMO cotton in some countries could lead to an increase in yields. On the other hand, a higher share of (lower-yield) organic cotton, scarcity of irrigation water, and fertilizer shortages or cost increases have the potential to counterbalance this development. Therefore, yields are expected to stay relatively stable or grow only incrementally. Short-term, the cotton planted area will continue to fluctuate with term planting decisions based on expected economics compared to competing crops, such as soybeans or corn. Additional volumes might come from mechanically recycled cotton, a stream so far limited in scale <0.3 million tons produced in 2020 [22], but with great potential. Long-term, the acreage is somewhat limited upwards given the decline of arable land globally [23], increase in population, and therefore competition with food crops. Cotton-growing is also exposed to risks caused by climate change. As a recent report warns, "by 2040 half of global cotton growing regions will face high or very high climate risk exposure to at least one climate hazard" [24]. This development could eventually lead

to both a smaller cotton-growing area and crop failure; developments already witnessed, e.g., as a result of the 2010/11 flooding in Pakistan, 2019 Australian wildfires, and repeated hurricanes and drought in Texas.

Other plant-based natural fibers such bast, flax, and hemp have recently gained popularity. However, similar to cotton, they compete with other crops and are therefore somewhat limited in their potential to grow further. Animal-based fibers are equally supply-constrained. Wool production declined from 1.5 million tons in 1995 to just above 1.0 million tons in 2021 [25], and no reversal of the trend is expected.

To summarize, while cotton and other natural fibers offer many benefits in being of renewable origin, friendly to the skin, and biodegradable at the end of their useful life, they are limited in their ability to help satisfy the additional 29 million tons of demand until 2030.

Another important aspect of cotton and other natural fibers is their price. To simply keep their 2021 combined ~28% share in the total fiber market, an output increase of 8 million tons until 2030 would be required, which – based on the above supply constraints – is unrealistic. Assuming consumers continue to ask for these fibers, and based on typical economies of supply and demand, one would expect their price to increase significantly.

Contrary to cotton and other natural fibers, the supply of synthetic fibers has grown disproportionately. While they accounted for only 18% of global fiber demand in 1970, their share increased to 66% by 2021 [26], reaching a total of close to 75 million tons. Several advantages have enabled this growth. They can be engineered to meet sought-after functional properties such as stretch or abrasion resistance. Polyester staple fibers and filament yarn – with 84% of production volume the most widely used synthetics – are particularly versatile and relatively inexpensive [27]. However, the use of synthetics has recently come under scrutiny from NGOs including Changing Markets [28, 29], the Ellen MacArthur Foundation [30], and the WWF [31]. Main points of criticism include their fossil origin and therefore depletion of finite resources, high emissions in their production process, lack of true fiber-to-fiber recycling, and release of polluting microfibers throughout their life. In response to this criticism from NGOs, but also encouraged by growing demand from consumers and in anticipation of potential future regulation, many apparel brands such as Levi Strauss [32], Reformation [33], and Patagonia [34] have committed to cutting their use of petroleum-based materials, or in a first step at least using more recycled rather than virgin synthetic fibers.

While their high strength and hydrophobic nature make synthetic fibers a good fit for applications such as sportswear and functional wear, they are not suitable for all applications. As outlined by Hämmerle [35], "The physiological performance of cellulose fibres – cotton or man-made – is unmatched by any other man-made fibre. They are hydrophilic and stand for absorbency and breathability." This is where wood-based cellulosic fibers (WBCF) such as viscose, modal, and lyocell can play a critical role in closing the so-called "Cellulose Gap" [36]. "Similar properties … make them the best substitute for cotton" [37] – not to compete, but to complement cotton and compensate for demand that cotton is unable to meet due to the above-mentioned supply constraints.

Wood-based cellulosic fibers have another major advantage, particularly when compared to synthetic fibers: their superior sustainability credentials in areas such as resource consumption, land use, and biodegradability – just to name a few. Wood-based cellulosic fibers – as their name suggests – are made of wood, which is rain-fed and does not require additional irrigation. While cotton in some countries is produced without irrigation, the "world average blue water used to produce a kilogram of lint was 1931 litres", summing up to a worldwide "estimated 48,338 trillion litres (…) used for cotton production in 2018-2019" [38]. Based on global average cotton yields, cotton requires 1.31 hectares to produce one ton of fiber. In the most productive countries Australia and China, that value is only 0.52 hectares per ton of fiber [39]. In comparison, lyocell produced from eucalyptus plantations requires only 0.2 hectares to produce one ton of fiber [40]. "It should also be noted that unless the cotton is certified organic, there will be high levels of pesticides, herbicides and fertilizers used in cotton production also. Note that the land use for cotton growing is of agricultural quality, whereas the land used for growing of the trees is marginal land, generally unsuitable for growing agricultural crops" [41]. At the end of their life, clothes made of synthetic fibers take up to 450 years to decompose into microfibers [42], while those made of wood-based fibers are fully biodegradable under soil, freshwater and marine environment, achieving "full biodegradation within a couple of months" [43]. Based on those considerations, products made of truly sustainable fibers – such as organic cotton, lyocell, and ecologically produced viscose – are in high demand, both by apparel brands as well as consumers.

Wood-based cellulosic fibers are not only produced from virgin wood pulp, but also recycled content such as pre- and post-consumer textile waste, that is processed into pulp [44]. At its launch in 2017, Lenzing's TENCEL™ Lyocell fiber with REFIBRA™ technology was the "first cellulose fiber featuring recycled material on a commercial scale" [45]. By 2025, fibers with REFIBRA™ technology are planned to include up to 50% recycled content – an important step towards a more circular textile industry.

Based on the above-mentioned advantages in fiber properties and sustainability, we expect wood-based cellulosic fibers to continue their growth above the 2-3% market average, at a rate of 4-6% until 2030. Among WBCF, similar absolute growth of around 2 million tons each is expected from viscose fibers and lyocell fibers (see Figure 6). Lyocell fibers – the latest generation of wood-based fibers with superior sustainability credentials and a closed-loop production process – are expected to show the highest rate of growth with 20-30% p.a., starting from a lower base of fewer than 0.4 million tons in 2021.

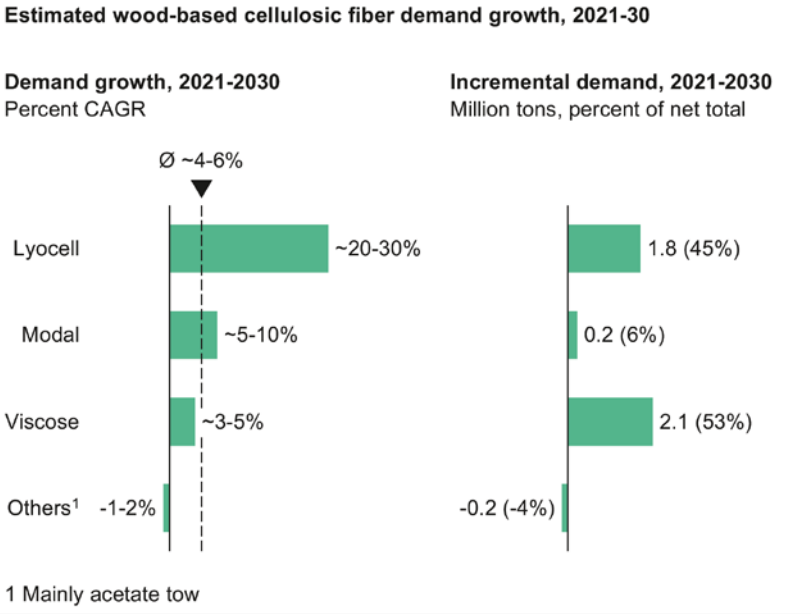
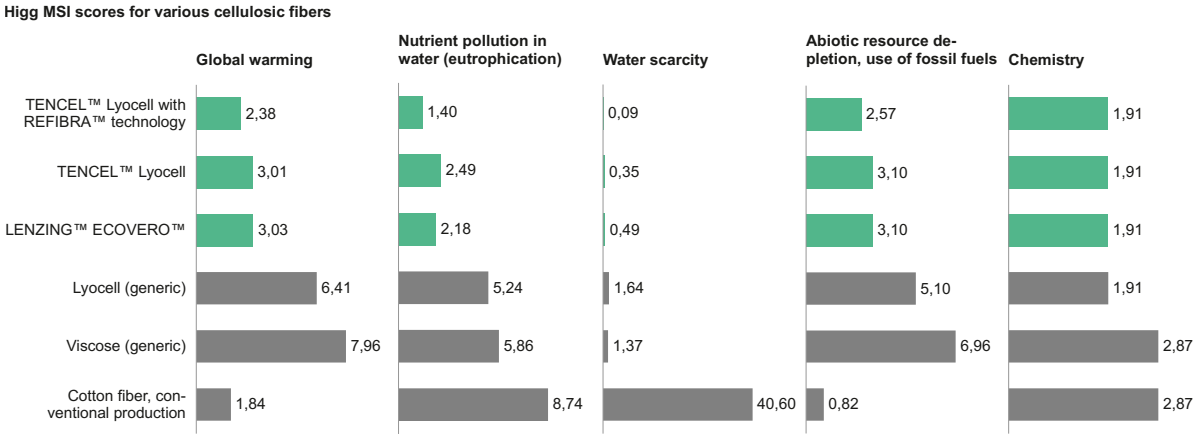


Figure 6: *Estimated wood-based cellulosic fiber demand growth, 2021-2030*

Not all wood-based cellulosic fibers are equally sustainable. Differences can be observed in areas such as wood sourcing or a plant's steam and electricity generation. Canopy's annual "Hot Button Report" aims at assessing the wood sourcing efforts and risks of viscose manufacturers. Its last edition for 2021 found "49.5% of all global supply of MMCF [man-made cellulosic fibers] is produced by producers who have attained a green shirt … with Birla and Lenzing retaining their dark green shirt status" [46] as leaders in the industry. On the other hand, many producers are criticized for "high risk sourcing" of wood pulp from ancient and endangered forests. A similar situation can be observed in utility generation of lyocell plants: while Lenzing's lyocell plants primarily utilize bio-based energy or natural gas to generate their steam and electricity demand, almost all Chinese lyocell plants exclusively run on coal. This difference in steam and electricity generation and utilization influences the carbon footprint of assets and products directly. Data for Life Cycle Assessment (LCA) comparisons between MMCF producers and products can be found via the Sustainable Apparel Coalition's Higg "Material Sustainability Index" (Higg MSI) tool [47]. Lenzing shares and periodically updates its LCA data with Higg MSI, while other lyocell producers are summarized under "Lyocell (generic)". Clearly, TENCEL™ Lyocell and LENZING™ ECOVERO™ fibers display a more favorable sustainability footprint than their generic equivalents (see Figure 7). In addition, Lenzing has set an ambitious science-based target (SBT) of a 50% reduction in CO_2 emissions (Scope 1, 2 and 3) per ton of product by 2030 compared to a 2017 baseline, which will help foster this leading position.



Note: These results were calculated using the Higg Material Sustainability Index (Higg MSI) tools provided by the Sustainable Apparel Coalition. The Higg MSI tool assesses impacts of materials from cradle-to-gate for a finished material (e.g. to the point at which the materials are ready to be assembled into a product). However, these figures only show impacts from cradle to fiber production gate (impact per kg/fiber). Higg MSI scores were calculated based on Higg MSI database V3.4 (June 2022).

Figure 7: *Higg MSI scores for various cellulosic fibers*

In addition to the well-known viscose, modal, and lyocell fibers, numerous additional technologies and processes for producing cellulosic fibers are under development. The most commonly mentioned ones are the carbamate process, ionic liquid-based process, and direct spinning using micro-fibrillated cellulose [48]. Many start-ups, particularly in Nordic countries work on commercializing those technologies, often with investment by pulp and established fiber manufacturers. While not all of their ambitious growth plans might eventually materialize, those developments can hopefully add to the supply of sustainable fibers in the future.

Conclusions

- Fiber demand continues to increase at 2-3% p.a., driven mainly by per-capita consumption
- Growth in fiber consumption comes with a significant environmental cost, CO_2 footprint, water and pesticide consumption, microplastic release, and products being landfilled at the end of their useful life
- Oil-based synthetic fibers, which have historically captured most of the growth, increasingly come under scrutiny for their fossil origin, high emissions in their production process, lack of true fiber-to-fiber recycling, and release of polluting microfibers into the ocean
- Cellulosic fibers meet the need of consumers in many applications of aesthetics and moisture management, which will gain in importance as climate change heats our planet
- Cotton, the main category of cellulosic fibers, is expected to remain supply constrained. Increasing concerns are voiced due to the significant use of water and pesticides, but also due to competing land use for food crops
- Among sustainable fibers, wood-based cellulosic fibers have the biggest potential for growth. Their properties are close to natural cellulosic fibers and they come with superior sustainability credentials in areas such as resource consumption, land use, and biodegradability
- Going forward, WBCF also provide a perfect base for a circular business opportunity for all cellulosic products, reducing the need for wood while giving a new life to cotton and other cellulosic fiber products
- Even among WBCF, differences in sustainability are significant, primarily based on their wood source as well as energy usage

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AustroCels Biorefinery in the Course of Time

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Abstract

The exceedance of planetary boundaries accompanied by an environmental crisis and increasing consumer demands for sustainable products calls for a thorough transformation of the industry. In particular, pulp and paper industry is at the forefront of a sustainable bioeconomy, by utilizing renewable raw materials.

In this article we provide a short overview of the AustroCel Hallein transformation from a traditional paper making company toward a modern biorefinery and provide some general considerations regarding the potential of pulp mills as biorefineries with a specific focus on bioethanol production.

History

The AustroCel biorefinery, located in Hallein, dates to the year 1889. In Manchester the industrialist Edward Partington and the Austrian chemist Dr. Carl Kellner founded the company "The Kellner Partington Paper Co. Ltd". One year later in 1890 the Austrian company was registered and the construction of the factory in Hallein started.

The location of the mill in Hallein had three major advantages for pulp and paper production. In the close surrounding there were large amounts of spruce wood available that could be transported on the river Salzach. The river Salzach also provided sufficient energy for the mill and the close salt mine provided brine for the required production of bleaching chemicals.

In 1898 the first paper-making machine was put into operation and in 1914 already 18.000 to of pulp and 4.500 to of paper were produced.

The period of the 1st WW, the interwar period and the 2nd WW posed a substantial challenge for the production.

However, at that time the efforts to establish a bioethanol production on site started. From 1941 on bioethanol based on the fermentation of sugars obtained during pulping was produced. These developments represent the initial groundwork of the novel bioethanol plant of AustroCel ramped up beginning of 2021.

After WW 2 "Kellner Partington" soon became one of the largest pulp producers in Austria. Beginning of the 1960s the mill had to struggle with the general overcapacity in the market for pulp and paper. Hence, the focus was shifted to high-quality print and writing paper. The highlight of this development was the implementation of a new paper-making machine, which was the largest "printing paper machine" in Austria.

Mid of the 1970s the mill reached a capacity of 85.000 to of pulp, 110.000 to of paper and 35.000 to of coated paper.

The 1980s can be summarized as the "environmental decade", including the implementation of a waste-water treatment plant, a novel pulp production, replacement of the chlorine bleaching, etc. and in 1990 the mill was the largest industrial company in the state of Salzburg with 1200 employees.

In 2009, due to the continuous decline of the demand for "coated papers" the paper production at the mill in Hallein was stopped and the last "Tambour" was delivered.

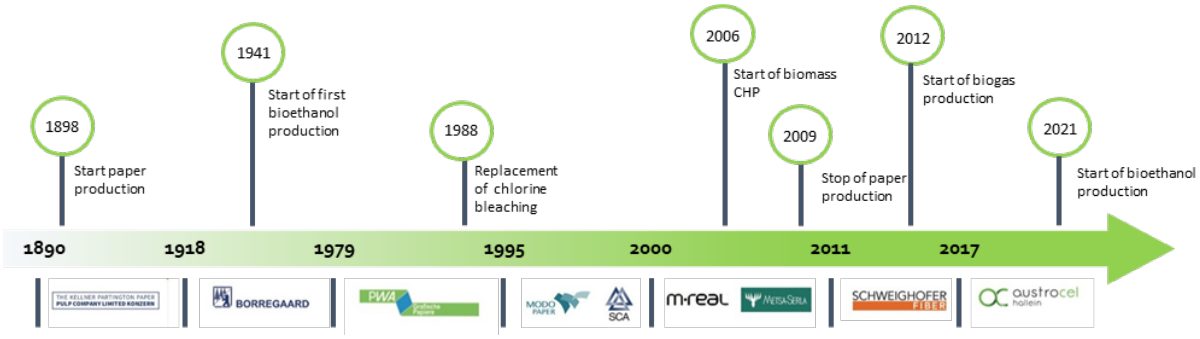

Figure 1: *Important Development steps of the AC biorefinery during different ownerships of the mill*

After this harsh cut, intensive consideration regarding possible future production scenarios took place and the decision was made to focus on the fabrication of high-quality dissolving pulp. After investments of around 60MEuro production started in 2013.

Within the last 15 years AustroCel invested substantially in biorefinery activities, including a biomass CHP; biogas production; and bioethanol production.

Wood based Biorefinery

The current climate crisis makes a thorough change of our current economic system, that is mainly based on the utilization of non-renewable resources, toward renewable resources necessary. Currently, refined fossil oil is the dominating resource for a multitude of products (e.g., polymers, energy, etc.).

In analogy to this conventional refining, it is the strategy of "biorefinery approaches", to utilize renewable agricultural and forestry feedstocks for material- and energy applications. The various biorefinery concepts mainly differ in the utilized feedstock. For example, there are sugar, or starch-based biorefineries. In the case of lignocellulosic biorefineries wood is utilized as the starting feedstock. Different processing steps of this biomass result in various intermediate or end products, such as chemicals, raw materials, or bioenergy.

Characteristic feature of biorefineries is the coupling of material- energy-based product pathways. Hence, biorefineries contribute to a sustainable transformation from two sides: from a material- and an energy perspective.

In principle, two main biorefinery concepts are used 1) "bottom up" and 2) "top down" systems. "Bottom-up" approaches represent typical extensions of existing biomass valorization plants such as pulp mills, whereas "top-down" facilities are designed from scratch to obtain a multitude of products from specific raw materials.

In this regard, the European pulp and paper industry is at the forefront of the increasing biorefinery efforts, by utilizing the renewable resource wood from sustainable sourced forests. Apart from the two main products pulp and paper, numerous other bio-based products are already fabricated.

According to a recent study, there are about 139 biorefineries active in Europe, mainly pulp and paper facilities. About 3% of the revenues of the pulp and paper industry result from biorefinery products, with an upward trend. [Cepi 2021]

The following part provides a brief overview and background regarding the potential of pulp mills based on acidic sulfite pulping for biorefinery approaches.

For dissolving pulp, it is necessary to obtain a very pure cellulose fraction. For that, the other wood constituents, including hemicelluloses, lignin and extractives needs to be removed. These constituents account for the potential side-streams and side products.

For example, during the acidic Mg-sulfite pulping around 47% of the mass of spruce wood is removed in side streams. Per to of spruce wood this accounts for 255 kg lignin, 140 kg galacto-glucomannan, 58 kg arabino-glucuronoxylan and 18 kg extractives. (Sixta in Handbook of pulp).

Most of these side products are in the brown liquor after the pulping process. The polysaccharides are hydrolyzed due to the acidic conditions. Different rates of hydrolysis yield oligo or monosaccharides. The hemicelluloses' structure determines the carbohy-

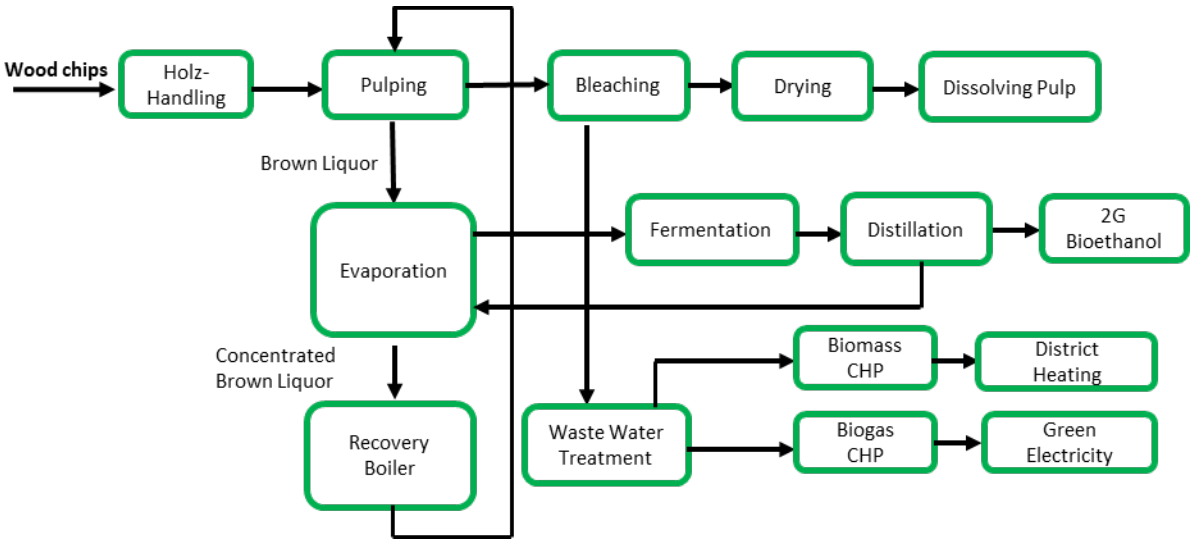

Figure 2: *Flow Scheme of the AustroCel Biorefinery*

drate profile in spent liquors. Galacto-glucomannan tends to be cleaved down to the monomers, whereas glucurono-xylan is resistant to total hydrolysis due to glucuronic acid side groups.

In the case of spruce wood, the brown liquor is mainly composed of mannose, galactose, glucose, xylose, and arabinose. The C6 sugars represent an ideal feedstock for fermentation approaches, for example bioethanol production.

In contrast to the alkaline Kraft-pulping the lignin is not split at the ether bonds; but rather sulfonated, which results in a solubilization of the Lignin.

The resulting lignosulfonate is characterized by a good water solubility; high MW and can be used for dispersing applications.

The fourth main constituent of wood are so-called extractives, which are lipophilic compounds and specific for the various wood species. For example, spruce extractives are rich in terpenes, including alpha-pinene (Sjöström, 1993). During sulfite pulping alpha-pinene is oxidized to p-cymene (Rydholm, 1965), an aromatic compound, which then can be isolated from cooking liquors and can be utilized in manifold applications.

After removal of the brown liquor the pulp undergoes a bleaching sequence. The hereby removed organic material can be used for biogas production. Typical components found in a bleaching effluent from a sulfite pulp mill are a mixture of hydroxycarboxylic acids, fatty acids, methoxybenzoic acids and other lignin-derived compounds (Bogolitsyna, Holzforschung 2012).

A rather novel development for pulp and paper industry is driven by the need to foster circularity of raw materials and covers strategies to re-use cellulose-based textiles.

In general, separation of cotton or regenerated cellulose fibers from synthetic fibers is complex and costly and the degree of polymerization (DP) of cellulose chains might be small due to the production process and numerous washing cycles.

One strategy for reuse of the cellulose fraction is its hydrolysis and fermentation while the synthetic fraction can be recycled in the fiber production cycle.

Hydrolysis and fermentation of cotton parts of textile waste offers one way to recycle the synthetic fraction in fabrics and convert the used cellulose to fermentable sugars. Deposition or incineration can be avoided, and the fermentation products are capable to replace fossil based raw materials.

AustroCel Biorefinery

The AustroCel Hallein GmbH (AC) currently employs around 290 people and is one of the leading producers of textile pulp from spruce wood. The biorefinery in Hallein produces up to 160.000 to dissolving pulp, 100GWh district heating and 100 GWh green electricity. By that AC provides green energy

for about 25 000 households and district heating for 10 000 households. Since 2021 AC runs the largest 2G bioethanol plant based on wood, with a capacity of up to 35 Mill liters per year.

Bioethanol [1]

The current EU policy for renewable energy including bioethanol is based on the EU Energy and Climate Change Package (CCP) and the Fuel Quality Directive (FQD). The so-called Renewable Energy Directive (RED) represents one part of the CCP and provides the specific requirements for liquid biofuels. (Flach, Lieberz, & Bolla, 2019). In 2019 an amendment, the RED-II, was published. It implies, that the share of renewable energy in the final energy consumption must be at least 14% by 2030.

Austrian blending mandates between 2012 and 2020 were 5.75% overall, divided in 6.3% biodiesel and 3.4% bioethanol. Since 2020, the overall percentage is 8.75% without division between fuels. The introduction of E10 was already discussed, but never enforced. Double counting is valid for waste materials and residual products from agricultural and forestry production including fisheries and aquaculture, residues from processing, cellulosic non-food materials or lignocellulosic materials. (Lieberz, 2019)

Further legislation, transposing RED-II into national law has yet to be created and will consitute the framework for targets beyond 2020. Setting specific targets for the use of advanced biofuels (eg. 2G bioethanol), according to RED-II, will increase market demand for advanced biofuels. RED-II foresees following targets for advanced biofuels: 0.2% by 2022, 1% by 2025 and 3.5% by 2030 of final consumption of energy in the transport sector.

The current production of advanced bioethanol in the EU is estimated at around 50 million litres. (Flach, Lieberz, & Bolla, 2019) Most advanced bioethanol producers utilize agricultural residues, such as wheat straw or corn stover. Borregaard and Domsjö Fabriker are utilizing brown liquor from wood pulping for their production, such as AustroCel Hallein. St1 is fermenting organic wastes to bioethanol. (ETIP Bioenergy, 2020)

Table 1 lists operational advanced bioethanol production facilities in Europe. The joint capacity amount to 63,420 t/y (equal 79.9 million litres).

Bioethanol at AustroCel Hallein

Already between 1941–1988 bioethanol was produced in the pulp mill in Hallein with a capacity of 6000l/d. This time established experience with brown liquor as substrate. From 2007–2009 a technical preproject, including a conceptual engineering was performed.

In 2011, the transformation from paper-to dissolving wood resulted in an increased sugar content in the spent sulfite liquor, which enabled a higher bioethanol yield.

Hence a new project was established, and it took 3,5 years from concept and basic engineering (July 2017) to full scale production in 2021.

AustroCel Hallein already conducted 60 fermentation and distillation trial runs in lab scale. Substrate and by-products were comprehensively analysed. Different yeast strains and their properties were cultivated and evaluated in a microbiology lab. Process parameters and their effects on sugar conversion rate and yeast viability were tested and a pilot fermentation plant was operating for more than 2 years.

30 million litres bioethanol per year, accompanied by substantial CO_2 saving, could substitute about 1% of gasoline demand by 2025.

[1] Parts of this chapter were previously published in the Biofit Case Study report, a Horizon 2020 project.

Table 1: *Other operational advanced bioethanol production facilities in Europe (status 2020)*

Company	Country	Start-up Year	Capacity t/y
Borregaard Industries	Norway	1938	15,800
Domsjö Fabriker	Sweden	1940	19,000
St1 Cellulonix Kajaani	Finland	2017	8,000
St1 Etanolix Jokioinen	Finland	2011	7,000
Chempolis Ltd. Biorefining Plant	Finland	2008	5,000
St1 Etanolix Gothenburg	Sweden	2015	4,000
Clariant Sunliquid	Germany	2012	1,000
St1 Etanolix Hamina	Finland	2008	1,000
St1 Etanolix Vantaa	Finland	2009	1,000
St1 Etanolix Lahti	Finland	2009	1,000
IFP Futurol	France	2016	350
SEKAB Biorefinery Demo Plant	Sweden	2004	160
Borregaard BALI Biorefinery Demo	Norway	2012	110

The bioethanol plant at AC has operational and investment advantages compared to a greenfield scenario. Feedstock and energy supply of the pulp production process provide ideal boundary conditions. Additionally, it is an investment for a sustainable bioeconomy and a step forward to fulfilment of blending mandates for advanced biofuels according to RED-II.

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Lyohemp™ Fibres from Hemp Shive Dissolving Pulp

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Abstract

Hemp shives, which were grown in various areas and harvested, disintegrated and treated using different methods, were investigated. First sulphur free alkaline digestions of these hemp shives were carried out after physical-chemical characterisation. The obtained pulp was washed and bleached without using chlorine compounds and complexing agents. All required target parameters (residual lignin, degree of polymerization, solubility in Cuoxam) comply with limits for metal contents) could be achieved after optimisation of the conditions during digestion and cleaning. A scale-up into large lab-scale was successful and the resulting pulp is suited for spinning tests. Therefore, the first filaments based on 100% of hemp shives pulp are ready for presentation. The production of a pulp for making continuous filament and staple fibres turned out well. The transfer of all investigated processes in the industrial scale is possible and the pulp can be produced as far as possible by environmentally compatible means.

Introduction

Cellulose man-made fibres (CMMF) relive a strong increase of market demand. About 10 million tons should be enquired until end of this decade [1]. Because of the shortage of wood as resources the interest in using agricultural residues as raw material for pulp production is growing. Hemp as an interesting agricultural plant for soil improvement can be used for nutrition, cosmetics and isolation material.
Figure 1 shows the typical composition of mechanical treated hemp straw [2].

The textile use of fibres has a long tradition, but cotton on one hand and man-made on other hand led to replacement of this material. In a former project the development of Lyohemp™ based on hemp fibres was successfully investigated [3, 4]. The aim of our research now was to develop an environmentally compatible method for pulping hemp shives as raw material for manufacturing of Lyohemp™ fibres.

Shives (amount about 55%) as a typical side product of hemp cultivation are the largest share of the hemp straw and so its material use seems very attractive. Actually hemp shives are used as animal bedding or for loam constructions inforcement. Nevertheless, the revenue is low. Goal of a collaboration project with FUDI Futtermittel und Dienstleistungs GmbH & Co. KG (Zeulenroda, Germany), MATRAK Service und Lohnarbeits GmbH (Auma-Weidatal, Germany), IPWC and TITK was the development of a technology for production dissolving pulp based on this raw material. The pulp should be suited for production of Lyocell, or better Lyohemp™ , and should be characterised by a completely environmentally friendly process. So a sulphur free digestion without anthraquinone as well as washing and bleaching without chlorine components and complexing agents were in the focus.

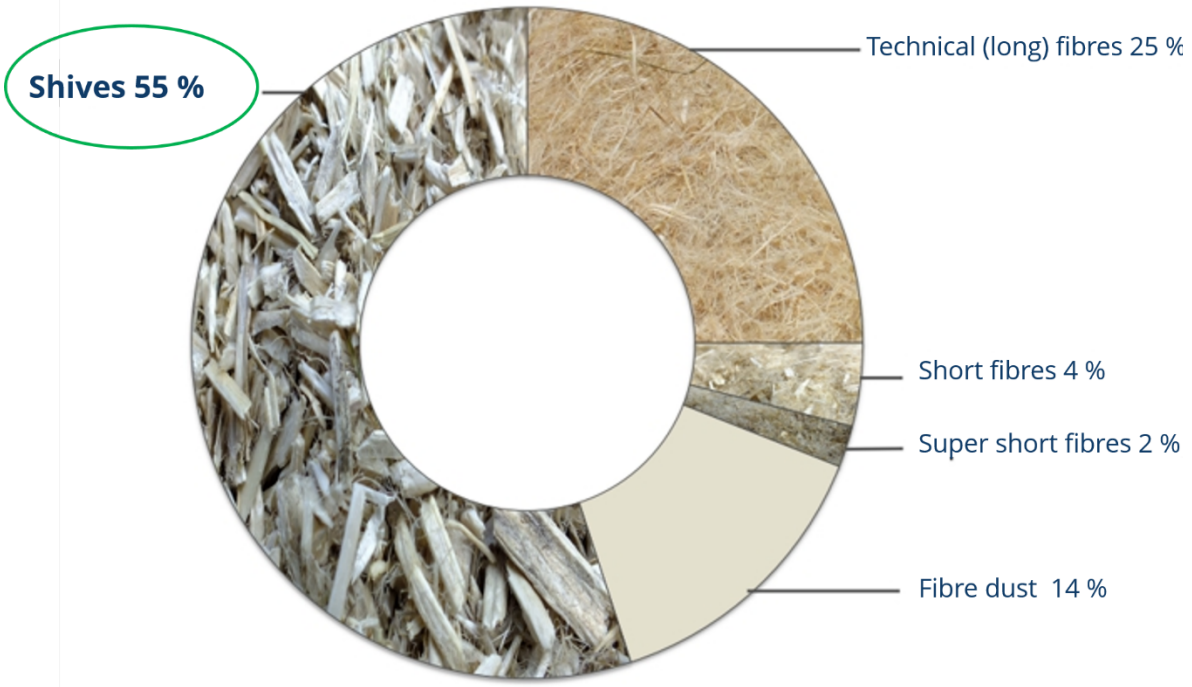


Figure 1: *Typical composition of mechanical treated hemp straw [2]*

Materials and methods

Characterization of raw material

In the frame of this project more than 20 hemp shive samples were investigated. The raw material was originated from various growing areas in Germany as well as in France and pre-treated in different ways.

For a first characterisation well known wet chemical methods were used. At first the dry content was determined. The following second step was an extraction in a 1:1-mixture of toluene and ethanol. Cellulose content (method by Kürschner and Hoffer, [5]), Klason-lignin content (method by Savard based on TAPPI T 222 om-83, [6]) and holocellulose content (water-insoluble carbohydrates, method by Wise, [7]) were determined using the extracted samples. The value for hemicellulose content is corresponding to the difference between holocellulose and cellulose. In addition, the ash content at a temperature of 575 °C was determined for all samples.

Digestion

Laboratory tests (2-Liter-autoclave)

For the first tests the digestion of the shredded hemp shives took place in 2-Liter-autoclave with temperature and pressure control as shown in figure 2.

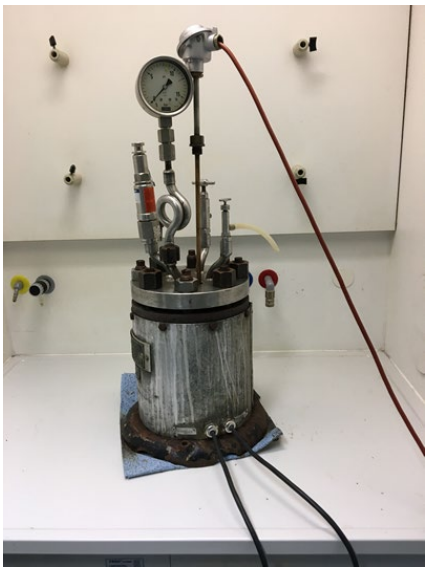


Figure 2: *2-Liter-autoclave (IPWC)*

About 170 g of raw material was covered by a freshly prepared sodium hydroxide solution (3.5 – 4.0% by weight). The liquor ratio, as consumed NaOH per dried raw material was 34% by weight. The optimal temperature ranged over 170 °C, the reaction time was 105 minutes, which leads to a H-Factor of about 1,500. The maximum process pressure increased to about 10 bar. For all tests the H-Factor as comparative value was calculated [8].

After finishing pulping, a multi-stage process of washing and bleaching steps followed. At first, the splinter content was separated. Characteristic is a washing step in 20% acetic acid (A) at the beginning, followed by bleaching applying 5% hydrogen peroxide (P) and repeating the acidic washing step(A). That means the optimal bleach sequence for the hemp shive pulp is A-P-A, carried out at a temperature of 85 °C. Finally, the pulp should be washed by deionized water to available a neutral pH-value.

Scale-up to 10-Liter-Digester

The first scale up took place in a 10-Liter-digester system with engineering and recirculation of alkaline solution control as shown in figure 3.

Figure 3: *10-Liter-digestor (IPWC)*

For this digestion 800 g shives are necessary. In contrast to pulping using the autoclave a preheating of cooking liquor is possible as well as an active cooling. So, the optimal parameters are a little bit different. The preferred liquor ratio is 40% by weight and the optimal temperature is 170 °C, too. The reaction time should be 150 min. So, a H-Factor of about 2,500 is necessary for reaching the pursued DP.

Characterization of pulp

The results of digestion were checked by TITK during an iterative evaluation procedure. Most important are the complete solution of the pulp in Cuoxam, a DP in the suited range (550 to 650), low metal ion contents (heavy, alkaline and alkaline earth metals) as well as a high α-cellulose amount [8ff]. Beside other criteria a Kappa-number lower than 5 was pursued.

The Kappa-number was determined following an internal standard at IPWC in accordance with ISO 302:2015-08. The dry content was determined by examining the loss in mass of the samples after drying at 105 °C. Pulp samples as well as cellulose samples regenerated from the dopes and from the spinning tests dissolved in Cuoxam were characterised by capillary viscometry for determination of the average degree of polymerization (Cuoxam-DP). The α-cellulose content was determined by investigation of the pulp amounts which are resistant to 17.5% sodium hydroxide solution at 20 °C.

The contents of heavy metal (Fe, Cu, Mn, Cr, Ni) as well as alkaline (Na, K) and earth alkaline- (Ca, Mg) ions were measured after microwave digestion according DIN EN ISO 11885 (E22) using ICP-OES. The ash content was determined after incineration at 900 °C.

Measurement of carboxyl group contents has been carried out by means of complexometric titration of zinc ions after removing of the metal ions from the cellulose at first and adding of zinc acetate solution in a second step. The carbonyl group contents were analysed by measurement of the absorbance at 530 nm after reaction with 2, 3, 5-triphenyltetrazoliumchloride solution.

The details of this cellulose characterisation were described in former publications. [9, 10]

Dope preparation and spinning tests

The preparation of cellulose dopes in small laboratory scale was carried out using a special vertical kneader system, linked with a RHEOCORD 9000 (HAAKE). Temperature, torque moment and revolutions per minute (rpm) vs. time were recorded on-line. The dopes were prepared, starting from an aqueous suspension of the treated pulp in 50 wt.-% aqueous NMMO, by removal of the excess water at elevated temperatures, higher shearing stress and low pressure during the dissolution processes (80-95 °C mass temperature, 800-40 mbar pressure, 5-20 rpm). 0.5 wt-% propylgallate, with regard to cellulose, were used for stabilisation of the NMMO solutions. After finishing of the excess water removal (achieving a NMMO monohydrate state), an after-dissolution kneading step (60 min, 90 °C mass temperature, 250 mbar) followed for homogenisation of the prepared dope.

An upscaling into 4 kg dope scale was carried out using planetary mixing machine PML 40 (Netzsch-Feinmahltechnik GmbH).

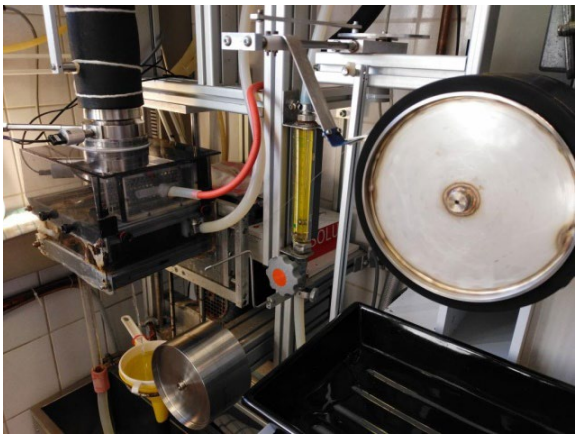
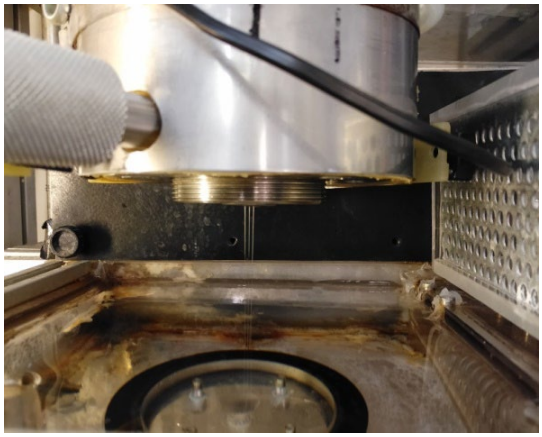
Small lab spinning tests were carried out by dry-wet spinning experiments for preparation of staple fibres of about 1.7 dtex fineness using a laboratory piston spinning equipment, which is described in former publication [11]. Spinning nozzles, containing 30 holes with capillary diameters of 100 μm were used for all laboratory spinning experiments. The spinning temperatures

were selected in each case according to the determined rheological properties of the used cellulose dopes.

Further semi-technical spinning tests using spinnerets with 6 x 80 capillaries (90 μm outlet diameter) were carried out for investigation of the spinning behaviour and stability. These trials were used for preparation of staple fibres and multifilament samples.

The equipment for the tests is shown in table 1.

Table 1: *Spinning equipment used for hemp shive pulp shaping*

 Small lab spinning equipment	 Large lab spinning equipment
 Small lab spinning nozzle (30 capillaries)	 Large lab spinning nozzle (6 x 80 capillaries)

Results and discussions

Technical basics and requirements

Some selected and the average values of plant analytics are compiled in table 2.

The cellulose content in shives (< 50%) is significant lower than in technical long fibres (> 70%), but in the same range like in wood or other agricultural residues. The chemical composition is almost independent on place or region of cultivation. A short retting time should be preferred because the cellulose content is higher and the lignin can be separated at lower temperatures and chemical consumption. For a good pulp quality also a satisfied separation from bast fibres, dust and fine content is reasonable. In the frame of our work we mainly used the mechanical pre-treated shives of the project partner FUDI, because they were available in sufficient quantity. Despite of the relative low cellulose content they had a good quality for pulping.

Optimization of digestion

Before optimisation the first challenge was to get a hemp shive based pulp using a sulphur free alkaline process. Compared with other agricultural residues the digestion requires harsher conditions; that means a

Table 2: *Plant analysis of different hemp shives*

Component/ region	Cellulose [%]	Hemicellulose [%]	Lignin [%]	Extraktives [%]	Ash content [%]
Brandenburg	43.9	33.6	16.4	2.8	1.6
Saxony	41.3	30.1	24.4	4.3	4.2
Mecklenburg	42.9	30.6	22.3	2.8	0.03
France	43.0	33.6	20.2	3.2	1.4
FUDI	37.3	34.5	21.7	4.2	1.6
range	37 - 46	30 - 34	16 - 25	2 - 7	< 0.1- 4

higher NaOH-concentration, higher temperatures and a longer cooking time are necessary. So the strived H-Factor is significant higher than below 1,000 as described in previous works [12, 13].

While in the 2-Liter-autoclave a H-Factor of 1,500 was optimal, in the 10-Liter-digestor a H-factor of 2,000 to 2,500 is required. Three digestions in this scale were required for manufacturing of larger pulp quantities for the large lab spinning test. The pulp could be used as a mixed sample. Therefore, a good reproducibility of pulping was notable.

As described above a three-step procedure for washing and bleaching after pulping is required for getting the target parameters. During the wash steps using de-ionized water and acetic acid (A) the metal ions were removed, target Kappa-number and DP could be adjusted via bleaching (P). Finally, all desired values were achieved.

The developed digestion and bleaching process permitted the preparation of hemp shives based pulps for Lyocell applications. The pulp parameters could be adapted for usage in fibre preparation by dry-wet spinning NMMO processes.

Dope preparation and spinning tests

Dope preparation and fibre spinning tests could be carried out successfully in both, small and large laboratory scale. The cellulose concentration used was in typical range of around 12%, also with regard to the rheological properties.

The prepared fibres and filaments showed well acceptable textile-physical properties, very comparable to industrially produced Lyocell fibres from wooden pulps [14].

Table 3: *Hemp pulp properties*

Parameter	Unit	Hemp pulp sample 1 2-Liter-autoclav	Hemp pulp sample 2 10-Liter-digestor
Cuoxam-DP		632	624
α-cellulose content	%	89.6	87.7
Carboxyl group content	µmol/g	n.m.	45.4
Carbonyl group content	µmol/g	13.6	18.8
Fe, Cu, Ni, Cr, Mn	ppm	14	45
Na, K	ppm	90 / 47	61 / 7
Mg, Ca	ppm	33 / 415	3 / 14
Ash content	%	< 0.1	n.m.

Table 4: *Spinning dope and fibre properties using hemp shive based pulps*

Dope characteristics	**Unit**	**Small lab spinning test**	**Large lab spinning test**	**Lyocell fibres from wood pulp [14]**
Zero shear viscosity (85°C)	Pas	10,620	13,270	
Cellulose concentration	%	11.6	11.9	
Fibre testing				
Fineness	dtex	1.8	1.7[a] / 1.6[b]	≤ 1.7
Fibre tenacity, cond.	cN/tex	36.0	39.7[a] / 51.5[b]	40 - 42
Elongation, cond.	%	16.3	12.3[a] / 7.2[b]	15 - 17
Loop tenacity	cN/tex	17.8	13.0[a] / n.m.[b]	
Cuoxam-DP		623	560	560 - 620
Photos of prepared fibres / filaments				

[a] staple fibre
[b] filament, single fibre testing

Conclusions

The aim of the studies was to evaluate the potential of hemp shives in conversion to high purity dissolving pulp grades those could be used for manufacturing Lyohemp™ fibre and further processing into textiles Shives as raw materials were successfully investigated by a modified soda cooking process and additional pulp bleaching and washing steps. So organic impurities and metal salt concentration could be decreased down to those levels which were compatible to the Lyocell process requirements. After adjustment and optimization, the hemp shive pulps prepared from different laboratory scales could be well dissolved in NMMO monohydrate and prepared dopes exhibited satisfying properties for air-gap spinning. The produced Lyohemp™ fibres proposed well sufficient mechanical properties for further textile processing. Yarns made of these fibres represent fine counts, high tenacity and low mass variation, which also benefit yarn dyeing and finishing procedures.

Unfortunately, a scale-up in technical standard could not be realized in the frame of this work, but it is assumed that manufactured Lyohemp™ fibres offer surprisingly good processing properties into yarns and fabrics as well as wearing and draping comfort in apparel application, too.

Acknowledgments

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Development of Sustainable Menstruation Pants using Speciality Viscose Fibres

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Abstract

Kelheim Fibres GmbH is one of the leading suppliers for fibres used in menstruation hygiene products. However, conventional menstruation products like sanitary pads, which are made from petrochemical fibres and raw materials, lead to a huge amount of non-biodegradable and non-recyclable waste. Therefore, it is necessary to engage in the development of more sustainable solutions. Here the development of a reusable menstruation pants mainly based on renewable cellulosic raw materials is described and the market potential of such a product is determined. In order to get to know the existing market for period pants a competitor product analysis of ten brands from the core markets Europe and North America was carried out. Simultaneously different cellulosic fabric materials were tested in order to design and assemble a prototype. The acquisition time and the rewet value of the products and material samples were tested. The best market product showed an outstanding performance but has drawbacks concerning the physical properties of the pants. The material tests showed that the performance is dependent on both, the material composition as well as the material structure. The most promising results showed CELLIANT® Viscose and Danufil® Fibres in the Topsheet, Galaxy® in the ADL layer, Bramante, a hollow viscose fibre, in the Absorbent Core and a water repellent woven fabric, a biodegradable PLA film or a sustainable coating as a Backsheet. Further work is planned to optimize the composition and the knitting structure of the individual layers.

Keywords: *Period Pants, Sustainability, Menstruation, Reusable products, Textiles, renewable fibres, cellulosic fibre, Circular economy*

Abbreviations:

- ADL: Acquisition distribution layer
- EU: European Union
- SUPD: Single Use Plastic Directive
- PLA: Polylactic acid

Conventional menstruation products like sanitary pads, are well established in the feminine hygiene sector. However, they have serious negative effects on the environment as they generate huge amounts of waste and cause marine pollution. With the Single Use Plastic Directive the European Union aims to substitute single use plastics and drive more attention towards this topic (European Parliament, 2019). As a result, menstruation products using synthetic materials have to be labelled accordingly. Due to the rising environmental consciousness, new, more sustainable products are on the rise. In the feminine hygiene sector, reusable products like menstruation pants have been developed and placed on the market. However most period pants use different raw materials, including synthetic fibres, in order to achieve an adequate performance. At the end of their product lifetime, these products contribute to the growing amount of textile waste, as they are hard to recycle and non-biodegradable. In this article the development of fully sustainable and biodegradable menstruation pants using predominantly cellulose based raw mate-

rials is displayed. In addition to that, the market prospects of such a product are showcased.

To determine the performance of menstruation pants and material combinations, an adaption of the acquisition time and rewet test method was used. The acquisition time is the time a defined liquid volume takes to be absorbed by the test specimen. The rewet value measures the amount of liquid that is given back by the test specimen to a filter paper in 10 seconds under the pressure of 1 kg. Both values should be as low as possible. The competitor product test was carried out with liquid volumes of 10, 15 and 20 ml. For the material test, a standardized sample size of 15 x 8 cm and a standard liquid volume of 10 ml were used. Further measurements of the thickness, the air permeability and the weight of all tested products and materials have been done as well.

Menstruation is a topic that affects half of the human population on earth at a certain time of their lives, which equals 3,865 billion people (United Nations, 2019). On average, the length of a menstruation cycle is 28 days and the amount of blood lost varies between 20 and 60 ml, with amounts over 80 ml classified as heavy menstruation (Mengel, 2020). As the subject of menstruation is still stigmatized and considered as a taboo topic, adequate communication is hard to achieve. As a result, informed choice of the product selection is not possible for a huge number of women and single use menstruation products are frequently disposed incorrectly leading to environmental and marine pollution. In 2017, 49 billion sanitary menstruation products that equal 590,000 tons were used alone in the 28 member states of the European Union (Van den Bossche , 2020). In addition to that single use sanitary pads are on average made 90% of synthetic materials (Van den Bossche, 2020) and therefore incorrect disposal endangers the marine environment through plastic waste and the creation of micro plastics. In contrast to that, tampons are made, up to 95% out of cellulosic material. In Germany the predominantly used menstruation products are tampons which are used by 71% of women closely followed by pads with 62% according to a study conducted in 2020 (Sparwelt GmbH, 2020). Only 7% of the study participants use sustainable alternatives to conventional single use menstruation products (Sparwelt GmbH,

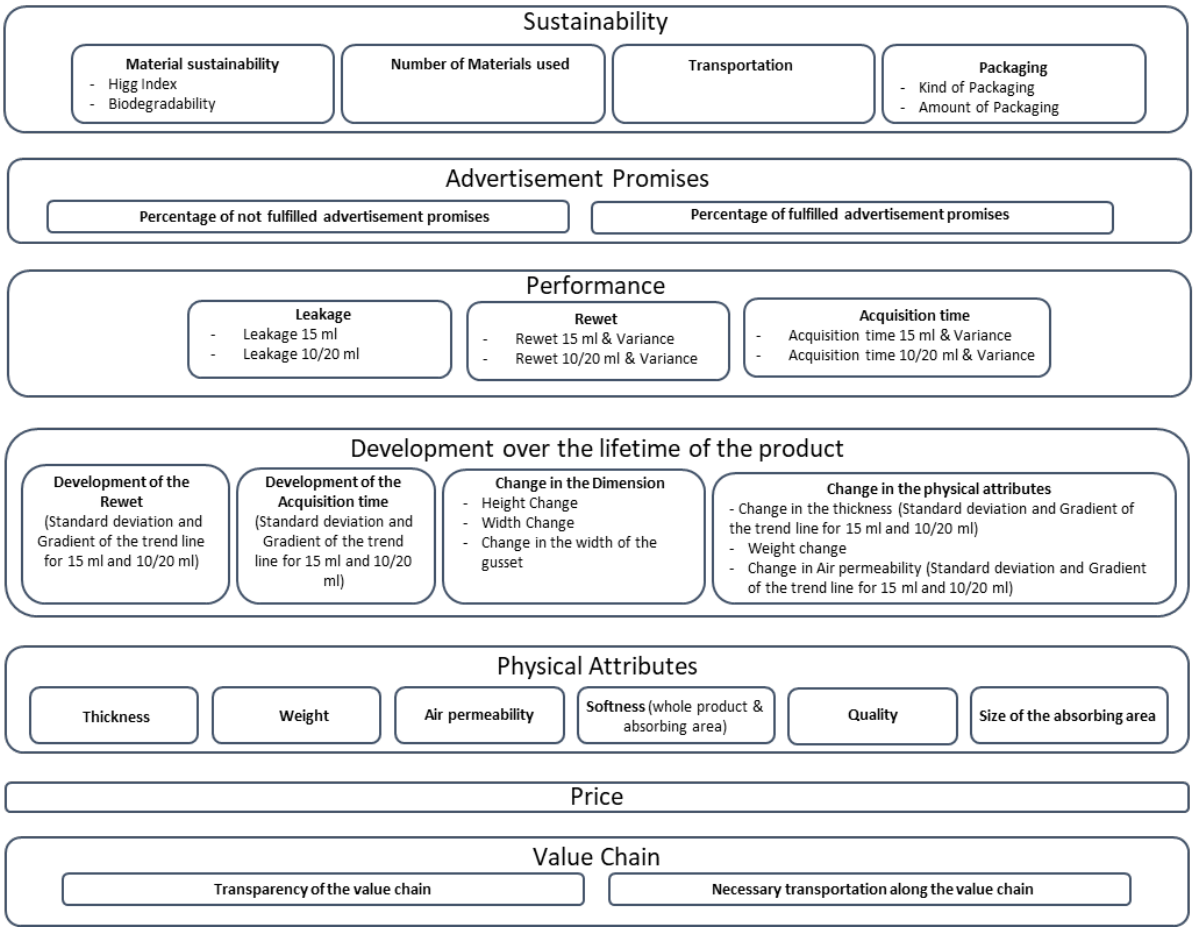

Figure 1: *Categories for the evaluation of the competitor product test of the menstruation pants*

2020). The main influencing factors found during the study for the use of sustainable products are the amount of the salary and the age of the woman. Women with a salary over 1500 Euro per month and an age between 20 and 29 are more likely to use sustainable alternatives (Sparwelt GmbH, 2020).

The competitor product analysis rates the products against each other in the categories sustainability, performance, physical attributes, development over the lifetime of the product, fulfilment of the advertisement promises, price and value chain. Each category is split up in sub-categories as displayed in figure 1. The categories performance and sustainability are graded double as they are the main interest of this paper.

The best product is rated with one point and the worst with 10. The brands were selected after an analysis of the worldwide market of period pants has taken place. In total 83 companies selling such products could be found in April 2021. The main markets identified are North America (21 companies) and Europe (56 companies) whereby France (30 companies) has the highest number of competing companies in the market. The overall period pant market is made up mainly by Start-Ups. Recently also more established brands are entering the market. This shows the rising trend towards more sustainable solutions in menstruation management. Especially large brands offer period pants that are only suitable as backup used together with a tampon or a menstruation cup. Especially in the European market, it has been observed that a transparent and local value chain is very important and that brands often engage in social projects regarding period positivity or saving the environment. The two most used advertisement categories worldwide are Sustainability/Environment and Comfort & Convenience. Possible new future market potentials can be seen in Asia especially in China and India as both countries have a growing population and a cultural climate that stigmatizes invasive menstruation products. In addition to that, Scandinavia and South-East Europe are not included in the period pants market so far but can have a huge market potential.

Criteria for the selection of the companies for the competitor product test are the reach on social media especially Instagram and Facebook, the online and offline retail, the location of the brand and that production on industrial scale can be detected.

The final rating of the competitive product test can be seen in table 1. The performance values of the winning German company are an average acquisition time of 3.73 seconds and an average rewet value of 3.77 g at a test liquid volume of 15 ml. Nevertheless, almost none of the products can performance wise compete with conventional pads. It is also noticeable that there is a huge variance in all measured values of the period pants over the testing span of 26 washing cycles which equivalents two years of usage. In addition to that there is a lack of a consistent unit for the amount of liquid a product can absorb. As a result, it is very difficult for consumers to choose the right product. Uncertainty is created which prevents women from trying period pants as an alternative to conventional products.

Table 1: *Evaluation of the competitor product test of the menstruation pants*

Position	Country of the brand	Sustainability	Advertisement promises	Performance	Lifetime Development	Physical Attributes	Price	Value Chain	Final Points
1	Germany	4.00	2.5	1.33	3.26	5.75	8	2.5	3.49
2	Spain	1.75	6.5	5.25	3.63	3.83	1	2	3.73
3	Germany	4.00	1.5	3.33	4.82	5.25	5	4	3.78
4	Canada	5.38	4	3.75	3.60	4	2	4.5	3.99
5	Germany	3.50	2	5	3.83	4.33	9	2	4.00
6	France	4.63	4	3.17	4.72	7.17	3	2.5	4.15
7	France	3.63	4	5	5.64	4.42	10	2	4.63
8	France	3.38	7.5	4.5	6,67	3.67	6	2	5.07
9	France	6.13	7.5	4.58	5.79	4.33	4	6	5.67
10	USA	5.25	6.5	6	5.76	5.33	7	5	5.85

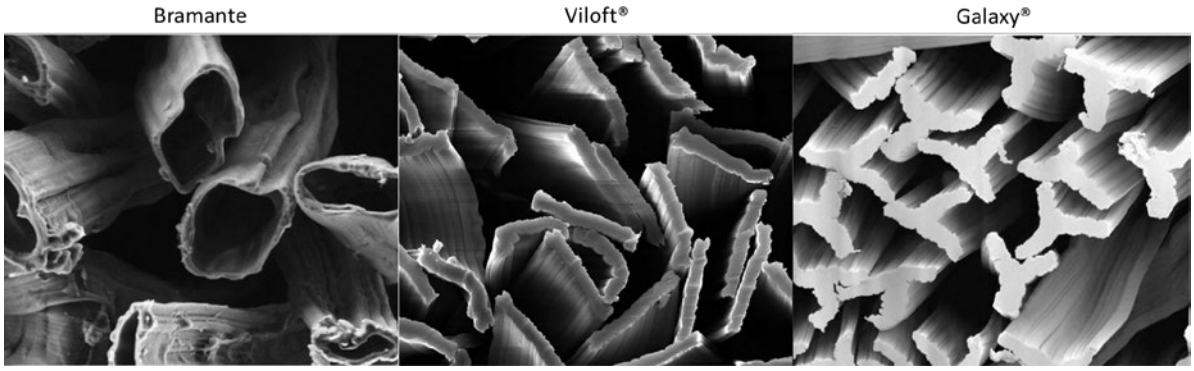

Figure 2: *Cross-sections of the Bramante, Viloft® and Galaxy® viscose fibres*

In order to develop a new, more sustainable product test with different materials and prototypes are carried out. The aim is to find an ideal material for each layers of menstruation pants. Menstruations pants are structured in four layers, the Topsheet, the ADL, the Absorbent Core and the Backsheet. The material tests aim to find the ideal fibre composition and textile structure for each of the first three layers. The prototype tests on the other hand target the Backsheet and the overall product assembly of the menstruation pants.

In figure 2 an ideal composition of a menstruation pant can be seen. It is assumed that the combination of the materials in the shown knitting structures will have an improved performance. In addition to that, the quality and the performance can be further increased by using the right manufacturing and seam technologies. In general, viscose is perfectly suited for the use in menstruation pants as it has a high water absorbing capacity. Another benefit is its biodegradability and the possibility to modify the fibre. The performance can be increased especially through the implementation of functionalized fibres. For the Topsheet a combination of CELLIANT® Viscose and Danufil® Viscose in a loose knitted structure with ideally implements tucks to create a perforation would be suitable. CELLIANT® Viscose is created by incorporating CELLIANT® powder into the viscose fibre. The Fibre has advanced well-being properties as it reflects the body heat back to the body in the form of infrared light. This property can help the wearer to a better wearing sensation even when period cramps occur. The ADL can be made from Galaxy®, a trilobal viscose fibre with good liquid transportation properties. Through the addition of an ADL using Galaxy® fibres the rewet value can be reduced and the pant has dry and improved wearing comfort. The Absorbing Core can be made from Bramante mixed with Viloft. Bramante is a hollow fibre ideal for the storage of liquids. As the textile construction using Bramante fibres cannot only store liquid in the fibre cavities but also inside of the fibre, more liquid can be absorbed. Furthermore, it enables the fibre to hold the liquid even under pressure which prevents leakage and creates and improved safety for the wearer. In addition to that a loop knitted structure is best suited for the absorbing core. In order to have a biodegradable Backsheet three options have been tested and should further be investigated. First using a PLA foil as a barrier layer, second a merino wool fabric woven under tension and third using sustainable finishing or coating chemicals offered by a German textile chemical company. However all three options have still some challenges that have to be overcome. The PLA foil rustles when sewn into a pant, the woven wool fabric is very expensive and the coating only showed washing permanence of up to 20 washing cycles. In the prototype an ADL is implanted. This layer is used frequently in conventional menstruation pads, but was not included in any of the tested reusable menstruation pants. Moreover, to avoid leakage in the menstruation pants a special seam design is used as shown in figure 3 in order to avoid penetrating the waterproof Backsheet.

In addition to that, Danufil® and Viloft are ideally suited as mixing components as the complement the function of the functionalized fibres. Viloft as a blending component for Bramante ensures that the Bramante fibre has enough space to expand during the liquid uptake and reach its full absorbency potential. In addition to that, both fibres help to optimize the processability of the specialized viscose fibres in the spinning and knitting process. Summarized it can be said that a product using a combination of different functionalized viscose fibres can be superior to other products as it can be designed thinner and more sustainable than currently available products as it enables the substitution of various synthetic and natural materials like cotton or polyester.

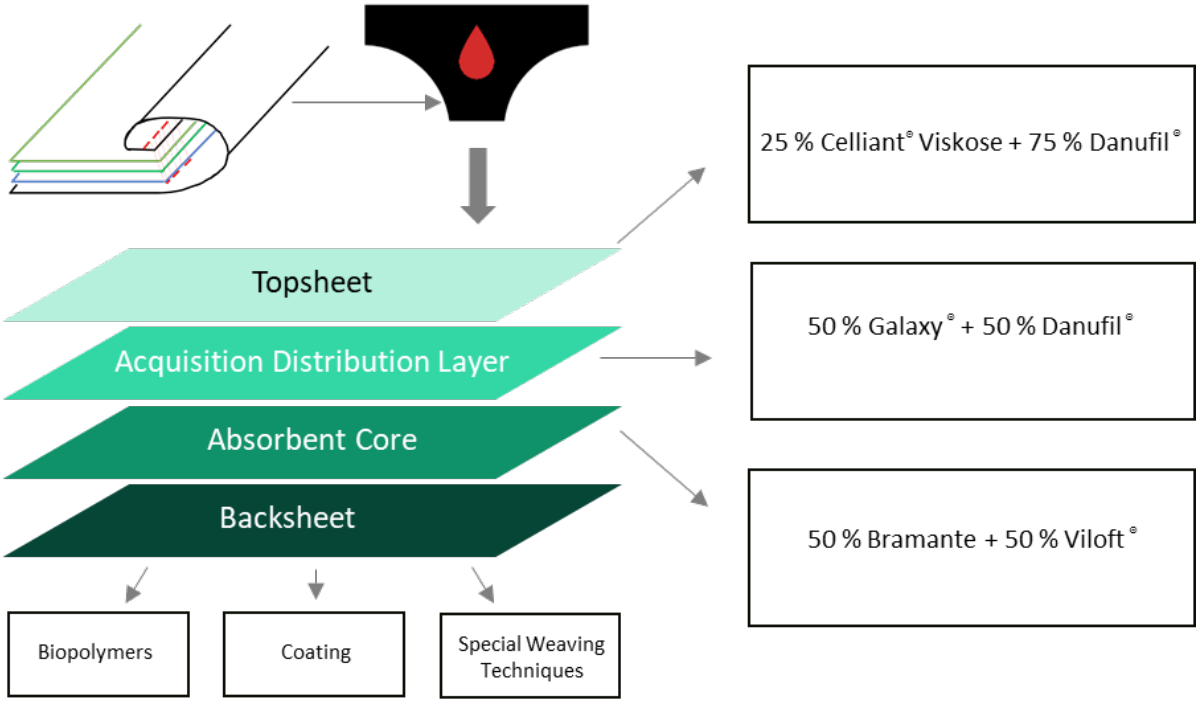

Figure 3: *Possibilities for an ideal composition for sustainable period pants*

To improve the prototype further tests have to be done for the Backsheet as well as for the other layers. In order to increase the performance, different knitted and woven structures have to be tested. In figure 3 an overview over possible knitting structures that might be suitable in a menstruation pant is given.

Using the material compositions shown in figure 2 and the knitted structures in figure 3 it is possible to find an ideal combination for a period pant. Advantages of the functionalized viscose fibres are that the thickness of the product can be decreased, the fibres are biodegradable and the number of different materials used in the product can be reduced drastically. In addition to that, the whole menstruation pant can be designed industrially biodegradable or recyclable. Furthermore, there are some possibilities to increase the sustainability of the product even further. When using Viscose fibres it is possible to produce spin dyed fibres by adding pigments to the spinning mass. With this technology the entire water intensive dyeing process can be skipped. Of course, the choice of pigments should be considered under environmental and human health aspects as well.

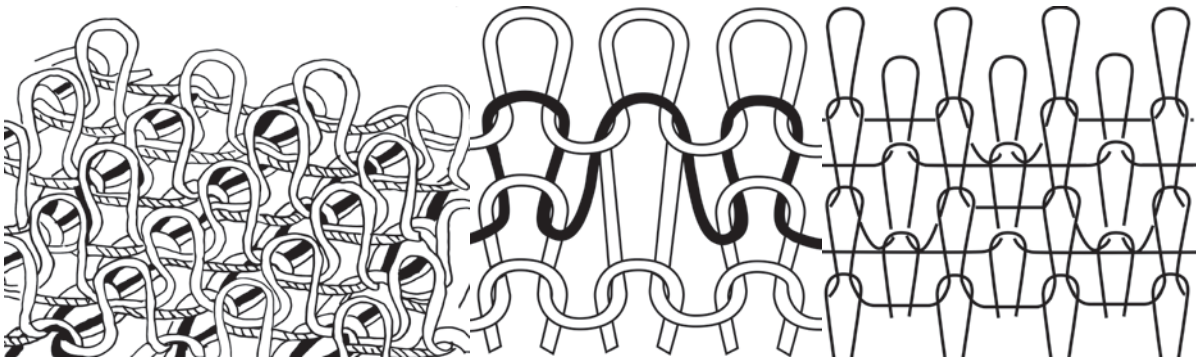

Figure 4: *Knitted structures probably suitable for the use in a menstruation pant: (1) pile loop knitted structure for the absorbing core; (2) Tuck knitted structure to create a perforation effect in the Topsheet; (3) Double pique knitted structure for the ADL layer*

The market potentials for a sustainable menstruation pants is high, as the menstruation pant market is expected to grow from a market volume of 79 million USD in 2018 (Jagtap, 2020) to the size of 180 million USD in 2021 (Future Market Insights, Department Consumer Product, 2021) and 580 million USD in 2025 (Jagtap, 2020). In addition to that, there are still countries in Europe that have not entered the menstruation pant market yet and could hold a potential for growing sales volumes. Especially Italy and the Scandinavian countries can be important in the next years. As mentioned before the SUPD can also be a driving force for more sustainable menstruation products on the European market. Outside of the core markets, China and India are expected to have a huge potential for period pants. However, when implementing reusable products like period pants it is important to educate the users accordingly on how to wash and clean them. As menstruation is highly stigmatized in these countries a risk can be seen for inadequate cleaning and drying of the product which might lead to increased bacteria growth.

The development of sustainable menstruations pants using predominantly biodegradable and renewable materials is a promising application that should be investigated further to minimize waste and to create a circular approach. However, it is important not to compromise on performance and comfort. The before described composition represents menstruation pants that combine sustainability, comfort and performance. The product is designed industrially biodegradable and can be seen as an example for a circular textile product. However, this path should not be limited to period pants but can be transferred to products in the incontinence and diaper market as well as to other textile applications.

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Lyocell Fibers from Pulps with High Mannan and Xylan Content – Part 2: Mechanical Properties

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Abstract

For sustainability reason, it is beneficial to utilize additional wood components like hemicelluloses for the production of cellulosic man-made fibers. In this study, we showed that mannan and xylan in softwood kraft pulps could be used successfully in the lyocell process at mill scale to spin holocellulosic fibers. Mannan was preserved throughout the process up to 96.6% while xylan was degraded more readily. Overall, fiber strength properties were in the range of market fibers. Dry elongation – reaching 11.7 to 14.3% - was even raised while dry tenacity – reaching 29 to 37cN/tex – was only slightly deteriorated by low molecular weight polymers. Wet elongation, in contrast, was clearly favored by a high share of long polymer chains. Overall, an indication was found that softwood-xylan showed to be a beneficial hemicellulose in lyocell fibers within the limits of our study.

Keywords: *kraft pulp, mannan, xylan, lyocell fiber, strength properties*

Introduction

About 20 years ago the US and European apparel industry was moving towards Asia due to significant cost advantages. But times are changing. 80% of the international chief procurement officers (CPOs) of the fashion industry now name speed-to-market and in-season-reactivity as the top priorities of their business *(Andersson et al. 2018)*. They have to meet customer desires of circular value chains with high sustainability and fast fashion at the same time. The pressure on profitability is enormous. The market is based on the pull-model, where products are produced in smaller charges and delivered on demand, and no longer on the push-model. Nearshoring and new delivery models are the key issues. Even if labor costs in China and Bangladesh are still lower compared to Western Europe and the US, the gap has decreased during the past decade and will continue to equalize. Due to savings in freight and duties, production sites are already now moving to Mexico, Turkey or Morocco. E.g., the production costs of denim for the European market can even be 3% lower when shifted from China to Turkey *(Andersson et al. 2018)*.

At the same time, a groundbreaking technology development for the sewing industry takes place: automated sewing technology using robotics is one example. Automating sewing is a problem by the nature of the fabric itself. These material problems like flexibility and stretchability have been overcome by a research group from Georgia Tech's Advanced Technology Development Center guided by Stephen Lang Dickerson *(US9085081B2, WO2008112842A2, US8573145B2, US2015122164A1)*. The new SewBot® technology can reduce production costs and the output will increase. As an example, only three to five workers can run 21 automated sewing lines. The production costs for a T-shirt made in the US are said to become comparable to those produced in Bangladesh. Additionally, the textile industry can realize on-demand replenishment easily.

Nearshoring and automation are two important factors for a circular economy. Sustainable raw material is the third and at least as important as the other two. According to the market volume, cellulose is the choice of renewable raw material. Certified wood or even textile waste produced in a biorefinery can provide dissolving pulp that is subsequently processed in the lyocell process to yield biodegradable and sustainable products. Lyocell fibers have always been an almost luxury fiber product with special properties. Why shouldn't we produce a new generation of fibers more suitable for the mass-market that will meet the requirements of circular value chain? This mill-study suggests an approach by using a bigger part of the raw material wood, not only cellulose but also hemicelluloses, creating holo-cellulosic man-made fibers.

Experimental

Material

Four different softwood market pulps were chosen for the production of lyocell fibers. Market pulps 1, 2 and 3 with their increased mannan and xylan content were produced by modified kraft pulping with subsequent ECF-bleaching. Pulp 1 and 3 originated from the same mill. The raw materials used were *Pinus ssp*.. Market pulp 4 was a standard dissolving pulp for lyocell fiber production with a low hemicellulose content made from *Picea abies*. The pulping process applied was the acid sulfite process combined with TCF bleaching.

All pulps were processed in the lyocell process at mill scale using NMMO as a solvent without major changes in production parameters. For this study, non-wovens fibers with a titer of about 1.8dtex have been chosen.

Methods

All analyses of pulp and fibers were performed according to Tappi-, ISO- and SCAN-standards.

The determination of neutral sugar monomers was performed by anion exchange chromatography (AEC) with pulsed amperometric detection (PAD) after a total hydrolysis with H_2SO_4 according to *Sixta et al. (2001)*.

Table 1: *Pulp properties of the hemicellulose rich softwood kraft pulps in comparison to a sulfite dissolving pulp.*

Analysis	Unit	Market pulp 1	Market pulp 2	Market pulp 3	Market pulp 4 - Reference
Wood		*Pinus ssp.*	*Pinus ssp.*	*Pinus ssp.*	*Picea abies*
Pulping process		Modified Kraft	Modified Kraft	Kraft	Sulfite
Glucan	%	84.1	85.7	82.2	96.0
Xylan	%	7.3	6.9	6.9	1.4
Mannan	%	5.5	5.1	5.7	1.3
Xylan/Mannan ratio	-	1.33	1.35	1.21	1.08
Arabinan	%	0.3	0.2	0.3	<0.1
Rhamnan	%	<0.1	<0.1	<0.1	<0.1
Galactan	%	0.2	0.2	0.2	<0.1
Sum of hemicelluloses	%	13.3	12.4	13.1	2.7
Brightness	%	90.4	89.1	91.6	94.2
Viscosity	mL/g	345	380	370	410
Kappa No.	-	0.3	0.7	0.3	0.1
R10	%	84.6	86.3	83.7	87.6
R18	%	88.2	89.8	87.4	94.5
COOH	µmol/g	40.0	33.5	44.1	25.2
Copper No	%	1.1	0.5	1.4	1.3
Acetone extractives	%	0.02	0.04	0.03	0.06
Ash 850°C	%	0.05	0.05	0.04	0.05
Fe	mg/kg	7.8	1.4	1.60	1.3

Results and Discussion

The three commercial softwood kraft pulps 1, 2 and 3 were not considered as dissolving pulps and so far have not been used for the lyocell process in plant scale. In general, they are used for other applications like e.g. fluff. For comparison, a sulfite dissolving pulp from softwood was added (market pulp 4). Pulp properties are described in detail in table 1. All softwood pulps showed the same level of viscosity required for application in the lyocell process. They differed in hemicellulose content and subsequently R18 and in the ratio of xylan to mannan. All other specifications like inorganics or extractive content satisfied the expectations of the demanding process.

The use of softwood pulps gave the advantage of observing effects from both hemicelluloses mannan and xylan, while hardwood as the raw material only gives the chance of monitoring mainly xylan. In this study, even a differentiation between both hemicelluloses was possible. A relative hemicellulose yield could be calculated by setting the cellulose yield to 100%. The results showed that xylan was more readily degraded than mannan during processing. This could be observed by lower relative xylan yields compared to relative mannan yields (see table 2). Even the reference pulp with its very low hemicellulose concentration, market pulp 4, showed the same tendency. The high relative mannan yield was observed for all pulps tested.

It is well known that mannan more readily associates with cellulose, which may lead to a stabilization of the polymer. *Corbett and Kidd (1958*) investigated the degradation behavior of mannan and xylan in akaline medium with 25 *N* NaOH at 100°C, which may give an indication for the lyocell process. Xylan was more readily removed from the pulp than mannan as observed in our study. *Ono et al. (2018)* suggested a more detailed hypothesis for mannan reaction mechanisms: The formation of non-phenolic lignin/polysaccharide α-ether bond structures is more likely for glucomannan than for xylan because of the steric hindrance of the C6-OH-group. The reaction occurs preferentially at high lignin concentration under acidic conditions at high temperature. This is the case during prehydrolysis before kraft pulping or during sulfite pulping. These bonds are subsequently cleaved under alkaline conditions via quinone methide formation in subsequent alkaline process steps like kraft pulping or oxygen delignification. In contrast, if the preceding acidic treatment is missing, the cellulose-mannan-structure can be assumed to result a more stable and more branched structure. This was the case for market pulps 1 to 3, and this may lead to a better incorporation of mannan into lyocell fibers compared to xylan. The mannan was obviously not much degraded due to

Table 2: *Lyocell fiber properties produced from hemicellulose rich softwood kraft pulps in comparison to a sulfite dissolving pulp.*

Analysis	Unit	Fiber 1	Fiber 2	Fiber 3	Fiber 4
Pulp used		Market pulp 1	Market pulp 2	Market pulp 3	Market pulp 4 - Reference
Titer	dtex	1.9	1.9	1.8	1.3
Tenacity dry	cN/tex	29.2	31.1	37.4	34.5
Elongation dry	%	11.9	11.7	14.3	12.6
Tenacity wet	cN/tex	24.9	28.7	32.0	29.3
Elongation wet	%	17.0	18.6	17.0	14.8
Tenacity wet/Tenacity dry	-	0.85	0.93	0.85	0.85
Elongation wet/Elongation dry	-	1.43	1.59	1.19	1.18
Xylan in fiber	%	6.8	5.9	7.1	1.4
Mannan in fiber	%	5.3	4.7	5.4	1.1
Sum of hemicelluloses	%	12.1	10.6	12.5	2.5
Xylan/Mannan in fiber	-	1.28	1.26	1.31	1.27
Xylan yield (rel.)	%	93.5	85.7	n. d.	85.7
Mannan yield (rel.)	%	96.6	92.8	95.4	87.0

Table 3: *Molecular weight distribution of hemicellulose rich lyocell fibers.*

Analysis	Unit	Fiber 1	Fiber 2	Fiber 3	Fiber 4
Mn	kg/mol	51	67	67	41
Mw	kg/mol	382	469	160	156
PDI	-	7.5	7.1	2.4	3.8
<DP80	%	5.2	3.3	2.5	6.2
<DP100	%	7.1	4.7	3.6	7.9
>DP2000	%	28.6	33.1	11.0	12.3

the mechanism described above while the xylan was degraded and partly dissolved in the spinning bath.

The mechanical properties of the resulting lyocell fibers were analyzed (see table 2). The tenacity decreased when pulp with high hemicellulose content was used (see figure 1). The difference of 8.2cN/dtex for a titer of 1.9dtex was significant. Despite high contents of hemicellulose of 10.6 to 12.5%. All the same, the strength data were still at the high level known for lyocell fibers. Although fiber 4 showed a much lower titer of 1.3dtex, it provides an indication of properties of conventional lyocell market fibers. These findings match exactly with the literature *(Nypelö et al. (2018), Ma et al. (2017), Sixta et al. 2006, Chen et al. (2015), Zhang and Tong (2007), Zhang et al. (2008)). Nypelö et al. (2018)* correlated the strength reduction with the lower cellulose content. Lower cellulose content leads to a decrease in polymer orientation, and therefore a loss in strength.

In our case, the cellulose content was decreased by increased hemicellulose content (see figure 1). Even the lyocell fiber produced from low hemicellulose sulfite pulp fitted into the scheme regarding the correlation of sum of hemicellulose and tenacity, but not regarding the share of low MW polymers. Obviously, this was due to the lower chain length of hemicelluloses and to their chemical structure, which may have hindered the formation of more oriented areas in the final fiber (see table 3). Our findings emphasize the explanation of *Nypelö et al. (2018)*.

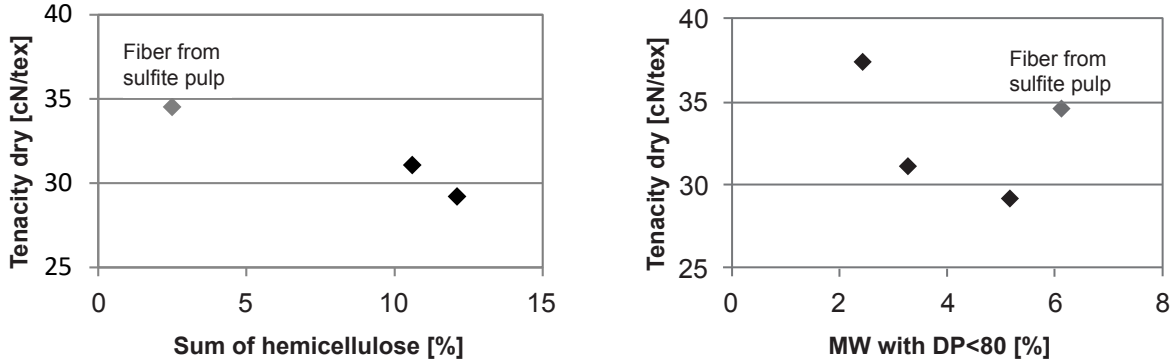

Figure 1: *Correlation of dry fiber strength with hemicellulose contents and low MW polymers of the lyocell fibers.*

For the lyocell process with NMMO, *Fink et al. (2004)* stated that there is no memory with respect to crystalline order of the starting pulp and that different pulp types and morphologies do not significantly alter the supramolecular fiber structure. They attributed variations in fiber strength to the influence of different DPs of the polymers although they used unbleached hardwood organosolv pulps with increased lignin and xylan content. No significant changes in fiber mechanical properties were observed assuming nearly equal supramolecular order and orientation.

In our study, there was a clear correlation between fiber tenacity and pulp viscosity determined by the SCAN-method, but not with Mw of the fibers determined from molecular weight distribution (see table 2 and 3). Overall, the dry tenacity was a function of cellulose chain length and cellulose content, and it was still at a high level despite a hemicellulose content of up to 12.5%.

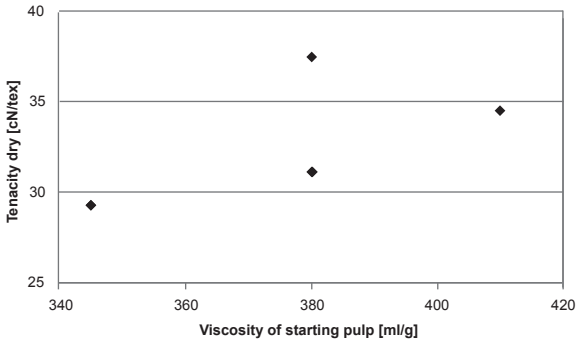

Figure 2: *Influence of pulp viscosity on fiber tenacity.*

The wet tenacity decreased with increasing hemicellulose content. Obviously, the water uptake was higher for lyocell fibers with higher hemicellulose content due to a higher content of accessible OH-groups originating from hemicelluloses. This fact resulted in lower wet tenacity.

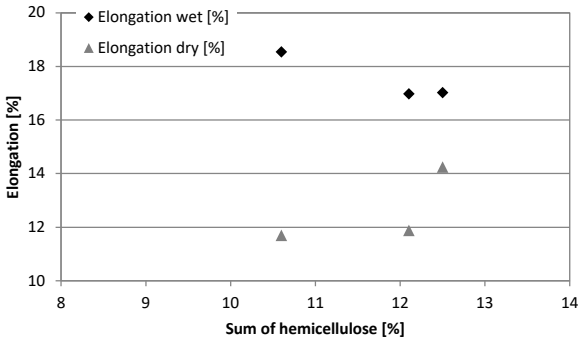

Figure 3: *Effects of hemicellulose contents of lyocell fibers on wet and dry elongation.*

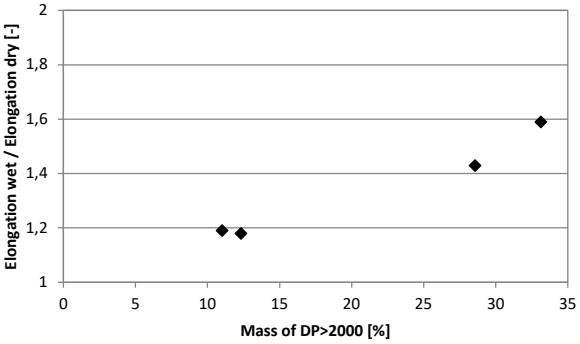

Figure 4: *Effects of high molecular weight polymers of lyocell fibers on wet to dry elongation.*

Within the range of this study, dry elongation was favored by higher hemicellulose content (see figure 3). Although *Nypelö et al. (2018)* described the elongation of lyocell fibers as almost constant using a lyocell process with an ionic liquid as solvent. Contradictory, wet elongation decreased with higher hemicellulose contents. This effect showed a more complex role of hemicelluloses in lyocell fiber strength buildup.

In general, wet fiber properties are strongly depending on the fiber structure. The more porous core swells stronger than the denser skin. Therefore, the skin hinders swelling of the core and thus helps for better wet properties. *Nypelö et al. (2018)* observed no skin for xylan and lignin enriched Ioncel F fibers. The core-shell-organization was revoked for lignin and xylan rich lyocell fibers. We described the same for our hemicellulose rich lyocell fibers *(Schild et al. (2019))*. This should have a negative effect on wet elongation. There may also be a second more prominent effect on wet elongation. Lyocell fibers have a high orientation and therefore a high dimensional stability, which results in a high wet modulus, which is observed from figure 3. Both properties were altered when we raised the concentration of hemicelluloses in the fibers. The skin was diminished and the orientation of the polymers was interfered. This may also explain lowered wet properties for fibers with higher hemicellulose content (see figure 3).

While dry strength properties increased with a lower part of low MW polymers wet elongation was favored by a high share of long chain polymers, here measured as DP>2000 (see figure 4). Fibers 1 and 2 showed a lower dry elongation due to the high amount of low MW polymers. But for wet elongation their values increased over proportionally due to their high share of high MW polymers (see table 2 and 3). The long polymer chains were presumably bridging between structure elements resulting in better elasticity in the wet state.

Nevertheless, a differentiation between xylan and mannan as natural wood polymers is of main interest. In this study, a higher share of xylan compared to mannan favored fiber strength properties, both dry fiber elongation and dry fiber tenacity as can be seen from figures 5 and 6. The fiber tenacity could be raised from about 30cN/tex to 37.4cN/tex. Elongation was increased significantly from 11.9% to 14.3% by only raising the ratio of xylan to mannan in the fiber. The effects on fiber properties were clearly recognizable taking into account that this study was not a single lab experiment, but it comprises mill trails over several days.

It is well known from polymer science, that short chain polymers have a positive effect on elasticity and give rise to higher elongation at break. At the same time, they affect tenacity negatively. Short chains do not participate in stiffness and buildup of strength. Mannan has the longer chain length of both hemicelluloses and associates more readily with cellulose. Therefore, it should be favorable for fiber formation

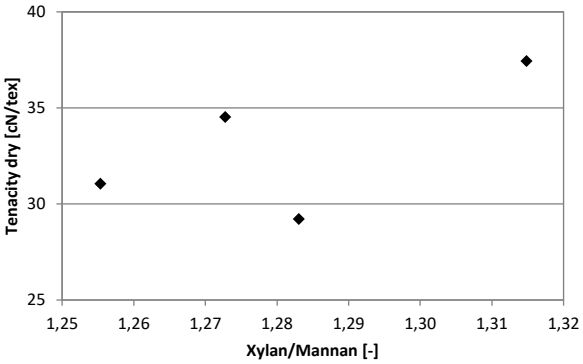

Figure 5: *Influence of xylan to mannan ratio on tenacity of dry lyocell fibers.*

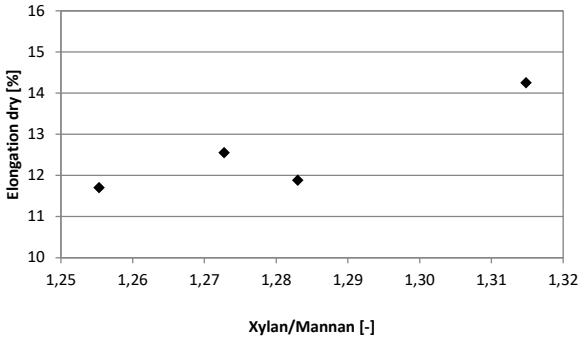

Figure 6: *Influence of xylan to mannan ratio on elongation of dry lyocell fibers.*

and strength properties. But the opposite was the case for our study. A higher share of xylan in the lyocell fibers showed a beneficial effect on fiber strength properties. It even seamed to compensate for negative impacts of mannan on elasticity.

The results of *Park et al. (2020)* indicated the same unexpected correlation for hardwood xylan and tensile strength. They used the hardwood *Liriodendron tulipifera* as raw material. Therefore, the predominant hemicellulose was xylan. Results showed that in the case of wet-spun cellulose nanofibers hardwood-xylan could increase the tensile strength.

Berglund et al. (2020) conducted more fundamental research on the role of softwood hemicelluloses in combination with cellulose hydrogels. They treated bacterial cellulose with galactoglucomannan (GGM) and arabino-4-*O*-methylglucuronoxylan (AGX) isolated from spruce wood. GGM interacted closely with cellulose, especially after alkaline treatment. This phenomenon facilitated cellulose crystallization and therefore, the overall crystallinity increased slightly. AGX, in contrast, hindered the packing of cellulose chains and by that reduced the crystallinity. This gave rise to a dramatic increase of elongation at break under tension. The purpose of this basic research by *Berglund et al.* was to elucidate the cell wall 2 (S2) and therefore, did not cover the process of fiber spinning. Nevertheless, this study explained two effects found in our mill trails: first, the loss of crystallinity for the lyocell fibers with high xylan content as published in *Schild et al. (2019)*; second, the increase of elasticity due to a higher xylan content. Still there is no explanation for the increase in fiber tensile strength by xylan in regenerated cellulosic fibers as described in our study and by *Park et al. (2020)*.

Although fundamental know how of hemicellulose structures and functions in native wood cell walls and regenerated fibers is available, an explanation of the role of different hemicelluloses during fiber forming seems not that simple. More investigations on this topic are important to generate a valid hypothesis.

Conclusions

Softwood kraft pulps with increased xylan and mannan contents could be demonstrated to be a promising new raw material for the production of holocellulosic lyocell fibers. Using additional wood polymers like hemicelluloses would increase resource efficiency and sustainability. Beneficial aspects of these holocellulosic fibers dominate since the overall yield will be higher at fiber strength levels comparable to conventional market fibers, and the chemical consumption during pulping will be lower. Therefore, the proposed new fiber type will be of significant economic interest. Production trials at industrial scale showed a realistic way of completion. Prolonged production trials are inevitable to study the impacts of hemicelluloses on closed process loops and recovery.

Nevertheless, lab investigations may be of interest for a better understanding of the roles of the different hemicelluloses xylan and mannan in fiber forming and their impacts on fiber properties.

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Mordanting of Cellulosics with Iron (III) Sodium Tartrate (FeTNa) Complexes for Coloration with Natural Dyes

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Abstract

The effects of cellulose pretreatment with iron (III) sodium tartrate (FeTNa) complexes on subsequent coloration with natural dyes (alizarin, madder root extract, tannic acid) was investigated. The pretreatment leaves iron residues in the cellulose, which functions as a mordant in the subsequent dyeing. The residual iron improves dye uptake and the wash and light fastness ratings of the dyed materials. Thus, pretreatments with FeTNa may be a method of improving cellulose coloration with natural dyes.

Keywords: *FeTNa, viscose, alizarin, tannic acid*

Introduction

Iron (III) sodium tartrate complexes (FeTNa)[1] exhibit strong interactions with cellulose through formation of coordination complexes between the transition metal ion and polymer hydroxyl groups [1]. Stable mixtures of FeTNa are achieved with iron (III), tartaric acid and NaOH in a molar ratio of 1:3:6 respectively [2, 3], but by increasing the relative proportions of tartaric acid and/or NaOH, the degree of FeTNa interaction with cellulose may be varied from swelling up to dissolution. Thus FeTNa formulations have been employed for such purposes as viscometric evaluation of cellulose degree of polymerization [4], structure modifications of cellulosics [5, 6], and the manufacture of membranes and fibers [7, 8].

In our investigations on the effect of FeTNa on structure and morphology of regenerated cellulosics [9-11], it was noticed that the treatments left iron residues in the treated fibers. Iron salts are among the commonly used "mordants" in coloration with natural dyes to improve dye uptake and the wash- and light-fastness of dyed substrates. In this paper, we report on the effect of FeTNa pretreatments on the dyeing of viscose fabrics with alizarin (1,2-dihydroxy anthraquinone), extracts of roots from the madder plant *(Rubia tinctorum)* the main colorant in which is also alizarin, and tannic acid.

[1] The names of these mixtures are abbreviated as "FeTNa" in English language publications and as "EWNN" in German language publications (for Eisen-Weinsäure-Natrium).

Materials

The substrate used in the work was a satin-woven fabric made of viscose fibers, with a mass/area of 79 g/m^2, kindly provided by Lenzing AG (Austria). Of the colorants used, the madder root was obtained from a local farmer, the tannic acid ($C_{76}H_{52}O_{46}$) was of >95% purity, and the alizarin was of microscopy grade (purity ≥ 98%). All other reagents were of purity 95% or higher unless mentioned otherwise, and deionized water (of conductivity less than 10 µS/m) was used in the formulation of solutions.

Methods

Demineralization of fabrics

As calcium in cellulose fibers may influence their dyeability [12], the fabrics were demineralized to remove any residual ions, by treating them with a 0.5% (w/w) solution of HCl at a liquor ratio of 1:40 for 1 hour at 40°C. They were then rinsed with deionized water, neutralized by immersion in 1 g/l CH_3COONa, rinsed again with deionized water, and line-dried.

Preparation of FeTNa solutions

The solutions were prepared as described previously [9, 11] with a basic formulation of $FeCl_3.6H_2O$, tartaric acid, and NaOH in a molar ratio of 1:3.28:9.56. Three variations of this basic formulation were investigated in this work, where the iron (III) concentration was always 0.25 mol/l, but the content of excess NaOH (i.e. in addition to the amount required for the basic formulation) varied between 0.8, 1.25 and 2.5 mol/l.

FeTNa mordanting of fabrics

The demineralized fabrics were padded with the FeTNa solutions at a nip pressure of 1 bar and roller speed of 1 m/min, rested at room temperature for 10 min, then rinsed twice with deionized water for 5 min, neutralized by immersion in 1 g/l CH_3COOH, rinsed again with deionized water, and line-dried.

Determination of iron content in mordanted fabrics

About 0.2 g pieces from FeTNa mordanted fabrics were subjected to extraction in 50 ml of 1 mol/l HCl for 30 min at 90°C, and the Fe content in extracts were photometrically determined with the 1,10-phenanthroline method (DIN 38406–1: 1983-05) as described elsewhere [13]. In brief, 5 or 10 ml of extracts were pipetted into a 100 ml volumetric flask and buffered to pH 5 with ammonium acetate buffer. To this was added 2 ml of 100 g/l $NH_2OH.HCl$ and 2 ml of 5 g/l 1,10-phenanthroline. A colored complex was formed, the photometric absorbance of which was measured at 510 nm. The Fe contents were derived from the measured absorbance using a calibration curve constructed over a concentration range of 0.5–5.0 mg/l Fe with ammonium ferrous sulfate.

Fabric dyeing

The dyeings were performed on 5 cm × 20 cm pieces from the FeTNa mordanted fabrics. In addition, a set of dyeings were performed on pieces from fabrics treated only with 0.8, 1.25 and 2.5 mol/l of NaOH.

With alizarin and tannic acid: Fabric pieces were immersed in dye formulations at a liquor ratio of 1:40, and agitated at 90°C for 1 h, then rinsed with deionized water and line-dried. The alizarin dye formulation contained 0.25±0.02 g/l of the colorant, 1 g/l CH_3COOH and 1 g/l CH_3COONa. The tannin dye formulation contained only 0.25±0.02 g/l of the colorant.

With madder root extract: The extract was obtained by immersing the plant material in deionized water heated to 90°C at a liquor ratio of 1:20 for 1 hour, and the residual solids were filtered out. The primary colorant in madder root extract is alizarin, and thus the extracts were diluted with deionized water to the extent that the dilutions exhibited the same photometric absorbance at 510 nm as a 0.25 g/l solution of the high purity alizarin. The diluted solutions, along with 1 g/l CH_3COOH and 1 g/l CH_3COONa, were used in the dyeing of fabric pieces, which was performed in the same manner as described above.

Determination of residual colorant content in solutions

Alizarin: A volume of 5 or 10 ml of the residual solution after dyeing was pipetted into a 100 ml volumetric flask containing 50 ml of 0.2 mol/l NaOH, and deionized water was added to make up the rest of the volume. The alizarin content was determined from photometric measurements of these solutions at 510 nm, using a calibration curve constructed with high purity alizarin in the concentration range of 0.001–0.050 g/l. The measurements were performed in triplicate.

Tannic acid: The residual tannic acid content in dye solutions was determined with the Folin–Ciocalteu (F–C) method. A 20 µl volume of residual solution was pipetted into a glass tube containing 1.58 ml deionized water and 100 µl of F–C reagent (Sigma-Aldrich). After a minute, a volume of 300 µl Na_2CO_3 solution was added, and the absorbance of solution was deter-

mined at 560 nm. The Na_2CO_3 solution contained 200 g of the salt dissolved in 800 ml deionized water, boiled, cooled, and then diluted up to 1 liter. The measurements were performed in triplicate.

Measurements on dyed fabrics

The reflectance spectra of the dyed pieces were measured on a d/8 spectrophotometer (Model CM 3610d, Konica Minolta, Japan). The measurement area was 8 mm in diameter and the specular component was excluded. The color coordinates on the CIELAB space were calculated for a D65 illuminant and 10° observer with the onboard software from the measured reflectance. The color depth was calculated with the Kubelka-Munk function from the reflectance measured at the wavelengths of maximum absorbance: 510 nm (samples dyed with alizarin and madder root extracts) and 560 nm (samples dyed with tannin).

The color fastness to washing was determined as per DIN 54014, by evaluating the color change in specimens washed with a detergent mixture containing 1.5 g/l of Ufarol™ NA 30 (30% formulation of sodium lauryl sulfate from Unger Fabrikker AS, Norway) and 1 g/l of Glucopon® EC 650 (50% formulation of C8-C16 alkyl polyglucosides from BASF AG, Germany) at 40°C for 30 min at a 1:50 liquor ratio. The color fastness to daylight was determined as per ISO 105-B01: 1999 by evaluating the color change in samples exposed for 24 h on a XENOTEST Alpha Lm device (Atlas-MTS, USA). The color changes in both tests were evaluated with grey scales as per ISO 105-A02: 1993.

Results and discussion

Table 1. *Iron content and L*, a*, b* coordinates in the CIELAB color space of fabrics pretreated with FeTNa containing different amounts of excess NaOH.*

Excess NaOH (mol/l)	Fe content (g/kg)	L*	a*	b*
0.8	2.2 ± 0.08	80.02	7.52	20.62
1.25	2.4 ± 0.16	79.72	7.25	22.47
2.5	3.4 ± 0.21	76.75	9.11	24.52

The iron content in samples increased with the content of excess NaOH in the FeTNa (see Table I), which may be attributed to the increase in substrate swelling with the change in NaOH amounts. From the CIELAB coordinates shown in the same table, it may be observed that the rise in Fe content coincided with a reduction in the L* value and an increase of the b* value, indicating that the fabrics acquired a dark yellowish hue with rising Fe content.

Table 2: *Residual dye contents in baths (g/l) after dyeing from an initial dyebath concentration of 0.25 g/l, forfabrics pretreated with FeTNa containing different amounts of excess NaOH, and fabrics pretreated with NaOH alone. The values shown in parentheses are the exhaustion percentages calculated from the concentration changes.*

Excess NaOH (mol/l)	Pretreatment[a]	
	FeTNa	NaOH alone
	Alizarin	
0.8	0.11 ± 0.01 (56%)	0.19 ± 0.01 (24%)
1.25	0.11 ± 0.01 (56%)	0.17 ± 0.01 (32%)
2.5	0.15 ± 0.01 (40%)	0.19 ± 0.01 (24%)
	Madder	
0.8	0.14 ± 0.01 (44%)	0.21 ± 0.01 (16%)
1.25	0.16 ± 0.01 (36%)	0.21 ± 0.01 (16%)
2.5	0.15 ± 0.01 (40%)	0.20 ± 0.01 (20%)
	Tannic acid	
0.8	0.06 ± 0.02 (76%)	0.20 ± 0.01 (20%)
1.25	0.06 ± 0.02 (76%)	0.20 ± 0.01 (20%)
2.5	0.06 ± 0.02 (76%)	0.20 ± 0.01 (20%)

[a] The residual dye contents in baths from dyeing of non-pretreated samples with alizarin, madder root extract and tannic acid were 0.17 ± 0.01 g/l, 0.20 ± 0.01 g/l and 0.18 ± 0.01 g/l corresponding to exhaustion percentages of 32%, 20% and 28% respectively.

The residual dye contents in baths after the dyeing experiments, and the calculated exhaustion percentages (i.e. the percent change in dye concentrations) are shown in Table 2. It may be observed that the FeTNa pretreated fabrics exhibited significantly greater dyebath exhaustion than the fabrics treated only with NaOH, which behaved similar to the non-pretreated samples. Thus, the residual Fe in the FeTNa treated fabrics exerted a positive uptake on the colorant uptake. Among the FeTNa treated fabrics, the exhaustion levels were greater with tannic acid as compared to the alizarin and madder extracts, which correlates with the significantly high propensity of tannic acid to form iron complexes [14]. Madder root extracts contain other components in addition to alizarin [15] that may interfere in alizarin complexation with iron, which may explain the lower exhaustion levels as compared to with pure alizarin.

Table 3: *L*, a*, b* coordinates in the CIELAB color space of fabrics dyed after pretreatment with FeTNa containing different amounts of excess NaOH and with NaOH alone.*

Excess NaOH (mol/l)	Pretreatment								
	FeTNa					NaOH alone			
	L*	a*	b*	K/S		L*	a*	b*	K/S
	Alizarin								
0.8	52.86	6.73	2.23	1.88		91.16	–1.10	11.39	0.05
1.25	50.65	7.12	4.66	2.40		91.26	–0.99	11.04	0.06
2.5	52.12	6.98	7.01	2.55		91.24	–1.02	11.18	0.07
	Madder								
0.8	54.36	10.89	1.10	2.22		75.28	12.11	19.33	0.33
1.25	52.95	11.28	1.23	2.14		76.18	11.85	19.14	0.35
2.5	53.89	10.59	0.88	2.63		75.70	11.94	18.90	0.41
	Tannic acid								
0.8	50.27	2.06	–1.59	1.84		—	—	—	—
1.25	50.06	2.05	–2.98	1.88		—	—	—	—
2.5	47.33	2.02	0.75	2.22		—	—	—	—

In Table 3 are listed the color coordinates and the shade depth (K/S) measured on fabrics after dyeing. The samples after dyeing were rinsed, whereupon all colorant was lost from the fabrics dyed with tannic acid after pretreatment with NaOH alone. Thus, their color coordinates and K/S values are not reported. From the other results, it may be observed that the FeTNa pretreated samples exhibit greater K/S values as compared to fabrics pretreated with NaOH alone, which is consistent with greater dye exhaustion values. Within the FeTNa pretreated samples, the K/S generally increases with the content of excess NaOH, and that is consistent with the rising Fe content in the samples. A rise in Fe content corresponded with a darker fabric color after the FeTNa pretreatment, and this is reflected even in the color after dyeing. The trends in L* values parallel those in the K/S. The differences in a* and b* coordinates between the FeTNa pretreated and NaOH pretreated fabrics reflect the color changes that occur on complex formation between the colorant and mordant. And the differences in a* and b* between the alizarin and madder root extract dyed samples reflect contributions of other components in the root extract in addition to the alizarin.

The wash- and light fastness ratings of the dyed samples are shown in Table 4 (1: poor to 5 and above: excellent). The wash fastness levels were consistently better in samples dyed after the FeTNa pretreatment, and the light fastness levels were generally better after the FeTNa pretreatment.

Table 4. *Wash and light fastness grades of fabrics dyed after FeTNa pretreatment and pretreatment with NaOH alone.*

Excess NaOH (mol/l)	Pretreatment			
	FeTNa		NaOH alone	
	Wash	Light	Wash	Light
	Alizarin			
0.8	3	>5	1	>5
1.25	2/3	>5	1	>5
2.5	2	>5	1	>5
	Madder			
0.8	3	>5	1	3
1.25	3	>5	1	3
2.5	3	>5	1	3
	Tannic acid			
0.8	3	3	—	—
1.25	3/4	3	—	—
2.5	3/4	3	—	—

Conclusions

It was observed that FeTNa pretreatments of cellulosics were effective in functioning a pre-mordanting treatment for the coloration of cellulosics with natural dyes (alizarin, madder and tannic acid). The pretreatment improved dye uptake and the light and wash fastness values. It is likely that apart from the Fe residues after the pretreatment, the swelling effect of FeTNa also contributed to the improved dye uptake and fastness levels, and thus such pretreatment may be useful in improving the performance of natural dyes.

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6-Deoxy-6-hydrogenocelluose: Synthesis and Characterization of Cellulose with Reduced Functionality

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Abstract

Cellulose *p*-toluenesulfonic acid esters (TsCell) as well as TsCell peracetate were treated with sodium borohydride in aprotic-dipolar solvents aiming at the synthesis of 6-deoxy-cellulose derivatives. The nucleophilic displacement reaction is incomplete at a reaction temperature of 50 °C, while a sample free of sulfur was obtained at 100 °C provided that the primary hydroxyl groups were tosylated only. The solubility of the TsCell turned from aprotic-dipolar solvents to water after the reaction. The reaction is selective towards the primary position, while secondary Ts groups remained unaffected. The solubility of the samples is governed by the remaining substituents, namely Ts and acetyl moieties. The formation of the 6-deoxy moiety was evidenced by the presence of a signal at18 ppm in the ^{13}C-NMR spectrum, which is characteristic for the 6-deoxy moiety.

Keywords: *6-deoxy-cellulose, hydrogen bonds, NMR spectroscopy, nucleophilic displacement, reduction*

Introduction

Conversion of cellulose into various derivatives is conducted due to different reasons. A commercially very import process is the fiber spinning via cellulose xanthogenate, where cellulose is transformed to a relatively unstable derivative and regenerated after a shaping process [1]. A variety of cellulose derivatives is produced in different quantities for a huge number of applications [2-4]. All in all, the hydrogen bond system, which renders the cellulose insoluble in the typical organic solvents and in water is broken up to a certain extent and the polymers become soluble. In this regard, the influence of the type of substituent, its degree of substitution, and -very importantly- its distribution within the repeating unit and along the polymer chain on the macromolecular properties has been discovered [5-7].

The repeating unit of cellulose contains three hydroxyl groups of different reactivity. Thus, conversions typically lead to products with random functionalization pattern provided that the steric demand of the reagent applied is low. Using reagents with high steric demand affords products with exclusive functionalization of easily accessible hydroxyl groups within the repeating unit. Selective blocking of hydroxyl groups can be used to influence the hydrogen bond system [8]. Thus, the proton of the hydroxyl group has to be replaced by another moiety. In this regard, so-called deoxy-celluloses are an interesting class of substances because the hydroxyl group has been substituted completely. Except deoxy-celluloses bearing heteroatoms at position 6 of the repeating unit, conversions of the primary hydroxyls to methyl groups are scarcely described in the literature [9].

6-Deoxy-6-chloro celluloses can be obtained by reacting cellulose with sulphuryl chloride in *N,N*-dimethyl formamide (DMF) [10]. Highly selective bromination of cellulose using lithium bromide and *N*-bromosuccinimide has been described [11]. The halogen renders the adjacent carbon atom susceptible for nucleophilic displacement reactions. Cellulose derivatives bearing the deoxy group at position 3 have been prepared by reduction of 3-deoxy-6-*O*-trityl cellulose with tributyl tin [12]. It could be demonstrated that the enzymatic degradation of 6-deoxy-cellulose by cellulases from *Trichoderma viride* and *Aspergillus niger* is slower compared with cellulose but still faster than the degradation of 6-halogeno-6-deoxy-celluloses [13]. The preparation of 6-deoxy-celluloses comprises the introduction of a leaving group, e. g. 6-deoxy-6-halogeno or *p*-toluenesulfonic acid (Ts) ester, followed by conversion with a hydride donor, e. g., sodium borohydride [14-16]. Pyrolysis reactions of cellulose derivatives afforded products having complex structures, e. g., unsaturated functionalities in addition to deoxy moieties [17]. Cyanoethyl cellulose had been converted to deoxy-celluloses by reduction with sodium in liquid ammonia [18-20]. The treatment of 6-deoxy-6-azido cellulose bearing residual Ts groups bound to the secondary hydroxyl groups of the cellulose with lithium aluminum hydride was found to remove those Ts groups in addition to the intended reduction of the azide moieties without mentioning the presence of deoxy structures [21].

Because most of the studies on deoxy-cellulose derivatives had been published many decades ago, the structure characterization of the products is limited to the methods available to date. Therefore, we attempted to revisit the synthesis of 6-deoxy-cellulose and their characterization with modern techniques like NMR spectroscopy.

Experimental

Materials

Microcrystalline cellulose (Avicel®, Sigma Aldrich) was dried in vacuum at 105 °C over potassium hydroxide. Lithium chloride (Sigma Aldrich) was dried in vacuum at 150 °C over potassium hydroxide. *p*-Toluenesulfonic acid chloride (Sigma Aldrich), sodium borohydride, DMF (over molecular sieves, Sigma Aldrich) and *N,N*-dimethylacetamide (DMA, over molecular sieves, Sigma Aldrich) were used as received. Triethylamine (Sigma Aldrich) was distilled from calcium hydride prior to use.

Measurements

FTIR spectra were recorded on a Nicolet Avatar 370 DTGS spectrometer using the KBr technique. The ^{1}H- and ^{13}C-NMR spectra were acquired with Bruker Avance 400 (400 MHz) spectrometer in dimethyl sulfoxide (DMSO)-d_6 or in D_2O at 60 °C with a concentration of at least 5% (w/w) of polymer in solution using the solvent peak as internal reference. Elemental analysis (C, H, S content) was carried out using a Vario EL III (Elementaranalysensysteme Hanau, Germany). The chlorine content was determined according to Schöniger's method [22].

Methods

Synthesis of cellulose p-toluenesulfonic acid esters (TsCell)

Cellulose *p*-toluenesulfonic acid esters (samples **1a**, degree of substitution of Ts groups, DS_{Ts} 0.94, degree of substitution of 6-deoxy-6-chloro groups, DS_{Cl} 0.11 and **1b**, DS_{Ts} 2.11, DSCl 0.18) were prepared by conversion of cellulose dissolved in DMA/LiCl Ts chloride in the presence of triethylamine according to a previously published method [23]. Sample **1c**, DS_{Ts} 0.98 (DS_{Cl} not determined) is a peracetylated Ts cellulose [24].

Reaction of TsCell 1a with NaBH4, typical example

TsCell (5.0 g, 0.016 mol, sample **1a)** was added to 50 mL dry DMF under stirring until complete dissolution of the polymer occurred. Sodium borohydride (2,14 g, 0.057 mol, 3.5 mol/mol modified AGU) was added and the mixture was stirred for 24 h at 50 °C. Gelation occurred and the mixture was diluted with 25 mL DMF. Diluted HCl (15%) was added to the cooled mixture in order to destroy excess sodium borohydride followed by pouring the mixture into 1 L ethanol/water (90:10, v/v). The precipitated polymer was collected by filtration, washed 5 times with 200 mL ethanol/water (90:10, v/v) each, once with 200 mL ethanol, and dried in vacuum at 60 °C.

Yield: 2.7 g

Elemental analysis: 47.96% C, 5.84% H, 0.17% N, 0.31% S.

The polymer dissolved in DMA, DMSO, DMF, and *N*-methylpyrrolidone (NMP) but not in water.

^{13}C-NMR spectroscopy (DMSO-*d6*, ppm): 145.48, 130.54, 128.07 ($C_{aromatics,\,Ts}$), 103.4 (C1), 98.40 (C1'), 84.50, 79.90, 75.03, 74.37, 73.98, 70.61 (C2,3,4,5), 60.96 (C6, OH), 21.60 ($CH_{3,\,Ts}$), 18.07 ($C6_{Deoxy}$).

FTIR spectroscopy (KBr, cm^{-1}): 3524 ν OH (weak), 3010-3000 ν =CH, 2960, 2904 ν CH, CH_3, 1600 ring vibration, 1376 δ CH_3, ν_{as} SO_2, 1252 δ CH (branched alkane), 1119, 1098, 1054 ν C-O-C(AGU), 842 δ =CH (strong).

Results and Discussion

The conversion of TsCell (sample **1a-1c**) was carried out with sodium borohydride in dry DMF or DMSO at elevated temperatures (Figure 1, Table 1). Gelation of the system was observed during the reaction that indicated a change of solubility of the polymer. Quenching with diluted HCl and precipitation in ethanol/water (90:10, v/v) afforded the corresponding 6-deoxy-celluloses. The products were characterized by elemental analysis as well as by FTIR- and NMR spectroscopy.

The FTIR spectrum of sample **2a** (not shown) showed the typical signals of the cellulose backbone and the

Table 1: *Conditions for and results of the reaction of cellulose p-toluenesulfonic acid esters (TsCell) with sodium borohydride for 24 h.*

Conditions				Results										
TsCell[a]	Amount (g)	Solvent[b]	Temperature (°C)	Product	Yield (g)	Elemental analysis (%)				Solubility[b]				
						C	H	N	S	H_2O	DMA	DMF	DMSO	NMP
1a	5.0	DMF	50	**2a**	2.7	47.96	5.84	0.17	4.31	-	+	+	+	+
1a	5.0	DMF	100	**2b**	1.37	44.79	6.39	0.76	0	+	-	-	-	-
1b	1.8	DMSO	100	**2c**	0.95	44.97	4.79	0	11.08	-	+	+	+	+
1c	1.50	DMF	100	**2d**	1.03	44.48	6.37	0.86	0.64	-	+	+	+	+

[a] **1a**: Degree of substitution of Ts groups, DS_{Ts}, 0.94, degree of substitution of 6-deoxy-6-chloro groups, DS_{Cl}, 0.11; **1b** DS_{Ts} 2.11, DS_{Cl} 0.18; **1c** DS_{Ts} 0.98, DS_{Cl} not determined.

[b] *N,N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), *N,N*-dimethylacetamide (DMA), *N*-methylpyrrolidone (NMP), soluble (+), insoluble

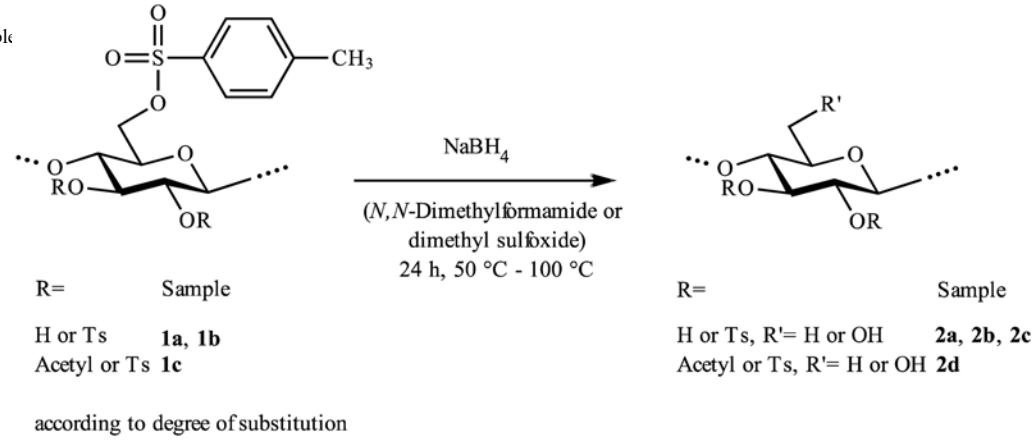

Figure 1: *Reaction scheme for the conversion of cellulose p-toluenesulfonic acid (Ts) ester with sodium borohydride.*

remaining Ts groups. Hydroxyl groups lead to a signal at 3524 cm^{-1}. Weak signals appearing in the range between 3000 cm^{-1} and 3100 cm^{-1} as well (=CH), a signal at 1600 cm^{-1} (ring vibration), 1497 cm^{-1} (ν_{as} SO_2), 1192 cm^{-1} (ν_s SO_2), 1376 cm^{-1} (δ CH_3), and 842 cm^{-1} (δ CH_3) were attributed to the Ts group. Moreover, a set of absorptions between 1054 cm^{-1} and 1120 cm^{-1} was assigned to the glycosidic bonds (ν C-O-C) of the polymer backbone. However, the FTIR spectroscopy did not give clear evidence for the presence of the intended 6-deoxy structure.

The elemental analysis indicated that the sulphur content of sample **2a** with 4.31% was below the sulphur content of Ts cellulose **1a** (9.73%). If the reduction took place at 100 °C, the sulphur practically disappeared

(sample **2b**). In case of higher DS_{Ts} of the starting material, some sulfur remained after the reaction with sodium borohydride (sample **2c**, 11.08% S after the reaction). This led to the conclusion that Ts groups bound to position 6 of the repeating unit had been replaced by hydrogen or by a hydroxyl group. As known, Ts groups attached to positions 2 and 3 are more stable and remain in the molecule (sample **2c**). This finding could also be observed by ^{13}C-NMR spectroscopy (Figure 2). The signal occurring in the spectra of all samples in the range around 18 ppm can be assigned to the 6-deoxy function (-CH_3) and agrees with the literature data on 6-deoxy-glucose [25]. The poor resolution of the DEPT135 NMR spectra of both samples did not allow reliable signal assignment. Nevertheless, all other structural features of the cellulose derivatives could be detected. In case of samples with low initial DS_{Ts} and high reaction temperature (samples **2b** and **2d**), the typical signals of the Ts moiety almost completely disappeared, which is in accordance with results of the elemental analysis. In case of samples synthesized at lower temperature (sample **2a**) and higher initial DS_{Ts}, signals at 21.5 ppm (methyl group of Ts, C8) and 128 ppm – 145 ppm (aromatic carbon atoms, C9,10,11,12) were detected. It must be pointed out that the intended nucleophilic displacement reaction of Ts by hydride ions apparently did not proceed completely. A signal in the range between 60 ppm and 61 ppm was clearly assigned as CH_2OH moiety, i. e. position 6 of the repeating group. Thus, hydrolytic cleavage of the Ts groups caused by traces of water or during workup instead of formation of deoxy moieties must also be considered. Moreover, a signal appearing at 45 ppm was detected in the ^{13}C-NMR spectrum of sample **2a** only. This chemical shift is characteristic for CH_2Cl, which is the result of a side-reaction occurring during the tosylation of cellulose. Obviously, the chlorine is acting as leaving group as well and is replaced by hydride or a hydroxyl group is formed during the reaction at 100 °C.

Only sample **2b** was found to be water-soluble and insoluble in organic solvents. All other samples retained their solubility in aprotic-dipolar media. It can be hypothesized that the influence of intermolecular hydrogen bond from/to OH group at

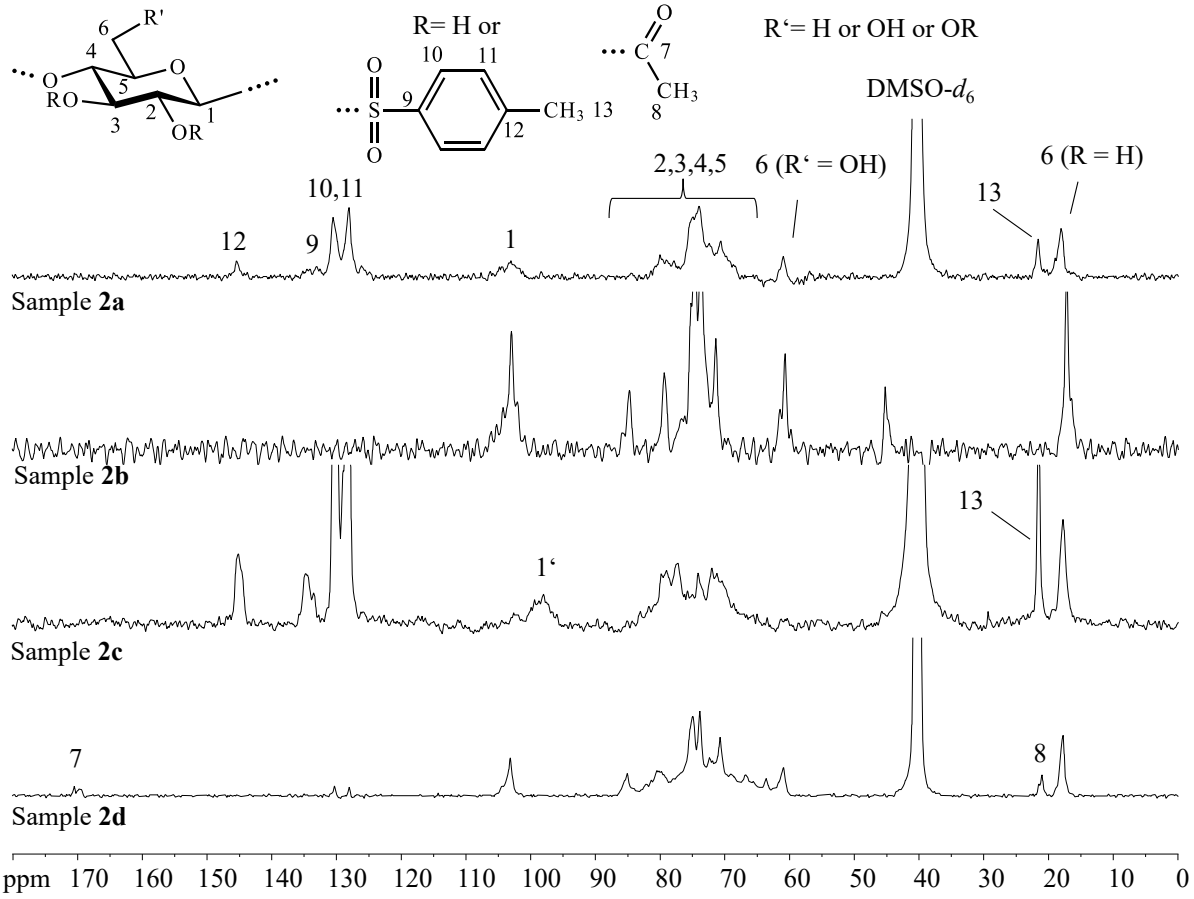

Figure 2: *^{13}C-NMR spectra of deoxy-cellulose derivatives obtained by reaction of cellulose-p-toluenesulfonic acid esters with sodium borohydride.*

position 6 is attenuated leading to solubilization of the polymer. On the contrary, the solubility of samples **2b-2c** in aprotic-dipolar solvents is governed by the nonpolar Ts groups and additional acetyl moieties in case of sample **2d**.

Conclusions

It could be demonstrated that 6-deoxy-celluloses can be prepared by treatment of Ts cellulose with hydride ions. The reaction occurs selectively at position 6 of the repeating unit. Thus, substituents bound to the secondary positions remained unaffected. The 6-deoxy group renders the polymer water soluble, which is a further proof of the importance of intermolecular hydrogen bonds for the dissolution behavior of cellulose. The cellulose derivatives with "reduced functionality" could be helpful in investigation of hydrogen bond-based phenomena.

Acknowledgements

We are indebted to Annett Pfeifer for providing the Ts celluloses and to Denny Renner for performing the syntheses.

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Synthesis of Novel Polygalacturonic Acid Hydrazones and their Rheological and Emulsifying Properties

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Abstract

Polygalacturonic acid, the main constituent of pectin, can be heterogeneously transformed into the hydrazide derivative. The content of hydrazide groups can be tailored easily. The water soluble and nucleophilic pectin derivative may react with long chain aliphatic aldehydes forming amphiphilic polygalacturonic acid hydrazones. Their aqueous solutions exhibit non-Newtonian flow behavior, which is shown by *n*-values < 1 in the Ostwald-de Waele equation. The amphiphilic polymers stabilize oil/water emulsions. As monitored by microscopy, the mixtures with oil droplets smaller than 10 μm obtained are stable over 56 days.

Keywords: *Polygalacturonic acid hydrazide, Hydrazone, o/w Emulsion, Nonanal, Pseudoplastic flow*

Introduction

The research interest in polysaccharides (PS) as renewable source is strongly increasing recently. Pectin as a polyanionic representative of these class of biopolymers is especially difficult to transform completely, due to its insolubility in organic solvents. Different ways of synthesis have been reported that can increase the hydrophobic character of pectins and lead to the formation of amphiphilic materials, which can be employed as emulsifiers [1]. Zheng et al. alkylated the carboxylate group of the repeating unit with long chain alkyl halides in a heterogeneous system containing aqueous NaOH/2 propanol [2]. Furthermore, acylation reaction at the hydroxyl groups at C2, C3 have been established, using carboxylic acid anhydrides and catalytic amounts of K_2CO_3 in a solvent free reaction [3]. The treatment can be used to render pectins soluble in organic solvents for further reactions. The procedure usually needs large amounts of organic solvent or a multistep protocol to render the samples soluble for synthesis. Recently, a heterogeneous synthesis for pectin hydrazide could be established, leading to water soluble and nucleophilic pectin derivatives very efficiently [4]. The degree of transformation of the carboxylic acid to a hydrazide group can be adjusted easily. The hydrazide formed can further react with electrophilic compounds. Thus, the question arose, if polygalacturonic acid hydrazides can be transformed to amphiphilic materials by combining the carboxylic acid group of the backbone with a long alkyl chain substituent at the hydrazide moiety. The reaction of pectin hydrazide with nonanal is reported. Infra red- and nuclear magnetic resonance spectroscopy as well as the rheological parameters and some emulsifying properties of these new pectin hydrazones are discussed.

Materials

Polygalacturonic acid hydrazide was synthesized by heterogeneous conversion of the polygalacturonic acid with hydrazine hydrate according to literature [4]. Hydrazine hydrate (80 w%) was obtained from Merck, ammonia (25 w%) from Carl Roth GmbH and nonanal (≥ 95%, FCC) from Sigma Aldrich. Polygalacturonic acid (Sigma Aldrich) had a content of galacturonic acid ≥ 90% (enzymatic) and M_n = 56.905 $gmol^{-1}$ determined by size exclusion chromatography corresponding to a degree of polymerization (DP) = 330. All other reagents were of analytical grade and have been used without further purification.

Methods

Fourier Transform Infrared (FTIR) spectra were recorded on a NICOLET AVATAR 370 DTG spectrometer using translucent KBR tablets. The NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer at 40 °C. The sample concentration was at least 60 $mgmL^{-1}$. Deuterium oxide and deuterated dimethyl sulfoxide (dmso-d6) was used for the measurements. Samples were referenced to 3-(trimethylsilyl)-propanesulfonic acid sodium salt. Size exclusion chromatography (SEC) was carried out with an Agilent 1200 Series LC equipment (SEC pump G1310A, RI detector G1362A, columns: PSS Gram30 and PSS Gram1000 in series, flow rate: 1 mL min^{-1}; eluent: H_2O / 0.08 M Na_2HPO_4 / 0.05% NaN_3 pH 9. The calibration standard was polyethylene glycole (PEG). A VARIO EL III CHNS analyzer (Elementaranalysensysteme GmbH) was used for elemental analyses. Flow curves have been obtained on a Haake Mars Modular Advanced Rheometer System. The temperature was controlled within 0.1 °C with a Haake UTM Controller. A microscope Axioskop 40 Zeiss was used to investigate the droplet sizes of the emulsions. All pictures have been taken with a Canon EOS 1300 D. The images were analyzed with the software ImageJ.

Emulsions have been formulated by combining the aqueous polymer solution with sunflower oil and mixing the compounds for 60 seconds at 4000 min^{-1} with a T25 Ultra Turrax. The samples were stored in a refrigerator at 7 °C or in a laboratory-type drying cabinet at 25 °C and 60 °C.

Synthesis

Polygalacturonic acid hydrazide was synthesized according to Literature[4]. In a typical procedure 5.0 g (28 mmol) polygalacturonic acid was suspended in 100 mL 2-propanol. Hydrazine hydrate (1.8 g, 56 mmol) was added, and the mixture was allowed to stir at room temperature for two hours. The solid was filtered, acidified with 50 mL 2-propanol/HCl conc. (95 vol%/5 vol%) and subsequently washed with 2-propanol/H_2O (80 vol% /20 vol%) 10x 50 mL. The yellow solid was dried in vacuum at 60 °C for 24 h. Yield: 4.92 g (99%)

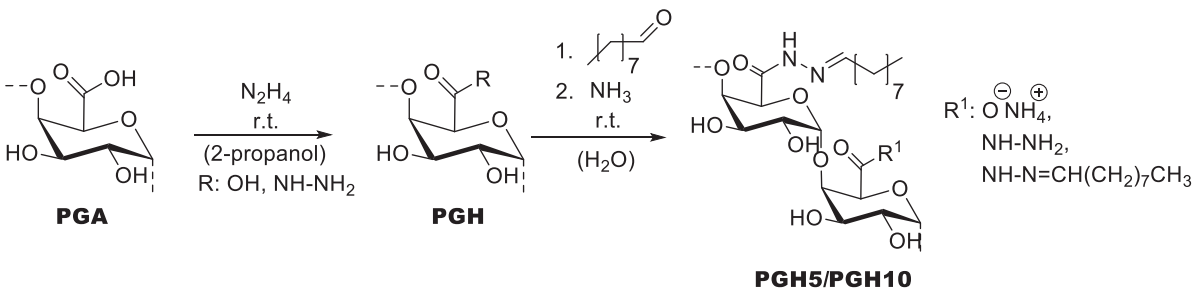
FTIR (KBr) v [cm^{-1}]: 3186 (v, OH), 1723 (v, COOH), 1579 (v, C(O)NH-), 1402, 1232, 1088, 1066, 1007, 944, 624. ^{1}H NMR (400 MHz, D_2O, ppm) δ: 5.05, 4.80, 4.40, 3.95, 3.74 (repeating unit). ^{13}C NMR (100 MHz, D_2O, ppm) δ: 174.7 (C6), 99.4 (C1), 78.3, 71.2, 68.8, 68.3. Elemental analysis (m%) (calc./found): C (37.9/ 35.2), H (5.3/ 5.7), N (14.7/ 1.0), hydrazide content: 7%.

In a typical procedure for the hydrazone formation, 2.0 g (11 mmol) polygalacturonic acid hydrazide was dissolved in 50 mL deionized water and 1.0 g (7 mmol) nonanal was added with stirring at room temperature. After 5 min 10 mL of conc. ammonia was added and the mixture was poured into ethanol (300 mL), the precipitating solid was filtered on a G3 glass filter, washed 3 times with ethanol (200 mL) and twice with ethylacetate (200 mL). The product was dried in vacuum at 60 °C for 24 h. Yield: 1.93 g (95%).

FTIR (KBr) v [cm^{-1}]: 3220 (v, OH), 1726 (v, COOH), 1590 (v, C(O)NH-), 1413, 1224, 1088, 1074, 1013, 949, 630. 1H NMR (400 MHz, D_2O, ppm) δ: 7.10, 5.01, 4.04, 3.76, 3.62, 1.26, 0.85.^{13}C NMR (100 MHz, D_2O, ppm) δ: 175.2, 102.4, 80.9, 73.0, 70.7, 66.9, 63.4, 60.1, 34.4, 31.8, 26.4, 25.2, 19.5, 16.4. Elemental analysis (m%) (calc./found): C (37.9/ 38.4), H (5.3/ 5.9), N (14.7/ 0.8).

Results and Discussion

The heterogeneous synthesis of reactive polygalacturonic acid hydrazides could be employed to prepare derivatives with low content of hydrazides in a tailored fashion [4]. A material with hydrazide content of 7% has been synthesized heterogeneously in 2-propanol. It was subsequently allowed to react with nonanal for 5 min and 10 min, which resulted in the pectin hydrazones **PGH5** and **PGH10** (Scheme 1).



***Scheme 1:**. Starting from polygalacturonic acid (PGA) a heterogeneous synthesis leads to polygalacturonic acid hydrazide (PGH), which was reacted for 5 min and 10 min in water with nonanal to form polygalacturonic acid hydrazones (samples **PGH5** and **PGH10**)*

The hydrazones formed have been neutralized with ammonia to increase their water solubility by forming the ammonium polygalacturonate. The materials thus obtained show different colors as depicted in Figure 1. The materials were dissolved in dist. water and their rheological behavior was characterized.

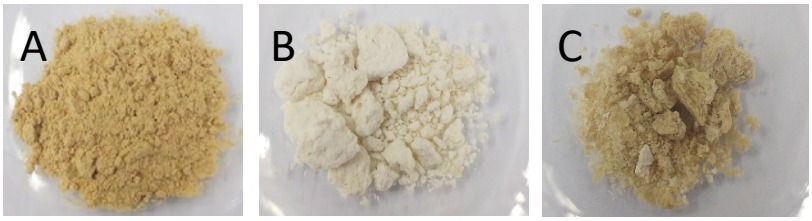


***Figure 1:**. Appearance of the starting material polygalacturonic acid (A) and the final polygalacturonic acid hydrazones **PGH5** (B) and **PGH10** (C).*

Rheological behavior

The flow curves of the pectin, pectin hydrazide, and the hydrazones **PGH5** and **PGH10** investigated show that all samples behave as non-Newtonian fluids (Figure 2). Their apparent viscosity decreases with increasing shear rate. This pseudo-plastic behavior is very pronounced for the polygalacturonic acid hydrazide in the range of shear rate from 1 s^{-1} up to 100 s^{-1}. The reorientation of the macromolecules along the direction of flow and the disentanglement of the molecule chains can explain these findings.

A general trend of decreasing apparent viscosity with increasing temperatures at shear rates above 100 s^{-1} can be seen for all samples. This temperature-viscosity behavior is especially seen in the range from 20 °C to 40 °C and less pronounced from 40 °C up to 60 °C for pectin, **PGH5** and **PGH10**. The PGH samples investigated show an increase in viscosity with increasing temperature at low shear rates (< 20 s^{-1}). At higher shear rates (> 20 s^{-1}), the viscosity decreases with increasing temperature. This effect is much less pronounced than for the other samples investigated.

The shear dependence of the viscosity can be further analyzed considering the Ostwald-de Waele power-law (Equation 1):

$$\tau = k \cdot \gamma^n \qquad (1)$$

Here τ is the shear stress, k is the consistency index and *n* is the power-law exponent [5]. For shear thinning fluids $0 < n < 1$ and for shear thickening fluids n is larger than 1. Plotting both the shear stress and the shear rate logarithmically, values for *n* and log *k* can be obtained (Figure 3). All obtained values are given in Table 1.

Figure 3 shows the linear graphs for $\gamma > 30$ s^{-1}. Therefore, equation 1 can be employed. It was found that the *n*-values are lower than 1 in the temperature range investigated (20 °C to 60 °C). This indicates indeed shear thinning behavior for all samples in the shear rate range considered. Polygalacturonic acid shows only a small change in n-value from $n = 0.94$ (20 °C) to $n = 0.93$ (60 °C). On the contrary, pectin hydrazide shows a clear decrease in n-value at increasing temperatures. Starting with the highest value $n = 0.87$ at 20 °C the exponent decreases to $n = 0.77$ at 40 °C and to $n = 0.71$ at 60 °C. The two pectin hydrazide derivatives ***PGH5*** and **PGH10** are showing significant different *n*-values. With increasing temperature, **PGH5** shows *n*-values of $n = 0.78$, $n = 0.83$ and $n = 0.77$ at 20 °C, 40 °C and 60 °C. For **PGH10** the *n*-values are comparable with the starting pectin ranging from $n = 0.97$, $n = 0.98$ and $n = 0.80$ at 20 °C, 40 °C and 60 °C, respectively.

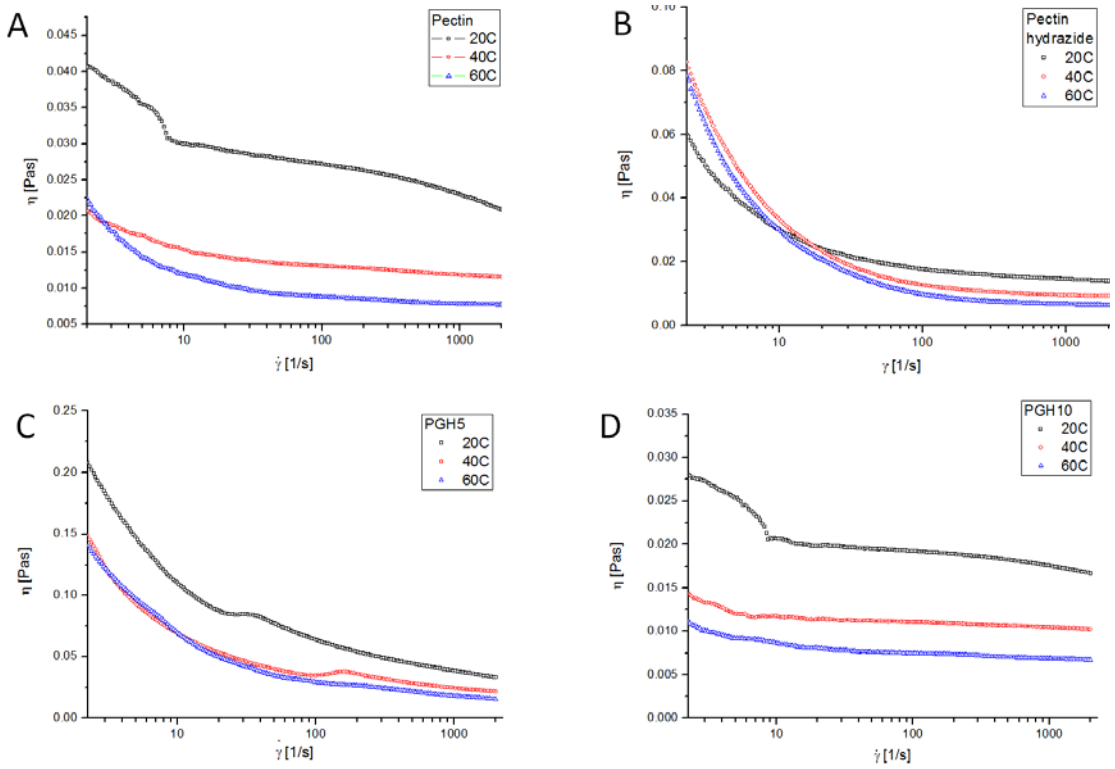


Figure 2: *Apparent shear viscosity versus shear rate of pectin (A), pectin hydrazide (B), PGH5 (C) and PGH10 (D) in aqueous solution at 20 °C, 40 °C and 60 °C respectively.*

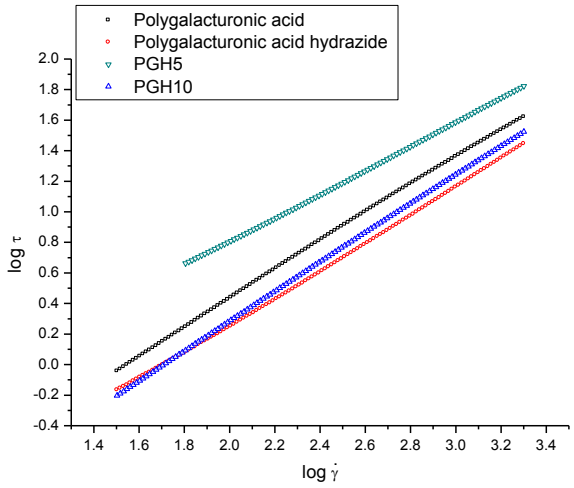


Figure 3: *Logarithm of shear stress versus logarithm of shear rate of polygalacturonic acid, polygalacturonic acid hydrazide, PGH5 and PGH10 at 20 °C.*

Emulsifying properties

The polygalacturonic acid hydrazide derivatives **PGH5** and **PGH10** synthesized show good water solubility. Thus, samples with 2.5 m%, 5 m%, and 10 m% polysaccharide derivative have been dissolved in deionized water and mixed with sunflower oil (up to 14 m%). The emulsions formed have been stored at different temperatures (7 °C, 25 °C and 60 °C) over a period of 56 days. The diameter change of the oil droplets over time was monitored with a microscope and photographs were taken to measure the size of the emulsified oil droplets. The samples stored at 60 °C separated within minutes after the storage temperature was reached. The samples stored at r.t. or 7 °C showed a clear trend. Samples with lower amount of PGH derivative separated faster than samples with higher mass% PGH derivative.

Table 1: *Rheological parameters of aqueous solutions of polygalacturonic acid, polygalacturonic acid hydrazide, PGH5 and PGH10.*

	T = 20 °C		T = 40 °C		T = 60 °C	
	n	k	*n*	k	*N*	k
Pectin	0.94	0.035	0.95	0.017	0.93	0.012
Pectin hydrazide	0.87	0.034	0.77	0.041	0.713	0.043
PGH5	0.78	0.179	0.83	0.083	0.77	0.090
PGH10	0.97	0.022	0.98	0.012	0.80	0.020

Table 2: *Mass ratios of polygalacturonic acid hydrazone derivatives with 14 m% sunflower oil and the time dependent change in diameter of the produced emulsion droplets after mixing. Samples have been stored a 7 °C.*

	PGH Derivative	Mass %	Droplet Diameter [μm]	
			Start	After 56 d
EM1	PGH5	2.5	8	23
EM2	PGH5	5.0	9	10
EM3	PGH5	5.0*	7	9
EM4	PGH10	5.0	7	8
EM4	PGH10	10.0	5	6

* Sample was mixed with 5 m% Glycerin

A picture of emulsion EM2 stable for the time of investigation (56 days) and the relative occurrence of the size of emulsified oil drops is shown in Figure 4. The influence of glycerin as a stabilizer was tested in one trial as well (EM3) but gave only marginal changes in the outcome of the droplet size and stability of the emulsion formed. Other additives for stabilization of the emulsion e.g. against microorganism growth have not been tested. It could be observed that a few samples started to mold after a few days, which lead to the destruction of the emulsion within a single day. These samples have been prepared again and showed to be stable on the second trial. No correlation could be found between the appearance of mold and the composition of the emulsion.

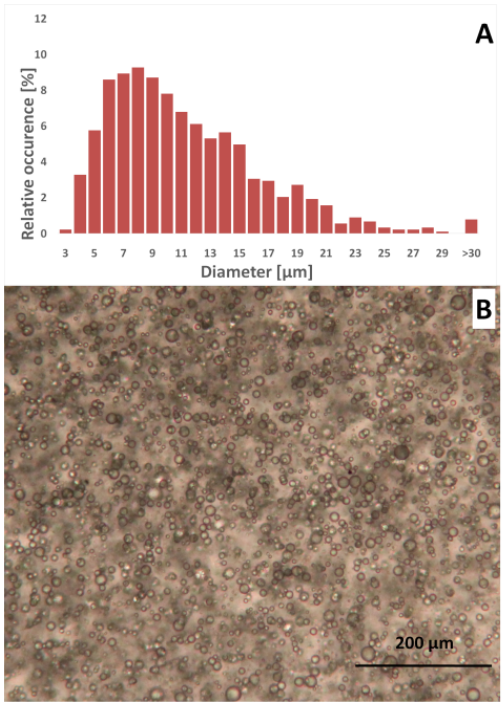


Figure 4: *Relative occurrence and diameter of emulsified oil droplets of sample EM2 (A) after 56 days of storage and (B) a magnified view of the same sample.*

Conclusions

Polygalacturonic acid hydrazide, as one of few nucleophilic polysaccharides, can be synthesized with a tailored hydrazide content. The nucleophilic group formed can be employed to synthesize hydrazones. Conversions with the hydrophobic aldehyde nonanal and subsequent neutralization of the carboxylic acid groups with ammonia lead to the formation of amphiphilic materials. The polymers investigated show non-Newtonian behavior. This is most evident for PGH. In contrast to the starting PGA and the final materials **PGH5** and **PGH10**, the PGH shows the smallest change in the apparent viscosity during measurement with shear rates above 100 s^{-1}. The apparent viscosity of PGH at low shear rates (< 10 s^{-1}) even shows a temperature-viscosity behavior reversed compared to the other samples investigated. The amphiphilic materials **PGH5** and **PGH10** show emulsifying properties and stabilize oil in water emulsions over several weeks with droplet sizes as low as 6 μm after 56 days of storage at 7 °C. Pectin hydrazide can function as a platform for new and interesting pectin hydrazones, shifting from petrochemical based to more sustainable resources. Future studies will show, how the stability of these amphiphilic materials can be increased over a larger temperature range. Moreover, the introduction of further functional groups via this path is under investigation and will expand the applicability of pectin and its derivatives.

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Side Reactions During the Homogeneous Esterification of Starch with Unsaturated Cinnamic Acid Derivatives in Molten Imidazole

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Abstract

The homogeneous synthesis of starch cinnamates was studied. Molten imidazole was employed as efficient reaction medium for the polysaccharide and cinnamoyl chloride as reagent. It was postulated that an activated cinnamoyl imidazolide is generated in the process, which would result in efficient esterification and high degrees of substitution (DS). It was found, however, that side reactions of the α,β-unsaturated carboxylic acid derivative and the reaction medium occurred. By spectroscopic experiments, it was demonstrated that imidazole reacts with the double bond by Aza-Michael addition reaction. Mixed esters of starch cinnamate and starch 3-(1*H*-Imidazol-1-yl)-3-phenyl-propanoate (StIPP) are obtained.

Keywords: *starch; imidazole; cinnamic acid derivatives; esterification; side reactions*

Introduction

In the context of current sustainability and climate change discussions, products made from renewable and biodegradable sources, in particular polysaccharides, gain increasing importance as alternatives to oil-based synthetic polymers [1,2]. The homogeneous modification of polysaccharides may lead to novel, advanced materials with tailored properties [3]. In this context, the chemical modification of starch, especially by esterification, opens a wide range of opportunities to obtain biopolymer-based materials with promising properties, e.g., for applications in the field of biodegradable plastics, adhesives, and additives for the adjustment of rheological and colloidal properties [4-7]. Dimethyl sulfoxide (DMSO) and certain aqueous media are usually employed as solvents for the esterification reactions [8-11]. Recently, a novel approach was found in which molten imidazole is used as reaction medium for the efficient homogeneous synthesis of long chain aliphatic and complex starch esters with thermoplastic properties [4,12]. Therein, imidazole acts not only as solvent for the polysaccharide but also as base as well as activation agent for the esterification reagent. The reaction proceeds by the *in situ* formation of a carboxylic acid imidazolide, which is formed by conversion of imidazole with an acid chloride or anhydride. Variation of the type and the amount of ester moieties as well as the starch used, opens up a wide range of products, with diverse properties and a broad range the melting temperatures [4]. In order to broaden the spectrum of thermoplastic starch esters and, hence, their properties, the introduction of unsaturated ester moieties is of great interest. The double bounds can be crosslinked after melting, e.g., by UV irradiation, making the melting irreversible ("thermosetting properties") [13-15]. Reactions of starch with the chlorides of unsaturated fatty acids

have been reported in the system *N,N*-dimethyl acetamide / LiCl in the presence of *N,N'*-carbonyldiimidazole (CDI) as activator [16]. Furthermore, studies on transesterification of starch with unsaturated fatty acid esters were carried out [17]. Cinnamates, i.e., esters of the aromatic α,β-unsaturated cinnamic acid, are very interesting compounds that can be crosslinked easily by irradiation with UV light [18,19]. Moreover, cinnamic acid and its derivatives are of great interest in the context of valorization of lignocellulosic biomass. Thus, aim of the present work was to study the homogeneous synthesis of starch cinnamates by conversion of the polysaccharide with cinnamoyl chloride in molten imidazole.

Experimental

Materials

Starch, FLOJEL 60 ACS 1240 (Mw = 454.29 g/mol, National Starch) was applied as starting polymer. It was dried in vacuum at 105 °C over potassium hydroxide for 24 h before use. Cinnamoyl chloride, imidazole, and isopropyl alcohol were purchased from Sigma Aldrich. Further chemicals and reagents were of analytical grade and were used as received.

Measurements

NMR spectra were acquired at room temperature and 60 °C on a Bruker Avance 250 MHz or 400 MHz spectrometer with 16 scans for ^{1}H-NMR spectroscopy and up to 20.000 scans for ^{13}C-NMR spectroscopy and at least 50 mg sample per ml dimethyl sulfoxide (DMSO)-d_6. 2D experiments were measured with the 400 MHz spectrometer. Elemental analysis (C, H, S content) was carried out using a Vario EL III (Elementaranalysensysteme Hanau, Germany). The chlorine content was determined according to Schöniger's method [20].

Methods

Dissolution of starch in imidazole

Starch (2.5 g, 15.4 mmol) was mixed with imidazole (22.7 g, 333.4 mmol) and the temperature was increased to 100 °C under stirring. After the melting of imidazole was completed, the mixture was stirred for 1 h at 100 °C to obtain a clear, slightly yellow solution of starch.

Homogeneous esterification of starch with cinnamoyl chloride (starch ester 1, typical example)

Directly before the synthesis, cinnamoyl chloride, which forms solids blocks in the storage bottle in which it is supplied, was heated to 60 °C. An appropriate amount of the liquid was removed, quenched, grinded into a powder, and weighted to obtain the amount of reagent required for each individual reaction. Cinnamoyl chloride (2.6 g, 15.4 mmol) was added to the above described solution of starch (2.5 g, 15.4 mmol) in imidazole (22.7 g, 333.4 mmol) at 100 °C. The reaction mixture was allowed to react for 1 h at 100 °C under mechanical stirring. The reaction was stopped by adding isopropyl alcohol (50 ml) and the reaction mixture was poured into an excess of isopropyl alcohol (200 ml). The precipitating product was removed by filtration, washed with isopropyl alcohol (four times with 200 ml each), and dried at room temperature under vacuum.

Mass yield: 4.8 g.

Elemental analysis: 47.3% C, 5.1% H, 4.1% N, 0.0% Cl.

^{13}C-NMR spectroscopy (DMSO-d_6, ppm): 170.2 (C-7), 166.1-167.0 (C-16), 145.4 (C-9), 140.3 (C-19), 137.2 (C-25), 134.0 (C-10), 130.5 (C-13, C-22), 128.7 (C-12, C-14, C-21, C-23), 127.3 (C-11, C-15, C-20, C24), 122.0 (C-26, C-27), 118.4 (C-8), 100.9 (C-1), 106.2 (C-1'), 82.2-77.0 (C-2, C-3, C-4, C-5), 64.1 (C-6), 60.7 ($C6_{substituted}$), 57.3 (C-18), 40.0 (C-17).

FTIR spectroscopy (KBr, cm^{-1}): 3400 ν OH, 3010 ν_{as} $CH_{aromatic}$, 1750 ν C=O, 1665 ν C=N, 3010 ν_{as} $CH_{aromatic}$, 1500 δ $CH_{aromatic}$, 1320 ν C-N, ν_{as} C-C(=O)-O- , 1022 ν C-O-C_{AGU}, 740 γ C=C.

Results and Discussion

It was found that molten imidazole can efficiently dissolve starch and that it can be employed as reaction medium for the homogeneous chemical derivatization of the polysachharide [4]. It likewise acts as solvent for starch and the reactants as well as base and activating agent (mediator) for esterification reactions. In the present work, starch was dissolved in imidazole at 100 °C and converted with different amounts of cinnamoyl chloride for 1 to 24 h. As has been described previously, the acid chloride first reacts with imidazole to form the corresponding activated carboxylic acid imidazolide (Figure 1) [4].

The reaction of the cinnamic acid imidazolide with starch dissolved in imidazole was carried out at different molar ratio of cinnamoyl chloride per anhydroglucose unit (AGU) from 1 / 1 to 5 / 1 (Table 1). The products obtained at lower molar ratios (up to 2 / 1) were soluble

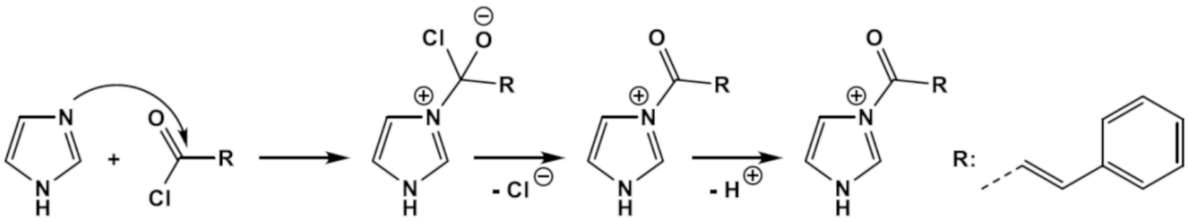

Figure 1: *Reaction scheme for the synthesis of carboxylic acid imidazolides by conversion of an acid chloride with imidazole.*

in chloroform, which was expectable considering the hydrophobicity of the aromatic ester moiety that was introduced. Interestingly, products obtained at higher molar ratios (3 / 1 and higher), i.e., derivatives with supposedly higher degrees of substitution (DS), did not dissolve in chloroform but in more polar solvents like dimethyl sulfoxide (DMSO). Despite rigorous washing and reprecipitation, the products showed a considerable amount of nitrogen (about 4 to 6%). This was unexpected based on previous studies using molten imidazole as medium for the synthesis of a broad variety of starch esters in which imidazole traces could be removed with low efforts to obtain pure products. Thus, it was speculated that side reactions with the reaction medium might occur in the present case.

Table 1: *Conditions for and results of the esterification of starch with cinnamoyl chloride in molten imidazole at 100°C.*

Reaction conditions		Product					
Reaction time, h	Molar ratio CCl / AGU[a]	#	Elemental composition			Solubility[b]	
			C	H	N	DMSO	$CHCl_3$
1	1 / 1	**1**	47.3	5.1	4.1	+	+
1	2 / 1	**2**	59.0	5.0	4.1	+	+
1	3 / 1	**3**	63.0	5.4	5.0	+	–
1	4 / 1	**4**	69.6	5.1	4.5	+	–
24	5 / 1	**5**	66.8	5.3	5.6	+	–

[a] Mol cinnamoyl chloride (CCl) per mol anhydroglucose unit (AGU).
[b] DMSO: dimethylsulfoxide, $CHCl_3$: chloroform, + = soluble, – = insoluble.

The analysis of the reaction products by FTIR spectroscopy revealed typical absorption bands for the polymer backbone (ν_{as} C-C(=O)-O-) around 1020 cm^{-1} and the ester moieties (ν C=O) around 1750 cm^{-1}. This clearly indicates the successful esterification of starch. However, additional absorption bands were observed in the FTIR spectra at 1320 cm^{-1} (ν C-N) and 1665 cm^{-1} (ν C=N) that are characteristic for the presence of aromatic tertiary amine moieties and imines, respectively. Thus, it is speculated that side reactions of the unsaturated carboxylic acid derivative and imidazole occurred that resulted in a covalent binding of the aromatic amine to the polysaccharide. It has been reported that the conversion of aliphatic α,β-unsaturated carboxylic acids such acrylic- and methacrylic acid with *N,N'*-carbonyldiimidazole (CDI) in DMSO can result in an addition of imidazole to the double bound [21, 22]. Aryl substituted, i.e., conjugated, α,β-unsaturated carboxylic acid like cinnamic acid did not show this side reaction and CDI activated esterification of starch in DMSO was feasible [21]. However, the present study indicates that this might be the case for conversion in molten imidazole. Based on the spectroscopic results, it is hypothesized that cinnamoyl chloride reacts with imidazole by a nucleophilic attack of the nitrogen atom of imidazole at the double bond according to Aza-Michael addition reaction (Figure 2). 3-(1*H*-Imidazol-1-yl)-3-phenyl-propanoyl chloride is formed, which can react with the hydroxy groups of starch to form starch 3-(1*H*-Imidazol-1-yl)-3-phenyl-propanoate (StIPP) esters. Comparable reactions have been described for low molecular weight organic compounds in the literature [23].

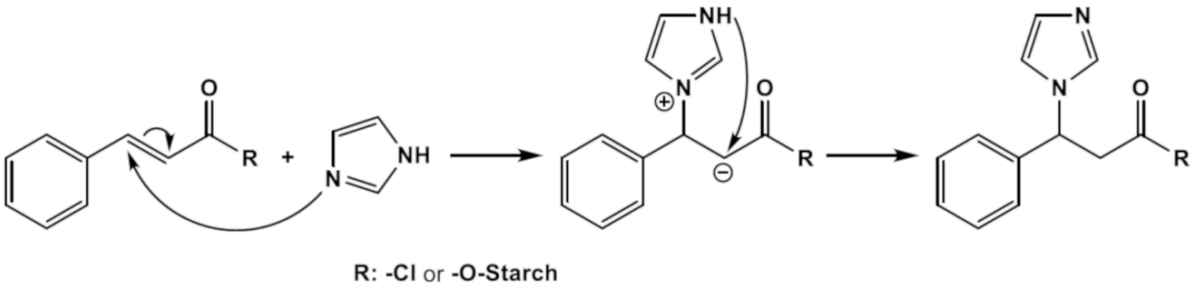

Figure 2: *Propose reaction scheme for addition of imidazole to the double bound of cinnamic acid derivatives.*

The starch derivatives obtained were well soluble in DMSO or chloroform. Thus, solution state NMR spectroscopy could be performed to elucidate the molecular structure of the derivatives. The ^{13}C-NMR spectrum of sample 1 is shown in Figure 3. It features the characteristic peaks of the carbon atoms C-1 to C-6 of the AGU between about 60 ppm and 106 ppm. The peak at 106.2 ppm can be attributed to the anomeric C-1 atom adjacent to C-2 with an unmodified hydroxy group. In addition, a less intensive peak at 100.9 ppm was observed that can be attributed to a C-1 atom in vicinity to an esterified C-2 position. Thus, it can be concluded that the position C-2 is partially converted to an ester moiety. Moreover, minor peaks corresponding to an esterified position C-6 were observed (60.7 ppm). In addition to peaks that could be attributed to the starch backbone, characteristic peaks for cinnamic ester moieties were identified between around 118 ppm and 170 ppm. Two separate peaks were observed in the "carbonyl region" at 170.2 pmm (attributed to StIPP that was introduced by a side reaction) and at about 167 ppm (attributed to starch cinnamate), which demonstrates that two different types of ester moieties are present. The downfield shift of the carbonyl related peak in StIPP can be explained by the loss of the electron donating +M-effect of the conjugated double bond that occurred upon addition of imidazole. Peaks attributed to the aromatic ring of the cinnamic acid substituent were observed in the range of about 140 ppm to 130 ppm. At 137.2 ppm, 140.3 ppm, and 57.3 ppm, additional signals were recorded that can be assigned to C-25 (bound imidazole moiety) as well as C-19 (aromatic carbon atom adjacent to C-18) and C-18 (former sp^2-carbon atom with a covalently bound imidazole group) of the StIPP substituent. The peak belonging to the α-carbon atom (C-17) of the StIPP is located around 40 ppm and covered by the DMSO solvent peak. The signals of carbons of the original double bond are located at 145.4 ppm (C-9) and 118.4 ppm (C-8), demonstrating the existence of the mixed ester of starch cinnamate and StIPP. The latter peak also includes the aromatic C-26 of the imidazole moiety.

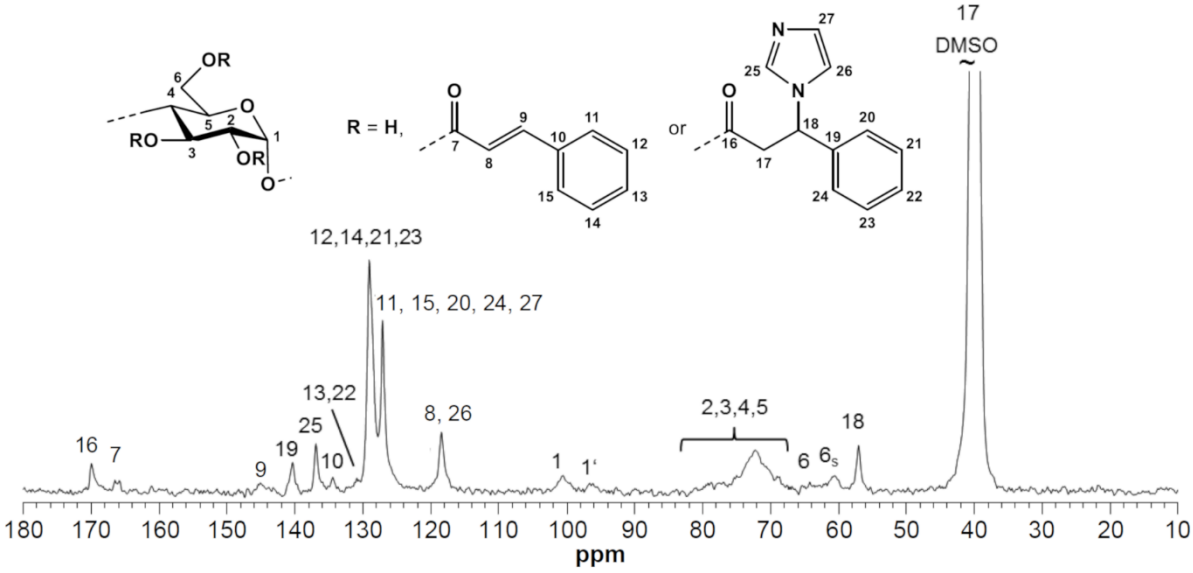

Figure 3: *^{13}C-NMR spectrum of the mixed starch ester derivative 1, recorded in DMSO-d_6 at 25 °C.*

The ^{1}H-^{1}H-COSY-NMR spectrum of sample **1** shows different cross peak patterns due to presence of a double bound that can potentially be in cis or trans configuration (Figure 4). The vicinal coupling constant describes two functional groups bound to two adjacent carbon atoms and it largely depends on the dihedral angle. Compared to cis isomers (~7 Hz), trans isomers exhibit significantly higher values of vicinal coupling constants (~16 Hz). The cross peaks of the two trans isomeric protons of the double bound were recorded at 7.6 ppm and 6.6 ppm, thus, demonstrating that a certain content of the original double bounds remained in the starch ester products.

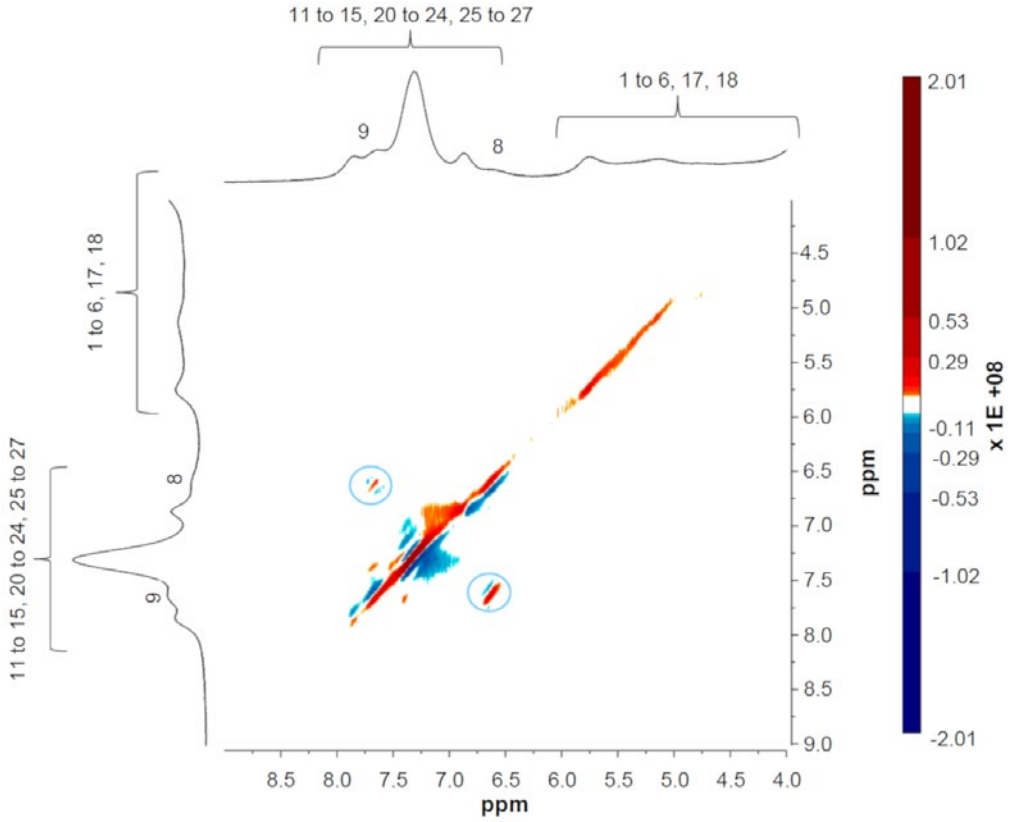

Figure 4: *^{1}H-^{1}H-COSY-NMR spectrum of the mixed starch ester derivative 1, recorded in DMSO-d_6 at 25 °C.*

Conclusions

In this work, it was found that the esterification of starch with cinnamoyl chloride, an unsaturated aromatic acid derivative, in molten imidazole leads to side reactions. The conversion results in the desired formation of the activated carboxylic acid imidazolide. Thus, esterification of starch occurred. However, imidazole also reacts with the double bound of the α,β-unsaturated carboxylic acid by Aza Michael addition reaction. It was demonstrated that the corresponding 3-(1*H*-imidazole-1-yl)-3-phenyl-propanoate moiety formed is also esterified to the polymer backbone. Based on these findings it can be concluded that other activation agents (e.g., tosyl chloride, oxalyl chloride / *N,N*-dimethylformamide) and / or reaction media should be employed for the synthesis of starch esters with α,β-unsaturated carboxylic acids.

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Part 1: In-Depth Investigation of Potential Growth Inhibitors for Microorganisms Used for the Production of Value-Adding Products from Spent Sulphite Liquor

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Abstract

The sulphite process yields not only dissolving pulp but also large quantities of spent sulphite liquor (SSL) rich in mono- and oligomeric sugars. Useful substances such as ethanol, butanol, polyhydroxyalkanoates (PHAs), etc. can be produced therefrom. In addition to the desirable high amounts of sugars process lyes also contain other substances, which can have inhibitory effects on the microorganisms. Part 1 of the study addresses the effect of organic acids, phenols, furan derivatives and alcohols on three strains of microorganisms *Thermoanaerobacter mathranii, Clostridium saccharoperbutylacetonicum and Halomonas halophila*. These results were compared with literature data. It was found that all three strains have a relatively high resistance to organic acids, furan derivatives and alcohols in the concentration range of the industrial samples. Some phenolic compounds caused inhibition of cell growth. Minimal differences in their structure lead to major differences in the inhibitory effect. Finally, the effect of mixtures of potential inhibitors on the growth of *Clostridium saccharoperbutylacetonicum* was investigated. However, only additive but no synergistic effects were observed.

Keywords: *Sulphite spent liquor valorisation, fermentation, growth inhibitors, extremophiles*

Introduction

Fermentation of sulphite spent liquor to produce ethanol or fodder yeast is an ancient art, which is nowadays practised by only a few pulp mills [1,2]. Often the economics of ethanol production are not favourable. However, fermentation can yield products with higher added value than the aforementioned and that was our motivation to investigate other microbial transformations, such as butanol or PHA production [3,4]. Another motivation was to improve the existing ethanol production. Employing thermophiles, which tolerate relatively high temperatures and can often metabolise C6-sugars and C5-sugars, in contrast to the now used *Saccharomyces cerevisiae* strains [5]. In our studies, we observed a pronounced inhibitory effect of spent sulphite liquors on the growth of the investigated microorganisms *Thermoanaerobacter mathranii, Clostridium saccharoperbutylacetonicum* and *Halomonas halophila*. To gain a deeper understanding of the nature of the inhibition, an extensive screening was performed.

A wide range of compounds is formed out of lignin

and hemicellulose during the digestion of lignocellulosics in the sulphite process. Some of them have inhibitory effects on the growth of microorganisms [6-13]. Depending on their origin, the inhibitors were identified: organic acids, phenols, furan derivatives and metal cations, which originate, for example, from the digester material [14-16]. The furan derivatives include furfural and hydroxymethylfurfural. They are formed as by-products in hydrolysis due to the degradation of pentoses and hexoses. Furan derivatives can influence cell replication so that the growth rate and the specific productivities are reduced [17,18]. They also cause the inhibition of glycolytic and fermentative enzymes, decrease levels of intracellular ATP and NAD(P)H, damage the repairing mechanism of cells and destroy cell membranes. In addition, furfural leads to the accumulation of reactive oxygen species, which causes damage to the mitochondria, vacuolar membranes, the actin cytoskeleton and the nuclear chromatin[19]. Further degradation of furan derivatives results in the formation of formic acid and levulinic acid. Formic acid, levulinic acid, and acetic acid are the most abundant weak organic acids in the spent liquors. Acetic acid is formed during pulping due to the cleavage of acetyl groups present in the native wood hemicelluloses. Undissociated organic acids can pass the cell membrane. There are several explanations for the inhibitory effect such as acidification of the cytoplasm, anion accumulation, membrane perturbation, ATP depletion and perturbation of metabolism [6,20]. Hydrophobic organic acids have a more inhibitory effect on the cells than less hydrophobic organic acids because they interact with the cell membrane [19]. A large number of different aromatic compounds with a variety of substituents makes the identification and quantification rather difficult. The harmfulness of phenols is said to increase with an increasing degree of hydrophobicity and with decreasing molecular weight. They can cause a loss of integrity of cell membranes and enzyme matrices affecting the cell growth and sugar assimilation [6,21]. Studies are showing that phenols block the pathway of the assimilation of organic acids and reduce cell growth and glucose utilization [22]. Other studies report that phenols induce reactive oxygen species in different parts of the cell. It results in cytoskeleton damage and DNA mutagenesis [19,23]. Moreover, synergistic effects of the inhibitors are described [15].

The majority of publications deal with these effects in the context of ethanol production from simultaneous saccharification and fermentation processes and related processes. Considerably fewer groups describe inhibitory effects and countermeasures concerning spent sulphite liquors [24-26]. Ethanol production from spent sulphite liquor is an ancient but declining art because on one hand, the use of the sulphite process is declining and on the other hand, the integration of an ethanol plant is often economically infeasible. In addition to the production of ethanol, processes for the production of butanol, succinic acid, fumaric acid, bacterial cellulose and polyhydroxyalkanoates from sulphite spent liquors are also described. These processes are still in the launch or even testing stages [27-31].

As mentioned above, we observed pronounced inhibitory effects of spent sulphite liquors on the growth of our microorganisms. Therefore, we performed an extensive screening of inhibitory effects of single compounds on the anaerobic ethanol producer *Thermoanaerobacter mathranii*, anaerobic butanol producer *Clostridium saccharoperbutylacetonicum* and the aer-

Table 1: *Concentration ranges of potentially inhibiting organic acids, alcohols and furan derivatives. (Data from six commercial spent sulphite liquors as well as literature data from wood hydrolysates).*

Potential inhibitors	Molar mass [mg/mmol]	Industrial samples		Literature data [32-35]	
		lowest concentration [mmol/l]	highest concentration [mmol/l]	lowest concentration [mmol/l]	highest concentration [mmol/l]
Organic acids:					
Formic acid	46.03	0.75	8.25	34.76	67.35
Acetic acid	60.05	81.35	160.51	39.97	43.30
Alcohols:					
Methanol	32.04	5,55	20.8	N/A	N/A
Ethanol	46.07	0.22	2.57	N/A	N/A
Furan aldehydes:					
Furfural	96.08	0.25	10.89	2.71	10.41
Hydroxymethylfurfural	126.11	0.50	5.32	3.89	46.78

obic PHA producer *Halomonas halophila*. The investigated concentration ranges of potentially inhibiting organic acids, alcohols and furan derivatives were derived from the analyses of spent sulphite liquor from six sulphite mills covering softwood and hardwood as well as paper pulp and dissolving pulp productions. The highest and the lowest values were identified irrespective of the wood species or the pulp produced.

Interestingly the concentrations achieved in lab-scale experiments are significantly higher for formic acid and hydroxymethylfurfural compared to the industrial samples and significantly lower for acetic acid (Table 1).

We relied on literature data [32-35] in the case of potentially inhibiting phenolic compounds. The highest concentration in the literature was 6.42 mmol/l for gallic acid, the lowest was <0.001mmol/l for p-coumaric acid. 3 mmol/l and 7 mmol/l were the concentrations chosen for our study to ensure that the highest concentration is covered and that concentration-dependent differences if any could be detected. Occasionally a wider concentration range was measured and the results were included in the study (see Supporting Information (SI)).

Table 2: *Concentration ranges of potentially inhibiting phenolic compounds in wood hydrolysates [12,32-37].*

Potential inhibitors	Molar mass [mg/mmol]	lowest concentration [mmol/l]	highest concentration [mmol/l]
Phenol	94.11	0.37	0.37
Catechol	110.10	0.02	4.00
Resorcinol	110.10	N/A	N/A
Hydroquinone	110.10	0.06	0.06
Pyrogallol	126.11	0.53	0.79
Gallic acid	170.12	4.28	6.42
Guaiacol	124.13	4.95	4.95
Vanillin	152.15	0.21	2.83
Syringaldehyde	182.17	0.18	1.17
4-Hydroxybenzoic acid	138.12	0.04	0.59
Vanillic acid	168.14	0.04	0.50
Apocynin	166.17	0.04	0.05
Homovanillic acid	182.17	0.03	0.03
Syringic acid	198.18	0.19	1.27
Coniferyl aldehyde	178.18	0.20	1.69
p-Coumaric acid	164.16	<0.001	<0.001
Ferulic acid	194.18	0.033	1.1
Ellagic acid	302.20	0.06	3.86

The inhibitory effect of a compound was judged by the changes in microbial growth and expressed as % of the microbial growth of the inhibitor-free cultivation. Taking into account the small sample volumes because the cultivation of experiments with *Clostridium saccharoperbutylacetonicum* and *Thermoanaerobacter mathranii* were performed in microtitre plates in 80 µl medium and cultivation of *Halomonas halophila* in shake flasks in 100 ml medium and the work with living systems, we decided not to compare absolute numbers, but to define growth ranges instead and illustrate these with the following colour code (Table 3):

Table 3: *Colour code for growth ranges of microorganisms after the addition of potential inhibitors from the spent sulphite liquor: red is toxic, yellow is moderately toxic, green is nontoxic, and dark green means a positive effect on the growth of cells.*

growth [%]	>100	67-100	33-66	0-33

The full set of experimental data and literature data [7-10,13,42] is provided in SI Tables S1-S3 and S5 applying the same colour code. The evaluation is exclusively based on the changes in growth assessed as final OD at a particular time compared to the control. Fermentation conditions such as aerobic or anaerobic, type of microbe (yeast, bacterium etc) and productivity were not part of the investigation/evaluation.

Materials and Methods

Thermoanaerobacter mathranii (DSM 11426)

Medium preparation

For the cultivation of *Thermoanaerobacter mathranii* (DSM 11426), the DSMZ 640 medium from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures was used. The medium contains 0.9 g/l sodium chloride, 0.4 g/l magnesium chloride hexahydrate, 0.75 g/l monopotassium phosphate, 1.5 g/l dipotassium phosphate, 2 g/l peptone from casein, 1 g/l yeast extract, 1 ml/l trace elements solution SL-10, 2.5 mg iron(III) chloride hexahydrate, 0.75 g/l L-cysteine hydrochloride monohydrate, 0,5 mg resazurin.

For the preparation of the trace elements solution SL-10, 1.5 g iron(II) chloride tetrahydrate was dissolved in 10 ml 7.7 M HCl and diluted with 990 ml deionised water. The following salts were added to the solution: 70 mg zinc chloride, 100 mg manganese(II) chloride tetrahydrate, 6 mg boric acid, 190 mg calcium chlo-

ride hexahydrate, 2 mg copper(II) chloride dehydrate, 24 mg nickel(II) chloride hexahydrate, 36 mg sodium molybdate dehydrate. Finally, it was made up to 1000 ml with deionised water.

The pH value of the solution was adjusted to 7.2. The medium was autoclaved for 10 min at 120 °C. Glucose (5 g/l) was used as a carbon source. The concentrated sterile glucose solution was added to the medium before the inoculation of microorganisms.

Cultivation

The work was carried out in a glovebox under forming gas atmosphere. 1 ml cryo stock (-80 °C) of cells in glycerol was thawed at room temperature and added to 9 ml medium. The cells were incubated with agitation for 12 h at 65 °C. To preserve vital cells culture was re-inoculated in a medium once again and incubated for 5 h. These cells were used for inhibitor experiments.

Inhibitor screening

For inhibitor experiments with alcohols, organic acids and furan aldehydes, 1 ml culture was added to 9 ml medium containing 5 g/l glucose and an aliquot of the inhibiting substance under forming gas atmosphere, whereby the organic acids were neutralized before addition to the medium. The cells were incubated with agitation for 8 h at 65 °C. At the same time, the medium without cells was incubated under the same conditions. The samples for the determination of the growth curve by OD measuring were taken from all solutions at regular intervals. Measurements were performed in triplicate.

The inhibitor experiments with phenolic compounds were carried out in microwell plates under forming gas atmosphere. 50 µl medium with 5 g/l glucose and with/without an aliquot of the respective phenolic compound were pipetted into each well. Thereafter, 30 µl of the culture was added to the solutions. The microtitre plate was sealed with a transparent oxygen-impermeable adhesive film. The cells were incubated for 10 h at 65 °C. The OD measurements were carried out at regular intervals. Measurements were performed in triplicate.

Halomonas halophila (DSMZ 4770)

Medium preparation

For the cultivation of *Halomonas halophila* (DSMZ 4770), the DSMZ 4340 medium from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures was used. The medium contains 81 g/l sodium chloride, 7 g/l magnesium chloride hexahydrate, 9.6 g/l magnesium sulfate hexahydrate, 0.477 g/l calcium chloride dehydrate, 2 g/l potassium chloride, 0.06 g/l sodium hydrogen carbonate, 0.026 g/l sodium bromide, 5 g/l peptone from casein and 10 g/l yeast extract. The pH value of the solution was adjusted to 7. The medium was autoclaved for 10 min at 120 °C. Glucose (1 g/l) was used as a carbon source. The concentrated sterile glucose solution was added to the medium before the inoculation of microorganisms.

Cultivation

1 ml cryo stock (-80 °C) of cells in glycerol was thawed at room temperature and added to 19 ml of medium. The cells were incubated with agitation for 14 h at 30 °C. To preserve vital cells culture was re-inoculated in a medium once again and incubated for 12 h. These cells were used for inhibitor experiments.

Inhibitor screening

For inhibitor experiments, 1 ml culture was added to 99 ml of medium containing 1 g/l glucose and an aliquot of the inhibiting substance, whereby the organic acids were neutralized before addition to the medium. The cells were incubated with agitation for 140 h at 30 °C. At the same time, the medium without cells was incubated under the same conditions. The samples for the determination of the growth curve by OD measuring were taken from all solutions at regular intervals. Measurements were performed in triplicate.

Clostridium saccharoperbutylacetonicum (DSMZ 14923)

Medium preparation

For the cultivation of *Clostridium saccharoperbutylacetonicum* (DSMZ 14923) was used a medium, which contains 0.3 g/l magnesium sulfate heptahydrate, 2 g/l yeast extract, 6 g/l peptone from casein, 3 g/l ammonium acetate, 1.5 g/l potassium dihydrogen phosphate, 1.2 g/l dipotassium hydrogen phosphate, 0.01 mg/l iron(II) sulfate heptahydrate, 0.5 g/l L-cysteine. The pH value of the solution was adjusted to 7. The medium was autoclaved for 10 min at 120 °C. Glucose (20 g/l) was used as a carbon source. The concentrated sterile glucose solution was added to the medium before the inoculation of microorganisms.

Cultivation

The work was carried out in a glovebox under forming gas atmosphere. 1 ml cryo stock (-80 °C) of cells in glycerol was thawed at room temperature and add-

ed to 9 ml medium. The cells were incubated with agitation for 24 h at 30 °C. To preserve vital cells culture was re-inoculated in a medium once again and incubated for 18 h. These cells were used for inhibitor experiments.

Inhibitor screening and tests of synergistic effects

The inhibitor experiments and experiments to study synergistic effects were carried out in microwell plates under forming gas atmosphere. 50 µl medium with 20 g/l glucose and with/without an aliquot of the respective inhibiting substance were pipetted into each well, whereby the organic acids were neutralized before addition to the medium. Thereafter, 30 µl culture was added to the solutions. The microtitre plate was sealed with a transparent oxygen-impermeable adhesive film. The cells were incubated for 10 h at 30 °C. The OD measurements were carried out every hour. Measurements were performed in triplicate.

OD measurements

For the reading of the optical density of microorganisms, the bacterial suspension was measured in a 96-well microtiter plate in Thermo Scientific™ Multiskan™ GO Mikrotiterplatten-Spectrophotometer at 600 nm. As a light source, the Xenon flash lamp was used. The microtiter plate was shaken for 5 s before the measurement.

Results and discussion

Inhibition effects of aliphatic acids, alcohols and furan aldehydes

Formic acid did not cause a significant inhibition in the concentration range of the industrial samples. Pronounced inhibitions started from about 27 mmol/l and caused complete inhibition of growth only at very high concentrations (380 mmol/l, *Escherichia coli* LY01). Acetic acid was responsible for some inhibition of the upper limit of the industrial samples (about 150 mmol/l) to complete inhibition at high concentrations. *Candida shehateae* ATCC 22984 proved to be a little more resistant to acetic acid than the other microorganisms (see SI Table S1).

Methanol is rather stimulating than inhibiting for the investigated concentrations for *Thermoanaerobacter mathranii* and *Clostridium saccharoperbutylacetonicum*. Also, the growth of *Halomonas halophila* was not inhibited in this concentration range. Ethanol is harmless up to 17 mmol/l. When investigating 10 to 100 times that amount, *Thermoanaerobacter mathranii* performs poorly compared to *Escherichia coli* LY01, which stops growing only above 1000 mmol/l (see SI Table S2).

In the case of furan aldehydes, the amount of inhibition strongly depends on the microorganisms in the range between 6 mmol/l and 30 mmol/l for furfural and between 8 mmol/l and 30 mmol/l for hydroxymethylfurfural. Both aldehydes seem to have a stimulating effect on the growth at rather low concentrations. Growth is strongly inhibited above 30 mmol/l (see SI Table S3).

Inhibition effects of phenolic compounds

For inhibitor tests, the substances were selected based on the variability of the substitution pattern on the aromatic ring and in the sidechain of the lignin monomers (phenylpropane units). The compounds (cf. SI Table S4) contain variations in the ortho-positions of the phenolic OH groups and/or in the sidechain as illustrated in Figure 1.

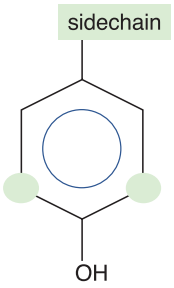


Figure 1: *Structural variations (green) of the phenylpropane units of lignins in Nature and our test programme.*

The following two tables exemplify how the substitution pattern of the aromatic ring and/ or the structure of the side chain influence the inhibitory potency of the compounds. The concentration ranges between 3 and 7mmol/l. The measure is the growth of the microorganisms. The data are extracted from SI Table S5. The colour code is as above (Table 3).

Phenols with up to three hydroxyl groups on the aromatic ring are mostly harmless sometimes even promoting growth in some cases (Table 4). Only *Escherichia coli* LY01 shows restricted growth at 6.4 mmol/l. Also, the orientation of the OH-groups at the ring does not seem to contribute to any significant differences in growth.

Phenols with an additional methoxyl-moiety at the aromatic ring are also mostly harmless and sometimes even promote growth. Phenols with two methoxyl-

Table 4: *Examples of harmless compounds – a variable number of OH-groups at the aromatic ring, no sidechain.*

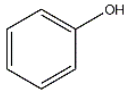
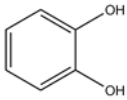
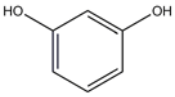
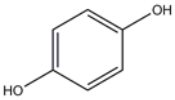
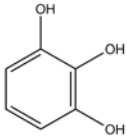
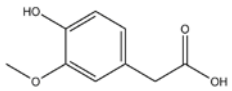
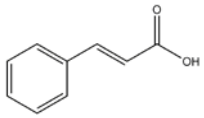
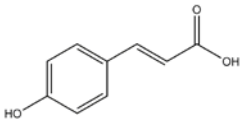
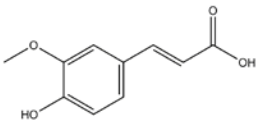
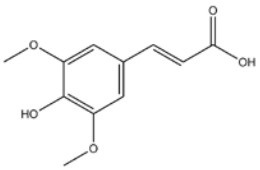
possible inhibitors	concen-tration [mmol/l]	growth [%]	microorganism/strain
phenol 	3.00	94	*Thermoanaerobacter mathranii* DSM 11426
	3.00	88	*Clostridium saccharoperbutylacetonicum* DSM 14923
	3.00	83	*Halomonas halophila* DSMZ 4770
	7.00	122	*Thermoanaerobacter mathranii* DSM 11426
	7.00	93	*Clostridium saccharoperbutylacetonicum* DSM 14923
	7.00	66	*Halomonas halophila* DSMZ 4770
	10.63	118	*Thermoanaerobacter mathranii* DSM 11426
catechol 	3.00	122	*Thermoanaerobacter mathranii* DSM 11426
	3.00	93	*Clostridium saccharoperbutylacetonicum* DSM 14923
	3.00	139	*Halomonas halophila* DSMZ 4770
	3.18	75	*Escherichia coli* LY01
	4.00	108	*Thermoanaerobacter mathranii* DSM 11426
	6.36	50	*Escherichia coli* LY01
	7.00	112	*Thermoanaerobacter mathranii* DSM 11426
	7.00	68	*Clostridium saccharoperbutylacetonicum* DSM 14923
	7.00	92	*Halomonas halophila* DSMZ 4770
	9.00	105	*Saccharomyces cerevisiae* Baker's yeast
	9.08	106	*Thermoanaerobacter mathranii* DSM 11426
resorcinol 	3.00	105	*Thermoanaerobacter mathranii* DSM 11426
	3.00	98	*Clostridium saccharoperbutylacetonicum* DSM 14923
	3.00	94	*Halomonas halophila* DSMZ 4770
	4.00	107	*Thermoanaerobacter mathranii* DSM 11426
	7.00	95	*Thermoanaerobacter mathranii* DSM 11426
	7.00	89	*Clostridium saccharoperbutylacetonicum* DSM 14923
	7.00	90	*Halomonas halophila* DSMZ 4770
hydroquinone 	3.00	122	*Thermoanaerobacter mathranii* DSM 11426
	3.00	95	*Clostridium saccharoperbutylacetonicum* DSM 14923
	3.00	141	*Halomonas halophila* DSMZ 4770
	3.50	137	*Thermoanaerobacter mathranii* DSM 11426
	4.00	121	*Thermoanaerobacter mathranii* DSM 11426
	4.54	75	*Escherichia coli* LY01
	6.36	50	*Escherichia coli* LY01
	7.00	144	*Thermoanaerobacter mathranii* DSM 11426
	7.00	80	*Clostridium saccharoperbutylacetonicum* DSM 14923
	7.00	88	*Halomonas halophila* DSMZ 4770
pyrogallol 	3.00	119	*Thermoanaerobacter mathranii* DSM 11426
	3.00	139	*Clostridium saccharoperbutylacetonicum* DSM 14923
	3.00	121	*Halomonas halophila* DSMZ 4770
	3.50	94	*Thermoanaerobacter mathranii* DSM 11426
	7.00	118	*Thermoanaerobacter mathranii* DSM 11426
	7.00	109	*Clostridium saccharoperbutylacetonicum* DSM 14923
	7.00	68	*Halomonas halophila* DSMZ 4770

Table 5: *Examples of harmful compounds - variations in the aromatic rings with long-chain carboxylic acids.*

possible inhibitors	concen-tration [mmol/l]	growth [%]	microorganism/strain
homovanillic acid 	2.42	106	*Thermoanaerobacter mathranii* DSM 11426
	3.00	108	*Thermoanaerobacter mathranii* DSM 11426
	3.00	108	*Clostridium saccharoperbutylacetonicum* DSM 14923
	3.00	90	*Halomonas halophila* DSMZ 4770
	5.49	70	*Thermoanaerobacter mathranii* DSM 11426
	7.00	61	*Thermoanaerobacter mathranii* DSM 11426
	7.00	93	*Clostridium saccharoperbutylacetonicum* DSM 14923
	7.00	91	*Halomonas halophila* DSMZ 4770
trans-cinnamic acid 	3.00	55	*Thermoanaerobacter mathranii* DSM 11426
	3.00	47	*Clostridium saccharoperbutylacetonicum* DSM 14923
	6.75	1	*Saccharomyces cerevisiae* Baker's yeast
	7.00	36	*Thermoanaerobacter mathranii* DSM 11426
	7.00	36	*Clostridium saccharoperbutylacetonicum* DSM 14923
p-coumaric acid 	3.00	50	*Thermoanaerobacter mathranii* DSM 11426
	3.00	37	*Clostridium saccharoperbutylacetonicum* DSM 14923
	6.09	63	*Saccharomyces cerevisiae* Baker's yeast
	7.00	34	*Thermoanaerobacter mathranii* DSM 11426
	7.00	18	*Clostridium saccharoperbutylacetonicum* DSM 14923
ferulic acid 	3.00	38	*Thermoanaerobacter mathranii* DSM 11426
	3.00	54	*Clostridium saccharoperbutylacetonicum* DSM 14923
	3.00	85	*Halomonas halophila* DSMZ 4770
	3.60	50	*Escherichia coli* LY01
	5.15	37	*Thermoanaerobacter mathranii* DSM 11426
	6.00	39	*Saccharomyces cerevisiae* Baker's yeast
	7.00	31	*Thermoanaerobacter mathranii* DSM 11426
	7.00	31	*Clostridium saccharoperbutylacetonicum* DSM 14923
	7.00	61	*Halomonas halophila* DSMZ 4770
sinapic acid 	3.00	31	*Thermoanaerobacter mathranii* DSM 11426
	3.00	29	*Clostridium saccharoperbutylacetonicum* DSM 14923
	7.00	12	*Thermoanaerobacter mathranii* DSM 11426
	7.00	9	*Clostridium saccharoperbutylacetonicum* DSM 14923

moieties at the aromatic ring seem to exercise a slightly higher inhibition than their counterpart with one methoxyl group. For example, syringaldehyde (two methoxyl-moieties) shows a growth-inhibiting effect at lower concentrations than vanillin (one methoxyl-moiety).

Phenols with an additional carboxyl-moiety at the aromatic ring are also mostly harmless and sometimes even promote growth. We could show this for p-hydroxybenzoic acid, gallic acid and also for the corresponding acids of vanillin and syringaldehyde namely vanillic acid and syringic acid.

The inhibitory effect is significant for aromatics with a propenyl carboxylic acid side chain (Table 5). Also, the substitution pattern of the aromatic ring seems to have an influence: trans-cinnamic acid (concentration range 1 mmol/l-7 mmol/l) exercises less inhibition than p-coumaric acid (strong inhibition at 7 mmol/l), which is comparable with ferulic acid. The most inhibitory acid is sinapic acid (concentration range 3 mmol/l-7 mmol/l). *Saccharomyces cerevisiae* Baker's yeast seems to differ in behaviour by showing the most inhibition when grown on trans-cinnamic acid (6.8 mmol/l, 1% growth) followed by ferulic acid (6 mmol/l, 39% growth) and showing the lowest inhibi-

tion when grown on p-coumaric acid (6 mmol/l, 63% growth) [24].

The comparison of the inhibitory potency of aromatic compounds with long-chain carboxylic acids with their aldehyde counterparts shows similar results as of aromatic compounds with short-chain carboxylic acids (cinnamaldehyde and trans-cinnamic acid, coniferyl aldehyde and ferulic acid). Aldehydes inhibit the growth of microorganisms stronger than their corresponding acids in a comparable concentration range.

In conclusion, the strongest inhibitors possess a propenyl side chain. Aldehydes are more inhibiting than their corresponding acids. *Clostridium saccharoperbutylacetonicum* proved to be to a certain extent more susceptible to inhibition than *Thermoanaerobacter mathranii* and *Halomonas halophila*.

Is the inhibition synergistic or additive?

In addition to the screening of single substances combinations of substances were screened for synergistic effects using the microtitre plate setup. *Clostridium saccharoperbutylacetonicum* was used for the tests because it is more susceptible to inhibition than *Thermoanaerobacter mathranii* and *Halomonas halophila* as stated above. Two scenarios were tested:

1) combinations of one "harmless" compound with one inhibiting compound, e.g. pyrogallol plus coniferyl aldehyde and phenol plus coniferyl aldehyde (Figure 2)
2) the combination of two "harmless" compounds, e.g furfural and 5-hydroxymethylfurfural HMF (Figure 3)

The parameter to measure the extent of inhibition is growth change compared to a control culture without any (potential) inhibitors. For the dilutions, a glucose-containing medium was used to ensure that all samples contained the same concentration of glucose to rule out any effects due to substrate limitation.

Our experiments show that within the investigated concentration ranges and for the investigated compounds additive effects occur, but no synergistic effects can be deduced from the data.

Conclusions

In summary, the anaerobic strains *Thermoanaerobacter mathranii* and *Clostridium saccharoperbutylacetonicum* and aerobic strain *Halomonas halophila* behave very similarly to the addition of potential inhibitors from the spent sulphite liquor. *Clostridium saccharoperbutylacetonicum* proved to be to a certain extent more susceptible to inhibition than *Thermoanaerobacter mathranii* and *Halomonas halophila*. Formic acid did not cause a significant inhibition in the concentration range of the industrial samples. Acetic acid was responsible for some inhibition at the upper concentration range of the industrial samples. Methanol is rather stimulating than inhibiting for the inves-

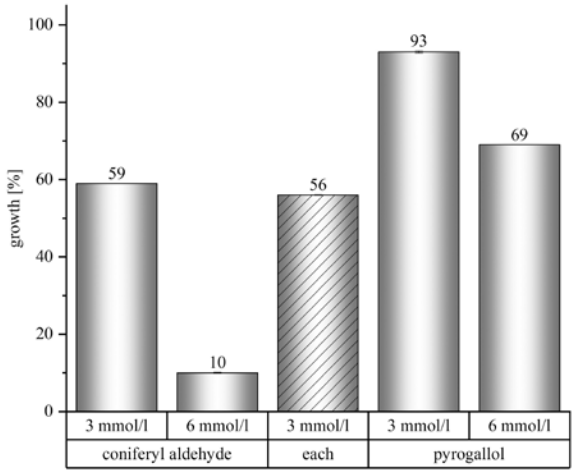


Figure 2: *Scenario 1 – growth of Clostridium saccharoperbutylacetonicum after addition of a harmless, harmful phenolic compound (pyrogallol and coniferyl aldehyde) and their mixture. The effect is additive, not synergistic.*

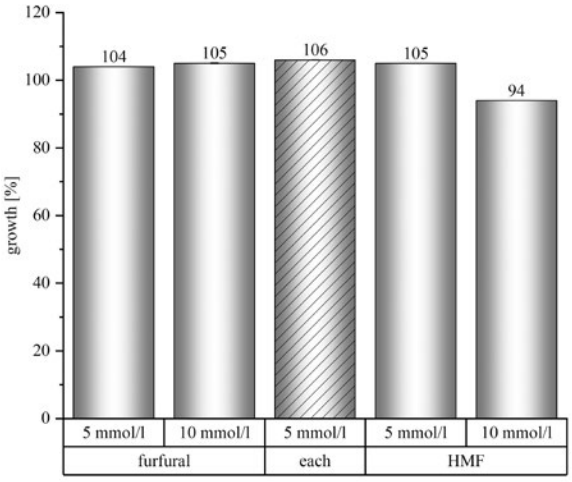


Figure 3: *Scenario 2 – growth of Clostridium saccharoperbutylacetonicum after the addition of two harmless compounds and their mixture: furfural and HMF. Two harmless compounds combined yield no inhibitory effect at all (see also SI Figure S1)*

tigated concentrations for *Thermoanaerobacter mathranii* and *Clostridium saccharoperbutylacetonicum*. Ethanol is also harmless in the concentration of interest. The experiments with phenolic components have shown that phenols with aldehyde groups are more inhibiting than their corresponding acids. The strongest inhibitors possess a propenyl side chain. These findings agree with the literature data.

The effect of the combination of different potential inhibitors from the spent sulphite liquor on the growth of *Clostridium saccharoperbutylacetonicum* was also investigated. Additive effects were observed, but no synergistic effects could be detected.

Acknowledgement

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Appendix

Supporting Information

Colour code for growth ranges of microorganisms

growth [%]	>100	67-100	33-66	0-33
	promoting growth	non-toxic	moderately toxic	toxic

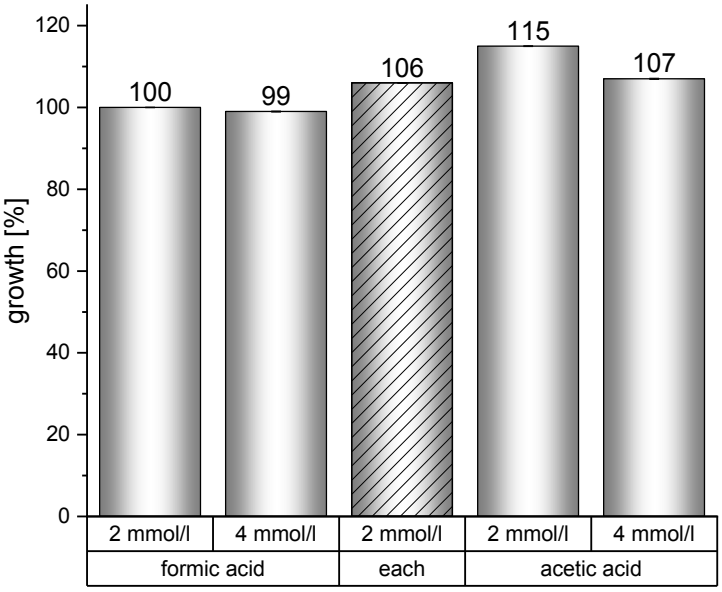


Figure S1: *Scenario 2 – growth of Clostridium saccharoperbutylacetonicum after the addition of two other harmless compounds and their mixture formic acid and acetic acid. Two harmless compounds combined yield no inhibitory effect at all.*

Table S1: *Inhibitory effects of aliphatic acids.*

substance	conc. [mmol/l]	growth [%]	microorganism/strain	reference
formic acid	0.65	97	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	2.06	98	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	3.41	95	Thermoanaerobacter mathranii DSM 11426	this paper
	3.48	91	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	3.48	89	Halomonas halophila DSMZ 4770	this paper
	6.95	77	Thermoanaerobacter mathranii DSM 11426	this paper
	6.95	81	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	6.95	95	Halomonas halophila DSMZ 4770	this paper
	24.99	75	Escherichia coli LY01	Zaldivar 1999(b)
	27.24	44	Thermoanaerobacter mathranii DSM 11426	this paper
	27.24	56	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	27.16	96	Halomonas halophila DSMZ 4770	this paper
	54.32	50	Escherichia coli LY01	Zaldivar 1999(b)
	217.27	20	Escherichia coli LY01	Zaldivar 1999(b)
	380.22	0	Escherichia coli LY01	Zaldivar 1999(b)
acetic acid	74.94	106	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	83.26	75	Escherichia coli LY01	Zaldivar 1999(b)
	83.26	96	Candida shehateae ATCC 22984	Delgenes 1996
	83.26	63	Pichia stipitis NRRL Y-7124	Delgenes 1996
	83.26	79	Saccharomyces cerevisiae CBS 1200	Delgenes 1996
	83.26	76	Zymomonas mobilis ATCC 10988	Delgenes 1996
	88.76	96	Thermoanaerobacter mathranii DSM 11426	this paper
	88.76	109	Halomonas halophila DSMZ 4770	this paper
	109.41	97	Thermoanaerobacter mathranii DSM 11426	this paper
	109.41	113	Halomonas halophila DSMZ 4770	this paper
	112.41	101	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	128.39	93	Thermoanaerobacter mathranii DSM 11426	this paper
	128.39	117	Halomonas halophila DSMZ 4770	this paper
	149.68	64	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	149.88	50	Escherichia coli LY01	Zaldivar 1999(b)
	166.53	84	Candida shehateae ATCC 22984	Delgenes 1996
	166.53	63	Pichia stipitis NRRL Y-7124	Delgenes 1996
	166.53	52	Saccharomyces cerevisiae CBS 1200	Delgenes 1996
	166.53	44	Zymomonas mobilis ATCC 10988	Delgenes 1996
	166.53	61	Thermoanaerobacter mathranii DSM 11426	this paper
	249.79	79	Candida shehateae ATCC 22984	Delgenes 1996
	249.79	64	Pichia stipitis NRRL Y-7124	Delgenes 1996
	249.79	56	Saccharomyces cerevisiae CBS 1200	Delgenes 1996
	249.79	26	Zymomonas mobilis ATCC 10988	Delgenes 1996
	238.10	20	Escherichia coli LY01	Zaldivar 1999(b)
	416.32	0	Escherichia coli LY01	Zaldivar 1999(b)

Table S2: *Inhibitory effects of alcohols.*

possible inhibitors	Conc. [mmol/l]	growth [%]	microorganism/strain	reference
methanol	4.68	122	Thermoanaerobacter mathranii DSM 11426	this paper
	4.68	106	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	4.68	91	Halomonas halophila DSMZ 4770	this paper
	11.70	107	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	18.73	109	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	46.81	127	Thermoanaerobacter mathranii DSM 11426	this paper
	46.81	94	Halomonas halophila DSMZ 4770	this paper
ethanol	0.22	107	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	1.19	110	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	1.72	100	Thermoanaerobacter mathranii DSM 11426	this paper
	1.72	88	Halomonas halophila DSMZ 4770	this paper
	2.17	106	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	17.15	88	Thermoanaerobacter mathranii DSM 11426	this paper
	17.15	84	Halomonas halophila DSMZ 4770	this paper
	171.50	19	Thermoanaerobacter mathranii DSM 11426	this paper
	260.48	75	Escherichia coli LY01	Zaldivar 1999(a)
	426.75	11	Thermoanaerobacter mathranii DSM 11426	this paper
	434.14	75	Escherichia coli LY01	Zaldivar 1999(b)
	499.26	50	Escherichia coli LY01	Zaldivar 1999(a)
	651.21	50	Escherichia coli LY01	Zaldivar 1999(b)
	933.39	20	Escherichia coli LY01	Zaldivar 1999(b)
	1193.88	0	Escherichia coli LY01	Zaldivar 1999(b)
	1302.41	0	Escherichia coli LY01	Zaldivar 1999(b)

Table S3: *Inhibitory effects of furan aldehydes.*

possible inhibitors	conc. [mmol/l]	growth [%]	microorganism/strain	reference
furfural	0.03	115	Thermoanaerobacter mathranii DSM 11426	this paper
	0.03	99	Halomonas halophila DSMZ 4770	this paper
	0.21	116	Thermoanaerobacter mathranii DSM 11426	this paper
	0.21	95	Halomonas halophila DSMZ 4770	this paper
	0.36	121	Thermoanaerobacter mathranii DSM 11426	this paper
	0.36	106	Halomonas halophila DSMZ 4770	this paper
	0.94	124	Thermoanaerobacter mathranii DSM 11426	this paper
	0.94	103	Halomonas halophila DSMZ 4770	this paper
	1.04	115	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	3.12	98	Thermoanaerobacter mathranii DSM 11426	this paper
	3.12	114	Halomonas halophila DSMZ 4770	this paper
	3.64	114	Halomonas halophila DSMZ 4770	this paper
	4.16	114	Halomonas halophila DSMZ 4770	this paper
	5.20	81	Candida shehateae ATCC 22984	Delgenes 1996
	5.20	75	Pichia stipitis NRRL Y-7124	Delgenes 1996
	5.20	53	Saccharomyces cerevisiae CBS 1200	Delgenes 1996
	5.20	82	Zymomonas mobilis ATCC 10988	Delgenes 1996
	5.72	118	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	10.41	62	Candida shehateae ATCC 22984	Delgenes 1996
	10.41	53	Pichia stipitis NRRL Y-7124	Delgenes 1996
	10.41	19	Saccharomyces cerevisiae CBS 1200	Delgenes 1996
	10.41	81	Zymomonas mobilis ATCC 10988	Delgenes 1996
	10.41	123	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	15.61	5	Thermoanaerobacter mathranii DSM 11426	this paper
	20.82	10	Candida shehateae ATCC 22984	Delgenes 1996
	20.82	1	Pichia stipitis NRRL Y-7124	Delgenes 1996
	20.82	10	Saccharomyces cerevisiae CBS 1200	Delgenes 1996
	20.82	44	Zymomonas mobilis ATCC 10988	Delgenes 1996
	20.82	75	Escherichia coli LY01	Zaldivar 1999(b)
	23.94	75	Escherichia coli LY01	Zaldivar 1999(a)
	24.98	50	Escherichia coli LY01	Zaldivar 1999(b)
	30.18	50	Escherichia coli LY01	Zaldivar 1999(a)
	36.43	0	Escherichia coli LY01	Zaldivar 1999(b)
	38.51	0	Escherichia coli LY01	Zaldivar 1999(b)
5-hydroxy-methyl-furfural	0.31	116	Thermoanaerobacter mathranii DSM 11426	this paper
	0.31	96	Halomonas halophila DSMZ 4770	this paper
	0.40	108	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	0.51	117	Thermoanaerobacter mathranii DSM 11426	this paper
	0.51	89	Halomonas halophila DSMZ 4770	this paper
	1.19	93	Halomonas halophila DSMZ 4770	this paper
	1.49	126	Thermoanaerobacter mathranii DSM 11426	this paper
	1.49	92	Halomonas halophila DSMZ 4770	this paper
	2.78	108	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	3.96	100	Halomonas halophila DSMZ 4770	this paper
	5.15	105	Clostridium saccharoperbutylacetonicum DSM 14923	this paper

possible inhibitors	conc. [mmol/l]	growth [%]	microorganism/strain	reference
	7.93	92	Candida shehateae ATCC 22984	Delgenes 1996
	7.93	95	Pichia stipitis NRRL Y-7124	Delgenes 1996
	7.93	35	Saccharomyces cerevisiae CBS 1200	Delgenes 1996
	7.93	51	Zymomonas mobilis ATCC 10988	Delgenes 1996
	12.69	104	Halomonas halophila DSMZ 4770	this paper
	18.24	75	Escherichia coli LY01	Zaldivar 1999(b)
	21.41	50	Escherichia coli LY01	Zaldivar 1999(b)
	23.79	75	Escherichia coli LY01	Zaldivar 1999(a)
	23.79	32	Candida shehateae ATCC 22984	Delgenes 1996
	23.79	31	Pichia stipitis NRRL Y-7124	Delgenes 1996
	23.79	17	Saccharomyces cerevisiae CBS 1200	Delgenes 1996
	23.79	69	Zymomonas mobilis ATCC 10988	Delgenes 1996
	30.13	50	Escherichia coli LY01	Zaldivar 1999(a)
	31.72	0	Escherichia coli LY01	Zaldivar 1999(b)
	35.68	0	Escherichia coli LY01	Zaldivar 1999(a)
	39.65	8	Candida shehateae ATCC 22984	Delgenes 1996
	39.65	1	Pichia stipitis NRRL Y-7124	Delgenes 1996
	39.65	11	Saccharomyces cerevisiae CBS 1200	Delgenes 1996
	39.65	33	Zymomonas mobilis ATCC 10988	Delgenes 1996

Table S4: *Structures of the lignin-derived substances tested as growth inhibitors.*

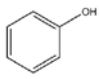
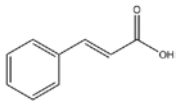
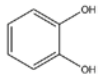
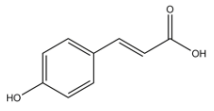
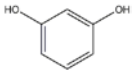
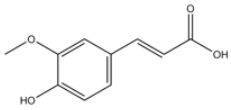
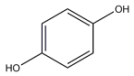
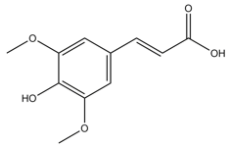
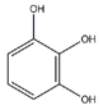
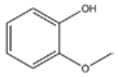
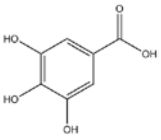
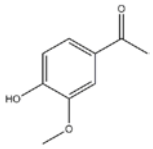
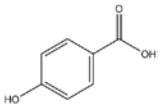
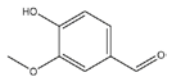
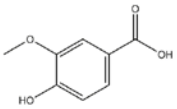
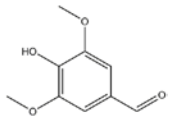
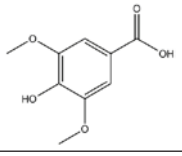
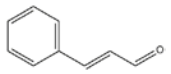
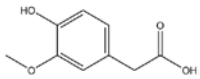
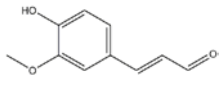
Phenol		trans-Cinnamic acid	
Catechol		p-Coumaric acid	
Resorcinol		Ferulic acid	
Hydroquinone		Sinapic acid	
Pyrogallol		Guaiacol	
Gallic acid		Apocynin	
p-Hydroxybenzoic acid		Vanillin	
Vanillic acid		Syringaldehyde	
Syringic acid		Cinnamaldehyde	
Homovanillic acid		Coniferylaldehyde	

Table S5: *Structures of the lignin-derived substances tested as growth inhibitors.*

possible inhibitors	concen-tration [mmol/l]	growth [%]	microorganism/strain	reference
phenol	3.00	94	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	88	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	3.00	83	Halomonas halophila DSMZ 4770	this paper
	7.00	122	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	93	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	7.00	66	Halomonas halophila DSMZ 4770	this paper
	10.63	118	Thermoanaerobacter mathranii DSM 11426	this paper
catechol	3.00	122	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	93	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	3.00	139	Halomonas halophila DSMZ 4770	this paper
	3.18	75	Escherichia coli LY01	Zaldivar 2000
	4.00	108	Thermoanaerobacter mathranii DSM 11426	this paper
	6.36	50	Escherichia coli LY01	Zaldivar 2000
	7.00	112	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	68	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	7.00	92	Halomonas halophila DSMZ 4770	this paper
	9.00	105	Saccharomyces cerevisiae Baker's yeast	Larsson 2000
	9.08	106	Thermoanaerobacter mathranii DSM 11426	this paper
	27.25	0	Escherichia coli LY01	Zaldivar 2000
resorcinol	3.00	105	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	98	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	3.00	94	Halomonas halophila DSMZ 4770	this paper
	4.00	107	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	95	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	89	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	7.00	90	Halomonas halophila DSMZ 4770	this paper
	9.08	104	Thermoanaerobacter mathranii DSM 11426	this paper
hydroqui-none	3.00	122	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	95	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	3.00	141	Halomonas halophila DSMZ 4770	this paper
	3.50	137	Thermoanaerobacter mathranii DSM 11426	this paper
	4.00	121	Thermoanaerobacter mathranii DSM 11426	this paper
	4.54	75	Escherichia coli LY01	Zaldivar 2000
	6.36	50	Escherichia coli LY01	Zaldivar 2000
	7.00	144	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	80	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	7.00	88	Halomonas halophila DSMZ 4770	this paper
	9.00	105	Saccharomyces cerevisiae Baker's yeast	Larsson 2000
	9.08	117	Thermoanaerobacter mathranii DSM 11426	this paper
	27.25	0	Escherichia coli LY01	Zaldivar 2000
pyrogallol	3.00	119	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	139	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	3.00	121	Halomonas halophila DSMZ 4770	this paper
	3.50	94	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	118	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	109	Clostridium saccharoperbutylacetonicum DSM 14923	this paper

possible inhibitors	concen-tration [mmol/l]	growth [%]	microorganism/strain	reference
	7.00	68	Halomonas halophila DSMZ 4770	this paper
gallic acid	3.00	127	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	91	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	3.00	86	Halomonas halophila DSMZ 4770	this paper
	3.50	122	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	91	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	76	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	7.00	58	Halomonas halophila DSMZ 4770	this paper
	13.52	75	Escherichia coli LY01	Zaldivar 1999(b)
	29.39	50	Escherichia coli LY01	Zaldivar 1999(b)
	146.96	20	Escherichia coli LY01	Zaldivar 1999(b)
	235.13	0	Escherichia coli LY01	Zaldivar 1999(b)
guaiacol	2.82	75	Escherichia coli LY01	Zaldivar 2000
	3.00	116	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	117	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	3.00	78	Halomonas halophila DSMZ 4770	this paper
	3.50	112	Thermoanaerobacter mathranii DSM 11426	this paper
	4.83	50	Escherichia coli LY01	Zaldivar 2000
	7.00	95	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	94	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	7.00	55	Halomonas halophila DSMZ 4770	this paper
	24.17	0	Escherichia coli LY01	Zaldivar 2000
vanillin	2.63	75	Escherichia coli LY01	Zaldivar 1999(a)
	2.89	87	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	101	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	72	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	3.00	91	Halomonas halophila DSMZ 4770	this paper
	3.29	50	Escherichia coli LY01	Zaldivar 1999(a)
	3.29	67	Candida shehateae ATCC 22984	Delgenes 1996
	3.29	12	Pichia stipitis NRRL Y-7124	Delgenes 1996
	3.29	49	Saccharomyces cerevisiae CBS 1200	Delgenes 1996
	3.29	62	Zymomonas mobilis ATCC 10988	Delgenes 1996
	5.92	50	Escherichia coli LY01	Zaldivar 1999(a)
	6.57	<100	Saccharomyces cerevisiae Baker's yeast	Larsson 2000
	6.57	9	Candida shehateae ATCC 22984	Delgenes 1996
	6.57	1	Pichia stipitis NRRL Y-7124	Delgenes 1996
	6.57	14	Saccharomyces cerevisiae CBS 1200	Delgenes. 1996
	6.57	37	Zymomonas mobilis ATCC 10988	Delgenes 1996
	6.57	70	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	66	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	45	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	7.00	55	Halomonas halophila DSMZ 4770	this paper
	9.86	0	Escherichia coli LY01	Zaldivar 1999(a)
	10.00	14	Thermoanaerobacter mathranii A3M4	Klinke 2001
	13.14	2	Candida shehateae ATCC 22984	Delgenes 1996
	13.14	1	Pichia stipitis NRRL Y-7124	Delgenes 1996
	13.14	9	Saccharomyces cerevisiae CBS 1200	Delgenes 1996
	13.14	12	Zymomonas mobilis ATCC 10988	Delgenes 1996

possible inhibitors	concen-tration [mmol/l]	growth [%]	microorganism/strain	reference
syring-aldehyde	1.03	89	Candida shehateae ATCC 22984	Delgenes 1996
	1.03	72	Pichia stipitis NRRL Y-7124	Delgenes 1996
	1.03	100	Saccharomyces cerevisiae CBS 1200	Delgenes 1996
	1.03	82	Zymomonas mobilis ATCC 10988	Delgenes 1996
	1.65	75	Escherichia coli LY01	Zaldivar 1999(a)
	2.74	38	Klebsiella pneumoniae	Nishikawa 1988
	3.00	96	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	57	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	3.00	74	Halomonas halophila DSMZ 4770	this paper
	3.29	50	Escherichia coli LY01	Zaldivar 1999(a)
	3.86	45	Candida shehateae ATCC 22984	Delgenes 1996
	3.86	38	Pichia stipitis NRRL Y-7124	Delgenes 1996
	3.86	39	Saccharomyces cerevisiae CBS 1200	Delgenes 1996
	3.86	72	Zymomonas mobilis ATCC 10988	Delgenes 1996
	5.49	94	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	55	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	28	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	7.00	61	Halomonas halophila DSMZ 4770	this paper
	7.72	5	Candida shehateae ATCC 22984	Delgenes 1996
	7.72	4	Pichia stipitis NRRL Y-7124	Delgenes 1996
	7.72	19	Saccharomyces cerevisiae CBS 1200	Delgenes 1996
	7.72	60	Zymomonas mobilis ATCC 10988	Delgenes 1996
	10.00	26	Thermoanaerobacter mathranii A3M4	Klinke 2001
	13.72	0	Escherichia coli LY01	Zaldivar 1999(a)
p-hydroxy-benzoic acid	2.90	75	Escherichia coli LY01	Zaldivar 1999(b)
	2.90	74	Klebsiella pneumoniae	Nishikawa 1988
	3.00	104	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	91	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	3.00	101	Halomonas halophila DSMZ 4770	this paper
	3.19	95	Thermoanaerobacter mathranii DSM 11426	this paper
	5.79	50	Escherichia coli LY01	Zaldivar 1999(b)
	7.00	88	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	93	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	7.00	97	Halomonas halophila DSMZ 4770	this paper
	10.00	75	Thermoanaerobacter mathranii A3M4	Klinke 2001
	18.10	20	Escherichia coli LY01	Zaldivar 1999(b)
	108.60	0	Escherichia coli LY01	Zaldivar 1999/(b)
vanillic acid	2.62	100	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	99	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	70	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	3.00	92	Halomonas halophila DSMZ 4770	this paper
	3.27	75	Escherichia coli LY01	Zaldivar 1999(b)
	3.57	64	Klebsiella pneumoniae	Nishikawa 1988
	6.84	50	Escherichia coli LY01	Zaldivar 1999(b)
	7.00	89	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	45	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	7.00	83	Halomonas halophila DSMZ 4770	this paper
	10.00	85	Thermoanaerobacter mathranii A3M4	Klinke 2001

possible inhibitors	concen-tration [mmol/l]	growth [%]	microorganism/strain	reference
	23.79	20	Escherichia coli LY01	Zaldivar 1999(b)
	89.21	0	Escherichia coli LY01	Zaldivar 1999(b)
apocynin	2.65	107	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	64	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	3.00	66	Halomonas halophila DSMZ 4770	this paper
	3.50	108	Thermoanaerobacter mathranii DSM 11426	this paper
	6.02	97	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	110	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	42	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	7.00	54	Halomonas halophila DSMZ 4770	this paper
	10.00	52	Thermoanaerobacter mathranii A3M4	Klinke 2001
homo-vanillic acid	2.42	106	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	108	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	108	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	3.00	90	Halomonas halophila DSMZ 4770	this paper
	5.49	70	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	61	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	93	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	7.00	91	Halomonas halophila DSMZ 4770	this paper
syringic acid	2.22	90	Thermoanaerobacter mathranii DSM 11426	this paper
	2.52	80	Klebsiella pneumoniae	Nishikawa 1988
	3.00	110	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	88	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	3.00	91	Halomonas halophila DSMZ 4770	this paper
	3.53	75	Escherichia coli LY01	Zaldivar 1999(b)
	5.05	79	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	72	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	79	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	7.00	96	Halomonas halophila DSMZ 4770	this paper
	8.07	50	Escherichia coli LY01	Zaldivar 1999(b)
	10.00	91	Thermoanaerobacter mathranii A3M4	Klinke 2001
	25.23	20	Escherichia coli LY01	Zaldivar 1999(b)
	88.30	0	Escherichia coli LY01	Zaldivar 1999(b)
cinnam-aldehyde	3.00	51	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	44	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	7.00	27	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	21	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
coniferyl-aldehyde	1.00	92	Saccharomyces cerevisiae Baker's yeast	Larsson 2000
	1.00	100	Saccharomyces cerevisiae pL+Ss	Larsson 2001
	2.47	52	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	33	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	8	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	3.00	74	Halomonas halophila DSMZ 4770	this paper
	7.00	17	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	6	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	7.00	42	Halomonas halophila DSMZ 4770	this paper

possible inhibitors	concen-tration [mmol/l]	growth [%]	microorganism/strain	reference
trans-cinnamic acid	1.35	41	Saccharomyces cerevisiae Baker's yeast	Larsson 2000
	3.00	55	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	47	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	6.75	1	Saccharomyces cerevisiae Baker's yeast	Larsson 2000
	7.00	36	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	36	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
p-coumaric acid	3.00	50	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	37	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	6.09	63	Saccharomyces cerevisiae Baker's yeast	Larsson 2000
	7.00	34	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	18	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
ferulic acid	1.03	64	Saccharomyces cerevisiae Baker's yeast	Larsson 2000
	1.80	75	Escherichia coli LY01	Zaldivar 1999(b)
	2.27	39	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	38	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	54	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	3.00	85	Halomonas halophila DSMZ 4770	this paper
	3.60	50	Escherichia coli LY01	Zaldivar 1999(b)
	5.15	37	Thermoanaerobacter mathranii DSM 11426	this paper
	6.00	39	Saccharomyces cerevisiae Baker's yeast	Larsson 2000
	7.00	31	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	31	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	7.00	61	Halomonas halophila DSMZ 4770	this paper
	15.45	20	Escherichia coli LY01	Zaldivar 1999(b)
	77.25	0	Escherichia coli LY01	Zaldivar 1999(b)
sinapic acid	3.00	31	Thermoanaerobacter mathranii DSM 11426	this paper
	3.00	29	Clostridium saccharoperbutylacetonicum DSM 14923	this paper
	7.00	12	Thermoanaerobacter mathranii DSM 11426	this paper
	7.00	9	Clostridium saccharoperbutylacetonicum DSM 14923	this paper

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Part 2: Elucidating the Interactions of Growth Inhibitors from Spent Sulphite Liquor with Aerobic and Anaerobic Microorganisms

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Abstract

During pulping large quantities of spent liquor are generated, which contain high amounts of degraded polysaccharides. These saccharides represent a good substrate for various biotechnological processes.

In addition to the mono- and oligomeric sugars, the waste liquors contain other substances that may have inhibitory effects on the microorganisms used in the fermentation processes. This work was designed to understand the interaction of potential inhibitors with living cells by employing NMR spectroscopy.

The investigation of the interaction of the anaerobic ethanol producer *Thermoanaerobacter mathranii* and the aerobic polyhydroxyalkanoate (PHA) producer *Halomonas halophila* with potential inhibitors shows that all substances with aldehyde moieties change their structure during the fermentation, while all other inhibitors remain unchanged. Furthermore, the reduction of the aldehyde group to the hydroxyl group takes place throughout the interaction with anaerobic microorganisms and the oxidation to the carboxylic acid throughout the interaction with aerobic microorganisms.

Finally, the effect of the corresponding alcohols and carboxylic acids formed during the fermentation on the growth of bacteria was investigated. The experiments proved that the newly formed substances have a less inhibitory effect on the cells than their parent components with aldehyde groups.

Keywords: *growth inhibitors, pulp industry, spent sulphite liquor, ethanol production, PHA production, Thermoanaerobacter mathranii, Halomonas halophila*

Introduction

Sulphite pulping is one of the major commercial pulping processes. In addition to the main product pulp, large quantities of spent liquor are produced during the process. The spent sulphite liquor (SSL) contains lignosulphonates, hemicelluloses, extractives and their degradation products [1-3]. It provides a carbon source for fermentative utilization, which does not compete with the food chain [4-7]. The fermentative desugarization of the spent liquors with the anaerobic strain *T. mathranii* for the ethanol production and the halophilic aerobic strain *H. halophila* for the production of polyhydroxyalkanoates would provide a substantial contribution to the profitability of the pulping process [8-11].

In addition to the desirable high amounts of mono- and oligomeric sugars, SSL also contains degradation products of lignosulfonates and additional degradation products of hemicellulose, cellulose: organic acids, phenol and furan derivatives, which can have inhibitory effects on the microorganisms [12-15]. In our previous investigations, the effects of these substances in concentrations corresponding to the concentrations from industrial SSLs were shown for *T. mathranii* and *H. halophila* amongst others. Both strains proved to have a relatively high resistance to organic acids and furan derivates. However, some phenol derivatives especially aromatic aldehydes cause inhibition of cell growth [16]. Witz explains this effect with the high reactivity of aldehydes and their ability to form covalent bonds with cellular nucleophilic groups [17].

Non-aromatic substances, on the other hand, are less toxic to microorganisms. Water-soluble lower alcohols and organic acids are fermentation products of many microorganisms. Cells developed protective mechanisms in the evolutionary process and can tolerate a relatively high amount of these compounds [18]. However, the situation is completely different for phenol derivatives. These compounds rarely occur in high concentrations under natural conditions. This is probably the reason why even small amounts of these substances are harmful to cells [19,20].

In Nature, there are microorganisms known to degrade lignin and its degradation products. There are several studies, in which the degradation reactions of substituted phenols from lignocellulosic hydrolysates by those microorganisms were investigated. For example, Harazono *et al*. found aromatics-degrading bacterial strains in the guts of a lower termite species. These strains were identified as *Burkholderia* sp. VE22 and *Citrobacter* sp. VA53 and can metabolize degradation products of lignin such as aromatic aldehydes. During this process, the corresponding aromatic alcohols or aromatic carboxylic acids, respectively, were formed as intermediate metabolites [21]. Falconnier *et al*. described the metabolism of ferulic acid to vanillin by the white-rot fungus *Pycnoporus cinnabarinus* 1-937, whereby coniferyl aldehyde was formed as an intermediate, which was then converted to coniferyl alcohol [22]. However, the exact mechanism of the inhibiting effect of these substances on the cells is not yet elucidated [21,23-25].

To achieve a deeper understanding of the behaviour of the strains we used for SSL valorisation, ^{1}H NMR spectroscopy was employed.

For studying the interaction of the anaerobic microorganism *T. mathranii* with different potential inhibitors from the pulp industry process lyes, these substances were characterized by ^{1}H NMR spectroscopy in a medium with glucose, which was used as a carbohydrate source in fermentation experiments. Then the cells were incubated directly in an NMR tube with the potential inhibitors in a medium with glucose and finally investigated by ^{1}H NMR spectroscopy. To ensure that no conversions of the tested substances take place in the medium without bacteria, the experiments were also carried out without the addition of microorganisms. The used concentrations of furan derivatives in these experiments correspond to the concentrations of these substances in spent sulphite liquors from different industrial partners. The range of phenolic test substances and their concentration was derived from the literature [1-3,13,26].

Since the aerobic strain, *H. halophila* is sensitive to oxygen limitation the cultivation could not be performed directly in the NMR tube. Instead, the experiments were carried out in small shake flasks. The potential inhibitors were incubated in a medium with and without cells. As above, glucose was the carbohydrate source. The samples for ^{1}H NMR measuring were taken from all solutions at regular intervals.

Materials and Methods

Thermoanaerobacter mathranii (DSM 11426)

Medium preparation

For the cultivation of *T. mathranii* (DSM 11426), the DSMZ 640 medium from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures was used. The medium contains 0.9 g/l sodium chloride, 0.4 g/l magnesium chloride hexahydrate, 0.75 g/l monopotassium phosphate, 1.5 g/l dipotassium phosphate, 2 g/l peptone from casein, 1 g/l yeast extract, 1 ml/l trace elements solution SL-10, 2.5 mg iron (III) chloride hexahydrate, 0.75 g/l L-cysteine hydrochloride monohydrate, 5 g/l glucose and 1 g/l sodium 2,2-dimethyl-2-silapentane-5-sulfonate, which was used as an internal standard. Water was substituted by D_2O. The pH value of the solution was adjusted to 7.2 with 35 % DCl. The medium was autoclaved for 10 min at 120 °C.

For the preparation of the trace elements solution SL-10, 1.5 g iron(II) chloride tetrahydrate was dissolved in 10 ml 7.7 M HCl and diluted with 990 ml deionised water. The following salts were added to the solution:

70 mg zinc chloride, 100 mg manganese (II) chloride tetrahydrate, 6 mg boric acid, 190 mg calcium chloride hexahydrate, 2 mg copper (II) chloride dehydrate, 24 mg nickel (II) chloride hexahydrate, 36 mg sodium molybdate dihydrate. Finally, it was made up to 1000 ml with deionised water.

Cultivation

The work was carried out in a Glovebox under forming gas atmosphere. 1 ml Cryo-Stock (-80 °C) containing 700 µl cells in glycerine and 300 µl D_2O was thawed at room temperature and then added to 9 ml medium. The cells were incubated with agitation for 12 h at 65 °C. To preserve vital cells the culture was re-inoculated in a medium once again and incubated for 10 h. These cells were used for the inhibitor screening experiments.

Inhibitor screening

For the inhibitor screening experiments, the medium containing an aliquot of one of the potential inhibitors was prepared and analysed by ^{1}H NMR spectroscopy. The organic acids were neutralized with 40 % NaOD solution before their addition to the medium. In the next step, 500 µl culture was added to an NMR tube with a 500 µl medium containing an inhibiting substance* and a sugar source. The solution was incubated with agitation at 65 °C. At the same time, the medium without cells was incubated under the same conditions. After 72h, the samples with and without cells were analysed by ^{1}H NMR spectroscopy.

* The used concentration of phenol derivatives was 7 mmol/l, furfural 4 mmol/l, HMF 4 mmol/l, formic acid 22 mmol/l, levulinic acid 10 mmol/l and acetic acid 89 mmol/l.

Monitoring of potential inhibitors with aldehyde groups during fermentation of T. mathranii

For the determination of changes of potential inhibitors with aldehyde groups during fermentation 1 ml culture was added to 9 ml medium. The cells were incubated with agitation for 72 h at 65 °C. At the same time, the medium without cells was incubated under the same conditions. The samples for ^{1}H NMR measuring were taken from all two solutions at regular intervals.

Coniferyl aldehyde was incubated in D_2O with agitation for 72 h at 65 °C.

Effect of fermentation products of potential inhibitors with aldehyde groups on cell growth of T. mathranii

For the determination of the effects of fermentation products of aromatic aldehydes on cell growth, the incubation of cells was carried out in the same way as in experiments of monitoring aromatic aldehydes during fermentation of *T. mathranii*, except for using deionized water instead of D_2O and HCl instead DCl. Further, DSS was not used in this experiment. The cells in medium with and without aromatic aldehydes as well as their fermentation products* were incubated for 77 h. The samples for the determination of the growth curve by OD measurement were taken from all solutions at regular intervals. The determinations were carried out in triplicate.

* The concentrations of potential inhibitors with aldehyde groups as well as their fermentation products are listed in Tables 2 and 3.

Halomonas halophila (DSMZ 4770)

Medium preparation

For the cultivation of *H. halophila* (DSMZ 4770), the DSMZ 4340 medium from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures was used. The medium contains 81 g/l sodium chloride, 7 g/l magnesium chloride hexahydrate, 9.6 g/l magnesium sulfate hexahydrate, 0.477 g/l calcium chloride dehydrate, 2 g/l potassium chloride, 0.06 g/l sodium hydrogen carbonate, 0.026 g/l sodium bromide, 5 g/l peptone from casein, 10 g/l yeast extract, 1 g/l glucose and 1 g/l sodium 2,2-dimethyl-2-silapentane-5-sulfonate, which was used as an internal standard. Water was substituted by D_2O. The pH value of the solution was adjusted to 7 with 35 % DCl. The medium was autoclaved for 10 min at 120 °C.

Cultivation

1 ml Cryo-Stock (-80 °C) containing 700 µl cells in glycerine and 300 µl D_2O was thawed at room temperature and added to a 19 ml medium. The cells were incubated with agitation for 14 h at 30 °C. To preserve vital cells the culture was re-inoculated in the medium once again and incubated for 12 h. These cells were used for the inhibitor screening experiments.

Monitoring of potential inhibitors during fermentation of H. halophila

For the inhibitor screening experiments, the medium containing an aliquot of one of the potential inhibitors was prepared and analysed by ^{1}H NMR spectroscopy. The organic acids were neutralized with 40 % NaOD solution before adding to the medium. In the next step 1 ml culture was added to a 250 ml Erlenmeyer flask with a 99 ml medium containing the inhibiting substance*. The solution was incubated with agitation for 143 h at 30 °C. At the same time, a medium without cells was incubated under the same conditions. The

samples for ^{1}H NMR measuring were taken from all solutions at regular intervals.

* The used concentration of phenol derivatives is 7 mmol/l, furfural 4 mmol/l, HMF 4 mmol/l, formic acid 22 mmol/l, levulinic acid 10 mmol/l and acetic acid 89 mmol/l.

Effect of fermentation products of potential inhibitors with aldehyde groups on cell growth of H. halophila

For the determination of the effects of fermentation products of aromatic aldehydes on cell growth, the incubation of cells was carried out in the same way as in experiments of monitoring aromatic aldehydes during fermentation of *H. halophila*, except for using deionized water instead of D_2O and HCl instead DCl. Further, DSS was not used in this experiment. The cells in medium with and without aromatic aldehydes as well as their fermentation products* were incubated for 150 h. The samples for the determination of the growth curve by OD measurement were taken from all solutions at regular intervals. The determinations were carried out in triplicate.

* The used concentrations of potential inhibitors with aldehyde groups as well as their fermentation products are listed in Tables 2 and 3.

NMR spectroscopy

All NMR measurements were carried out on a 300 MHz Bruker Advance III NMR spectrometer with 5 mm probe at 25 °C. Chemical shifts are given in ppm. The ^{1}H NMR spectra were recorded with the following acquisition parameters: 8.25 s 90° pulse, 5.5 s acquisition time, 20 ppm spectral width and a relaxation delay of 10 s. 16 scans were accumulated. The spectrum was referenced to the DSS peak at 0.00 ppm. The numbering of hydrogens is according to the numbering shown in Fig. 1-3. The hydrogens of furfural, coniferyl aldehyde as well as their fermentation products have the following chemical shifts:

^{1}H NMR (D_2O, 300 MHz) of furfural: δ(ppm) H= 9.49 (1H, s, H_a), 7.91 (1H, dd, J_1=1.6 Hz, J_2=0.7 Hz, H_b), 7.57 (1H, dd, J_1=3.7 Hz, J_2=0.7 Hz, H_c), 6.75 (1H, dd, J_1=3.7 Hz, J_2=1.6 Hz, H_d).

^{1}H NMR (D_2O, 300 MHz) of furfuryl alcohol: δ(ppm) H= 7.51 (1H, dd, J_1=1.8 Hz, J_2=0.9 Hz, H_e), 6.43 (1H, dd, J_1=3.2 Hz, J_2=1.8 Hz, H_f), 6.40 (1H, dd, J_1=3.2 Hz, J_2=0.9 Hz, H_g), 4.54 (2H, s, H_h).

^{1}H NMR (D_2O, 300 MHz) of 2-furoic acid: δ(ppm) H= 7.63 (1H, dd, J_1=1.6 Hz, J_2=0.5 Hz, H_e), 7.01 (1H, dd, J_1=3.5 Hz, J_2=0.5 Hz, H_f), 6.58 (1H, dd, J_1=3.5 Hz, J_2=1.6 Hz, H_g).

^{1}H NMR (D_2O, 300 MHz) of coniferyl aldehyde: $\delta(ppm)_H$ = 9.48 (1H, d, J=8,2, H_{a1}), 7.68 (1H, d, J=15,7, H_{b1}), 7.32 (1H, d, J=1,8, H_{c1}), 7.25 (1H, dd, J_1=8.2, J_2=1,8, H_{d1}), 6.96 (1H, d, J=8.2, H_{e1}), 6.70 (1H, dd, J_1=15,7, J_2=8,2, H_{f1}), 3.90 (3H, s, H_g).

^{1}H NMR (D_2O, 300 MHz) of coniferyl alcohol: δ(ppm) H= 7.15 (1H, d, J=2.0, H_{k1}), 6.98 (1H, dd, J_1=8.2, J_2=2.0, H_{i1}), 6.89 (1H, d, J=8.2, H_{j1}), 6.57 (1H, d, J=16.0, H_{k1}), 6.30 (1H, m, H_{l1}), 4.23 (2H, dd, J_1=6.0, J_2=1.3, H_{m1}), 3.91 (3H, s, H_g).

^{1}H NMR (D_2O, 300 MHz) of coniferyl aldehyde dimer: $\delta(ppm)_H$ = 9.48 (1H, s, J=8,2, H_{a2}), 7.68 (1H, s, H_{b2}), 7.32 (1H, d, J=1.8, H_{c2}), 7.25 (1H, dd, J_1=8.2, J_2=1.8, H_{d2}), 6.96 (1H, d, J=8.2, H_{e2}), 3.90 (3H, s, H_{g2}).

Measuring optical density

The optical density of the bacterial suspensions was measured in a 96-well microtiter plate in a Thermo Scientific™ Multiskan™ GO Mikrotiterplatten-Spectrophotometer at 600 nm. As the light source, the Xenon flash lamp was used. The microtiter plate was shaken for 5 s before the measurement.

Results and discussion

Inhibitor screening T. mathranii

The investigation of the interaction of *T. mathranii* on the potential inhibitors shows that organic acids and phenolic components with carboxyl, methoxyl and hydroxyl functional groups do not change during fermentation while furan derivates and phenolic components with aldehyde groups change their structure (Table 1). These changes were closely monitored. For this purpose, the fermentations were carried out on a larger scale in 15 ml tubes and the samples for NMR analysis were taken at regular intervals. The changes in furfural and coniferyl aldehyde are described in more detail below.

Monitoring of potential inhibitors with aldehyde groups during fermentation of the anaerobic strain T. mathranii

Furfural remains unchanged in the medium before the incubation with the microorganism. The addition of *T. mathranii* leads to changes in its structure (Figure 1). The peak at 9.49 ppm corresponding to the aldehyde proton of furfural disappears. The chemical shifts of H_b at 7.91 ppm, H_c at 7.57 ppm and H_d at 6.75 ppm change. New signals arise at 7.51, 6.43, 6.40 and 4.54 ppm. These signals correspond to furfuryl alcohol

Table 1: *Interaction of T. mathranii (TM) and H. halophila (HH) on different potential inhibitors during fermentation*

potential inhibitor	structural change	
	TM	HH
organic acids:		
formic acid	N	-*
acetic acid	N	-*
levulinic acid	N	N
furan derivatives:		
furfural	**Y**	**Y**
HMF	**Y**	**Y**
phenol derivatives:		
guaiacol	N	N
phenol	N	N
catechol	N	N
resorcinol	N	N
hydroquinone	N	N
pyrogallol	N	N
homovanillic acid	N	N
ferulic acid	N	N
4-hydroxybenzoic acid	N	N
gallic acid	N	N
m-coumaric acid	N	N
apocynin	N	N
coniferyl aldehyde	**Y**	**Y**
syringaldehyde	**Y**	**Y**
vanillin	**Y**	**Y**

*These compounds were metabolized by microorganisms

formed during the fermentation. A strong signal at 4.78 ppm in both spectra is indicative of water. The peak at 5.22 ppm is the α anomeric proton and the peak at 4.63 ppm is the β anomeric proton of glucose. They disappear during fermentation indicating the complete digestion of the glucose as expected. The signals in the range from 7.42 to 7.31 ppm are from the medium.

This experiment shows that the aldehyde moiety of furfural was reduced to the alcohol moiety throughout the fermentation with *T. mathranii*, i. e. furfuryl alcohol was formed from furfural.

All other potential inhibitors with aldehyde groups displayed the same behaviour as furfural. They were reduced to the corresponding alcohols during the fermentations with *T. mathranii*. Before the addition of the bacteria to the medium, no changes in the investigated compounds were observed. Coniferyl aldehyde, however, behaved differently. In addition to the change in the medium with bacteria, there occurred an expected dimerization after dissolving the aldehyde in the medium without any cells. To test its stability, coniferyl aldehyde was also dissolved under the same conditions in pure D_2O. In D_2O, coniferyl aldehyde remained unchanged. This means that one or more media components caused the dimerization of the co-

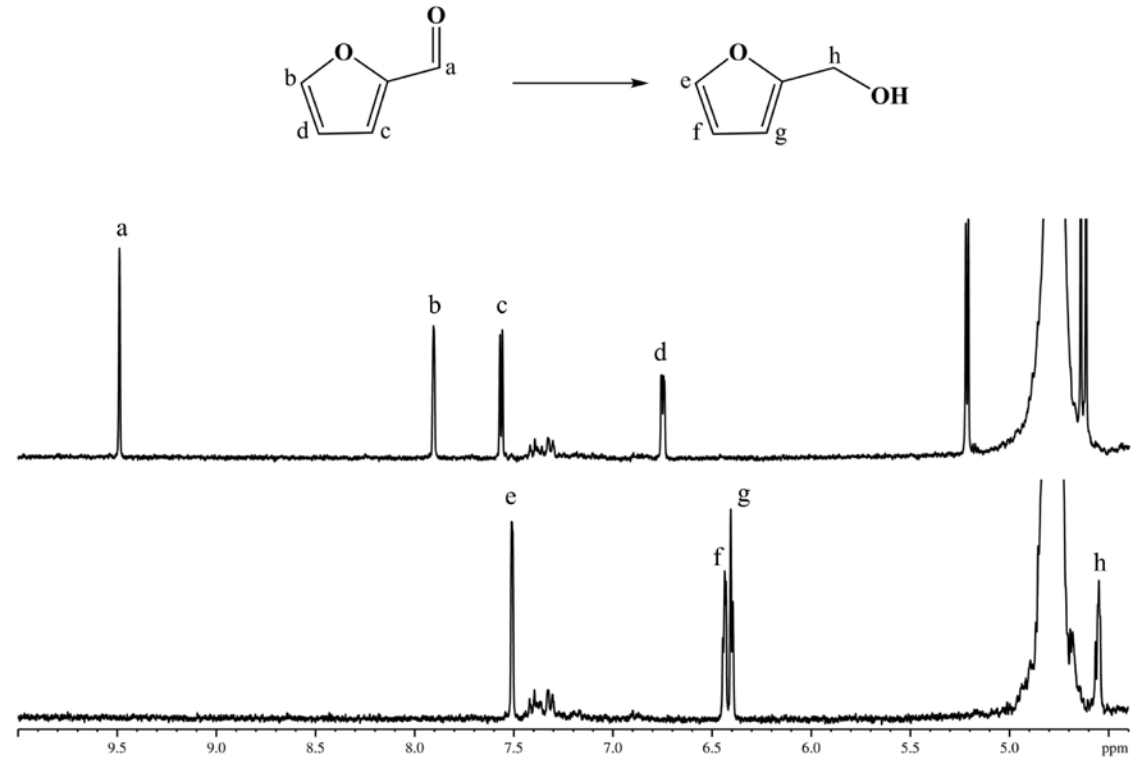

Figure 1: *^{1}H NMR spectra (D_2O, 300 MHz) of medium with furfural before the incubation with T. mathranii (top), and after fermentation for 72 h at 65 °C (bottom).*

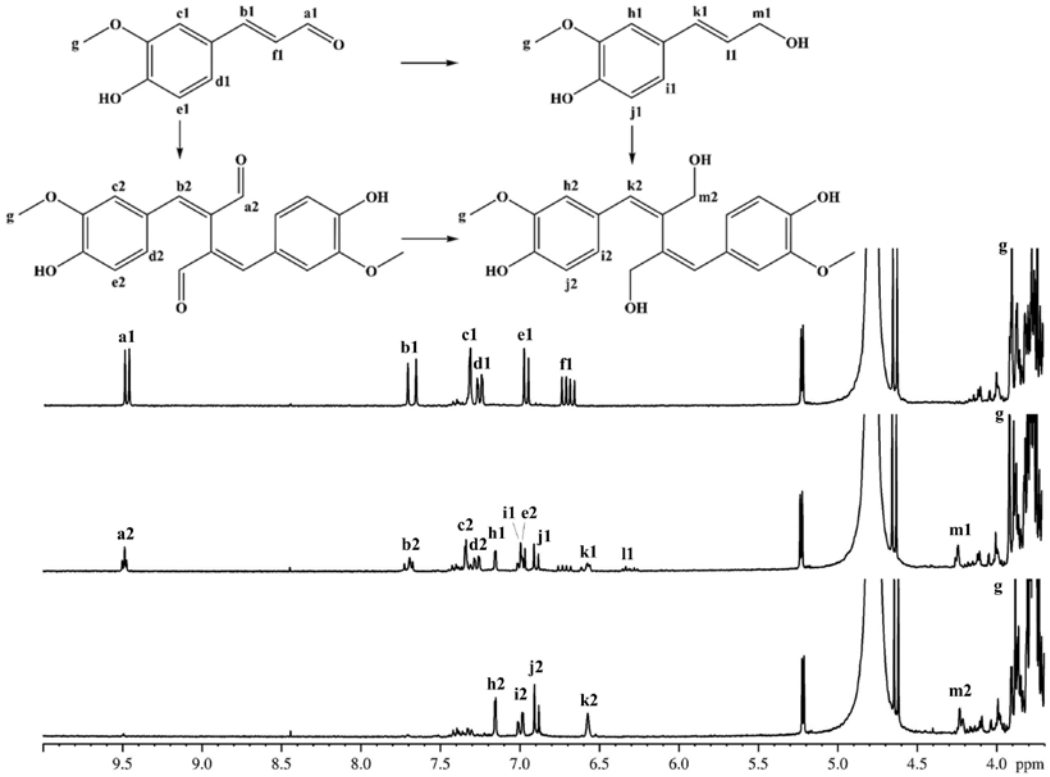

Figure 2: *^{1}H NMR spectra (D_2O, 300 MHz) of medium with coniferyl aldehyde without the addition of T. mathranii (top), medium with coniferyl aldehyde after addition of T. mathranii after incubation at 65 °C for 13 h (middle) and 72h (bottom).*

niferyl aldehyde. Further investigations to that extent were beyond the scope of our studies.

Figure 2 shows the changes of coniferyl aldehyde during fermentation of *T. mathranii*. The new signals appear in the spectrum after the incubation with microorganisms. These signals are assigned to coniferyl alcohol.

In this case, as well, the dimerization reaction of coniferyl aldehyde took place. In addition, further changes in signal patterns were observed. The doublet of doublets at 4.23 ppm (H_{m1}) converts into a doublet (H_{m2}), and the doublets at 6.57 ppm (H_{k1}) convert into a singlet (H_{k2}) and the signal at 6.30 ppm (Hl1) disappears. These changes indicate the additional dimerization reaction of coniferyl alcohol or the reduction of the aldehyde moieties in the dimer or both.

The signals of the anomeric glucose protons at 5.22 and 4.63 ppm remain visible in the spectrum of coniferyl aldehyde after the incubation with cells for 72h. As reported previuously, coniferyl aldehyde has the strongest inhibiting effect of all tested substances [16]. As a result, the growth of microorganisms is more inhibited, so the consumption of glucose during the fermentation with coniferyl aldehyde is much lower than during the fermentation with furfural, which does not inhibit the growth of *T. mathranii* at the given concentration.

A dimerization reaction in the medium was observed only when coniferyl aldehyde was used. All other phenol derivatives did not change their structure in the medium. However, the addition of *T. mathranii* caused the reduction of substances with aldehyde moieties to the corresponding alcohols.

Monitoring of potential inhibitors during the fermentation of the aerobic strain *H. halophila*

The contents of formic and acetic acid decreased during the fermentation of *H. halophila*. The reason for this is that these substances were metabolized by the microorganism [27]. Otherwise, Table 1 shows structural changes of the same compounds during the fermentation as observed with *T. mathranii:* levulinic acid, phenolic components with carboxyl, methoxyl and hydroxyl functional groups did not change during fermentation while furan derivates and phenolic components with aldehyde groups change their structure. In contrast to the fermentations with *T. mathranii*, with *H. halophila* the aldehydes were not reduced but

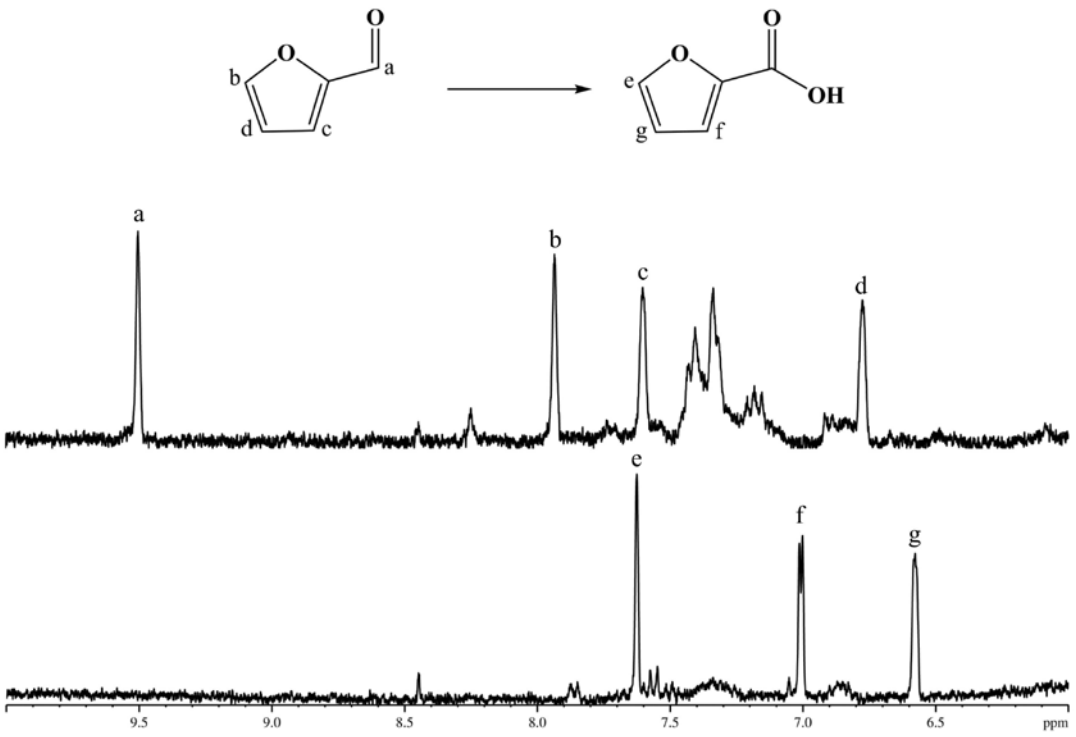

Figure 3: *^{1}H NMR spectra (D_2O, 300 MHz) of medium with furfural without the addition of H. halophila (top), medium with furfural after addition of H. halophila after incubation for 143 h at 30 °C (bottom)*

oxidised yielding carboxylic acids instead of alcohols. Using the example of furfural, Figure 3 shows the structural changes caused throughout the interaction with *H. halophila*.

As with the interaction of furfural with *T. mathranii*, the aldehyde proton corresponding to the peak at 9.49 ppm (H_a) disappears and the peak pattern of H_b at 7.91 ppm, H_c at 7.57 ppm and H_d at 6.65 ppm changes. New signals arise at 7.63, 7.01 and 6.58 ppm. These signals correspond to 2-furoic acid formed during fermentation. The signals at 8.45, 8.25 and 7.73 ppm as well as in the ranges from 7.43 to 7.15 and from 6.92 to 6.81 ppm are from the medium.

The analysis of the other spectra of potential inhibitors with aldehyde groups during fermentation with *H. halophila* showed that all these substances were oxidized to the corresponding carboxylic acids.

Effect of fermentation products of potential inhibitors with aldehyde groups on cell growth of *T. mathranii* and *H. halophila*

The effect of the substances derived during the fermentation from potential inhibitors with aldehyde groups on cell growth was also investigated (Tables 2 and 3). The cells were incubated under the same conditions a) with the addition of aromatic aldehydes or b) with the addition of their corresponding alcohols in the case of *T. mathranii* or c) with the corresponding carboxylic acids in the case of *H. halophila*. The change in the optical density of all bacteria suspensions was measured to determine the growth of the microorganisms. For determination of the effect of aldehydes, alcohols or carboxylic acids on cell growth the ΔOD (optical density) of microorganisms in medium only was compared with the ΔOD of bacteria in the medium containing aldehydes, alcohols or carboxylic acids. The ΔOD of bacteria in the respective medium without inhibitors was defined as 100% growth.

The addition of coniferyl aldehyde to the medium led to a decrease in cell growth to 63 % compared to *T. mathranii* cells in medium only. The presence of coniferyl alcohol in the medium does not have any significant impact on cell growth (Figure 4).

The investigations show that substances with alcohol and carboxylic moieties have no inhibiting effect or a weaker inhibiting effect on the growth of the microorganisms than the components with aldehyde moieties (Tables 2 and 3).

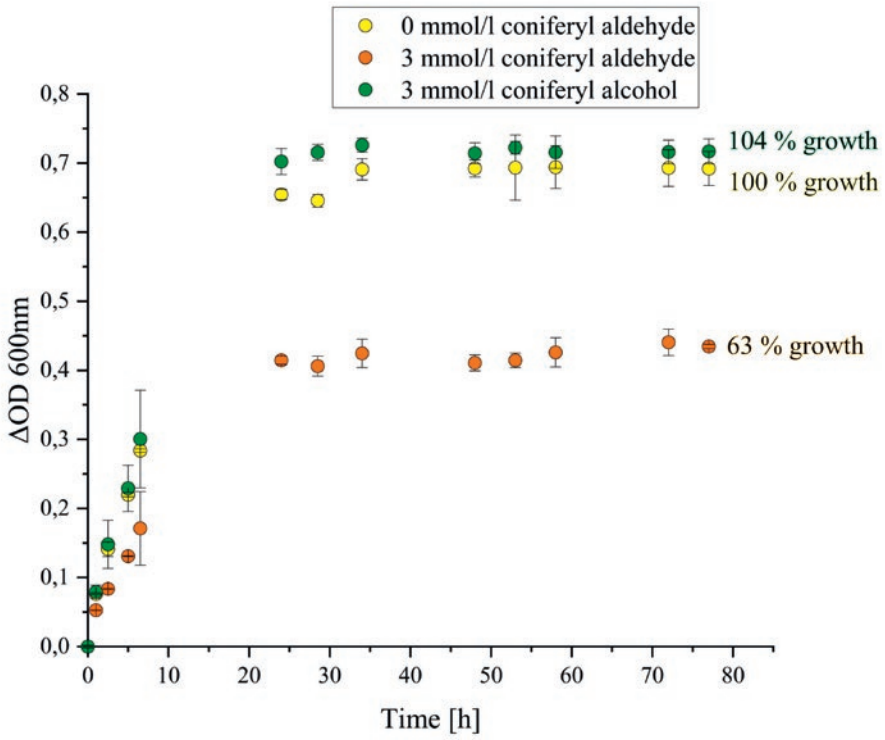

Figure 4: *Growth behaviour of T. mathranii during incubation in medium only (yellow curve) and in a medium with coniferyl aldehyde (orange curve) or coniferyl alcohol (green curve)*

The results are generally in good agreement with the results from other groups. Wang *et al*. reported that the yeast *Saccharomyces cerevisiae* can convert aldehydes generated during the pretreatment of lignocellulose to the corresponding alcohols by multiple aldehyde reductases [28]. Several reductases of the gram-negative bacterium *Zymomonas mobilis* are responsible for reducing phenolic aldehydes from lignocellulose pretreatment to the corresponding phenolic alcohols [29]. Both strains are usually cultivated under anaerobic conditions. Similarly, the reduction of furan aldehydes as a detoxification process of the microorganisms was described by several authors [30,31]. Ruettimann *et al*. reported that the aerobic strain *Streptomyces viridosporus* T7A oxidizes low molecular weight lignin-related aldehydes to the corresponding acids [32].

Conclusions

The study of the structural changes of potential inhibitors from the pulp industry spent liquors during fermentation with the anaerobic ethanol producer

Table 2: *Effects of potential inhibitors with aldehyde groups and their corresponding alcohols on the fermentation of T. mathranii (orange: impact on growth, green: no significant impact).*

concentration [mmol/l]	potential inhibitors with aldehyde groups	growth [%]	substances forming during fermentation	growth [%]
3	Vanillin	101±0,05	Vanillyl alcohol	90±0,01
7	Vanillin	65±0,02	Vanillyl alcohol	97±0,03
3	Syringaldehyde	111±0,01	Syringyl alcohol	93±0,01
7	Syringaldehyde	78±0,04	Syringyl alcohol	106±0,01
3	Coniferyl aldehyde	63±0,03	Coniferyl alcohol	104±0,018
7	Coniferyl aldehyde	19±0,04	Coniferyl alcohol	84±0,01
0.3	Furfural	113±0,00	Furfuryl alcohol	107±0,01
3	Furfural	98±0,04	Furfuryl alcohol	104±0,05
0.3	HMF	116±0,03	2,5-Bis(hydroxymethyl)furan	112±0,02
1.5	HMF	121±0,01	2,5-Bis(hydroxymethyl)furan	120±0,03

Table 3: *Effects of potential inhibitors with aldehyde groups and their corresponding carboxylic acids on the fermentation of H. halophila (colour code same as above).*

concentration [mmol/l]	potential inhibitors with aldehyde groups	growth [%]	substances formed during fermentation	growth [%]
3	Vanillin	91±0,08	Vanillic acid	92±0,03
7	Vanillin	55±0,04	Vanillic acid	83±0,1
3	Syringaldehyde	74±0,004	Syringic acid	91±0,20
7	Syringaldehyde	61±0,04	Syringic acid	96±0,23
3	Coniferyl aldehyde	74±0,03	Ferulic acid	85±0,01
7	Coniferyl aldehyde	42±0,01	Ferulic acid	61±0,13
0.3	Furfural	105±0,06	2-Furoic acid	113±0,03
3	Furfural	111±0,04	2-Furoic acid	115±0,01
0.3	HMF	96±0,02	5-hydroxymethyl-2-furoic acid	99±0,07
1.5	HMF	94±0,03	5-hydroxymethyl-2-furoic acid	101±0,04

T. mathranii and the aerobic PHA producer *H. halophila* shows that most substances do not change their structure during the interaction with the microorganisms. However, exceptions are compounds with aldehyde moieties. They are either reduced to the corresponding alcohols in case of the anaerobic microbial strain or oxidized to the corresponding carboxylic acids in case of the aerobic microbial strain. In both cases, the microorganisms convert a toxic substance to a less toxic substance. We think that these detoxification processes are performed at the expense of cell growth.

Acknowledgement

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Part 3: Detoxification of Spent Sulphite Liquor to Remove Growth Inhibitors in Microbial Transformations

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Abstract

Within the framework of this work, the spent sulphite liquor (SSL) was detoxified through different detoxification technologies, which help selectively to remove toxic substances and thereby improve fermentation. To determine the most effective method for SSL detoxification, the growth of *Clostridium saccharoperbutylacetonicum* was observed after the addition of different amounts of detoxified SSL. The comparison of these techniques shows that the detoxification with hydrogen peroxide and peroxidase as a catalyst is the most effective method for the detoxification of the SSL. The second-best methods are detoxifications with activated carbon and lignin, respectively. The effect of ion exchangers is less significant, whereby the detoxification with anion exchangers yields better results than with cation exchangers. The comparison of the alkaline pretreatments shows that treatment with calcium hydroxide is more effective than treatment with ammonium hydroxide and magnesium hydroxide.

Keywords: *Sulphite spent liquor valorisation, fermentation, growth inhibitors, detoxification of hydrolysates*

Introduction

During sulphite pulping large amounts of spent sulphite liquor are produced containing lignosulphonates, extractives hemicelluloses, monosaccharides and various degradation products. The monosaccharides from the SSL are valuable carbohydrate sources for various biotechnological processes [1-5].

In previous investigations, we screened the effect of organic acids, phenols, furan derivatives and alcohols contained in the SSL on several microorganisms. Certain phenol derivatives show pronounced inhibiting effects on those microorganisms during fermentation. In this work, we screened for the best detoxification method using a microtiter plate setup. Our working hypothesis was that those techniques that remove phenolic compounds should be most successful. *Clostridium saccharoperbutylacetonicum* was used for the tests. In earlier works, this strain proved to be more susceptible to inhibition than the other two strains of the investigations *Thermoanaerobacter mathranii* and *Halomonas halophila* [6].

In the literature, various detoxification methods for hydrolysates of lignocellulosic materials are described. Depending on the starting material and the type of hydrolysis, biological, chemical, physical, or combined processes are used (Table 1).

Table 1: *Detoxification methods in ethanol production*

Detoxification	Process lye	Conditions	Effect	Reference
Peroxidase	Model inhibitors in model medium	0.01 µM of enzyme	p-Coumaric acid, ferulic acid, vanillic acid, and vanillin, the removal efficiency ≈100%	[7]
Laccase	Willow, impregnated with SO_2, steam-treated	1mM laccase, pH 5.3, 30°C, 12h	Removal of monoaromatic phenols, improved fermentation	[8]
Coniochaeta ligniaria	Model inhibitors in model media, corn stover dilute acid hydrolysate	Selected on feedstock phenols and furfural	*Coniochaeta ligniaria* NRRL30616 metabolises furfural, HMF*, aromatic, aliphatic acids and aldehydes	[9,10]
Trichoderma reesei	Willow, impregnated with SO2, steam-treated	Shake flasks, 30°C, 350rpm	*Trichoderma reesei* digests pentoses, produces cellulolytic enzymes and detoxifies the hydrolysate	[11]
Ureibacillus thermosphaericus	Waste, wood hydrolysate	Incubated at 50°C, 24h	Removes furfural, HMF; increases fermentability by *S. cerevisiae*	[12]
Activated carbon	SSL from acid hydrolysis pre-treated *Eucalyptus globulus* wood chips,	a) 2.5g AC/l hydrolysate, 1 day, at RT; b) 2%, pH 7, 1h	a) Improved fermentability; b) low benefit	[13,14]
Anion exchange chromatography	Norway spruce, impregnated with sulfuric acid, steam-treated	pH 10	Removal of phenols, furan derivatives and organic acids	[15,16]
Lignin	Spruce dilute acid hydrolysate	Treated with lignin residue	Removal of 53% phenolic compounds, 68% furan derivatives, improved fermentability	[17]
Alkaline treatment	Dilute acid hydrolysate	pH 9 /80°C-pH 12 /30°C, NaOH for 3h	Improved fermentability	[14,15,18-20]
Reducing agents	Spruce/sugar cane bagasse, thermochemical treatment	dithionite and sulphite 5.0-17.5 mM	Improved fermentability (SHF, SSF)	[21]
Ethyl acetate extraction of wood	Steam exploded (SE) poplar wood	H_2O /ethyl acetate (~1/5) added to the wet SE-poplar wood	Ineffective	[18]
Ethyl acetate extraction of hydrolysate	Steam exploded (SE) aspen wood	SE-hydrolysate four times extracted with ethyl acetate	Increased fermentability	[22]
Trialkylamine extraction	Corn stover prehydrolysate	30% trialkyl amine, 50% n-octanol, 20% kerosene	Removal of 73.3% acetic acid, 45.7% HMF and 100% furfural, improved fermentability	[23]
Supercritical CO_2 extraction	Spruce hydrolysate	CO_2 pressure 200 bar, density 0,84 g/ml, 40°C	Increased fermentability as well as lower concentrations of inhibitors such as phenolics and furan derivatives	[24]
Evaporation	Spruce dilute acid hydrolysate	a) Evaporation 10%; b) evaporation 90%	a) Ineffective; b) improved fermentability	[15]
Heat treatment	Yellow poplar dilute acid + stream treatment	Heat treatment (75°C, 95°C 10 min, 140°C 2,5-3h) + ion exchange	Reduction of acetic acid, phenols	[20]

*5-hydroxymethylfurfural

In biological detoxification techniques, hydrolysates are treated with specific enzymes or whole cells. Enzymatic detoxification is described as one of the most effective methods for removing phenols from lignocellulosic substrates. Mostly laccases and peroxidases are used for this purpose. A distinct advantage of enzyme use is that carbohydrates are not consumed. Cho *et al*., for example, investigated six phenolic model substances that inhibit the production of butanol. Complete removal of these phenols was achieved with a peroxidase from *Coprinus cinereus*. The treatment significantly increased the butanol yields [7]. In the work of Jönsson et al., the effects of a laccase, a phenoloxidase and lignin peroxidase from *Trametes versicolor* were studied on real substrate namely the hydrolysate from willow pretreated with SO_2 and steam. The treatment with laccase and lignin peroxidase resulted in the removal of phenolic compounds and improved ethanol fermentability of the hydrolysate [8].

Some microorganisms can metabolise lignin as well as furan derivatives and acetic acid. Lignocellulosic substrates detoxified with those microorganisms can be hydrolysed more easily into fermentable sugars in further stages of the pretreatment process. This results in reduced demand for chemicals and process heat as well as shorter hydrolysis times. Treatment with the fungus *Coniochaeta ligniaria* NRRL30616, which can metabolize furan derivatives, aromatic and aliphatic acids and aldehydes, resulted in an improvement in the fermentation efficiency of corn stover hydrolysate [9,10]. To improve the efficiency of ethanol production from hydrolysate obtained after steam-pretreatment of willow, the fungus *Trichoderma reesei* is used. This fungus utilizes pentoses, simultaneously removes water-soluble inhibitors and, as an additional benefit produces cellulolytic enzymes [11]. Another example of a microorganism used for detoxification is the thermophilic bacterium, *Ureibacillus thermosphaericus*, which degrades toxic compounds in the hydrolysate of waste house wood. It does not metabolize sugars and improves ethanol production by *Saccharomyces cerevisiae* or the ethanologenic recombinant *Escherichia coli* KO11 [12].

The chemical and physical methods for the detoxification of the hydrolysates lignocellulosic materials are manifold. They can roughly be summarised in the following categories:

a) Interaction with a carrier material, such as adsorption to activated carbon or interaction with an ion-exchange resin
b) Extraction with (organic) solvents
c) Overliming
d) Chemical modification
e) Other

Using **activated carbon**, Parajo *et al*. reduced the concentrations of phenols and acetic acid in acid hydrolysis pre-treated *Eucalyptus globulus* wood [13]. Whereas in the work of Helle *et al*., this method was not the most effective [14]. Tesfaw et al. also saw some effect when using activated carbon alone, but in combination with overliming it was more effective [25]

A low-cost and effective detoxification method is the solid-phase extraction of hydrolysates with **lignin**, which is produced in large quantities as a by-product during pulp production. Its hydrophobic properties make the separation of aromatic and furan derivatives possible [17].

The use of **anion exchange resins** is an effective method for the removal of phenols, organic acids and furan derivatives. Unfortunately, it causes an undesirable sugar loss of up to 75 %, which can be reduced to 1 % by the addition of sodium sulphate [15-16]. The combination of heat treatment and treatment with ion exchangers is well suited for the separation of acetic acid and phenolic compounds from lignocellulosic hydrolysate [20].

The **extraction** with ethyl acetate was used for the removal of low molecular weight phenolic substances. Cantarella *et al*., extracted sulphite and steam-treated poplar wood with water and ethyl acetate. Despite the removal of a large number of phenols the desired improvement in fermentability was not achieved [18]. In contrast, the fermentability of steam-pretreated aspen wood hemicellulose could be improved by extraction with ethyl acetate. Not only phenolic compounds but also other inhibitors (except for acetic acid) were removed in this process [22]. The difference in those two processes is that Cantarella *et al*. applied the treatment to solid biomass, whereas Wilson *et al*. treated the hydrolysates. Although the latter treatment was very efficient, using ethyl acetate on large scale requires an explosion-proof installation and thus high capital expenditure. Therefore, ethyl acetate was ruled out from this investigation.

Zhu *et al*. used a mix of trialkyl amines, n-octanol and kerosene as extracting agents for the treatment of the corn stover prehydrolysate. This method removed acetic acid, HMF, and furfural, which significantly improved the fermentation [23]. However, those mol-

ecules are not our detoxification targets and the recovery of the solvent mixture does not seem feasible in large-scale production. A promising solvent represents supercritical CO_2, which is perfectly suited to the separation of phenols and furan derivatives. The advantage of the use of supercritical CO_2 is that no sugar loss and pH value changes take place [24].

Overliming or alkaline treatment is also an important detoxification method. Generally, it describes the alkaline treatment of acidic hydrolysates which promotes precipitation of low molecular weight components, for example, phenols or furan derivatives [18]. Most commonly used are $Ca(OH)_2$, NaOH or NH_4OH to achieve a basic pH (about 10), followed by readjustment to a neutral pH usually using H_2SO_4. [14,15,19]. This procedure largely enhances the fermentability although the rationale behind this effect is yet to be understood [20]. $Mg(OH)_2$ was included in the tests since the Mg^{2+}ion is a common counterion in nowadays sulphite processes. This way the introduction of another ion species complicating the recovery process would be avoided.

Also **reducing agents** were used as pretreatment agents. The number of furan derivatives in lignocellulose hydrolysates was reduced with dithionite and sulphite, which improved the fermentability of these hydrolysates. The detoxification can be carried out directly in a fermenter at the same reaction conditions (pH value and temperature) which prevail during the fermentation [21]. Although bisulfite and dithionite are used as bleaching agents in mechanical pulping they are not used in chemical pulping. Moreover, we decided against using potentially hazardous chemicals for detoxification purposes.

Another example of detoxification is evaporation. Evaporation helps to remove volatile components, such as acetic acid, formic acid, and furfural [15]. However, this method is not suited for the removal of the inhibiting phenolic substances identified in earlier studies [6]. Also, membrane separation processes were not included in our study because of the expected loss of significant amounts of the monomeric sugars while trying to remove the inhibitor molecules that are in the same size range.

Among the above-listed processes, Fernandez *et al*. consider adsorption an advantageous technique in terms of cost, environmental impact and detoxification performances [26]. Zhang *et al*. concluded that sequential treatment of overliming and active carbon of prehydrolysates was necessary to vastly improve the fermentation performance of *C. saccharobutylicum* [27]. Tesfaw *et al*. came to similar conclusions [25].

While the use of adsorbents to detoxify lignocellulosic hydrolysates was the object of a wide number of studies, the specific implementation of adsorption to detoxify SSL was only studied by a limited number of authors. Xavier *et al*. developed a two-step adsorption process on ion-exchange resins [28] for subsequent ethanol fermentation with *Pichia stipitis*. SSL was initially treated with a cation-exchange resin to remove Mg^{2+} and other cations from the pulping process. In the second step, organic acids, polyphenols, and lignosulphonates were separated from carbohydrates by employing an anion-exchange resin. This process led to a dilute, almost transparent solution containing mainly neutral monomeric sugars. Takahashi *et al*. studied the adsorptive removal of inhibitors from a model SSL in the production of ethanol with *Saccharomyces cerevisiae*. They compared the effectiveness of activated carbon, precipitated calcium carbonate (PCC) or XAD-4 resin. Activated carbon proved to be the most effective adsorbent by removing 100 % furfural, 48 % acetic acid, and 70 % lignosulfonate from SSL [29].

Materials and Methods

Detoxification

For all applied detoxification methods, the SSL solution was sterile-filtered before the fermentation.

Enzymatic detoxification

345.000 u/l horseradish peroxidase was added to 10 ml SSL. 1.3 µl H_2O_2 (8 mmol) was diluted with 98.7 µl deionised water. The H_2O_2 solution was added dropwise to the SSL. The mixture was stirred for 1 h at 100 rpm at room temperature.

Detoxification with lignin

Lignin (Indulin AT) was washed with deionised water until the wash liquor was colourless and had a neutral pH. 2, 5 and 10 wt. % Indulin AT were added to 10 ml SSL. The mixtures were stirred for 1 h at 100 rpm at room temperature. Thereafter, the lignin was removed by filtration.

Detoxification with activated carbon

2, 5 and 10 wt. % activated carbon was added to 10 ml SSL. The mixtures were stirred for 1 h at 100 rpm at room temperature. Thereafter, the activated carbon was removed by filtration.

Detoxification with cation exchange
2 g cation exchange (Lewatit, 1368 Ca/320, Lanxess) was washed with deionised water and added to 10 ml SSL. The mixture was stirred for 1 h at 100 rpm at room temperature. Thereafter, the cation exchange resin was filtered off.

Detoxification with anion exchange
2 g anion exchange (AG 1-X8, 20-50 mesh, chloride form, Bio-Rad) was washed with deionised water and added to 10 ml SSL. The mixture was stirred for 1 h at 100 rpm at room temperature. Thereafter, the anion exchange resin was filtered off.

Detoxification with $Ca(OH)_2$
$Ca(OH)_2$ was added to 10 ml SSL and the pH was adjusted to 11. The mixture was stirred for 3 h at 100 rpm at 60 °C. Thereafter, the precipitate was filtered off.

Detoxification with $Mg(OH)_2$
$Mg(OH)_2$ was added to 10 ml SSL and the pH was adjusted to 8.5. The mixture was stirred for 3 h at 100 rpm at 60 °C. Thereafter, the precipitate was filtered off.

Detoxification with NH_4OH
NH_4OH was added to 10 ml SSL and the pH was adjusted to 8.5. The mixture was stirred for 3 h at 100 rpm at 60 °C. Thereafter, the precipitate was filtered off.

Growth experiments with *Clostridium saccharoperbutylacetonicum* (DSMZ 14923)

Medium preparation
For the cultivation of *C. saccharoperbutylacetonicum* (DSMZ 14923) was used a medium, which contains 0.3 g/l magnesium sulfate heptahydrate, 2 g/l yeast extract, 6 g/l peptone from casein, 3 g/l ammonium acetate, 1.5 g/l potassium dihydrogen phosphate, 1.2 g/l dipotassium hydrogen phosphate, 0.01 mg/l Iron(II) sulfate heptahydrate, 0.5 g/l L-cysteine. The pH value of the solution was adjusted to 7. The medium was autoclaved for 10 min at 120 °C. Glucose (20 g/l) was used as a carbon source. The concentrated sterile glucose solution was added to the medium before the inoculation of microorganisms.

Cultivation
The work was carried out in a glovebox under forming gas atmosphere. 1 ml cryo stock (-80 °C) of cells in glycerol was thawed at room temperature and then added to 9 ml medium. The cells were incubated with agitation for 24 h at 30 °C. To preserve vital cells culture was re-inoculated in a medium once again and incubated for 18 h. These cells were used for the microtiter plate experiments.

Microtiter plate experiments
The effect of various detoxification methods on the growth of *Clostridium saccharoperbutylacetonicum* was tested in a microtiter plate under forming gas atmosphere. For the determination of detoxification effects on the growth of *C. saccharoperbutylacetonicum*, the ΔOD (optical density) of microorganisms in a medium with the sugar mixture was compared with the ΔOD of microorganisms in the non-detoxified as well as detoxified SSL. The sugar mixture in the medium corresponds to the composition of the SSL (1.4 mg/l arabinose, 2.9 mg/l galactose, 6.8mg/l glucose, 20.1 mg/l mannose and 8.0 mg/l xylose). The pH of the non-detoxified, as well as detoxified SSL, was set to 7. A dilution range of the SSL from 1:2 to 1:30 (Figure 1) was prepared. For the preparation of the dilutions, the medium with a sugar content corresponding to the SSL sugar concentration was used. 50 µl medium with sugar mixture or SSL were pipetted into each well. Thereafter, 30 µl cultures were added to the solutions. The microtiter plate was sealed with a transparent oxygen-impermeable adhesive film. The cells were incubated for 10 h at 30 °C. The OD measurements were carried out at regular intervals in triplicate.

Results and discussion

Spent sulphite liquor from a dissolving pulp mill was subjected to the following detoxification treatments:

a) Enzymatic: horseradish peroxidase (HRP)/ H_2O_2 because HRP is significantly cheaper than laccase
b) Adsorption on 2%, 5% and 10% (w/v) Indulin AT
c) Adsorption on 2%, 5% and 10% (w/v) activated carbon (AC)
d) Cation and anion exchange, respectively
e) Alkaline treatment with $Ca(OH)_2$, $Mg(OH)_2$ and NH_4OH; $Mg(OH)_2$ is particularly interesting for SSLs derived from Mg-sulphite pulping because it does not introduce new chemicals into the SSL.

The detoxified SSLs were then tested in a microtitre plate setup with *C. saccharoperbutylacetonicum* as the reference microorganism. The growth of microorganisms was investigated after the addition of different amounts of SSLs, which were detoxified by the

above-mentioned techniques. Growth was monitored by OD measurements and expressed as % growth compared to the inhibitor-free control (=100%). Because of the experimental setup, the highest amount of SSL possible is 63%. The remaining volume is taken up by the inoculum.

Figure 1 summarises the results of all types of treatments. Changing the concentration of the SSL is not very effective. Lowering the concentration to 1/30 only triples the growth of the microorganisms. It has practical disadvantages as well. Dilution means additional water use in the pulp mill, which is nowadays undesired. All pulp mills try to significantly reduce the amount of process water they use. In addition, a huge energetic effort is required to evaporate the added water for further use of the sugar-free SSL. Last but not least an external carbohydrate source is required to make up for lowering the monosaccharide concentration as well. The figure also shows that the treatment with peroxidase/H_2O_2 is the most effective, followed by activated carbon. Lignin seems to have somewhat better effectiveness than ion exchange resin and may also be the more economical solution of the two. Overliming has a very limited positive effect on bacterial growth.

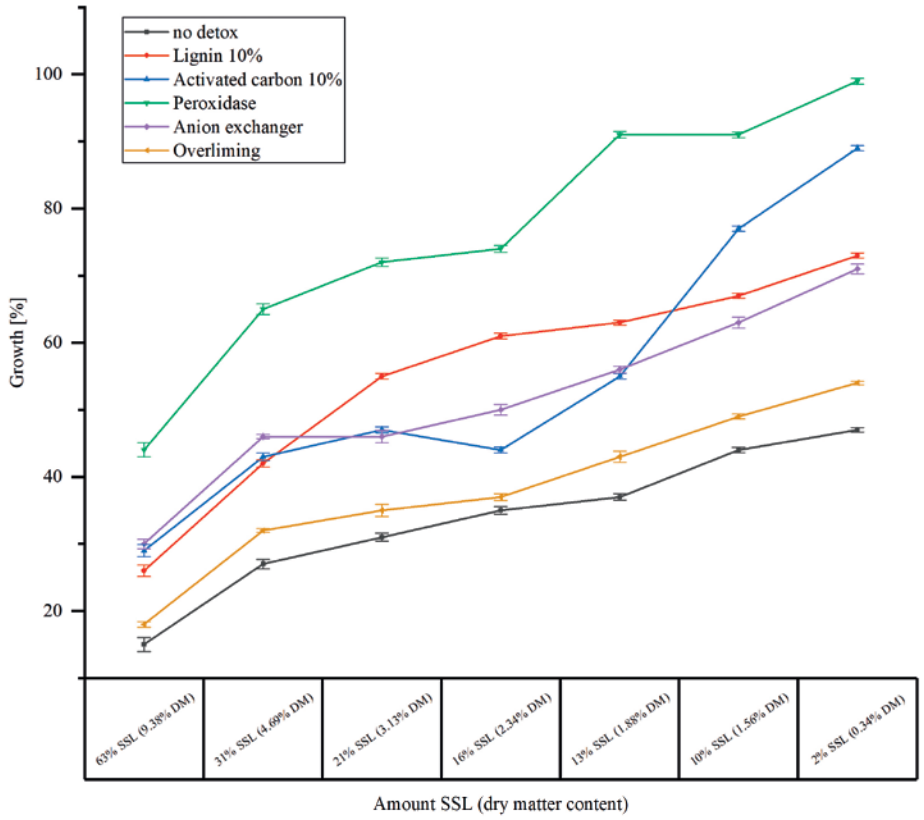

Figure 1: *Summary of the effects of detoxification on microbial growth with lignin, activated carbon, horseradish peroxidase/H_2O_2, ion exchange resin and overliming in combination with different amounts of SSL.*

Figure 2 summarises the results of all three bases used for the alkaline treatment. Overliming with $Ca(OH)_2$ results in slightly higher microbial growth than treatment with NH_4OH. $Mg(OH)_2$ had no effect at all, which is a little disappointing. From a practical point of view in a magnesium sulphite process only an alkaline treatment with $Mg(OH)_2$ could be easily implemented.

Figure 3 depicts the detoxification effectiveness of ion exchange resins in detail. The anion exchanger performed a little better than the cation exchanger. The detoxification with lignin (Figure 4) is somewhere in the range of the anion exchange, even slightly better. Data on the influence of the amount of lignin applied are inconclusive.

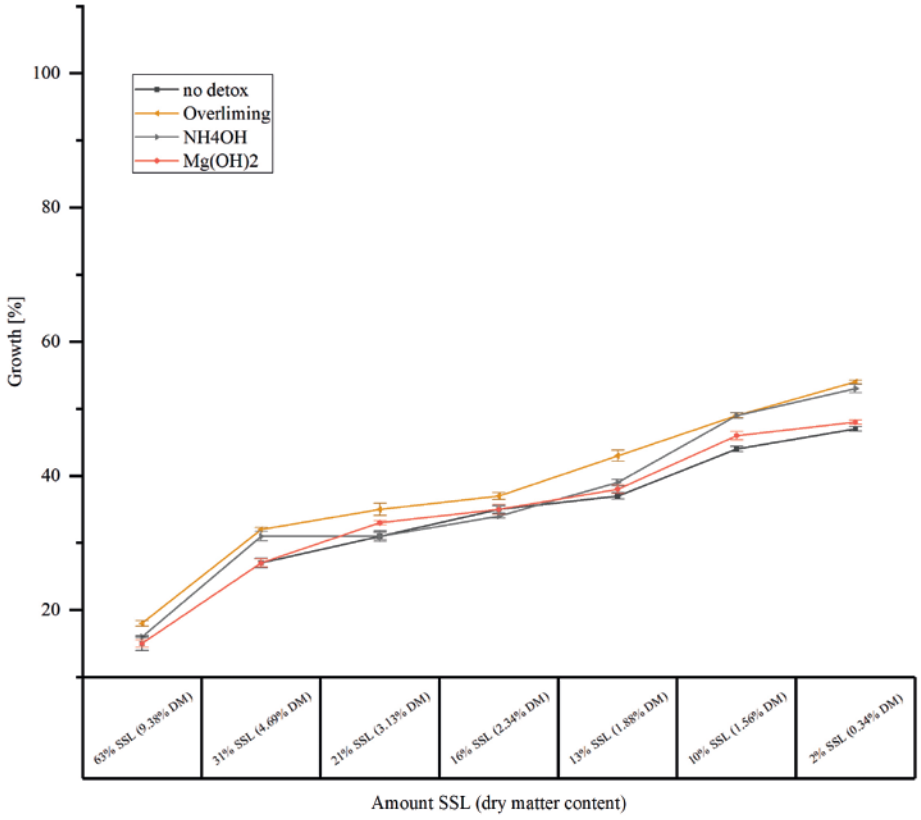

Figure 2: *Alkaline treatment with $Ca(OH)_2$ (overliming), NH_4OH and $Mg(OH)_2$.*

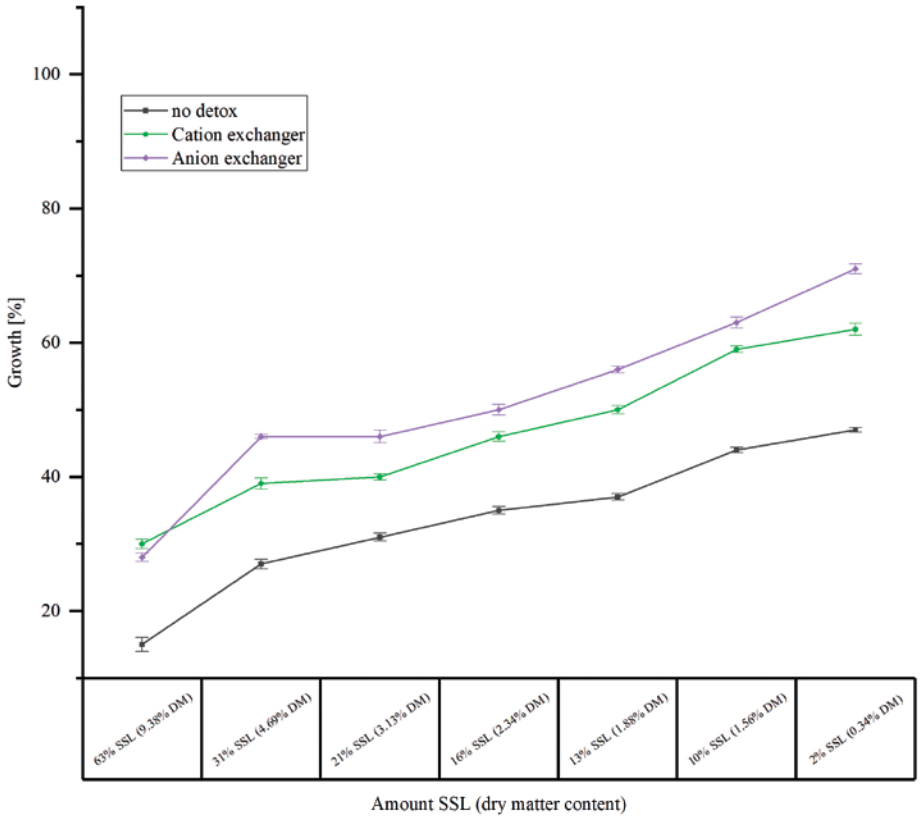

Figure 3: *Treatment of SSL with anion and cation exchangers.*

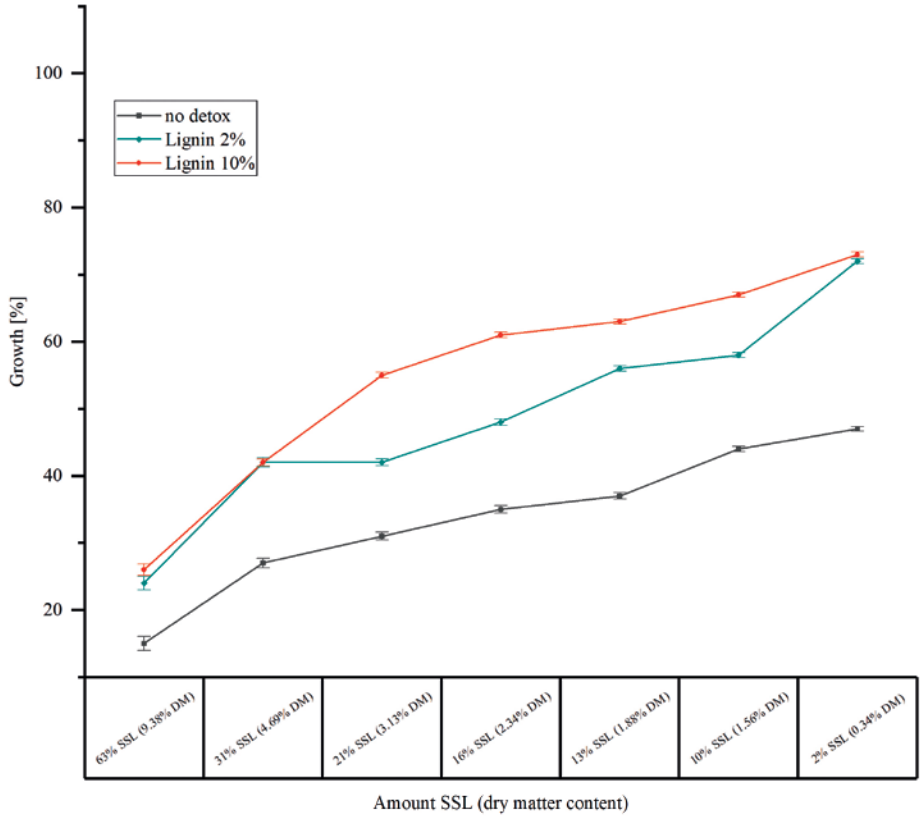

Figure 4: *Treatment of SSL with different amounts of (solid) lignin.*

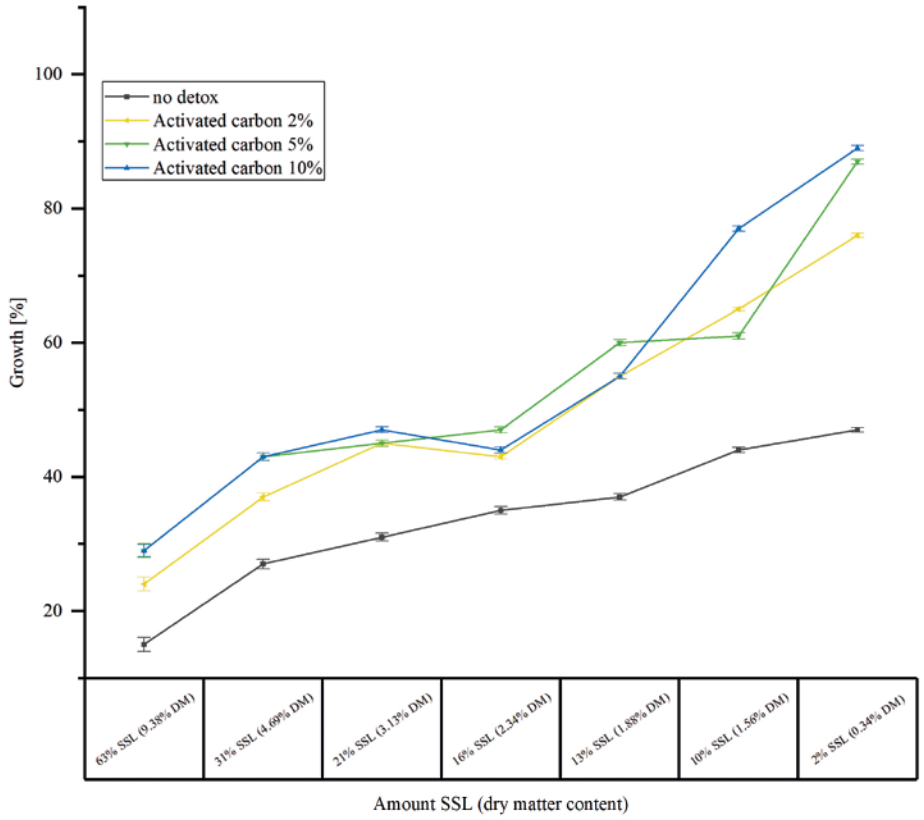

Figure 5: *Treatment of SSL with different amounts of activated carbon.*

The treatment with 10% activated carbon shows the second-best detoxification result. The steep rise at the end of the 5% and 10% curves in Figure 5 allow us to speculate that there is room for improvement in the ratio of activated carbon to dry matter content.

The results are partly in agreement with the trends in the literature and partly not, which is not surprising. Lignocellulose sources, digestion processes and the respective microorganisms vary widely in the literature and, thus, limit comparability.

Conclusions

The most effective detoxification method for *C. saccharoperbutylacetonicum* fermentations of SSL is employing horseradish peroxidase/H_2O_2. The second-best method is the adsorption onto activated carbon closely followed by adsorption onto lignin. Ion-exchange resins show some effect. Alkaline treatment has a very limited effect in the case of $Ca(OH)_2$ and no effect at all for $Mg(OH)_2$ as a base. The results corroborate our working hypothesis that techniques that remove phenolic compounds are the most effective

The next steps are to elaborate the economically and technologically most rewarding detoxification method or to grow microorganisms better adapted to the SSL.

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Lignin-Containing PMDI-Binder for Wood Particle Board Production

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Abstract

Comprehensive testing of various routes of lignin pretreatments and the final adhesive preparation and performance allowed a better understanding of kraft lignin properties and margins within which this lignin can be utilized in the application with pMDI.

The prospects and properties of adhesive formulations prepared, such as homogeneity, viscosity, stability and mechanical properties are discussed in detail.

Introduction

Application of technical lignins, such as kraft, lignosulfonates, organosolv and other biorefinery lignins, in various adhesive systems to bind different wood composites has so far been among the most promising lignin utilization scenarios and at the same time one of the hardest challenges [1]–[3].

The pulp and paper industry is still the largest biorefinery branch and generates with the kraft process around 80% of the lignin theoretically available for material use. Prior to further application, lignin has to be isolated from the black liquor, which is produced as one of the intermediate product streams. Generally, the black liquor is burned to recover the cooking chemicals and to generate energy for the process. The recovery may be a bottleneck in the entire process, hindering further expansion of the pulp production. Hence, an increase in the capacity of the recovery boilers incinerating black liquor can resolve such limitations to a certain extent. Another workaround, namely the isolation of kraft lignin from the black liquor, can significantly decrease the load on the recovery boiler, allowing to proportionally increase the production and potentially reach higher profit by implementing isolated lignin as a high value-added raw material in various applications. The latter, of course, depends on whether the isolated lignin can be sold, and this implies that there is a certain market for applications. High-tonnage scale and economically feasible kraft lignin isolation technology has lately become industrially available [4]. The range of companies, such as Valmet, Stora Enso (Lineo®), Domtar (BioChoice™), UPM (BioPiva™) or Suzano to name a few, offering lignin produced with LignoBoost™, LignoForce™ or SLRP™ technology at a ton scale is constantly expanding.

The application of lignin in different adhesive formulations allows achieving certain advantages, such as improving the thermal properties, modulus of elasticity and water resistance, as well as to achieve good bonding strength [5]. Lignin-containing materials would offer a higher portion of renewable materials in existing applications, although a full substitution of all adhesive components is currently not conceivable on industrial scale.

Usually, positive aspects often come at the expense of some negative side effects. For lignin in adhesives this is a curing rate reduction, an increase in viscosity of the adhesive, and a limited lignin solubility, to name but a few [6].

The physicochemical properties and reactivity of technical lignins is highly dependent on the source of the lignin, the process of its extraction from lignocellulosic biomass, i.e. pulping conditions, as well as further isolation and purification protocols. There is one particular reason why an effective utilization of technical lignins in industrial applications is still scarce [7], [8]. The rather broad molecular weight distribution, and the accompanying high dispersity of lignins with regard to functional group profiles are among the fundamental parameters which influence the lignins´ behavior and final product performance [9].

Lignin depolymerization was proposed to improve lignin reactivity [8]. However, modern understanding of lignin applications, based on aspects of economical sustainability and viability, rather prefers maintaining the macromolecular nature of lignin. As an alternative to depolymerization methods, lignin fractionation by means of different approaches has been investigated [8], [9]. As already mentioned above, an essential parameter of lignin is its composition and content of functional groups. Because of a relatively high content of hydroxy (–OH) and carboxy (–COOH) groups, lignin usually has a higher affinity towards polar matrices (solvents or other polymers) and, therefore, usually possesses a limited compatibility with non-polar matrices, which generally lead to poor mechanical properties of the final composite and requires significant adjustment of process parameters, such as temperature, reaction time and others [10]. At the same time, the –OH and –COOH groups can readily be used as starting points for chemical modifications, such as: alkylation, esterification, acetylation etc., towards decreasing the lignin´s hydrophilic character and, therefore, improving its incorporation into the required matrix [8]. However, one should be aware, that masking those functional groups will inevitably also lead to the decrease of lignin reactivity in polymerization reactions, e.g., with diisocyanates.

To improve lignin reactivity, many other structure modification strategies, targeting different functions in aromatic or aliphatic moieties, have been proposed [6], [7]. Chemical modifications, such as: demethylation, methylolation, phenolation and others, allowing generation of additional –OH groups and/or unoccupied positions in aromatic rings, have been confirmed to have a positive impact on the lignin´s reactivity with diisocyanates [7].

Undoubtedly, any additional lignin pretreatment predictably leads to the increase of process complexity and increase in costs. In order to improve the economic competitiveness and rationality of lignin modification in an effective utilization an integrated or concerted process is proposed. The simultaneous generation of vanillin and oxidized and degraded kraft lignin is an example of such an approach, which can further be applied in combination with polyurethane manufacture [11].

In general, the elaborated knowledge on lignin structure and reactivity, wide variety of approaches for its modification, new methods for fast and precise lignin characterization as well as testing of lignin-based composite materials, provide strong flexibility in future development of lignin utilization strategies [12].

Beside lignin properties and reactivity, the utilization of a proper crosslinking agent comprises another key factor influencing successful applications of lignin within adhesive formulations [7]. One of the most common crosslinkers is polymeric 4,4’-diphenylmethane diisocyanate (pMDI), which is already widely applied as a sole adhesive in wood panel industry in production of oriented strand board (OSB), and seldom in medium-density fiberboard (MDF) and highly specialized particle boards [5], [12]. pMDI represents a fast- curing, formaldehyde-free adhesive, providing quite high mechanical bonding at relatively low charge, and a high resistance against water [12]. The direct reaction of pMDI´s isocyanate groups and lignin hydroxy groups, leading to the formation of urethane bonds, has been found to proceed comparatively slow. It is considered that pMDI applied as an adhesive also reacts with water constituent, present as a moisture in wood, being one of the most crucial parameters [13]–[15].

The selection of an appropriate lignin type is the key for lignin implementation into materials or substitution of oil-based chemicals. Unlike lignosulfonates, which global production and market availability are comparably limited, kraft lignin is currently the only type of technical lignin potentially available in large quantities. Its relative simplicity of isolation, possible high purity, decently strong interest of pulp and paper companies in lignin stream separation and utilization as well as a rather high abundance of kraft mills made this lignin type a good candidate for the present investigation. Though phenol in phenol-formaldehyde adhesives can be successfully substituted - at least partially - by kraft lignins, the corresponding binders still suffer from toxicity concerns. The alternative adhesive approach is cross-linking of the wood particles by polymeric diisocyantes (polymeric diphenylmethane diisocyanate – pMDI) [16]. The chemical, although toxic as the starting compound, forms unproblematic polymers. Although the overall amount of pMDI used relative to the wood particles ranges around 2%, it is still desirable to partially exchange pMDI with lignin [16].

The aim of this research was to elaborate a binder formulation, comprised predominantly of polymeric diphenylmethane diisocyanate (pMDI) and technical lignin, with a superior performance compared to current adhesives and to achieve an economically attractive decrease in the pMDI consumption in wood particle board production.

Experimental

Materials and chemicals

Indulin AT (MeadWestvaco, USA) lignin, as one commercially available kraft lignin, was selected as a reference lignin in this project, serving as a standard, well-characterized kraft lignin.[14]. In addition, a range of kraft lignins of various European mills have been applied as well (Lignin-A, B and low molecular weight (LMW) Lignin-A, B).

A solvent-free polymeric diphenylmethane diisocyanate (pMDI; ONGRONAT WO 2750) with average functionality of 2.7 and an NCO content of 30.0 – 32.0 wt% from BorsodChem Zrt. (Kazincbarcika, Hungary) was used. The viscosity of 220 ± 5 mPa·s was determined with a cone plate rheometer at 20 °C with a shear rate of 100 s^{-1}.

Propylene carbonate (PC), glycerol 1,2-carbonate (GC), polyethylene glycol 200 (PEG), poly(ethylene glycol) diglycidyl ether (PEGDGE), acetyl triethyl citrate and other chemicals were of p.a. quality and were purchased from Sigma-Aldrich.

Analysis methods

Phosphorus-31 NMR experiments and sample preparation were done according to [17], [18]. The samples were dried in a vacuum oven prior to dissolution in the standard mixture of chloroform-*d* / pyridine-d_5 followed by derivatization step with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane. The NMR experiments were performed on a Bruker Avance II 400 (at 162 MHz for ^{31}P) equipped with a 5 mm cryoprobe (Prodigy) with z–gradients at room temperature (standard Bruker pulse programs; Bruker, Rheinstetten, Germany). Data were collected with 64 k data points and apodized with an exponential window function (lb = 5) before Fourier transformation. A 0.6 s acquisition time and a relaxation delay of 15 s were used and 256 scans were collected. The content of hydroxyl groups was obtained by integration of the following spectral regions: internal standard (152.4-151.3 ppm), total aliphatic -OH (150.0-144.6 ppm), total phenolic -OH (144.6-137.0 ppm) and carboxylic acids (136.0-133.6 ppm).

Size-Exclusion Chromatography (SEC) was performed on a Dionex UltiMate 3000 system (Thermo Fisher Scientific, Germany), including degasser, autosampler, column oven, UV detector, and refractive index (RI) detector (Shodex RI-101) according to [19]. Dry lignin samples were dissolved in DMSO/LiBr (0.5%). Prior to SEC analysis, the solutions were filtered through a 0.45 μm PTFE syringe filter. Separation was performed on three Agilent PolarGel M columns (7.5 × 300 mm) with dimethyl sulfoxide (DMSO) containing LiBr (0.5% w/v) as the mobile phase. Columns were calibrated with polystyrene sulfonate standards of known molecular weight: Mw = 1100, 1920, 3610, 6520, 14900, 29100, 63900, 148000 g·mol^{-1}, ĐM <1.20. The analysis parameters were: flow rate 0.5 mL·min^{-1}; column temperature 40°C; injection volume 10 µL. Data evaluation was performed with Chromeleon software, version 6.80.

The differential scanning calorimetry (DSC) and thermogravimetric analyses (TGA) were carried out using NETZSCH STA 409 PG instrument in nitrogen atmosphere. The temperature range was 30–500°C, the heating rate 10°C·min^{-1}, the purge gas velocity 25 ml·min^{-1}, and the sample weight 5-10 mg.

Oxyalkylation of lignin

Lignin (1.0 g; approx. 6 mmol -OH·g^{-1}) was dissolved in approx. 6 g of propylene carbonate (PC) or glycerol

carbonate (GC), and approx. 90 mg of K_2CO_3 were added. The mixture was allowed to react at 170°C and 140°C, in case of PC and GC, respectively, for 3 h under stirring in N_2 atmosphere. After the completion of the reaction, the mixture was cooled to room temperature and added to the 10-fold amount of deionized acidified (pH 2) water. The precipitated product was isolated and thoroughly washed with 5 × 50 ml water by means of centrifugation. The isolated product was first freeze-dried and finally dried in a vacuum oven at 40°C.

Alkylation

Methylation and isopropylation of hydroxy groups was done with the corresponding alkyl halides according to a procedure adopted from [20]. Complete or partial derivatization was achieved by using either a strong or a weak base catalyst, e.g. NaOH or K_2CO_3. Lignin was dissolved in DMSO, finely ground basic catalyst and the corresponding alkyl halide were added (8h, RT). Ethylation of lignins was performed with diethyl sulfates according to [21]. A lignin sample was dissolved in acetone and 30% NaOH solution was slowly added with vigorous stirring. Dialkyl sulfate was added dropwise to the lignin solution. The temperature of the reaction medium was kept at around 20 °C. The mixing was continued for 16 hours. Acetone was partially evaporated on a rotary evaporator. Further isolation was the same for all alkylation experiments, i.e. the lignin solution was diluted with water 3-4 times and acidified with 1 M HCl solution to pH 2. The precipitated lignin was centrifuged off, thoroughly washed with excess deionized water until almost pH-neutral, and freeze-dried.

Acetylation

Isolated lignins were quantitatively acetylated by adding a mixture of freshly distilled acetic anhydride and dry pyridine (4.7:4.0 v/v, respectively) and stirring for 10 days at room temperature in the dark, according to [21].

Ultrafiltration

Sequential lignin ultrafiltration (U.F.) has been done according to [22] using a stirred ultrafiltration cell (Model 8200, 200 mL, Millipore Amicon). Regenerated cellulose (RC) membranes with polypropylene support from Millipore Corp. (USA) were used (thickness: 230 µm, diameter: 63.5 mm). Three suitable membranes were selected, producing four lignin fractions, i.e.: >100, 100-30, 30-1 and <1 kDa.

Indulin lignin was dissolved in water at pH 12, which was adjusted with 1M NaOH. The ultrafiltration process was started with the membrane having the highest cutoff, followed consecutively by filtration through membranes with decreasing cutoff. Each filtration step was stopped when the color of the permeate became almost colorless. Approximately two liters of water with pH 12 were used for automatic refilling of the cell containing the retentate, to ensure complete fractionation. The permeates were concentrated on a rotary evaporator at 40°C and used for the next step of fractionation. The retentates and the permeate remaining after the last step of fractionation were acidified to pH 2 with 1 M HCl. The precipitated lignin fractions were isolated by centrifugation, thoroughly washed with deionized water and lyophilized to a constant weight.

Bonding strength development tested on wood

To evaluate the bonding strength of the freshly cured adhesives, a self-constructed bonding strength tester was used, according to [12]. The adhesive formulations were tested on birch veneer with a thickness of 1.5 mm and an overlapping area of 10 x 34 mm. 114 $g \cdot m^{-2}$ adhesive were applied on the wood stripes and pressed at 120°C for 5 minutes. This test was used as a fast comparison of the bonding strength of different lignin/pMDI binder combinations. The homogeneity and applicability of the formulations were evaluated by visual inspection and microscopy.

Visual evaluation of resin mixtures

In addition to the macroscopic optical assessment of the adhesive mixtures, microscopic images were also taken for analysis. The evaluation was carried out using a light microscopy with software support (Olympus digital microscope DSX-1000, Olympus Hamburg, Germany). The images were taken from samples on object slides at 20x and 30x magnification using transmitted light.

Particle board production and testing

Particle boards with dimensions of 50x50x1.4 cm^3 were pressed in a laboratory press (G. Siempelkamp GmbH &CO. KG., Krefeld, Germany). The two-component adhesive was sprayed onto middle-layer particles with an air gun and then manually distributed into a pressing mold. After hot-pressing at 220°C using a press factor of 9.3 $s \cdot mm^{-1}$, the test boards were conditioned for 14 days at a standard climate (20°C and 65% relative humidity). The target density of the particleboards was 650 $g \cdot cm^{-3}$. The determination of internal bond strength perpendicular to the plane (IB) was done according to EN319 on a universal testing machine (Shimadzu Europe GmbH, Duisburg, Germany) [23].

Results and Discussion

The general requirements for the binder formulation in wood particle boards had to meet following requirements: a low viscosity (<1500 mPa·s), homogeneity, reasonably high content of lignin (at least 20-30% w/w), sufficiently long shelf life of the final formulation, no malodorous smell and cost-efficient preparation procedure. Integration of technical lignin into the existing pMDI adhesive system usually relies on several conditions, such as lignin reactivity, ability to form a homogeneous mixture or solution, stability of the chemical and physical properties and others. In order to meet the above mentioned requirements, different lignin modifications, such as alkylation, oxyalkylation and acetylation, were tested [1], [6]. To investigate the influence of the molecular weight of lignin, indulin was fractionated by ultrafiltration and the fractions prepared were partially alkylated and tested in several formulations. In addition, a high-speed homogenization approach was investigated. The tests were benchmarked against pure pMDI and also non-modified lignins.

Oxyalkylation

Technical lignins contain considerable amounts of aromatic and aliphatic hydroxyl groups, and can, therefore, be applied as a polyol in polyurethane formulations. However, direct application does not guarantee the desired behavior and acceptable adhesive performance. Therefore, it was suggested to modify lignin by means of an oxyalkylation reaction leading to the formation of so-called chain-extended hydroxyalkylated lignins [1]. This lignin modification uses a substitution of aromatic hydroxyls with an aliphatic chain carrying aliphatic hydroxyl groups. The latter are supposed to be more reactive in the reaction with pMDI than the original aromatic hydroxyls. Lignin oxyalkylation has already been confirmed to be effective in lignin-polyurethane synthesis [1].

The conventional approach to oxyalkylation involves a reaction with different alkylene oxides, such as propylene oxide [24], [25]. Necessary precautions upon handling the volatile, flammable, carcinogenic propylene oxide made this approach less attractive, especially considering an industrial perspective. However, recently it was proposed to substitute alkylene oxides with cyclic aromatic carbonates possessing low toxicity, high boiling point, biodegradability and other “green” chemical properties [26]–[28].

A range of lignin samples was oxyalkylated with propylene carbonate (PC) and glycerol 1,2-carbonate (GC) according to a procedure adopted from literature (Table 1 and 2, samples 1-9) [28]. The concept of this lignin modification for adhesive preparation was not just an increase of lignin reactivity, but also the simultaneous preparation of a more homogeneous liquid lignin polyol formulation.

Due to the formation of new OH groups, multi-oxyalkylation was reported to be a side reaction. The extent of this unwanted pathway strongly depends on the reaction conditions, i.e., catalyst, temperature, time etc. In practice, if the reaction conditions applied are not optimized for a specific lignin, not just an additional consumption of cyclic aromatic carbonate, but also a noticeable increase of the lignin´s molecular weight, which causes e.g., an undesired increase in viscosity, must be expected [29].

The analysis of the hydroxyl and carboxyl groups in the oxyalkylated samples was performed by means of ^{31}P NMR [17], [18]. The comparison of the originally present hydroxy and carboxy groups with their content and composition after oxyalkylation confirmed complete substitution of aromatic hydroxy and carboxy groups and a doubling of the necessary aliphatic hydroxyls in all lignins investigated (Table 1). At the same time, a decrease in the total hydroxy groups content indicated a lignin polymerization reaction during oxyalkylation. This observation was confirmed by analysis of the molecular weight distribution (Table 2) [19], [30].

Targeting the increase in reactive aliphatic hydroxyl groups in lignin, also a set of experiments on oxyalkylation of lignin with glycerol carbonate was performed. Due to the additional hydroxy group present in glycerol carbonate, the alkyl chain introduced into the lignin eventually contains two aliphatic hydroxyl groups. As anticipated, the content of aliphatic hydroxy groups was approximately two times higher than upon oxyalkylation with propylene carbonate (Table 1, samples 3, 6 and 9). Significant increase (approximately four times) of the molar mass showed the extent of the undesired polymerisation, which appeared to be about two times larger than upon oxyalkylation with propylene carbonate (Table 2, samples 3, 6 and 9).

Thus, the efficiency of the oxyalkylation of lignin with propylene and glycerol carbonate was considered successful and quite promising for certain applications. The concept was modified in order to im-

Table 1: *Content of hydroxyl and carboxyl groups (mmol·g^{-1}) in lignins before and after chemical modifications*

Sample		Aliphatic -OH	Aromatic -OH	-COOH
(1)	Indulin	2.16	3.56	0.30
(2)	Indulin (PC)	3.88	0.00	0.00
(3)	Indulin (GC)	5.55	0.00	0.00
(4)	Lignin-A	2.14	4.19	0.60
(5)	Lignin-A (PC)	3.42	0.00	0.00
(6)	Lignin-A (GC)	8.45	0.00	0.00
(7)	Lignin-B	1.74	4.37	0.63
(8)	Lignin-B (PC)	3.64	0.00	0.00
(9)	Lignin-B (GC)	5.70	0.00	0.00
(10)	Indulin homogenized in (PC)	2.49	3.88	0.24
(11)	Indulin methylated	0.17	0.05	0.00
(12)	Indulin methylated partially	1.43	0.23	0.02
(13)	Indulin ethylated completely	0.22	0.12	0.36
(14)	Indulin ethylated partially 90%	0.26	0.27	0.33
(15)	Indulin ethylated partially 80%	0.47	0.54	0.36
(16)	Indulin ethylated partially 60%	0.82	1.28	0.35
(17)	Lignin-B ethylated completely	0.15	0.12	0.53
(18)	Indulin isopropylated	1.00	0.13	0.02
(19)	Lignin-B isopropylated	0.60	0.12	0.10
(20)	Indulin acetylated	0.08	0.23	0.43
(21)	Lignin-B acetylated	0.07	0.21	0.38
(22)	Indulin U.F. >100 kDa	1.93	2.95	0.54
(23)	Indulin >100 kDa methylated partially	1.55	0.22	0.03
(24)	Indulin U.F. 100-30 kDa	3.02	3.62	0.40
(25)	Indulin U.F. 100-30 kDa methylated partially	1.30	0.22	0.05
(26)	Indulin U.F. 30-1 kDa	1.95	3.61	0.84
(27)	Indulin U.F. 30-1 kDa methylated partially	1.24	0.28	0.06
(28)	Indulin U.F. <1 kDa	1.57	3.49	1.29
(29)	Indulin U.F. <1 kDa methylated partially	0.79	0.18	0.02
(30)	LMW lignin-A	1.34	4.44	0.81
(31)	LMW lignin-A methylated partially 85%	0.34	0.50	0.51
(32)	LMW lignin-A methylated partially 50%	0.68	2.23	0.52
(33)	LMW lignin-B	1.17	4.05	0.66
(34)	LMW lignin-B ethylated partially 11%	0.75	3.89	0.10

Table 2: *Statistical moments of molar mass distribution of lignins before and after derivatization based on PSS calibration (note that all statistical moments are largely underestimated by this calibration).*

Sample		Mn	Mp	Mw	Mz	Mw/Mn
(1)	Indulin	170	1000	3900	17800	22.9
(2)	Indulin PC	1100	2800	17150	108400	15.6
(3)	Indulin GC	1700	7400	20500	110200	12.1
(4)	Lignin-A	100	750	2700	14800	27.0
(5)	Lignin-A (PC)	950	2900	7400	29000	7.8
(6)	Lignin-A (GC)	1200	3400	14800	118800	12.3
(7)	Lignin-B	950	2100	5200	20300	5.5
(8)	Lignin-B (PC)	950	2500	8800	42000	9.3
(9)	Lignin-B (GC)	1500	3800	19000	127000	12.7
(22)	Indulin U.F. >100 kDa	1923	6000	13500	82590	7.0
(24)	Indulin U.F. 100-30 kDa	1772	2740	3530	6050	2.0
(26)	Indulin U.F. 30-1 kDa	735	1190	1410	2325	1.9
(28)	Indulin U.F. <1 kDa	270	315	500	940	1.9
(30)	LMW lignin-A	480	1215	2031	6586	4.2
(33)	LMW lignin-B	393	473	1300	8600	3.3

prove the overall economy by avoiding a very high demand of carbonates. In general, a low lignin dosage combined with additional costs for carbonates will not be economically successful.

Further experiments have shown that under the reaction conditions recommended in the literature, the only viable ratio between lignin and propylene or glycerol carbonate is 1:2. A higher content of lignin is not possible due to the very high viscosity of the resulting mixture.

The oxyalkylation of lignin and the behavior of carbonates at the selected ratio were additionally investigated by means of a simultaneous temperature analyzer (STA). In those experiments the oxyalkylation reaction conditions were accurately simulated. It was shown that oxyalkylation and/or condensation occur also without the commonly used K_2CO_3 catalyst, just being driven by external heating. Similar TG and DSC patterns were observed for PC and GC blank reactions, which have suggested that the self-condensation reaction of propylene and glycerol carbonates may occur even at moderate temperatures (100ºC), especially in the presence of a catalyst.

Moreover, the presence of catalyst was found to play a crucial role with respect to the "pot life" of the final adhesive mixture. As it was observed, the presence of even minor amounts of catalyst in the final formulation with pMDI dramatically increased the curing rate, leaving nearly no time slot for the resin to be applied.

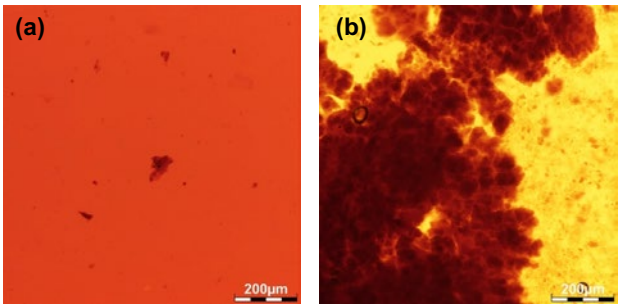
An attempt to run lignin oxyalkylation with propylene or glycerol carbonates without catalyst at a ratio 1:2 under otherwise identical conditions was tested. The products were isolated by precipitation in water and characterized by ^{31}P NMR (Table 1, sample 10). As anticipated, the content of lignin hydroxy groups has almost not increased, which indicated that lignin has experienced homogenization rather than the desired chemical modification. Meanwhile, based on STA experiments, the carbonates underwent self-condensation to certain degree, which eventually noticeably increased the viscosity of the polyol prepared.

Lignin Homogenization

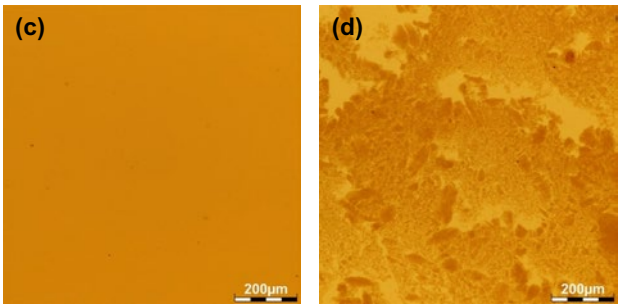
Application of a solvent is highly important not just for lignin homogenization, but also for the regulation of the viscosity of the final adhesive. At the same time, the selected solvent should not react with lignin or pMDI at ambient conditions before the adhesive is applied.

Experiments have shown that lignins can be successfully homogenized in various solvents, such as PC, GC, PEG and PEGDGE, even without application of long-term heating and mixing (Figure 1). Moreover,

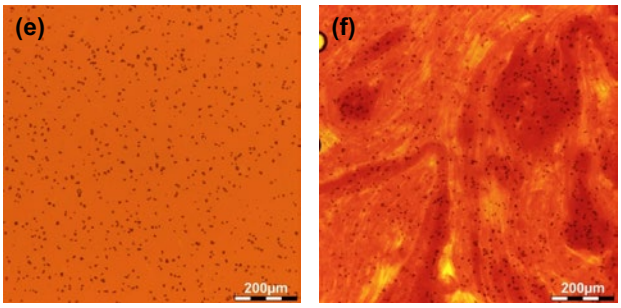
Indulin **(1)** in PC (left) and PC/pMDI (right)



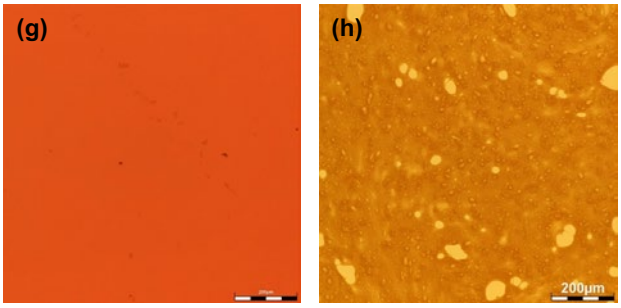
Indulin partially ethylated **(15)** in PC (left) and PC/pMDI (right)



LMW lignin-B **(33)** in PC (left) and PC/pMDI (right)



LMW lignin-B partially ethylated **(34)** in PC (left) and PC/pMDI (right)



LMW lignin-B **(33)** in pMDI (left) and LMW lignin-B partially ethylated **(34)** in pMDI (right)

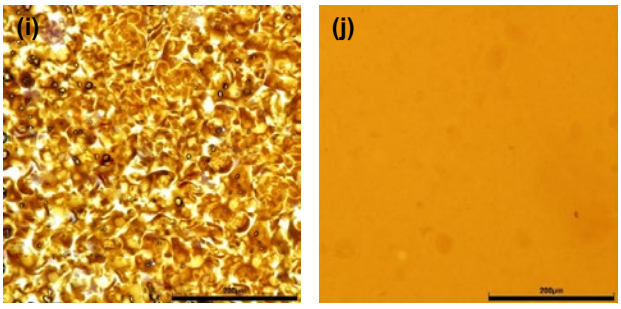


Figure 1: *Microscopic pictures of lignin samples being homogenized in the PC, PC/pMDI and pMDI.*

those solvents may additionally act as crosslinkers upon adhesive curing. However, not all of these solvents were found to be suitable for the preparation of pMDI formulations. For example, PEGDGE, being a very good solvent for kraft lignin, could not at all provide a stable homogeneous adhesive when lignin-PEGDGE was mixed with pMDI.

Lignin polyols were prepared by homogenizing lignins in a corresponding solvent at a 1:2 ratio, and then the lignin polyols were intensively mixed with pMDI at a 1:1 ratio and immediately the bonding strength after hot pressing was tested with bonding strength tester. Taking into account that the tensile shear strength of pMDI binders depends significantly on the press temperature and the moisture content of the veneers, those two parameters were strictly controlled at the optimized levels [13].

Though all tests showed fairly high tensile shear strength compared to just pMDI applied as sole adhesive, e.g., reaching 5.7 and 3.8 $N \cdot mm^{-2}$ in case of PC and GC, respectively, they also revealed that lignin polyol – pMDI formulations had a rather low shelf life and tended to react and re-slurry after short standing (Figure 1, a and b). This phenomenon negatively influenced the overall adhesive performance, bringing it to a state of unacceptably high viscosity, which to some extent would render the adhesive inapplicable in wood particle board production. Thus, it was concluded that the lignin polyol-pMDI adhesives required fundamental optimisation of the proportions, utilization of some viscosity reducers or application of specifically selected or chemically modified lignins.

Complete and Partial Alkylation of Lignin

It is known that lignins have amphiphilic properties mainly due to the more hydrophobic aromatic and aliphatic skeleton on the one side and numerous hydrophilic hydroxy, carboxy and other functional groups on the other side. It was necessary to investigate the level of influence of hydrophilic groups on adhesive preparation and behaviour. For this purpose, a hydrophobization of lignin by alkylation and acetylation was performed. It was expected that such modifications would not just improve lignin homogeneity in pMDI, but at the same time might increase lignin

reactivity due to its better dissolution and thus accessibility. Thus, lignin samples were modified by means of complete and partial alkylation with dimethyl, diethyl and diisopropyl sulfates (Table 1, samples 11-19) [21], [25], [31]. The degree of alkylation was controlled by the amount of the derivatizing agent and implementation of basic auxiliaries, NaOH and K_2CO_3. While NaOH allowed for complete -OH and -COOH group derivatization due to its high basicity, K_2CO_3 as a much weaker base permitted alkylation only at the more acidic aromatic hydroxy and carboxy groups.

In order to investigate if the alkyl chain length and branching have some influence on the lignin solubility, suspendability and eventually reactivity, a set of reactions with diethyl and isopropyl alkylating agents was carried out to obtain ethylated and isopropylated lignins, respectively (Table 1, samples 13-19).

Lignin modification by acetylation with acetic anhydride was selected as an alternative to methylation, providing completely derivatized hydroxy groups and improved lignin solubility in non-polar solvents (Table 1, samples 20-21) [32]. Acetylation was further used to confirm the blocking of hydroxyl groups and thus improvement of hydrophobic interactions in the pMDI system.

Both complete and partial lignin alkylation as well as acetylation made the lignins noticeably more suspendable and miscible in polyols, such as PC and GC, which allowed a significant reduction of the PC portion (Figure 1, c, d, g, h and j). Moreover, it was observed that already the partially alkylated lignins start to completely dissolve in just pMDI at quite high proportions (Figure 1, j). Completely ethylated and partially isopropylated lignins required some short-term, moderate heating (e.g. 50ºC) to facilitate dissolution in pMDI.

Based on previous experiments, PC was chosen for lignin homogenization as the cheapest and most available reagent on an industrial scale. Modified lignin: PC polyols were prepared first in ratios of 1:2 or 1:1 and then mixed with pMDI in proportions of 1:1 or 3:1, which resulted in 20, 25 and approximately 37% of lignin in the final formulations ((lignin:PC):pMDI = (1:2):3, (1:2):1 and (1:1):2, (1:1):0.67), based on dry mass. The performance of the adhesives prepared was evaluated by means of tensile shear strength. Nearly all adhesive systems tested demonstrated significantly better performance compared to just neat pMDI. The performance of the partially methylated lignin similar or even slightly better than that of completely methylated lignin. The decrease of PC considerably raised the viscosity of the adhesives, but did not influence the strength of the binder as much as the amount of pMDI. The average tensile shear strength was approx. 7.8 and dropped by about 25% down to 5.9 $N \cdot mm^{-2}$, when pMDI was reduced two times.

The performance of the adhesives containing ethylated lignins was quite similar to that with methylated lignins. It was confirmed that the partially ethylated lignins formed homogeneous and stable adhesive mixtures which had a relatively low viscosity, on average ~1200 mPa·s, and therefore they were much more convenient to be applied on the wood surface. The tensile shear strength of the freshly cured adhesive with (lignin:PC):pMDI ratio (1:1):2 clearly demonstrated that approximately 60% masking of the hydroxy groups is already sufficient to form a homogeneous formulation providing even higher tensile shear strength than that obtained with completely ethylated lignin (Table 1, Figure 2).

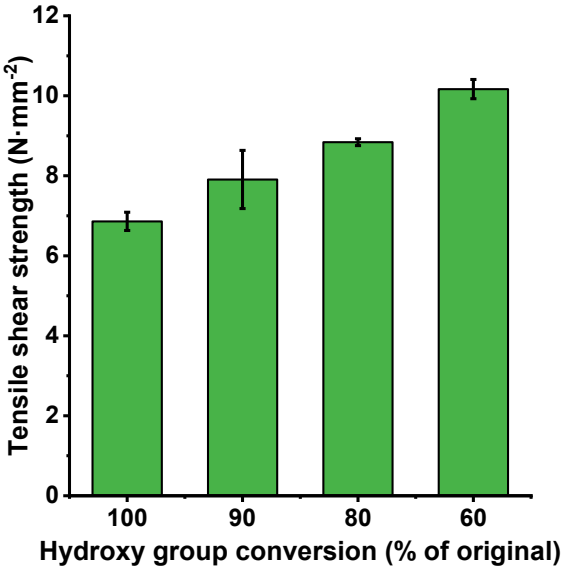


Figure 2: *Evaluation and comparison of adhesive performance containing perethylated and partially ethylated Indulin with (lignin:PC):pMDI ratio (1:1):2*

Compared to methylated and acetylated samples, the isopropylated lignin – PC – pMDI formulations obtained (at the same ratios used with other lignins) showed a comparably low viscosity, on average approx. 600 mPa·s. The tensile shear strength was quite similar for all lignins and similar to that obtained with methylated and ethylated lignins.

As anticipated, lignin acetylation, which allowed complete derivatization of all hydroxy groups, confirmed the positive effect of hydrophobization. All adhesive systems containing acetylated lignins with (lignin:PC):pMDI ratio (1:1):2 showed a very good lignin homogenization capability and formation of stable formulations in PC and pMDI with comparably moderate viscosity (1300 mPa·s), similar to that obtained with ethylated lignins. A subsequent testing of the tensile shear strength demonstrated high strength values for all adhesives, which in average reached 7.4 $N \cdot mm^{-2}$ at 100% wood failure.

Though alkylation and acetylation of lignin as pretreatment steps prior to adhesive preparation cannot be applied in a large-scale production due to the high price of reagents and a possible malodorous smell of the product, they unambiguously indicated the positive effect of the modification of hydroxyl groups on the viscosity of the pMDI adhesive system.

Fractionation of Lignin

One of the most crucial lignin characteristics that influences solubility and reactivity is the molar mass and its distribution. In order to verify the influence of lignin molecular weight on its homogenization in a solvent and further in pMDI, and to estimate the efficiency of the corresponding adhesive, several lignin fractions out of Indulin with relatively narrow molar mass distributions were prepared.

There are several techniques available for lignin fractionation [22], [33]–[35]. Ultrafiltration through a membrane of known cut-off can be considered as one of the most powerful and straightforward methods. The average yields of fractions >100, 100-30, 30-1 and <1 kDa determined in two parallel experiments were 43.2, 22.6, 12.7 and 5.7% of dry mass of starting Indulin sample, respectively.

Size-exclusion chromatography of the fractions confirmed an efficient fractionation and a narrow molar mass distribution (Table 1 and 2, samples 22, 24, 26 and 28) [19], [30]. As anticipated, the determined molecular weight distribution of the lignin fractions did not perfectly correspond to the molecular weight cut-off of ultrafiltration membranes applied, however, a strong correlation was evident [22]. Also, as expected, the content of hydroxyl groups changed depending on the change in molecular weight (Table 1 and 2) [22]. This was especially evident in the case of the fraction smaller than 1 kDa. The lignin fractions obtained were further tested in various formulations with pMDI.

A set of lignin fractions prepared was additionally modified by means of partial methylation as described before. Analysis of functional groups confirmed a nearly complete methylation of aromatic hydroxyl and carboxyl groups and approximately 25-50% (depending on the molecular weight of the fraction) of aliphatic hydroxyl groups (Table 1, samples 23, 25, 27 and 29).

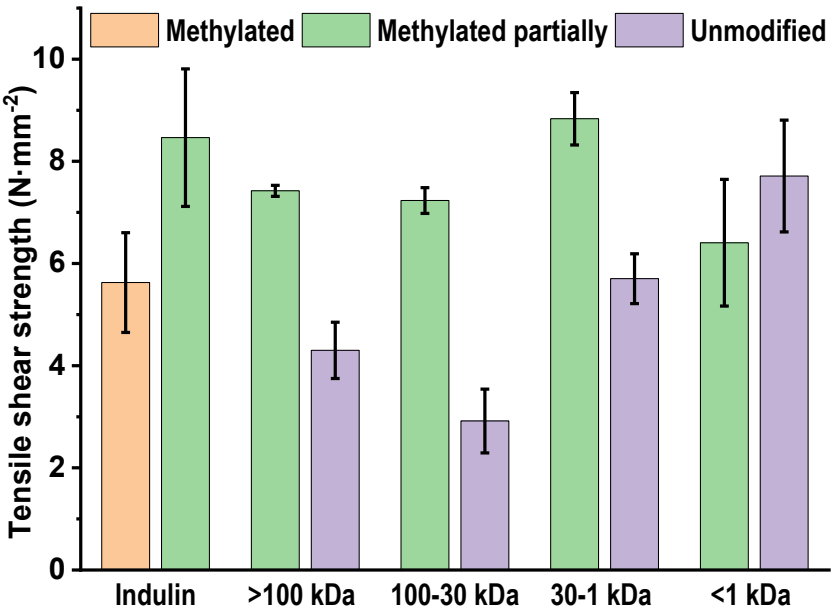


Figure 3: *Tensile shear strength determined for methylated and partially methylated unfractionated Indulin (ratio of (lignin:PC):pMDI (1:1):1), its fractions and partially alkylated fractions*

Indulin lignin fractions prepared directly after ultrafiltration and those after partial methylation were homogenized in PC at the ratio of 1:1 and then mixed with pMDI at the ratio of 1:1 (viz. lignin polyol: pMDI).

Good homogeneity in PC and then in pMDI was observed only in case of low molecular weight fractions below 1 kDa and 1-30 kDa, especially after partial methylation. All other fractions, when dissolved in PC, aggregated after pMDI addition, leading to phase separation and difficulties in applying them to the wood surface. Such behavior can be explained by the strong influence of lignin molecular weight on its solubility and insufficient dosage of the semi-polar PC solvent in the mixture. Nevertheless, it was possible to apply each adhesive formulation on wood to perform tensile shear strength test for bonding strength comparison (Figure 3). The tensile shear strength results showed very high values for the Indulin reference. However, it must be emphasized that applying the reference formulation to a wooden surface resulted in a non-homogeneous, clumpy surface, which makes industrial implementation practically impossible with general equipment used currently in particle board production. Only with the partially methylated lignin, a uniform application of the lignin-pMDI resin became possible and satisfactory strength values were achieved.

The low-molecular weight lignin fractions after partial methylation showed the best performance in the binder. The results for partially methylated lignin represent a significant improvement of adhesive formulation performances. This effect was much larger for the high molar mass fractions.

However, it was quite important to further corroborate the effect of complete or partial alkylation of lignin and the influence of molar mass on real industrial lignin samples. Various technical lignin samples supplied from different pulp mills were screened to match the desired low molecular weight. LMW lignin-A (30) had a weight-average molecular weight of approximately 2000 $g \cdot mol^{-1}$ (Table 2). A good miscibility in PC and then in pMDI was observed after partial ethylation (85 and 50% hydroxy group conversion), offering adhesive formulations with reasonably low viscosity of 1400 mPa·s. The strength test demonstrated that both adhesives provided high tensile shear strength of up to 7.7 $N \cdot mm^{-2}$ with 100% wood failure and confirmed that for such LMW lignin-A already 50% of hydroxy group derivatization was sufficient to achieve adhesive with good performance.

Particle Board Pressing and Testing

Though ethylation of lignin with diethyl sulphate might be considered less attractive as a pre-treatment step prior to adhesive preparation in the light of modern biorefinery and "green chemistry" approaches, it was highly important to evaluate the influence of the lignin alkylation degree on the lignin-PC-pMDI adhesive performance, to be tested by the most realistic method, i.e., particle board pressing itself.

Experiments on modified lignins homogenization in PC and then in pMDI at previously optimized ratios clearly demonstrated that complete alkylation was not required and that in the case of implementation of LMW lignin it was possible to achieve a good compromise between lignin molecular weight and its hydrophobicity, sufficient to allow preparation of a homogeneous and stable adhesive formulation. Additional technical lignin screening allowed finding

Table 3: *Formulation tested and particle board characteristics*

Formulation		Ratio	Adhesive amount	pMDI amount	Average board density	Average internal bond strength
			% w/w on dry wood	% w/w on dry wood	$kg \cdot m^{-3}$	$N \cdot mm^{-2}$
pMDI Reference (1)		-	4	2	621	0.51
pMDI Reference (2)		-	2	4	626	0.39
(a)	pMDI:PC	3:1	4	3	659	0.66
(b)	pMDI:PC	1:1	4	2	682	0.57
(c)	pMDI:PC:Indulin partially ethylated	1:1:1	4	1.3	629	0.52
(d)	pMDI:PC:LMW lignin-B ethylated (partially 11%)	1:1:1	4	1.3	650	0.50

a sample LMW lignin-B (33) with even smaller molecular weight compared to the one (30) discussed above (Table 2). Thus, two lignin samples were prepared, a partially ethylated Indulin (15) and LMW lignin-B (34) with 80 and 11% hydroxyl group masking, respectively (Table 1, Figure 1, c, d, g and h). The effect of implementation of PC without lignin in the formulation was investigated (Table 3). All boards prepared surpassed the required strength criterion for P2 class boards (furniture and interior design in dry conditions). A two-fold decrease of applied pMDI reduced the bonding strength by 20% (Table 3). At the same time, it was observed that addition of even small portions of propylene carbonate increased adhesive bonding strength significantly. Though bonding strengths of adhesives containing lignins were somewhat lower compared to pMDI-PC formulations, it was possible to achieve a significant reduction of pMDI and PC (Table 3).

Conclusions

Our study has demonstrated a strong interconnection between lignin properties and the final pMDI adhesive formulation. It was shown that not every technical lignin would be appropriate in such an application. A wide range of technical lignin specimens was prepared and examined, and various chemical modifications, namely fractionation, oxypropylation, acetylation, methylation, ethylation and *iso*-propylation were evaluated. Obviously, not all common concepts discussed in the literature, regarding what the best properties of lignin applied in polyurethane formulations should be, were applicable.

Lignin oxyalkylation usually is intended to improve lignin reactivity towards isocyanate groups present, in particular in pMDI. However, our tests showed that this approach has the least attractiveness and application prospects in particle board production.

A more viable approach for a considerable reduction of pMDI in the particle board production has been proposed: utilization of technical kraft lignin and application of a cyclic carbonate, in particular propylene carbonate, as a solvent, diluter and reagent at the same time. A preferably low-molecular weight isolated kraft lignin can be easily dissolved in an equal amount of carbonate and then homogenized in pMDI. The formulations prepared for use as a binder in particle boards have shown superior properties and allowed noticeable reduction of pMDI consumption.

Another crucial issue studied within this research, which dramatically influences the overall adhesive performance, was the ratio of components and the way the adhesive system was prepared. Mechanical homogenization is a good alternative to the chemical pre-treatment of lignin and, in combination with the use of certain diluents, can provide formulations of acceptable viscosity.

Tensile shear strength tests and orientation experiments provided first insights how to utilize lignin in its genuine form in pMDI formulations. The decrease of pMDI demand while improving the bonding strength was confirmed to be possible, although with the need to use a solvent, such as propylene carbonate, to achieve the desirable homogeneity and improved viscosity. The results allowed a better grasp of ideas regarding what lignin characteristics should look like and which issues would be critical upon adhesive preparation.

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Appendix:
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Removal of Spa Oil Stains from Cotton and Lyocell Sheets as Assessed by Reflectance Analysis

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Abstract

Bedsheet fabrics used in wellness and spa sector are often exposed to staining by spa oils, which could lead to early exclusion of the fabric in case of permanent staining, or due to damages by repeated bleaching. In this study, the removal of spa oil from lyocell and cotton bedsheets after an industry-typical washing process was investigated by the means of reflectance analysis of the stained surface, showing apparent better removal of spa oil from lyocell bedsheet than from regular cotton.

Introduction

Spa Oil Application

A representative component for a typical spa massage oil is grapeseed oil [1]. Being a non-volatile unsaturated triglyceride, if deposited on fabric and not removed or decomposed it will, in theory, most likely persist as a liquid. Over time, it may oxidise or polymerise to form stiff inclusions within the fabric.

On fabrics, liquids that wet the fibres can wick substantially by moving between and through the fibres until the interfacial and capillary forces that push the wicking process come to balance [2, 3]. All in all, for a finite volume deposited on a large area of fabric, wicking slows over time. By allowing a dose of oil to spread through a fabric until wicking has effectively ceased, the fibre and oil approach a state of interfacial and capillary equilibrium, essentially ensuring that, barring the effects of the wash on interfacial tension, the liquid oil remains relatively localised.

Equilibration is an essential process also because continued wicking therefore indicates an excess of oil. In some sense, during wicking the oil is not optimally supported by the fibre and it is oversaturated. The excess oil is the most trivial to remove because wicking is the process of seeking a more stable state, and the effective fraction of oil not seeking this is relatively stable. Ergo any oil load must be allowed to equilibrate.

Washing Process

As oils frequently spread to cover other materials, a typical wash process begins by stripping the bulk of oils and fats with an emulsifier to expose other soils. These are hydrated by the main detergent load, which continues to emulsify some residual fats to a certain degree while the mechanical action of the wash breaks apart the soils. The detergent, as well as any suspending agents and continued mechanical action, together help suspend the soils so they do not redeposit. Any remaining soil traces on the fibre are bleached to make them colourless to the naked eye before a softener is deposited on the fibre to restore the skin-feel or other mechanical properties of the fabric. Any residual chemicals are then rinsed away, and the process is complete.

Reflectance, Vision & Oil

While the term "reflection" usually invokes the idea of mirror-like objects, *diffuse* reflection is the matte-effect: the scattering of light [4]. Most simple everyday objects show some combination of both. Fabrics are good diffuse reflectors because they contain a high proportion of tiny surfaces to cause chaotic reflections that add up to diffuse reflection. When a colourless liquid such as water or oil wicks into a white fabric such as cotton, it appears darker or greyer (or brighter, if held up to a light) because it is more transparent: the air spaces in the fibre are filled with liquid, reducing the efficiency of those reflections so that light passes through instead of being reflected. Reflectance is a measurement of the relative intensity of light that is diffusely reflected from a surface. By measuring the reflectance at a set of wavelengths relative to some standard material, we obtain a reflectance spectrum.

The CIELAB unifying colour space breaks colours down into three component axes of human perception: L (lightness scale), a (green-red axis) and b (blue-yellow axis). The value of L, for colourless or near-colourless materials ($a \approx b \approx 0$), corresponds almost perfectly to its reflectance [5]. For white, grey and black materials, a reflectance spectrum will appear flat, measuring a constant (or near-constant) value of reflectance in the visible range of wavelengths. Therefore, when measuring the reflectance of white fabrics with and without oil, the reflectance directly quantifies the perceived lightness of the fabric and, consequently, the perception of cleanliness.

The means to convert between several colour measurement scales, including Lab and RGB, are well known and readily available in a variety of software packages, many of which are free. Therefore, in imaging, we can convert standard RGB images to Lab-approximation images and, extracting a monochrome image of L, begin to visualise impact.

Materials

Fabric bolts were provided by Lenzing. The fabrics are described in table 1.

Table 1: *Fabrics used for the study.*

Name	Description	/g m^{-2}
Cotton 100%	100% Cotton, bleached, mercerised, non-brightened fabric	130
Lyocell 100%	100% Lenzing Lyocell, non-brightened fabric	130

Experimental

Spa Oil Application

Grapeseed oil (0.5 ml) was applied along one short edge of a 5 cm by 20 cm swatch of each fabric and allowed to spread and equilibrate for 30 minutes. In this way, the oil was found to spread over no more than one half of the overall surface area of the fabric. The "oil-free" end of the fabric was a hemmed edge for reference.

Washing Process

All steps of the washing protocol maintained a constant dip level and mechanical action in a Girbau HS6013 industrial washing machine. Dummy load was 4.06 kg white PET, cottons and polycottons.

The washing and drying parameters are listed in table 2.

Table 2: *Parameters for drying and washing.*

Stage of Wash	T (°C)	T (s)	Chemicals	Notes
Emulsifier to Strip Oil	60	600	Premium Emulsifier, 15 ml/kg.	—
Main Detergent Wash	45	600	Atlas Pro, 15 ml/kg.	—
Peroxide Bleach	60	600	Hydrogen Peroxide, 10 ml/kg.	—
Softener	Ambient	300	Simply Soft, 10 ml/kg.	—
Rinse & Spin	Ambient	300	—	Spin cycle, max spin 60 s.
Rinse & Spin	Ambient	300	—	Spin cycle, max spin 300 s.

A Girbau ED340 industrial dryer ran for 30 minutes at an outlet temperature of 70 +/– 5 °C for 25 minutes to dry the load after each wash.

Reflectance Measurement & Analysis

An OceanOptics Maya2000Pro USB fibre-optic spectrometer was coupled to a HS2000 standard broadband white light source through a bespoke non-contact reflectance head. The spectrometer was controlled in MATLAB® with bespoke software. The reflectance standard was fused Teflon.

Reflectance spectra of fabrics were collected for pre- and post-wash samples. Relative reflectance is reported relative to a sample's oil-free region.

The mean reflectance value in the 750—795 nm region of the spectrum was chosen to represent the overall reflectance of a spectrum. The degree of recovery W = 1 – ((1-P)/(1-B)), where P is the post-wash relative reflectance of oily fabric and B is the baseline pre-wash relative reflectance of oily fabric. Thus, W takes values from 0—1 where 0 represents complete oil saturation and 1 represents levels of oil comparable to a blank.

The values of P and B directly convey the apparent brightness of an oily spot on fabric relative to its unstained surroundings.

Dye Contrast Photography

After washing treatments, fabric was dyed in a 1% aqueous solution of C.I. acid green 25, a water-soluble sulfonated anthraquinone dye, for 10 minutes before rinsing clear in deionised water and hanging up to dry. This dye has affinity for cellulose and is capable of dyeing fibre in spite of oil coverage, as demonstrated by the levelness of the dye after stripping the oil content with chloroform. It is insoluble in grapeseed oil, chloroform and mixtures of the two. The dye exacerbates the visual appearance of oil stains.

An oil-free section of the dry, dyed fabric was saturated with oil and the whole of each swatch was photographed under a halogen lamp shining through a white Teflon sheeting diffuser. The camera used a static white point and fixed ISO (automatic white correction disabled). The workup took place in GNU Image Manipulation Program 2.10.12. For the purposes of illustration, images were internally white-level-corrected using a mean value for the oil-free portion of fabric. Thus, any sample patches are made directly comparable. Sample patches were selected from the corrected images to represent the oil-free, washed oil and oil-saturated sections of fabric, converted to show the L-value of the CIELAB colour space.

Results and Discussion

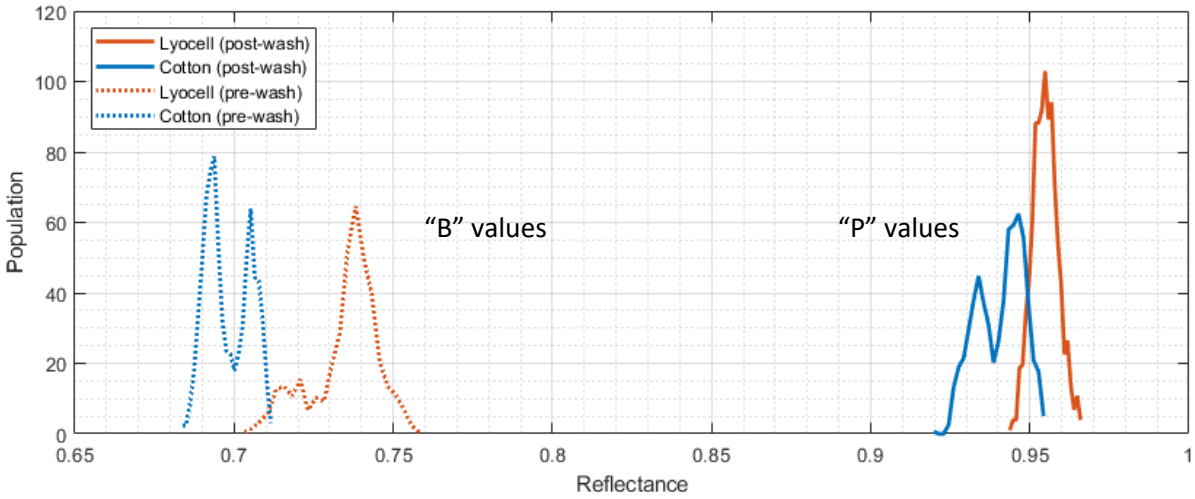

Figure 1: *Visualisation of the degree of recovery W, showing the lessened visibility of an oil stain on lyocell, relative to cotton.*

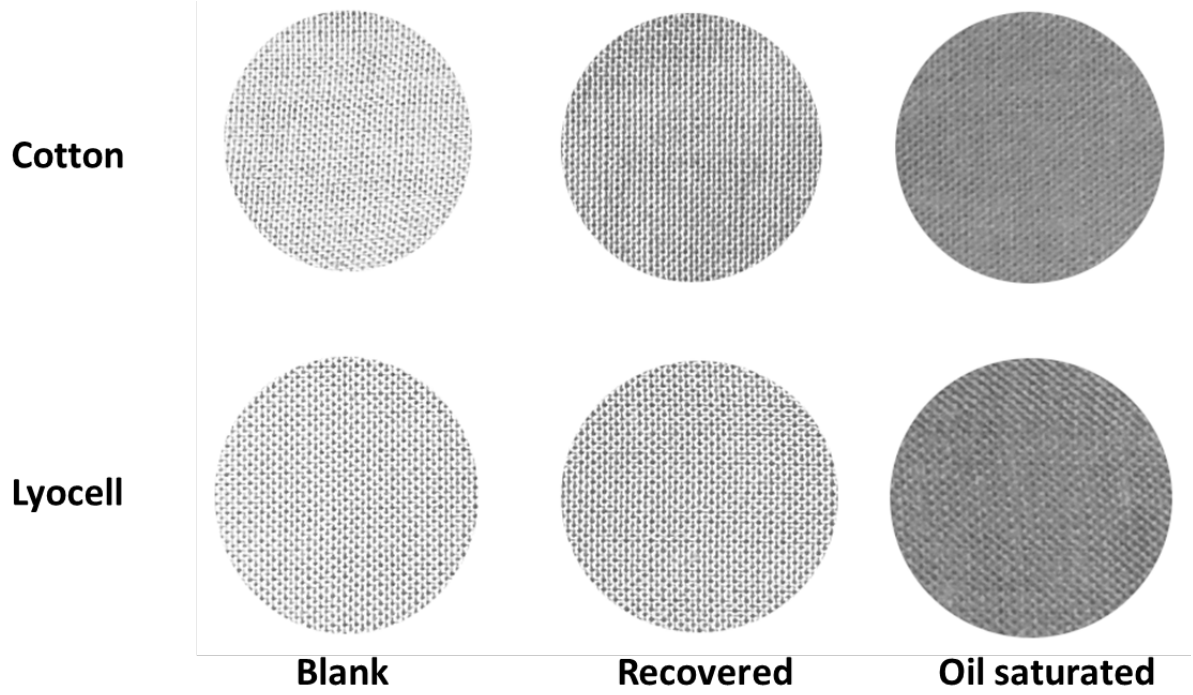

Figure 2: *L-values of cotton and lyocell fabrics. Compared to a fully saturated sample, a washed oil stain on cotton appears darker than an oil stain on lyocell washed under the same conditions.*

The degree of recovery W scales from 0 (no visible difference from oil saturation) to 1 (no visible difference from the blank). It averages 0.74 for cotton and 0.85 for lyocell. We can see in figure 1 that not only is W higher for lyocell than for cotton, lyocell both stains less visibly (higher "B" values, dotted traces on figure 1) and recovers better (higher "P" values, solid traces on figure 1).
The distributions of values in figure 1 are well-resolved, giving confidence that the values are distinct.

An imaging L-value is shown in figure 2. The L-imaging exacerbates the already-visible effect in a way that makes the presence of oil stains easy to capture and convey with a camera. After recovery, the Lyocell is consistently more similar visually and instrumentally to its blank than not, while the cotton standard is appreciably different. In reality, there is a visible "tide mark" of oil on both swatches.

The method provides an optical evaluation of the staining on the fabric surface, regardless of actual mass retention, i.e. the amount of oil still present in the fibre. It can be reasoned that pure lyocell could still give better consumer satisfaction in terms of "clean" perception than pure cotton in this application.

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Viscose and Lyocell Fibers from Bamboo Dissolving Pulp – A Scientific Review on Claimed Properties

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Abstract

There is quite some confusion at the market about so-called "bamboo fibers". Mostly, viscose and lyocell fibers from bamboo dissolving pulp are misleadingly named "bamboo fibers", in contrast to virgin and natural bamboo fibers. In the EU, USA and Canada, these products consequently have to be labelled as "viscose or rayon"; the appendix "from bamboo" is optional. This review of scientific literature enlightens the origin and properties of viscose and lyocell fibers from bamboo dissolving pulp in more detail. The mechanical fiber properties, UV≈protection and comfort properties are discussed, and the literature shows that they are not unique because they are comparable to those of regenerated cellulosic fibers from any other dissolving pulp. Differences in UV protection could be ascribed to inconsistent measurement methods. Especially, antimicrobial effects have been reported by producers and were attributed to the natural resistance of bamboo plants. No differentiation was made between bacteriocidal and bacteriostatic. Responsible bio-chemicals are mainly stored in the leaves and therefore, removed even before pulping. Several research teams disproved the fact of special antimicrobial fiber properties. They stated that a preservation of antimicrobial properties associated with the raw material is not very likely. In contrast, the literature gives evidence that variations in antimicrobial performance of the regenerated cellulosic fibers are more likely related to residual chemicals from the viscose production process, e.g. sulfur.

Introduction

The wording "bamboo fiber" is not defined nor consistently applied. Three different groups of fibers could be identified in the literature:

The first and dominant group comprises regenerated cellulosic fibers made from bamboo dissolving pulp. They have the biggest market share compared to other "bamboo fibers". They are produced in the viscose process. However, customers are confused and misled, because the wording is easily mixed up with natural and virgin bamboo fibers as described below. In the EU, USA and Canada, these products consequently have to be labelled as "viscose or rayon (from bamboo)" and no longer "bamboo fiber" (*Nayak and Mishra 2016*). These regulations have consistently been pursued e.g. by the Federal Trade Commission, USA or by higher regional courts in the EU. Several companies have so far been sued for using misleading wording. The same is true for lyocell fibers from bamboo dissolving pulp, although, they have a minor market share. Some bamboo-lyocell fibers offered at the market are even blended products and not lyocell fibers made from bamboo pulp.

Virgin and natural bamboo fibers are the second group. In contrast, these original bamboo fibers have only a minor market share. They are processed by a mechanical separation process like flax or hemp. Experts can easily distinguish natural bamboo fibers from viscose fibers because they are hollow, round, much smaller and contain only cellulose I in contrast to solid viscose fibers with their typical cloudy shape and cellulose II (see figure 1 and *Wang and al. (2010)*).

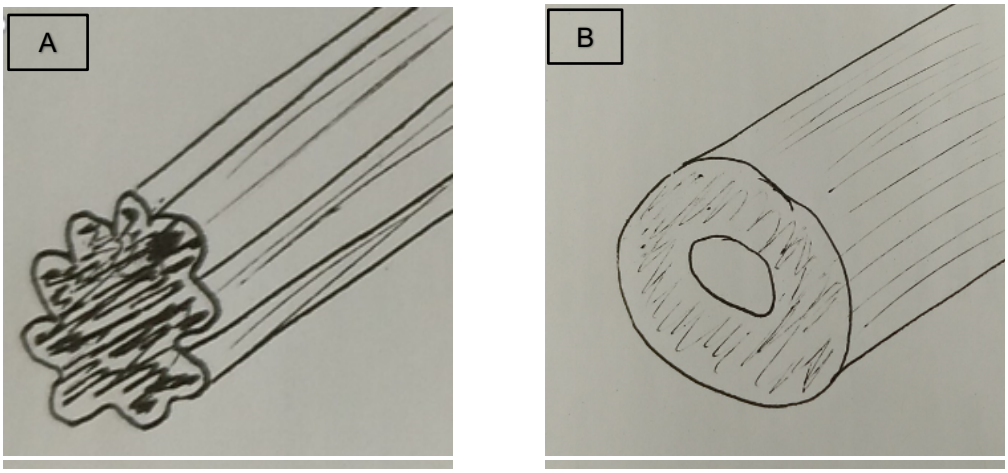

Figure 1: *Schematic drawing of the cross section of regenerated and natural bamboo fibers; A: shape of a viscose fiber from bamboo dissolving pulp; B: shape of a natural virgin bamboo fiber.*

The size of the two fiber types is similar in diameter, but very different in length: A standard viscose fiber with a titer of 1.3 dtex has a diameter of 10.5 μm and a length of 30 to 40 mm while a single isolated bamboo fiber depicts a diameter of 10 to 30 μm and a length of 1 to 4 mm *(Rusch et al. 2019)*. Their mechanical properties are about >50% less compared to the properties of viscose fibers from bamboo pulp.

A third group of so-called "bamboo fibers" are charcoal bamboo fibers. This composite fiber is produced by the addition of charcoal from bamboo to cellulose acetate production (e.g. by *Shanghai Tenbro Bamboo Textiles Co. Ltd,* China) or mixed with polyester (e.g. by Quanzhou Shengxin Fibre Co. Ltd, China). Charcoal is a by-product of the production of bamboo acetic acid. Bamboo carbon layers are often used for three layered fabrics for e.g. wetsuits. Astonishingly, Asian producers state that charcoal from bamboo has the same properties as viscose fibers from bamboo pulp with respect to their antimicrobial behavior. They attribute this to the organic extractives content of the plant. It is hardly convincing that the bamboo ash containing carbon and minerals should have the same properties as the non-burned plant. The burning of the wood surface, charring, is a very old procedure to improve durability especially in the contact zone with the ground for construction wood. Carbon and minerals of the ash contribute to an improvement of antimicrobial properties, more precisely a bacteriostatic effect, but they do not have a bactericidal effect. One may therefore deduce that the charcoal has a bacteriostatic effect that has nothing to do with the properties of the organic molecules of the original plant. The article of *Nayak and Mishra (2016)* provides a detailed description of all types of so-called bamboo fibers and their production processes.

Verification or disproof of claimed properties by scientific literature

What properties are claimed for viscose and lyocell fibers from bamboo?

Producers were identified by an internet search, and their claims on viscose and lyocell fibers made from bamboo dissolving pulp were analyzed. Interestingly, they all claim the same facts in a similar wording on their homepages, and they all attribute these properties to a non-specified sum of extractives of bamboo that are called "kun" or "khun". The properties are:

- Organically grown bamboo, eco-friendly
- UV protection
- Excellent moisture absorption and permeability
- Soft, smooth and good drapability
- Bright color and special luster; good dyeing properties and color fastness
- Thermal control
- Anti-bacterial and bacteriostatic
- Producers of lyocell fibers from bamboo claim the same properties and insist that they are superior to viscose made from bamboo and normal lyocell fibers.

Hereinafter, this list of properties was compared to results from scientific literature.

What is so special about the plant bamboo?

Bamboo is a grass and not a timber, endemic to all continents except Europe *(Nayak and Mishra 2016)*. Its many species depict a broad variety of properties, and its uses have a very long history and are plentiful from flooring to medical. In its natural environment, the plant itself shows many benefits like soil stabilization, water pollution treatment, carbon capturing, improving soil fertility and more. On natural sites, bamboo does not need any fertilizers, watering or replanting. The growth rate outraises wood by far. In tropical forests, the annual growth rate of bamboo is about 4 tons per hectare, in plantations, up to 20 to 36 tons per hectare. For comparison, the annual growth of a beech forest in Denmark is about 11 tons per hectare *(Scholz 2019)*, and it is constantly decreasing in Europe from the 1950s. Nevertheless, severe problems with fertilization and pesticides occur in any kind of plantations.

If there is any bactericidal or bacteriostatic performance of viscose fibers originating from bamboo pulp as claimed by the producers, these substances should be present in the raw material itself. Here we have to differentiate between the culms and the leaves of the bamboo plant. The bamboo wood or culm, which is the raw material for pulping, is classified class 5 natural durability according to DIN-EN 350-2. This is the lowest and less durable class. For instance, oak is categorized class 2 and spruce class 4. The classification describes the natural durability of the material against fungi that means that there is almost no fungistatic activity of natural bamboo *(Schmidt et al. 2015)*.

Extractives from the culm range from 3.4% – in average – to over 16% for the inner layer of the internodes of a certain bamboo species *(Wahab et al. 2013)*. There are plenty of bamboo species with a broad variety of extractives. More than 40 components have been identified in the literature. About half of the extractives are soluble in hot water and an additional portion is soluble in alkali, which means that they will not survive pulping. *Afrin et al. (2012)* investigated the natural bamboo plant and used 20% DMSO to xtract hemicelluloses, and 80% dioxane water for lignin extraction. They found weak antibacterial activity for the first sample characterizing hemicelluloses and high antimicrobial activity against *Escherichia coli* for the isolated lignin sample. The authors attributed this to the aromatic and phenolic functional groups of lignin in bamboo. It is well known that lignin in wood and annual plants has a general antimicrobial effect, and it is widely used as a natural preservative *(Espinoza-Acosta et al. 2016, Gabov et al. 2017, Dong et al. 2011)*. In general, bamboo shows a low lignin content, even lower than eucalypt. Published data range from 10.2% to 22.4% lignin for bamboo and 26.6% for eucalypt from Brazil *(Wang et al. (2010), Ribas Batalha et al. (2012)*), which was confirmed by the data of *Nayak and Mishra (2016)* for bamboo from Indonesia. This implies that the natural antimicrobial property of the bamboo culm must be low because of the low lignin concentration in the raw material, and furthermore, that it will be removed after alkaline pulping with its typical harsh conditions. Regarding the raw material, there is no indication that bamboo pulp, viscose or lyocell fibers should depict special antimicrobial properties that can be ascribed neither to the extractives nor to the lignin of the raw material of natural bamboo culms.

Nevertheless, bamboo is known as an allelopathic plant. This means that the plant produces biochemicals that work like natural herbicides and pesticides to protect the plant actively from diseases. The so-called allelochemicals are produced and stored in the leaves of the plant *(Rawat et al. 2017)*. Especially, younger bamboo plants develop a moderately strong allelopathic activity *(Ogita and Sasamoto 2017)*. This is the reason why no pesticides and herbicides are necessary for natural sites of bamboo. These extractives are used for medical or cosmetic purposes separated from bamboo leaves. All the same, leaves are not used for pulping; only culms are.

Producers argue that the claimed antimicrobial properties of the final product can be attributed to the extractives of the bamboo culm. This is obviously a misleading interpretation of the natural resistance against pests and the suppression of undergrowth because it results from the leaves that are removed during harvesting before pulping.

However, bamboo has a perspective for future uses, especially for non-wood pulping. The enormous growth rate, easy harvesting and the high cellulose content of 50%+ combined with a low lignin content make it a promising raw material, not only for dissolving pulp production. Nevertheless, major drawbacks for dissolving pulp production are the high ash content of up to about 2%, a silica content up to about 1.6% of the raw material and disintegration problems

of the nodes. In some areas, harvesting can only be carried out seasonally because of the monsoon. All the same, the benefits make it worth to work on these challenges.

Which are the mechanical properties, UV protection factor and comfort properties of viscose fibers from bamboo pulp?

The U.S. Federal Trade Commission (FTC), an autonomous government organization, measured Fourier Transform Infrared Spectroscopy (FTIR) of conventional viscose fibers and viscose fibers from bamboo pulp. The spectra matched identically showing that there is no chemical difference between viscose fibers from bamboo or wood pulp. They state that the cellulose is the same *(Nayak and Mishra 2016)*.

Mechanical Properties

Mechanical properties of viscose and lyocell fibers from bamboo pulp have been compared to conventional viscose and lyocell several times. Table 1 gives a comparison of different literature data. *Erdumlu and Ozipek (2008), Lipp-Symonowizc et al. (2011)* and *Büyükakinci (2010)* demonstrated that normal viscose and viscose from bamboo pulp showed comparable strength properties. The strength properties are more related to the production process, the molecular weight of the pulp and its distribution. The same was confirmed for lyocell fibers from bamboo compared to lyocell fibers from wood by *Yang et al. (2009)*.

UV Protection

Gambichler et al. (2001) investigated the UV protection of a broad variety of fabrics and found a UV protection factor UPF of more than 70% for wool, polyester, and fabric blends, and only less than 30% for cellulosic fibers like cotton, linen, and viscose fabrics. Naturally, the color of the fabrics showed a great influence: fabrics with black, navy-blue, white, green, or beige colors provided a higher UPF. The authors as well complained about the non-standardized testing procedure, which renders the comparison of results from different research groups very difficult. *Hatua et al. (2013) and Mishra et al. (2012)* from the University of New Delhi, India, measured the UV protection

Type of fiber	Type of pulp	Titer dtex	Tensile strength cN/dtex	Elongation %	Literature
Viscose	Beech pulp	1.3	25	18.3	*Schild and Sixta (2011)*
Viscose	Eucalypt pulp	1.3	26	18.2	*Schild and Sixta (2011)*
Viscose	Wood pulp	1.7	25-31	14-18	*Bambrotex*
Viscose	Bamboo pulp	3.3	21	19.7	*Yang et al. (2009)*
Viscose	Bamboo pulp	3.1	16	16.8	*Erdumlu and Ozipek (2008)*
Viscose	Bamboo pulp	2.5	16	16.2	*Erdumlu and Ozipek (2008)*
Viscose	Bamboo pulp	2.0	15	15.3	*Erdumlu and Ozipek (2008)*
Viscose	Bamboo pulp	1.7	22-25	14-18	*Bambrotex*
Viscose	Bamboo pulp	Density 1.51g/cm^3	16	17.1	*Lipp-Symonowizc et al. (2011)*
Lyocell	Eucalypt pulp	1.3	36	13.5	*Schild et al. (2020)*
Lyocell	Eucalypt pulp	1.8	32	12.9	*Schild et al. (2020)*
Lyocell	Eucalypt pulp	1.4	41	12.8	*Schild and Sixta (2011)*
Lyocell	Wood pulp	2.8	35	8.6	*Yang et al. (2009)*
Lyocell	Bamboo pulp	2.9	36	9.3	*Yang et al. (2009)*

Table 1: *Literature data of mechanical properties of viscose and lyocell fibers from bamboo pulp and wood pulp compared with natural virgin bamboo fibers.*

of viscose fabrics made from bamboo pulp and compared it to a cotton fabric. They found the same UPF if the cover percentage and areal density of the two fabrics was similar. Comparable findings have been reported by *Gericke and Pol (2011)* and *Sarka and Appidi (2009)*. In contrast, an Indian producer of modal fibers and Chinese producers of viscose fibers made from bamboo dissolving pulp claim high UPF values of up to 97.5% plus *(Bambrotex 2003, Aditya Birla Group 2017)*. Scientists explain the apparently higher UPF of bamboo viscose fabrics by a higher cover percentage and areal density instead of bamboo's inherent UV protective property.

Comfort Properties

Gericke and Pol (2010) from the University of Stellenbosch, South Africa, investigated the comfort properties of fabrics made from viscose, cotton and viscose made from bamboo. Thermal resistance, water vapor resistance, water absorbency and moisture permeability showed very similar results (tab. 2). They could not find any evidence that viscose from bamboo was superior to any of the other fabrics tested. *Cimilli et al. (2010)* even favored regular modal and viscose over viscose from bamboo with respect to comfort properties of knitted socks.

Are there any antimicrobial properties?

What does antimicrobial mean? Bacteriostatic vs bactericidal

Per definition, bacteriostatic is something that prevents the growth of bacteria e.g. keeps them in the stationary phase of growth. In contrast, bactericidal means that it actively kills bacteria. Only a reduction of bacteria on a sample of close to 100% is considered as bactericide. Anti-bacterial or antimicrobial is a broader term referring to both, killing microorganisms and/or stopping their growth.

Internationally standardized testing methods exist and are widely accepted and used: Antibiotic Resistance and Sensitivity Testing of Bacteria from the American Type Culture Collection. ATCC was established in 1925 with headquarters in Virginia, USA *(ATCC 2021)*. It is a nonprofit organization, which collects, stores, and distributes standard reference microorganisms, cell lines and other materials for research and development. Taking into account differences in sample preparation and selection of bacteria strains, the standardized method usually allows a comparison of results.

Only very few producers of viscose or lyocell from bamboo pulp mention bactericidal effects. The majority refers to their fibers being anti-bacterial/antimicrobial and bacteriostatic. This may be used misdirecting because retail customers usually will not distinguish between killing and stopping of microorganisms.

What happens to extractives during pulping of bamboo?

The processes used for production of dissolving pulp from bamboo are mostly prehydrolysis kraft or soda/AQ pulping followed by conventional or elemental chlorine free bleaching, sometimes combined with enzyme treatment *(Yuan et al. 2017, Ma et al. 2011, Ribas Batalha et al. 2012)*. *Sugesty et al. (2015)* tested four Indonesian bamboo species for production of dissolving pulp in lab scale. The properties of the final bleached pulps were comparable to market pulps from wood. The ash content in the raw material ranged from 1.7 to 6.1% and dropped to 0.08% in the final pulp. The final ash content of the bleached pulp was very low, which may be due to a high water consumption as usual during lab procedures. The same happened to the extractives content; it decreased from 3.4 - 8.4% in the raw material to 0.06 - 0.09% in the pulp. A significant decrease was visible although different solvents had to be used due to the form of the sample. However, this article gives an important answer with respect to antimicrobial features of regenerated fibers:

Viscose fabric made of	Thermal resistance m^2K/W	Water vapor resistance m^2Pa/W	Water absorbency %	Moisture permeability index, Im
Wood pulp	0.181	21.19	2.16	0.52
Bamboo pulp	0.189	20.74	2.02	0.55

Table 2: *Measurements of comfort properties by Gericke and Pol (2010).*

The components said to be responsible for the antimicrobial properties are claimed to be part of the extractives. The extractive content was significantly lowered by pulping and bleaching to the level of market dissolving pulps. Therefore, extractives said to have an antimicrobial effect have been removed to a very high percentage by pulping and bleaching.

Components of the bamboo plant ensuring its natural durability are extractives and lignin. Their concentrations in the culm, the raw material for pulping, are low compared to wood. Furthermore, they are to a great extend removed during the production of dissolving pulp. In this point of view, there is no advantage of dissolving pulp from bamboo over wood. Additionally, no special component in bamboo pulp could be identified in the literature that would give rise to special fiber properties.

Is there a proof of the antimicrobial claims for viscose and lyocell fibers?

This is probably where the story of antimicrobial viscose from bamboo began: Bambrotex, part of *Hebei Jigao Chemical Fiber,* China, published test results for anti-bacterial effects from their own lab and from the *Japan Textile Inspection Association*. The Japanese tests showed a reduction of >70% of bacteria, while their own tests, which were only conducted with the single bacteria strain *Staphylocccus areus,* showed a reduction of >96.5%. While the Japanese results would stand for a bacteriostatic effect, the Chinese results would imply a bactericidal property *(Bambrotex 2007)*. Different companies argue that the antimicrobial effect originates from a non-specified group of extractives from the plant called “kun” or “khun” which is said to be tightly combined with the cellulose persisting throughout the production process. This may theoretically be true for natural mechanically processed bamboo fibers. In contrast, for regenerated fibers from bamboo pulp, the *U.S. Federal Trade Commission* (FTC) states that “once the cellulose is simply cellulose, the source cannot be differentiated” *(Nayak and Mishra 2016)*.

Some additional Chinese literature reported bacteriostatic or even bactericidal properties for regenerated fibers from bamboo dissolving pulp. Nevertheless, results are not consistent. *Yang et al (2009)* found an antimicrobial activity for viscose and lyocell fibers from bamboo compared to viscose from wood pulp, which showed no activity at all. The samples were washed with ethanol that probably did not remove chemical residues from the production processes, which might have caused the antimicrobial activity of the sample. Controversy, a researcher team from the Colorado State University, USA, tested 100% bamboo viscose knit single jersey with no previous finishing treatment from China. The sample showed only minimal antimicrobial activity towards *S. aureus* and *E. coli (Sarka and Appidi (2009)*). A second group *(Gericke and Pol (2011)*) found a similar, also minor antibacterial effect of both viscose fabrics from bamboo and wood pulp; both did not eliminate or prevent bacterial growth. They identified sulfur residues on their fabrics that are known to have an antimicrobial effect. This article is a strong indication that there is only a weak antimicrobial activity of viscose fibers no matter if they are made from bamboo or wood. In contrast, the bacteriostatic effect is obviously induced by residual chemicals from the process and not by the raw material. A bactericidal property was not measured in any of the literature.

Hardin et al (2009) were even more rigorous and accused the producers of market fraud. They investigated the antimicrobial behavior of a wide range of market samples from several fabrics and non-woven products from viscose made from bamboo from different suppliers in comparison to normal viscose from wood. Using the standardized test method from ATCC with three different types of bacteria, they found no antimicrobial activity in any of the samples. *Xi and Qin (2012)* finally gave a proof of the abovementioned assumptions. They demonstrated that natural bamboo fibers did not show any activity against bacteria, but regenerated fibers depicted up to 76% bacteriostatic rate against *Staphylococcus aureus*. They applied three different bacteria with the standard-test described by *Hardin et al (2009)* and used other annual plants as reference like jute and flax and antibacterial cotton. Xi and Qin used solvents typical for the separation of extractives like hot water, benzene and 1% NaOH during sample preparation. These results confirm that extractives from natural bamboo do not provide any antimicrobial impact. They argued: “The antibacterial performance of regenerated bamboo fiber may largely come from the use of a large amount of chemicals in manufacturing process.” The same arguments are valid for the lyocell process. Although, tradeKorea shows a brochure of *Fujian Hongyuan,* China, with documents for antimicrobial effects of lyocell fibers *(tradeKorea 2021)*. Additionally, *Acelon (2013)*, Taiwan, claims the antimicrobial properties of their lyocell fibers in the patent TW201437444A.

What patents have been filed?

It is an interesting approach to study the patent landscape because the data of patent claims have to withstand careful scrutiny. There are plenty of patents covering the application of bamboo viscose in textile blends or covering bamboo charcoal in multiple layered fabrics. Antimicrobial properties are mainly attributed to bamboo charcoal or to fibers with special antimicrobial treatment (addition of e.g. metals). Only six producers were identified holding patents that deal with the production of regenerated cellulose fibers from bamboo pulp (tab. 3). The listed patents represent patent families and not single patents. No patents for the production of viscose fibers from bamboo dissolving pulp are granted. The patents cover modal and lyocell fiber production only. Two of these patent applications have been withdrawn by the applicants (CN101857983A, CN102234849A), and four have been granted by the patent authorities (CN103556281B, CN100503907C, TW201437444A, AT505492B1). Out of these, only Acelon (TW201437444A) claims special properties for their lyocell fibers made from bamboo pulp. Bamboo pulp is mixed with coffee residues, dissolved and spun in NMMO. They list a "natural antimicrobial property of bamboo cellulose fiber with natural antibacterial, deodorizing and negative ion functions" in the description and in the claims. In example 3, the authors describe the antimicrobial property of the fiber as bacteriostatic, not bactericide. They used *Staphylococcus aureus* and *Klebsiella pneumoniae* from ATCC as test strains, but not an internationally standardized testing method nor an internationally standardized data analysis. Additionally, they only discuss the effect of bamboo and not the antimicrobial effect of coffee grounds, especially, because coffee grounds are widely known as home remedies for various pests in the garden and household.

Certificates

The labelling advertised in the internet is often elusive. Producers from China, India and Taiwan claim OCIA, FSC and OEKO-TEX 100 certificates. In their article "No Such Thing as Organic Bamboo Clothing", *The Epoch Times* marks the Chinese certificates for organic bamboo as false and even as global market fraud by referring to the *Organic Crop Improvement Association* (OCIA) *(Vos 2014)*.

The *Forest Stewardship Council* (FSC) issues a certificate for the wood production from socially and environmentally compatible forestry. OEKO-TEX® offers two certifications for textiles: OEKO-TEX® 100 for products and OEKO-TEX® 1000 for production sites and factories. OEKO-TEX® labels ensure that these fibers do not contain allergenic dyestuffs or other banned chemicals that are harmful to human health. Thus, FSC and OEKO-TEX 100 certificates maybe correct.

The *Global Organic Textile Standard* (GOTS) is a measure for textiles made from organic fibers. It includes textile processing, manufacturing, packaging, labeling, exportation, importation and distribution, but not the cultivation of the plant. Any kind of regenerated fiber is not organically grown and can therefore not be certified by GOTS. In a blended fabric with up to 10% regenerated fibers, the certificate may be used if the viscose is made from organically grown bamboo. In general, GOTS certificates are consequently not available for regenerated cellulosic fibers from bamboo or any other pulp source.

Conclusions and Outlook

There is quite some confusion on the market about "bamboo fibers", their origin and properties. This literature and patent review gives a scientific overview on so-called "bamboo fibers" and sums up the following facts:

Most so-called "bamboo fibers" are viscose fibers made from bamboo dissolving pulp like any other viscose fibers made from eucalypt or other wood dissolving pulp. They should not be mixed up with natural virgin bamboo fibers.

Although viscose fibers are simple to distinguish from natural fibers for an expert, customers are easily cheated by false information. Therefore, the product has to be named "viscose/rayon fiber (from bamboo)" in the EU, USA and Canada.

Mechanical properties, UV protection and comfort properties of viscose and lyocell fibers and fabrics from bamboo dissolving pulp are reported in the literature to be similar to viscose and lyocell fibers from any other dissolving pulp. Therefore, there is no unique selling position (USP) for viscose or lyocell fibers from bamboo pulp.

The producers attribute the so-called unique properties of fibers from bamboo pulp especially to the antimicrobial behavior of the raw material itself. Scientific literature shows that the natural resistance of

bamboo plants results from biochemicals produced and stored in the leaves, which are removed before pulp production. Only culms are used for pulping. Additionally, the harsh conditions during dissolving pulp production remove extractives and lignin from the raw material. A preservation of antimicrobial properties associated with the raw material was disproved by scientific literature.

The literature gives evidence that variations in antimicrobial performance of the regenerated cellulosic fibers are more likely related to residual chemicals from the production process like e.g. sulfur from the viscose process.

The same facts are valid for lyocell fibers from bamboo pulp. Although some sources report antimicrobial performance of lyocell fibers from bamboo, this seems very unlikely.

Certificates for organic fibers have been identified as frauds in the literature. GOTS certificates are generally not available for regenerated fibers of any kind.

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