

Valorization of different types of biomass via polyoxometalates to formic acid derivatives and desired solid products

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Abstract

This dissertation deals with the utilization of various biomass using polyoxometalate (POM) catalysts. Formic acid as well as its derivatives and solid valuable products like proteins from the ProFA process and cellulose from the POM-ionosolv process were produced. The solid value products depended on the starting biomass used. A cellulose-rich solid was produced from lignocellulosic biomass and a protein-rich solid from algae-based biomass. The first part of the dissertation deals with the production of cellulose-rich solids and formic acid or its derivatives. For this purpose, the lignocellulosic biomass was first fractionated using different methods (organosolv and ionosolv) which were subsequently compared with regard to further oxidation using POM catalysts. The ionosolv method proved to be a promising method as more formic acid could be produced. Using a tributylmethylphosphonium methylsulfate ionic liquid (IL) and methanol as co-solvent, a white, cellulose-rich solid with over 90 % glucan was successfully produced. Furthermore, the production of formic acid/formic acid derivatives using POM catalysts in different solvents was investigated. In particular, the solvent-catalyst interaction of the solvent best suited for the production of cellulose-rich solids was investigated using various analytical techniques. It was found that a POM-IL salt forms in the IL/methanol solvent *in-situ*, which has different selectivities to methyl formate as a formic acid derivative depending on the IL.

In the second part of the dissertation, the production of protein-rich solids from macroalgae was investigated using the OxFA (**O**xidation of lignocellulosic biomass to **f**ormic **a**cid) process. Different types of macroalgae (red algae (*Porphyra dioica*), brown algae (*Fucus vesiculosus*) and green algae (*Ulva fenestrata*)) as well as two different POM catalysts ($\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ and $\text{H}_8\text{PV}_5\text{Mo}_7\text{O}_{40}$) were screened. Applying the most promising combination, *Porphyra dioica* and $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$, an optimization study was performed using a Box-Behnken Design of Experiments. This allowed the formic acid yield to be increased from 8.6 % to 16.4 % in combination enabling a protein recovery of 59.5 %. In order to further increase the protein recovery, three promising extraction methods (alkali hydrolysis, ultrasound-assisted extraction and IL extraction) were tested and optimized. Ultrasound-assisted extraction was identified as the best method enabling a protein recovery of 87.2 %.

Abstract

The findings of this dissertation have helped to evaluate the possibilities of POM catalysts in biorefinery-like concepts and to further develop these concepts.

Zusammenfassung

Die vorliegende Dissertation beschäftigt sich mit der Nutzung von verschiedenen Biomassen in Kombination mit Polyoxometallat (POM) Katalysatoren. Hierbei wurden sowohl Ameisensäure als auch ihre Derivate und ein festes Wertprodukt hergestellt. Die festen Wertprodukte ergaben sich nach der eingesetzten Ausgangsbiomasse. Bei lignocellulotischer Biomasse wurde ein cellulose-reicher Feststoff hergestellt und bei algen-basierter Biomasse ein protein-reicher Feststoff. Der erste Teil der Dissertation beschäftigt sich mit der Herstellung von cellulose-reichem Feststoff und Ameisensäure/Ameisensäure-Derivaten. Hierzu wurde zunächst die lignocellulotische Biomasse mit verschiedenen Methoden (organosolv und ionosolv) fraktioniert und diese in Hinblick auf die weitere Oxidation mit POM Katalysatoren verglichen. Hierbei stellte sich die ionosolv Methode als vielversprechende Methode heraus, da mehr Ameisensäure produziert wurde. Mit Hilfe einer Tributylmethylphosphonium methylsulfat IL (ionische Flüssigkeit) und Methanol als Co-Lösungsmittel konnte ein weißer, cellulose-reicher Feststoff mit über 90 % Glucan hergestellt werden. Im Weiteren wurde die Produktion von Ameisensäure/Ameisensäurederivaten mit Hilfe von POM Katalysatoren in unterschiedlichen Lösungsmitteln untersucht. Hierbei wurde im besonderen auch die Lösungsmittel-Katalysator-Interaktion des für die Produktion von cellulose-reichem Feststoff am besten geeigneten Lösungsmittels mit Hilfe verschiedener Analytikmethoden untersucht. Es stellte sich heraus, dass sich im IL/Methanol-Lösungsmittel *in-situ* ein POM-IL Salz bildet, welches je nach IL unterschiedliche Selektivitäten zu Methylformiat als Ameisensäure-Derivat aufwies.

Im zweiten Teil der Dissertation wurde die Herstellung von protein-reichem Feststoff aus Makroalgen mit Hilfe des OxFA Prozesses (**O**xidation von lignocellulotischer Biomasse zu Ameisensäure (engl. **f**ormic **a**cid)) untersucht. Hierbei wurden verschiedene Makroalgentypen (Rotalge (*Porphyra dioica*), Braunalge (*Fucus vesiculosus*) und Grünalge (*Ulva fenestrata*)) und zwei POM-Katalysatoren ($\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ und $\text{H}_8\text{PV}_5\text{Mo}_7\text{O}_{40}$) getestet. Mit der vielversprechendsten Kombination, *Porphyra dioica* und $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$, wurde eine Optimierungsstudie mit Hilfe eines Box-Behnken Design of Experiments durchgeführt. Dadurch konnte die Ameisensäureausbeute von 8,6 % auf 16,4 % in Kombination mit einer

Zusammenfassung

Proteinrückgewinnung von 59,5 % gesteigert werden. Um die Proteinrückgewinnung weiter steigern zu können, wurde ein Screening und eine Optimierung von drei vielversprechenden Extraktionsmethoden (Alkalihydrolyse, Ultraschall-unterstützte Extraktion und IL Extraktion) durchgeführt. Hierbei wurde die Ultraschall-unterstützte Extraktion mit einer Proteinrückgewinnung von 87,2 % als beste Methode identifiziert.

Die Erkenntnisse dieser Dissertation haben dazu beigetragen die Möglichkeiten von POM-Katalysatoren in Bioraffinerie-ähnlichen Konzepten zu evaluieren und weiterzuentwickeln.

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Nomenclature

Acronyms / Abbreviations

AE	Alkaline extraction
AIL	Acid insoluble lignin
AIL	Aprotic ionic liquid
As	Arsenic
ASL	Acid soluble lignin
ASS-F	Atom emission spectroscopy-flame
B	Boron
C/S	Catalyst/substrate ratio
CAB	Cellulose acetate butyrate
C	Carbon
CCA	Cr ^{III} , Cu ^{II} , and As ^V
CCl	Chlorine chloride
CMC	Carboxymethyl cellulose
CNCs	Cellulose nano crystals
CNFs	Cellulose nano fibrils
CO ₂	Carbon dioxide
Cr	Chrome

Nomenclature

Cu	Copper
DMBA	Methanolic DMBA solvent [DMBA][HSO ₄]/MeOH 30 wt.%/70 wt.%
[DMBA][HSO ₄]	N,N-dimethylbutylammonium hydrogen sulfate
DoE	Design of Experiment
dw	dry weight basis
EC	Ethylcellulose
[Emim][DBPI]	Ethyl methyl imidazolium dibutyl phosphate
EtOH	Ethanol
EU	European Union
FA	Formic acid
FID	Flame ionization detector
FT-IR	Fourier-transformation infrared spectroscopy
GC	Gas chromatography
H ₂ SO ₄	sulfuric acid
[H ₁ Cim]Cl	1-methylimidazolium chloride
HEC	Hydroxyethyl cellulose
He	Helium
H	Hydrogen
HMF	Hydroxy-methyl furfural
HPA-2	Two-fold vanadium substituted Keggin structured polyoxometal- late H ₅ PV ₂ Mo ₁₀ O ₄₀
HPA-5	Five-fold vanadium substituted Keggin structured polyoxometal- late H ₈ PV ₅ Mo ₇ O ₄₀
HPA-Mn2	Two-fold manganese substituted Keggin structured polyoxometal- late H ₁₁ PMn ₂ Mo ₁₀ O ₄₀

HPA-Mn	One-fold manganese substituted Keggin structured polyoxometal- late $H_7PMnMo_{11}O_{40}$
HPA-VMn	vanadium and manganese substituted Keggin structured polyox- ometallate $H_8PVMnMo_{10}O_{40}$
HPA	Hetero polyanions
HPC	Hydroxypropyl cellulose
HPLC	High-performance liquid chromatography
HPMC	Hydroxypropyl methylcellulose
ICP-OES	inductively coupled plasma optical emission spectrometry
ILE	Ionic liquid extraction
IL	Ionic liquid
IMTA	Integrated Multi-Trophic Aquaculture
IPA	Iso polyanions
KOCH ₃	Potassium methoxide
LAP	Laboratory analytical procedure
MCC	Microcrystalline cellulose
MC	Methylcellulose
MeOH	Methanol
Mn	Manganese
Mo	Molybdenum
N ₂	Nitrogen (gas)
NaCMC	Sodium carboxymethyl cellulose
NaOCH ₃	Sodium methoxide
NaOH	Sodium hydroxide

Nomenclature

Nb	Niobium
NC	Nanocellulose
NMR	Nuclear magnetic resonance spectroscopy
NREL	National Renewable Energy Laboratory
O ₂	Oxygen (gas)
O	Oxygen (element)
OxFA	Oxidative production of Formic Acid
PDA	Photo dode array detector
pH	Pondus Hydrogenii, negative decadic logarithm of the hydrogen ion concentration
PIL	Protic ionic liquid
PI	Analogue pressure gauge
PIR	Digital pressure transducer
POM	Polyoxometalate
P	Phosphorous
<i>P</i>	Pressure (bar)
pTSA	Para-toluene sulfonic acid
RI	Refraction index detector
RuBisCo	Ribulose 1,5-bisphosphate carboxylase/oxygenase
<i>S</i>	Selectivity
SCXRD	single-crystal X-ray diffraction
SDS	Sodium Dodecyl Sulfate – PolyAcrylamid Gel Electrophoresis
Si	Silican
SOSSEC	Submersible Offshore Shellfish and Seaweed Cage

S	Sulfur
SWV	Square wave voltametry
<i>T</i>	Temperature (°C)
Ta	Tantalum
[TBMP][MeSO ₄]	Tributylmethylphosphonium methyl sulfate
TBMP	Methanolic TBMP solvent [TBMP][MeSO ₄]/MeOH 30 wt.%/70 wt.%
TCD	Thermal conductivity detector
[TEA][HSO ₄]	Triethylammonium hydrogen sulfate
TEA	Methanolic TEA solvent [TEA][HSO ₄]/MeOH 30 wt.%/70 wt.%
TIC	Temperature indicated control
UAE	Ultrasound assisted extraction
V	Vanadium
W	Thungsten
<i>X</i>	Conversion
XPS	X-ray photoelectron spectroscopy
XRD	Powder X-ray diffraction
<i>Y</i>	Yield

Chapter 1

Introduction

Global warming and the associated severe weather events are one of the greatest challenges of our time. With the Paris Climate Agreement in 2015, politicians from 195 countries declared their intention to limit global warming to 1.5 °C, reducing emissions and adapting to climate change and channeling financial resources in line with climate protection goals.^[2] 2024 the global average temperature was exceeded by 1.6 °C compared to the pre-industrial level.^[3] If the next years will follow this trend, the goal to reduce the global warming of 1.5 °C will fail and even the new threshold of 2 °C can not be realized. The Met Office forecast indicates that there is a 47 % chance that the next five-years average will exceed the 1.5 °C threshold.^[3] However, any limitation of greenhouse gas emissions will help prevent the earth from warming further and reduce the risk of irreversible tipping points.

The climate protection program of the German government has the goal to reduce the greenhouse gas emissions by 2030 to 119 million t CO₂-equivalents in the industrial sector and be greenhouse gas neutral by 2050.^[4] In 2022, the industrial sector was responsible for 164 million t CO₂-equivalents of greenhouse gas emissions. Hereby, the chemical industry contributes 5.2 million t CO₂-equivalents to Germany's ecological footprint in the year 2022 which is about 4 % of the total CO₂ emissions of the country.^[5] It is important to drive forward the transition to green chemistry in research and the development in order to reduce both CO₂ emissions from the chemical industry and the use of finite fossil raw materials. Biomass, as a renewable raw material, is a promising alternative. In October 2022, the Federal Ministry of Food and Agriculture presented the key points of a national biomass strategy. The strategy aims to ensure the sustainable, climate-friendly and resource-efficient production and use of biomass. This also means that waste and residual materials from the biomass produced should be used as efficient as possible.^[6]

Introduction

To this end, three guiding principles have been formulated:^[6]

- Prioritisation of material use,
- Prioritising multiple uses,
- Prioritising the use of the biomass fraction of biogenic waste materials.

This study contributes to possible solutions within the framework of the national biomass strategy.^[6] It examines different types of biomass, such as lignocellulosic and algal biomass, for their use as a source of cellulose or protein, and investigates the conversion of residues to formic acid. According to the first principle of the national biomass strategy^[6], this thesis investigates the use of biomass to produce platform chemicals like formic acid to bind the carbon in long term industrial goods or in cellulose and its further use. According to the second principle of the national biomass strategy^[6], the material stream which is traditionally used for energy generation within the cellulose production is now used on a material basis for the production of formic acid and therefore ready for a multiple use case. In case of the protein production the valorization of the carbohydrates as waste stream to formic acid brings a potential multiple use case. To investigate this ideas the present work is separated into two different parts:

- Using lignocellulosic biomass for the production of cellulose and formic acid derivatives,
- Using algal biomass for the production of protein and formic acid.

These parts are summarized in Figure 1.1. The first part of the present work is divided into two studies. One deals with the investigation of a two-step concept to produce formic acid and a cellulose-rich solid residue. Firstly, screening of an effective fractionation method of lignocellulosic biomass into cellulose-rich solids and a further fraction is investigated. In the second step, this fraction is converted to formic acid and its derivatives using polyoxometalate catalysts. In the second study, the fractionation with ionic liquids (IL) in combination with organic solvents like methanol for lignocellulosic biomass is investigated. The fractionation concept is combined with the oxidative catalysis using polyoxometalates (POMs) to produce *in-situ* methyl formate as formic acid derivative.

The second part of the present work includes a study investigating the production of proteins and formic acid from algal biomass using POMs. Hereby, the OxFA (**O**xidation of lignocellulosic biomass to **f**ormic **a**cid) process was optimized using a Design of Experiment (DoE) to maximize the amount of protein-rich solid residue and formic acid. The OxFA process is catalyzed by POMs to produce fromic acid under oxidative conditions from biomass. In

a second step the protein from this protein-rich solid residue was extracted using various extraction methods. Another aim of this study is to find the best combination of OxFA process as acid treatment and extraction method for producing proteins.

These two different parts of the present work investigate the possibility of using biomass for the production of the platform chemical formic acid or formic acid derivatives and a solid valuable product in one process maximizing the valuable output of the biomass valorization.

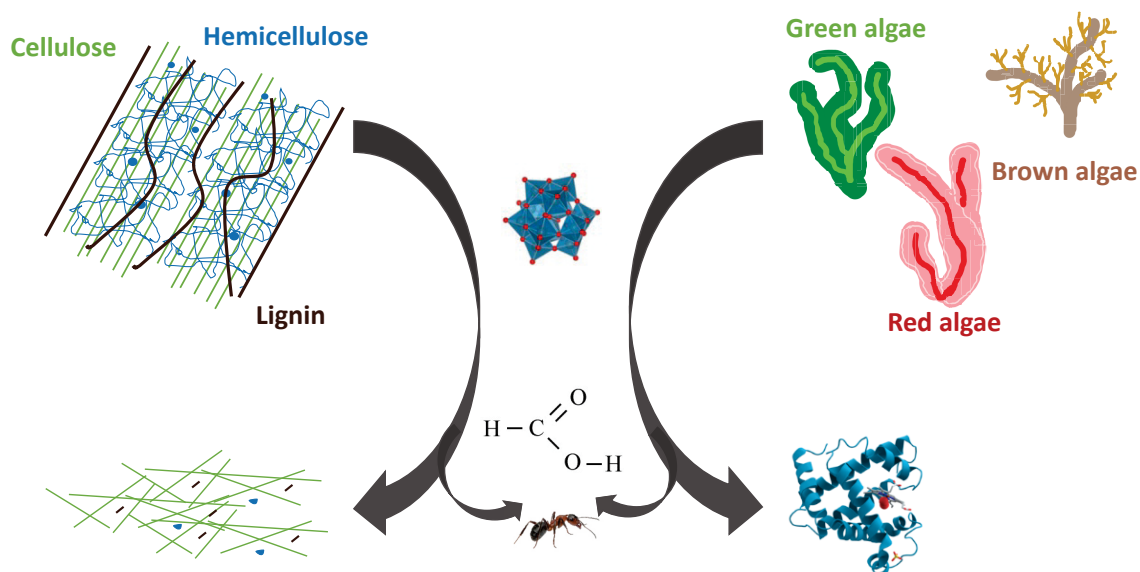


Fig. 1.1 Graphical summary of the thesis.

Chapter 2

Theoretical Background

This chapter presents the theoretical background of this thesis. Firstly, the various types of biomass that can be utilized as a source of renewable carbon and protein are elucidated (vide infra 2.1). Subsequently, the various biomass pre-treatment methods, including fractionation techniques such as ionosolv and organosolv, as well as protein extraction methods employing alkaline, ultrasonic, and ionic liquid extraction are addressed (vide infra 2.2). The third chapter addresses the class of catalysts employed in this study, namely polyoxometalates (vide infra 2.3). It elucidates their structure and properties, as well as the known processes they catalyze. The final section concerns the valuable products processed in this thesis (vide infra 2.4). On the one hand, the liquid product formic acid and its utilization are explained; on the other hand, proteins and cellulose as solid products, as well as their utilization are discussed.

2.1 Biomass as a Carbon and Protein Source

According to Kaltschmitt^[7], the term biomass covers in principle all substances of organic origin. This means that biomass includes plants, bacterias, fungi and animals, their residues and by-products, dead but non-fossil phytomass and zoomass such as straw as well as all substances of biogenic origin that have been created through technical conversion or material utilization. The latter includes paper and pulp, black liquor, slaughterhouse waste and organic household waste. Biomass is distinguished from the fossil energy source peat, the fossil secondary product of decay of organic matter, so peat is no longer counted as biomass.^[7]

Biomass can basically be divided into three groups: Primary, secondary and tertiary biomass. Primary biomass is created through the direct photosynthetic utilization of solar energy, i.e. this includes the entire plant mass as well as agricultural and forestry products or plant residues and by-products from agriculture and forestry. Secondary biomass obtains its energy

Theoretical Background

only indirectly from the sun, i.e. it is formed through the decomposition or conversion of organic matter in higher organisms (e.g. animals). This includes the entire zoomass and its excrement (e.g. liquid manure). Tertiary biomass is produced by one or more technical processing steps of primary or secondary biomass. This category therefore includes paper and pulp, wooden furniture, etc.^[7]

Biomass as abundantly available renewable resource can be used to generate several valuable products such as biofuels, biopolymers, platform chemicals etc. in a carbon neutral manner. Therefore, the greenhouse gas emissions are mitigated.^[8,9] Different types of biomass can be used. In the next two sections, two types of primary biomass are explained in more detail: Lignocellulosic biomass (vide infra section 2.1.1) as source of carbon, the building block for various chemicals and algal biomass (vide infra section 2.1.2) as source for proteins among other molecules as building block for biopolymers and as carbon source as well.

2.1.1 Lignocellulosic Biomass

Lignocellulosic biomass are plants and their components mainly consisting of varying proportions of the carbohydrate polymers cellulose, hemicellulose and the aromatic polymer lignin.^[10,11] These include hardwood, softwood and grasses, for example. Hardwood, in general, is used as term for wood from angiospermous trees and therefore deciduous species. Some examples are oak, maple, hickory, birch or beech. The term softwood, however, is used synonymously for wood originating from gymnospermous trees, which are coniferous trees. Some well-known trees of this category are pine, fir, spruce and juniper. Some deciduous species like poplars, willows categorized as softwoods when differentiated according to density. Furthermore, hardwood is denser, dries slower, lasts longer while burning and contains more total heat per volume compared to softwood. However, softwood has a higher heat content per unit of mass because it contains more resin than hardwood.^[12] The composition of the lignocellulosic biomass vary between the numerous species even depending on the climate, soil conditions and the location they are originated from.^[12,13]

The composition of softwoods is reported to approximately consist of 38 to 52 wt.% of cellulose, 15 to 30 wt.% of hemicellulose and 25 to 39 wt.% of lignin.^[14] In case of hardwoods, the proportion of cellulose is reported to be in the range of 40 to 55 wt.%, while the range for hemicellulose is 24 to 40 wt.% and for lignin, 18 to 25 wt.%. The cellulose content of different grasses, including wheat straw, miscanthus, and switchgrass, has been reported to range from 30 to 60 wt.% of cellulose, while their hemicellulose content is reported to range from 20 to 50 wt.%. The lignin content of these grasses is 12 to 30 wt.%.^[9,15] The chemical characteristics of the three main components of lignocellulosic

biomass are outlined in the following subsections. In order to provide a visual representation of the structure of lignocellulosic biomass, a schematic drawing is presented in Figure 2.1.

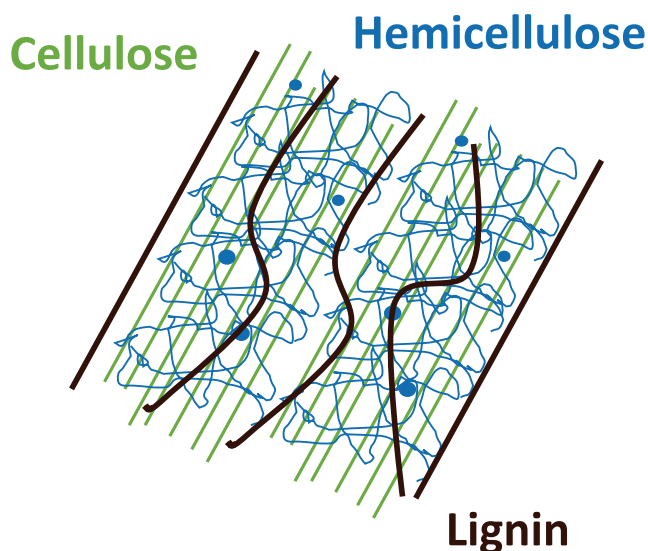


Fig. 2.1 Schematic drawing of the structure of lignocellulosic biomass.

Cellulose

Cellulose is one of the most abundant natural biopolymers found on earth.^[9,16–18] Cellulose is composed of D-glucose subunits linked by β -1,4 glycosidic bonds and plays a crucial role in strengthening and structuring the cell wall of plants. The structure can be seen in Figure 2.2. It consists of unbranched and parallel arranged long polysaccharide chains held together by inter- and intramolecular hydrogen bonds and van-der-Waals forces. The cellulose chains are aggregated together with a lateral dimension of 3-5 nm. These elementary fibrils are a bundle of cellulosic crystals along the fibril axis alternated with disordered domains. Bundle of these chains are known as cellulose microfibrils (shown as green lines in Figure 2.1) with a cross-sectional width of 5-20 nm and a length of several micrometers.^[19] This results in a rigid crystalline or amorphous structure. Depending on the cellulose origin and its treatment, the degree of polymerization ranges from 1000 to 15000. The degree of crystallinity also depends on the origin of the cellulose: Wood-based and plant-based cellulose usually ranges from 40 % to 60 % while cellulose from other origins shows higher degree of crystallinity between 80 % to 100 %.^[9,16–18,20–22] The origin of cellulose is not only lignocellulosic biomass, but also bacteria-based, algae-based, and tunicate-based cellulose exist.^[20] The cellulose chains are packed in layers as nanofibrils which are held together by hemicellulose and lignin matrix.^[20] Cellulose is not soluble in aqueous or organic solvents.

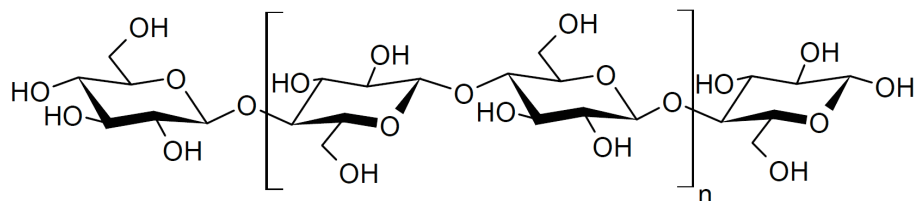


Fig. 2.2 Chemical structure of cellulose, according to Alonso et al.^[23]

Hemicellulose

Hemicellulose is capable of forming a cross-linked network of a heterogeneous mixture of pentoses and hexoses when it binds with cellulose microfibrils, lignin and pectin. Its structure can be seen in Figure 2.3. This occurs within the cell walls. Its composition varies in hardwood (mainly xylans and glucomannans) and softwood which contains glucomannans, xylans, arabinogalactans, xyloglucans and glucans. Together, these pentoses (xylose, arabinose) and hexoses (mannose, glucose, galactose) and sugar acids (4-o-methyl-glucuronic, galacturonic and glucuronic acids) form a complex carbohydrate. These sugars are linked to each other by β -1,4-glycosidic and β -1,3-glycosidic bonds forming a branched polymer (shown as blue lines in Figure 2.1).^[9,24–26]

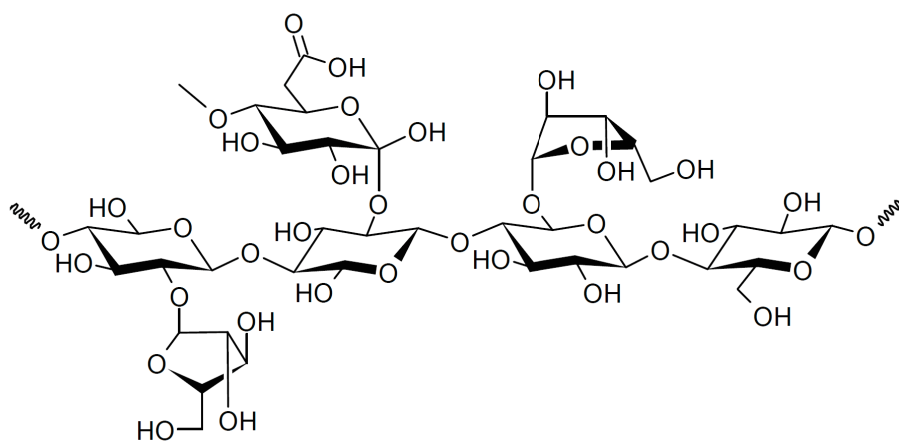


Fig. 2.3 Chemical structure of hemicellulose, according to Alonso et al.^[23]

Depending on the starting material, the main strand has different side chains that vary in type, frequency and position. These branches characteristically influence the physical and chemical properties of hemicellulose. Hemicellulose is highly soluble in alkaline solutions and easily hydrolyzed by acids. In plant cell walls, hemicellulose is partially covalently bound to lignin via the side chains and intertwined with the cellulose fibers.^[23,27,28]

Lignin

Lignin is a complex, amorphous hetero-biopolymer comprising phenylpropane units connected by carbon-carbon and aryl-ether linkages. The structure can be seen in Figure 2.4. Lignin is formed by the oxidative coupling of three monolignols: trans-p-coumaryl alcohol, trans-coniferyl alcohol and trans-sinapyl alcohol (shown as brown lines in Figure 2.1). This phenylpropanoid unit of lignin represents the main bottleneck to the depolymerization of lignocellulosic biomass, providing structural support, impermeability and protection against microbial invasion. Lignin is considered alongside cellulose to be the most abundant biopolymer in nature.^[9,16,29–31]

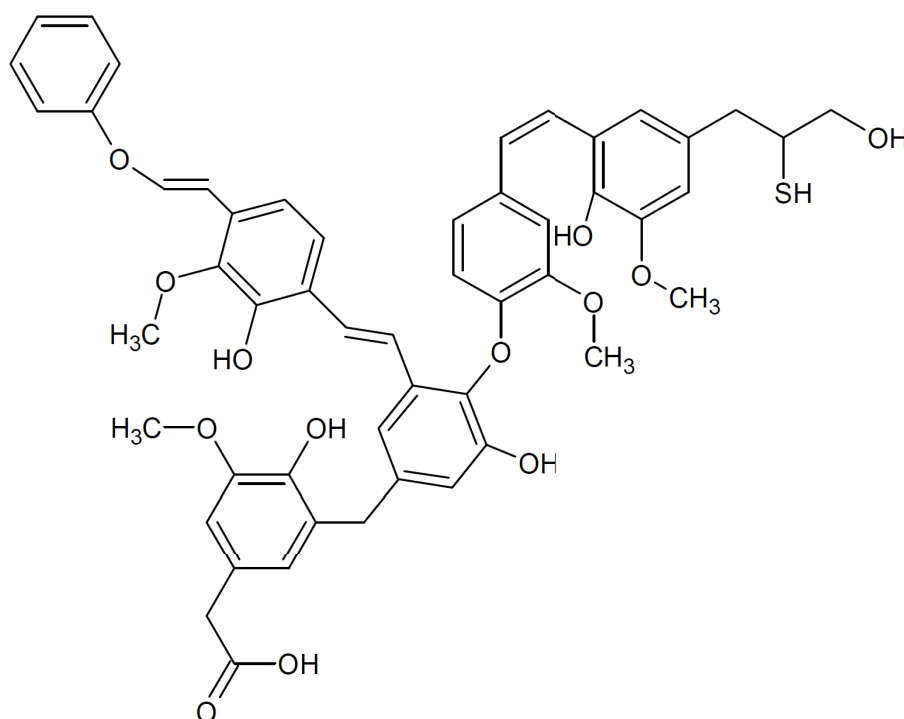


Fig. 2.4 Chemical structure of lignin, according to Alonso et al.^[23]

Annually, about 10 to 50 billion tons of lignocellulosic biomass is produced worldwide.^[9,32] Due to its complexity, lignocellulosic biomass is inherently recalcitrant to enzymatic conversion. Thus, the utilization of the various components of lignocellulosic biomass described above necessitates pre-treatment.^[33,34] The different pre-treatment methods are explained in Section 2.2.

Theoretical Background

Production of Lignocellulosic Biomass

This section deals with forest management and the associated timber production as well as the provision of stalk-like biomass. The global forest area is around 4 billion hectares, which corresponds to approximately one third of the global land area. Around 80 % of the total global biomass is found in forests, which contributes three quarters of global production.^[10,35,36] This means that the total living above- and below-ground biomass of forests amounts to 600 Gt or an average of $149 \text{ t} \cdot \text{ha}^{-1}$.^[10,37] In many parts of the world, natural forests are being degraded by unsustainable wood utilization or cleared by land-use changes. To counteract this problem, the pan-European criteria for sustainable forest management were formulated. These can be found in Table 2.1.^[10,35–39]

Table 2.1 Pan-European criteria for a sustainable forest management.^[39]

Criteria	Description
1	Conservation and appropriate enhancement of forest resources and their contribution to global carbon cycles
2	Maintaining the health and diversity of forest ecosystems
3	Maintaining and enhancing the production function of forests (i.e. timber and non-timber)
4	Maintaining, conserving and appropriately enhancing biodiversity in forest ecosystems
5	Preservation and appropriate enhancement of protective functions in forest management (especially soil and water)
6	Preservation of other socio-economic functions and conditions

Timber production is focused on high-quality trees, and diseased or inferior trees are removed during thinning. In a coniferous stand (usually softwood), the first thinning is carried out when there are no more green branches at a height of approx. 4 m. During these thinnings, wood chips and wood chippings can be obtained as an energy source. After several decades, mature trees are available, i.e. the diameter at breast height is approx. 50 cm for spruce, pine and larch, approx. 60 cm for beech and approx. 70 cm for oak in a deciduous forest. These trees are then harvested for end use in the furniture industry, as construction timber or in the paper industry.^[7,10,40]

There are also so-called short rotation plantations in mid-latitudes, which are planted on arable land for timber production. The rotation period of these plantations is between three

and six years. Furthermore, agroforestry systems are created by planting trees in strips between the fields in order to produce a timber harvest here as well.^[41,42]

Grasses such as miscanthus or the fully grown silphie are cultivated on arable and/or grassland areas. The harvesting technique differs little from the conventional harvesting technique for arable crops.^[7] The use of arable land means that energy grasses compete with food production on this land. However, agroforestry farming systems as a mixed system of crop and energy grass harvesting can improve soil fertility and diminish soil erosion on arable land. Such systems can be used for carbon capture. Additionally, the benefits of agroforestry to the water regime improve the yield of food crops as less wind on the ground lead to less evaporation and therefore more water for food crops between the agroforestry stripes.^[43]

Another research field for the climate friendly production of lignocellulosic biomass is the restoration of peatlands and wetlands which should lead to a new biomass source except from classical agricultural harvesting.^[43,44]

2.1.2 Algal Biomass

Algae are a diverse group of photosynthetic organisms living in marine and fresh water environments. Overall, the annual area-specific CO₂ assimilation rates of algae are up to five times higher than those of higher (terrestrial) plants.^[7,45] Algae can be divided into unicellular algae (microalgae or phytoplankton) and multicellular algae (macroalgae or filamentous).^[46–50] This biomass group covers about 55000 species and more than 100000 strains of marine, brackish, fresh water and terrestrial algae.^[47,51,52] In contrast to that broad diversity, only a dozen species are commercially cultivated worldwide.^[53] Macroalgae as comparatively large, multicellular and auxotrophic organisms belong to the lower plants.^[47–49] They are able to grow up to 60 m in length and are classified mainly into three groups: green (Chlorophyta), red (Rhodophyta) and brown (Phaeophyta).^[47,50,53,54] Microalgae as primitive forms of plants are unicellular or simple-multicellular photosynthetic microorganisms with a size of < 400 µm and a diameter of 1 to 30 µm.^[46,47,52] Besides the classification of algae according to their size, they can be divided into various groups based on the photosynthetic pigmentation variation. For microalgae, these groups are green, blue-green, red, brown and golden algae.^[46,47,54,55] Similar to lignocellulosic biomass, the pigment, growth rate, size, weight, and chemical composition of an algae is effected by their habitat environment (for example light, temperature, pH, salinity, nutrient, pollution, etc.).^[47,49,53] Macroalgae are sources of proteins and carbohydrates as well as various biologically active compounds such as carotenoids and phycobilins. The highest protein content is observed in red macroalgae (max. 47 % dw (dry weight basis)), followed by green macroalgae (max. 26 % dw) and brown macroalgae (max. 15 % dw).^[56] The carbohydrate content also varies based on the type of

Theoretical Background

macroalgae: Rhodophyta (30 to 60 % dw), Phaeophyta (30 to 50 % dw), and Chlorophyta (25 to 50 % dw). Typically, the amount of carbohydrates and proteins of macroalgae varies over the year. In winter, the protein content appears to be higher due to the low photosynthetic activity. This causes a lower production and storage of carbohydrates.^[57]

One of the most significant challenges associated with the utilization of macroalgae proteins is the complexity of their extraction. This is due to the fact that some of these proteins are present intracellular, while others are found along with polysaccharides on the cell wall. The scalability of macroalgae protein extraction processes and the necessity for environmentally friendly methods also restrict the applicability of these proteins. To prevent protein degradation, the macroalgae is typically utilized immediately after harvesting or drying, or frozen for subsequent use. A combination of extraction and purification techniques is commonly employed to obtain high protein yields.^[56]

In Asia, macroalgae are known as ‘nori’ (*Porphyra* spp.), ‘wakame’ (*Undaria pinnatifida*) and ‘kombu’ (*Laminaria saccharina*) and are used for the provision of food. Cultivation and harvesting of such macroalgae for human consumption are carried out manually on ropes / lines in so-called mari cultures, for example. In Europe, algae production (which has only been realized to a very limited extent) has so far mainly carried out manually via wild harvesting. In places near the coast, algae are used as fertilizer and animal feed. Today, hydrocolloids such as carrageenan and alginate are also separated from macroalgae and used in the food and pharmaceutical industries due to their gel-forming properties.^[7,58,59]

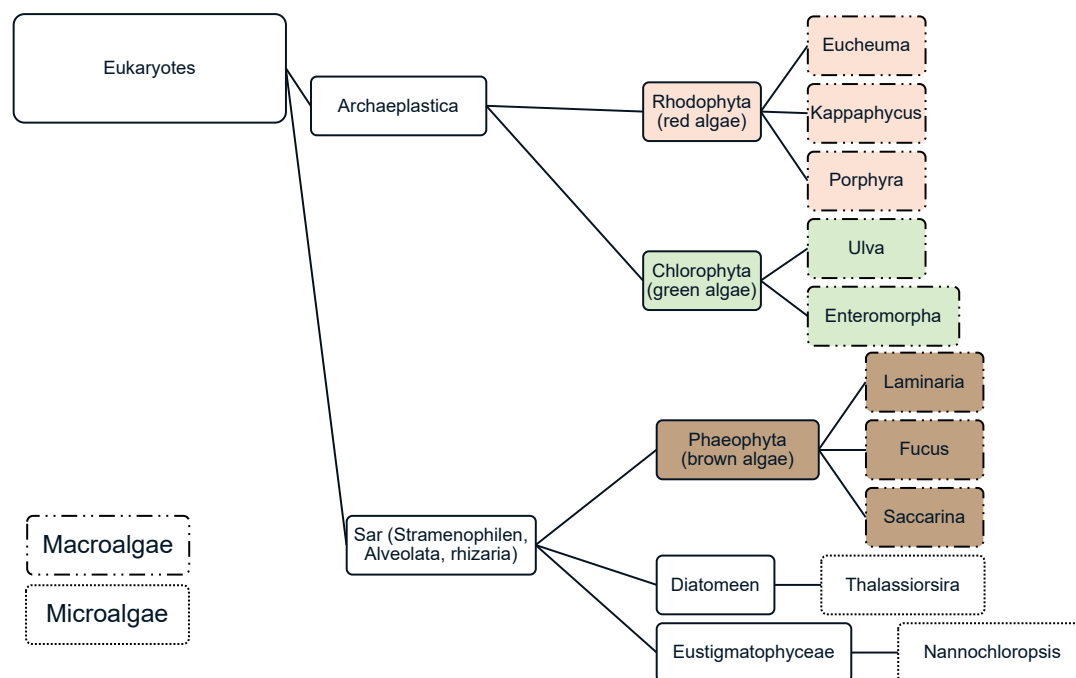


Fig. 2.5 Scheme of the different eukaryotic algae according to Kaltschmitt et al.^[7]

Most relevant for this thesis is the group of macroalgae highlighted in Figure 2.5. In the Figure, the different eukaryotic classes are shown. The three groups of macroalgae and the most common harvesting methods are described in more detail in the following subsections.

Green Algae - *Ulva fenestrata*



Fig. 2.6 *Ulva fenestrata* an example of a green algae.^[60]

Green algae (Chlorophyta or seaweeds) is a diverse group to which more than 1500 macroalgae species are assigned to. Currently, the output of green macroalgae aquaculture represents only a fraction of < 1 % of global aquafarming. Nevertheless, this aquaculture witnesses an increasing trend in productivity and commercial diversification over the last decades.^[61] Green algae are less sensitive to climate change than the other two macroalgal groups (red and brown algae).^[62,63] Seaweeds are known to have favorable harvesting properties and diverse polysaccharides to use them in biorefineries and thus enabling the production over a variety of sustainable products. One group of interesting species are *Ulva* spp. They are renowned for their opportunistic nature, characterized by high growth rates and the potential to cause green tides globally. However, their commercial utilization remains largely confined to direct food use.^[64,65] In this thesis, the green algae *Ulva fenestrata* (see Figure 2.6) was used as a substrate combining a high protein content of 15 % to 20 % on a dry weight basis and a wide range of polysaccharides like cellulose, starch, syloglucan, glucouronan, and ulvan.^[65–67]

Red Algae - *Porphyra dioica*



Fig. 2.7 *Porphyra dioica* as an example of red algae.^[68]

The Rhodophyta (red algae) are one of the most ancient and sizable groups of eukaryotic algae, with a registered total of more than 10000 species distributed across the globe. These algae are the most extensively cultivated and commercialized in the world, and are utilized as a source of food ingredients, hydrocolloids, fertilizers and animal feed, amongst numerous other applications.^[62,63] A prominent representative of Rhodophyta is the *Porphyra dioica* (Figure 2.7). This algae grows in shallow waters at moderate temperatures of 25 to 30 °C. The phycobiliproteins color the *Porphyra dioica* in their typical red color. Due to its high protein content, this species of red algae is one of the most extensively cultivated and economically significant macroalgae.^[69] The protein content of the *Porphyra dioica* is up to 47 % dw and the carbohydrate content about 40 to 50 % dw. The main carbohydrate present in the *Porphyra dioica* is porphyran.^[70,71]

Brown Algae - *Fucus vesiculosus*

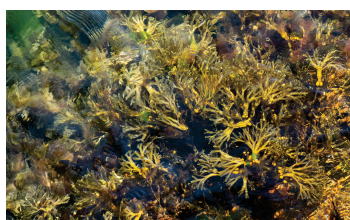


Fig. 2.8 *Fucus vesiculosus* as an example of brown algae.^[72]

Brown algae are a large and diverse class named Phaeophyta growing almost exclusively within marine habitats of cold temperate zones.^[62,73] The pigment fucoxanthin results in the characteristic color of these macroalgae. The applications of brown algae are mainly in food industry, cosmetic and pharmaceutical sector as the hydrocolloid alginate can be produced from them.^[62] The brown algae *Fucus vesiculosus* is used in this thesis as one of

2.1 Biomass as a Carbon and Protein Source

the most prominent brown algae of the genus *Fucus* (Figure 2.8).^[74,75] The natural habitat of *Fucus vesiculosus* is in cold to temperate waters along rocky shorelines of the Northern hemisphere.^[62,76] Alongside with the use in cosmetics, *Fucus vesiculosus* is applied in biofertilizer production, animal feed, and pharmaceutical industry.^[75] Fucoindans from the fucan family are polysaccharides and characterized as bioactive compounds unique to brown algae.^[77] Additionally, these algae are rich in fatty acids, proteins, and vitamins. The protein content varies between around 6 % to 10 % of dry weight.^[78,79]

Cultivation and Harvesting Methods of Macroalgae Biomass

There are various ways of producing macroalgae for commercial applications on an industrial scale, such as aquaculture or wild harvesting. In aquaculture systems, macroalgae are cultivated either on land in a tank or in open waters (nearshore and offshore). In principle, it makes sense to cultivate only native species in such aquaculture systems so that the impact on the ecosystem remains as low as possible. Primary production is only possible in the so-called photic zone, the upper layer of water that is flooded with light, as this is where effective photosynthesis can take place. This must be taken into account when cultivating on lines or grids, as the light penetration of the surface decreases with increasing biomass growth.^[7,80]

In the sea, the natural colonization of algae on specially laid lines can be exploited, but one disadvantage here is that this method cannot be expected to produce a homogeneous harvest, as different species will colonize. Alternatively, targeted cultivation can also be carried out with species immobilized and cultivated on ropes. These are first cultivated on ropes in an indoor breeding facility and later, after one to two months, transferred to an outdoor marine farm.^[7,81] There are various options for offshore seaweed production to withstand the harsh conditions at sea, which can cause entire systems to drift away or biomass to be torn off despite being anchored. These options include the longline system, which consists of lines that are attached with anchors and buoys. Another option is the offshore ring, where an approx. 5 m ring is attached to a buoy to tension the culture lines. There is also the Submersible Offshore Shellfish and Seaweed Cage (SOSSEC) system. This is a cultivation technique in which a lowerable steel cage is attached to the foundation of an offshore wind turbine for cultivation. This makes it easier to load it with organisms and lower it back down to the appropriate height. The cultivation method often used for the red algae *Porphyra dioica* is the Integrated Multi-Trophic Aquaculture (IMTA). This concept is based on the symbiosis of different species from different trophic levels. Meaning that fish, mussels and macroalgae can be cultivated in parallel. Ensuring economic and ecological sustainability because, for example, the different organisms can feed on each other's excrement.^[7,82–84]

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At present, the cultivated macroalgae are sown and harvested manually. Automation and further development of these processes is required in order to minimize production costs. Depending on the cultivation method, the macroalgae are harvested on land (rings) or offshore (lines). In the case of wild harvesting, the macroalgae are harvested mechanically with special boats or manually near the shore by divers from the existing stocks. With these techniques, however, the entire algae is torn from its substrate, thereby destroying the population. This represents a damaging intervention in the ecosystem. The subsequent regeneration time required can be four to five years (mechanical harvesting) and depends on the intensity of utilization and the harvesting technique. With manual harvesting, on the other hand, the ecosystem regenerates after one to two years.^[7,85]

After harvesting, the biomass must be cleaned of sand, stones, living organisms and other impurities such as plastic. As already described, the time of harvesting is essential for the composition of the biomass. After washing the biomass, it is dried, frozen or ensiled (preservation by lactic acid fermentation). This depends on the target application and species.^[7]

2.2 Biomass Pre-treatment Methods

Due to the complexity and diversity of different biomasses, it is necessary to pre-treat the biomass to make it accessible for either microorganisms, enzymes or catalysts in different processes. Pre-treatment is used to break down the biomass in order to release the various components for further utilization and conversion. In the case of lignocellulosic biomass, pre-treatment is used, among others, to fractionate the biomass into its basic components: cellulose, hemicellulose and lignin. For algae, pre-treatment is used to fractionate proteins, lipids, carbohydrates or pharmaceutically and cosmetically interesting products such as polyphenols, peptides or pigments.^[86]

2.2.1 Fractionation Methods for Lignocellulosic Biomass

Lignocellulosic biomass is a complex renewable plant resource mostly composed of polysaccharide cellulose, hemicellulose and the aromatic polymer lignin (vide intra Section 2.1.1). Cellulose, for example, is one of the most abundant renewable polysaccharides on earth and the main component of plant cell walls.^[9] This component has received great research interest as an environmentally friendly feedstock for synthesizing chemicals, biofuels or bio-based products.^[87,88] To use lignocellulosic components the most effective way, the raw material has to be fractionated. Several approaches of biorefineries show the idea of using all fractions of the lignocellulose separately. Biorefineries are the equivalent to petroleum refineries with the difference that biomass is the main resource used to generate chemicals, materials, and energy.^[89] This fractionation method is a crucial point in biorefineries as this effects significantly the technical and economic impact of the process.^[90] In general, biological, physical, and chemical methods can be applied to fractionate lignocellulosic biomass. Each method does have specific advantages and disadvantages which are summarized in Table 2.2. The aim of the pretreatment process is to reduce the crystallinity of cellulose, improve the accessible area for the extraction of desirable components to enhance the rate of hydrolysis after the pretreatment.^[91] Thus, the pretreatment disrupt the matrix of the lignocellulosic biomass.^[92,93] This enhances the accessibility of hydrolytic enzymes.^[92,94]

Theoretical Background

Table 2.2 Summary of advantages and disadvantages of different types of pretreatment methods for lignocellulosic biomass.^[95]

Pretreatment methods	Advantages	Disadvantages	References
Physical method (e.g. Milling, Steam explosion, Liquid hot water, Irradiation)	– Reduce particle size of biomass – Environmentally friendly	– Requires more Energy – Higher capital investment – Insufficient recovery of lignin	[96,97]
Chemical method (e.g. Acid, Alkaline, organosolv, ILs)	– Easily degrading lignocellulosic structure – Short pretreatment time	– Special equipment required – Some chemicals are toxic – Special disposal of chemical waste	[98–100]
Physiochemical method (e.g. Ultrasonic-assisted alkaline)	– Enhances recovery of biopolymers	– Consume energy – Requires more steps	[100,101]
Biological (e.g. Enzyme)	– Environmentally friendly – Saves energy – No addition of chemicals	– Longer pretreatment times	[100,102]

For this thesis the chemical fractionation methods are used as they easily degrade the lignocellulosic structure. The most common techniques for fractionating lignocellulosic biomass are alkaline and acid treatment.^[95,103] With acid pretreatment, mostly with sulfuric acid (H_2SO_4), the hydrogen bonds between cellulose and hemicellulose could be hydrolyzed. Thus, alkaline (mostly sodium hydroxide (NaOH)) solubilizes the lignin.^[95,104] The advantage of these methods are the cheap solvents. However, using these solvents, special equipment is necessary due to a corrosion risk. Another disadvantage is the neutralization of pH for further downstream processes.^[95,105] This thesis focuses on two other fractionation methods with the potential of recycling these solvents: the organosolv and ionosolv method.^[106,107] These two will be discussed in the following Subsections.

Organosolv Method

The organosolv method in general solubilizes lignin and hemicellulose in the liquid phase and a high-purity cellulose fraction remains in the solid phase. The organosolv process combines some desired requirement for an effective fractionation method such as: highly digestible cellulose, low production of inhibitors, non-corrosive, potentially recyclable solvents and high component selectivity.^[108] organosolv also allows a non-contaminating fractionation in contrast to Kraft or sulfite pulping to recover high-quality lignins.^[109]

The solvents employed in organosolv processes are organic compounds, with a concentration ranging from 30 to 100 vol.% in water and are utilized with or without the presence of a catalyst. The process temperature is maintained within the range of 100 to 250 °C, with the residence time varying from 30 minutes to several hours. The operational conditions

are contingent upon the specific solvent and the biomass being processed. Typical solvents include aliphatic alcohols such as methanol, ethanol and butanol, polyols like ethylene glycol or propylene glycol, and organic acids such as formic acid and acetic acid, as well as ketones or ethers.^[90,110] As catalysts, alkalis, acids and salts have been investigated.^[110] One of the most used solvents in organosolv processes is ethanol due to its low toxicity, low cost, and possibility of recycling for reuse.^[90,111] By reducing the viscosity of water, ethanol enhances the solvent penetration into the cell wall.^[90,107] Using ethanol high delignification rates between 45 % and 96 % with different biomasses like wood and agro-industrial residues can be achieved. Another common low-chain aliphatic alcohol used in organosolv pretreatments is methanol. With a low price and a low boiling point, the advantages of methanol are similar to those of ethanol. Nevertheless, methanol has a higher toxicity which is a main drawback of using methanol. Another drawback in the operation using ethanol and methanol are their inflammability demanding safety procedures to avoid accidents and their low boiling points. Therefore, using these two alcohols would need high-pressure reactors and consequently high investments costs.^[90,111] The low boiling point is advantageous for the potential recycling of the solvents, as this would be less energy-intensive than for high boiling solvents. On the other hand, like described above, the low boiling point would lead to cost-intensive equipment.^[90] Nevertheless, organosolv is a promising method for the pretreatment of lignocellulosic biomass, also in the concept of whole biorefineries.

Ionosolv Method

The ionosolv pretreatment method is based on ionic liquids as solvents for the valorization of biomass. Ionic liquids (ILs) are salts with melting points below 100 °C. They are formed by the combination of different organic cations and organic or inorganic anions and exhibit excellent thermal stability, medium-high polarity, and negligible vapor pressure.^[112,113] ILs can be categorized into two groups: aprotic ionic liquids (AILs) and protic ionic liquids (PILs).^[113,114] At the PIL structure, a free proton is present which appears to be the main difference between the two groups of ILs. In a biomass processing context, two main approaches with ILs are possible. The first approach involves the dissolution of the entire lignocellulosic biomass structure, with the objective of recovering the pretreated biomass. This process of solubilization and precipitation has been shown to enhance subsequent yields. The second approach involves the selective dissolution of lignin and hemicellulose, while leaving the cellulose largely intact. This approach enables the potential valorization of the different biomass fractions on an individual basis. The latter is known as the ionosolv process.^[113,115] Mainly low-cost and thermally stable PILs mixed with water are used in the ionosolv process.^[116] This process was successfully applied on various lignocellulosic

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biomass like miscanthus,^[116,117] sugarcane bagasse,^[118] pine,^[119] beech wood^[120,121] and willow^[122,123]. Triethylammonium hydrogen sulfate [TEA][HSO₄] is one of the PILs used in this process with estimated costs of \$0.78 kg⁻¹.^[123–125] Using the PIL 1-methylimidazolium chloride [H₁Cim]Cl (prize approx. \$3 kg⁻¹^[123,124,126]) even Cr^{III}, Cu^{II}, and As^V (CCA)-treated wood could be fractionated and decontaminated via the ionosolv process. The cellulose-rich pulp could be used via enzymatic saccharification with a saccharification yield of 75 %.^[123,127] This proves the broad substrate range which can be used in the ionosolv fractionation technique. The two main products from the ionosolv treatment are highly crystalline cellulose which potentially can be used as bulking agent in food or pharmaceutical industry or for enzymatic hydrolysis of cellulose to hexoses achieving high yields and lignin.^[116,128,129] This lignin fraction is a potential sustainable source of chemical building blocks for renewable aromatic or functional carbon materials.^[129,130] A schematic process description is shown in Figure 2.9.

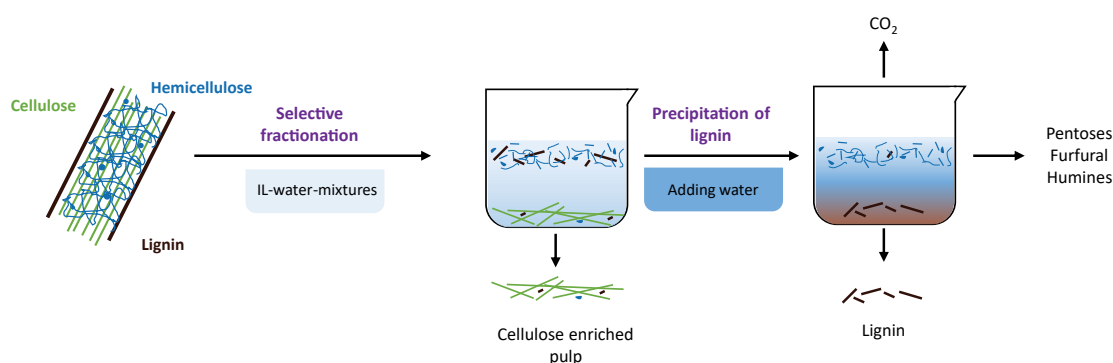


Fig. 2.9 Schematic process flow-chart of the ionosolv process, according to Bukowski.^[131]

The anions role in the solubilization process is disrupting hydrogen bonds among the lignocellulosic components and forming new hydrogen bonds particularly with the cellulose hydroxyl groups. In contrast, the potential role of the cation in the dissolution of polymers is linked to the size and the hydrophobic properties of the cation.^[113,132] Therefore, the IL system is highly tunable for distinguished purposes applying different cation and anion combinations. For using the IL in a biorefinery like context, the recovery and reuse is crucial.^[113,133] The performance of IL pretreatment can be affected by the presence of impurities, including soluble lignin particles and/or carbohydrate degradation products such as furfural or HMF (hydroxy methyl furfural). In addition, the thermal stability of ILs can also be problematic, making its reuse a major challenge.^[113,134] Theoretically, successful IL recycling requires complete product recovery, elimination of impurities and long-term IL stability. However, this is not the case in the solubilization of lignocellulosic biomass,

especially when more pH-sensitive biopolymers such as proteins and hemicelluloses are present. One strategy to overcome this problem is to perform a pre-extraction of the proteins and/or hemicelluloses to ensure that this fraction can be properly utilized. However, it should be noted that this approach necessitates additional steps in the process, which can result in an increase in the overall investment cost. Another strategy might be the utilization of that impurities within the IL.^[113] This strategy was applied in the POM-ionosolv concept, which is described in more detail in Section 2.3.3.

2.2.2 Protein Extraction Methods

The fractionation of macroalgae can be carried out with regard to various components such as lipids or proteins. Since proteins represent a valuable product in this work, only the extraction of these will be discussed. For the extraction of proteins several methods can be applied: Enzyme hydrolysis, microwave assisted extraction, ultrasound assisted extraction, pulse electric field and solid-liquid extraction in different solvents like acid, alkaline or ionic liquid.^[135] Table 2.3 shows the advantages and disadvantages of the different extraction methods.

Table 2.3 Protein extraction techniques to disrupt cell walls.^[135]

Extraction Method	Advantage	Disadvantage	References
Enzyme hydrolysis	– Limited use of organic solvents – Specific and mild technique for extracting protein and bioactive components	– Long extraction times – Enzyme preparation: expensive and requires specific temperature and pH	[135–139]
Microwave-assisted extraction	– No organic solvents – Short extraction times	– Use in dried algal biomass limited	[135,137,140,141]
Ultrasound-assisted extraction	– Fast processing time – Low energy consumption – Thermal sensitive technique – Limited use of organic solvents	– Potential structural changes within polysaccharide structure	[135,140,142,143]
Pulse electric field	– Non-thermal – Energy efficient technique	– Limitations on scaling up	[135,140,144–146]
Solid–liquid extraction	– Simple and cost effective	– Partial degradation of the proteins and bioactive components – Time consuming	[135,147–150]

In this section, only a selection of protein extraction techniques will be discussed in detail. Firstly, the alkaline treatment is explained. Secondly, the ultrasonic-assisted treatment is discussed and in the next Subsection, the IL extraction method as novel approach is described.

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Alkaline Treatment

Alkaline hydrolysis is a conventional extraction technique used for the purpose of obtaining algal protein. Algal proteins are conventionally extracted by using aqueous, acidic, or alkaline solutions, followed by a process of centrifugation. In addition, the application of enrichment techniques such as ultrafiltration and/or chromatography is also a common practice. In order to preserve the structure, stability, and bioactive nature of the extracted proteins, the experiments are often conducted at mild extraction conditions at fixed pH ranges and at temperatures below 80 °C.^[56] An aqueous extraction is considered to be one of the most straightforward and environmentally friendly protein extraction techniques. The resulting yield is often low in comparison to extraction with acids or alkalis. Using this techniques certain factors like time, temperature, and weight to volume ratio of the algal biomass have to be optimized.^[135,151] Among these, alkali is found to have the highest yield, with 56 % protein extracted from *Ascophyllum nodosum* using 0.4 M NaOH, as reported by Kadam et al.^[57] Consequently, acid or alkali extraction has been extensively employed for macroalgae protein extraction, with sequential acid-alkali extraction being identified as the most effective method. The application of acid before an alkali solution has been shown to enhance the liberation of polysaccharides and proteins within the cell wall matrix, thereby increasing the solubilization of proteins in the alkali medium and improving the protein recovery.^[143] Kadam et al.^[57] reported that the sequential extraction increased the protein yield from 56 % to 59 % from *Ascophyllum nodosum*. Apart from acid and alkali, different reagents such as ammonium sulfate or trichloroacetic acid and organic solvents can also be used for the solubilization or precipitation of macroalgae proteins.^[57] Some studies investigate the application of the alkaline treatment together with a physical treatment method like the ultrasonic method (vide infra next Subsection)) for an enhancement of the protein recovery.^[152]

Ultrasonic Treatment

The ultrasonic treatment extracts protein through the use of acoustic cavitation generated by soundwaves causing microturbulences in form of air bubbles. These bubbles create mechanical energy in the form of shear forces by bursting. These forces break down the macroalgal cell wall polymers enhancing the release of target molecules.^[153–156] The ability to generate progressively high-intensity cavitation zones increases its suitability for scale-up.^[157] This method is environmental friendly because it uses lower amounts of solvents and can also be conducted with water as solvent, furthermore it requires low energy.^[140] This treatment method can be applied also together with other treatment methods, for example

the alkaline treatment (vide intra previous Subsection). Depending on the operation power and sonication parameters the extraction of protein can be enhanced to 70 % dw and 86 % dw protein recovery from *Ulva* sp. and *Gracilaria* sp. respectively.^[158] With this method, higher extraction yields can be achieved in shorter processing times with lower temperatures making it suitable for the extraction of thermolabile compounds.^[56,159]

Ionic Liquid Extraction

IL extraction is a novel technique for protein extraction. The IL interacts with the cell wall and disrupt the intermolecular H-bond in the cellulose of the cell wall causing their dissolution. Thus, the cell walls are partially or completely damaged.^[56] ILs can be synthesized from natural sources increasing its sustainability. Furthermore, some ILs used for the extraction of protein are less environmental toxic. Some examples for ILs used as solvent for the protein extraction are Choline Chloride (ChCl), Ethyl methyl imidazolium dibutyl phosphate ([Emim][DBP]) and Tributylmethylphosphonium methylsulfate ([TBMP][MeSO₄]).^[150,160] Currently, the IL recovery and reuse after protein extraction with different techniques like adsorption, extraction, and membrane processes are under investigation.^[150]

To visualize the above described methods 2.10 shows a scheme of every extraction done in lab scale.

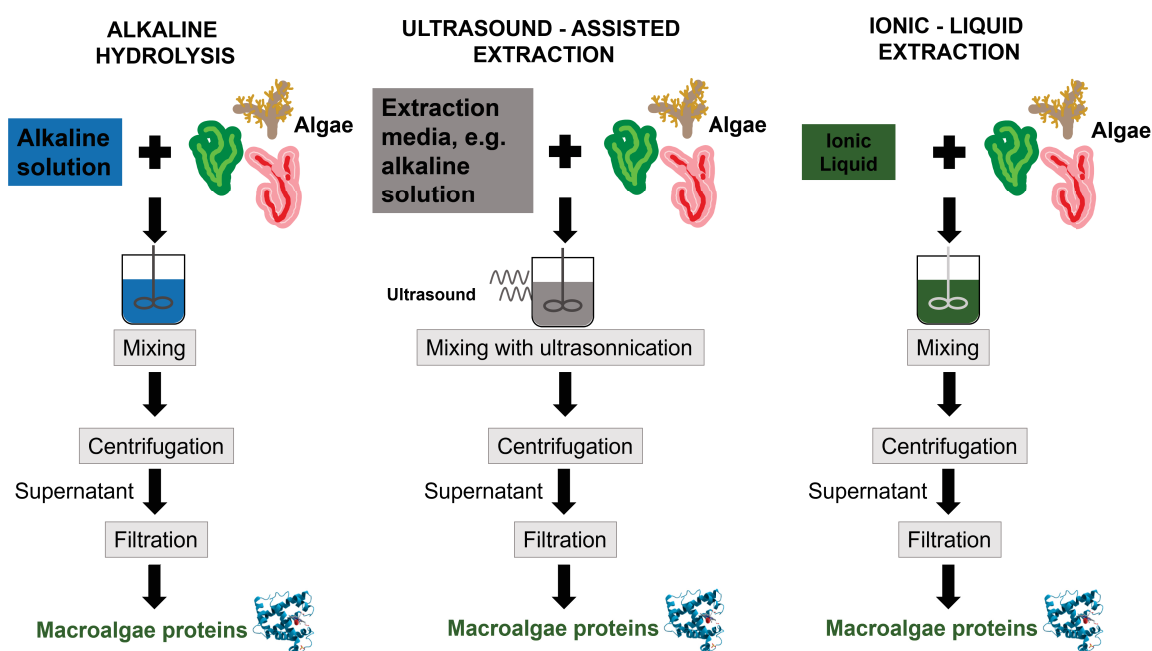


Fig. 2.10 Schematic flowchart of the different extraction methods at lab scale.

2.3 Polyoxometalates as Oxidation Catalysts

Polyoxometalates (POMs) are a group of inorganic, polyanionic cluster compounds that are predominantly associated with elements of the fifth (vanadium (V), niobium (Nb) and tantalum (Ta)) and sixth group (molybdenum (Mo) and tungsten (W)) of the periodic system of elements, each in their highest oxidation state.^[161] This group of polyanionic cluster is characterized by a high structural versatility and therefore share a great variety of chemical characteristics which can be used for catalysis. This will be explained in Section 2.3.1 and 2.3.2. Subsequently, two selected processes where POMs are used will be described in Section 2.3.3 with its Subsection.

2.3.1 Structure of Polyoxometalates

In general, POMs can be classified in two main categories: iso-polyanions (IPAs) and hetero-polyanions (HPAs).^[161,162] These categories are based on different structural types which will be explained later. IPAs, generally, are described with the formula $[M_mO_o]^{p-}$ made of the d^0 metal cations and oxide anions or addenda metals and oxygen. Addenda atoms have the capacity to alter their coordination number with oxygen from 4 to 6 through the process of polymerization in an acidic solution. Additionally, they possess a high positive charge.^[161] The only structural type in this category is the Lindqvist-POM ($[M_6O_{19}]^{2-}$). This POM has only one transition metal atom in its structure. Six $[MO_6]$ polyhedra constructing the octahedral hexametalate $[M_6O_{10}]^{n-}$ (when $n=2$, $M = Mo$ or W , when $n = 8$, $M = Nb$ or Ta) with an edge-sharing linkage. In this structure, each metal center forms one $M=O$ bond with the oxygen on the surface of the molecule. Therefore, each of the six terminal oxygens occupies the corner of an octahedron with high symmetry (O_h).^[161,163–165] This structure can be seen in Figure 2.11 top left.

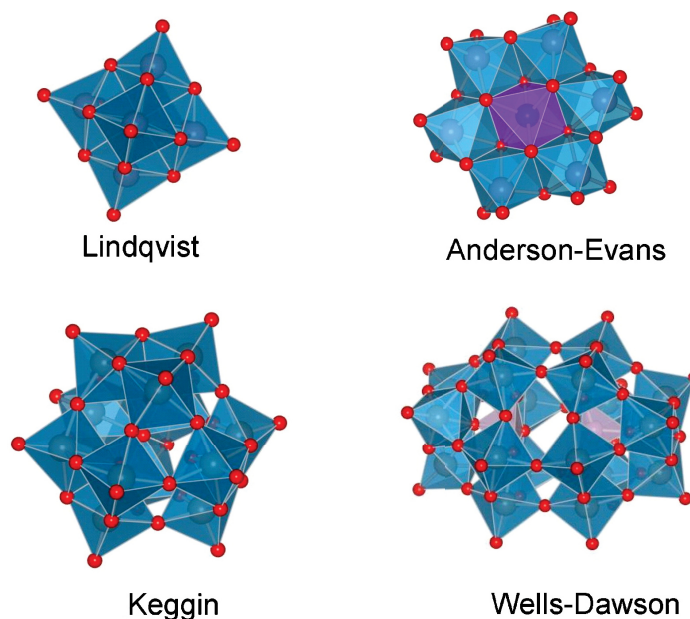


Fig. 2.11 Most common POM structures. IPA: Lindqvist structure (top left), HPAs: Anderson-Evans (top right), Keggin (bottom left), Wells-Dawson (bottom right). Color code: oxygen atoms (red) and metals (blue), heteroatoms (violet).^[166]

HPAs, on the other hand, have d^0 metal cations and oxide anions together with heteroatoms. Their general formula is $[X_xM_mO_o]^{q-}$. M is the metal cation and X the heteroatom such as Si(V), P(IV), B(III), etc.^[161,167] Here, various structure types such as Keggin ($[XM_{12}O_{40}]^{n-}$), Wells-Dawson ($[X_2M_{18}O_{62}]^{n-}$), Anderson-Evans ($[XM_6O_{24}]^{n-}$), Dexter-Silverton ($[XM_{12}O_{42}]^{n-}$) or Allman-Waught ($[XM_9O_{32}]^{n-}$) are known.^[161] Hereby the ones shown in Figure 2.11 are the most common types, namely, Keggin, Wells-Dawson and Anderson-Evans. Therefore, these structure types are shortly explained more precisely. In the Anderson-Evans structure the hetero element is octahedrally coordinated in the center of the structure by six oxoligands.^[168] This makes the structure highly flexible and provides different modifications including: the incorporation of a large number of different heteroatoms with different size and oxidation state or the incorporation of organic and inorganic cations and molecules with different coordination motifs.^[161,169–172]

The Wells-Dawson structure is formed by two XO_4 tetrahedra each coordinating one M_6O_{13} triad cap and one M_6O_{14} belt linking to another M_6O_{14} belt by sharing corner oxygen atoms.^[161]

The most famous structure type of POMs is the Keggin. Within this structure, twelve MO_6 octahedra are linked around a central XO_4 tetrahedron, whereby each of the four oxygen atoms coordinate on the central heteroatom X. This structure is part of a M_3O_{13} triad.^[173]

Through synthesis methods like the self-assembly method and the Lacunary method different

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transition-metals can be incorporated into the POM structure or existing metals can be substituted.^[174–176] The Lacunary method is based on the Lacunary POM structures which are not an independent POM structure type. They are derived from any POM structure type (e.g. Keggin or Wells-Dawson) in which one or more MO_x polyhedra are missing.^[174,177–179] Due to the resulting vacancies, Lacunary structures prove to be particularly reactive towards themselves or foreign metal ions.^[180] Through these method, the elemental compositions, skeleton structure, valance state, and atomic number of the synthesized POMs and therefore its catalytic properties can be varied.^[181] These properties will be discussed in the following Section 2.3.2.

2.3.2 Properties for Catalysis

POMs possess intriguing properties that favor their use as catalysts. Compared to classical metal oxides, they exhibit a high anionic charge, an enormous molecular size combined with a high molar mass, and a defined structure and size. POMs have controllable functions, including adjustable solubility of the acidic forms in water and other oxygen-rich compounds such as ethers or alcohols. Additionally, they demonstrate strong electrolytic properties, rendering them suitable for electrochemical applications.^[182] Due to their various properties, POMs can also be applied in medical catalysis, for instance in chemodynamic therapy and as antibiotic.^[183,184]

The suitability for redox reaction can be explained by the reversible or pseudoreversible redox activity of POMs typically ascribed to the empty and non-bonding d-orbital of the metal centers. For instance, all metalate centers (predominately at the high oxidation state) with a polyhedral structure of MO_2L_4 in C_s symmetry contain a single unshared oxygen atom. This generally possesses reversible redox ability. Subsequent to a reductive process, the resulting mixed-valence POMs distribute delocalized electrons over the metal atoms, such as Lindqvist, Keggin, or Wells-Dawson-type anions.^[185,186] Due to the fact that all M atoms are identical, the electrons are delocalized over all MO_x cluster at room temperature by rapid intramolecular electron transfer. The reduction results in an increase in negative charge density at the POM/HPA anions, thereby enhancing their basicity. Consequently, the reduction can be accompanied by protonation, contingent on the pK_a of the produced anions. Thus, the potentials of the reversible redox pairs of HPA anions are significantly affected by pH. In a neutral aqueous or organic solution where no protonation can occur, both Keggin- and Wells-Dawson-type POM anions may undergo several one-electron reductions.^[187,188] Furthermore, POMs have a intrinsic resilience to oxidative decomposition, elevated thermal stability, remarkable sensitivity to light and electricity, and tunable acidity.^[188] The various possible applications in catalysis are substantiated in their multiple active sites like protons,

oxygen atoms, and metals. It has been demonstrated that POMs can act as Brønsted acids, facilitating acid-catalyzed reactions. Some oxygen atoms on the surface of POM anions, particularly those on Lacunary sites of Lacunary POM anions with a high negative charge, exhibit sufficient basicity to react with protons, even to abstract active protons from organic substrates. Consequently, these surface oxygens of POM catalysts can be identified as the active sites in base-catalyzed reactions.^[188] However, the most credits for the catalytic activity of POMs should be given to the metals. They are active sites in all oxidative reactions, acid-catalyzed reactions and most other reactions. Nearly all of the used POMs for oxidation reactions are W or Mo based compounds. Sometimes other transition metals are incorporated. For example, incorporating metals with strong Lewis acidity, like Al, Zr, Hf or Ln, the POM properties can be tuned accordingly.^[188–191] Another widely incorporated atom in POM structures is vanadium. Several studies describe the high oxidation ability of these vanadium-substituted POMs.^[192–199] Two examples for the use of a vanadium substituted polyoxometalate will be explained in the following section.

2.3.3 State of the Art Processes using Polyoxometalates as Catalysts

As stated in the previous sections, POMs have various chemical properties which make them useful catalysts in redox-reactions and Brønsted acid catalyzed reactions. Two examples will be explained in the following subsections. The OxFA process in which the biomass is oxidized to formic acid is described in Section 2.3.3. The second Section deals with the combination of the ionosolv fractionation method and the OxFA process in the so called POM-ionosolv concept.

OxFA Process – Oxidation of Biomass to Formic Acid

One promising application for POMs is the oxidation of biomass to biogenic formic acid (FA) via the OxFA process. This process was first developed and optimized at the University of Erlangen-Nuermberg by Wölfel^[200] and Albert^[197]. The process is commercially applied by the OxFA GmbH. One schematic flow-chart of this process is shown in Figure 2.12. The catalyst development in this process is an example for the selective tunability of the POM properties. The first generation of POM catalyst for this process was a double vanadium substituted Keggin-polyoxometalate $H_5PV_2Mo_{10}O_{40}$ (HPA-2). By optimizing the catalyst properties via further substitution of addenda metals (Mo) using vanadium atoms increasing the degree of substitution, the redox-activity of the POM could be tuned. This tuning resulted in higher space-time-yields compared to HPA-2. This catalyst is the five-fold vanadium substituted Keggin-POM HPA-5 ($H_8PV_5Mo_7O_{40}$).^[194,201,202] Expanding the scope

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of biomass from mono-saccharides like glucose or xylose to complex waste biomass or lignocellulosic biomass under mild reaction conditions (90 °C and 20 bar oxygen) a solubility promotor, namely p-toluene sulfonic acid (pTSA) have to be applied. pTSA also serves as reaction accelerator enhancing the efficiency of the OxFA process.^[196,198] In detail, different lignocellulosic as well as algae feedstock could be converted to FA in high yields up to 55 % (for pomace as substrate).^[196,203] Even, secondary feedstocks like coffee husks, palm rachis or sugarcane bagasse could be effectively oxidized in similar yields.^[198] Additionally, the undesired by-product of the production of levulinic acid, humins, can be valorized via the OxFA process to formic acid. As formic acid can also be used as a catalyst in the production process of levulinic acid, the two processes have the potential to be applied in a tandem.^[204,205]

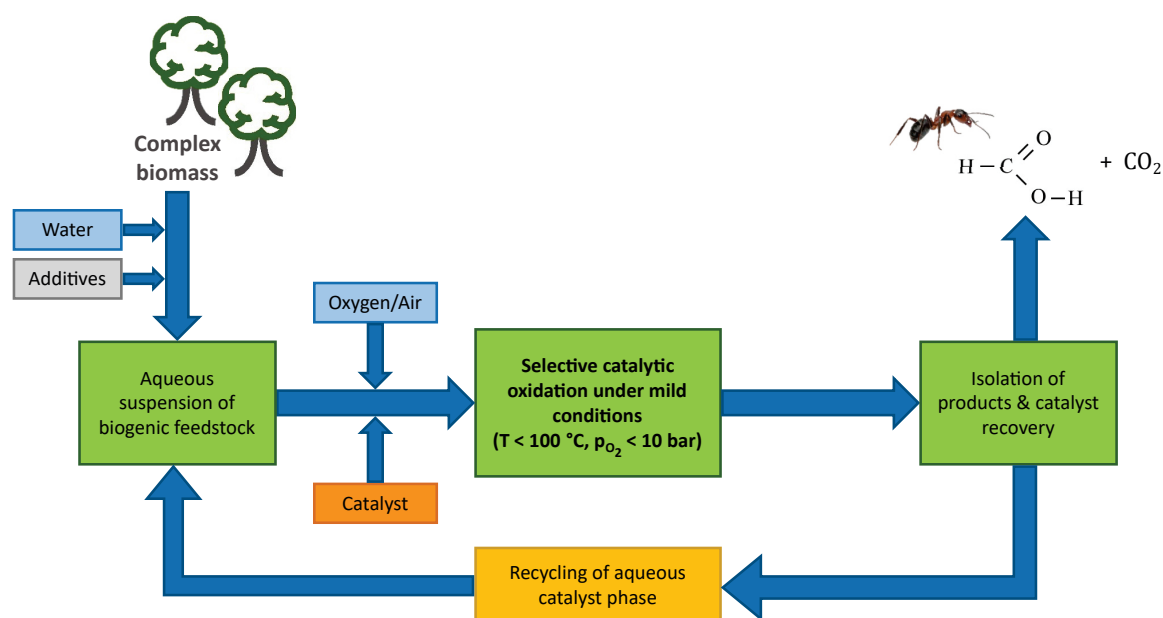


Fig. 2.12 Schematic flow-chart of the OxFA process, according to Albert.^[197]

The efficiency of the OxFA process can be improved by adding an *in-situ* extracting agent like the linear alcohols 1-hexanol and 1-heptanol. These extract the produced formic acid *in-situ* and prevent the reaction mixture to become too acidic for the POM catalyst. With that biphasic system, yields of up to 85 % of FA from glucose and up to 61 % from complex lignocellulosic biomass like beech wood are possible.^[206] This system could also be tested in a continuous mini-plant by Voß et al.^[207]

Investigating new solvent systems, a selectivity enhancing effect of methanolic solutions was found. Applying the OxFA process in a methanolic solution enables the formation of FA and

respective methyl formate (through *in-situ* esterification) in almost perfect selectivity towards FA/methyl formate of > 99 %.^[199,208]

POM-ionosolv concept

The POM-ionosolv concept combines the fractionation of lignocellulosic biomass using ILs (vide infra Section 2.2.1) and the OxFA process. Hereby, Bukowski et al.^[120,121,131] pioneered this novel combined concept by demonstrating its efficiency in several studies.^[120,121,131] The POM-ionosolv concept is a biorefinery-like approach with an *in-situ* oxidation using a POM catalyst to produce cellulose-enriched pulp and formic as well as acetic acid as valuable liquid products. The cellulose-enriched pulp is further processed via enzymatic saccharification to valorize the glucose units of the cellulose. This approach is illustrated in Figure 2.13.

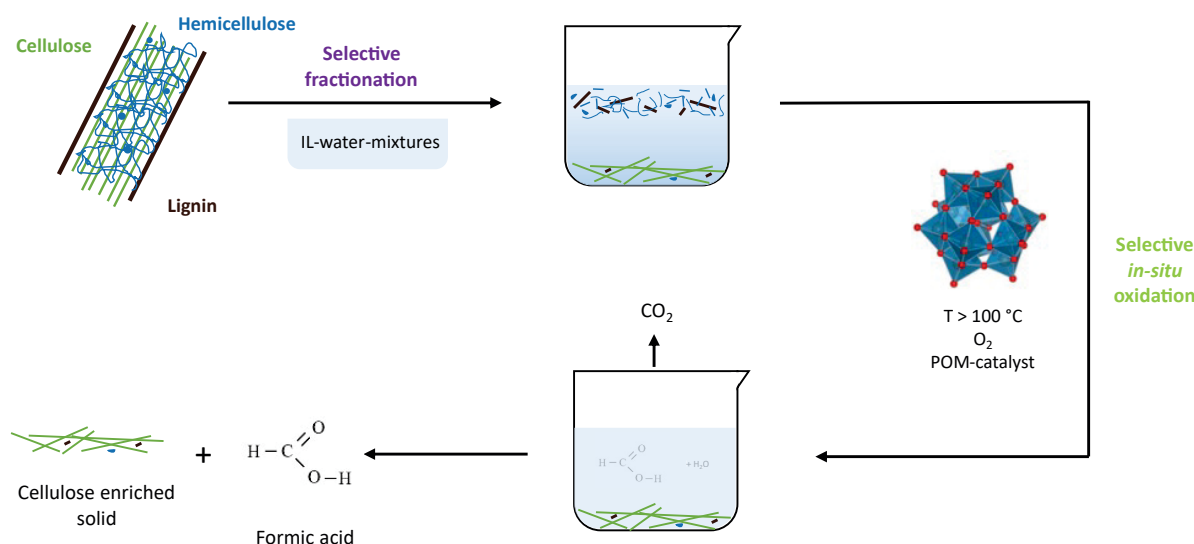


Fig. 2.13 Schematic flow-chart of the POM-ionosolv process, according to Bukowski.^[131]

By screening different catalysts, the five-fold vanadium substituted Keggin-type POM HPA-5 was found to be the most effective catalyst in terms of yield of formic acid (23 %). For this studies, [TEA][HSO₄] as IL with water as co-solvent in different ratios was used. This approach works for all kinds of lignocellulosic biomass, like beech wood (hardwood), pine wood (softwood) or miscanthus (grass).^[121] The POM-ionosolv concept works under relatively mild reaction conditions such as 125 °C reaction temperature and 30 bar oxygen. A sensitivity analysis determined the temperature of 125 °C and a ratio of 30 wt.% IL in 70 wt.% water as the optimum for a high formic acid yield.^[120] This has an influence on the composition of the cellulose-enriched pulp. Furthermore, the fractionation step is the

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rate-determining step in the hole concept.^[120] One merit of the POM-ionosolv approach is the recycling of the catalyst and the purity of the cellulose-enriched solid residue in terms of catalyst traces and cellulose proportion.^[120] Overall, this concept is a promising approach for the complete valorization of lignocellulosic biomass.

2.4 Valuable Products from Biomass

From the two described raw materials lignocellulosic biomass (vide intra Section 2.1.1) and algal biomass (vide intra Section 2.1.2) various products for plenty of applications and industries can be manufactured. Figure 2.14 gives a brief overview of the industries where the different fractions of biomass can be used.

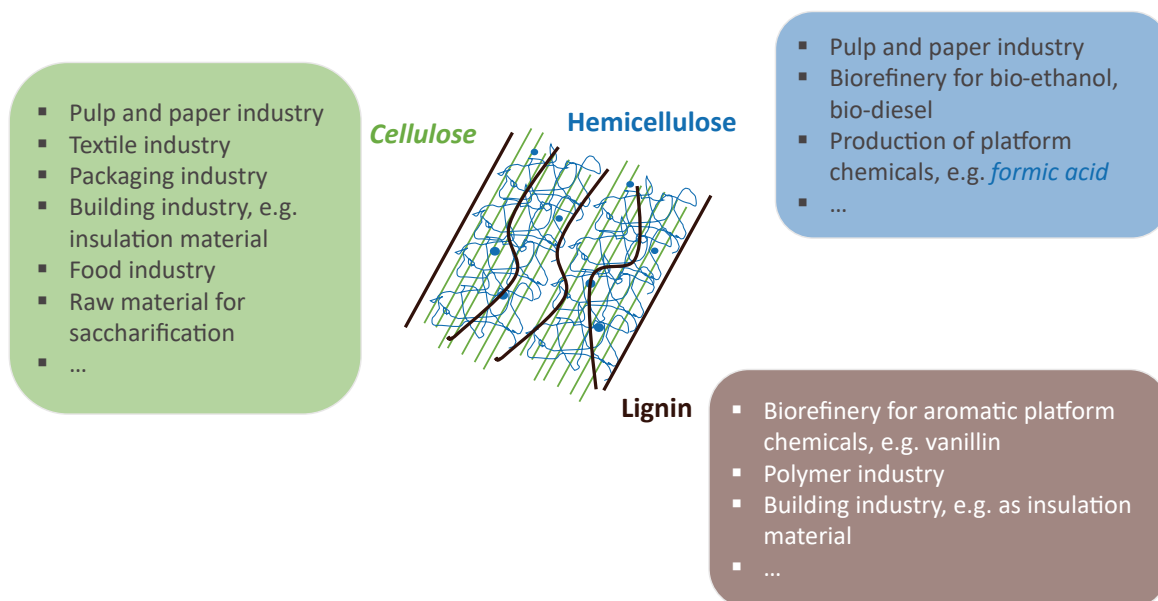


Fig. 2.14 Overview of the different industries which can use the different fractions of lignocellulosic biomass.

Lignin possesses a high calorific value (23 GJ t^{-1}), thus classifying it as a suitable propellant. A significant proportion of the lignin produced in various wood pulping processes is utilized for energy production purposes, with up to 98 % being allocated for this purpose. Lignin sulphonates are the predominant material employed in this field, functioning as dispersing or binding agents in the production of pellets. Synthesis gas can be produced by gasifying lignin, and a mixture of different hydrocarbons is produced by pyrolysis. The hydrogenation of lignin produces various phenol derivatives. The chemical use of lignin is also of particular interest due to the fact that it is the only plant-based ingredient that already has aromatic structures. However, the processes for producing for example vanillin are very complex and cost-intensive. The exploration of further aromatic compounds from lignin is a current research focus. Frequently, model systems are employed to investigate novel syntheses; however, these models do not accurately reflect natural lignin.^[209–211]

The presence of varying sugar components and elevated structural diversity within hemicellulose renders large-scale industrial utilization challenging. Nevertheless, this diversity

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concomitantly offers significant potential for the utilization of hemicellulose as a biopolymer raw material. The process of hydrolysis can be utilized to obtain simple sugars such as xylose from hemicellulose. Xylose can be converted to furfural or fermentatively to bio-ethanol.^[212] In Figure 2.14 the relevant products for this thesis formic acid (vide intra Section 2.4.1) and cellulose (vide intra Section 2.4.2) are marked.

For the algal biomass, Figure 2.15 gives an overview about the industry and products. Macroalgae extracts (carrageenan, alginate, and agar) are used in the food industry making 40 % of the market of hydrocolloids. Furthermore, high valuable components such as polysaccharides, proteins, polyphenols and pigments are the main components of macroalgae.

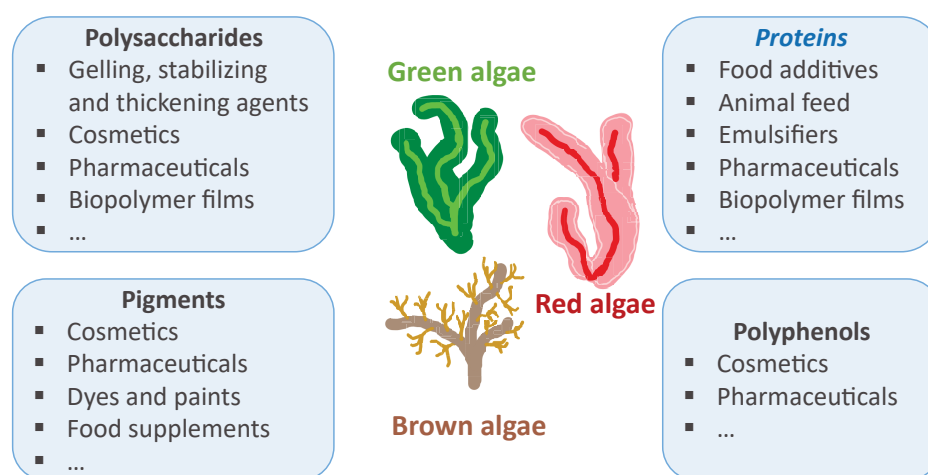


Fig. 2.15 Overview of the different industries which can use the different fractions of algal biomass, according to Kammler et al.^[62]

Polysaccharides include alginate, laminarin, fucoidan from brown algae, carrageenan and agar from red algae and ulvan from green macroalgae. Alginate is used for the production of sponges, gels, porous scaffolds, microcapsules, fibers and micropheres.^[62,213,214] These products are widely used in the pharmaceutical industry or packaging industry. Carrageenan is used in food industry as thickener and stabilizer, in the pharmaceutical industry because it is anticoagulant, antithrombotic, antiviral, antitumor and cholesterol lowering.^[62,214,215] Agar forms thermally reversible gels and is therefore used as gelling or thickening agent. Due to its thermo-reversible properties it is also used as dental impression material, culture media in laboratories or for gel electrophoresis or chromatography.^[62,214,216] Pigments like carotenoids and phycobiliproteins or fucoxanthin and lutein are antioxidants, anti-inflammatory, anti-viral, neuroprotective, anti-obesity, anti-angiogenic and anti-cancer.^[62,217,218] Therefore, they are used in pharmaceutical or nutraceutical industry.

Polyphenols are applied among others in the cosmetic industry as anti-aging agent for the skin.^[62,219,220]

Proteins, which are relevant products for this thesis, are described in depth in Section 2.4.3.

2.4.1 Formic Acid as Liquid Phase Product

Formic acid is the simplest carboxylic acid and also known as methanoic acid with the formula HCOOH . Naturally, formic acid occurs in the glands of ants (Formicidae) and in the stinging hairs of nettles. Formic acid is a colorless, corrosive liquid with a melting point of $8.3\text{ }^{\circ}\text{C}$ and a boiling point of $100.8\text{ }^{\circ}\text{C}$ (at 1013 hPa).^[221] This boiling point is relatively high for such a short-chain organic compound due to the intermolecular hydrogen bonds and the resulting association as dimers. In formic acid vapor at ambient temperature 95 % is dimerized.^[221] Formic acid is miscible with many polar solvents and in any ratio with water. With the latter it forms a high boiling azeotrope containing 22.4 wt.% water and 77.6 wt.% formic acid with a boiling point at $107.6\text{ }^{\circ}\text{C}$ at 1013 hPa.^[221–228]

Analogues to the higher homologous of carboxylic acids, formic acid can undergo reactions such as esterification and amidation. Due to the high acidity of formic acid, simple esterifications need no mineral acid as catalysts and are comparatively fast. Due to the aldehydic carbonyl group formic acid can have a reducing effect like aldehydes.^[221,224,225,227,228]

The global production capacity of formic acid was valued one million tons per year in 2019.^[225] The market for formic acid is estimated growing by 3.0 % to 5.6 % annually.^[221,225,228–230] Industrially, formic acid is produced following two process routes, the acidolysis of formates (salts from formic acid) or the hydrolysis of methyl formate. The latter process has a market share of 80 % to 90 %.^[221,230] This route has two process stages shown in Figure 2.16. The first one is the carbonylation of methanol to methyl formate at elevated temperatures of $80\text{ }^{\circ}\text{C}$ and a pressure of 45 bar with a basic catalyst (sodium methoxide or potassium methoxide (NaOCH_3 or KOCH_3)).^[221,228] The second stage is the hydrolysis of the formed methyl formate to formic acid via autocatalysis. In order to shift the hydrolysis equilibrium to the formation of formic acid and methanol an excess of water is used. Additionally, additives like tertiary amines can be used to form adducts with the free acid and thus removing it from the equilibrium. The methanol formed in the second stage is reused in the first stage again.^[221,228,230,231]

Theoretical Background

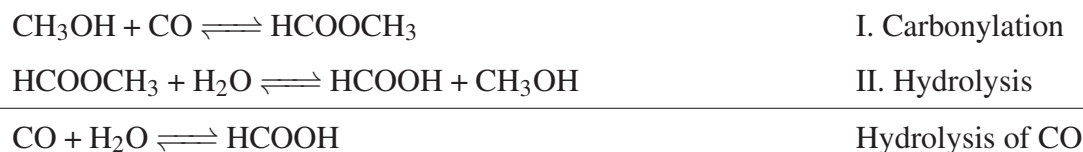


Fig. 2.16 Reaction system of methyl formate hydrolysis to formic acid, according to literature.^[231]

In order to shift to more sustainable production routes, several companies and scientists develop new approaches for the synthesis of formic acid. Such approaches include the OxFA process (vide intra Section 2.3.3) or carbon dioxide-based approaches via electrochemistry in alkaline medium or catalytic hydrogenation.^[228,229,232–236]

Formic acid has a wide range of applications like food additive, preservative in silage and animal feed, as well as textile, leather, and rubber industries. In textile industry, formic acid is used to regulate the pH value producing synthetic fibers or neutralizing alkaline solutions. In the pharmaceutical and food sector, it is employed as synthetic intermediary for synthetic insulin, caffeine, aspartame, and vitamin B1.^[221,225,230,232]

Formic acid has some interesting properties for hydrogen storage in addition to its material use in various industries. It has a low toxicity, low flammability, and is liquid at ambient conditions. All these advantages make formic acid a promising hydrogen storage component. About 4.4 wt.% of hydrogen can be stored gravimetrically in one formic acid molecule. However, formic acid achieves a high volumetric storage density due to its high density of 1.22 g mL^{-1} which stores up to 53 g of hydrogen per liter. The low enthalpy of reaction for the dehydrogenation of formic acid allows the production of hydrogen under moderate conditions of below 100°C . For this reaction metals like platinum, copper, or nickel can be applied. Additionally, the use of formic acid as liquid synthesis gas equivalent or fuel for a direct formic acid fuel cell are being under research.^[225,228,229,237–241]

2.4.2 Cellulose and Cellulose Derivatives as Solid Products

Cellulose is a highly versatile material, exhibiting a range of mechanical and physical properties that are a result of its ability to adopt a variety of molecular structures, molecular weights (or levels of polymerization), crystallinity, chain and fibril dimensions, and aspect ratios for detailed chemical information (vide intra Subsection 2.1.1). These factors collectively influence the material's final macroscopic properties, including its mechanical, optical, and thermal characteristics.^[242] In addition to its beneficial properties, cellulose is inexpensive, biodegradable and has a low density. The porous and interconnected nature of cellulosic materials can be tailored, rendering them suitable for a variety of applications. To address

undesirable traits or to create new desired characteristics, cellulose can undergo a chemical alteration process that involves replacement of its native hydroxyl groups with functional groups, such as certain acids, chlorides, and oxides. The inherent hierarchical structure of cellulose endows it with a natural flexibility and high specific strength. Examples of cellulose-based materials include regenerated cellulose (cellophane and rayon), microcrystalline cellulose, and nanocellulose or cellulose derivatives.^[88,243]

Cellulose has a wide range of applications. For instance, cellulose from cotton, jute or flax is used in textile production, while in paper production, cellulose is required in a particularly pure form. Cellulose is also used in composite or other materials, such as cellophane. Cellulose esters are used as fibers or films in cigarette filters or food packaging.^[88] The latest research has also tested the use of cellulose in aerogels which is summarized in the review of Zhang et al.^[244]

Regenerated cellulose is created by transforming natural cellulose into soluble cellulosic derivatives and further regeneration. This results either in fiber (like rayon) or a film (like cellophane). Some application of this cellulose type are HPLC (High performance liquid chromatography) filters, paper, cellophane, rayon, and biofuels (cellulosic ethanol).^[88]

One of the most important cellulose derivatives is microcrystalline cellulose (MC) with some applications like multipurpose pharmaceutical excipient, reinforcing element in green composites or starting material for crystalline nanocellulose. Nanocellulose (NC) is rod-shaped cellulose ranging in length from nanometers to microns with a diameter under 100 nm.^[245] NC from lignocellulosic material can be divided into two main types: cellulose nanofibrils (CNFs) and cellulose nanocrystals (CNCs). Nanocellulose can be applied in a wide range of nanomaterials and -composites.^[88]

The cellulose derivatives are obtained via modification of cellulose by altering its chemical structure introducing various chemical groups to impart specific properties.^[20,88] The detailed chemical composition and the different possible origins of cellulose are described in Section 2.1.1. This Section will deal with the applications of different cellulosic materials. The derivatization of cellulose is achieved through chemical reactions between a reagent that owns the desired functional groups and the cellulose. Common cellulose derivatives are ethers and esters like methylcellulose (MC), ethyl cellulose (EC), hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), hydroxypropyl methylcellulose (HPMC), carboxymethyl cellulose (CMC), and sodium carboxymethyl cellulose (NaCMC) for ethers and cellulose acetate, cellulose acetate butyrate (CAB), cellulose nitrate and cellulose propionate for esters.^[88,246] In the following, the different cellulose derivatives are shortly explained by their application and origin.

MC is obtained by reacting alkali-cellulose with an etherifying agent like dimethyl sulfate,

Theoretical Background

iodomethane and chloromethane. MC is non-digestible, nontoxic, and nonallergic and approved for food application globally. In the European Union (EU), it is used as emulsifier, texturing agent in bakery products, or retent the shape and reduces fat as well as crystal development in ice cream for example.^[247]

CMC is the ether of chloroacetic acid or its sodium salt and alkaline cellulose. It is used in industrial applications like detergents, paper, adhesives, ceramics, cosmetics, food, pharmaceuticals, and textiles, as well as tissue engineering.^[88,248,249]

HPMC is a synthetic derivative of the natural polymer cellulose and is used as a food ingredient and additive in food industry. Furthermore, HPMC was tested as biofilm for packaging applications and in oil recovery from oil reservoirs, textiles and pharmaceutical industry to produce tablets and pills.^[88,250]

Cellulose acetate as most important cellulose ester is produced by treating cellulose with acetic anhydride and a catalytic amount of sulfuric acid. It is used in the production of plastics, fibers and eyeglass frame material as well as cigarette filters and synthetic fibers.^[88,251]

Cellulose nitrate is a highly flammable compound derived from cellulose also known as nitrocellulose or guncotton. Thus right now, the use has been restricted due to safety concerns.^[88]

Cellulose and its derivatives have attracted attention for applications in the biomedical field, pulp and paper industry, food packaging and electronics.^[88,252–254] Another application of cellulose and its derivatives are hydrogels for various medical applications due to their natural and unique encapsulation and binding capabilities. Hydrogels are three-dimensional crosslinked macromolecular cellulose networks which are hydrophilic possessing the ability to absorb and release large amounts of water. The use of these gels are as biomaterials in drug delivery vectors, carriers for cells in tissue engineering and biosensors.^[88,255]

2.4.3 Proteins as Solid Products

Proteins from algal biomass are predominantly structural and enzymatic, as exemplified in photosynthetic reactions. They exhibit significant variation in terms of charge, complexation and hydrophobicity, and can be categorized into two distinct groups: soluble or insoluble proteins. Soluble proteins from water-based biomass are predominantly constituted of RuBisCo (ribulose 1,5-bisphosphate carboxylase/oxygenase), while insoluble proteins are primarily membrane proteins in conjunction with lipids, pigments and carbohydrates, and range in size from less than 5 kDA to more than 60 kDA.^[62,256–262]

Proteins are essential for animal and human food and the estimated protein required by Europe in 2054 will be 56 million tons.^[135,263] To face this demand, alternative protein sources have to be found. One potential protein source are the mirco- and macroalgae proteins. Macroalgal proteins are used traditionally as food source in many Asian countries, whereas

2.4 Valuable Products from Biomass

the potential of these source of protein in the Western countries is not widely applied yet.^[264] These have the advantage to not require land for cultivation.^[135,265] The protein content in macroalgae (8 to 47 % dw) is reported to be higher than conventional protein sources like milk (3.4 %).^[135,266–268] 40 % of the total amino acids in the algae are essential amino acids and therefore the protein quality is considered high.^[62,260] Additionally, in macroalgae exist some bioactive peptides with potential health benefits.^[135,269] These peptides could play a major role in humans regulatory processes like the immune, cardiovascular, and nervous system.^[135,270] Some amino acids present in algal biomass are also considered cytoprotective, anti-aging, anti-inflammatory and anti-tumor. Consequently, studies are being conducted on the utilization of algal proteins within the pharmaceutical and cosmetic industries.^[62,271] Furthermore, proteins can be used to produce biofilms or protein-polymer particles.^[272,273] Proteins can also form or stabilize gels and films, foams, emulsions and sols.^[56,274] To release these proteins the breakdown of polysaccharides in the cell wall of the macroalgae is important. Different methods are described in Section 2.2.2.

Chapter 3

Motivation

Biomass is a source of sustainable carbon that has the potential to replace fossil fuels such as crude oil in the long term. This study investigates potential applications using POMs as biomass oxidation catalysts. Due to their adjustable redox activity and Brønsted acidity, various applications using POM catalysts in biomass conversion processes are demonstrated, for example, the oxidation of humins or lignocellulosic biomass in the OxFA process.^[194,196,205,275] In order to expand the broad field of application of POM catalysts with regard to the valorization of biomass, various biorefinery-like concepts are further developed and investigated in this dissertation. In order to be able to use biomass effectively, it is important to be able to break it down into its components. For cellulose as one biomass component various applications, e.g. in the pulp and paper industry, are known. For the other components like hemicellulose or lignin still an energetic use over a material use is present. Therefore, this thesis explores the material valorization of hemicellulose or lignin to formic acid or its derivatives. In this way, the individual components of the biomass can be valorized separately. This leads to the first research question of this dissertation:

- Which fractionation method for lignocellulose in combination with oxidation using POM catalysts is the most promising regarding to the quality of the cellulose-rich solid as a valuable product?

Formic acid and its derivatives have been identified as a further valuable product in biorefinery-like concepts investigated in this dissertation. Formic acid serves as a platform chemical and is traded in the literature as a liquid synthesis gas equivalent.^[195] Its derivatives can be converted into formic acid. This leads to the second research question:

- In which solvent can most of the dissolved components of the lignocellulosic biomass be converted into formic acid or its derivatives using a POM catalyst?

Motivation

In this dissertation, the idea of producing a solid valuable product and formic acid as a liquid valuable product in an oxidative POM-catalyzed process is extended to another biomass category, algae-based biomass. In this case, the solid valuable product is a protein-rich solid, which is further purified in a second step – the extraction. Consequently, two further research questions arise here, which are to be answered:

- Is the oxidation of algae-based biomass using polyoxometalate catalysts suitable for the production of protein-rich solids and formic acid?
- Which extraction technique is the most promising technique for the highest protein yield?

The objective of this dissertation is to answer the research questions raised and thus contribute to the effective use of biomass of different categories using POM catalysts in biorefinery-like concepts.

Chapter 4

Materials and Methods

The studies and experiments in this thesis were conducted in two different test plants on laboratory scale (from 20 mL to 600 mL volume) and with various chemicals (vide intra Section 4.1). Both of these reactor setups and the typical working procedure using them are explained in more detail in Section 4.2. Depending on the objective of the study, different analytical measurement methods were applied. These are described characterizing the applied device, method and purpose of the measurement in Section 4.3. This Section is divided into three parts: Measurements of the gaseous phase (vide intra Section 4.3.1), of the liquid phase (vide intra Section 4.3.2) and of solid material (vide intra Section 4.3.3). The latter category is explained for organic and inorganic solid material. In Section 4.4 the basic calculations used in this dissertation are shown.

4.1 Chemicals

This Section will list the used chemicals divided into gases, liquid chemicals and solid materials and their supplier. Table 4.1 lists the used gases. Table 4.2 the used liquid chemicals and Table 4.3 the solid materials including the used catalysts and substrates.

Materials and Methods

Table 4.1 List of gases.

Entry	Gas	Type	CAS	Purity /%	Supplier
1	oxygen	reaction gas	7782-44-7	99.999	Westfalen AG
2	nitrogen	inert gas	7727-37-9	99.999	Linde AG
3	hydrogen	torch gas	1333-74-0	99.999	Linde AG
4	argon	carrier gas	7440-37-1	99.996	Linde AG
5	helium	inert gas	7440-59-7	99.996	Linde AG

Table 4.2 only lists the liquids which were used in high amount in the different studies. For the minor used liquids, the supplier and other details can be found in the respective experimental details in the different studies.

Table 4.2 List of liquids.

Entry	Chemical	Type	CAS	Purity /%	Supplier
1	water	solvent	-	deionized	University of Hamburg
2	methanol	solvent	67-56-1	> 99.9	VWR Chemicals
3	ethanol	solvent	64-17-8	99.8	VWR Chemicals
4	tributylmethyl-phosphonium methylsulfate	solvent	69056-62-8	> 95	Iolitec Ionic Liquids Technologies GmbH
5	N,N-dimethylbutyl-ammonium hydrogen-sulfate	solvent	-	-	synthesized at Imperial College London
6	triethylammonium hydrogensulfate	solvent	-	-	synthesized at University of Hamburg according to literature ^[276]
7	choline chloride	extraction solvent	67-48-1	99	Merck KGaA
8	acetone	washing solvent	67-64-1	chemically pure	Walter-CMP GmbH & Co. KG
9	dimethyl sulfoxide	washing solvent	67-68-5	99.5	Grüssing GmbH

Materials and Methods

Table 4.3 List of solid materials.

Entry	Solid	Type	Supplier
1	$H_8[PV_5Mo_7O_{40}]$	catalyst	synthesized at University of Hamburg according to literature ^[194,277–280]
	$H_5[PV_2Mo_{10}O_{40}]$		
	$H_8[PVMnMo_{10}O_{40}]$		
	$H_{11}[PMn_2Mo_{10}O_{40}]$		
	$H_7[PMnMo_{11}O_{40}]$		
2	beech wood	substrate	mushrooms & equipment shop
3	birch wood	substrate	Räucherspan.de
4	spruce wood	substrate	Räucherspan.de
5	pine wood	substrate	Jbach GmbH
6	miscanthus	substrate	Silwood Park campus (Imperial College London, UK)
7	porphyra dioica	substrate	ALGApplus Ltd
8	ulva fenestrata	substrate	ALGApplus Ltd
9	fucus vesiculosus	substrate	ALGApplus Ltd

4.2 Laboratory Test Plants

The following Sections describe the used laboratory plants and the associated experimental procedure for each setup. The experimental Sections of the respective studies (vide intra Sections 5.1.2, 5.2.2 and 5.3.2) give more detailed information.

4.2.1 Ten-fold Screening Plant – 30 mL Volume

The high pressure screening plant (see Figure 4.1) with a volume of 10 mL of liquid phase was used for small scale experiments. The ten plant reactors are made of Hastelloy C276. All pipes, valves and fittings are made out of stainless steel 1.4751. Each reactor is equipped with a gasket out of Teflon. The total volume of each reactor including the reactors with the number 1, 5, 7, 8 is about 32.58 mL and the volume of the other reactors (2, 3, 4, 6, 9 and 10) is about 31.22 mL. Each high-pressure autoclave is equipped with a rupture disk with a burst pressure of 90 bar ($\pm 10\%$, 20 °C). The outlet of the rupture disc is connected via a steel flex line to the laboratory exhaust. Likewise, at each reactor an analogue pressure gauge

(PI 1 to PI 10) and a digital pressure transducer (PIR 1 to PIR 10, *Keller AG*) is installed. The autoclaves can be individually shut off from the gas supply via a ball valve. This supply is provided via steel flex lines, which are connected to the individual reactor heads by means of quick-release couplings. Via these steel flex lines and a three-way valve V4, all reactors can be individually filled with either inert gas (N₂ or He) or reaction gas (O₂) or the process gas can be released in the exhaust gas. Needle valve V1 is located in the gas line for precise gas dosing. Thermal energy is fed into the reactors via a heating plate, which is controlled by a *Horst GmbH* controller (TIC 2). Below the heating plate is a magnetic stirring plate (*IKA Werke GmbH & Co. KG*), which can be infinitely adjusted with a speed of 0...1000 rpm. For a typical experiment, the autoclaves were filled with substrate, catalyst, solvent, and a magnetic stirring bar and sealed with the corresponding reactor head. It has to be ensured that the Teflon seal is intact before the reactor is closed. After the reactors had been placed in the heating block and the steel flex lines were attached to the bursting discs, the gas supply lines to the reactors were attached via a plug-in connection. The adapters for digital pressure recording were mounted to the digital pressure sensors. In order to guarantee the necessary purity of the gas phase within the reactors, the autoclaves were first purged three times with the reaction gas. To fill the reactors, the valve on the reactor head and the valves V2 and V3 were opened. For adjusting the pressure precisely to the desired initial pressure, the needle valve V1 was used. After the desired pressure was reached, the valves on the reactor heads were closed and the gas in the pipes of the plant was released to the off-gas with the aid of the three-way valve V4. After setting the initial pressure, the temperature at the heating block was set to the reaction temperature. During the heating process, the stirrer speed was adjusted to 300 rpm and after reaching the reaction temperature, the stirrer speed was set to 1000 rpm. After the reaction time, the reaction was stopped by setting the temperature of the heating block to 0 °C to prevent the heating block from further heating and take the reactors out to cool them at room temperature. After the cool-down, the current pressure and temperature of the reactors was measured and noted. The reaction gas was filled into a gas bag as a gas sample. For this purpose, an adapter tube was attached between the reactor head and the gas bag to release the pressure from the reactor by slowly opening the ball valve. Afterwards, the reactors were opened and liquid samples were filtered through a 125 µm filter. In this filters any solid material could be separated from the liquid phase.

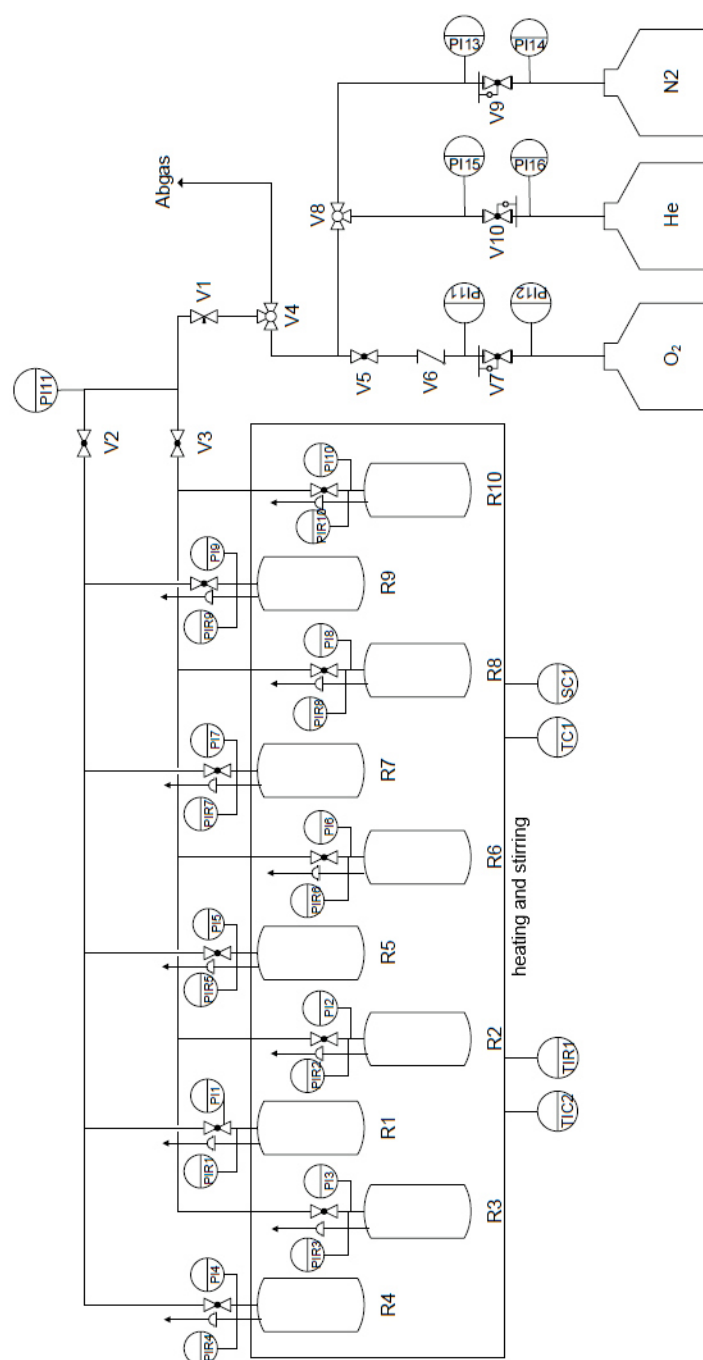


Fig. 4.1 Flow diagram of the high-pressure ten-fold screening plant.

4.2.2 High Pressure Autoclave – 600 mL Volume

In Figure 4.2 the flow diagram of the high-pressure 600 mL autoclave is shown. The 600 mL reactor vessel, the gas entrainment stirrer (both devices from *Parr Instruments*), the cooling coil, the dip tube and the rupture disc are constructed from the nickel alloy Hastelloy C276. The reactor vessel is furnished with a glass liner, which is used for the weighing of the reaction mixture. All additional components, including pipes, screw connections and valves, are manufactured from 1.4571 stainless steel. The flat Teflon ring seal, manufactured by *Parr Instruments*, is capable of withstanding temperatures up to 250 °C. The gas entrainment stirrer is driven by a controllable three-phase motor from *Cemp International*. The reactor is equipped with a heating jacket from *Parr Instruments*, which can be regulated by two Pt-100 thermocouples (TIC) integrated within the reactor and between the reactor and the heating jacket through a cascade control mechanism. A *Lauda* cryostat is linked to the cooling coil for the purpose of cooling. This serves to prevent any potential overheating and to shorten the cooling phase. The system incorporates a gas storage tank, enabling the conveyance of gas from either the storage tank or the house line via the bypass. The gas may be conveyed by an MFC from *Bronckhorst*, with a range of 0-80 NmL/min. The reactor is equipped with three lines, namely a nitrogen line, a helium line and an oxygen line. Pressure gauges have been installed on the reactor and the storage tank in order to display the pressure. Furthermore, the reactor pressure is gauged by an *Ashcroft-type G2* pressure sensor. In order to safeguard against the occurrence of an overpressure situation, a rupture disc has been installed on the reactor and a pressure relief valve on the gas line. The system permits the acquisition of both gas and liquid samples. The pressure can be fully released from the reactor into the exhaust air via valves V-9 and V-10. The high-pressure autoclave can be controlled via a control box with regulation and control elements from *Siemens AG*, which is connected to a computer. The temperature and pressure of the reactor, as well as the pressure in the storage tank, can be monitored and recorded. Additionally, the target temperature can be set and the pressure in the reactor can be regulated via the MFC. Prior to filling the reactor, the functionality of the Teflon seal on the reactor head, the efficacy of the exhaust air, the functionality of the cryostat and the pre-pressure of the oxygen line are evaluated. Subsequently, the glass liner is filled with the requisite catalyst, solvent, and substrate. Once the reactor pot with the inserted glass liner has been properly assembled, the heating jacket is attached to the reactor and connected to the power supply. Subsequently, the reactor is purged on three occasions with reaction gas via V-6, thus ensuring that a pure gas atmosphere is guaranteed for the reaction. Valves V-9 and V-10 are employed for the release of oxygen. Subsequently, the inlet pressure within the reactor is adjusted via the utilization of bypass valve V-6. The heating phase is initiated by setting the stirrer speed to 300 rpm and establishing the desired temperature.

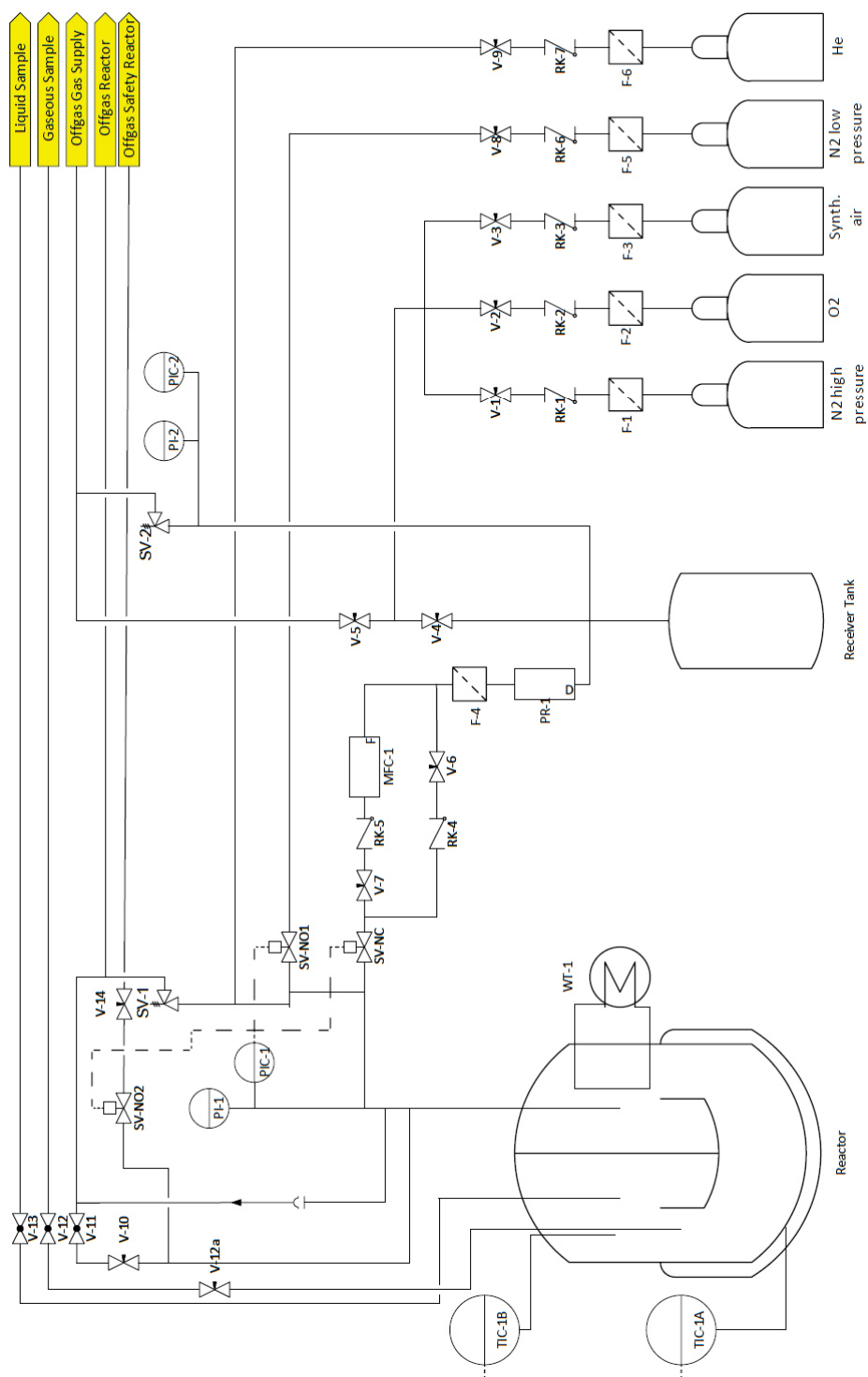


Fig. 4.2 Flow diagram of the high-pressure 600 mL plant.

Furthermore, the monitoring software is initiated and the desired recording interval is selected. During this interval, the reactor pressure, temperature, MFC flow rate and upstream reactor pressure are recorded. Upon reaching the desired temperature, the stirrer is set to 1000 rpm. This allows the ingress of gas into the medium, thereby initiating the reaction. Following the completion of the reaction time, the system is then terminated, the stirrer speed is set to 300 rpm and the temperature is set to 0 °C. This marks the commencement of active cooling. Once the system has cooled sufficiently, a gas sample is taken into a gas bag by opening valves V-12 and V-12a. After the stirrer has been switched off, the pressure was released into the off-gas. Now, the reactor vessel was dismantled and the system was cleaned.

4.3 Measurement Setups

This Section and the different Subsection describe the used measurement devices. The detailed measurement methods can be seen in the studies themselves (vide intra Chapter 5).

4.3.1 Measurement of Gaseous Phase

The gas samples were analyzed via a gas chromatography (GC) with a Variant 450-GC. This GC is equipped with a Shin-Carbon-ST-column (2 m x 0.75 mm) and a 250 µL sample loop. As carrier gas argon at 4.82 bar was used. The detectors were a flame ionization detector (FID) and a thermal conductivity detector (TCD).

4.3.2 Measurement of Liquid Phase

For the analysis of the liquid phase different measurement methods were applied. The measurement using a high-performance liquid chromatography (HPLC) for aqueous systems and for alcoholic systems or examining the catalyst nuclear magnetic resonance spectroscopy (NMR) of the nuclei ^1H , ^{13}C , ^{31}P and ^{51}V were applied. The description of the equipment is shown in Table 4.4.

Materials and Methods

Table 4.4 List of measurement devices for liquid phase analysis.

Entry	Name of measurement method and device	Description	Analysis of
1	HPLC	device: Shimadzu SCL System controller with LC-40 D pump, SIL 40 auto sampler, RID 20 A RI-detector, SPD M40 PDA-detector and VTO-40 S oven column: Biorad Aminex HPAX-87H (300~mm x 7.8~mm) detectors: refraction index detector (RI), photo diode array detector (PDA)	Product analysis in aqueous phase
2	NMR	device: Bruker Avance III HD 600~MHz basis frequency: 600.13~MHz 5~mm BBFO Smart Probe with ATM and z-gradient probe changer: SampleXpress Lite	Product analysis in non-aqueous phase nuclei: ^1H , ^{13}C Catalyst analysis nuclei: ^{31}P , ^{51}V

4.3.3 Measurement of Solid Material

In the studies of this thesis two different solid materials were analyzed. To differentiate these two materials the following Subsections deal with the organic solid material originating from the substrates and the inorganic solid material originating from the catalyst.

Organic Solid Material

Various substrates were used in these studies, which can be categorized into two main groups, namely algae and lignocellulosic biomass. The analysis methods for both substrates are briefly summarized in Table 4.5. For substrates from lignocellulosic biomass, CHNS, laboratory analytical procedures (LAP) from NREL (National Renewable Energy Laboratory) and enzymatic saccharification were performed. Substrates from algal biomass were analyzed with CHNS, moisture analysis, SDS-PAGE (Sodium Dodecyl Sulfate – PolyAcrylamid) Gel Electrophoresis and Bradford Assay. Detailed information about the analysis methods are described in the experimental parts of the studies shown in Section 5.

4.3 Measurement Setups

Table 4.5 List of measurement devices and methods for organic solid material analysis.

Entry	Name of measurement method and device	Description	Analysis of
1	CHNS	device: Euro Vector EA 3000	products for carbon (C), nitrogen (N), hydrogen (H), and sulfur (S) content
2	NREL	analysis prepared according to LAP NREL/TP-510-42618 ^[281]	product composition
3	Encymatic saccharification	analysis prepared according to LAP NREL/TP-5100-63351 ^[282]	analysis for the ability of the solid product to be saccharified by enzymes
4	Moisture analysis	device: Mettler Toledo HE53 moisture analyzer	moisture content
5	SDS PAGE (Sodium Dodecyl Sulfate – PolyAcrylamid) Gel Electrophoresis	SDS gel: SERVAGel™ TG PRiMETM 12 % Gel	quantification of protein content
6	Bradford Assay	according to Bradford quantification method ^[283]	quantification of protein content

Inorganic Solid Material

In the study "Boosting POM-Ionosolv biorefining of lignocellulosic biomass by using redox-balanced polyoxometalate catalysts in methanolic ionic liquid reaction media"^[284] described in Section 5.2 investigations on an inorganic solid material originating from the catalyst were done. The following Table (4.6) shows the short summary of the analytical methods applied to that inorganic solid material, more detailed information about the methods can be found in the experimental Section of this study (vide intra Section 5.2).

Materials and Methods

Table 4.6 List of measurement methods and devices for the analysis of the inorganic solid material originating from the catalyst.

Entry	Name of measurement method	Description	Analysis of
1	Fourier-transformation infrared spectroscopy (FT-IR)	device: Shimadzu IR Spirit	catalyst POM structure
2	inductively coupled plasma optical emission spectrometry (ICP-OES)	device: ARCOS ICP-OES from Spectro (V, P, Mo)	elemental composition elements: V, P, Mo
3	atom emission spectroscopy-flame (ASS-F)	device: Solaar S Series ASS-F (Mn)	elemental composition element: Mn
4	X-ray photoelectron spectroscopy (XPS)	device: Thermo Scientific system	catalyst POM structure
5	X-ray powder diffraction (XRD)	device: Huber G670 powder diffractometer	catalyst POM structure
6	square wave voltammetry (SWV)	device: Metrohm - Autolab PGSTAT101 scan rate: 5~mV/s modulation amplitude: 20 mV frequency: 25 Hz	POM redox-activity
7	single-crystal X-ray diffraction (SCXRD)	device: SuperNova, dual source (Cu K α X-ray), Atlas diffractometer	catalyst POM structure

4.4 Calculations

This Section describes the most important calculations in its basic forms that were used in this thesis. The detailed formula are explained in each study separately in the experimental Sections (vide intra Sections 5.1.2, 5.2.2 and 5.3.2).

Every study calculated the yield (Y) after a variation of the following basic formula 4.1:

$$Y_p = \frac{n_{p,out} - n_{p,in}}{n_{s,in}} \left| \frac{V_s}{V_p} \right| \quad (4.1)$$

Where p is a abbreviation for product and s for substrate, n is the molar amount of substance and v is the stoichiometric coefficient.

The conversion X or in terms of the POM-ionosolv studies dissolution called approach is in general described by the formula 4.2.

$$X_i = \frac{n_{s,in} - n_{s,out}}{n_{s,in}} = 1 - \frac{n_{s,out}}{n_{s,in}} \quad (4.2)$$

Where s is a abbreviation for substrate and n is the molar amount of substance.

In two studies (vide intra Section 5.1.2 and Section 5.3.2) the selectivity S was as well calculated via a variation of the equation 4.3

$$S_p = \frac{Y_p}{X_s} = \frac{n_{p,out} - n_{p,in}}{n_{s,in} - n_{s,out}} \left| \frac{v_s}{v_p} \right| \quad (4.3)$$

Where p is a abbreviation for product and s for substrate, n is the molar amount of substance and v is the stoichometric coefficient.

Chapter 5

Cumulative Part of the Dissertation

The present Chapter constitutes the central component of the dissertation, offering an overview of the various studies conducted as part of this endeavor. Each study is initiated by an introductory statement, and as delineated in Chapter 3, the dissertation explores the question of how biomass can be utilized effectively. To this end, two studies were conducted, employing lignocellulosic biomass as a substrate. The first study (vide intra Section 5.1) investigates the most effective fractionation method for lignocellulosic biomass in combination with an oxidative process step to produce cellulose-rich solids, and subsequently to formic acid and formic acid derivatives, in a two-step process using a POM catalyst. The second study, described in Section 5.2, deals with a combination of different fractionation methods, namely the ionosolv and the organosolv method, and how the solvent system can be effectively used to produce a cellulose-rich solid and methyl formate using a POM catalyst. The third study utilizes macroalgae as a substrate, with a focus on the production of protein-rich solids and formic acid through the utilization of POM catalysts. This study is elaborated upon in detail in Section 5.3.

5.1 Sequential fractionation and polyoxometalate-catalyzed oxidation of lignocellulosic biomass improves cellulose purity and formic acid yield

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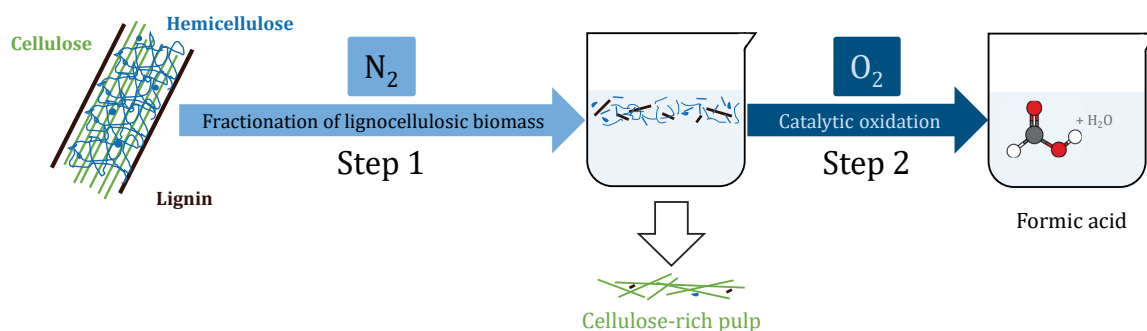


Fig. 5.1 Graphical abstract of the first study of this dissertation: "Sequential two step fractionation of lignocellulosic biomass and POM catalyzed oxidation to formic acid improves cellulose purity and formic acid yield".^[285]

5.1.1 Summary

In the study "Sequential fractionation and polyoxometalate-catalyzed oxidation of lignocellulosic biomass improves cellulose purity and formic acid yield"^[285] a two-step approach for the valorization of lignocellulosic biomass was developed. The concept is based on former studies of Bukowski et al.^[120,121] and the developed POM-ionosolv concept explained in Section 2.3.3. The first step was the fractionation of beech wood as industrial relevant substrate under nitrogen atmosphere using an organosolv or ionosolv approach. The second step includes a homogeneous oxidation of the dissolved lignocellulosic biomass to formic acid and its derivatives using POM catalysts. For the organosolv fractionation method aqueous methanol mixtures and aqueous ethanol mixtures were used and for the ionosolv fractionation method triethylammonium hydrogen sulfate [TEA][HSO₄] as well-known solvent. Different water to solvent ratios (30/70 and 60/40) were compared as well as just for the organosolv-method different temperatures (125 °C and 170 °C). All other reaction conditions (18 g beech wood chips (630 µm), 30 bar nitrogen, 1,200 rpm stirrer speed, 6.5 h reaction time) remained constant to realize a fair comparison. All received cellulose-enriched pulps from the tested combinations were analyzed through compositional analysis after NREL^[286] and processed via enzymatic saccharification^[282]. The cellulose-enriched pulp of the ionosolv method with a 60/40 ratio of IL to water had the highest glucose content with 73.5 % compared to the pulp of the applied organosolv concepts. However, the highest yield of glucan after

the saccharification of the cellulose-enriched pulp could be achieved with the 30/70 ratio organosolv-method. Hereby, the solvent seems not to have a significant influence as the yields do not differ at all (78.3 % for methanolic high-temperature organosolv and 78.2 % for ethanolic high-temperature organosolv). This is a 11-times higher yield than for the untreated beech wood (7.7 %). The pulp of ionosolv fractionation resulted in a lower saccharification yield of 68 %. With the fractionation methods 30 % (organosolv) and respectively 45 % (ionosolv) of the total carbon content of the lignocellulosic biomass could be dissolved into the liquid phase. The latter could be converted to the valuable product formic acid or in case of the organosolv solvent formic acid esters with a five-fold vanadium incorporated Keggin-type POM catalyst in the second step. At reaction conditions of 125 °C, 1,200 rpm, 30 bar oxygen and 6 h reaction time, a yield of formic acid of 27 % of the dissolved carbon content could be achieved. Increasing the reaction time, full conversion and a higher yield of formic acid would be achieved. In general, the concept led to a cellulose-enriched pulp without any interactions with the POM catalyst which increased the quality of the pulp. Separating the two steps led to a safer process as the risk which is followed by high temperatures and oxygen pressures in combination with organic solvents could be avoided due to lower temperatures in the second catalytic step.

5.1.2 Article I

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Sequential fractionation and polyoxometalate-catalyzed oxidation of lignocellulosic biomass improves cellulose purity and formic acid yield

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Abstract: Lignocellulosic biomass is an important source of renewable chemicals and materials but its full valorization is necessary in order to achieve economic and sustainability goals. This study proposes a two-step approach for the valorization of lignocellulosic biomass combining solvent-assisted fractionation and catalytic oxidation with molecular oxygen to generate cellulose and formic acid. An industrially relevant lignocellulosic substrate, beech wood, was fractionated under a nitrogen atmosphere using either an organosolv (methanol or ethanol) or ionosolv (ionic liquid) aqueous solvent to produce dissolved hemicellulose and lignin as well as a cellulose-rich solid. The dissolved components were oxidized catalytically to formic acid and its derivatives in a second step using the fivefold vanadium substituted Keggin-type polyoxometalate $H_8[PV_5Mo_7O_{40}]$. Enzymatic hydrolysis of the cellulose-enriched solid generated purified glucose higher in yields than the single-step comparison method. The organosolv-fractionation increased enzymatic saccharification to 78% whereas the ionosolv-fractionation increased the saccharification yield to 68%, in comparison with 18% for the one-step conversion and only 8% for the untreated beech wood. In the oxidation step, a 28% formic acid yield was achieved under 125 °C, 1200rpm, 30bar oxygen, and 6.5h reaction conditions. The improvements observed for the two-step approach are attributed to the influence of the solvent on the catalyst preventing the latter from interacting with the cellulose-enriched solid, which improved the quality of the pulp and increased the overall formic acid yield. © 2025 The Author(s). *Biofuels*, *Bioproducts* and *Biorefining* published by Society of Industrial Chemistry and John Wiley & Sons Ltd.

Supporting information may be found in the online version of this article.

Key words: lignocellulosic biomass; polyoxometalate; homogenous catalysis; ionic liquid; organosolv

Introduction

Lignocellulosic biomass, one of the world's most abundant bioresources, is derived from plants. Annually, approximately 200×10^9 tonnes of biomass waste from the food and agro-based industries could be commercially exploited at low cost.¹ In 2018 alone, 8 million tonnes of softwood and hardwood biomass were discarded in Germany.^{2,3} However, only 3% of agricultural waste biomass and 20% of waste wood are processed into useful materials, with the rest incinerated,^{3,4} resulting in a waste of resources.

Lignocellulosic biomass is composed of cellulose (30% to 50%), lignin (15% to 40%), and hemicellulose (25% to 35%). Cellulose is a promising source of glucose, which can be converted into valuable products such as bioethanol, methanol, and butanol,^{5–12} but its structural complexity limits glucose accessibility. Nonhydrolyzed cellulose can also be used in various applications, such as packaging and textiles. The other components, lignin and hemicellulose, can be converted into value-added chemicals and materials like organic acids, biochar, bio-oil, and phenolic compounds.^{8,13} The abundance of this feedstock and the range of potential products make lignocellulose a promising alternative to natural gas and crude oil in the chemical industry. To maximize biomass utilization, fractionative pretreatment can separate cellulose, hemicellulose, and lignin.^{8,14} Solvent-based methods, such as ionic liquids, deep eutectic solvents, organic solvents, aqueous acid or alkali, and liquid hot water, can be employed, often assisted by physical (e.g., solvent expansion, ultrasound, extrusion) or biological (e.g., using bacteria or fungi) pretreatment methods.⁸

A combination of different pretreatments is generally used in a biorefinery process, starting with milling. Each pretreatment method has some merits and demerits, which Prasad *et al.*¹⁴ have summarized in a review of the key pretreatment techniques.^{7,14} Three approaches are known for dissolving lignin alongside hemicellulose in the pretreatment step: alkaline hydrolysis, ionosolv fractionation and organosolv fractionation. Alkaline hydrolysis requires a long residence time and the alkali needs to be neutralized after pretreatment, producing additional waste.¹⁴ Ionosolv pretreatment uses ionic liquids (ILs), which are salts with a melting point below 100 °C, characterized by high polarity and low vapor pressure in combination with water.¹⁵ Protic ILs are preferred for use for lignocellulose fractionation by

the ionosolv method. Such protic ILs include, for example, *N,N*-dimethylbutylammonium hydrogen sulfate [DMBA] [HSO₄], or triethylammonium hydrogen sulfate [TEA] [HSO₄], which offer a good compromise between solubility of lignin and cost-effective fractionation.^{16–19} Lignin solubility is influenced strongly by the cation-anion combination, with ILs containing anions that form strong hydrogen bonds leading to better lignin dissolution. Cations that can interact directly with aromatic rings also enhance lignin solubility.¹⁷ Organosolv fractionation employs mixtures of water and organic solvents such as methanol, ethanol, acetone, or ethylene glycol.¹⁴ Pretreatment is usually carried out at temperatures between 100 °C and 250 °C.¹⁸

Using alcohol as reaction media in organosolv-treatment the lignocellulosic biomass undergoes three breakdown mechanisms: the hydrolysis of the internal lignin and bonds of lignin and hemicellulose; the glycosidic bond hydrolysis in hemicellulose, and condensation between lignin and reactive aldehydes. Hence, the lignin fractions in alcohol undergo depolymerization, condensation, and redistribution frequently. Monosaccharides break down into 5-hydroxymethyl furfural if an additional acid catalyst is used.¹⁹ The recovery and recycling of the solvents is mandatory for the feasibility of large-scale applications. To minimize energy consumption for the evaporation of the solvents in distillation columns, low boiling point solvents are preferred. These volatile and flammable solvents require careful control under high pressure to reach the target reaction conditions and avoid explosions.

This study investigated organosolv and the ionosolv fractionation, as alkaline hydrolysis requires an additional neutralization step before the liquid fraction can be used further, making the approach inherently wasteful. The dissolved hemicellulose and lignin can be used to produce short-chain carboxylic acids like formic acid and acetic acid.^{20,21} Formic acid is utilized in many industries including textiles, agriculture or pharma and is discussed as energy carrier or fuel for fuel cells.^{20–23} In an alcohol-containing medium, formic acid undergoes esterification to methyl formate with methanol and ethyl formate with ethanol, which can be hydrolyzed to formic acid and the alcohol in aqueous acidic media.

A sequential biorefinery approach was investigated for the production of a cellulose-enriched solid through an initial fractionation step using either organosolv or ionosolv

methods, followed by catalytic *in situ* oxidation to formic acid using a polyoxometalate (POM) catalyst. Polyoxometalates are anionic metal oxide clusters with diverse structural properties, known for catalyzing the oxidation of biomass to platform chemicals such as formic acid.²⁰ The combination of ionosolv fractionation and POM-catalyzed oxidation was previously demonstrated by Bukowski *et al.*,²⁴ employing a single-step approach termed the POM-ionosolv concept. In this method, ionosolv fractionation was combined with a highly redox-active POM catalyst to convert hemicellulose and part of the lignin concurrently into formic and acetic acids.^{25,26} The second product was a cellulose-rich pulp amenable to enzymatic saccharification. Bukowski *et al.*²⁴ demonstrated the suitability of the POM-ionosolv concept for various biomass types, including miscanthus, pine, and beech wood. The fivefold vanadium-substituted Keggin-type phosphomolybdate $H_8[PV_5Mo_7O_{40}]$ (HPA-5) was found to yield the highest formic acid production (23%).²⁴ HPA-5 is also the state-of-the-art catalyst for biomass oxidation to formic acid under aqueous conditions in the so-called OxFA process, achieving formic acid yields of up to 28.7% from beech wood.^{27–29} The yield was further increased to 32% with *in situ* extraction using 1-heptanol.^{30,31} A correlation between carbohydrate solubilization and carboxylic acid yield was observed in the POM-ionosolv process. The proof-of-concept study showed that a compromise between the optimal conditions for fractionation (140–170 °C) and oxidation (90–120 °C) was necessary.²⁴ In a subsequent study, Bukowski *et al.*³² conducted a sensitivity analysis and found that 70 wt% water content yielded the highest formic acid production (39%) in the presence of HPA-5 and O_2 at 125 °C. Improved lignin and hemicellulose extraction was also observed in stand-alone ionosolv fractionation, based on compositional

analysis of the cellulose-rich solid. Previous work had indicated that catalyst traces remained on the cellulose-rich solid after POM-ionosolv treatment, necessitating Soxhlet washing with methanol.²⁶ To address this, the present study separated the integrated POM-ionosolv concept into two sequential steps: initial fractionation under nitrogen, followed by catalytic oxidation under oxygen, without the need for solvent separation or exchange. In addition to [TEA][HSO_4]-water mixtures previously used in ionosolv fractionation, methanol- and ethanol-water mixtures as organosolv solvents were also investigated. This study combined, for the first time, the oxidation of dissolved biomass fragments by a POM catalyst with different fractionation methods.

Results and discussion

Comparison of ionosolv and organosolv fractionation methods in the first step

This study first characterized the hardwood substrate beech. Beech wood was chosen as the substrate as it is a well defined tree genus that is abundant in Euroasia and North America, is grown for its timber, and is used in industry and research.^{24,32} Beech wood was also the substrate in previous studies investigating POM mediated oxidation of lignocellulose, enabling a direct comparison.^{25,26} The beech wood was fractionated using two different main approaches, the ionosolv and the organosolv approaches.

For the organosolv approach, two short chain alcohols, methanol and ethanol, were used and [TEA][HSO_4] was used for the ionosolv approach. Water was used as a co-solvent in both cases. The cellulose-enriched solid was characterized using National Renewable Energy Laboratory (NREL)

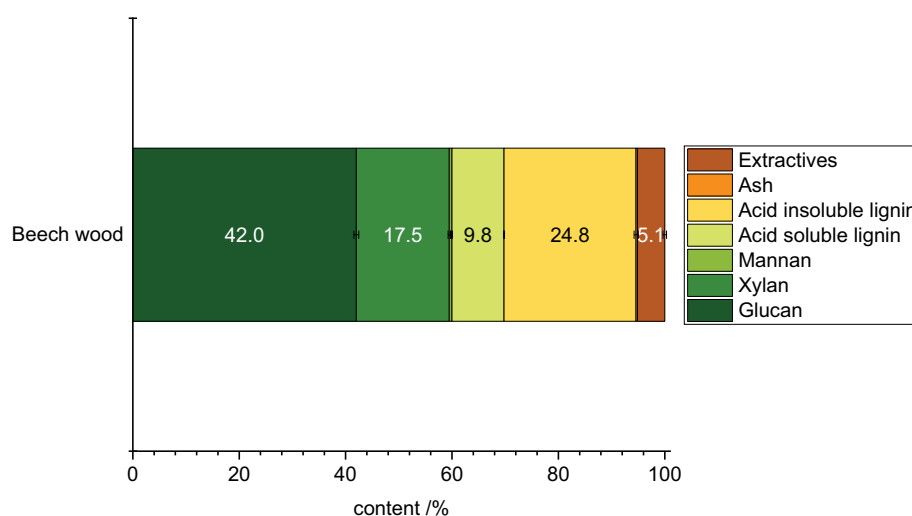


Figure 1. Compositional analysis of the raw material beech wood according to LAP NREL/TP-510-42618.²⁸

protocols and elemental analysis. The composition of beech wood was determined using the compositional analysis method developed by the NREL as shown in Fig. 1. A glucose content of 42.0% ($\pm 0.5\%$) was assigned to the cellulose present in the biomass. The hemicellulose content was 18%, consisting of 17.5% ($\pm 0.2\%$) xylan and 0.51% ($\pm 0.04\%$) mannan. The total lignin content was determined as 34.6% ($\pm 0.30\%$), with an acid insoluble lignin (AIL) content of 24.8% ($\pm 0.4\%$) and an acid soluble lignin (ASL) content of 9.8% ($\pm 0.1\%$). The beech wood also contained 5.1% ($\pm 0.3\%$) water or ethanol-soluble extractives. Extractives include nonstructural components of biomass samples that could potentially interfere with the downstream analysis of the biomass samples – for example, lipids.²⁷ Based on the compositional analysis, an elemental composition of 46.78 wt% carbon and 6.34 wt% hydrogen (Supporting Information, Table S1) was calculated, which is useful information for investigating conversion and yield in the oxidative conversion step.

In order to compare the different fractionation approaches, the same loading of 18 g beech wood chips (particle size $\leq 630\ \mu\text{m}$) in 200 g of liquid phase were processed under the optimized fractionation conditions (125 °C, 1200 rpm, 30 bar nitrogen, 6 h reaction time) determined by a previous study.³² As organosolv fractionation typically requires higher temperatures than ionosolv fractionation, additional experiments at 170 °C were conducted. The temperature of 170 °C was chosen to be a good compromise between effectiveness of organosolv fractionation and energy savings within the 6 h reaction time.

Two different triethylammonium hydrogen sulfate (named TEA)/water ratios (30/70 wt% and 60/40 wt%) were chosen based on the POM-ionosolv experiments in the previous studies and the highest xylose dissolution in the liquid during ionosolv fractionation.^{25,32} The same alcohol:water ratios were also tested for comparison employing the same reaction conditions. This might not have been the optimal organosolv conditions for beech wood but they are consistent with conditions reported in the literature for achieving effective fractionation with 50/50 vol% ratio.¹⁸ For comparison reasons, no further catalyst was added within the organosolv approach.

The effectiveness of the fractionation with the various solvent–water mixtures was evaluated by measuring the dissolution of the carbon fraction from the solid beech wood into the liquid phase ('dissolution %TCC', Fig. 2), relative to the total carbon content (TCC) of the biomass. In both organosolv methods at 125 °C, carbon dissolution remained below 10 %TCC, which could be attributed to the low processing temperature. Unlike other reported organosolv-fractionation methods,^{18,29} which operate under autogenous

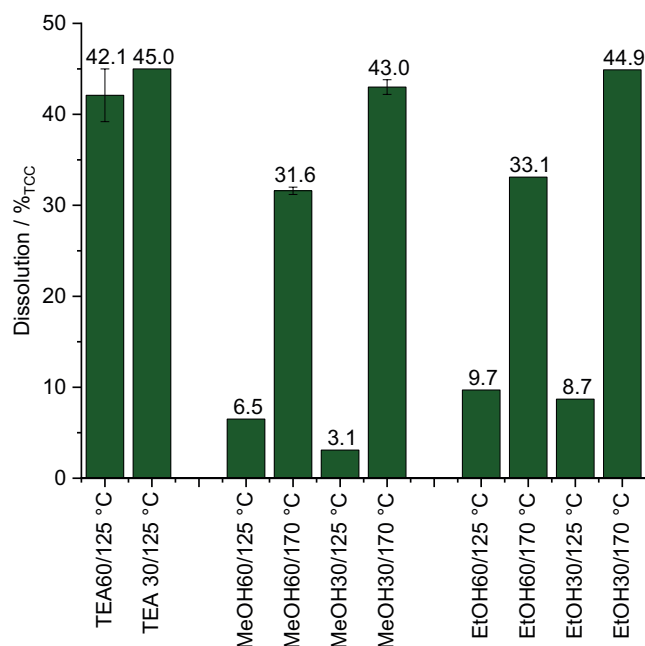


Figure 2. Dissolved carbon in the liquid phase (dissolution) related to the TCC. Reaction conditions: 200 g liquid phase (TEA60 – 60 wt% of [TEA][HSO₄], TEA30 – 30 wt% of [TEA][HSO₄], MeOH60 – 60 wt% of methanol, MeOH30 – 30 wt% methanol, EtOH60 – 60 wt% of ethanol, EtOH30 – 30 wt% of ethanol, the rest being water), 18 g beech wood chips ($\leq 630\ \mu\text{m}$), 30 bar nitrogen at 125 °C/170 °C, 1200 rpm stirring speed, 6 h reaction time.

pressure, the organosolv process in this study was conducted under 30 bar nitrogen to maintain the solvents in the liquid phase and ensure better comparability between fractionation methods. At the higher temperature of 170 °C, carbon transfer improved to 43.0 %TCC for MeOH60/170 °C and 44.9 %TCC for EtOH30/170 °C. In the ionosolv fractionation, the highest carbon transfer under both conditions was comparable, at 42% to 45% ($\pm 0.5\%$ %TCC).

Figure 3 shows the composition of the solid material after fractionation with different approaches. In general, the low-temperature organosolv-fractionation (at 125 °C) showed minor (below 3%) extraction of hemicellulose and nearly no extraction of lignin (below 1%). These findings are in line with the dissolution related to the TCC for low-temperature organosolv experiments shown in Fig. 2. The AIL decreased slightly but, in contrast, the ASL increased in comparison with the raw beech wood. This might be because of a known systematic interference within the NREL lab procedures with the extractives in the raw biomass. These extractives were sometimes assigned to be ASL which explains the slight increase. The high-temperature organosolv fractionation extracted a higher percentage of the hemicellulose content.

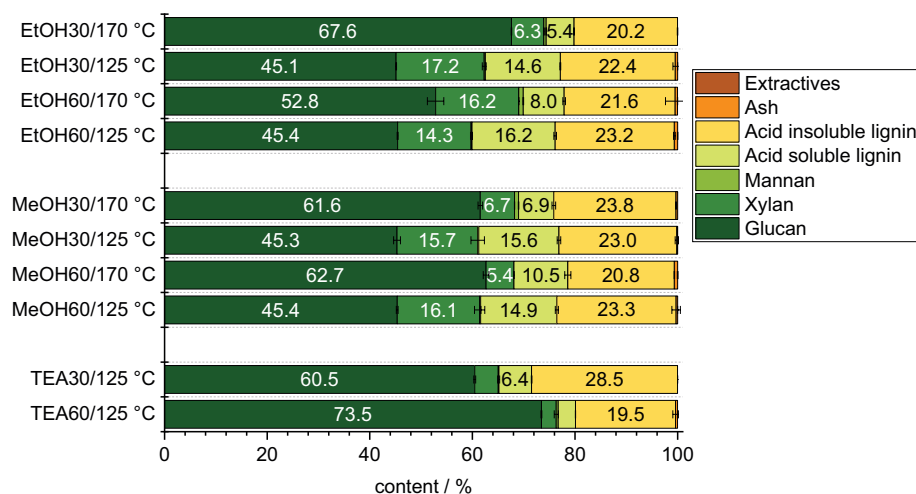


Figure 3. Composition of cellulose-rich solids after the first step. Glucose content refers to cellulose content, xylan content to hemicellulose content. Acid soluble lignin (ASL), acid insoluble lignin (AIL), ash, and mannose are shown. The procedure was conducted in accordance with LAP NREL/TP-510-42618²⁸. Reaction conditions: 200 g liquid phase (TEA60 – 60 wt% of [TEA] [HSO₄], TEA30 – 30 wt% of [TEA][HSO₄], MeOH60 – 60 wt% of methanol, MeOH30 – 30 wt% methanol, EtOH60 – 60 wt% of ethanol, EtOH30 – 30 wt% of ethanol, the rest being water), 18 g beech wood chips ($\leq 630 \mu\text{m}$), 30 bar nitrogen pressure at 125 °C/170 °C, 1200 rpm stirring speed, 6 h reaction time.

The methanolic organosolv fractionation MeOH60/170 °C increased the solubilization of xylan to 12.1 wt% (5.4 wt% $\pm$ 0.1 wt%) in the cellulose-enriched solid compared with 17.5 wt% ($\pm$ 0.2 wt%) in the raw beech wood. The ethanolic version EtOH60/170 °C only extracted 1.3 wt% of xylan (16.2 wt% $\pm$ 0.01 wt%) in the cellulose-enriched solid. The highest glucose content in the cellulose-rich solid for an organosolv solvent was achieved with the ethanolic organosolv fractionation method EtOH30/170 °C, with 67.6 wt% ($\pm$ 0.05 wt%) glucose content in the solid compared with only 52.8 wt% ($\pm$ 0.2 wt%) for the higher ethanol content EtOH60/170 °C. In contrast, the methanolic organosolv solvent MeOH60/170 °C yielded a glucose content of 62.7 wt% ($\pm$ 0.6 wt%), which was not significantly higher than that of the MeOH30/170 °C solid (61.6 wt% ($\pm$ 0.5 wt%)). The ethanolic organosolv-fractionation was affected substantially by the water to ethanol ratio. A higher water content (EtOH30/170 °C) was related to a lower xylan content (6.3 wt% $\pm$ 0.03 wt%) compared with beech wood (17.5 wt% $\pm$ 0.2 wt%) and therefore a higher hemicellulose dissolution and cellulose content.

For the ionosolv fractionation, TEA60/125 °C achieved the highest cellulose content at 73.5 wt% ($\pm$ 0.08 wt%), whereas TEA30/125 °C yielded only 60.5 wt% ($\pm$ 0.05 wt%), indicating a clear benefit from higher TEA loading. Elemental analysis of carbon, hydrogen, nitrogen, and sulfur (CHNS) was performed on the solid after fractionation to identify any

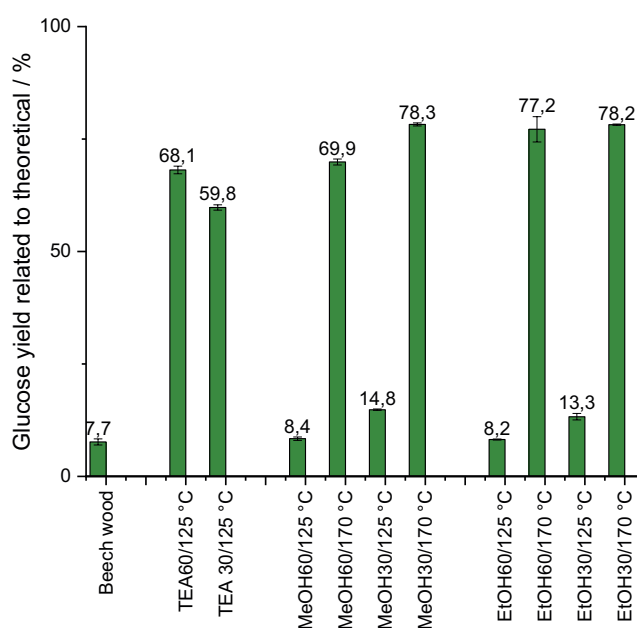


Figure 4. Enzymatic saccharification yields for the cellulose-rich solid obtained with organosolv and ionosolv fractionation. Reaction conditions: 200 g liquid phase (TEA60 – 60 wt% of [TEA][HSO₄], TEA30 – 30 wt% of [TEA] [HSO₄], MeOH60 – 60 wt% of methanol, MeOH30 – 30 wt% methanol, EtOH60 – 60 wt% of ethanol, EtOH30 – 30 wt% of ethanol, the rest being water), 18 g beech wood chips ($\leq 630 \mu\text{m}$), 30 bar nitrogen at 125 °C/170 °C, 1200 rpm stirring speed, 6 h reaction time.

changes; the data are provided in the Supporting Information, Table S1.

Additional enzymatic saccharification of the cellulose-rich material was performed to assess the potential use of solid material after fractionation. Generally, a higher glucose content is correlated with improved accessibility for enzymes that hydrolyze the cellulose to glucose, which can further be used for biofuels like bioethanol or other value-added biobased chemicals.^{30,31,33} However, residual ionic liquid remaining on the solid could affect the enzymatic hydrolysis performance as well as the compositional analysis. Before performing the enzymatic saccharification, all cellulose-rich solids were therefore washed with deionized water to minimize interference with the solvents used for the fractionation. The washing procedure was a significant advantage in contrast with the developed one-step POM-ionosolv approach, which required washing with boiling methanol in a Soxhlet extraction to make sure that no catalyst traces remained on the cellulose-rich solid as this resulted in converting glucose to mannose and inhibited the enzymes in the saccharification assay.²⁴ Figure 4 shows the glucose yields which probe the accessibility of glucose in the cellulose-rich solid to the cellulase enzymes. The data shows that all tested fractionation methods enhanced glucose release from the cellulose-rich solid. For the organosolv fractionations, the higher extraction of hemicellulose and lignin correlated with a higher glucose yield. Another trend was that higher water content led to higher glucose yield; however, this effect was more pronounced for methanol than for ethanol. This correlation was most apparent in the low-temperature organosolv experiments with MeOH30/125 °C and EtOH30/125 °C, where the glucose yield could be nearly doubled ($14.8\% \pm 0.2\%$ and $13.3\% \pm 0.7\%$) in comparison with the raw beech wood ($7.7\% \pm 0.7\%$). Even the extraction of hemicellulose had a significant influence on the glucose yield after enzymatic saccharification. As shown in a previous study,³² the higher the water content, the higher the dissolution of hemicellulose, which led to the phenomenon described above. Nevertheless, the increase in the fractionation temperature enhanced the enzymatic saccharification significantly from $7.7\% (\pm 0.7\%)$ to $78.3\% (\pm 0.3\%)$ in methanol organosolv fractionation. In contrast with the organosolv fractionation, the ionosolv-fractionation with the lower water content gave the highest glucose yield of $68.1\% (\pm 0.8\%)$, correlating with the glucose content in the two cellulose-rich solids. In general, organosolv fractionation resulted in higher glucose yields than the ionosolv fractionation in the selected conditions. The positive correlation between lignin extraction and glucose saccharification yield with a higher IL:water ratio fits well

with previous investigations.^{34,35} The difference between the enzymatic saccharification ability of the ionosolv-processed solid and the organosolv-processed solids could be explained by the different solvents used. Owing to the differing solvation mechanisms of hemicellulose and lignin, the accessibility of the remaining cellulose to cellulase varied. It has been reported that the polymorphic nature of cellulose had a significant impact on the adsorption behavior between the cellulose and the cellulase. The higher the crystallinity, the higher is the binding affinity.^{36,37} The lignin dissolution in the ionosolv concept depends on disrupting hydrogen bonds among the lignocellulosic components. The cation's role in the dissolution of biomass is connected with the size of the cation and its hydrophobic properties.^{8,38} In an acidic environment due to the protic IL the alpha-aryl-ether bonds in the biomass are likely to break.¹⁸ Protic ILs often promote dehydration reactions, leading to the formation of furfural, which is a volatile and reactive compound.⁸ This could be a drawback in context of a biorefinery approach as furfural can further produce humins in the solvent system. In this study, the oxidation step using a POM turns this drawback into an advantage, as the POM converts furfural to formic acid as a valuable product. For the organosolv method, this is not an issue as the used alcohol is neutral. During the organosolv approach, the solvent became slightly acidic due to the autocatalytic process releasing acetic acid from the biomass.¹⁸ In that slightly acidic environment the alpha-aryl-ether bonds also break easily. The organic solvent helps to solubilize the aromatic macromolecules of lignin based on the polymer solubility theory.^{18,39–41}

In general, the yields of enzymatic saccharification depend highly on the substrate and the pretreatment method as Dziekońska-Kubczak *et al.*³⁵ show. In this study, different pretreatment methods such as steam explosion, dilute acid, and alkali pretreatments were tested on different hardwoods and glucose yields up to 79.6% were achieved with an alkali treatment and aspen chips as substrate. Asada *et al.*⁴² even reported a glucose yield of 89% with beech wood as substrate and steam explosion as pretreatment. The cellulose-rich solid can also be valorized to nanocellulose, ionogels, hydrogels, and so forth.^{8,43}

Figure 5 shows the yield of the different products of interest in the liquid phase after the fractionation step, which could be detected by high performance liquid chromatography (HPLC). They were xylose, furfural, and acetic acid as well as CO₂, which was detected by gas chromatography (GC). The yield shown on the left side of the diagram is related to the dissolution (see Eqn (1)). Some reaction products were either missed or could not be measured, which prevented a full accounting of all carbon-containing materials before and

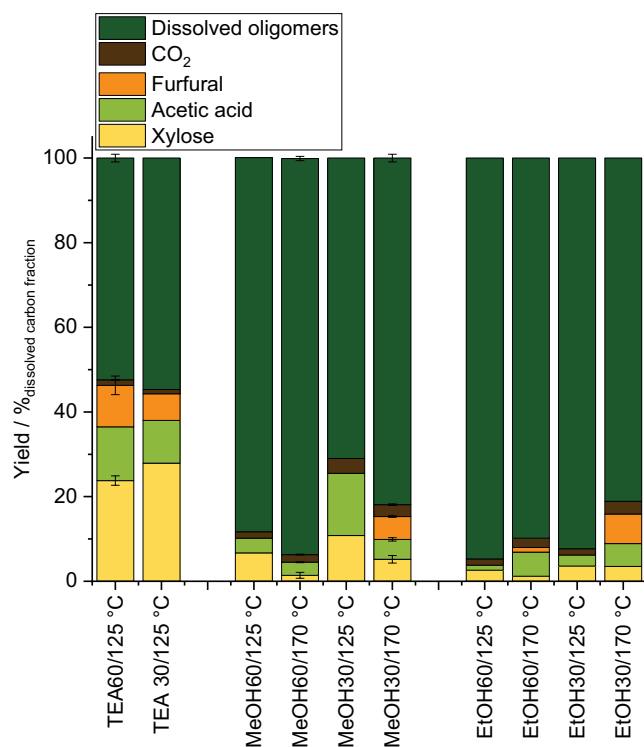


Figure 5. Carbon compounds extracted into liquid phase after fractionation. The yield is related to dissolved carbon fraction of detectable products. Reaction conditions: 200 g liquid phase (TEA60 – 60 wt% of [TEA][HSO₄], TEA30 – 30 wt% of [TEA][HSO₄], MeOH60 – 60 wt% of methanol, MeOH30 – 30 wt% methanol, EtOH60 – 60 wt% of ethanol, EtOH30 – 30 wt% of ethanol, the rest being water), 18 g beech wood chips ($\leq 630 \mu\text{m}$), 30 bar nitrogen at 125 °C/170 °C, 1200 rpm stirring speed, 6 h reaction time.

after the process. Gel permeation chromatography (GPC) analysis showed that oligomers with molar masses of 100 to 2000 g $\cdot\text{mol}^{-1}$ were also present after fractionation (see Supporting Information, Fig. S1) – these were dissolved oligomers. The oligomers extracted with ethanolic organosolv fractionation had the lowest average molecular weight (M_w of 101.6 g $\cdot\text{mol}^{-1}$) whereas the methanolic organosolv solvent contained oligomers with the highest average molecular weight (M_w = 336.0 g $\cdot\text{mol}^{-1}$) and the oligomers extracted with ionosolv solvent had an intermediate M_w of 178.7 g $\cdot\text{mol}^{-1}$ (Supporting Information, Tables S2–S4). Figure 5 shows that these low-molecular-weight oligomers were a major fraction of the liquid phase (over 60%) and might also be a promising substrate for the second oxidation step to formic acid in addition to monomeric xylose.^{32,44}

Figure 5 also shows that a higher water content led to a higher concentration of monomeric xylose in the liquid phase. For example, the xylose content increased from 23.8% to 27.9% dissolved carbon fraction in the ionosolv

fractionation. This trend was also observed for the organosolv fractionation with methanol and ethanol but was less prominent. One explanation might be the higher solubility of hemicellulose sugars in water in comparison with the short-chain alcohols and therefore the more likely hydrolysis of oligomers if the sugars are more soluble in the ionic liquid than in the alcohols. Water also assists in the hydrolysis of amorphous glycosidic bonds within hemicellulose and facilitates the separation of hemicellulose from cellulose by mobilizing the connecting hydrogen bonds.^{26,45}

Another reason for the stronger effect observed with ionosolv fractionation may be that short-chain alcohols are non-acidic, whereas the anion in [TEA][HSO₄] is a moderate acid (pK_a = 2), promoting hemicellulose disruption and hydrolysis. To improve xylose yield in the liquid phase during organosolv pretreatment, acid addition may be beneficial.⁴⁶ In this study, no acid was added during organosolv fractionation, as the polyoxometalate (POM) catalyst introduced in the second step also acts as an acid. Tatibouët⁴⁷ demonstrated the synergistic effect of POM redox sites and acids in methanol oxidation; however, this effect was not observed with HPA-5 in a previous study.⁴⁸ As shown in Supporting Information, Table S5, the acidity of the HPA-5 POM catalyst was too low to enable significant methanol oxidation, confirming earlier findings.^{48,49} The positive effect of higher water content on xylose yield has also been reported by Bukowski *et al.*²⁴ and Abouelela *et al.*³⁴ for beech wood and miscanthus.

Additional GC-MS analysis of the liquid organosolv-solutions after fractionation also showed that methyl- and ethyl esters are formed as formic acid derivatives despite them several other species could be detected but not quantified (Supporting Information, Figs S4 and S5). As expected, the higher the alcohol:water ratio, the more esterification was observed for the organosolv solutions. Different C5 and C6 sugar esters carrying methyl- and ethyl- groups were also found. These measurements could not be performed for the liquid phase of the ionosolv fractionation due to the nonvolatile nature of the IL. From the analysis of the dissolved products, all fractionation methods showed promising results for further oxidation to formic acid (esters) in the second step.

Investigations on the catalytic oxidation step to formic acid and its derivatives

In the second step of the two-step approach, the liquid phase generated in the first fractionation step was separated from the solid and used as a substrate. The reaction solution from the first step was filtered and the liquid phase that was received was used for catalytic oxidation using the HPA-5 catalyst and molecular oxygen as oxidant according to

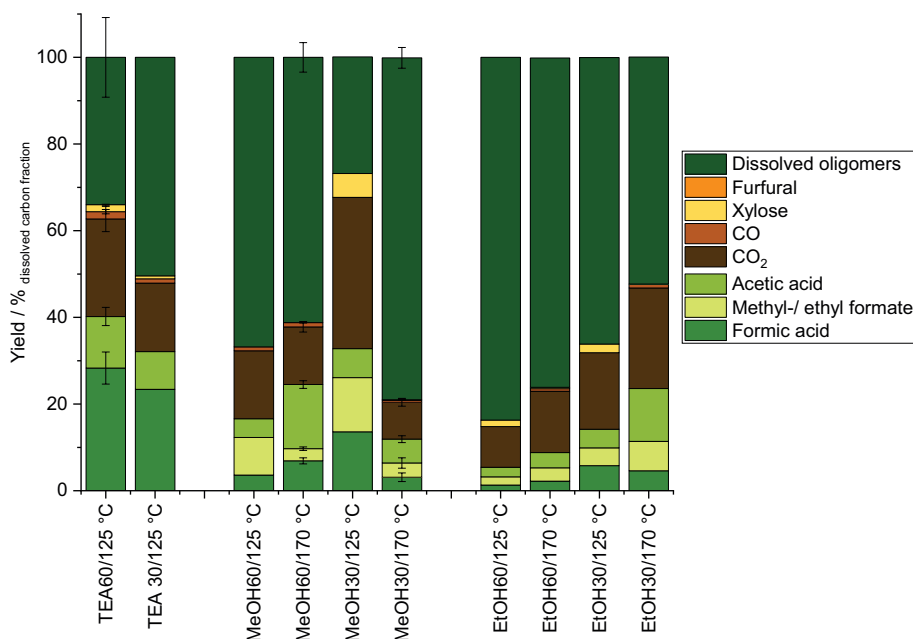


Figure 6. Product yields in the liquid phase after the second oxidation step. Reaction conditions: liquid phase of first step (TEA60 – 60 wt% of [TEA][HSO₄], TEA30 – 30 wt% of [TEA][HSO₄], MeOH60 – 60 wt% of methanol, MeOH30 – 30 wt% methanol, EtOH60 – 60 wt% of ethanol, EtOH30 – 30 wt% of ethanol, rest being water), 0.5 $\frac{\text{mmol}}{100 \text{ g liquid phase}}$ of HPA-5, 30 bar oxygen, 125 °C, 1200 rpm stirrer speed, 6.5 h reaction time.

previous studies.^{24,32} Thus, 1 g of HPA-5 catalyst was added to the liquid phase. Details of the experimental procedure can be found in the experimental section. The target oxidation products were formic acid and its esters, methyl formate and ethyl formate in the case of the organosolv fractionations.

Figure 6 presents the yields of various oxidation products, determined by HPLC or GC–MS in the liquid phase and by GC in the gas phase. The highest formic acid yield, 29%, was achieved after ionosolv fractionation with high IL content (TEA60/125 °C) as the first step. In addition to the complete oxidation of xylose and furfural to formic acid, a significant portion of the dissolved oligomers was also converted. However, a substantial amount of material was fully oxidized to CO₂.

The product yields obtained in the second step essentially followed the dissolution yields of the first step. If high xylose and furfural content was achieved after fractionation, the yields of formic acid and its derivatives were also high. The results for control experiments without lignocellulosic biomass but with the catalyst and the different solvents used are shown in Supporting Information, Table S5 to evaluate potential degradation of the used fractionation solvent under oxidative conditions, showing that only a negligible degradation (below 0.2% of the solvent) took place. It is known that the selectivity to formic acid and methyl formate is significantly improved (>90%) during the degradation of

sugars when methanol is used as a co-solvent and HPA-5 as a catalyst because overoxidation to CO₂ can be efficiently suppressed.⁴³ There is an ongoing scientific discussion on the mechanism responsible for that effect.^{43–45,50} Nevertheless, the selectivity-boosting effect of methanol on HPA-5 can be seen in the comparison of the selectivity of formic acid derivatives in the two different organosolv solvents.

Table 1 presents the selectivity for formic acid, its esters, and CO₂ resulting from the catalytic oxidation step. The selectivity ratio of liquid products (formic acid + esters) to gaseous by-products (CO₂) is given as a measure of value relative to waste. The ionosolv fractionation showed the highest selectivity ratio, reaching 1.49 for the low IL content sample (TEA30/125 °C). As far as we are aware, the detailed mechanistic interactions between HPA-5 and [TEA][HSO₄] have not yet been investigated. It is known, however, that POM-ILs can activate CO₂ and serve as effective catalysts for various oxidation reactions, such as oxidative desulfurization, oxidative cleavage of olefins, and alcohol oxidation to carboxylic acids, as summarized in the reviews by Martinetto *et al.*⁴⁶ and Wang *et al.*⁴⁷. Methanolic organosolv fractionation also exhibited significantly higher selectivity toward formic acid and its esters (0.78) than ethanolic organosolv fractionation (0.56), an effect referred to as selectivity boosting.⁴³ In all fractionation methods, higher water content influenced selectivity positively toward formic acid and its esters.

Table 1. Selectivities for formic acid, formic acid esters and CO₂. The selectivity is related to the dissolved carbon.

#	Name	Selectivity /%			Selectivity ratio formic acid + esters/CO ₂
		Formic acid	Methyl formate	Ethyl formate	
1	TEA60/125 °C	26.5			1.26
2	TEA30/125 °C	23.4			1.49
3	MeOH60/125 °C	3.6	8.7		0.78
4	MeOH60/170 °C	6.9	2.8		0.73
5	MeOH30/125 °C	13.6	12.5		0.74
6	MeOH30/170 °C	3.1	3.3		0.76
7	EtOH60/125 °C	1.3		1.9	0.33
8	EtOH60/170 °C	2.2		3.1	0.37
9	EtOH30/125 °C	5.8		4.1	0.56
10	EtOH30/170 °C	4.6		6.8	0.48

Conclusions

A two-step biorefinery concept, comprising sequential hydrolytic fractionation of lignocellulosic biomass under an inert atmosphere followed by HPA-5-catalyzed oxidation under an oxygen atmosphere, was investigated in detail. The cellulose-enriched solid from ionosolv fractionation exhibited the highest cellulose content (73.5%) using a 60 wt% IL to 40 wt% water ratio. However, the highest glucose yields upon enzymatic saccharification (78.3% and 78.2%) were obtained from solids produced by high-temperature organosolv fractionation (170 °C) with 30 wt% methanol or ethanol and 70 wt% water. Both high-temperature organosolv and ionosolv fractionations dissolved between 30% and 45% of the total carbohydrate content (TCC) into the liquid phase. In ionosolv fractionation, higher IL content (60 wt%) led to greater dissolution of both hemicellulose and lignin, an effect less pronounced in organosolv fractionation for both methanol and ethanol. As expected, increased extraction of hemicellulose and lignin enhanced glucose yields during enzymatic saccharification.

Ionosolv fractionation also produced the highest yields of xylose (26%), acetic acid (10%), and furfural (8%) among the dissolved carbon fractions, with the remainder comprising unspecified oligomers. In the subsequent oxidation step, ionosolv-derived fractions yielded more formic acid (28%) and acetic acid (10%) than organosolv-derived fractions, which exhibited higher overoxidation to CO₂ (up to 35%). Generally, IL/water mixtures enabled a higher degree of oxidation than alcohol/water mixtures.

The two-step approach offers several advantages: the cellulose-enriched solid requires no extensive solvent washing, as fractionation (cellulose production) and

oxidation (formic acid production) are separated, preventing catalyst contamination of the solid. Consequently, the cellulose was protected from catalytic degradation, leading to higher quality material as demonstrated by enzymatic saccharification. Further improvements to this two-step concept, particularly in enhancing formic acid yields, represent a promising area for future research. Investigating catalyst interactions in the various solvents would contribute to a deeper understanding of the oxidation step and enable further optimization of the biorefinery process.

Experimental details

Chemicals

All chemicals and feedstocks are commercially available and were used without further purification. Sulfuric acid (>95%) and triethylamine (99%) were purchased from Grüssing GmbH (Filsum, Germany). Methanol (>99.9%, HPLC grade) and ethanol (99.8%, HPLC grade) were supplied by VWR LLC (Radnor, Pennsylvania, USA).

Beech wood chips were provided from the Mushrooms & Equipment Shop (Münster, Germany). The water was provided as deionized water from the University of Hamburg (Hamburg, Germany).

Syntheses of catalyst and ionic liquid

The vanadium-substituted HPA-5 (H₈[PV₅Mo₇O₄₀]) POM-catalyst was synthesized as specified in the literature.⁴⁵ The synthesis route is described in detail in the review of Raabe et al.⁴⁸ The elemental composition determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) of the catalyst batch that was used as well as the structural characterization by Fourier transform infrared spectroscopy

(FTIR) can be found in the Supporting Information (Table S6 and Fig. S6). The ionic liquid triethylammonium hydrogen sulfate [TEA][HSO₄] was synthesized by the addition of sulfuric acid to triethylamine: 2.5 mol triethylamine (253.0 g) was placed into a round-neck flask cooled with an ice bath and the sulfuric acid (5 M, 500 mL, 2.5 mol) was added slowly. The resulting ionic liquid was concentrated on a rotary evaporator (75 mbar, 90 °C, overnight) cooling to room temperature until a colorless, crystalline solid was formed. The water content of the [TEA][HSO₄]-ionic liquid crystals was determined to be 2.5% by Karl Fischer titration (Metrohm 831 KF coulometer with Metrohm 728 stirrer (Metrohm, Herisau, Switzerland)).

Fractionation experiments

All fractionation experiments were conducted in a 600 mL Hastelloy autoclave (Parr Instrument GmbH (Moline, Illinois, USA)) equipped with a gas entrainment stirrer. The reactor was charged with 18 g of ground and sieved substrate ($\leq 630 \mu\text{m}$) and 200 g of liquid phase, consisting of varying ratios of IL, ethanol, or methanol to water. After sealing, the reactor was purged three times with nitrogen (30 bar) and prepressurized to approximately 20 bar. It was then heated to the target temperature (125 °C or 170 °C) while stirring at 300 rpm. Upon reaching the reaction temperature, the nitrogen pressure was adjusted to 30 bar and the stirrer speed increased to 1200 rpm to ensure effective mixing. After a reaction time of 6 h, the stirrer speed was reduced to 300 rpm, and the reactor was cooled to room temperature. A gas sample was collected from the cooled gas phase and analyzed by GC before depressurizing the reactor. The solid residue was filtered, washed with deionized water, and dried, then subjected to CHNS analysis, compositional analysis, and enzymatic saccharification. The liquid phase was analyzed by HPLC and GPC.

Catalytic oxidation experiments

Experiments for the second step (catalytic oxidation) were performed in the same reactor as the fractionation experiments. The substrates (liquid phase from fractionation step containing dissolved biomass) and the HPA-5 catalyst at a concentration of 0.5 mmol per 100 g of liquid phase were added. The reactor was purged three times with 30 bar oxygen and prepressurized with oxygen. The same procedure was applied as in the fractionation experiments except for 6.5 h reaction time and a stirrer speed of 1200 rpm was used from the beginning. After the experiment, a gas sample was taken from the reactor and analyzed by GC. The liquid phase was analyzed using HPLC and GC-MS.

Analytatics

The gas phase was analyzed using a Varian GC 450 (Varian Inc. (Palo Alto, CA, USA)) equipped with a Shin-carbon column (2 m $\times$ 0.75 mm), a thermal conductivity detector (TCD) (Varian Inc. (Palo Alto, CA, USA)), and a flame ionization detector (FID) (Varian Inc. (Palo Alto, CA, USA)). The yields (Y) of CO and CO₂ were determined according to Eqn (1):

$$Y_i = \frac{n_i}{n_{\text{dissolved carbon fraction}}} \times 100\% \quad (1)$$

with $i = \text{CO}_2 \text{ or CO}$

The liquid phase was analyzed using a Shimadzu (Kyōto, Japan) HPLC equipped with an Aminex Biorad (San Francisco Bay Area, USA) 87H column and a RI detector (RID-20A, Shimadzu). The sample was diluted 1:1 with 5 mM H₂SO₄ and filtered through a 0.2 μm Micropur PET (Polyester) filter (Altman Analytic (Munich, Germany)) before analysis. The eluent of the HPLC was sulfuric acid (5 mM) and used a 0.5 mL $\cdot \text{min}^{-1}$ flow rate. The column oven temperature was set to 45 °C. The main products xylose, furfural, formic acid and acetic acid were quantified by HPLC. The alcoholic esters of formic acid – methyl formate and ethyl formate – were quantified by GC-MS. Tert-butanol was used as an internal standard. The yields of the different products were determined using Eqn (2).

$$Y_i = \frac{n_i}{n_{\text{dissolved carbon fraction}}} \times 100\% \quad (2)$$

with $i = \text{xylose, furfural, acetic acid, formic acid, methyl formate, ethyl formate}$

The selectivity of different components was determined using Eqn (3):

$$S_i = \frac{Y_{i, \text{total carbon}}}{\text{dissolution}} \times 100\% \quad (3)$$

with $i = \text{component}$

The elemental composition of the solid was analyzed using an Euro Vector EA 3000 (EuroVector (Pavia, Italy)) to determine the carbon (C), nitrogen (N), hydrogen (H) and sulfur (S) content.

The compositional analysis of the cellulose rich solid was carried out according to the laboratory analytical procedure (LAP) NREL/TP-510-42618²⁸ and the enzymatic saccharification according to LAP NREL/TP-5100-63351.⁴⁹ The amount of C in the different samples had to be calculated using Eqn (4). The dissolved carbon fraction was determined

using Eqn (5) and the amount of dissolved C ($n_{\text{dissolved,C}}$) via Eqn (6).

$$n_C = \frac{m * w_C}{M_c} \quad (4)$$

dissolution =

$$\left(1 - \frac{m_{\text{solid residue after fractionation}} \times w_{C, \text{solid residue after fractionation}}}{m_{\text{beech wood, initial}} \times w_{C, \text{beech wood initial}}} \right) \quad (5)$$

×100%

$$n_{\text{dissolved carbon fraction}} = n_{C, \text{beech wood}} - n_{C, \text{solid residue}} \quad (6)$$

Gel permeation chromatography (GPC) was performed using a Knauer K-4002 degasser and a Smartline 3800 autosampler, equipped with a PLgel 10 µm guard column and two PLgel 10 µm MIXED-B columns (Agilent), with pure methanol as the eluent at a flow rate of 1 mL·min⁻¹ and 35 °C, using a refractive index (RI) detector. The oven contained a Suprema 5 µm 8 × 50 mm guard column and two PSS Suprema Linear M 5 µm 8 × 300 mm columns. All samples were filtered through a syringe filter, diluted 1:1 with methanol, and analyzed using polyethylene glycol and polyethylene oxide as internal standards.

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5.2 Boosting POM-Ionosolv biorefining of lignocellulosic biomass by using redox-balanced polyoxometalate catalysts in methanolic ionic liquid reaction media

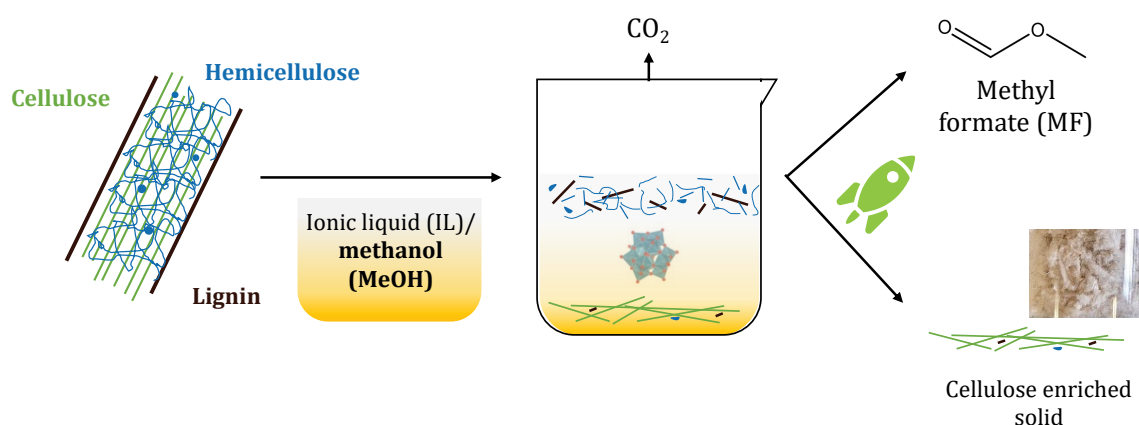


Fig. 5.2 Graphical abstract of the second study of this dissertation: "Boosting the POM-ionosolv concept by using ionic-liquid-alcoholic reaction media and polyoxometalate catalysts".^[284]

5.2.1 Summary

In contrast to the concept of the study in Section 5.1, this study^[284] investigated a one-pot system based on the first idea of the POM-ionosolv concept by Bukowski et al.^[121] to maximize the yield of the liquid products. The novelty of this study is the usage of a new solvent system of an IL and methanol. Methanol is well known boosting the selectivity of POM catalysts towards formic acid respectively methyl formate to nearly 100 %.^[208] Therefore the idea was to combine this effect together with the fractionation properties of ILs to boost the POM-ionosolv concept to a next level. The same lignocellulosic biomass as in study I (vide intra Section 5.1), beech wood, was used as a substrate. Screening three different ILs (*N,N*-dimethylbutylammonium hydrogen sulfate ([DMBA][HSO₄]), triethylammonium hydrogen sulfate ([TEA][HSO₄]) and tributylmethylphosphonium methyl sulfate ([TBMP][MeSO₄])) according to solvent stability based on methyl formate using different Keggin-type POMs was the start to find a suitable combination of solvents. The best system of solvents and catalyst was found to be the redox-balanced H₈PVMnMo₁₀O₄₀ Keggin-POM combined with the [TBMP][MeSO₄]/MeOH solvent mixture. This combination achieved the highest yields of methyl formate (19.8 % of the dissolved beech wood). Furthermore, the cellulose-enriched pulp was characterized via compositional analysis according to NREL^[286]

5.2 Boosting POM-Ionosolv biorefining of lignocellulosic biomass by using redox-balanced polyoxometalate catalysts in methanolic ionic liquid reaction media

and processed through enzymatic saccharification^[282] to evaluate the quality of the received pulp. Hereby, the advanced POM-ionosolv concept generated a white pulp with over 90 % of glucose content. A saccharification yield of 77 % from that pulp was achieved. In this pulp, over 60 % of the initial cellulose could be extracted. Moreover, the occurred catalyst-IL precipitate was deeply studied via NMR, FT-IR, XPS, and X-ray diffraction measurement. The structure of that precipitate could be fully clarified through these methods. Furthermore, it could be demonstrated by heating up the reaction solution in a glass tube that the reaction mixture at the choosen reaction conditions is homogeneous. To separate and purify the products, a washing procedure with acetone for the cellulose-enriched pulp and a proof of concept distillation for the methyl formate were established within the study. Finally, the novel developed concept was applied to several other lignocellulosic substrates like birch, spruce, pine wood and miscanthus.

This newly concept is a promising approach for generating a highly pure cellulose-enriched pulp and methyl formate as a second product in liquid phase.

5.2.2 Article II

StefanieWesinger, Aleksandra Rabiner, Suhaib Nisar, Leonhard Schill, Mariusz Grzegorz Kubus, Maximilian J. Poller, Anders Riisager, Agnieszka Brandt-Talbot, Jason P. Hallett, Jakob Albert, ChemSusChem **2025**, DOI 10.1002/cssc.202501034.

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Boosting POM-Ionosolv Biorefining of Lignocellulosic Biomass by Using Redox-Balanced Polyoxometalate Catalysts in Methanolic Ionic Liquid Reaction Media

Stefanie Wesinger, Aleksandra Rabiner, Suhaib Nisar, Leonhard Schill, Mariusz Grzegorz Kubus, Maximilian J. Poller, Anders Riisager, Agnieszka Brandt-Talbot, Jason P. Hallett, and Jakob Albert*

This article presents an advanced iteration of the polyoxometalate (POM)-Ionosolv concept to generate biobased methyl formate in high yield and a bleached cellulose pulp from lignocellulosic biomass in a single-step operation by using redox-balanced POM catalysts and molecular oxygen in alcoholic ionic liquid (IL) mixtures. The performance of the three Ionosolv-ILs triethylammonium hydrogen sulfate ([TEA][HSO₄]), *N,N*-dimethylbutylammonium hydrogen sulfate ([DMBA][HSO₄]), and tributylmethylphosphonium methyl sulfate ([TBMP][MeSO₄]), mixed with methanol (MeOH) (30/70 wt%), is evaluated by methyl formate yield from extracted hemicellulose and lignin as well as purity of the bleached cellulose pulp in the presence of various Keggin-type POMs. The

redox-balanced H₈PVMnMo₁₀O₄₀ POM catalyst in [TBMP][MeSO₄]/MeOH emerge as the most effective combination, achieving 20% methyl formate yield from commercial beech wood. The glucan content in the bleached cellulose-enriched solid consisted is over 90%, demonstrating that the use of MeOH drastically improved lignin extraction in parallel with full hemicellulose extraction. The cellulose is highly susceptible to enzymatic hydrolysis, generating a pure and concentrated cellulosic glucose stream. The formed solid catalyst complex is examined in detail to reveal its chemical nature as POM-IL-complex. The approach is applicable to disparate types of lignocellulosic biomass, including hardwood, softwood, and grass.

1. Introduction

Considering the acceleration of global warming in recent years, it is imperative for the chemical industry to transition to the utilization of renewable resources. A particularly promising renewable resource is lignocellulosic biomass, chiefly comprised of cellulose,

hemicellulose, and lignin.^[1] Cellulose is a polysaccharide consisting of β -D-glucopyranose units linked together by β -1,4-glycosidic bonds, constituting 23–50 wt% of lignocellulosic biomass. Cellulose has gained significant interest as an environmentally friendly and renewable feedstock for the synthesis of biobased materials, biofuels, and chemicals.^[1–3] Its abundance, high mechanical strength, biodegradability, and renewability makes it attractive in a variety of applications, including the packaging, paper and textiles, bioplastics, and scaffolds for biomedical products and other advanced materials.^[1–3] Hemicellulose is a complex branched group of polysaccharides, consisting of heterogeneous mixtures of pentoses (xylose, arabinose) and hexoses (mannose, glucose, galactose) as well as acids such as acetic, galacturonic, and glucuronic acid. β -1,4-glycosidic, and occasionally, β -1,3-glycosidic bonds link the subunits. Hemicellulose constitutes 20–40 wt% of lignocellulosic biomass. The hemicellulose in hardwoods contains mostly xylans and some glucomannans, while softwood mainly contains glucomannans and some xylans, arabinogalactans, xyloglucans, and glucans.^[3–5] The third major biopolymer in lignocellulosic biomass is lignin. It consists of phenylpropanoid units with one or two main aromatic subunits, guaiacyl and syringyl, and a range of aliphatic linkages, such as β -O-4 ether, β - β (resinol), and β -5 (phenylcoumaran). Lignin has a random structure, nonhydrolysable linkages, is hydrophobic, and self-reactive (lignin condensation), which protects against microbial invasion and obstructs lignin extraction.^[6–9] Every component in lignocellulosic biomass can be valorized into products used for various applications, including biofuels, biochemicals, biopolymers, and pharmaceuticals.^[10–12]

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The occurrence of multiple components necessitates fractionative pretreatment of lignocellulosic biomass, which is carried out in the presence of solvents, such as water, organic solvents (organosolv), or organic salts, including ionic liquids (ILs) and deep eutectic solvents.^[13] Among the various fractionation approaches, the use of low-cost IL water mixtures is a promising avenue (ionosolv fractionation) due to the low volatility of the ILs, which facilitates solvent recovery.^[14] ILs are salts that exist as liquids typically below 100 °C. They consist of large, asymmetric carbon-containing cations and small, typically nonorganic anions.^[13,15] Some examples of ILs used successfully in lignocellulosic biomass fractionation are low-cost salts, such as triethylammonium hydrogen sulfate [TEA][HSO₄] and *N,N*-dimethylbutylammonium hydrogen sulfate [DMBA][HSO₄].^[14,16] Another IL known for effectively dissolving lignin is tributylmethylphosphonium methyl sulfate, [P₁₄₄₄][MeSO₄] or [TBMP][MeSO₄].^[13]

Previous studies have demonstrated the efficacy of combining ionosolv fractionation and in situ oxidation utilizing a polyoxometalate (POM) catalyst and molecular oxygen.^[17–19] POMs are anionic polynuclear metal-oxo clusters that exhibit a large structural diversity and tunable properties. Several studies have investigated the valorization of biomass using POM catalysts to produce value-added chemicals, such as formic acid, lactic acid, and levulinic acid.^[20–23] The combination of [TEA][HSO₄] and water (30:70 wt%) enabled extraction of hemicellulose and lignin from various biomass, which were oxidized in situ to formic acid in the presence of the HPA-5 (H₈PV₅Mo₇O₄₀) POM catalyst and molecular oxygen, while generating a cellulose-enriched solid with a high glucan content as a second product. So far, the purity of the cellulose-enriched solid has been a limiting factor for further utilization in biotechnology, which could be due to the low solubility of lignin in water.^[19,24]

The combination of ethanol as cosolvent with [TEA][HSO₄] for ionosolv-organosolv pretreatment was found beneficial in a previous study,^[19] while MeOH has also demonstrated to be a superior extraction solvent for lignin from lignocellulosic biomass in several studies.^[25,26] MeOH has also been investigated as a suitable solvent for POM-catalyzed biomass oxidation, showing that methyl formate as a promising building block in C₁ chemistry^[27] was obtained as the only oxidation product at exceptional >99% selectivity,^[28] resulting from manipulation of the HPA-5 catalyst by forming a stable vanadate-methanol-complex in solution completely suppressing undesired CO₂ formation.^[29] Hence, the combination of MeOH with IL as solvent for combined lignocellulose fractionation

and in situ POM-catalyzed oxidation has the potential for a step-change in biorefining of lignocellulosic biomass. **Table 1** underlines the importance of the different combined systems in this study.

The present study investigates the potential of this advanced POM-Ionosolv concept applying an efficient POM catalyst in an IL/MeOH reaction mixture for the production of a highly cellulose-enriched solid and methyl formate as a liquid product with high yield and selectivity in a single-step process. The use of several ILs displaying high hemicellulose and lignin extraction was examined in combination with methanol as a co-solvent and redox-balanced POM catalysts to avoid over-oxidation of the dissolved fraction to CO₂. The interaction between the IL and the POM catalyst was subjected to rigorous investigation, and the purification and separation of the solid product were demonstrated. Moreover, a diverse range of lignocellulosic biomass, including softwood, hardwood, and grass, were evaluated as potential substrates.

2. Results and Discussion

2.1. Selection of a Suitable Solvent for the Enhanced POM-Ionosolv Concept

A limitation of the biomass fractionation via the original POM-Ionosolv concept, as demonstrated by Bukowski et al.^[17,18] and Wesinger et al.,^[19] is the low extraction efficiency of lignin into the employed [TEA][HSO₄] with water as a cosolvent. Conversely, methanol has been suggested as a highly effective solvent for dissolving lignin.^[29,30] Therefore, an improved alcoholic POM-Ionosolv 2.0 concept was developed, combining the ionosolv fractionation method and the organosolv fractionation method using methanol. To the best of our knowledge, the combination with a POM catalyst and this novel fractionation method was not applied before.

Three different ILs were selected and investigated for their ability to enhance hemicellulose and lignin extraction: [TEA][HSO₄] as a benchmark from previous studies,^[17,18] [DMBA][HSO₄],^[31] and [TBMP][MeSO₄].^[32] The first two ILs are well-known, low-cost IL options established for ionosolv fractionation, with [DMBA][HSO₄] typically demonstrating improved lignin extraction relative to [TEA][HSO₄].^[31] The third IL was recognized for its effective lignin extraction^[16] but is not as cost-effective as the other two ILs. However, phosphonium ILs are known for their high stability

Table 1. Importance of different systems combined in this study.

	Methanol	ILs	POM catalyst
Function	Organosolv-fractionation	Ionosolv-fractionation	Catalytic oxidation
Advantages	- Increasing lignin fractionation^[25,26] - Suppressing undesired CO₂ formation in combination with POM catalyst^[28,29]	- Effective fractionation of complex biomass^[13] - Low volatility—enhancing the reaction safety^[14] - High quality of cellulosic residue after fractionation^[13,14,16]	- Effective in in-situ oxidation of biomass in different solvents, like water, methanol or aqueous ILs^[17–23] - Valorization of the liquid fraction of the combined fractionation method without a second step

against oxidizing agents.^[32] All ILs were mixed with MeOH in a ratio of 30 wt% IL and 70 wt% MeOH, based on the optimal IL-cosolvent ratio determined by Bukowski et al.^[18] For clarity, the solvent mixtures are named as follows: [TEA][HSO₄]/MeOH (30 wt%/70 wt%) is referred to as methanolic TEA solvent, [DMBA][HSO₄]/MeOH (30 wt%/70 wt%) as methanolic DMBA solvent, and [TBMP][MeSO₄]/MeOH (30 wt%/70 wt%) as methanolic TBMP solvent. Beech wood was chosen as the substrate to ensure better comparability with previous studies on the POM-Ionosolv concept.^[17–19]

After fractionating biomass in the three solvents without oxidative catalyst, the solids' composition, morphology, and elemental composition were determined (Figure 1). The compositional analysis of the solids and the untreated beech wood indicates that glucan could be enriched with all three solvents. Notably, the methanolic TEA solvent performed worse than the other two solvents, with 52.1% glucan in the solid compared to 42.0% glucan in the raw beech wood, showing only limited cellulose enrichment. In contrast, the other two solvents extracted lignin and hemicellulose near-quantitatively, with the glucan content in the DMBA and TBMP treated solid exceeding 90% (91.7% and 90.6%, respectively).

This high glucan content also correlated with the morphology of the solids (see Figure S1, Supporting Information). While the solid processed with methanolic TEA solvent had an appearance similar to unprocessed beech wood, the solids processed with methanolic DMBA and methanolic TBMP solvents appeared white and defibrillated, similar to cotton balls. The appearance suggests that combining the IL with MeOH as a fractionation medium in the presence of oxygen generated a bleached cellulose pulp consisting of over 90 wt% glucan. It is possible that the cellulose content is underestimated, which is a function of the laboratory analytical procedure (LAP) compositional analysis protocol, which was developed for raw lignocellulosic biomass. To investigate the accessibility of this cellulose-enriched solid to cellulase enzymes, an enzymatic saccharification according to LAP NREL/TP-5100-63351^[51] was performed. Figure 2 shows that all processed solids

generated a higher glucose yield than the unprocessed beech wood. The methanolic TEA solid generated the lowest glucose yield (38%), consistent with its lower glucan content (52.1 wt%) compared to the other IL/MeOH solvents tested. Conversely, the highest glucose yield of 87.6% was achieved with methanolic TBMP, while the methanolic DMBA solvent generated a glucose yield of 76.8%.

2.2. Combining Suitable Solvents with the HPA-5 Catalyst

To valorize the extracted hemicellulose and lignin, the HPA-5 POM catalyst was employed, as several studies have shown that this compound is highly promising for the in situ oxidation of lignin and hemicellulose in various solvents.^[17,18,20,22,33] All three methanolic IL solvents were screened with HPA-5 to oxidize the dissolved hemicellulose and lignin into the C₁ building block methyl formate. The comparison of the results obtained with and without catalyst for the different solvents is shown in Figure 3. The dissolution of the carbon fraction from beech wood was determined via the carbon content of the substrate determined by carbon, hydrogen, nitrogen, sulfur (CHNS) elemental analysis and the solid phase product and is shown on the right, while the methyl formate yield is shown on the left. Detailed calculations can be found in the Experimental Section.

Dissolution of carbon can be used to evaluate the fractionation performance while methyl formate yield is used to quantify the effectiveness of oxidative conversion of the dissolved compounds. Figure 3 shows that the methanolic TEA solvent extracted about 30% of the carbon fraction in the original biomass. Adding the HPA-5 catalyst enhanced the dissolution to about 50%, likely due to the additional Brønsted acidity provided by the POM catalyst, which facilitates the cleavage of carbon–carbon (C–C) bonds in the lignocellulosic biomass. In general, the other two solvents showed a higher dissolution whereby the enhancement was less pronounced. The difference in the dissolution of the carbon fraction with and without HPA-5 catalyst in the methanolic DMBA solvent

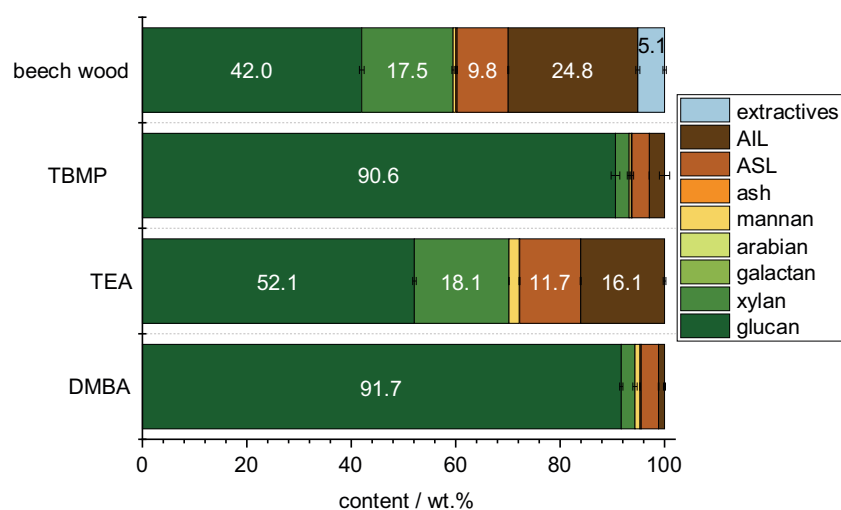


Figure 1. Compositional analysis of pure beech wood and the processed solids using different IL/MeOH solvents via NREL/TP-510-42618.^[47] Reaction conditions for preparing the solids: 120 °C, 24 h, 1000 rpm, 30 bar oxygen at reaction temperature, 0.5 g beech wood, and 10 g solvent (either methanolic TEA solvent, methanolic DMBA solvent, or methanolic TBMP solvent). ASL = acid soluble lignin, AIL = acid insoluble lignin.

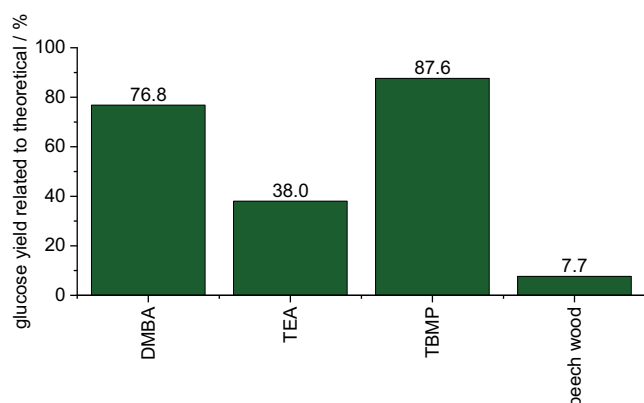


Figure 2. Enzymatic saccharification yields of pure beech wood in comparison to cellulose-enriched solid processed using different IL/MeOH solvents analyzed via LAP NREL/TP-5100-63351.^[51] Reaction conditions producing solids: 120 °C, 24 h, 1000 rpm, 30 bar of oxygen at reaction temperature, 10 g solvent (either methanolic TEA solvent, methanolic DMBA solvent, or methanolic TBMP solvent), 0.5 g beech wood.

was 1.9% (64.4% without catalyst and 66.3% with catalyst), within a standard deviation of 5%. For the methanolic TBMP solvent, the difference was 4.3% (above the standard deviation of 1.5%) with 65.8% dissolution without catalyst and 69.1% with catalyst.

The yield of methyl formate increased in the presence of the HPA-5 catalyst in both the methanolic DMBA solvent (from 2.9% to 5.9%) and the methanolic TBMP solvent (from 2.9% to 6.1%).

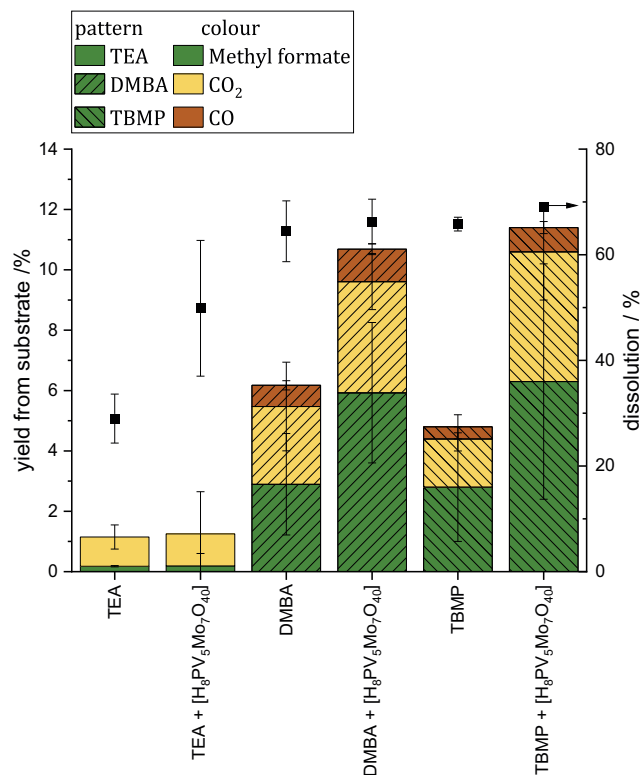


Figure 3. Product yields using different IL/MeOH solvents with the reference HPA-5 catalyst. Reaction conditions: 120 °C, 24 h, 1000 rpm, 30 bar oxygen at reaction temperature, 10 g solvent (either methanolic TEA solvent, methanolic DMBA solvent, or methanolic TBMP solvent), 0.5 g beech wood, 0.18 g catalyst HPA-5 (1 mmol g⁻¹ catalyst to solvent).

However, this was not the case for the methanolic TEA solvent, as it showed only a negligible methyl formate yield. The formation of the undesired byproduct CO₂ also increased with the presence of HPA-5 catalyst in both the methanolic DMBA solvent (from 2.6% to 3.7%) and the methanolic TBMP solvent (from 1.4 to 3.2%).

The stability of the IL/MeOH solvents under reaction conditions with HPA-5 present was further investigated in the absence of substrate. As evident in the ¹³C nuclear magnetic resonance (NMR) spectra of the reaction mixtures (Figure S2–S4, Supporting Information), all the IL/MeOH solvents formed traces of methyl formate resulting from acid-catalyzed MeOH oxidation, as previously shown by Tatibouët et al. using a vanadium-incorporated POM catalyst (H_{3+n}PV_nMo_{12-n}O₄₀ on K₃PMo₁₂O₄₀).^[34] Consequently, the methyl formate yields were corrected accordingly as described in the Experimental Section. In the tested methanolic IL solvents, acidic sites were present in the IL as well as the POM catalyst, and the latter also contributed redox-active sites. The termination of the reaction depends on both acidity and redox activity, hence, it is assumed that the partial oxidation of MeOH to methyl formate was facilitated by the high redox activity of HPA-5, due to the incorporation of five vanadium atoms into the POM structure, in combination with the acidic ILs. Therefore, balancing both redox-active and Brønsted acid sites within the POM structure is required. The acidity of the solvent media play also an important role for the overall reaction media stability.

As the methanolic TEA solvent generally showed lower performance in terms of hemicellulose and lignin dissolution and methyl formate yield, only the composition of the processed solids formed in the DMBA and TBMP solvents with HPA-5 catalyst were analyzed by near infrared (NIR) spectroscopy.^[35] As shown in Figure 4, the catalyst influenced the composition of the processed solids, resulting in a slightly lower glucan content with the catalyst than without it (see Figure 1) (methanolic DMBA solvent: 2.3% lower, methanolic TBMP solvent: 5.5% lower). This could be due to catalyst precipitation (elaborated in more detail in the next section), which also affected the compositional analysis of the solid.

The cellulose-enriched solid of the methanolic TBMP + HPA-5 solvent resulted in a higher glucose yield after enzymatic saccharification compared to the cellulose-enriched solid of the methanolic DMBA + HPA-5 solvent (Figure 5). However, addition of the redox-active HPA-5 catalyst slightly reduced the glucose yield after fractionation with the methanolic TBMP + HPA-5 solvent to 76.4%, compared to 87.6% after fractionation using the solvent without catalyst. For the methanolic DMBA + HPA-5 solvent, addition of the redox-active HPA-5 catalyst had an even more drastic effect, resulting in only 21% glucose yield after saccharification, significantly lower than the 76.8% yield obtained without the HPA-5 catalyst. This effect might be related to interference from the precipitated catalyst.

2.3. Selection of a Redox-Balanced POM Catalyst for the Enhanced POM-Ionosolv Concept

To introduce lower redox activity while maintaining the Brønsted acidity of HPA-5, other redox-active metals, such as manganese

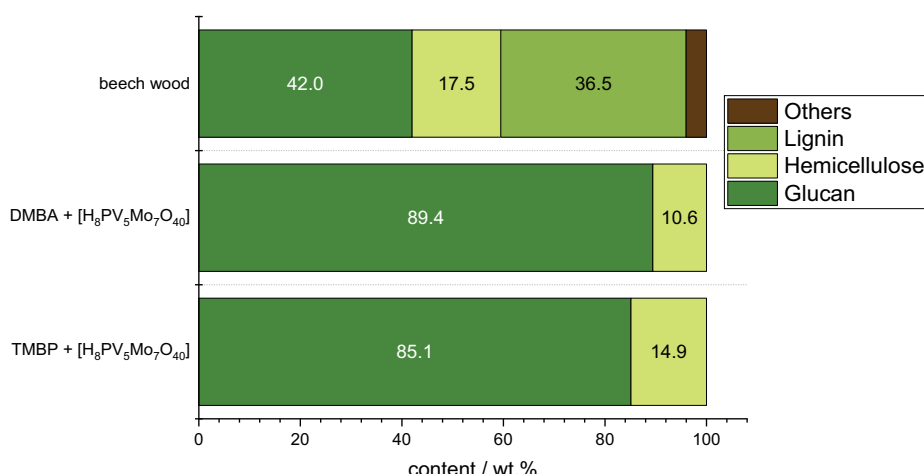


Figure 4. Compositional analysis of pure beech wood in comparison to the processed cellulose-rich solid analyzed by NIR spectroscopy after reaction with the HPA-5 catalyst. Reaction conditions: 120 °C, 24 h, 1000 rpm, 30 bar oxygen at reaction temperature, 10 g solvent (either methanolic TEA, methanolic DMBA, or methanolic TBMP), 0.5 g beech wood, 0.18 g catalyst HPA-5 (1 mmol g⁻¹ catalyst to solvent).

(Mn), can be introduced into the POM structure, or a lower vanadium content than the reference system can be used. The catalysts surveyed in this study in this regard were various Keggin-type structured POMs: HPA-2, a twofold vanadium-substituted POM (H₅PV₂Mo₁₀O₄₀); HPA-VMn, a vanadium- and manganese-substituted POM (H₈PVMnMo₁₀O₄₀); HPA-Mn₂, a twofold manganese-substituted POM (H₁₁PMn₂Mo₁₀O₄₀); and HPA-Mn, a onefold

manganese-substituted POM (H₇PMnMo₁₁O₄₀). The order of the catalysts in terms of redox activity is HPA-5 > HPA-2 > HPA-VMn > HPA-Mn₂ > HPA-Mn,^[36] as highlighted by square wave voltammetry (SWV) for the different catalysts (Figure S5, Supporting Information). Every peak in SWV represents a redox pair of the catalyst, and a larger number of peaks indicates higher redox activity. The higher the voltage of the pair, the higher the redox potential. It is not yet understood which pair(s) are responsible for the biomass oxidation in these POMs and clarifying this would exceed the scope of this study.

Like the HPA-5 catalyst, the alternative V- and Mn-containing POM catalysts were tested for solvent stability and formation of methyl formate under typical reaction conditions. All catalysts were also tested about their stability in the respective solvent system and found to be stable. This can be seen in the fourier transformed infrared spectroscopy (FT-IR) spectra after the reaction showing the typical Keggin-POM bands (see Figure S7, S9, and S11, Supporting Information). As shown in Figure S6, S8, and S10, Supporting Information, the methanolic TEA solvent was also unstable under reaction conditions with the alternative catalysts, despite their lower redox activity. Therefore, the methanolic TEA solvent was excluded from further studies. Instability was also observed in the methanolic DMBA and TBMP solvents under reaction conditions with highly redox-active POMs, like HPA-5 and HPA-2 (Figure S8 and S10, Supporting Information). However, certain IL-catalyst systems with less redox-active POMs were identified as stable (HPA-VMn, HPA-Mn₂, HPA-Mn) as they do not show any methanol oxidation under reaction conditions, hence, these systems were studied further. For the methanolic DMBA solvent, the liquid- and gas-phase analysis of the catalyst screening is shown in Figure 6. The highest yield of methyl formate (8.3%) was obtained with the HPA-VMn catalyst, which has medium redox activity. This catalyst contains vanadium as a highly redox-active metal and manganese to reduce the redox activity.^[36] For the biomass fractionation, no clear trend was identified with the different alternative catalysts, but the most extensive dissolution

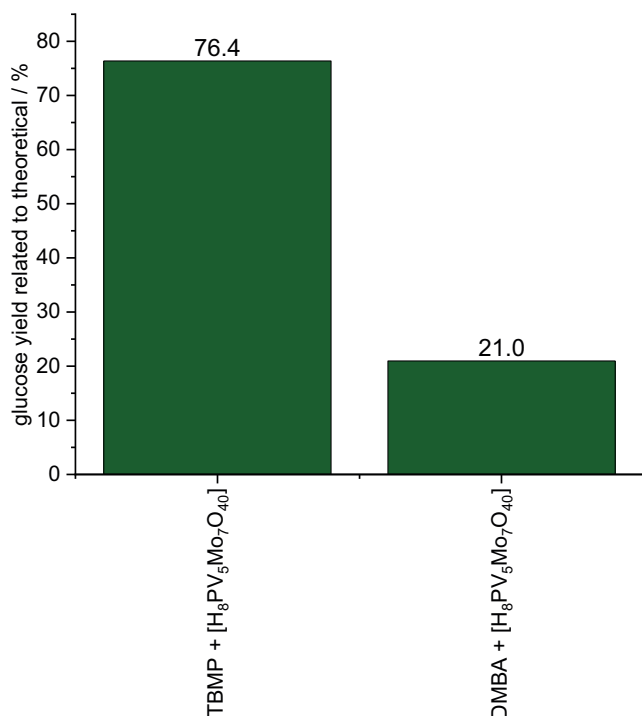


Figure 5. Enzymatic saccharification yield of processed cellulose-rich solid using different IL/MeOH solvents and HPA-5 as a catalyst analyzed via LAP NREL/TP-5100-63351.^[51] Reaction conditions: 120 °C, 24 h, 1000 rpm, 30 bar oxygen at reaction temperature, 10 g solvent (either methanolic TEA, methanolic DMBA, or methanolic TBMP), 0.5 g beech wood, 0.180 g HPA-5 (1 mmol g⁻¹ catalyst to solvent).

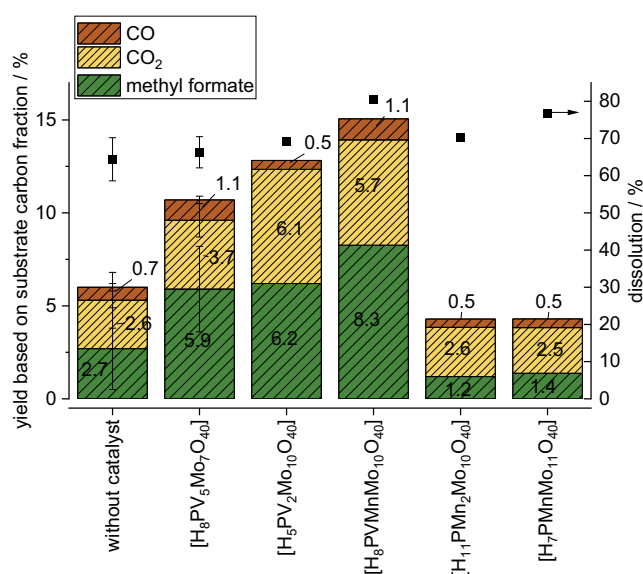


Figure 6. Product yields for the oxidation of beech wood in DMBA/MeOH solvent using HPA-5 ($\text{H}_8\text{PV}_5\text{Mo}_7\text{O}_{40}$), HPA-2 ($\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$), HPA-VMn ($\text{H}_8\text{PVMnMo}_{10}\text{O}_{40}$), HPA-Mn2 ($\text{H}_{11}\text{PMn}_2\text{Mo}_{10}\text{O}_{40}$), and HPA-Mn ($\text{H}_7\text{PMnMo}_{11}\text{O}_{40}$) as catalyst. Reaction conditions: 120 °C, 24 h, 1000 rpm, 30 bar oxygen at reaction temperature, 10 g methanolic DMBA solvent, 0.5 g beech wood, 0.180 g catalyst (1 mmol g⁻¹ catalyst to solvent).

(>70%) was achieved with the methanolic DMBA solvent containing the HPA-VMn and HPA-Mn catalysts.

Figure 7 presents the product yields obtained from the catalyst screening with the methanolic TBMP solvent. No clear trend in dissolution (ranging from 60% to 69%) was observed with respect to the redox activity of the catalysts. However, the highest methyl formate yield of 19.8% was achieved with the HPA-VMn catalyst in the methanolic TBMP solvent, which is more than double the yield obtained in the methanolic DMBA solvent. The HPA-2 catalyst also produced a good methyl formate yield (17.8%) in the methanolic TBMP solvent, but the solvent was unstable under reaction conditions (Figure S10, Supporting Information), and the product-to-waste ratio (methyl formate vs. CO₂) was lower (71.2%) compared to the HPA-VMn catalyst (79.5%). Therefore, the HPA-VMn catalyst and the methanolic TBMP solvent were identified as the best combined system for the alcoholic POM-Ionosolv concept, and all further investigations proceeded with this combination. However, an inorganic precipitate was identified on every cellulose-rich solid, regardless of the catalyst used which will be investigated in detail in the next section.

For a better comparison the turnover frequency (TOF) for methylformate formation using various POM catalysts in the different ILs DMBA and TBMP is shown in **Table 2**. The TOF confirms that the most efficient tested reaction media is TBMP with the HPA-VMn as catalyst ($\text{TOF} = 3.23 \times 10^{-4} \text{ s}^{-1}$).

2.4. Investigating Catalytic Behavior under Reaction Conditions

An inorganic precipitate was identified in every cellulose-rich solid after oxidative fractionation, regardless of the catalyst used. Pictures

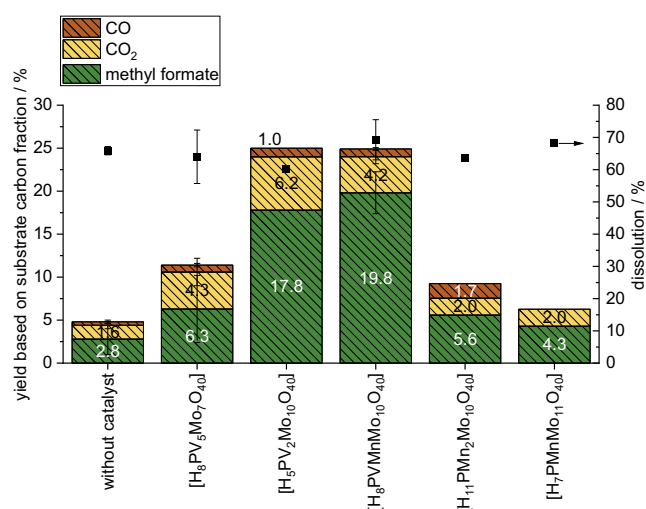


Figure 7. Product yields for the oxidation of beech wood in TBMP/MeOH solvent using HPA-5 ($\text{H}_8\text{PV}_5\text{Mo}_7\text{O}_{40}$), HPA-2 ($\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$), HPA-VMn ($\text{H}_8\text{PVMnMo}_{10}\text{O}_{40}$), HPA-Mn2 ($\text{H}_{11}\text{PMn}_2\text{Mo}_{10}\text{O}_{40}$), and HPA-Mn ($\text{H}_7\text{PMnMo}_{11}\text{O}_{40}$) as catalyst. Reaction conditions: 120 °C, 24 h, 1000 rpm, 30 bar oxygen at reaction temperature, 10 g methanolic TBMP solvent, 0.5 g beech wood, 0.180 g catalyst (1 mmol g⁻¹ catalyst to solvent).

of the catalysts before the reaction and of the precipitates after the reaction are shown in Figure S12, Supporting Information. The occurring color changes for the catalysts HPA-5, HPA-Mn, and HPA-Mn2 can be explained by the color change of the redox-active metal in the POM. The identified preferred catalyst HPA-VMn and its precipitate were investigated further. The nature of the precipitate formed in the preferred methanolic HPA-VMn/TBMP solvent was examined using multiple analytical techniques. First, the elemental composition of the HPA-VMn catalyst before the reaction and the inorganic precipitate after the reaction (separated from the mixture without substrate) was compared using inductively coupled plasma optical emission spectroscopy (ICP-OES) measurements (Table S1, Supporting Information). In the inorganic precipitate, the vanadium stoichiometry decreased by about 50%, and the phosphorus content increased fourfold when normalized to the Mo content, whereas manganese was not detected. The high phosphorus content indicates that the inorganic precipitate could be a combination of POM fragments and phosphonium cations from the IL.

FT-IR spectra of the inorganic precipitate were compared with the spectra of the initial catalyst HPA-VMn and the methanolic

Table 2. Comparison of TOF for methyl formate as single valuable catalytic product using various catalysts in the different reaction media of DMBA and TBMP.

Catalyst	DMBA solvent TOF [s ⁻¹]	TBMP solvent TOF [s ⁻¹]
HPA-5	1.04×10^{-4}	8.57×10^{-5}
HPA-2	1.12×10^{-4}	2.67×10^{-4}
HPA-VMn	1.46×10^{-4}	3.23×10^{-4}
HPA-Mn2	2.06×10^{-5}	8.66×10^{-6}
HPA-Mn	2.47×10^{-5}	4.07×10^{-5}

TBMP solvent (Figure 8). Characteristic bands for the Keggin-type POM structure and the TBMP-IL can be recognized (marked with colored areas). The Keggin-type structure bands are located at 1055 cm^{-1} (P=O vibration), 954 cm^{-1} (M=O_t vibration), and 874 and 767 cm^{-1} (M—O—M vertex and edge vibration).^[36] The methanolic TBMP solvent bands are at 2938 , 2961 , 2878 , 2835 , 1458 , 1418 , 1250 , 1209 , 1008 , 940 , and 737 cm^{-1} . This fingerprint was also identified by Albert et al.^[33] The bands at 2835 and 940 cm^{-1} could not be assigned, but the other bands of the methanolic TBMP solvent are evident in the FT-IR spectrum of the inorganic precipitate, indicating that the precipitate is a salt with a Keggin-type-structured POM anion and likely the TBMP-IL cation.

A single-crystal X-ray diffraction (SCXRD) measurement of the precipitate reveals the molecular structure (Figure 9), corroborating the composition suggested by FT-IR spectroscopy and elemental analysis. The crystal structure consists of typical Keggin-type POM structures composed of Mo and O atoms with a P atom as the central atom, surrounded by TBMP-IL cations. The asymmetric unit contains three Keggin anions and nine phosphonium cations, with 12 formula units in the unit cell. The residual anionic charge is most likely compensated by protons, which were not modeled. Due to the highly disordered cation, detailed modeling

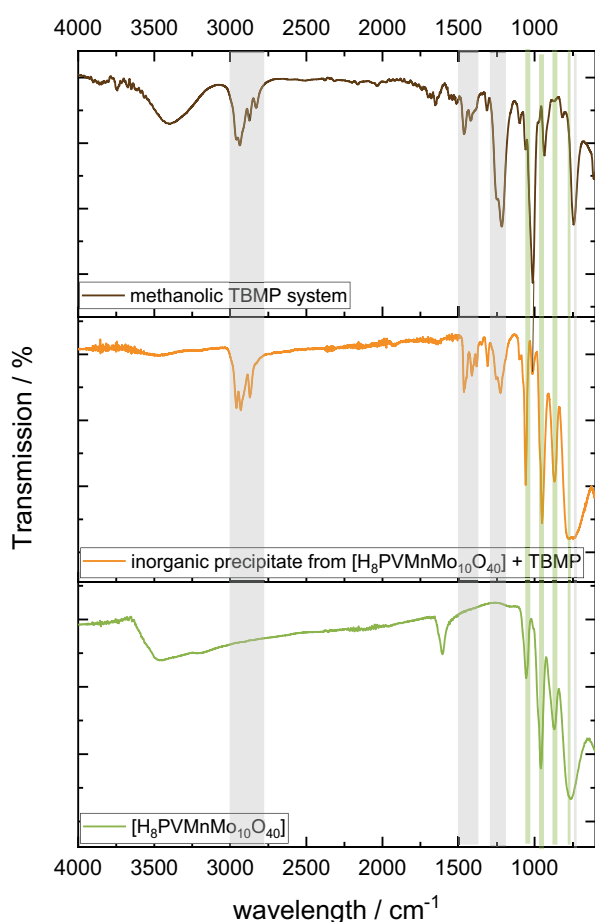


Figure 8. FT-IR spectra of the methanolic TBMP solvent (top), the HPA-VMn catalyst ($\text{H}_8\text{PVMnMo}_{10}\text{O}_{40}$) (bottom), and the inorganic precipitate from HPA-VMn in the TBMP/MeOH solvent (middle). The colored areas mark bands which appear similar in different spectra.

of the elemental composition of the metal sites was not deemed useful as it does not significantly improve the model. The elemental distribution can be seen in the ICP-OES data (Table S1, Supporting Information). Detailed information about the solid-state structure is provided in Table S2 and S3, Supporting Information.

The valence charge of Mn and V ions plays a major role in the bond-breaking and oxidation processes. Therefore, X-ray photoelectron spectroscopy (XPS) was used to determine the electronic structure of the surface elements (Mn, V, and Mo) of the HPA-VMn catalyst and the inorganic precipitate before and after a blank reaction (Figure 10 and 11, Figure S13, and Table S4, Supporting Information). Three peaks were observed and fitted for the Mn $2p_{3/2}$ band for the HPA-VMn catalyst and the inorganic precipitate prior to reaction, with binding energies around 641.72 , 643.56 , and 645.7 eV corresponding to Mn^{2+} , Mn^{3+} , and Mn^{4+} species, respectively.^[37–39] After the blank reaction (heating the reaction mixture up to 120°C), manganese was not detected in the inorganic precipitate, which aligns with the ICP-OES analysis results which showed no detectable amount of Mn after the reaction. Therefore, the manganese might dissolve into the liquid phase of the methanolic TBMP solvent during the reaction. Mixed Mn valence states were likely present during the redox reaction, as the interconversion between Mn^{2+} , Mn^{3+} , and Mn^{4+} enables efficient electron transfer between the reactants and the catalyst, thereby enhancing catalytic activity (see Figure 10).^[37–39] Interestingly, the manganese seems to have been present in different redox-active states even before any possible reaction of the catalyst.

In contrast, the vanadium ions on the surface of the unused catalyst showed a single peak at a binding energy of 517 eV , fitted from the V $2p_{3/2}$ bands and assigned to V^{5+} species.^[40] During the formation of the inorganic precipitate, the vanadium was reduced to V^{4+} (peak at binding energy of 516 eV fitted from the V $2p_{3/2}$ band). After the blank reaction, the inorganic precipitate shows a third peak at around 515 eV , fitted from the V $2p_{3/2}$ band, which can be correlated to a V^{3+} species. This indicates that during the blank reaction, a redox reaction took place (see Figure 11), which might be the consecutive degradation of MeOH to methyl formate, as previously established. Although the end point of the reaction pathway of methyl formate and CO_2 could not be detected via ^{13}C -NMR spectroscopy (Figure S10, Supporting Information), the surface of the inorganic precipitate shows a redox reaction of vanadium.

The methanolic TBMP solvent containing the HPA-VMn catalyst was yellow, while the methanolic TBMP solvent alone was colorless. Figure S14 and S15, Supporting Information, shows ^{31}P and ^{51}V NMR spectra of the reaction solution and the methanolic TBMP solvent, as well as NMR spectra of the removed catalyst and the inorganic precipitate after dissolution in deuterated acetonitrile. The ^{31}P NMR spectrum of the liquid reaction solution reveals the presence of a characteristic signal for the POM at -3.80 ppm and two small signals at -3.65 and -3.60 ppm , suggesting that not the entire POM catalyst was precipitated. The ^{31}P NMR spectrum of the dissolved inorganic precipitate displays a TBMP-IL-specific signal at 31.8 ppm ,^[41] as well the same signals of the POM, just slightly shifted downfield by 0.4 ppm , thereby substantiating a cation exchange between the IL and the POM. The ^{51}V NMR spectra

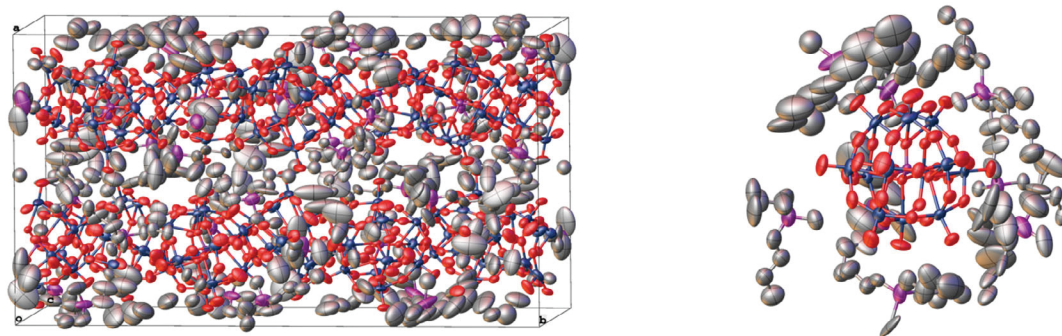


Figure 9. Solid-state structure of the inorganic precipitate: unit cell (left) and single Keggin-type anion surrounded by phosphonium cations (right). Color assignment: Metals (Mo, V, Mn) dark blue, phosphorus purple, oxygen red, carbon gray. Thermal ellipsoids are drawn at a probability level of 50%. Hydrogen atoms have not been modeled. The data for the structure is available through the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service (deposition number: 2422752)

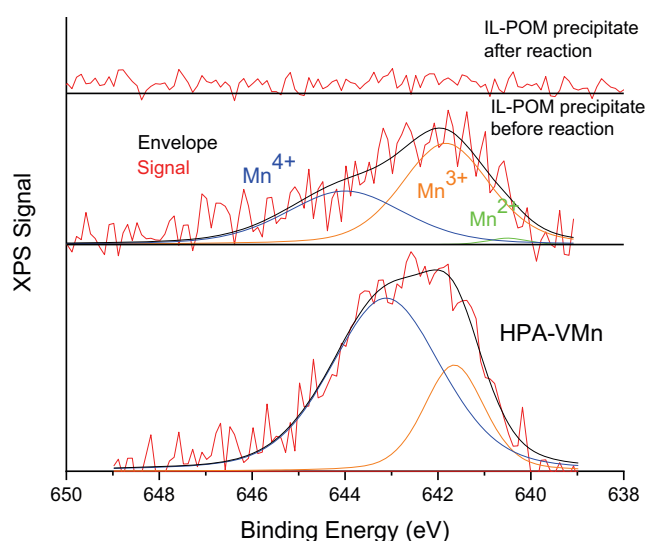


Figure 10. Comparison of the XPS signal of the Mn2p bands of the inorganic precipitate (IL-POM precipitate) before and after the reaction and the initial HPA-VMn POM catalyst. Reaction conditions: 120 °C, 24 h, 1000 rpm, 30 bar oxygen at reaction temperature, 10 g methanolic TBMP solvent, 0.180 g catalyst HPA-VMn (1 mmol g⁻¹ catalyst to solvent).

of the different phases show the presence of distinct vanadium species. In the methanolic TBMP solvent, the vanadium species exhibited peaks at –459 ppm (major) and at –539 ppm (minor), whereas the initial HPA-VMn dissolved in deuterated acetonitrile exhibits one prominent peak at –554 ppm and smaller peaks between –568 and –556 ppm. The inorganic precipitate displayed only one prominent peak at –525 ppm and a small peak at –539 ppm. The latter peak was also present in the spectrum of the liquid reaction solution. Keggin-type POMs are known to isomerize, and these isomers can be attributed to different signals. For a mono-substituted vanadium POM, all positions in the Keggin framework are equivalent, and therefore only one signal occurs.^[36,42] The shift of the prominent peak shows that the vanadium atom was situated in a different environment than that observed in the initial HPA-VMn catalyst, suggesting that different catalyst species were present.

To determine whether the catalytic reaction was homogeneous or heterogeneous, the methanolic TBMP solvent with

HPA-VMn was heated in a sealed glass pressure tube and observed during the heating process. A comparison of the reaction solvent at room temperature with that at 95 °C is provided in Figure S16, Supporting Information, and differential scanning calorimetry (DSC) measurements showing a transition around 85 °C for the POM-IL precipitate is shown in Figure S17, Supporting Information. At the elevated temperature, the inorganic precipitate completely dissolved in the reaction solution, resulting in a homogeneous system. Therefore, the reaction system of the methanolic POM-Ionosolv concept with the TBMP-IL and HPA-VMn can be classified as a homogeneous reaction.

Figure 12 illustrates the catalytic performance of the various states of the HPA-VMn catalyst. A reaction solution in which the HPA-VMn was weighed into the methanolic TBMP solvent, as with other reactions presented previously (HPA-VMn), is compared to a reaction solution in which the inorganic precipitate was used as a catalyst (inorganic precipitate). Moreover, a solution of the methanolic TBMP solvent that had been previously filtered to remove any inorganic precipitate was used as a reaction solution (filtrate). Additionally, a reaction conducted without a catalyst is illustrated in the figure (without catalyst). Figure S18, Supporting Information, explains the different experiments and shows the catalysts in pictures for a better understanding. As the yield of methyl formate nearly doubled in the filtrate compared to the reaction without a catalyst, it can be inferred that the amount of catalyst dissolved in the methanolic TBMP solvent at room temperature had significant catalytic activity. The reaction involving the HPA-VMn catalyst and the inorganic precipitate as the catalyst exhibited a strikingly similar performance profile. Accordingly, it can be concluded that the inorganic precipitate can be reused as a catalyst. Further studies on the recycling of the catalyst may be required to ascertain the performance after several reactions.

2.5. Time Dependent Optimization Study for the Enhanced POM-Ionosolv Concept

To optimize the formation of the methyl formate product, a time course study was performed for the reaction with the HPA-VMn catalyst in the methanolic TBMP system. The analysis of the gas

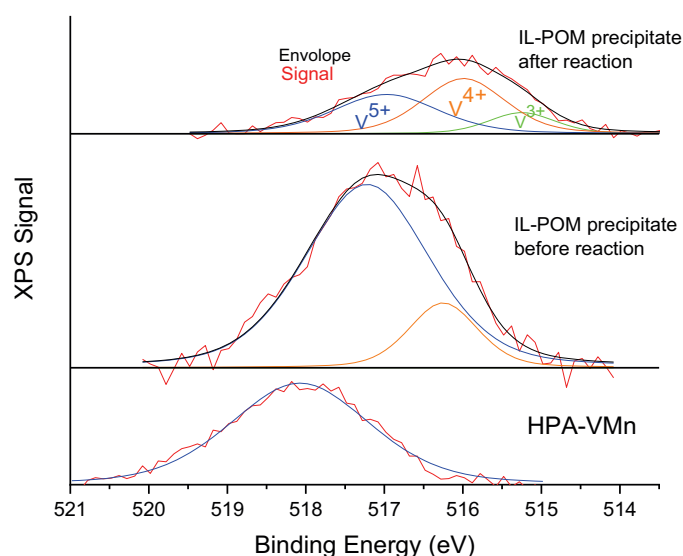


Figure 11. Comparison of the XPS signals of the V 2p bands for the inorganic precipitate (IL-POM precipitate) before and after the reaction and for the initial HPA-VMn POM catalyst. Reaction conditions: 120 °C, 24 h, 1000 rpm, 30 bar oxygen at reaction temperature, 10 g methanolic TBMP solvent, 0.180 g catalyst HPA-VMn (1 mmol g⁻¹ catalyst to solvent).

and liquid phases is illustrated in **Figure 13**. Regarding the production of methyl formate, the results indicate that a reaction time of 12 h was favorable, as the product yield declined with a longer

reaction of 24 h, during which the CO₂ yield concurrently increased to 4.9%. The yield of CO remained ≤1% at all reaction times, and the dissolution gradually increased to 64% over the 24 h period.

To evaluate the efficacy of a reaction time of 12–24 h, the cellulose-enriched solid was also characterized (Figure S19 and S20, Supporting Information). Compositional analysis showed that 92.1% and 91.3% glucan was obtained in the solids after 12 and 24 h, respectively. However, the enzymatic saccharification increased by around 40% between 12 and 24 h, making the reaction time of 24 h preferable. Extending the reaction time would lower the selectivity and the methyl formate yield even further and was therefore not tested.

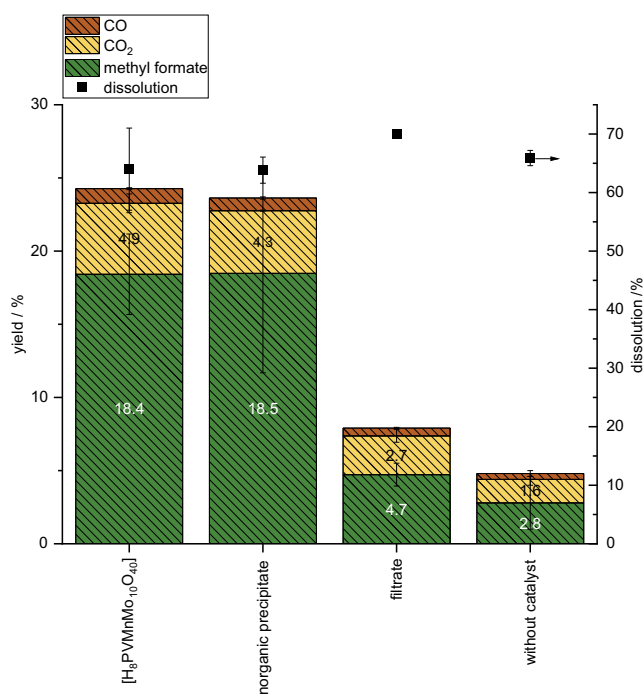


Figure 12. Product yields obtained from fractionative beech wood oxidation using different states of the HPA-VMn catalyst: The filtered methanolic TBMP solvent where the HPA-VMn catalyst is dissolved, the mixture of the methanolic TBMP solvent and the HPA-VMn, and a previously precipitated inorganic precipitate in the methanolic TBMP solvent. For comparison reasons, the reaction without any catalyst is shown as well. Reaction conditions: 120 °C, 24 h, 1000 rpm, 30 bar oxygen at reaction temperature, 10 g methanolic TBMP solvent, 0.180 g HPA-VMn or inorganic precipitate (1 mmol g⁻¹ catalyst to solvent).

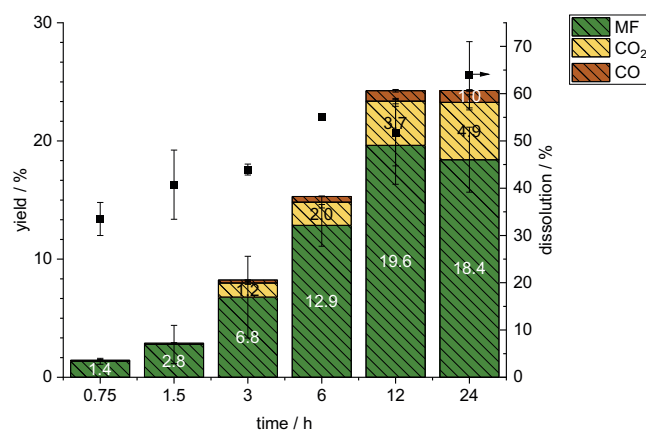


Figure 13. Product yields for fractionative beech wood oxidation with different reaction times. Reaction conditions: 120 °C, 24 h, 1000 rpm, 30 bar oxygen at reaction temperature, 10 g methanolic TBMP solvent, 0.180 g HPA-VMn (1 mmol g⁻¹ catalyst to solvent).

2.6. Separation and Purification of Reaction Products for the Enhanced POM-Ionosolv Concept

2.6.1. Processed Solid

Due to the presence of precipitated catalyst on the cellulose-enriched solid, a washing procedure was established to remove the precipitate. The established washing procedure^[17,18] used the volatile cosolvent (MeOH) to wash off nonvolatile IL and catalyst from the cellulose-enriched solid. However, this led to a high amount of vanadium on the solid as shown in Table S5, Supporting Information. Molybdenum is the main metallic component in the catalyst, and therefore, a good indicator to check for the presence of catalyst precipitate on the cellulose-enriched solid. The other elements of the catalysts were also measured with ICP-OES, and the results show a similar result for the molybdenum content (Table S5, Supporting Information). The molybdenum content in the cellulose-enriched solid after washing with MeOH was determined to 14.4% by ICP-OES. Hence, a second washing step was investigated utilizing dimethylsulfoxide (DMSO) or acetone, as both solvents are expected to dissolve the catalyst precipitate without interfering with the cellulose-enriched solid. Importantly, DMSO could reduce the amount of molybdenum on the cellulose-enriched solid below the detection limit (0.03%), whereas acetone washing reduced the amount of molybdenum to 0.06%.

The Keggin-type structure of the HPA-VMn catalyst was also visible in the FT-IR spectrum after the first MeOH wash (marked in Figure 14 with POM) with characteristic bands at 1059 cm^{-1} (P=O vibration), 962 cm^{-1} (M=O_t vibration), and 877 and 744 cm^{-1} (M—O—M vibrations), even though the former two bands overlapped with bands from the cellulose-enriched solid. As expected, the characteristic bands were not detected after the second washing step, confirming the effectiveness of the overall pulp washing procedure. The recycling of the catalyst from the washing solutions was not investigated in this study, potential methods are distillation or precipitation from the washing solution.

One disadvantage of DMSO is its high boiling point, which makes distillation of the solvent energy intensive. Therefore, the cellulose-enriched solid was contaminated with DMSO after washing, necessitating a third washing step to remove DMSO. To emphasize this, comparative CHNS analysis was performed on the washed solids, and the measured sulfur contents were compared. The sulfur content after the first MeOH washing was 0.27 and 0.23 wt% after the second washing with acetone. In contrast, the sulfur content increased to 30.43 wt% after washing with DMSO (Table S5, Supporting Information). The corresponding compositional analysis data of the two cellulose-enriched solids are shown in Figure S21, Supporting Information. The glucan content of both samples was above 90% (91.3% after acetone washing, 94.5% after DMSO washing), so no preference can be derived from the compositional analysis. Therefore, the two cellulose-enriched solids were evaluated using enzymatic saccharification. The residual DMSO in the cellulose-enriched solid significantly influenced the yield of glucose from enzymatic saccharification, resulting in a yield (42.6%) that was around 30% lower than that of the acetone-washed solid (76.6%) (Figure 15). This lower

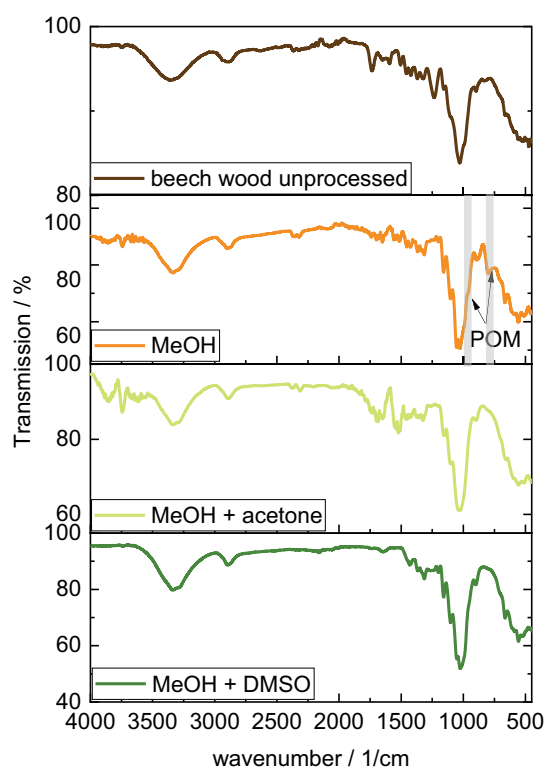


Figure 14. Comparison of different FT-IR spectra recorded of unprocessed beech wood and cellulose-enriched solid washed with methanol (MeOH), methanol and acetone (MeOH + acetone), and methanol and dimethyl sulfoxide (MeOH + DMSO).

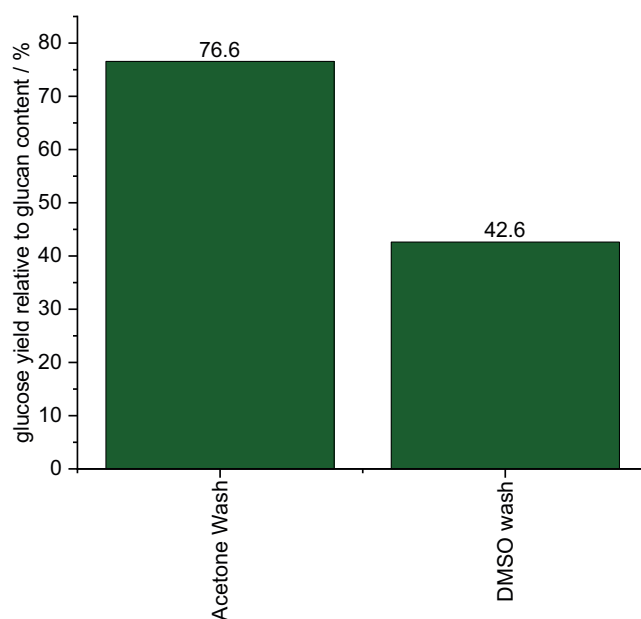


Figure 15. Comparison of enzymatic saccharification of the processed cellulose-enriched solid washed with acetone and DMSO analyzed via LAP NREL/TP-5100-63351.^[51] Reaction conditions: 120 °C, 24 h, 1000 rpm, 30 bar oxygen at reaction temperature, 10 g methanolic TBMP solvent, 0.5 g beech wood, 0.180 g HPA-VMn (1 mmol g⁻¹ catalyst to solvent).

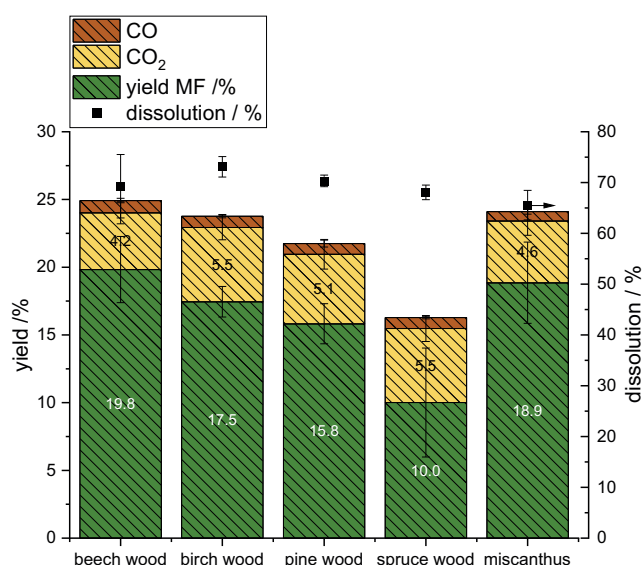


Figure 16. Product yields and dissolution of different types of lignocellulosic biomass as substrate using the methanolic TBMP solvent. Reaction conditions: 120 °C, 24 h, 1000 rpm, 30 bar oxygen at reaction temperature, 10 g methanolic TBMP solvent, 0.180 g HPA-VMn (1 mmol g⁻¹ catalyst to solvent).

performance might be explained by the suppression of endo-1, 4-β-glucosidase in the cellulase mixtures.^[43] Thus, the preferred washing agent for the second washing procedure was acetone, and this was used in further analysis.

2.6.2. Liquid Reaction Products

After purifying the cellulose-enriched solid, the separation of the liquid phase product methyl formate was investigated. An ex situ distillation in a round-bottom flask was performed to test the separation of methyl formate from the solvent, preferably from the IL. Methyl formate has a boiling point of 32 °C and MeOH of 65 °C.^[44,45]

As the methyl formate had a moderate concentration in the experiments, it proved beneficial to codistill the methyl formate and the MeOH at the boiling temperature of MeOH. The distillation allowed for the recovery of 96% of methyl formate with only MeOH as the solvent in the head, while the IL remained in the flask. ¹³C NMR spectra of the two distillation fractions obtained from a reaction solution (Figure S22 and S23, Supporting Information) confirm that distillation is a useful method for isolating the desired methyl formate product.

2.7. Substrate Scope for the Enhanced POM-Ionosolv Concept

The ionosolv-fractionation can be affected by the type of lignocellulosic biomass.^[17,20,46] To evaluate this effect, the substrate scope was investigated using birch wood as a second hardwood, pine and spruce wood as representative softwoods, and miscanthus as grass biomass. The focus was on the yield of methyl formate, the glucan content in the cellulose pulp, and glucose yield obtained with enzymatic saccharification. **Figure 16** presents the yields of the liquid phase product methyl formate and the gaseous phase products CO₂ and CO, as well as the dissolution of the substrate. A variation in the yield of methyl formate between 10.0%–19.8% was found, with the hardwoods and miscanthus showing higher yields (17.5% birch, 18.9% miscanthus, 19.8% beech) than the softwoods (15.8% pine, 10.0% spruce). Conversely, the yield of CO₂ (4.2%–5.5%) and CO (≈1%) did not differ much between substrates. The substrate dissolution in the methanolic TBMP-IL solvent was also similar (between 60% and 70%), with no recognizable trend between the substrate types.

Figure 17 shows the composition of the different feedstock pulps compared to the composition of the original biomass. All cellulose-enriched solids obtained after the POM-Ionosolv concept with the methanolic TBMP solvent had a glucan content of >90%, and the cellulose yield for all substrates was >60%, with the highest yield (73%) achieved with miscanthus. Detailed data can be

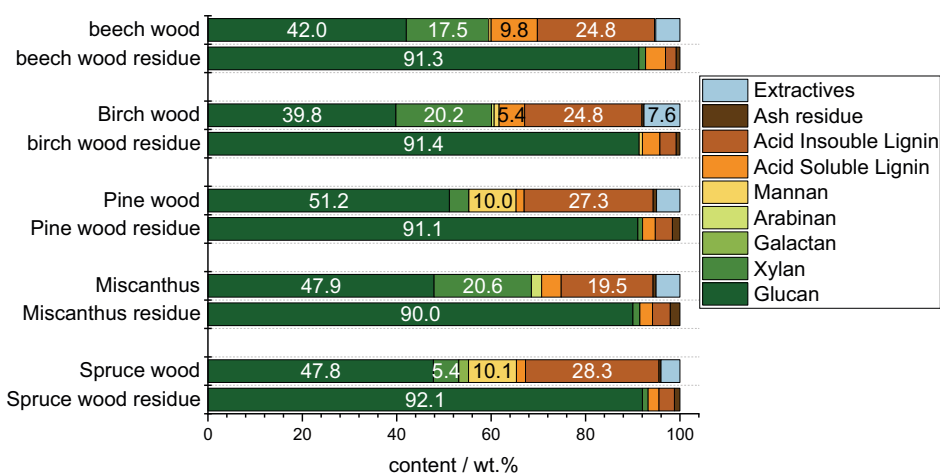


Figure 17. Compositional analysis of different types of lignocellulosic biomass and the processed cellulose-enriched solid done via NREL/TP-510-42618.^[47] Reaction conditions for preparing the solids: 120 °C, 24 h, 1000 rpm, 30 bar oxygen at reaction temperature, 0.5 g beech wood, 10 g methanolic TBMP solvent, 0.180 g HPA-VMn (1 mmol g⁻¹ catalyst to solvent). ASL = acid soluble lignin, AIL = acid insoluble lignin.

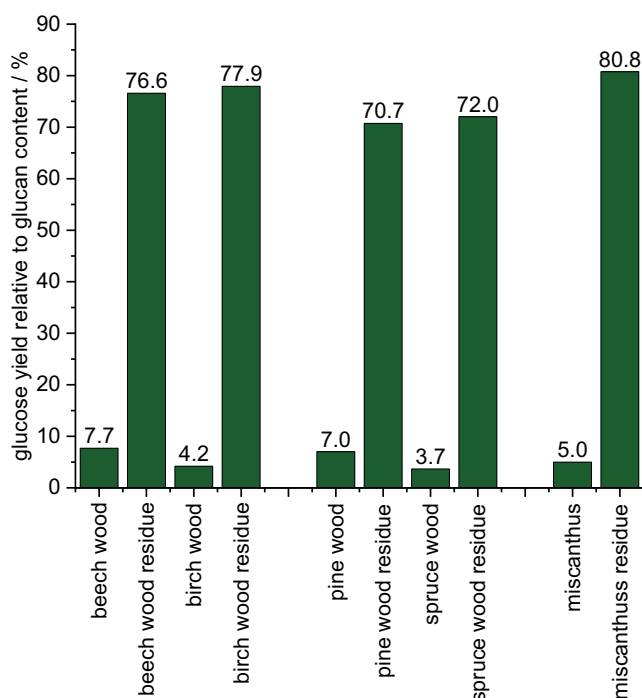


Figure 18. Enzymatic saccharification yield for different lignocellulosic biomass types compared to enzymatic saccharification of cellulose-rich solids generated by using methanolic POM ionosolv. Reaction conditions: 120 °C, 24 h, 1000 rpm, 30 bar oxygen at reaction temperature, 10 g methanolic TBMP solvent, 0.5 g beech wood, 0.180 g HPA-VMn (1 mmol g⁻¹ catalyst to solvent).

found in Figure S24, Supporting Information. For beech wood as an example this would mean: From 1 g of beechwood, 0.67 g of cellulose and 0.07 g of methyl formate can be produced meaning a high utilization of the whole biomass, only 0.01 g are not valorized as gaseous products (CO and CO₂). The glucose yield after enzymatic saccharification of the cellulose-enriched solids from different substrates were >70%, as shown in **Figure 18**. Even the treated miscanthus solid achieved a glucose yield of 80.8%, proving the versatility of the updated POM-Ionosolv 2.0 concept using a methanolic TBMP-IL solvent and HPA-VMn for various types of biomass.

3. Conclusions

Production of high methyl formate yields of 20% from beech wood and a cellulose-enriched solid with a glucan content exceeding 90% via an improved POM-Ionosolv concept has been demonstrated by utilizing MeOH as a co-solvent and identifying a redox-balanced HPA-VMn-POM catalyst. Different MeOH/IL (70 wt%/30 wt%) solvent mixtures were employed for the fractionation of the lignocellulosic feedstock, while simultaneously oxidizing the dissolved compounds to methyl formate via the homogeneous POM catalyst. The study identified the phosphonium-based IL [TBMP][MeSO₄] as the most suitable IL for the conversion. The cellulose-enriched solid retained 77% of the glucan contained in the beech biomass for subsequent enzymatic saccharification.

Furthermore, it was demonstrated that the IL/MeOH reaction media is stable against oxidation under the utilized reaction conditions. A catalyst screening of POMs with various redox activity identified HPA-VMn (H₈PVMnMo₁₀O₄₀) as the optimal catalyst for achieving the highest yield of methyl formate (≈20%). A comprehensive examination of the interactions between the catalyst and IL revealed that an inorganic POM-IL salt, which serves as a homogeneous catalyst in the system under operating conditions, precipitated out at room temperature. The POM-IL salt structure was elucidated through comprehensive analysis employing SCXRD, XPS, and FT-IR spectroscopy and ICP-OES.

To the best of our knowledge, this is the first instance of such a POM-IL structure being characterized via SCXRD measurements. With an optimal reaction time of 24 h, HPA-VMn in [TBMP][MeSO₄] enabled dissolution of 70% of beech wood in the liquid phase, representing an optimal compromise between the usability of cellulose-enriched solid and the yield of methyl formate. The separation of methyl formate from the IL and catalyst via distillation was demonstrated, and an optimized washing procedure for the purification of the cellulose-enriched solid with MeOH and acetone was established. Importantly, the catalytic system also proved effective for the conversion of softwood (pine and spruce) and grass biomass (miscanthus), with yields of 10% (spruce) to 19% (miscanthus) of methyl formate. All cellulose-enriched solids exhibited a glucan content exceeding 90% and an enzymatic saccharification accessibility exceeding 70%. In these pulps, over 60% of the cellulose in the initial substrates could be retained in the cellulose pulp, with 73% of the cellulose content retained for Miscanthus.

In conclusion, the previous water-based POM-Ionosolv concept has been enhanced by the introduction of MeOH as a co-solvent and the incorporation of a novel phosphonium-based IL, along with a new redox-balanced POM catalyst with a redox activity tuned for the application. Additionally, the catalyst has been subjected to comprehensive characterization through a range of analytical techniques. With further optimization of the catalyst recycling process, this system demonstrates high potential for application in biorefineries.

4. Experimental Section

Chemicals

All chemicals and feedstocks are commercially available and were used without further purification. The chemicals used are shown in **Table 3** with their purity and supplier.

Experimental Setup and Typical Working Method

All catalytic experiments were performed in 20 mL Hastelloy C276 autoclaves equipped with a magnetic stirrer. The catalyst (0.1 mmol), fractionated substrate ($x \leq 630 \mu\text{m}$, 0.5 g), and 10 g of different IL/MeOH mixtures with a ratio of 30 wt%/70 wt% were loaded into the reactor. The reactor was then closed and purged twice with 20 bar of oxygen before being repressurized with oxygen to get 20 bar pressure at reaction temperature. While the reactor was

Table 3. Used chemicals with purity and supplier.

Chemical	Supplier	Purity
Beech wood	Mushrooms and equipment shop	–
Miscanthus	Silwood Park campus (Imperial College London, UK).	–
Birch wood	Räucherspan.de (URL: https://www.raeucherspan.de/)	–
Spruce wood	Räucherspan.de (URL: https://www.raeucherspan.de/)	–
Pine wood	Jbach GmbH	–
Methanol	VWR chemicals	>99.5%
Acetone	Walter cMP GmbH & Co. KG	Pure
Dimethylsulfoxide (DMSO)	Grüssing GmbH	99.5%
Tributylmethylphosphonium methyl sulfate ([TBMP][MeSO ₄], TBMP)	Iolitec Ionic Liquids Technologies GmbH	>95%
<i>N,N</i> -Dimethylbutylammonium hydrogen sulfate ([DMBA][HSO ₄], DMBA)	Synthesized at Imperial College London, UK according to literature ^[52]	–
Triethylammonium hydrogen sulfate ([TEA][HSO ₄], TEA)	Synthesized at University of Hamburg according to literature ^[53]	–
Polyoxometalate catalysts (H ₈ PV ₅ Mo ₇ O ₄₀ (HPA-5), H ₅ PV ₂ Mo ₁₀ O ₄₀ (HPA-2), H ₈ PVMnMo ₁₀ O ₄₀ (HPA-VMn), H ₁₁ PMn ₂ Mo ₁₀ O ₄₀ (HPA-Mn2), H ₇ PMnMo ₁₁ O ₄₀ (HPA-Mn1))	Synthesized according to literature ^[22,36,54–56]	–

heated, the stirrer was set to 300 rpm, and at reaction temperature, the stirrer was adjusted to 1000 rpm. After the reaction time, the reactor was cooled to room temperature, and a gas sample was taken and analyzed via gas chromatography using a flame ionization detector (FID). The reactor content was filtered to separate the solid and the liquid phase. The solid was washed and analyzed via National Renewable Energy Laboratory (NREL) method, FT-IR spectroscopy, and CHNS elemental analysis and subsequently processed via enzymatic saccharification. The liquid phase was analyzed for reaction products via ¹H NMR spectroscopy with an internal standard of tert-butanol and MeOD in a ratio of 1:5 with the sample. For additional analysis, ¹³C NMR, ⁵¹V NMR, and ³¹P NMR were performed.

Catalyst Characterization

The catalyst and the catalyst precipitate were analyzed via FT-IR spectroscopy, ICP-OES analysis, XPS, SCXRD, and XRD. The liquid phase containing the catalyst was analyzed via ⁵¹V NMR and ³¹P NMR. The catalyst was also analyzed via SWV.

Washing Procedure

The solid after the reaction was first washed with 50 mL MeOH as a standard procedure, followed by washing with 100 mL dimethyl sulfoxide (DMSO) or acetone.

Distillation

A classic distillation setup consisting of a four-neck flask and a distillation bridge was initially used for distillation. A KPG stirrer from Heidolph (RZR 2021) was used for stirring. Water was used as a coolant for the distillation bridge. The four-neck flask was heated with a heating block to 75 °C and stirred at 200 rpm. The temperature inside of the flask was measured to be 67 °C, corresponding to the boiling temperature of MeOH. The product flask (the head of the distillation) was cooled with an ice water bath. For the experiment, three different methanolic TBMP solvent experiments with HPA-VMn as catalyst (reaction conditions: 120 °C, 24 h, 1000 rpm, 30 bar of oxygen at reaction temperature, 10 g solvent [TBMP][MeSO₄]/MeOH 30 wt%/70 wt%

(TBMP), 0.180 g HPA-VMn (1 mmol g^{−1} catalyst), 20 mL Hastelloy autoclave) were combined into 30 mL of feed solution and distilled.

Analytics

The solid and the substrates were analyzed using a Euro Vector EA 3000 to determine the carbon (C), nitrogen (N), hydrogen (H), and sulfur (S) content. The FT-IR spectroscopy was performed with an IR-Spirit from Shimadzu, and all data were smoothed using an 80-point Savitzky-Golay smooth. The typical wavelengths for the Keggin-type POM structure are: $\tilde{\nu}$ [cm^{−1}] = 3481 (w, O–H, H₂O), 1603 (O–H, lattice H₂O), 1055 (w, P–O), 959 (me, M=O), 871 ((M–O–M)_{vertex}), 762 ((M–O–M)_{edge}).^[36] The compositional analysis was prepared according to the LAP NREL/TP-510-42618.^[32] The enzymatic saccharification was prepared according to the LAP NREL/TP-5100-63351.^[47]

The gas phase was analyzed using a Varian GC 450 equipped with a Shin-carbon column (2 m × 0.75 mm) and both a thermal conductivity as well as a FID.

SWV data were measured with a concentration of 1 mmol L^{−1} in acetonitrile and a scan rate of 5 mV s^{−1} on an Ivium Potentiostat. The working electrode was glassy carbon (diameter: 3 mm), the reference electrode was Ag/Ag⁺, and the counter electrode was platinum. All SWV measurements were done with a scan rate of 5 mV s^{−1}, a modulation amplitude of 20 mV, and a frequency of 25 Hz. The elemental composition for Mn, Mo, P, and V was determined using a Solaar S Series ASS-F (atom emission spectroscopy-flame) from Thermo (Mn) and an ARCOS ICP-OES from Spectro (V, P, Mo). The liquid phase after the reactions was analyzed via NMR spectroscopy. All NMR spectra were measured with a Bruker Avance III HD 600 MHz. The ⁵¹V NMR was recorded with the following parameters: number of scans 4096, receiver gain 2050, relaxation delay 0.5, and pulse width 11.13. The ³¹P NMR was recorded with the following parameters: number of scans 1024, receiver gain 1820, relaxation delay 1.0, and pulse width 11.2. The ¹³C NMR was recorded with the following parameters: number of scans 512, receiver gain 2050, relaxation delay 1.0, and pulse width 9.31. The ¹H NMR was measured with a scan number of 16, a receiver gain of 2.8, a relaxation delay of 1.0, and a pulse width of 10.37.

XPS analyses were performed using a Thermo Scientific system at room temperature with AlK α radiation (1484.6 eV) and a spot size of 400 μ m. A flood gun was used to reduce sample charging effects,

and the obtained spectra were further corrected by setting the C1s binding energy at 284.8 eV. Data processing was done using the Advantage 4.87 software. Mn2p_{3/2} and V2p_{3/2} signals were each deconvoluted into three peaks using mixed Gauss–Lorentz functions (L/G: 30%–70% mix) using the Powell fitting algorithm. The peaks assigned to Mn²⁺, Mn³⁺, and Mn⁴⁺ were constrained to binding energies of 640.0 ± 0.5 eV, 641.5 ± 0.5 eV, and 643.5 ± 0.5 eV respectively, with their full-width at half-maximum (FWHM) allowed to vary between 1 and 3 eV. The peaks assigned to V³⁺, V⁴⁺, and V⁵⁺ were constrained to binding energies of 515.0 ± 0.25 eV, 516.0 ± 0.25 eV, and 517.0 ± 0.25 eV respectively, with their FWHM allowed to vary between 1 and 3 eV.

XRD measurements were carried out on a Huber G670 powder diffractometer using Cu Kα radiation within a 2θ range of 5°–70° in steps of 0.005° at a speed of 0.02° s^{−1}.

For the SCXRD experiment, a suitable single crystal of C₃₉Mo₁₂O₄₀P₄ was selected and mounted on a SuperNova, dual source (Cu Kα X-ray), Atlas diffractometer. The crystal was kept at 120.0(1) K during data collection. Using Olex2,^[48] the structure was solved with the SHELXT^[49] structure solution program using Intrinsic Phasing and refined with the SHELXL^[50] refinement package using Least Squares minimization. The crystal structure of C₃₉Mo₁₂O₄₀P₄ contains a highly disordered network of organic ligands. To get a complete overview of the ligand distribution in the structure, molecules were modeled with restraints and constraints: RIGU, SADI, DFIX, DANG, DELU, SIMU, and ISOR.

For DSC measurements, about 40 mg of sample was weighed into a Mettler Toledo DSC 1 and measured with a temperature program starting at −50 °C, followed by heating at 10 K min^{−1} to 150 °C with a final hold at 120 °C for 100 s.

Formulas for Calculation

The following equations show the formulas for the basic calculations done in this study. The molar amount of carbon (n_c) in a sample was calculated via Equation (1) with m as mass, w_c as mass fraction of carbon measured via CHNS analysis, and M_c as molar mass of carbon.

$$n_c = \frac{m * w_c}{M_c} \quad (1)$$

The amount of carbon fraction dissolved into the reaction media ($n_{C,dissolved}$) was determined via Equation (2).

$$n_{C,dissolved} = n_{C, substrate} - n_{C, solid residue} \quad (2)$$

The dissolution of the carbon fraction (dissolution) is the amount of carbon not retained in the solid, which therefore was dissolved in the reaction solution. This is shown in Equation (3).

$$\text{dissolution} = \left(1 - \frac{m_{\text{solid residue after reaction}} * w_{C, \text{solid residue after reaction}}}{m_{\text{substrate, initial}} * w_{C, \text{substrate initial}}} \right) * 100\% \quad (3)$$

As some solvents were susceptible to oxidation with some of the POM catalysts, a new molar amount of product i was introduced in Equation (4). This $n_{i,new}$ is the difference between the amount of product i ($i = \text{MF}, \text{CO}_2, \text{CO}$) found in the reaction with substrate ($n_{i,substrate}$) and the amount of product i found in the reaction without substrate (blank reaction).

$$n_{i,new} = n_{i,substrate} - n_{i,blank} \quad (4)$$

The yield of the products is calculated via Equation (5) using the new amount of product ($n_{i,new}$) in relation to the amount of dissolved carbon fraction ($n_{C,dissolved}$), as this is the only amount which can be converted into liquid and gaseous products.

$$Y = \frac{n_{i,substrate,new}}{n_{C,dissolved}} * 100\% \quad (5)$$

The value to waste ratio is calculated via Equation (6).

$$\text{value to waste ratio} = \frac{Y_{\text{methyl formate}}}{Y_{\text{CO}} + Y_{\text{CO}_2}} \quad (6)$$

The TOF is calculated via Equation (7)

$$\text{TOF} = \frac{n_{\text{methyl formate}}}{n_{\text{catalyst}} * t} \quad (7)$$

where t is the time of the reaction in seconds, n_{catalyst} is the molar amount of catalyst used, and $n_{\text{methyl formate}}$ is the molar amount of methyl formate.

Supporting Information

The supporting information is accompanied by various additional results and analytical data of catalysts and reaction solutions.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: cellulose pulp • green chemistry • lignocellulosic biomass • methyl formate • polyoxometalate • polyoxometalate-ionosolv biorefining

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5.3 ProFA - Valorization of macroalgae biomass as source of proteins and formic acid

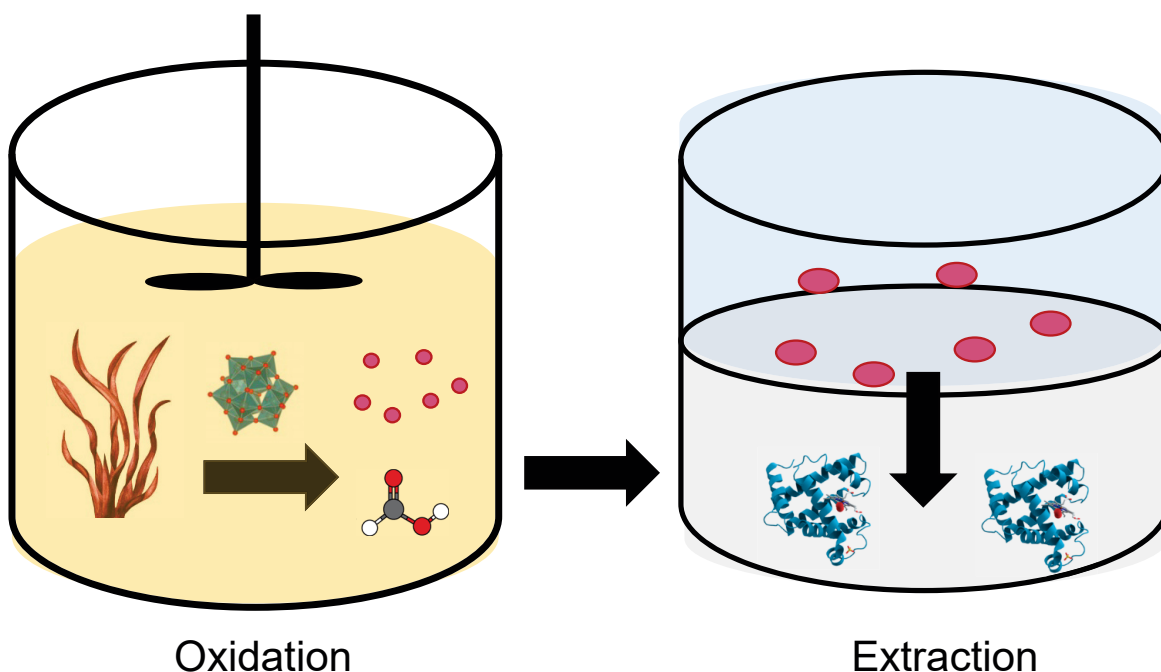


Fig. 5.3 Graphical abstract of the third study of this dissertation: "ProFA - Valorization of macroalgae biomass as source of proteins and formic acid".^[287]

5.3.1 Summary

In contrast to the other two studies in Sections 5.1 and 5.2, the third study investigates the possibility of using macroalgae as biomass for biogenic formic acid production. This study focuses on resource utilization and innovation using the OxFA process in combination with the extraction of proteins from macroalgae. The aim was to find out which macroalgae is best suited to produce formic acid and protein-rich solids. To this purpose, three different algae from three different groups were tested. The brown algae *fucus vesiculosus*, the green algae *ulva fenestrata* and the red algae *porphyra dioica* were investigated as substrates and the latter was identified as the most suitable tested algae due to the highest yield of formic acid and protein recovery. In addition, the most suitable tested catalyst was selected from two polyoxometalate catalysts $H_5PV_2Mo_{10}O_{40}$ and $H_8PV_5Mo_7O_{40}$ well known for the oxidation of biomass to formic acid. By tuning the redox-activity of the OxFA catalyst with the vanadium content, the amount of protein-rich solid residue, could be increased to 13.7 % of the total algae with a protein content of 36.4 % dw, while achieving a FA yield of 8.6 %

using $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ as catalyst. After porphyra dioica proved to be a promising substrate and the catalyst $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$ was identified as the most suitable one, the parameters temperature (80 °C – 120 °C), time (18 h – 30 h) and catalyst/substrate ratio (0.05 to 0.5) were tested for their statistical influence using a Box-Behnken Design of Experiments. The resulting model was used to optimize the protein content and formic acid yield. These optimized conditions were 80 °C, 30 hours and a catalyst-to-substrate ratio of 0.5. The conditions resulted in a protein yield of 59.5 % (before 36.4 % dw) and a yield of 16.4 % (before 8.6 %) of formic acid. Subsequent to the optimization of the OxFA process, the next step was the identification of the best protein extraction technique. Since the OxFA process (vide intra Section 2.3.3) already serves as acidic pre-treatment, three non-acidic methods were examined: alkali extraction (AE), ultrasound-assisted alkali-based extraction (UAE), and a novel method using choline chloride as ionic liquid (ILE) (vide intra Section 2.2.2). All extraction methods resulted in a protein recovery of more than 40 % dw from solid residues, with ultrasound-assisted extraction yielding the highest protein recovery of 87.2 % dw (at 100 % sonication amplitude). UAE resulted in 30 % higher protein recovery than alkaline hydrolysis and 40 % higher protein recovery than extraction with ionic liquid extraction. Therefore, the combination of the OxFA process with UAE was found to be the most efficient way to obtain protein and formic acid from macroalgae. Analysis of the extracted proteins showed that their composition is suitable for applications such as the production of biopolymer films. In summary, this process provides a possibility for the sustainable and holistic utilization of algal biomass producing valuable protein and formic acid.

5.3.2 Article III

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ProFA – valorization of macroalgae biomass as a source of proteins and formic acid†

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Macroalgae are promising feedstocks for the sustainable production of bioplastic films from proteins or platform chemicals such as formic acid (FA) as they are valuable sources of proteins and carbohydrates. This study explores the possibility of using macroalgae as biomass for biogenic FA production using the OxFA process in combination with the extraction of proteins from macroalgae with a focus on resource utilisation and innovation. Herein, the extraction of proteins and the utilisation of the other components of macroalgae to produce FA are linked for the first time. The aim was to find out which macroalgae is best suited to produce FA and protein-rich solids. For this purpose, three different algae were tested: the brown alga *Fucus vesiculosus*, the green alga *Ulva fenestrata* and the red alga *Porphyra dioica*. In addition, the most suitable catalyst for this study was selected from the two polyoxometalates H₅PV₂Mo₁₀O₄₀ (HPA-2) and H₈PV₅Mo₇O₄₀ (HPA-5) known for their suitability in the OxFA process. After *Porphyra dioica* proved to be a promising substrate, the parameters such as temperature (80–120 °C), reaction time (18–30 h) and catalyst/substrate ratio (0.05 to 0.5) were evaluated for their statistical influence using a Box–Behnken design of experiments. The resulting model was then used to optimise the protein content and FA yield. The optimal conditions were determined to be 80 °C, 30 hours and a catalyst-to-substrate ratio of 0.5 resulting in a protein yield of 59.5% and a formic acid yield of 16.4%. For protein extraction from the solid residues, three different methods such as alkaline hydrolysis, ultrasound-assisted extraction (UAE) and ionic liquid extraction (ILE) were investigated. All extraction methods resulted in a protein recovery of more than 40% dw, with UAE yielding the highest protein recovery of 87.2% dw (at 100% sonication amplitude) showing 30% higher protein recovery than alkaline hydrolysis and 40% higher protein recovery than ILE. It turned out that the OxFA process followed by protein extraction using UAE gave a high protein recovery and a promising yield of formic acid.

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Sustainability spotlight

To realize the goal of a carbon-neutral society, the closing of carbon and nitrogen cycles in platform chemicals would be extremely important. Macroalgae are promising feedstocks for the sustainable production of bioplastic films from proteins or platform chemicals such as formic acid as they are valuable sources of proteins and carbohydrates. This study explores the possibility of using macroalgae as biomass for biogenic formic acid production with the OxFA process in combination with the extraction of proteins from macroalgae with a focus on resource utilisation and innovation.

Introduction

Biomass is an important feedstock to target the problem of shifting the chemical industry to more sustainable processes. Algal biomass is an abundant renewable source of carbon (C) and nitrogen (N) combined within one species. Specifically,

macroalgae represent a vast and underexplored resource for protein extraction, with benefits such as rapid growth rates and minimal environmental footprint. Recently, macroalgae proteins have gained significant scientific interest for application in non-food industries such as cosmetic and pharmaceutical sectors.¹

Macroalgae are, on the one hand, important sources of proteins, lipids, vitamins, and other biologically active compounds like phycobilin and carotenoids.² On the other hand, using the carbohydrates from macroalgal biomass led to products like biogas,³ bioethanol or biobutanol.⁴ In addition, the proteins, polysaccharides and lipids found in macroalgae can be utilised for the production of biopolymers.⁵ The

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utilisation of macroalgae not only meets the growing demand for alternative protein sources, but also does so with minimal impact on the environment associated with the cultivation of macroalgae.^{1,6,7} Therefore, protein extraction from macroalgae is an important process for sustainable resource utilisation and innovative industrial application of products. Macroalgae are divided into three different types: red, brown and green macroalgae. One example for a red alga is the species *Porphyra dioica*, which is known for its high protein content (25–30% dw). These species are found especially in warm waters like in Portugal, New Zealand, South Africa, etc. and in freshwater and saltwater.^{8–10} One example for a brown alga is the species *Fucus vesiculosus* also known as Bladderwrack mainly present in cold-temperature water regions. These algae have a protein content of around 6% dw to 10% dw.^{11,12} For a green alga *Ulva fenestrata* is an example which can be found in many parts of the world in polluted and pure water as well as open or protected areas. This species is considered an important protein source with a protein content of 15% dw to 20% dw and a wide range of polysaccharides like cellulose, starch, glucan and ulvan.^{13–15}

Various protein extraction techniques such as liquid extraction and ultrasonic extraction as well as novel methods such as ionic liquid extraction and two-phase separation have been investigated for macroalgae proteins.¹ In general, a combination of extraction techniques together with a pre-treatment increases the protein yield. The fundamental principle of extraction techniques is the disruption of the cell wall. Alkaline hydrolysis is a conventional extraction technique for algal protein extraction.¹ The application of acid before alkali extraction enhances the liberation of polysaccharides and proteins within the cell wall matrix and can increase the solubilization of proteins.¹⁶ Ultrasound-assisted extraction (UAE) is a technology that utilizes sound waves at frequencies higher than 20 kHz to destroy the cell wall matrices of the macroalgae.¹⁷ During the treatment, smaller cavitation bubbles within the liquid surrounding the macroalgae cell collapse which results in higher shear stress and eventually break down of the cell wall.¹⁸ UAE results in lower solvent consumption and is in general a simple, efficient and inexpensive extraction method. Nevertheless, using ultrasound could lead to excessive heat generation which might interfere with the protein structure.¹⁹ A

more novel protein extraction approach is Ionic Liquid Extraction (ILE). Ionic liquids are known to be efficient extraction media for complex biomolecules especially lignin or hemicellulose in lignocellulosic biomass.²⁰ These ILs interact with the macroalgae cell walls while disrupting intermolecular H-bonds in the cellulose present in the cell wall causing their dissolution. This results in partial or complete damage of the cell wall.¹ Utilizing ILs synthesized from natural sources can increase the biocompatible nature and sustainability by using ILs with less toxic environmental impacts.^{21,22}

Different extraction approaches can be combined to improve the extraction of valuable components and the remaining fractions can be used as raw materials to produce other compounds, thus minimising waste streams. This concept increases the value of macroalgae as a bioresource and promotes sustainable development. Therefore, the selection of the most effective technology for the separation of proteins from macroalgae is crucial.^{1,19}

If proteins are extracted from the macroalgae, carbohydrates are usually waste products. In order to increase the total added value of macroalgae, these carbohydrates can be converted into platform chemicals. One possible value product is formic acid (FA). FA is widely used in various industries such as the chemical, agricultural, leather and rubber industries.²³ The latter is also discussed as a hydrogen carrier and as a liquid syngas equivalent.^{24,25} Biogenic formic acid can be produced from biomass especially carbohydrates under mild conditions (<100 °C) using the OxFA process.²⁶ Herein, biomass is selectively oxidised using a polyoxometalate (POM) catalyst to produce FA and carbon dioxide. Macroalgae, which are rich in polysaccharides, can also be used to produce biogenic FA using the OxFA process and therefore represent a sustainable alternative to fossil fuels.^{27–29}

This study focuses on combining the OxFA process with protein extraction methods for the complete valorization of macroalgae. In the OxFA process, carbohydrates are oxidized to FA, leaving a solid residue ideally consisting of mainly non-water-soluble proteins in unreacted macroalgae remains. We investigated various methods to extract these proteins therefore increasing the utilization of the algae. Three different macroalgae species, namely *Porphyra dioica*, *Ulva fenestrata* and *Fucus*

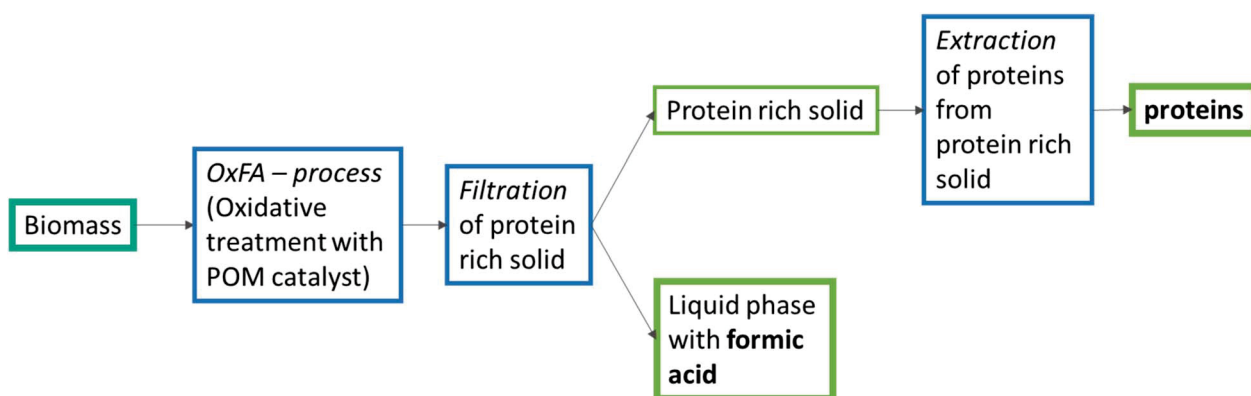


Fig. 1 Schematic flow-chart of the ProFA process. The unit operations are marked in blue and the reactants and products are colored greenish.



vesiculosus were tested for their use in this biorefinery concept. By applying a Box–Behnken design of experiments (DoE), the statistical influences on the OxFA process with the most promising macroalgae species are analyzed. Following the optimization, the solid residue obtained is utilized for protein extraction. This study also explores various protein extraction methods, emphasizing both traditional and novel techniques such as solid–liquid extraction, ultrasound-assisted extraction (UAE) and ionic-liquid based extraction (ILE). Additionally, it investigates the factors influencing protein recovery for each extraction method. Understanding these aspects is crucial for optimizing protein extraction protocols with maximized protein recovery. A simplified process scheme is shown in Fig. 1.

Experimental

Substrates and catalysts

The macroalgae used for the study, the red alga *Porphyra dioica*, the green alga *Ulva fenestrata* and the brown alga *Fucus vesiculosus*, were cultivated by the company ALGApplus in the coastal lagoon of Ria de Aveiro (40°38' N, 8°44' W) based on an open Integrated Multi-Tropic Aquaculture system. After harvesting, the macroalgae biomass was washed with seawater, centrifuged to remove the excess water, and dried in an air tunnel at low temperature by ALGApplus. To make sure that different particle sizes have no influence on the outcome of the reaction, the samples were milled to 250 µm using a Retsch ZM 200 (Retsch, Germany) at 18 000 rpm in Hamburg.

The used catalysts HPA-2 ($\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$) and HPA-5 ($\text{H}_8\text{PV}_5\text{Mo}_5\text{O}_{40}$) were synthesized according to a procedure described by Albert²⁸ and Raabe *et al.*³⁰ Further information about the synthesis and characterization of the catalysts is given in the ESI (Table S1 and Fig. S1 to S4).†

Catalytic oxidation experiments

All catalytic oxidation experiments were performed in a 600 mL Hastelloy C276 autoclave (Parr Instruments, USA) equipped with a gas entrainment stirrer (Cemp International, Germany). The reactor was filled with 10 g of algae and 200 g of water. The amount of catalysts varied between 0.5 g and 5 g according to the desired catalyst/substrate ratio of 0.05 and 0.5 $\text{g}_{\text{catalyst}}/\text{g}_{\text{substrate}}$. The reactor was closed and purged three times with molecular oxygen (30 bar). To set up the reaction conditions the reactor was pre-pressurized with *ca.* 20 bar of oxygen, heated up to the desired reaction temperature (80 °C or 120 °C) and a stirrer speed of 300 rpm was set. The screening experiments for the different algal substrates were conducted non-isothermally and isochore, and therefore the reaction temperature is the starting temperature of the experiment. The DoE experiments are carried out in an identical, but isothermal, set-up. At the reaction temperature, the desired pressure of 30 bar oxygen was adjusted and the stirrer speed was increased to 1000 rpm. After the reaction time (between 18 h and 30), the stirrer speed was set to 300 rpm and the reactor was allowed to cool down to room temperature. A gas sample was taken from the cooled down gas phase and analyzed *via* gas chromatography (GC). The

reactor was depressurized. The solid residue was filtered and washed with deionized water and dried overnight at 40 °C. The dry solid residue was weight and analyzed *via* organic elemental (CHNS) analysis for protein content. The liquid phase was analyzed *via* High Performance Liquid Chromatography (HPLC). The benchmark experiments were conducted as described with a temperature of 90 °C, a reaction time of 24 h and an amount of catalyst of 1 mmol (1.67 g (HPA-5) and 1.88 g (HPA-2)).

Protein extraction methods

Alkaline hydrolysis. Alkaline hydrolysis was based on the method described by Harnedy *et al.*³¹ with some modifications. For the extraction, 0.1 g of solid residue was suspended in NaOH solution (concentration was varied between 0.08–0.14 M) at a weight : volume ratio of 1 : 15, containing 0.2 g/100 mL of *N*-acetyl-L-cysteine (NAC). NAC is a reducing agent and as reported by Harnedy *et al.*³¹ its addition can increase the yield of alkaline soluble protein during extraction. Alkaline extraction was performed in a thermoshaker (Grant Instruments, United Kingdom) at 750 rpm and 20 °C for 4 h. The extract was separated by centrifugation at 10 000 rpm for 20 minutes and was analyzed for protein content. The extraction was performed in duplicate ($n = 2$).

Ultrasound-assisted extraction (UAE). The ultrasound-assisted extraction (UAE) was performed in an alkali medium. For the extraction, 0.6 g of solid residue was suspended in 0.4 M NaOH solution with a ratio of 1 : 15. An HD 2700 ultrasound machine (Bandelin, Germany) with an MS 73 probe was used. The samples were kept in an ice bath throughout the experiment to prevent heating up. An extraction time of 10 min and frequency of 20 kHz was used, and the amplitude was regulated between 20–100%. The highest frequency (100%) is a power of 70 W. The resulting solution was centrifuged at 5000 rpm for 15 min at 4 °C. The extract was separated and analyzed for protein content.

Ionic liquid extraction (ILE). The third purification approach is based on the methods described in Eppink *et al.*²¹ with some modifications. The solid residue was treated with choline chloride (concentration varied between 10 wt% and 50 wt%). A sample weight of 0.1 g was mixed with 1.5 mL of choline chloride (ratio of 1 : 15). The solution was mixed using a Vortex mini (Janke and Kunkel, Germany) and extracted for 10 min at 25 °C in a thermoshaker (Grant Instruments, United Kingdom) at 1000 rpm. The mixture was then centrifuged at 3000 rpm for 10 minutes at 4 °C. The extract was separated and analyzed for protein content.

Analytical methods

For the pre-characterization of the algal biomass, the protein, amino acid, carbohydrate, lipid, moisture and ash contents and the elemental composition were analyzed. For product analysis GC was used for the gas phase and HPLC for the liquid phase. The solid residue was analyzed for proteins and elemental composition.



Elemental composition – CHNS analysis. The elemental composition (CHNS-content) was determined *via* a Vario Macro Cube NCHS analyzer (Elementar, Germany) with an internal thermal conductivity detector.

Protein and amino acid analysis. The nitrogen content measured *via* CHNS-analysis was used for the quantification of the protein content by multiplying by a factor of 6.25.³² The amino acid profile was estimated *via* HPLC by hydrolyzing the proteins to their respective amino acids by the procedure followed by Lamp *et al.*³³

Carbohydrate and ash content. The carbohydrate and ash contents were estimated using the LAP procedure established by the National Renewable Energy Laboratory (NREL – TP-510-42618).³⁴ In short, the ash content of the sample was determined in a muffle furnace with a temperature profile between 105 °C and 575 °C. For carbohydrates, 1.5 mL of 72% sulfuric acid for hydrolysis were added to approximately 150 mg of the sample in a pressure tube. This tube was incubated for 60 min at 30 °C. After the hydrolysis step, 42 mL of deionized water was added to dilute the sulfuric acid to 4% and autoclaved for 60 min at 121 °C. The hydrolysates were filtered and analyzed for formic acid and acetic acid by HPLC. The HPLC was operated at a flow rate of 0.6 mL min^{−1} at 65 °C for 60 min with 5 mM sulfuric acid as the mobile phase in a Biorad Aminex HPX-87H column. For the carbohydrate content the filtrate was neutralized with calcium carbonate to a pH of 5–6 and analyzed *via* HPLC. This HPLC used a Shodex sugar SP0810 column operated at a flow rate of 0.6 mL min^{−1} at a temperature of 85 °C for 35 min with HPLC grade water as the mobile phase.

Lipid analysis. The lipid content was analyzed by a gravimetric method using Soxhlet extraction with *n*-hexane (Carl Roth GmbH, Germany). The extraction was carried out for 6 h with 5 g of the substrate using 150 mL *n*-hexane. The lipid extract was dried at room temperature for 24 h followed by solvent removal in a rotary evaporator at 40 °C and 250 bar pressure.

Moisture analysis. The moisture analysis was performed by a gravimetric method using a Mettler Toledo (USA) HE53 moisture analyzer.

Gas phase analysis. The gas samples from the catalytic reaction were analyzed using GC to measure the volume percentage of CO₂ produced. The GC used was a Varian 450 GC gas chromatograph equipped with a Shin-Carbon-ST-Column of 2 m × 0.75 mm. The samples were injected through a 250 µL sample tube and were passed through the column in a stationary phase with an argon gas flow at 4.8 bar.

Liquid phase analysis. The aqueous phase of the catalytic reaction was analyzed by HPLC to determine the amounts of formic and acetic acid formed. The reaction solution was filtered through a 0.25 µm filter and sampled for HPLC. The system was equipped with an Aminex HPX-87H column (Bio-Rad Laboratories, Inc., USA) and a RI-detector. The eluent was 5 mM H₂SO₄ with a flow rate of 0.5 mL min^{−1} at a temperature of 45 °C.

SDS-PAGE gel electrophoresis. SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel) electrophoresis analysis was

conducted to detect the protein content by applying a voltage for the sample to pass through a polyacrylamide gel placed in sodium dodecyl sulphate solution. The sample was compared with standard markers to estimate the size of the protein content in the sample. For the analysis, 20 µL of the sample extract and 5 µL of buffer (SERVA Tris-glycine/LDS sample buffer) were added. The mixture was heated to 95 °C in a thermoshaker (Grant Instruments, United Kingdom) at 700 rpm for 5 min followed by centrifugation at 10 000 rpm for 45 s. Around 5 µL of the samples were run through an SDS gel (SERVAGel™ TG PRiMETM 12% gel – 12 sample wells gel) alongside a standard marker ranging from 11 to 180 kDa. After completion of electrophoresis, the gel was removed from the gel tray and stained with a protein stain (SERVA Quick Coomassie Stain) for about 24 h. The gel was washed with water to remove excess stain for another 24 h and was used to obtain and compare the results.

Bradford assay. Bradford assay is a method used for the quantification of proteins by spectrophotometry.³⁵ Ionic liquids have been observed to cause negligible interference with the Bradford quantification method, and hence it was used for the quantification of proteins in the extraction method using ionic liquids.²¹ For the analysis, the samples were mixed with Bradford stock solution, which produced a specific color reaction with proteins and was analyzed with a spectrophotometer. To 40 mL of Bradford reagent (an acidified solution of Coomassie G-250), 160 mL of distilled water was added and mixed to form the Bradford stock solution. To establish a calibration curve, a standard Bovine Serum Albumin (BSA) was used. A concentration of 1 mg mL^{−1} BSA-stock solution was prepared, and it was diluted in the range of 0–1000 µg mL^{−1} to obtain the calibration curve. To measure the absorbance a plate reader with a UV-vis spectrophotometer was used. To a sample amount of 50 µL, 200 µL of Bradford solution was added and after 5 min of incubation at room temperature, the solutions were analyzed, and the absorbance was measured at 590 nm and 450 nm. The quotient 590 nm/450 nm was used for the calculation and the calibration graph obtained is given in ESI Fig. S5.†

Design of Experiments (DoE). Within the Design of Experiments (DoE), multiple factors are changed simultaneously so that the information about the statistical influence of these factors can be obtained. Based on this information an optimization can be carried out. This study used a Box–Behnken design to analyze the quadratic effects between the chosen factors. The software used for this study was Design Expert Version 11 from Stat-Ease, USA.³⁶ All experiments for this study were carried out in the above-described reactor set-up with the red macroalga *Porphyra dioica* as a substrate. The varied parameters are the reaction time (18 h to 24 h), catalyst/substrate ratio (0.05 g_{catalyst} g_{substrate}^{−1} to 0.5 g_{catalyst} g_{substrate}^{−1}) and reaction temperature (80 °C to 120 °C). All other reaction parameters like stirrer speed (1000 rpm), oxygen pressure (30 bar), solvent (water), solvent mass (200 g), catalyst (HPA-2) and mass of substrate (10 g) were kept constant. The catalyst/substrate ratio was varied through the mass of catalyst used. Table S4 in the ESI† shows the list of experiments screened for the design.



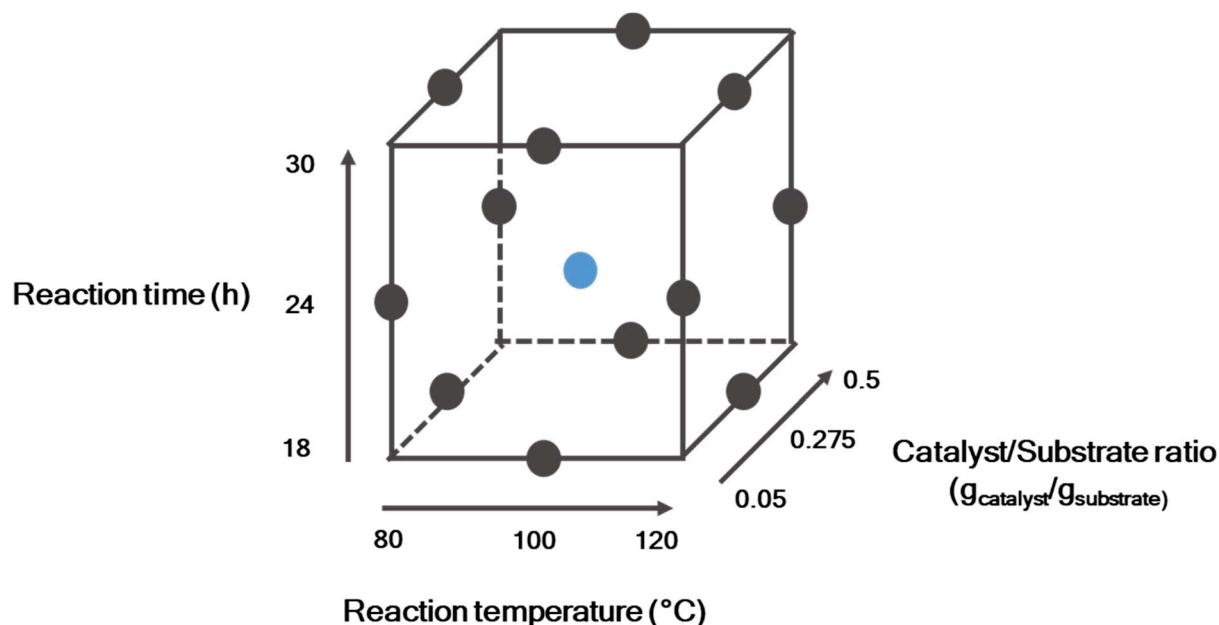


Fig. 2 Parameter room for the designed Box–Behnken–DoE. The ranges of variables used are found at the edges.

The parameter room in which the statistical influences are determined is shown in Fig. 2. The dots represent the screened experiments.

Results and discussion

Macroalgae characterization

In order to assess the potential of different types of macroalgae for valorization *i.e.* production of FA and extraction of proteins, they were first characterized in detail. The protein, lipid, carbohydrate, and moisture contents as well as the ash content were analyzed. These values can be seen in the diagram in Fig. 3.

The ash content is very high in all samples. For the algae *Porphyra dioica* and *Fucus vesiculosus* it is 43.4% ($\pm 1.0\%$) and 43.1% ($\pm 1\%$) respectively. Only the tested green alga *Ulva fenestrata* has an ash content of 27.3% ($\pm 0.2\%$) below 30%. The lowest percentage in the composition of the macroalgae is the lipid content. For all substrates tested, this is less than 10%, for the red alga *Porphyra dioica* it is even less than 1%. The moisture content before drying of the algae is only above 10% (11.2%) ($\pm 0.2\%$) for the green alga *Ulva fenestrata* and otherwise below 10%. The most interesting values for this study are the protein content and the carbohydrate content, as both have an influence on the later value products of the study. With a protein content of just under 30% (29.8%), the red alga *Porphyra dioica* has the highest tested value in the study. The other algae have a protein content of 16.0% for the green alga *Ulva fenestrata* and 6.8% for the brown alga *Fucus vesiculosus*. The higher the carbohydrate content, the higher the expected yield of the target product FA can be. Therefore, the macroalgae *Ulva fenestrata* and *Fucus vesiculosus* are good candidates for the production of FA with around 45% of carbohydrates (46.9% and 45.3%). All these characterization data lay well in line with other

studies of these three macroalgae.^{6,37–47} The characterization of *Porphyra dioica* in this study shows a 5% higher protein content and a relatively low carbohydrate content compared to that in other studies by Pimental *et al.*⁶ and Teles *et al.*³⁸ It is known that the growth phase at harvest and the growth conditions have a significant influence on the composition of the macroalgae. The location and climatic conditions at the cultivation area also have an impact on the composition of the biomass.¹⁰ Therefore, the deviations of the values from the cited studies are not surprising. Some reports for *Porphyra dioica* show even higher protein contents.¹⁰ On the one hand, with all the data of the three macroalgae, the reported protein content of *Porphyra dioica* in this study has the potential to result in high amounts of protein extracted from these macroalgae increasing the potential usage for a protein source. On the other hand, FA as the second target product is derived through the carbohydrates existing in the macroalgae samples. Therefore, *Fucus vesiculosus* and *Ulva fenestrata* with high carbohydrate content are the most promising substrates for the production of FA.

To complete the characterization of the three different macroalgae species, the amino acid profile and the CHNS-elemental composition results are shown in Fig. S2–S4 and Table S1.† The amino acid profile helps to understand the distribution of various amino acids present in the biomass, which is important for potential further use as a substrate for biopolymer film development. There are two groups of amino acids present in the macroalgae samples – hydrophobic and hydrophilic amino acids. The hydrophilic amino acids restrict protein-based biopolymer film applications in packaging of wet materials. Hence, it is beneficial to have a higher distribution of hydrophobic amino acids in the extracted proteins. Hydrophobic amino acids are Val (valine), Met (methionine), Ile (isoleucine), Leu (leucine), Phe (Phenylalanine) Gly (glycine),



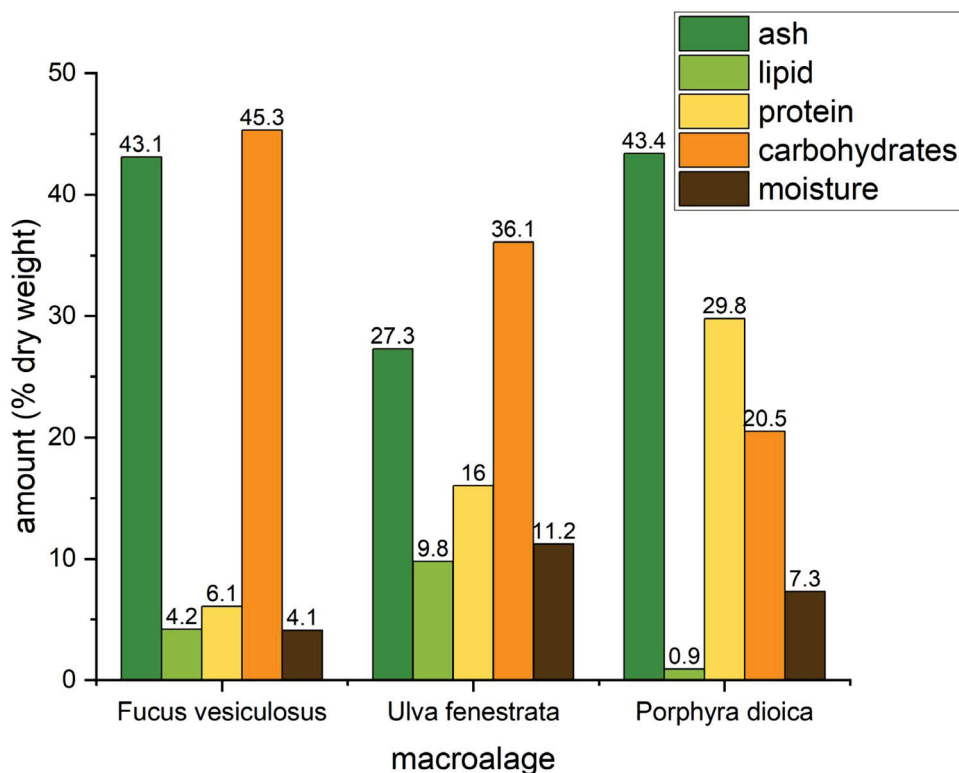


Fig. 3 Characterization of different macroalgae – *Fucus vesiculosus* (brown alga), *Ulva fenestrata* (green alga), and *Porphyra dioica* (red alga).

and Ala (alanine).⁴⁸ The amino acid profile of the used algae and a short description can be found in Fig. S6–S8.† By using the OxFA process as a pre-treatment method, the amino acid distribution can change significantly. The next step will therefore focus on the catalytic oxidation of the three macroalgae substrates introduced.

Catalytic oxidation of macroalgae using the OxFA process

The catalytic conversion of the three macroalgae is aimed at pretreating these algae for protein extraction and obtaining FA as a valuable product. Therefore, all macroalgae were oxidized under similar reaction conditions at a temperature of 90 °C, a pressure of 30 bar oxygen, a stirrer speed of 1000 rpm, and a reaction time of 24 h using 10 g macroalgae together with a catalyst amount of 1 mmol. The used catalyst is the well-established HPA-5 which has been proven to be an efficient catalyst for biomass oxidation especially the OxFA process.²⁶ Fig. 4 shows the OxFA-reaction produced FA (dark green) as well as the by-products CO₂ (yellow) and acetic acid (light green). Overall, a high conversion was observed for all macroalgae with 95.3% for *Fucus vesiculosus* and *Porphyra dioica* and 85.2% for *Ulva fenestrata*. However, FA-yields of 4.6% for *Ulva fenestrata* and *Fucus vesiculosus* as well as 6.5% for *Porphyra dioica* are comparatively low compared to that using sugars or lignocellulosic biomass as substrates for the OxFA process. The FA-yield depends on the substrate and the number of oxygen functionalities in the carbon framework.²⁷ The most favorable substrate in the study of Albert *et al.*²⁷ is pomace. Remarkably, the two

macroalgae with the highest carbohydrate content *Fucus vesiculosus* and *Ulva fenestrata* (Fig. 3) result in the lowest FA-yield. For the brown alga *Fucus vesiculosus* the oxidation reaction results in a high overoxidation to CO₂ of 37.6%. With FA and acetic acid as valuable products, this leads to a value to waste ratio of only 0.25. With a value to waste ratio below 1, the waste product CO₂ is more likely to be produced than valuable products. For the other two screened algae, the value to waste ratio lies under 1 as well, with a value of 0.42 for *Porphyra dioica* and 0.46 for *Ulva fenestrata*. In terms of the value to waste ratio, *Ulva fenestrata* is the best starting material, but regarding FA-yield *Porphyra dioica* leads to the highest yield amongst the screened macroalgae.

As the idea of this study is to generate two valuable products, namely FA and proteins, we decided to maximize the amount of solid residue, *i.e.* lower the conversion to preserve the protein content. The analysis of proteins in the reaction broth is difficult as the homogeneous POM catalyst interferes with the usual analytical methods. Esser *et al.*⁴⁹ showed the possibility of membrane separation through a nanofiltration membrane for the POM catalyst. However, this works because the target product FA or other small compounds can diffuse through the membrane and the catalyst is retained. Since proteins are much larger molecules, a new process has to be established for the separation of the proteins or amino acids from the catalyst, which warrants a study of its own. Therefore, this study focuses on the proteins left in the solid residue after the reaction as these proteins might be more hydrophobic and therefore a good feedstock for the production of biopolymer films. Since the



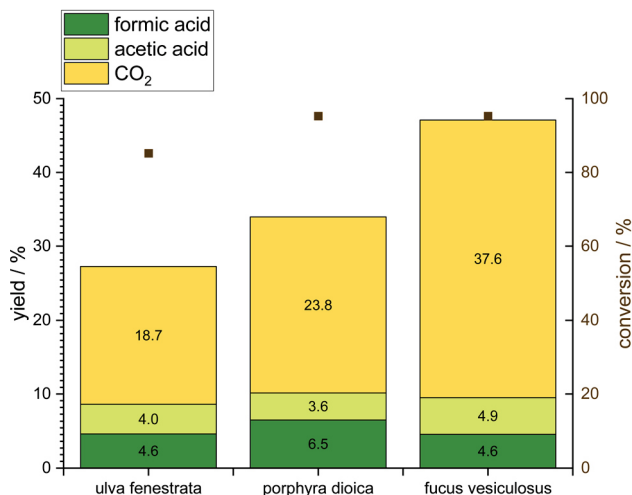


Fig. 4 Screening of different macroalgae in the OxFA process for their product yield (left y-axis) and algae conversion (right y-axis) using the HPA-5 catalyst. Reaction conditions: HPA-5 at an initial temperature of 90 °C, non-isothermal reaction, 30 bar oxygen pressure, 1000 rpm stirrer speed, 24 h reaction time. The liquid phase consisted of 200 g of water with 10 g of suspended macroalgae and 1.89 g of dissolved catalyst (1 mmol, HPA-5).

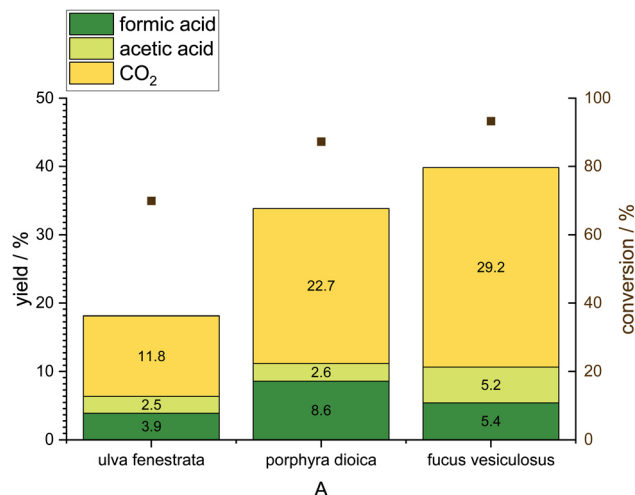


Fig. 5 Screening of different macroalgae in the OxFA process for their product yield (left y-axis) and algae conversion (right y-axis) using the HPA-2 catalyst. Reaction conditions: HPA-2 at an initial temperature of 90 °C, non-isothermal reaction, 30 bar oxygen pressure, 1000 rpm stirrer speed, 24 h reaction time. The liquid phase consisted of 200 g of water with 10 g suspended macroalgae and 1.95 g of dissolved catalyst (1 mmol, HPA-2).

initial experiment showed that the conversion with the five-times vanadium substituted Keggin-type POM-catalyst HPA-5 is too high for an investigation of the solid residue, we chose a less active catalyst for the next experiments. The next catalyst tested was the two-times vanadium substituted Keggin-type POM HPA-2. Raabe *et al.*⁵⁰ and Esser *et al.*⁵¹ showed in different studies the variable redox-activity of different vanadium-substituted POMs. Due to the different V-contents (5 vanadium atoms in HPA-5 and 2 vanadium atoms in HPA-2), the redox-activity could be tuned to lower the redox-potential with HPA-2 (Fig. S9 & S10†). As shown by Krueger *et al.*,⁵² the requirement of having V atoms adjacent to each other leads to an activity difference as well. The trends of the three macroalgae are similar, as basically the same chemistry is applied, just with a lower redox potential. The conversion of the three tested macroalgae could be reduced to 93.3% for *Fucus vesiculosus*, 87.3% for *Porphyra dioica* and 70% for *Ulva fenestrata* (Fig. 5). The highest reduction of conversion with nearly 15% could be achieved with *Ulva fenestrata*. A significant reduction of 8% was observed for *Porphyra dioica*; however with *Fucus vesiculosus* a reduction of just 2% could be achieved. Interestingly, the HPA-2-catalyst suppresses the overoxidation to CO₂ and reduces the yield of CO₂ to about 7% or 8% for *Ulva fenestrata*, and *Fucus vesiculosus*, with just 2% yield of CO₂ from *Porphyra dioica*. Another noteworthy point is the FA-yield, which increases in the case of *Porphyra dioica* up to 8.6% (before 6.5%) and *Fucus vesiculosus* up to 5.4% (before 4.6%) and decreases in the case of *Ulva fenestrata* to 3.9% (before 4.6%).

To obtain a complete picture of the process, the solid residue after the reaction was characterized for protein content by HPLC and for the amino acid profile. Exemplarily, the amino acid profiles for the red alga *Porphyra dioica* and its solid residue

after the OxFA process are shown in Fig. 6 and all other comparison diagrams are shown in Fig. S11 and S12.† The comparison between the solid residue and the neat *Porphyra dioica* shows that in general the relative content of most amino acids is increasing. Specifically, the hydrophobic amino acids (Val, Met, Ile, Leu, Phe, Gly and Ala) are more abundant, which is desirable for the production of biopolymer films. The highest increase among these was noticed for leucine (13%) and valine (16%). The other hydrophobic amino acids increase with 8% Phe, 4% Gly, 7% Ala, and 9% Ile, respectively. The only hydrophobic amino acid which decreases is Met from 1.7% to 0.9%. A similar trend can be seen in the other macroalgae samples. For *Ulva fenestrata* (Fig. S11†) the highest increase of hydrophobic amino acids was found for Ala (17%) and for Leu (12%). For the amino acid methionine, the same decreasing trend is found.

For *Fucus vesiculosus*, valine (14%) and leucine (19%) have the highest increase in hydrophobic amino acids. Remarkably, in the solid residue of the *Fucus vesiculosus* sample methionine is increasing compared to that in the raw macroalgae sample from 1.6% to 2.1%. The protein content between the macroalgae samples and the solid residue samples is depicted in Fig. 7. The elemental composition of the solid residues is shown in Table S3 in the ESI.† Clearly, the amount of protein increases after the pretreatment with the OxFA process in the solid residue. The highest total amount of protein content is with the red macroalgae *Porphyra dioica* and also its solid residue. For the alga *Fucus vesiculosus* an increase of the protein content by about 11% from 6% initially to 17% was achieved after the pretreatment.

Therefore, the initial proof of concept was successful: the OxFA process can serve as a useful pretreatment for protein extraction from macroalgae. It increases the protein content in



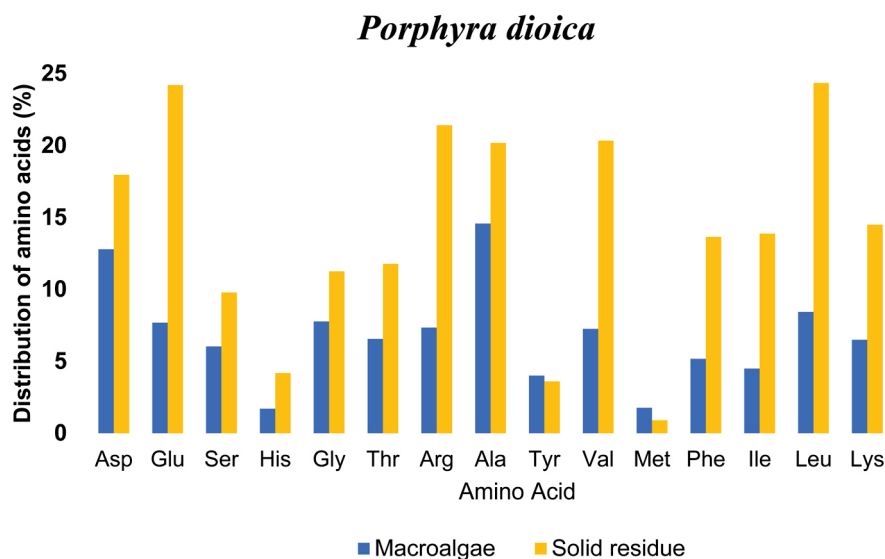


Fig. 6 Amino acid profile for the macroalga *Porphyra dioica* and its solid residue after the OxFa process. Reaction conditions: initial temperature 90 °C, non-isothermal reaction, 30 bar oxygen pressure, 1000 rpm stirrer speed, 24 h reaction time. The liquid phase consisted of 200 g of water with 10 g suspended macroalgae and 1.95 g of dissolved catalyst (1 mmol, HPA-2).

Protein content comparison

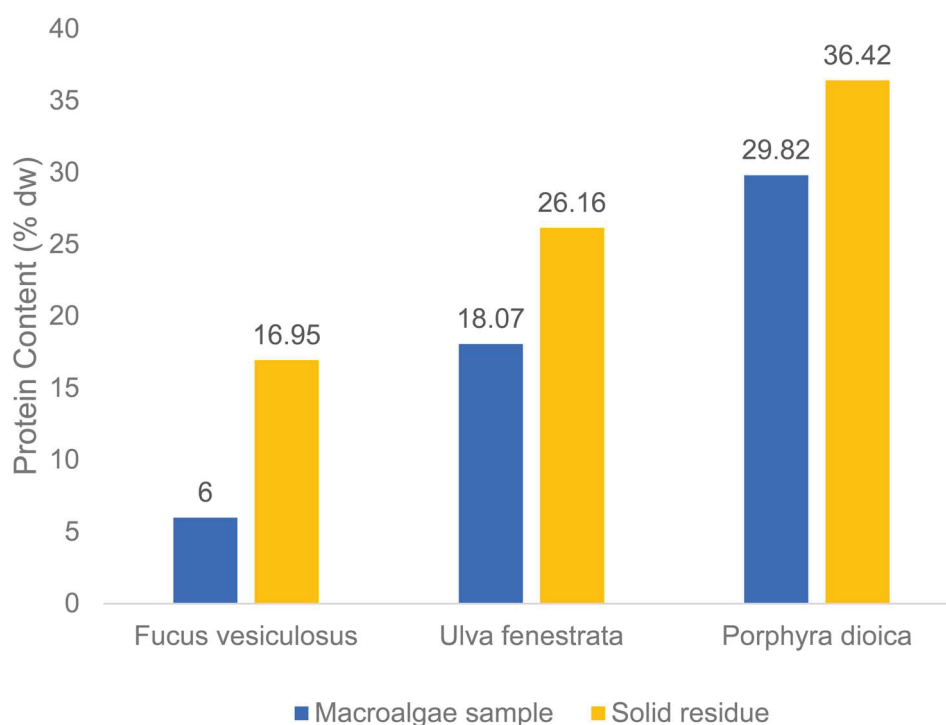


Fig. 7 Protein content comparison of the solid residues of the macroalgae samples after the OxFa process using the HPA-2 catalyst. Reaction conditions: $T = 90\text{ }^{\circ}\text{C}$, $p = 30\text{ bar oxygen}$, $t = 24\text{ h}$, stirrer speed = 1000 rpm.

the sample by converting the carbohydrates to a second valuable product FA. Nevertheless, to valorize the proteins in the solid residue an extraction method has to be applied on the solid residue. The standard method for protein extraction from

biogenic solids is alkaline hydrolysis, which was also applied to this solid residue. Table 1 shows the obtained protein quantity for all three macro algae solid residues after alkali extraction, which was measured *via* SDS-PAGE gel electrophoresis. The



Table 1 Protein concentration in g L⁻¹ in the supernatant of alkali extraction of the solid residue

Alkaline extract	Protein quantity (g L ⁻¹)
<i>Porphyra dioica</i> solid residue	18.3
<i>Ulva fenestrata</i> solid residue	10.0
<i>Fucus vesiculosus</i> solid residue	3.88

same trend that has already been shown for neat algae and solid residues can also be observed here, namely that *Porphyra dioica* provides the highest protein yield and is therefore the best source of protein.

These results provide a promising proof-of-concept for the combination of the OxFA process and subsequent protein extraction from algae. Nevertheless, a FA-yield of 8.6% and a protein quantity of 18.3 g L⁻¹ require further improvement for an economic process. The same applies for the high yield of CO₂ waste, which limits the valorization of the carbon content. For this reason, the next section identifies the statistical influence of various parameters on the OxFA process for valorization of macroalgae via a Box–Behnken design of experiments and provides a further optimization of the protein recovery and FA-yield.

Optimizing protein recovery and FA-yield via Box–Behnken DoE

For the DoE, a new batch of *Porphyra dioica* from the same supplier and region was used. To account for the significant differences in the composition, the new batch has also been fully characterized using the above-described methods, as

shown in Fig. S13–S15 and Table S3.† The second batch used has a high carbohydrate content of 48% dw which is on the upper end of the reported literature values between 46.4% dw by Noda *et al.*⁵³ and 45.40% dw reported by Smith *et al.*⁵⁴ The other valuable product in this study is proteins. Here, the second batch of *Porphyra dioica* has a 3% higher protein content compared to the first batch (Fig. S13†). The value of 33% dw of proteins is well in line with the literature value with a reported range of 25–47% dw.^{1,55,56} As already discussed, the variations in the biomass composition can be attributed to various factors like environmental conditions such as light, temperature, nutritional availability, and the harvesting seasons as well as the harvesting method. Another influential factor is the life cycle stage in which the macroalgae are harvested.^{10,52,53} Hence, understanding the detailed composition of the biomass is essential for exploring its potential for utilizing the macroalgae in this process.

The sensitivity analysis was performed using the statistical method of Box–Behnken DoE with the help of the software Design Expert.³⁶ The influence of three parameters was investigated on three levels. Furthermore, two-dimensional interactions between these factors were determined.⁵⁷ The Box–Behnken design was also used for response surface methodology for optimization, because the parameters are estimated using quadratic models, a sequential design is built and the detection of the lack of fit is possible for the model.^{33,57} For this purpose the temperature was varied between 80 °C and 120 °C where 80 °C is the lowest temperature reported in the literature where a catalytic reaction is activated.⁵⁸ The expectation for the lowest temperature is a high solid residue and therefore high protein content. The highest temperature of 120 °C is chosen because of the expected high FA-yield and a temperature above 120 °C would lead to the denaturation of proteins. Time as

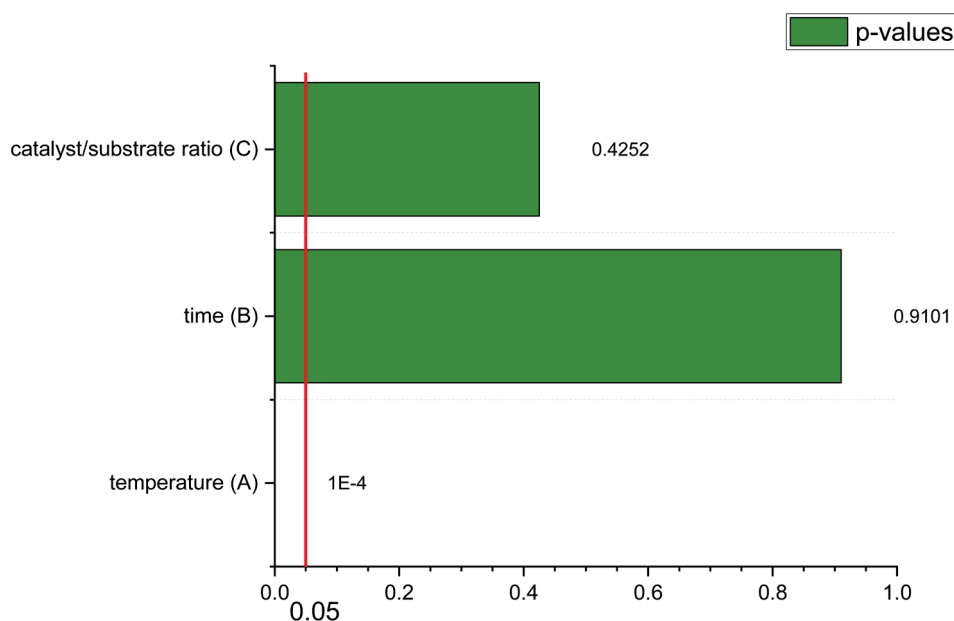


Fig. 8 Pareto chart resulting from the Box–Behnken DoE for the linear model of protein recovery. The red line indicates the significance limit of $p = 0.05$ which shows if the value is significant or not. A value below the significance level is significant.



a parameter was ranged between 18 h and 30 h because it is known that for complex water-insoluble biomass like beech wood an initial time is needed to get significant high yields of FA.⁵⁸ The screening time of 24 h was therefore set to the middle point and it was varied from around 6 h to 18 as shown in Fig. 2.

The response surface obtained is displayed in Fig. 9. The model indicates that protein recovery is only dependent on temperature which is clearly seen in Fig. 8. Decreasing the temperature increases the protein recovery especially when the temperature decreases below 80 °C. This is mainly due to the low conversion of the macroalgae as well as less denaturation of the proteins due to lower temperature. However, the OxFA process requires a minimum temperature of 80 °C for an effective activity of the POM catalyst. Even if a large amount of solid residue and therefore a large amount of protein in the solid residue is favorable, the OxFA process helps as a pre-treatment method for the proteins. This fact will be addressed later in detail with the screening of different further protein extraction methods.

The same Box–Behnken design was also used to analyze significant parameters for FA production. The suggested model here is a quadratic one with a sequential p -value of 0.0026. The statistical evaluation ANOVA is shown in Table S6.† The model is significant with a p -value < 0.0001. The lack of fit with a p -value of 0.0096 is significant, which means that the model does not fit well enough to the data. To get a non-significant lack of

fit a p -value higher than 0.05 is required. To verify this, two verification runs were performed. Fig. S17† displays the parameter room for the verification points as green dots. The experiments are run with the following parameters: for the first verification run a reaction time of 30 h, a reaction temperature of 80 °C and a catalyst/substrate ratio of 0.5 were chosen. For the second verification run a temperature of 120 °C, a reaction time of 30 h and a catalyst/substrate ratio of 0.5 were picked. The other reaction parameters were kept constant. The points were set to the edges of the parameter space. Fig. S18† shows the model predicted values and the actual experimental values. These experiments are well in line and the two verification points (marked with black circles) fit very well to the model and the model is validated. Therefore, the model was used for further interpretation and optimization.

The model found the following parameters to be significant: the temperature A , the catalyst/substrate ratio (C) and the quadratic interactions of these two A^2 and C^2 as well as the quadratic interactions between the two parameters AC . This is visualized in the Pareto chart (Fig. 10) where every parameter which is below the red line (significance limit) is deemed statistically significant.

The response surface for the FA-yield is depicted in Fig. 11. From the model, the temperature and the catalyst/substrate ratio are found to be the most influential factors. The best FA-yield was obtained for a catalyst/substrate ratio of 0.5. This can

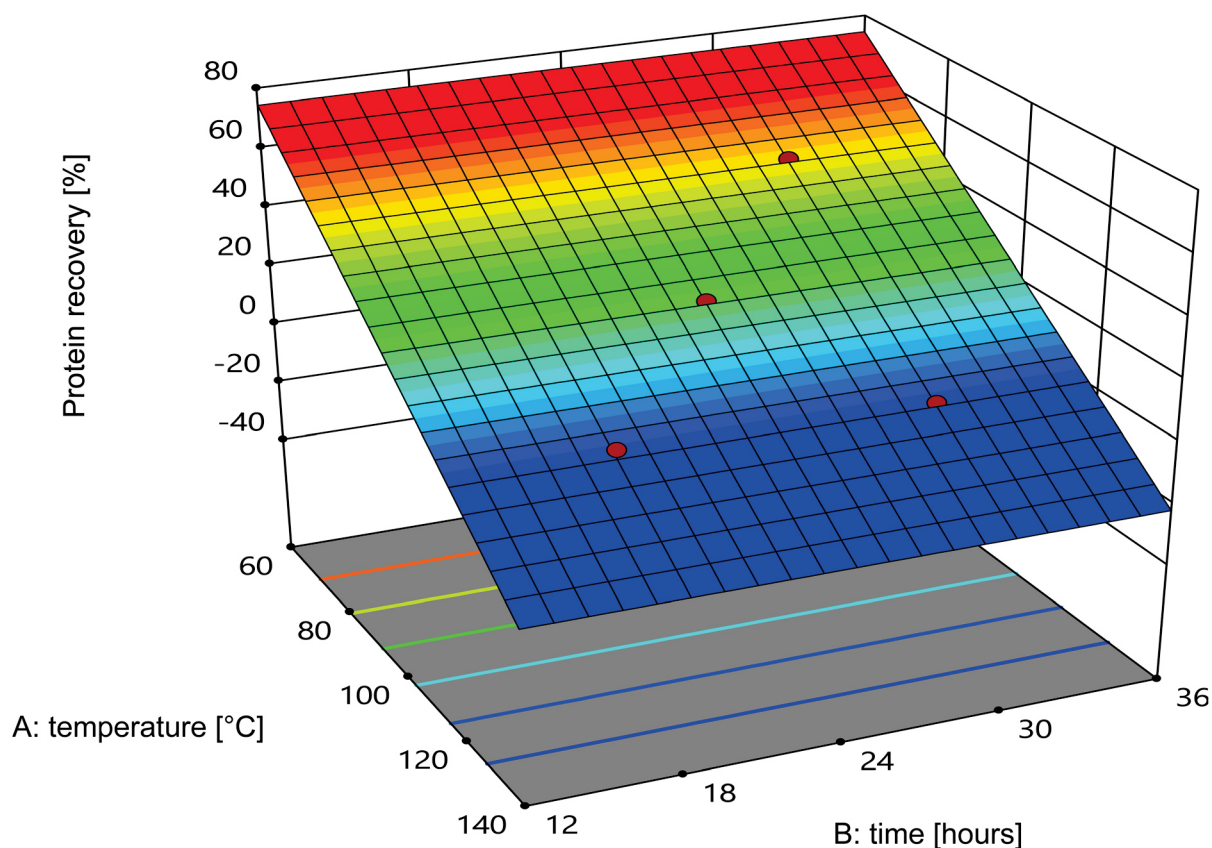


Fig. 9 The 3D response surface for protein recovery as result of the Box–Behnken DoE. The red dots depict the run points above the surface.



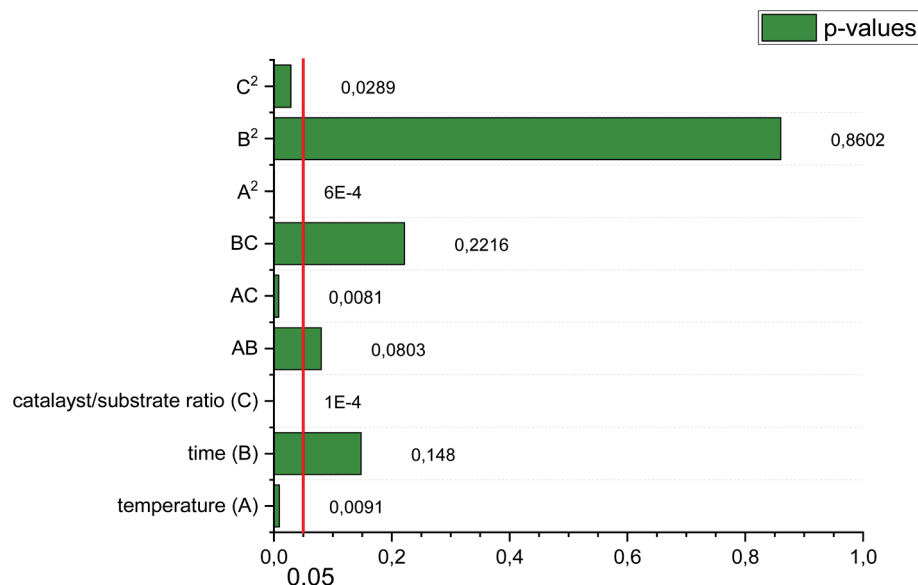


Fig. 10 Pareto chart resulting from the Box-Behnken DoE for the quadratic model for the FA-yield. The red line indicates the significance level of $p = 0.05$ which shows if the value is significant or not. A value below the significance level is significant.

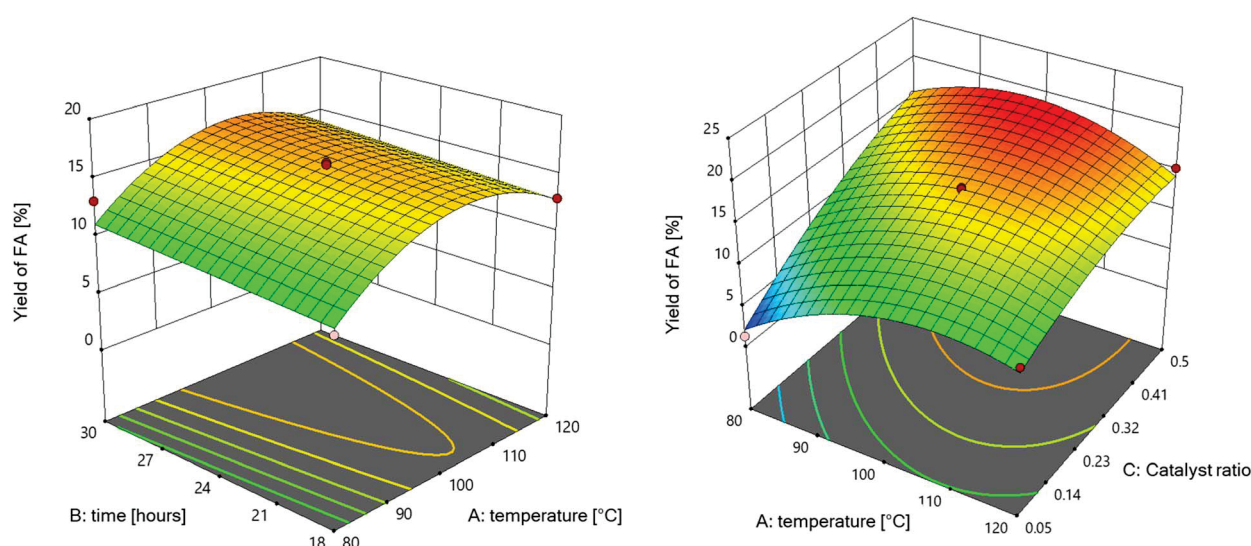


Fig. 11 The 3D response surfaces for FA-yield as result of the Box-Behnken DoE. The red dots depict the run points above the surface and the pink dots depict the run points below the surface.

be seen by the red colored region in the FA-yield (FA) – temperature-catalyst ratio plot in Fig. 11 on the right side. The model indicates that a temperature value between 90 °C and 110 °C produces the highest FA-yield. Interestingly, time is found to be an insignificant factor. Reichert *et al.*⁵⁸ reported that in the OxFa process, the formation of formic acid is much higher within the first 14 h of the reaction. After that the carbon dioxide formation reduces the formic acid selectivity.⁵⁹ This effect is probably the increasing amount of formic acid coupled with the decreasing pH that reduces the catalyst activity and thereby the FA production.^{60,61} To obtain a maximum conversion a reaction time of more than 18 h was initially chosen.

Therefore, the influence of time is found to be an insignificant factor. The diagram on the left side of Fig. 11 illustrates this very well.

The analysis of the influence parameters on the FA-yield shows a big contrast to the best parameters for protein recovery. As the latter favors a low temperature of 80 °C, high temperatures are more favorable for a high FA-yield. On the other hand, the second significant parameter for FA production, the catalyst/substrate ratio has no significant influence on protein recovery. Nevertheless, optimizing this process is a challenge due to the contrary influences of the temperature. It requires a compromise between FA-yield and protein recovery. The



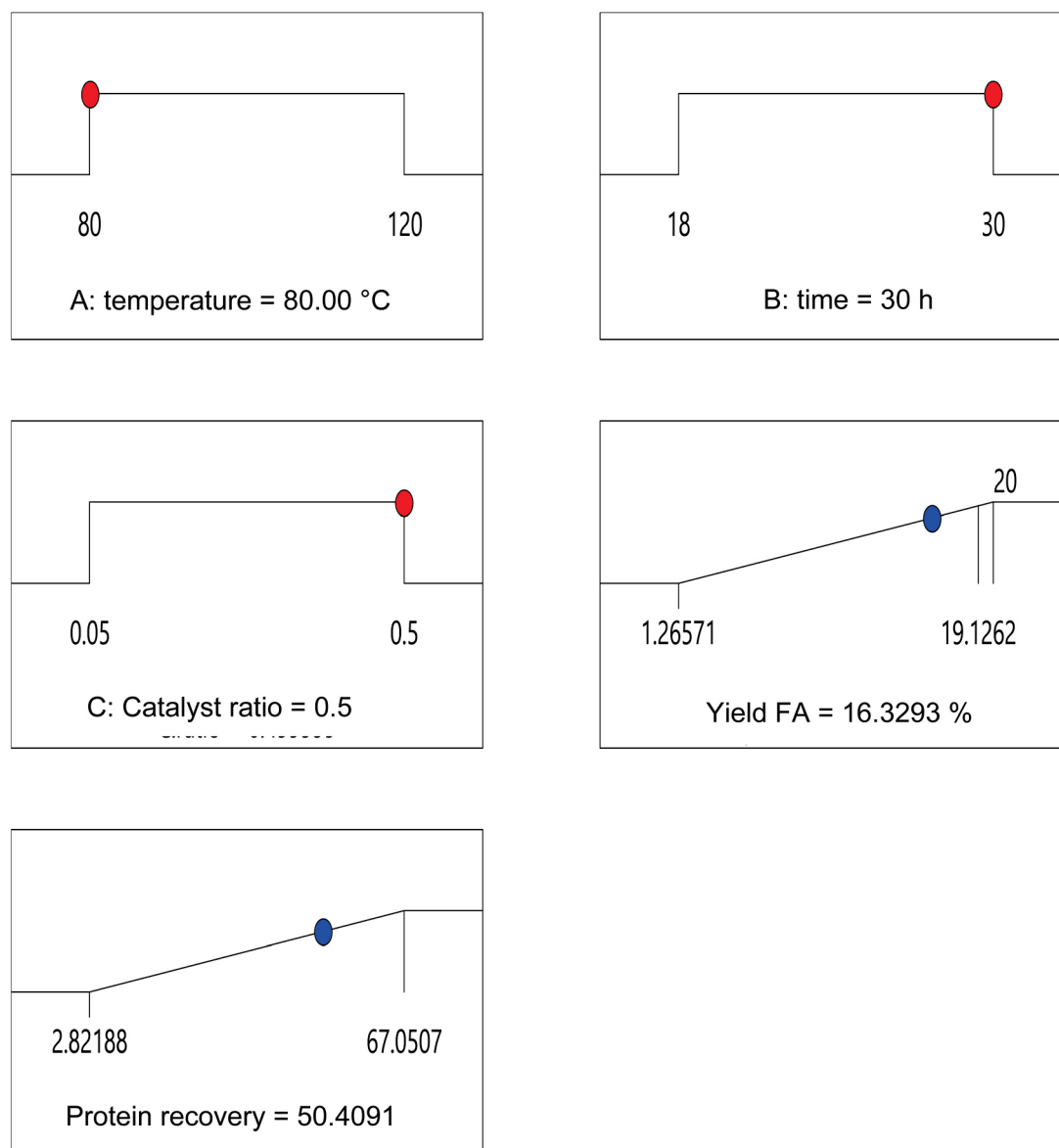


Fig. 12 The optimized conditions resulting from the Box–Behnken DoE maximizing FA-yield as well as protein recovery.

Design Expert software³⁶ can provide optimized conditions finding a compromise between FA-yield and protein recovery, these are shown in Fig. 12. The red dots show the suggested reaction conditions which are the following: 80 °C, 30 h, 0.5 $g_{catalyst} g_{substrate}^{-1}$ with the fixed reaction parameters of 1000 rpm stirrer speed, 30 bar oxygen pressure, 10 g of *Porphyra dioica*, and 200 g of water as solvent. The blue dots visualize the actual values and these are also shown in Table 2.

The experiment resulted in 59.55% protein recovery and 16.41% FA-yield which is a 7.8% higher FA-yield as that after the screening of different macroalgae. A comparison of the optimized FA-yield with *Porphyra dioica* as the substrate is shown in Fig. 13. In general, algal biomass results in FA-yields less than 30%.²⁷ The FA-yield depends on the number of oxygen functionalities in the substrate which is higher for lignocellulosic biomass like pomace.²³ Lignocellulosic biomass consists mainly

Table 2 The comparison between the predicted optimized values by the software design expert on the basis of a Box–Behnken model and the actual values obtained in the experiment^a

	Predicted	Actual
FA-yield (%)	19.13	16.41
Protein recovery (% dw)	50.40	59.55

^a Reaction conditions: 30 bar oxygen pressure, 1000 rpm stirrer speed, 30 h reaction time, 80 °C, 200 g of water with 10 g suspended macroalgae and a catalyst/substrate ratio of 0.5, catalyst: HPA-2.

of cellulose, hemicellulose, and lignin which also affect the FA-yield. Algae reportedly have negligible amounts of lignin and low carbohydrate (48%) compared to these biomasses (over 60%). The data shown in Fig. 13 indicate that only algae *Chlorella* sp. results in a higher FA-yield of 21.6% compared to



the *Porphyra dioica* in this study which resulted in a yield of 16.41%. Based on the biomass characterization, *Porphyra dioica* has a higher protein content of 33% dw compared to *Chlorella* sp. with a protein content of 19.3%dw.⁶² Subsequently, the overall carbohydrate content of *Chlorella* sp. might be higher than that of *Porphyra dioica* (49.9% dw). Since the aim of the study was to obtain a maximum FA-yield along with maximum recovery of proteins, *Porphyra dioica* with its high protein content is a better substrate for the aim of this study. Furthermore, since proteins can also be extracted from the remaining solid residue the usage of macroalgae as biomass is advantageous even with low FA-yields.

Protein extraction from the solid residue of the OxFA process

The solid residue after the reaction is most likely unreacted algae and potentially lipids, and not utilized carbohydrates and therefore further extraction is necessary. To investigate the best extraction approach for the solid residue three extraction methods were tested: alkaline hydrolysis as a state-of-the-art method,⁵⁵ and ultrasound-assisted extraction and ionic liquid extraction as novel approaches. Since the solid residue was already exposed to acidic conditions during the OxFA process, the choice of the tested extraction method excludes an acid extraction. It is important to keep in mind that every extraction method is the second part of a two-step extraction of proteins from a macroalga with the first step of an acid treatment with the valuable by-product FA.

For the first approach, the alkaline hydrolysis, the solid samples were extracted using NaOH based on the study by Harnedy *et al.*³¹ which reported that alkaline solutions can help in the extraction of non-water-soluble proteins of macroalgae. According to Kadam *et al.*,⁶³ the percentage of the extracted protein could be enhanced from 16.90% to 56.35% using 0.4 M NaOH instead of 0.4 M HCl as the extraction medium. According to a study by Harnedy *et al.*,³¹ the concentration of NaOH is a critical parameter in alkali-based extraction. Therefore, the concentration of NaOH in this study varied between 0.08 M and 5 M NaOH. The yield of alkali-soluble nitrogen can be increased by adding a reducing agent like NAC (*N*-acetyl-L-cysteine). Hence, 2 g per L NAC was used for the extraction as well. The extracts were analyzed for protein content and the protein recovery was calculated (equation 10) with respect to the protein content in the solid residue. To confirm the presence of proteins in the extract an initial SDS PAGE gel electrophoresis was conducted (Fig. S19†). A qualitative assessment of the protein profile shows the presence of protein with the range of 11 to 180 kDa with a regular intensity throughout the range. The intensity of the bands increases in the low molecular weight regions, and this indicates large amounts of smaller-sized proteins possibly in the form of peptides. The profile indicates higher amounts of protein content for 0.14 M NaOH concentration visible as a darker band. In contrast to the study of Harnedy *et al.*³¹ where the protein recovery remained constant when the NaOH concentration was increased from 0.12 M to 0.14 M, this study observed an increasing trend in the protein recovery. This trend

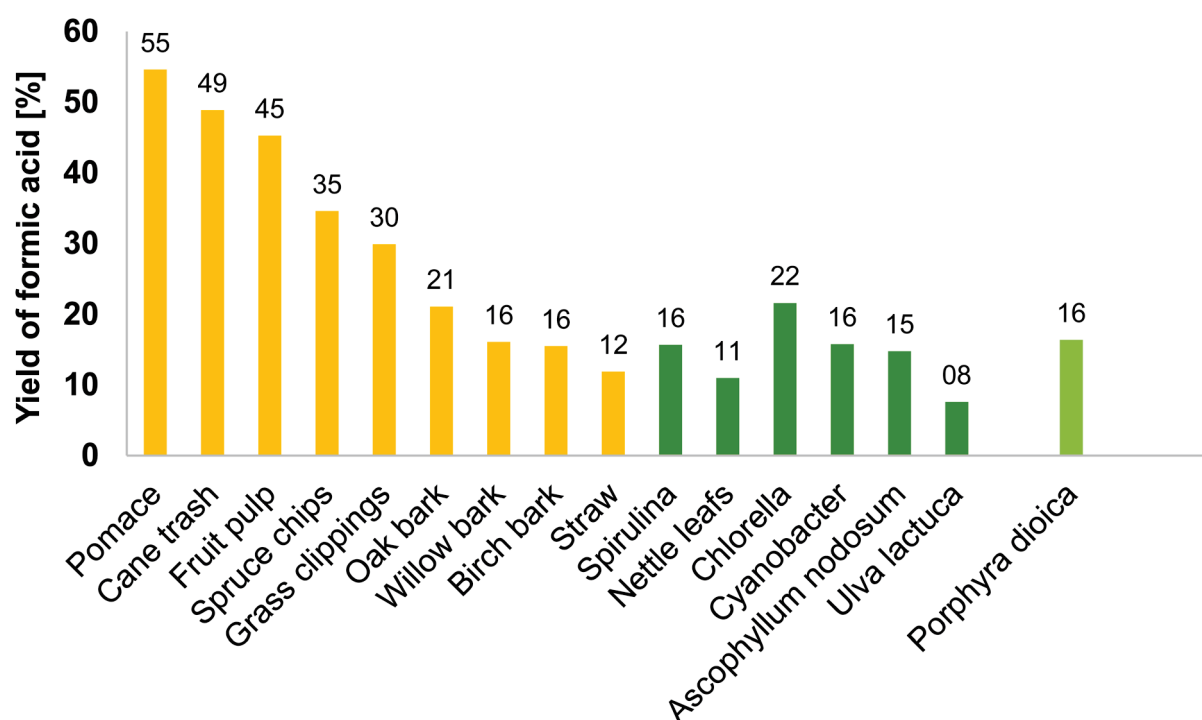


Fig. 13 Graph for comparing the FA-yield for different biomass substrates for the OxFA process (yellow and dark green): reaction conditions: 3.3 g substrate, 1.9 g (11 mmol) additive *para*-toluene sulfonic acid and 1.74 g (0.9 mmol) HPA-2 catalyst dissolved in 100.0 mL H₂O, 30 bar O₂, 90 °C, 24 h, 1000 rpm;²⁷ light green: optimized reaction conditions for protein and FA production from *Porphyra dioica*: 80 °C, 30 h, 0.5 g_{catalyst} g_{substrate}⁻¹, 30 bar oxygen pressure, 1000 rpm stirrer speed, 10 g of *Porphyra dioica* as the substrate, 200 mL of water as the solvent.



was also confirmed in our experiments (Fig. 13). The protein recovery is increasing with an increase in the NaOH concentration up to 1 M NaOH (57.8%) with a slight stagnation between 0.4 M and 0.8 M NaOH (47%). The maximum value of 57.8% protein recovery is similar to the value of 59.75% for *Ascophyllum nodosum* obtained by Kadam *et al.*⁶³ A further increase in the NaOH concentration to 5 M decreases the protein recovery to 35.8%. Lu *et al.*⁶⁴ reported a similar trend for protein recovery with NaOH concentration, wherein the protein recovery initially increases with increasing concentration, reaching a peak, and subsequently decreases. The reason for the increasing effect is that high alkali concentrations can promote a breakdown of hydrogen bonds and dissociation of hydrogen from proteins, enhancing protein solubility. The decreasing effect after a concentration of 1 M NaOH could be explained by the fact that strong alkali solutions and therefore elevated pH-values can disrupt the protein structure and cause denaturation leading to a loss of solubility of proteins.⁶⁴

The second approach tested was the Ultrasound-Assisted Extraction (UAE). This UAE was applied to the solid residue in an alkali medium of 0.4 M NaOH. Hildebrand *et al.*⁶⁵ reported this method to be an efficient approach for protein extraction if it is combined with an alkali medium. A study by Garcia-Vaquero *et al.*⁶⁶ analyzed the influential parameters for UAE and

reported that the sonication amplitude impacts the amount of protein extracted into the medium. Therefore, this study analyses the influence of this parameter as well which is shown in Fig. 14. In line with the study by Garcia-Vaquero *et al.*⁶⁶ the sonication amplitude in our study was varied between 20% and 100% with a fixed extraction time of 10 minutes in an alkali medium with a concentration of 0.4 M NaOH. The time was fixed to 10 min as a study from Hildebrand *et al.*⁶⁵ showed that a longer duration of ultrasonic treatment has a negative effect on the protein recovery (73.6% after 18 h compared to 76.6% after 10 min). The extracted samples were analyzed *via* SDS-PAGE gel electrophoresis to confirm the presence of proteins, and these results are shown in Fig. S20.† The SDS-PAGE results show increasing protein content with an increase in the sonication amplitude. Like the alkali extraction approach the bands in the SDS-PAGE gel electrophoresis are spread throughout a region between 11 to 180 kDa. The regions with lower molecular weights show a stronger intensity and therefore indicate a high number of smaller-sized proteins. Fig. 14 shows the comparison between the sonication amplitudes of 20%, 50% and 100%, respectively. The sonication amplitude of 100% clearly shows the best protein recovery of 87.2%. Ultrasound treatment induces cavitation within the liquid medium which leads to cell wall damage within the structure and an increased surface area enhancing the protein recovery. Hence, alkali

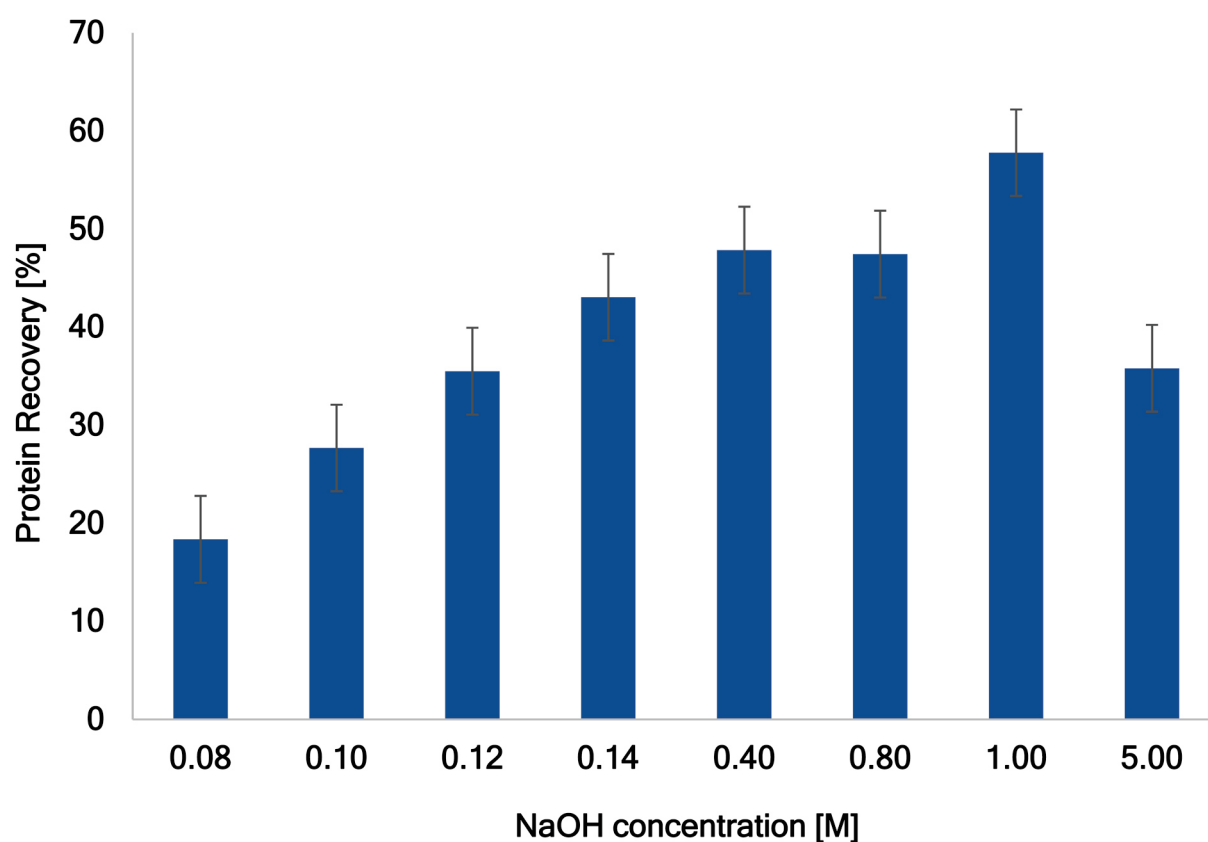


Fig. 14 The mean protein recovery of alkaline hydrolysis from the solid residue after the OxFA process for the concentration of NaOH from 0.08 M to 5 M. Reaction conditions of the OxFA process: 30 bar oxygen pressure, 1000 rpm stirrer speed, 30 h reaction time, 80 °C, 200 g of water with 10 g suspended macroalgae and a catalyst/substrate ratio of 0.5, catalyst: HPA-2.

extraction could be boosted with ultrasound. For comparison, non-ultrasound assisted alkali extraction with 0.4 M NaOH leads to a protein recovery of 47.8% and ultrasound assisted alkali extraction has a protein recovery of 68.2% with the lowest tested sonication amplitude of 20%. The maximum protein recovery in this study (87.2%) was even higher than previous reported values by Hildebrand *et al.* (76.6%)⁶² and Kadam *et al.* (57.2%)⁶³ with ultrasonic assisted extraction in an alkali medium. This can be attributed to the OxFA process, which works as an acidic pre-treatment of the macroalgae sample, resulting in an overall higher value for the protein extraction in this study.

The third extraction approach is ionic liquid extraction. The IL used in this study was choline chloride (ChCl), a green, cheap and environmentally friendly solvent.²¹ To test the influence of the concentration of ChCl in the extraction media, IL/water concentrations of 10–50 wt% of IL are tested. Higher concentrations of IL pose challenges in handling and processability as well as cause interference in analytical measurements.²¹ The nitrogen atoms in the ChCl solution affected the protein content estimation by the nitrogen content method, which was applied in the other tested extraction methods, and therefore a Bradford assay was used to quantify the proteins extracted by the IL. It is reported in a study by Suarez Garcia *et al.* that the Bradford assay method for quantification of the proteins is not impacted by ChCl.²¹ The resulting protein recovery with this approach is shown in Fig. 15. A clear trend is visible throughout

an increase in the ChCl content. The higher the IL content in the extraction media, the higher the protein recovery. This could achieve an increase in the recovery from 5.0% at 10 wt% of ChCl to 47.6% at 50 wt% of ChCl, which is a 9.5-fold increase. Similar trends were observed by Suarez Garcia *et al.*²¹ and Martins *et al.*⁶⁷ ChCl acts as a solubilizing agent helping the protein release from the macroalgae matrix and stabilizes the protein during extraction preventing denaturation. According to studies by Martins *et al.*,⁶⁷ the extraction of protein from macroalgae is also due to the salting-in effect caused by the addition of ChCl, especially in the case of proteins such as phycobili-proteins whose solubility increases with even a slight addition of CCl. However, the protein recovery obtained in this study was higher than the values reported in these previous studies. This is likely due to the OxFA process causing a pretreatment effect as mentioned above.

After investigating three different extraction techniques for the solid residue of the red macroalga *Porphyra dioica* after the OxFA process, Fig. 16 shows the comparison of these extraction techniques. It can be clearly seen that the best protein recovery approach with the highest value for protein recovery of 87.2% is UAE. Since the proteins are extracted into an alkali medium further purification is needed to utilize these proteins for applications like biofilm production. Ultrafiltration might be the best possible purification technique as other techniques like chromatography would need further treatment of the extraction broth (Fig. 17).

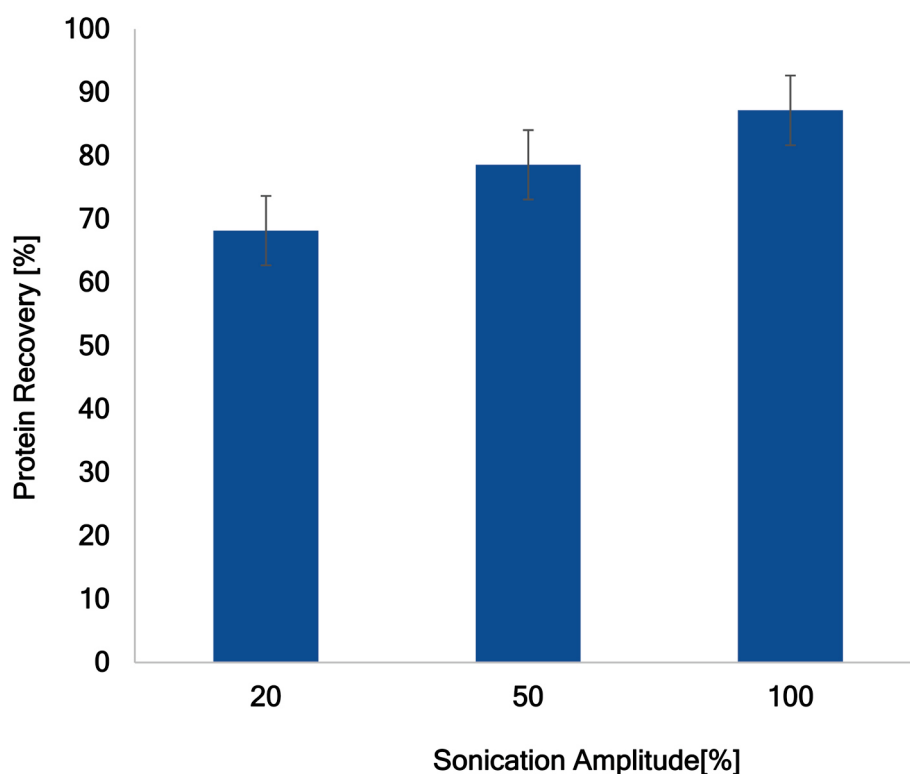


Fig. 15 The mean protein recovery of UAE from the solid residue after the OxFA process for varying sonication amplitudes of 20%, 50% and 100%. Reaction conditions of the OxFA process: 30 bar oxygen pressure, 1000 rpm stirrer speed, 30 h reaction time, 80 °C, 200 g of water with 10 g suspended macroalgae and a catalyst/substrate ratio of 0.5, catalyst: HPA-2.



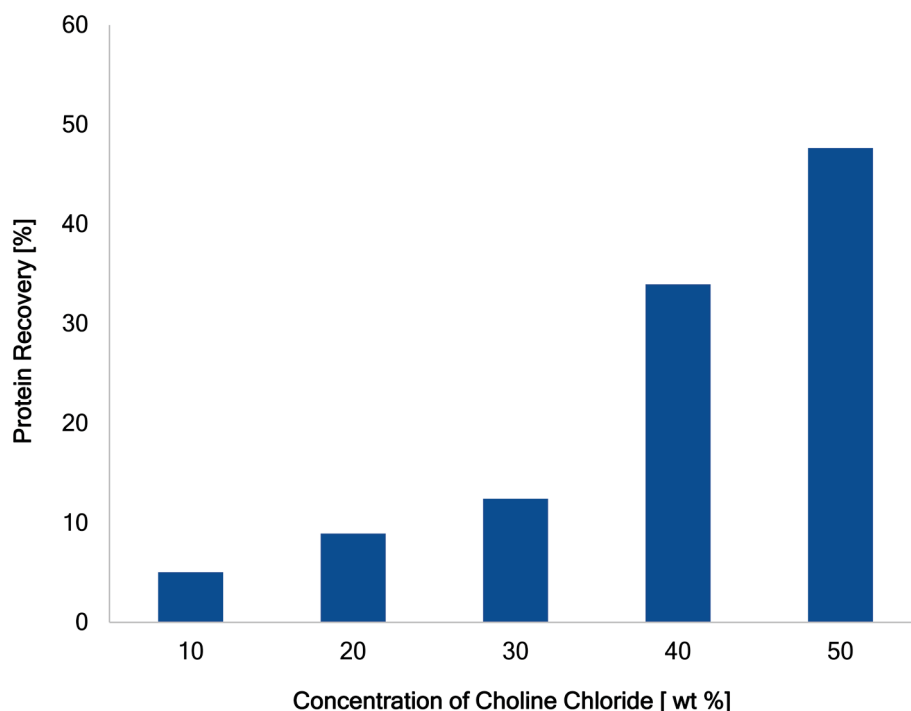


Fig. 16 The mean protein recovery for ILE from the solid residue after the OxFA process for different concentrations of choline chloride between 10–50 wt% (in water). Reaction conditions of the OxFA process: 30 bar oxygen pressure, 1000 rpm stirrer speed, 30 h reaction time, 80 °C, 200 g of water with 10 g suspended macroalgae and a catalyst/substrate ratio of 0.5, catalyst: HPA-2.

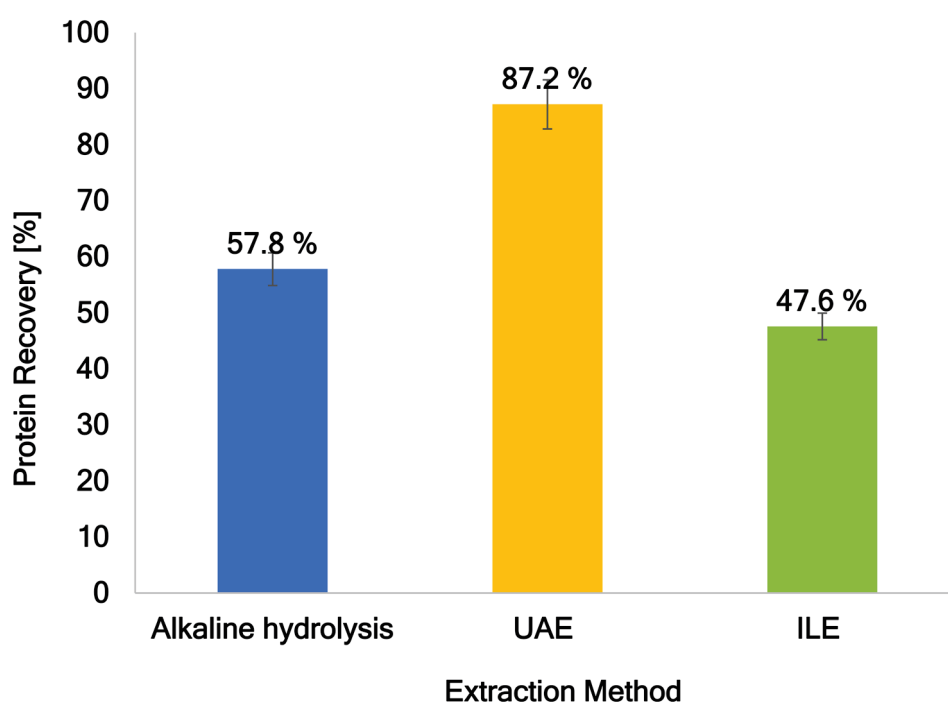


Fig. 17 The mean protein recovery from the solid residue after the OxFA process is presented. Comparison of the three methods alkaline hydrolysis (at 0.1 M NaOH), UAE (at 100% power input), and ILE (at 50 wt% CCl) is shown. The highest protein recovery was compared in each case.

The presented results clearly show that the combination of the OxFA process and UAE is a promising approach for the valorisation of macroalgae, specifically *Porphyra dioica*,

producing proteins and FA as valuable products. While the OxFA process is obviously an efficient pre-treatment method for protein extraction, some protein is lost during this treatment.



Conclusions

This study investigated the combined use of the OxFA process and different protein extraction methods for the production of proteins and formic acid from macroalgae (ProFA process). Three different subtypes of algae, specifically a red alga (*Porphyra dioica*), a brown alga (*Fucus vesiculosus*), and a green alga (*Ulva fenestrata*) have been tested. Out of these screened algae the red alga *Porphyra dioica* provided the best results. By tuning the redox-activity of the OxFA catalyst, the amount of protein-rich solid residue could be increased to 13.7% of the total algae with a protein content of 36.4% dw of protein, while achieving a FA yield of 8.6%.

After the identification of the most promising substrate, the OxFA process (in combination with an alkali extraction method) was further enhanced to optimize protein recovery next to FA yield. For this purpose, a Box–Behnken–DoE was applied and identified the temperature to be a significant parameter for protein recovery, while both the temperature and catalyst/substrate ratio had a significant influence on the FA yield. Using the optimized reaction parameters (80 °C, 30 h, 0.5 g_{catalyst} g_{substrate}^{−1}) *Porphyra dioica* as the substrate, and 200 mL of water as the solvent a yield of 16.4% with a protein recovery of 59.6% was achieved.

Following optimization of the OxFA process, the next step was the identification of the best protein extraction technique. Since the OxFA process already serves as acidic pre-treatment, three non-acidic methods were examined: alkali extraction (AE), ultrasound-assisted alkali-based extraction (UAE), and a method using choline chloride as ionic liquid (ILE). UAE was found to be the most effective protein extraction technique with a protein recovery of 87.2%. Therefore, the combination of the OxFA process with UAE was found to be the most efficient way to obtain protein and formic acid from macroalgae. Analysis of the extracted proteins showed that their composition is suitable for applications such as the production of biopolymer films.

In summary, this process provides a possibility for the sustainable and holistic utilization of algal biomass producing valuable proteins and formic acid.

Data availability

Various additional results and analytical data of catalysts and reaction solutions are included in the ESI.† Further raw measurement data and calculation files are available at Zenodo at <https://doi.org/10.5281/zenodo.15605969>.

Conflicts of interest

The authors declare no conflicts of interest.

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Chapter 6

Discussion of Research Questions

In the following, the results obtained in the course of this work are discussed with regard to the research questions raised in the motivation (vide intra Chapter 3). To end, the individual studies are placed in relation to each other and compared where possible.

As previously outlined, the fractionation of biomass is imperative for the effective utilization of its diverse components, irrespective of the specific category of biomass in investigation. Within the scope of this dissertation, two distinct biomass categories were employed for the experimental studies. However, the initial research question focuses exclusively on lignocellulosic biomass, given the comprehensive in-depth studies conducted on this subject within the thesis. The third research question addresses the fractionation of algae-based biomass. The first research question is:

Which fractionation method for lignocellulose in combination with oxidation using POM catalysts is the most promising regarding to the quality of the cellulose-rich solid as a valuable product?

In the study “Sequential fractionation and polyoxometalate-catalyzed oxidation of lignocellulosic biomass improves cellulose purity and formic acid yield”^[285] (vide intra Section 5.1), two fractionation methods promising for biorefinery were used to fractionate beech wood as a representative of lignocellulosic biomass. These two fractionation methods were the organosolv method with aqueous MeOH or EtOH as solvent and the ionosolv method with aqueous triethylammonium hydrogen sulfate ([TEA][HSO₄]) as solvent. The POM-ionosolv concept developed by Bukowski^[121] was the basis for these studies. The novelty was the use of a two-stage approach to minimize the influence of the POM catalyst on the cellulose-enriched solid. The organosolv approach was tested under the same conditions as the ionosolv approach (6 h reaction time, 18 g beech wood chips, 200 g liquid phase, 1200 rpm, 30 bar nitrogen).

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Except for the temperature where the organosolv approach needed a higher temperature of 170 °C instead of the 125 °C for the ionosolv approach to have a significant high dissolution. The organosolv approach was also tested at the temperature level of 125 °C (low-temperature organosolv) but the dissolution of the hemicellulose and lignin represented as dissolution of carbon fraction of the biomass was below 10 %. A hint for the optimum result for the dissolution gave the compositional analysis of the beech wood. This revealed 42 % of glucan content which was referred to cellulose. Therefore about 60 % of the biomass should be ideally dissolved into the liquid. The shown dissolution of the whole biomass illustrates this. Therefore, in this discussion the low-temperature organosolv approach was not be taken into consideration. The highest dissolution of carbon fraction for the tested approaches in the first study ranged between 32 % (methanolic high-temperature organosolv approach using 60 wt.% of methanol) and 45 % (ionosolv approach using 30 wt.% of [TEA][HSO₄]). In general the high-temperature organosolv approaches using MeOH and EtOH and the ionosolv fractionation methods came close to the desired maximum dissolution. To evaluate the quality of the cellulose-enriched solid after the fractionation, a compositional analysis was conducted. The results can be seen in Figure 6.2. In comparison Figure 6.1 shows the compositional analysis of the untreated beech wood.

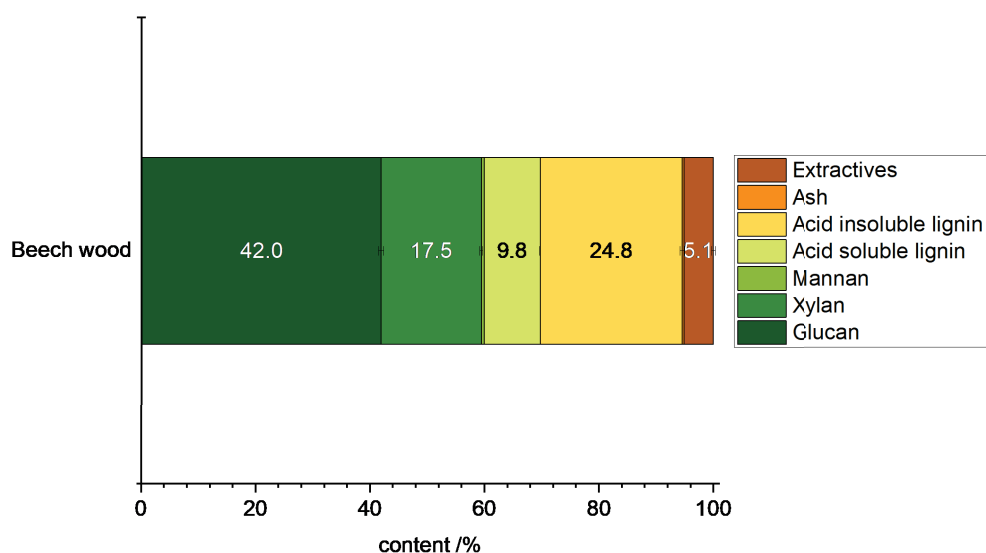


Fig. 6.1 Compositional analysis of the raw material beech wood according to LAP NREL/TP-510-42618.^[286]

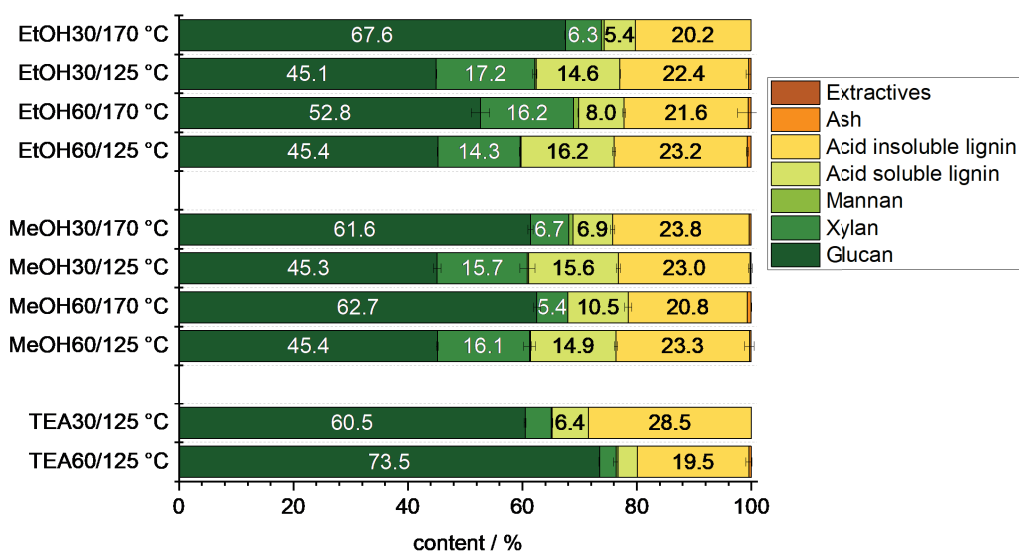


Fig. 6.2 Compositional analysis of cellulose-rich solids in comparison. Glucan content refers to cellulose content, xylan content to hemicellulose content. Acid soluble lignin (ASL), acid insoluble lignin (AIL), ash, and mannose are shown. The procedure was done after LAP NREL/TP-510-4261836.^[286] *Reaction conditions: 200 g liquid phase (TEA60 - 60 wt.% of [TEA][HSO₄], TEA30 - 30 wt.% of [TEA][HSO₄], MeOH60 - 60 wt.% of methanol, MeOH30 - 30 wt.% methanol, EtOH60 - 60 wt.% of ethanol, EtOH30 - 30 wt.% of ethanol, rest water), 18 g beech wood chips (630 μ m, dw), 30 bar nitrogen pressure at 125 °C/170 °C, 1200 rpm stirrer speed, 6 h reaction time.*

The trend of the dissolution is clearly visible in the composition of the different cellulose-enriched solids. The solvents with the highest dissolution (namely TEA30/125°C (45 %), EtOH30/170°C (44.9 %) MeOH30/170°C (43.0 %), TEA60/125°C (42.1 %), EtOH60/170°C (33.1 %) and MeOH60/170°C (31.6 %)) showed the highest glucan content. The other dissolution results are shown in Section 5.1. Interestingly, the ionosolv fractionation with more IL (TEA60/125°C) revealed the highest glucan content and therefore the highest amount of cellulose in the cellulose-enriched solid. For some applications like the use of cellulose as platform chemical the ability of enzymes to break down the cellulose structure is a key factor. Hence, the enzymatic saccharification according to the NREL procedure LAP NREL/TP51000-63351^[282] was investigated. The results are shown in Figure 6.3.

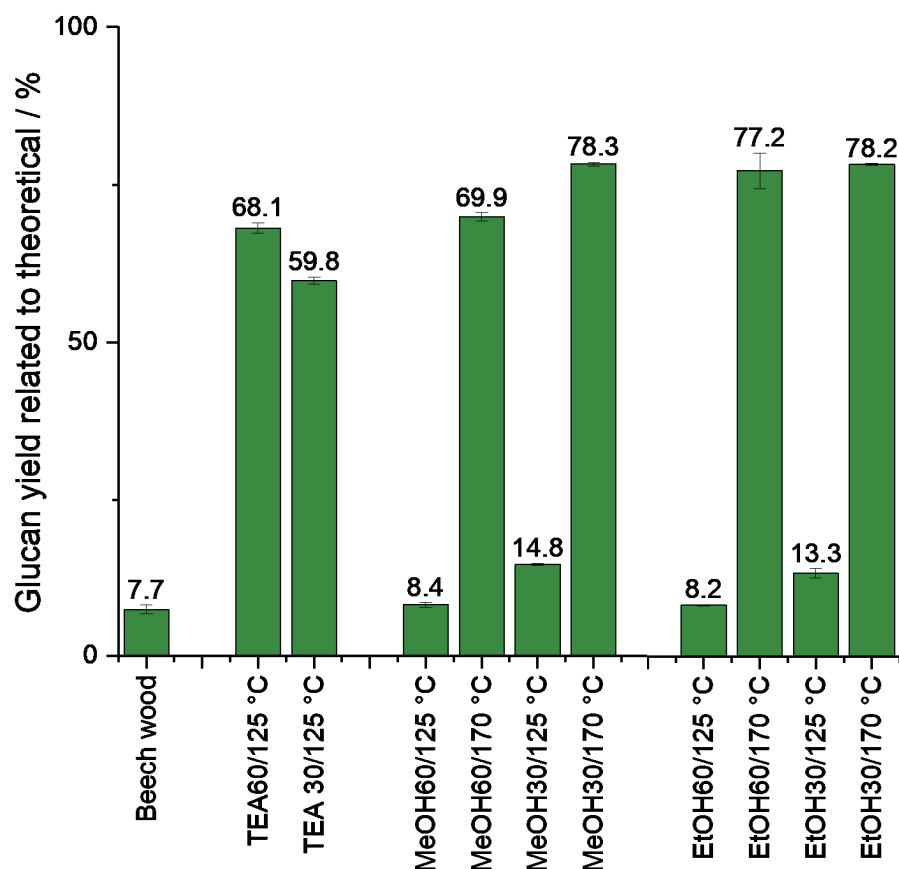


Fig. 6.3 Enzymatic saccharification of the different treated cellulose-rich solid residues after LAP NREL/TP-5100-6335149.^[282] Reaction conditions: 200 g liquid phase (TEA60 – 60 wt.% of [TEA][HSO₄], TEA30 – 30 wt.% of [TEA][HSO₄], MeOH60 – 60 wt.% of methanol, MeOH30 – 30 wt.% methanol, EtOH60 – 60 wt.% of ethanol, EtOH30 – 30 wt.% of ethanol, rest water), 18 g beech wood chips (630 μ m, dw), 30 bar nitrogen pressure at 125 °C / 170 °C, 1200 rpm stirrer speed, 6 h reaction time.

The trend is visible that a higher glucan content leads to higher ability of enzymes for the saccharification. Interestingly, the organosolv-treated solids as well as the ethanol-treated solids showed the highest glucan yield of 77.2 % (EtOH60/170°C) and 78.2 % (EtOH30/170°C). Also, the methanolic organosolv method MeOH30/170°C resulted in a high glucan yield of 78.3 %. The ionosolv treated solids led to about 10 to 20 % lower yields compared to the ethanolic organosolv approach. The difference between the yield of the organosolv-treated solids and the ionosolv-treated solids could be explained by the structure of the cellulose. The different solvents underwent a different lignin solvation mechanism. The ionosolv concept dissolves the lignin by disrupting the hydrogen bonds among the lignocellulosic components.^[113,288] The organosolv concept broke the alpha-aryl-ether-bonds in the biomass due to a slightly acidic environment generated by acetic acid released from the

biomass through an auto-catalytic process. Additionally, the organic solvent solubilized the aromatic macromolecules of lignin based on the polymer solubility theory.^[90,289–291] The understanding of dissolution of lignin is important as a positive linear correlation between delignification and saccharification is reported.^[292] The crystallinity of the cellulose also had an impact on the binding affinity of the cellulase enzymes for the saccharification.^[293,294] This correlation is not yet fully understood, as cellulase is a mixture of enzymes resulting in a various arbitrarily complex mechanisms.^[295,296]

To combine the positive effects of both methods, the high dissolution of lignin and hemicellulose of the ionosolv method and the high glucan yield after the enzymatic saccharification, a combined organosolv and ionosolv fractionation method was tested in the second study "Boosting POM-Ionosolv biorefining of lignocellulosic biomass by using redox-balanced polyoxometalate catalysts in methanolic ionic liquid reaction media"^[284] (vide intra Section 5.2). Hereby, the choice of the organic solvent has been made a favor for methanol for catalytic reasons. The used POM catalyst is known to be boosted in selectivity of formic acid/methyl formate if methanol is used as a solvent.^[199,208] For the solvent system, different promising ILs were tested. The solvents were the well known [TEA][HSO₄] IL, [DMBA][HSO₄] as 2nd generation of ionosolv-ILs and [TBMP][MeSO₄] known for a high lignin solvation ability.^[128,297,298] Figure 6.4 shows the comparison of the compositional analysis of the processed solids using the methanolic ionosolv systems with the different ILs.

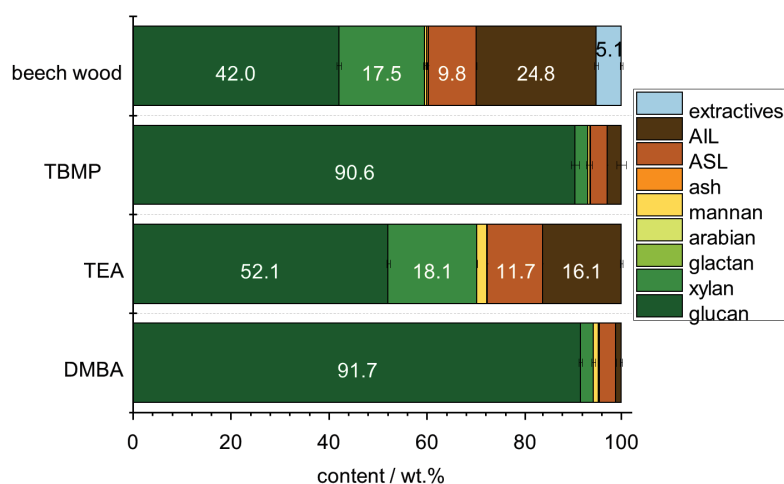


Fig. 6.4 Compositional analysis of pure beech wood and the processed solids using different IL/MeOH solvent systems via NREL/TP-510-4261832.^[286] *Reaction conditions: 120 °C, 24 h, 1000 rpm, 30 bar of oxygen at reaction temperature, 10 g solvent ([TEA][HSO₄]/MeOH 30 wt.%/70 wt.% (methanolic TEA system), [DMBA][HSO₄]/MeOH 30 wt.%/70 wt.% (methanolic DMBA system), [TBMP][MeSO₄]/MeOH 30 wt.%/70 wt.% (methanolic TBMP system)), 0.5 g beech wood (dw).*

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For simplification, the names are shortened: TBMP is the methanolic ionosolv system with 30 wt.% of [TBMP][MeSO₄] and 70 wt.% MeOH. DMBA is the short name for the methanolic system with the same ratio of IL/MeOH like the TBMP system but [DMBA][HSO₄] as IL. The same naming system was used for the methanolic ionosolv solvent with [TEA][HSO₄] which is consistently named TEA. The TEA system was not enhanced using methanol as co-solvent as a comparison between the methanolic TEA system and the aqueous TEA system (see Figure 6.2) shows. Hereby, a lower glucan content about 8 % was found for the methanolic TEA system. This is in clear contrast to the other two screened ILs which give a nearly pure (over 90 % of glucan) cellulose-enriched solid. The methanol in the system enhanced the lignin dissolution like other alcoholic co-solvents tested in the study of Chen et al.^[106] To evaluate the digestibility of the cellulose-enriched solid, enzymatic saccharification was conducted and the results are shown in Figure 6.5.

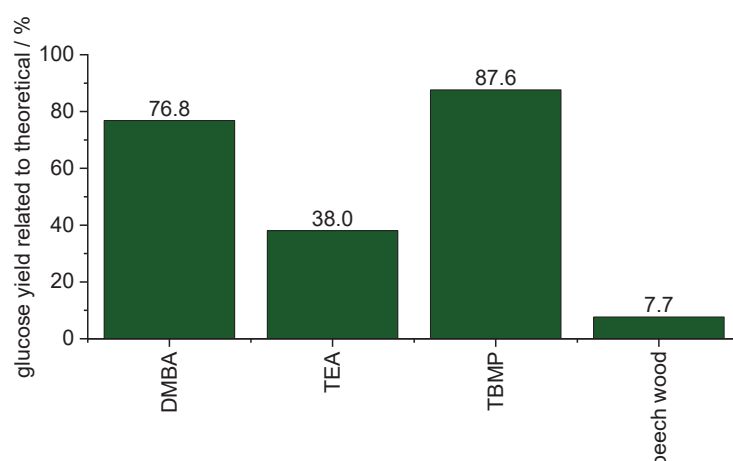


Fig. 6.5 Enzymatic saccharification yields of pure beech wood in comparison to cellulose-enriched solids processed using different IL/MeOH solvent systems. *Reaction conditions: 120 °C, 24 h, 1000 rpm, 30 bar of oxygen at reaction temperature, 10 g solvent ([TEA][HSO₄]/MeOH 30 wt.%/70 wt.% (methanolic TEA system), [DMBA][HSO₄]/MeOH 30 wt.%/70 wt.% (methanolic DMBA system), [TBMP][MeSO₄]/MeOH 30 wt.%/70 wt.% (methanolic TBMP system)), 0.5 g beech wood (dw).*

The highest yield of glucose/glucan related to theoretical accessible glucose was achieved using the TBMP system which was even about 10 % higher than the best organosolv system in the first study (vide intra Section 5.1). The second largest yield of 76.8 % (DMBA system) was about the same yield as the organosolv systems in the first study. Related to the low amount of dissolved hemicellulose and lignin and the accordingly large amount

in the cellulose-enriched solid residue of the methanolic TEA system, the yield was about 20 % lower than the aqueous TEA system of the first study. Based on these findings, the methanolic TBMP system as a combined organosolv-ionosolv fractionation method was the most promising tested method for the production of high-quality cellulose-enriched solid. Using oxygen as reaction gas, a bleaching effect was found within the organosolv-ionosolv systems generating a white, fluffy solid (vide intra Section 5.2). This solvent was also tested using different types of biomass like softwood (pine and spruce), hardwood (birch) and grass (miscanthus) revealing similar results (vide intra Section 5.2).

The second research question of this thesis deals with the oxidation of the dissolved components using POMs and hence the analysis of the corresponding liquid phase for the fractionation methods.

In which solvent can most of the dissolved components of the lignocellulosic biomass be converted into formic acid or its derivatives using a POM catalyst?

For answering the second research question, investigations on the dissolved fractions and the solvent system had to be conducted. For this purpose, the solvents containing the dissolved components were analyzed in the first study with GC, HPLC and GPC to find the dissolved monomers like xylose or furfural or organic acids like acetic acid and a glimpse of the dissolved oligomers. The CO₂ measured with GC is an indicator of how much dissolved carbon was over-oxidized during the oxidation step. Most important for the oxidation to formic acid using POMs are monosaccharides like xylose as these can be easily oxidized.^[120] The GPC analysis showed unspecified oligomers with molar masses between 100 g mol⁻¹ to 2000 g mol⁻¹ for all promising used solvent systems (ionosolv and high-temperature organosolv). Figure 6.6 shows the composition of the liquid phases. A clear trend is visible, that a high water content favored the dissolution of xylose. The ionosolv solvent and the methanol-organosolv solvent showed a higher xylose yield than the ethanol-organosolv solvent. In this study (vide intra Section 5.1) a two-step concept was used.

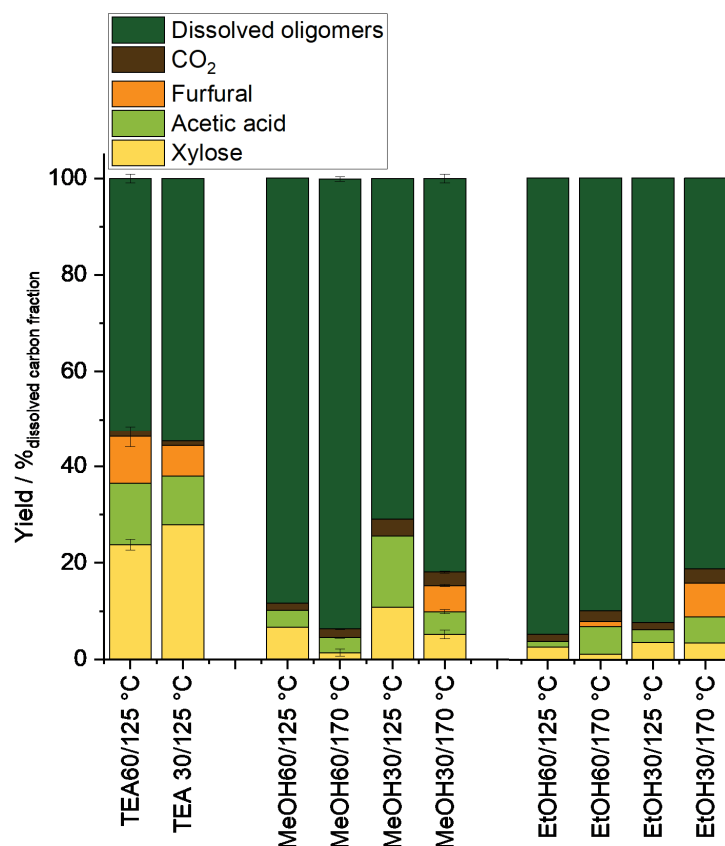


Fig. 6.6 Composition of liquid phase after fractionation, yield related to dissolved carbon fraction of detectable products – furfural, acetic acid, xylose, CO₂ and the not further specified dissolved oligomers in different solvents. *Reaction conditions: 200 g liquid phase (TEA60 – 60 wt.% of [TEA][HSO₄], TEA30 – 30 wt.% of [TEA][HSO₄], MeOH60 – 60 wt.% of methanol, MeOH30 – 30 wt.% methanol, EtOH60 – 60 wt.% of ethanol, EtOH30 – 30 wt.% of ethanol, rest water), 18 g beech wood chips (630 μm, dw), 30 bar nitrogen pressure at 125 °C / 170 °C, 1200 rpm stirrer speed, 6 h reaction time.*

After the first fractionation step, the cellulose-enriched solid was filtered and the liquid phase was analyzed. After that an oxidation of the dissolved components using the HPA-5 (H₈PV₅Mo₇O₄₀) POM was conducted. The HPA-5 Keggin POM was used because previous studies from Bukowski et al.^[120,121] had shown that this is the most suitable catalyst for the POM-ionosolv concept. The results of the oxidation reaction of the second step are shown in Figure 6.7.

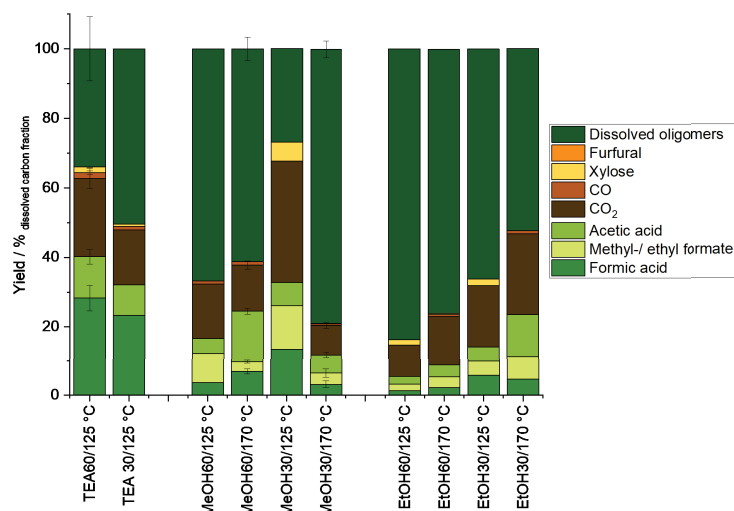


Fig. 6.7 Composition of liquid phase of the second step oxidation with a polyoxometalate catalyst. *Reaction conditions: liquid phase of first step (TEA60 – 60 wt.% of [TEA][HSO₄], TEA30 – 30 wt.% of [TEA][HSO₄], MeOH60 – 60 wt.% of methanol, MeOH30 – 30 wt.% methanol, EtOH60 – 60 wt.% of ethanol, EtOH30 – 30 wt.% of ethanol, rest water), 0.5 mmol/(100g(liquid phase)) of catalyst HPA-5, 30 bar oxygen pressure, 125 °C temperature, 1200 rpm stirrer speed, 6.5 h reaction time.*

The highest yield of formic acid of 26.6 % which could be achieved in the second step was within the ionosolv fractionation TEA60/125°C. As byproducts, CO₂ (22.5 %), acetic acid (11.9 %) and CO (1.7 %) were present. For the high-temperature organosolv concept with methanol (MeOH60/170°C) the highest accumulated yield of methyl formate and formic acid at 9.7 % was achieved. Which led to similar byproducts like the ionosolv concept with the following yields: acetic acid - 14.8 %, CO₂ - 13.3 %, and CO - 1 %. For the ethanolic organosolv (EtOH30/170°C) an accumulated yield of formic acid and ethyl formate of 11.4 %, a yield of CO₂ of 23.2 %, a yield of acetic acid of 12.2 % and no CO was found. The stability of all solvent-systems was tested with the HPA-5 catalyst, without any dissolved components, and found to be stable within the reaction conditions in terms of methyl formate. This means that no methyl formate as reaction product could be detected after the stability tests.

In the second study (vide intra Section 5.2), a one-pot system with three different ILs was tested. [TEA][HSO₄] was selected as benchmark from previous studies.^[120,121]

[DMBA][HSO₄] as second generation of low-cost ionosolv-IL^[116] and [TBMP][MeSO₄] for the good extraction of lignin^[298] were chosen. All ILs were mixed with methanol as co-solvent to further enhance the lignin extraction of the solvent mixture.^[299,300] The ILs were mixed with MeOH in a ratio of 70 wt.% MeOH to 30 wt.% IL according to the best

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IL water ratio determined by previous studies.^[120] In the following discussion the different solvent mixtures are abbreviated to TEA, DMBA and TBMP as explained previously. First, the stability of the solvent mixtures was also tested with the established HPA-5 catalyst. More details can be found in Section 5.2. The methanol within the system was found to be oxidized to methyl formate in the presence of the different acidic ILs and HPA-5. Hence, the solvent systems were not stable in terms of the reaction conditions according to methyl formate. As a consequence, an additional catalyst screening was done. The focus of the catalyst selection was to find a less redox-active catalyst so that the solvent system is stable in terms of methyl formate while the substrate is oxidized to methyl formate as desired product. The stability of the solvent system was a key parameter to ensure a possible recycling. The two main influences on the stability were the catalyst and the IL. The chosen ILs do have a different acidity, one influence on the production of dimethoxymethane or dimethyl ether or formaldehyde which serve as intermediates to methyl formate according to Tatibouët et al.^[301] The second influence on the stability of the solvent mixture was the redox activity of the POM catalyst. It was found that all solvent mixtures were not stable in terms of methyl formate with the established HPA-5 catalyst. Therefore, different POMs with a lower redox-activity were screened. The redox-activity can be tuned due to the capability of the POM catalyst to incorporate different metals. The chosen catalysts were:

- $\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$, a two-fold vanadium substituted Keggin-type POM (HPA-2)
- $\text{H}_8\text{PVMnMo}_{10}\text{O}_{40}$, a vanadium and manganese substituted Keggin-type POM (HPA-VMn)
- $\text{H}_{11}\text{PMn}_2\text{Mo}_{10}\text{O}_{40}$, a two-fold manganese substituted Keggin-type POM (HPA-Mn2)
- $\text{H}_7\text{PMnMo}_{11}\text{O}_{40}$, a one-fold manganese substituted Keggin-type POM (HPA-Mn)

The order of the catalysts in terms of redox activity is $\text{HPA-5} > \text{HPA-2} > \text{HPA-VMn} > \text{HPA-Mn2} > \text{HPA-Mn}$.^[277] TEA was not stable in terms of the formation of methyl formate with all the chosen catalysts and hence was not longer part of the investigation. DMBA and TBMP were found to be stable applying HPA-VMn and all the manganese incorporated POMs. For HPA-2 and HPA-5 the redox-activity was too high for a stable solvent mixture. Nevertheless, in the catalyst screening all tested catalysts are shown with a yield which is only based on the substrate carbon fraction and the methyl formate from the unstable solvent system was deducted (vide intra Section 5.2). The results of the catalyst screening for DMBA are shown in Figure 6.8 and for TBMP in Figure 6.9.

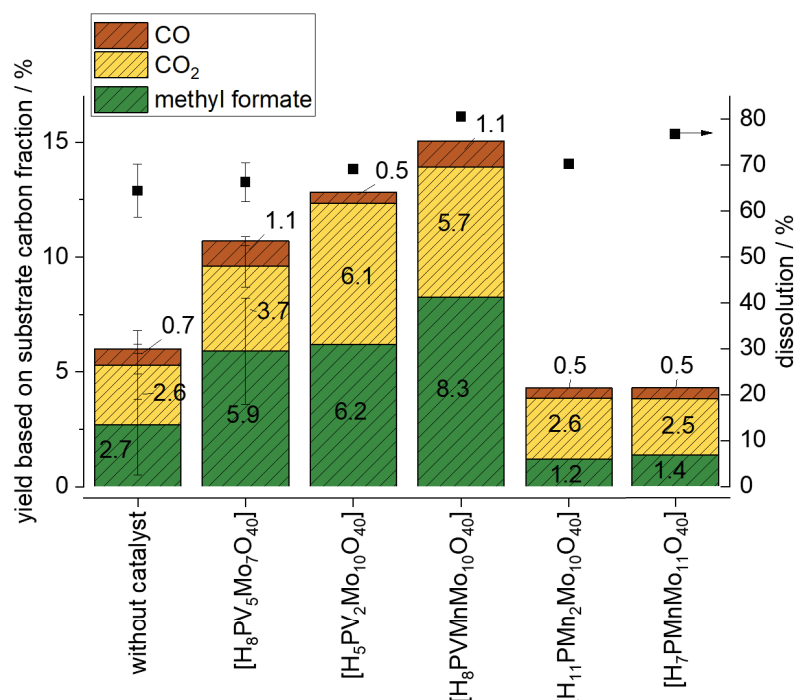


Fig. 6.8 Product yields for the oxidation of beech wood in the methanolic DMBA solvent mixture using HPA-5, HPA-2, HPA-VMn, HPA-Mn2, HPA-Mn as catalyst. *Reaction conditions: 120 °C, 24 h, 1000 rpm, 30 bar of oxygen at reaction temperature, 10 g solvent [DMBA]/[HSO₄]/MeOH 30 wt.%/70 wt.% (methanolic DMBA solvent), 0.5 g beech wood, 0.180 g catalyst (1 mmol g⁻¹ catalyst to solvent).*

For the dissolution no specific trend for a catalyst can be found in the screening. The dissolution of both solvents are ranged between 65 % to 80 % for DMBA and 60 % to 69 % for TBMP. In both catalyst screenings the HPA-VMn showed the highest yield of methyl formate. Within TBMP as solvent mixture the yield of methyl formate could be more than doubled from 8.3 % (DMBA) to 19.8 % (TBMP) and even the yield of CO₂ could be decreased to 4.2 % (TBMP) instead of 5.7 % (DMBA). Therefore, the selectivity to methyl formate using the HPA-VMn catalyst increased significantly (10 % for methyl formate with HPA-VMn in DMBA, 29 % for methyl formate with HPA-VMn in TBMP).

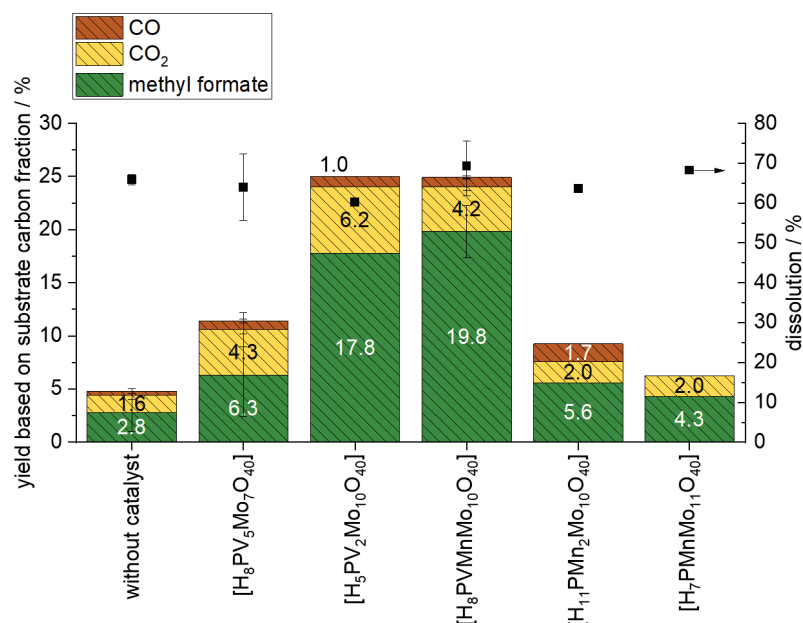


Fig. 6.9 Product yields for the oxidation of beech wood in the methanolic TBMP solvent mixture using HPA-5, HPA-2, HPA-VMn, HPA-Mn2, HPA-Mn as catalyst. *Reaction conditions:* 120 °C, 24 h, 1000 rpm, 30 bar of oxygen at reaction temperature, 10 g solvent [TBMP]/[MeSO₄]/MeOH 30 wt.%/70 wt.% (methanolic TBMP solvent), 0.5 g beech wood, 0.180 g catalyst (1 mmol g⁻¹ catalyst to solvent).

To investigate this selectivity boost, the catalyst-solvent interactions were in depth analyzed. It was found, that the POM and the IL built a solid precipitate which is a salt consisting of the cation of the TBMP-IL and the anion of the POM catalyst. This was investigated via ICP-OES, FT-IR, ⁵¹V-NMR and ³¹P-NMR, XPS and SCXRD (vide intra Section 5.2). The SCXRD (see Figure 6.10) revealed the structure of the TBMP-HPA-VMn salt. The Keggin-structure of the HPA-VMn was kept intact and the cations of the IL were surrounding the structure. By heating up the reaction solution with the solid *in-situ* forming POM-IL salt, it was found that the salt was dissolved at around 80 °C and therefore the reaction itself was homogeneous.

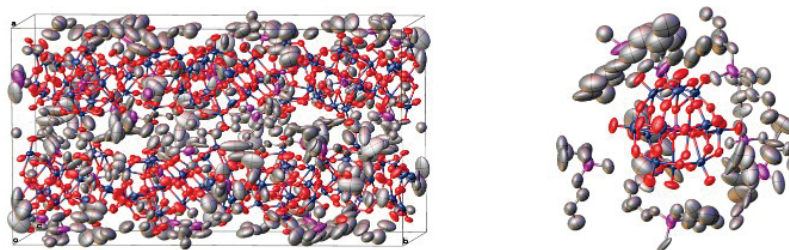


Fig. 6.10 Solid-state structure of the inorganic precipitate: unit cell (left) and single Keggin-type anion surrounded by phosphonium cations (right). Color assignment: Metals (Mo, V, Mn) dark blue, phosphorus purple, oxygen red, carbon gray. Thermal ellipsoids are drawn at a probability level of 50 %. Hydrogen atoms have not been modeled. The data for the structure is available through the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service (deposition number: 2422752).

Comparing the two studies of this thesis to find an answer for the second research question, the two-pot TEA/water system with HPA-5 as catalyst resulted in a higher yield of formic acid of 26.6 % compared to the best one-pot TBMP/MeOH system with HPA-VMn as catalyst (yield of methyl formate 19.8 %). As the latter solvent was the best for producing a cellulose-enriched solid residue, further development of the HPA-VMn TBMP/MeOH system is highly interesting for future studies.

The second big topic of this thesis is the valorization of algae biomass using POM catalysts. To investigate this potential the following research question is raised:

Is the oxidation of algae-based biomass using polyoxometalate catalysts suitable for the production of protein-rich solids and formic acid?

To answer this question, three different types of algae were characterized and oxidized using HPA-5 to analyze their potential as substrate for the production of formic acid and to analyze the protein content in the remaining solid (vide intra Section 5.3). The three types were a red algae (*Porphyra dioica*), a green algae (*Ulva fenestrata*) and a brown algae (*Fucus vesiculosus*). After an in depth characterization of the ash, lipid, protein, carbohydrate and moisture content, the macroalgae were screened with HPA-5 and a less redox active HPA-2 catalyst in water as solvent. Figure 6.11 and 6.12 show the results of these screenings. As a result *porphyra dioica* as substrate was identified for further investigation due to the high yield of formic acid (8.6 % with HPA-2) and high protein content (36.42 %, vide intra Section

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5.3) of the solid residue after processing. In all tested algae types an enhancing effect in the protein content of the applied OxFA process could be identified.

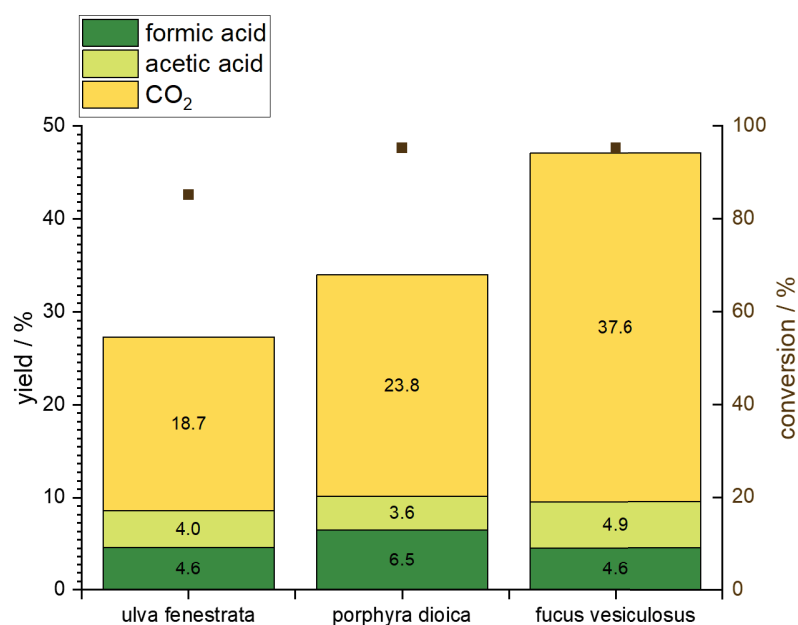


Fig. 6.11 Screening of different macroalgae (green (ulva fenestrata), red (porphyra dioica), brown (fucus vesiculosus)) with HPA-5. *Reaction conditions: $H_8PV_5Mo_7O_{40}$ at initial temperature 90 °C, non-isothermal reaction, 30 bar oxygen pressure, 1000 rpm stirrer speed, 24 h reaction time. The solvent was 200 g of water with 10 g suspended macroalgae and 1.886 g of dissolved catalyst (1 mmol, $H_8PV_5Mo_7O_{40}$).*

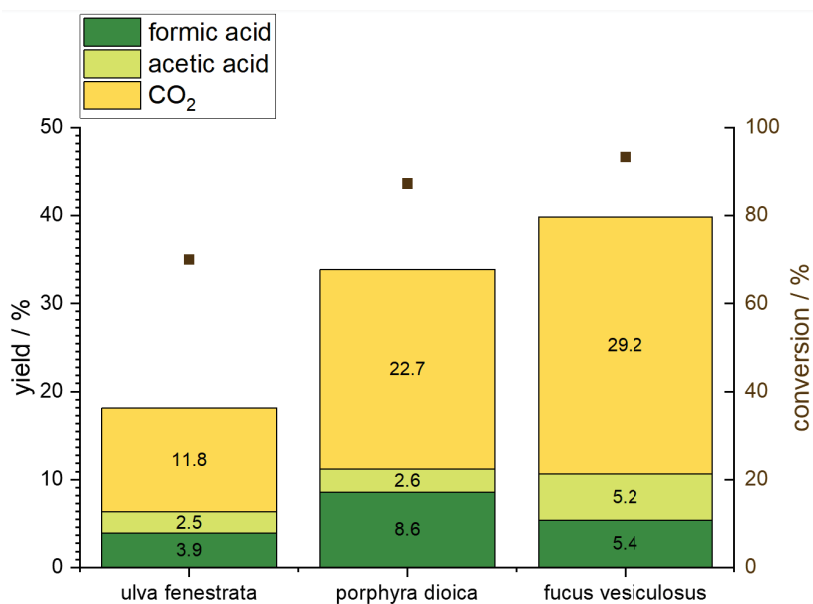


Fig. 6.12 Screening of different macroalgae (green (ulva fenestrata), red (porphyra dioica), brown (fucus vesiculosus)) with HPA-2. *Reaction conditions: $H_5PV_2Mo_{10}O_{40}$ at initial temperature 90 °C, non-isothermal reaction, 30 bar oxygen pressure, 1000 rpm stirrer speed, 24 h reaction time. The solvent was 200 g of water with 10 g suspended macroalgae and 1.952 g of dissolved catalyst (1 mmol, $H_5PV_2Mo_{10}O_{40}$).*

To further optimize this process, a Box-Behnken Design of Experiment was applied. The influence of three parameters, catalyst/substrate ratio, time and temperature, was investigated on three levels. Using the Box-Behnken Design of Experiments two-dimensional interactions between these factors could be determined as well. The temperature was varied between 80 °C to 120 °C, where 80 °C is the lowest temperature reported where the catalyst is activated.^[201] And the highest temperature was set to 120 °C as a higher temperature would denature the proteins. The time was varied between 18 h and 30 h because of the complex water-insoluble biomass used. In other reported issues an initial time of 18 h with complex water-insoluble biomass is necessary to get significant high yields of formic acid. The catalyst/substrate ratio was varied between $0.05 \text{ g}_{\text{catalyst}} \text{g}_{\text{substrate}}^{-1}$ and $0.5 \text{ g}_{\text{catalyst}} \text{g}_{\text{substrate}}^{-1}$. The optimization had two different targets – the yield of formic acid and the protein recovery. The analysis showed that temperature was a significant influence on both the yield of formic acid and the protein recovery. Besides temperature (T), the catalyst/substrate ratio (C/S) and the quadratic interactions of both (T^2 , C/S^2 , $C/S \cdot T$) were significant influences on the yield of formic acid. Unfortunately, the influence of the temperature was opposed for the yield of formic acid. The temperature should be high and for the protein recovery a low temperature was favorable. These correlations are shown in the Figures 6.13 and 6.14 which show the Pareto charts of the models for the protein recovery and for the yield of formic acid.

Discussion of Research Questions

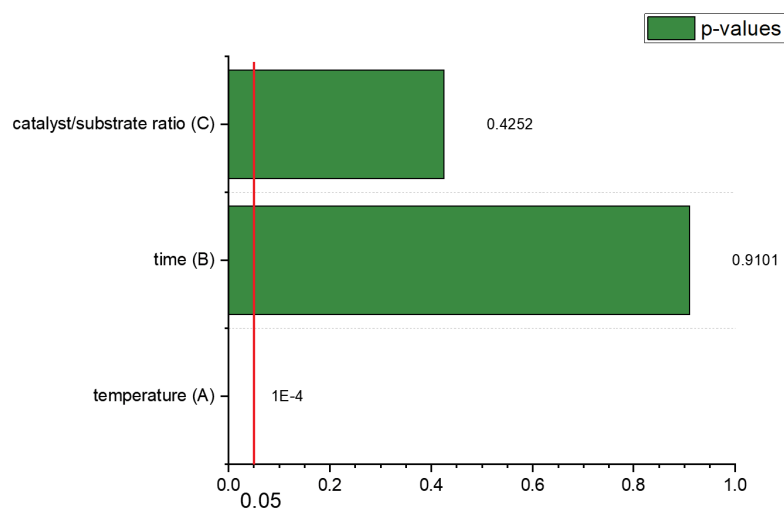


Fig. 6.13 Pareto chart for the linear model of the protein recovery. Red line indicates the significance limit of $p = 0.05$ which shows if the value is significant or not. A value below the significance level is significant.

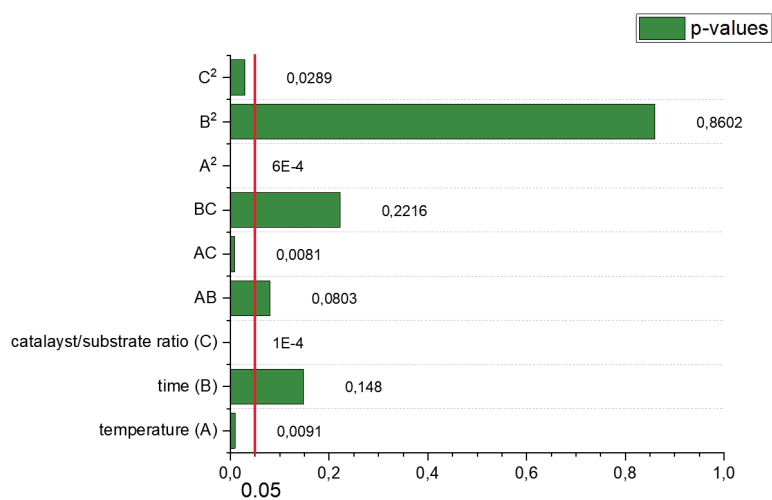


Fig. 6.14 Pareto chart for the quadratic model for the yield of formic acid. Red line indicates the significance level of $p = 0.05$ which shows if the value is significant or not. A value below the significance level is significant.

The 3D response surfaces which are shown in Figure 6.15 for the yield of formic acid and in Figure 6.16 for the protein recovery make the influence more graphically visible.

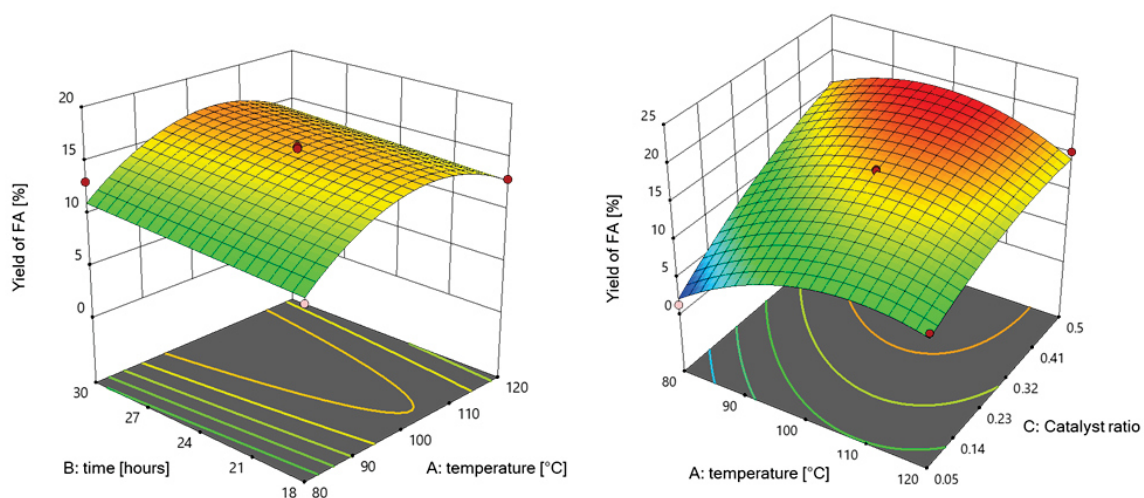


Fig. 6.15 The 3D response surfaces for yield of formic acid. The red dots depict the run points above the surface and the pink dots depict the run points below the surface.

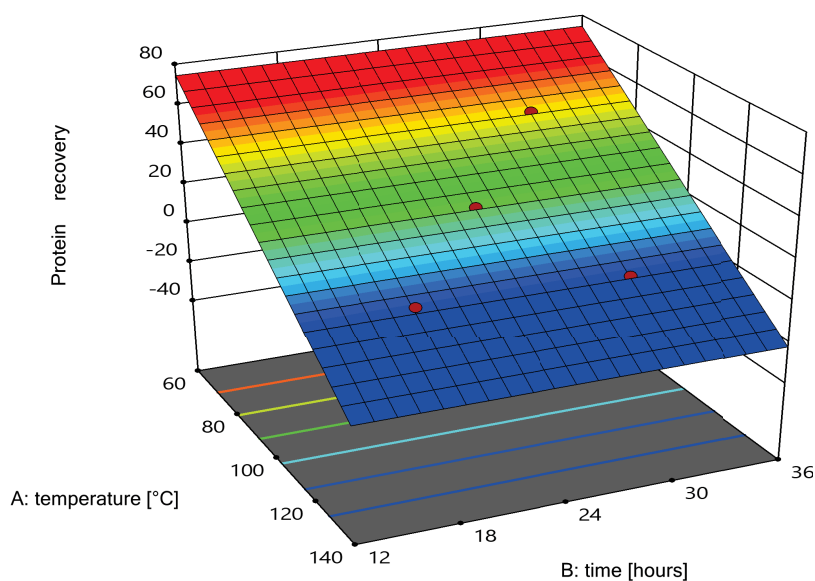


Fig. 6.16 The 3D response surface for protein recovery. The red dots depict the run points above the surface.

The optimization of the ProFA process was a challenge as the contrary influence of the temperature required a compromise between formic acid yield and protein recovery. The optimal conditions could be identified to 80 °C, 30 h reaction time, $0.5 \text{ g}_{\text{catalyst}} \text{g}_{\text{substrate}}^{-1}$ and the fixed reaction conditions of 1000 rpm stirrer speed, 30 bar oxygen pressure, 10 g of substrate

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and 200 g of water as solvent. With that optimized conditions a formic acid yield of 16.41 % and a protein recovery of 59.55 % could be achieved. This answers the research question, as the study could prove that algae biomass is a promising substrate for formic acid and proteins.

In this optimization study the protein was recovered with alkaline extraction. This led to the last research question:

Which extraction technique is the most promising technique for the highest protein yield?

For answering this question, three different extraction methods were chosen and optimized concerning the yield of protein from the solid residue after the OxFA process. Alkaline hydrolysis (AE) was used as state-of-the-art method,^[135] ultrasound-assisted extraction (UAE) and Ionic Liquid extraction (ILE) as novel approaches. During the OxFA process, the solid residue was already exposed to acidic conditions and therefore, the acid extraction was excluded from the tested extraction methods. All details of the tested extraction methods can be found in Section 5.3.

For the AE, sodium hydroxide (NaOH) was used in different concentrations between 0.8 M to 5 M to find the most suitable NaOH concentration. The protein recovery in that set of experiments raised until 1.0 M of NaOH (57.8 %) and decreased after that to 35.7 % at 5.0 M NaOH. This effect could be explained by a elevated pH-value which was likely to disrupt the protein structure and cause denaturation and hence leading to a loss of solubility of proteins.^[302]

For the second approach, the UAE was applied in an slightly alkali medium of 0.4 M NaOH. This was reported in literature to be an efficient method for protein extraction.^[303] Here, the sonication amplitude was varied between 20 % to 100 %. The highest extraction was shown at the sonication amplitude of 100 % with a protein extraction of 87.2 %.

For the third extraction approach the ILE was chosen. The IL used in the study was choline chloride (ChCl) as a green, cheap and environmentally friendly solvent.^[150] The concentration of ChCl was varied between 10 wt.% to 50 wt.% in water. Higher concentrations of IL would lead to handling and processability challenges in the analytical measurements^[150] and were therefore not tested. The extraction tests showed that with increasing ChCl concentration the protein recovery could be enhanced to 47.6 % at 50 wt.% of ChCl. This result was higher than other reported values in the literature (vide intra Section 5.3)^[150,304] and insists that the OxFA process as acid treatment had an enhancing effect on this extraction

method. Figure 6.17 shows the highest values of the different extraction methods tested in comparison.

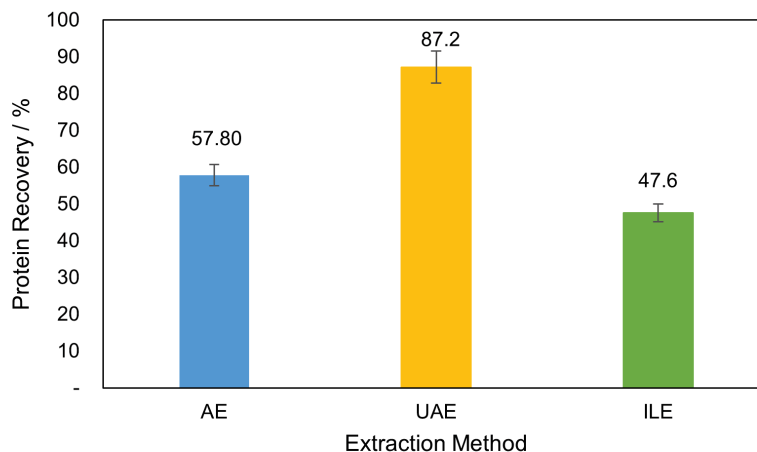


Fig. 6.17 The mean protein recovery comparison of the three methods Alkaline hydrolysis (at 0.1 M NaOH), UAE (at 100 % power input), and ILE (at 50 wt.% ChCl). The highest protein recovery was compared in each case.

It can be seen, that the UAE with an extraction of 87.2 % was the most promising approach for extraction of proteins in context of the valorization of macroalgae via the OxFA process. Analysis of the extracted proteins showed that their composition was suitable for applications such as the production of biopolymer films.

Chapter 7

Summary and Future Perspectives

This thesis has the potential to contribute to the expansion of the field of POM catalyst applications in biomass valorization. The study proposes two concepts for biomass valorization, utilizing distinct types of biomass (algal biomass and lignocellulosic biomass). These concepts have undergone enhancement and development.

The findings revealed that the employment of diverse fractionation methods can facilitate the synthesis of two valuable products: cellulose-enriched solid and formic acid/methyl formate. Comparing different fractionation methods, the ionosolv fractionation method is the most promising method to be applied with a consecutive or *in-situ* oxidation using POM catalysts. The findings of this dissertation indicate that a combination of tributylmethylphosphonium methyl sulfate ([TBMP][MeSO₄]) and methanol as co-solvent enhance the fractionation of lignocellulosic biomass by producing a highly pure, white cellulose-enriched solid residue (>90 % of glucan) which retain 77 % of its glucose content for subsequent enzymatic saccharification. This enhances the further use of the cellulose-enriched solid residue.

It has been demonstrated that the HPA-5 catalyst, when employed in combination with a two-step biorefinery concept and an oxidation as the subsequent step, attains 26.6 % formic acid. The recently discovered TBMP-methanol solvent system using H₈PVMnMo₁₀O₄₀ (HPA-VMn) as catalyst shows a yield of 19.6 % methyl formate. As this solvent produced a highly pure, white cellulose-enriched solid residue and a good yield of formic acid, this concept should be improved further. Insights into the *in-situ* forming POM-IL salt were revealed through the utilization of various analytical methods like ICP-OES, XPS, FT-IR and SCXRD. The catalyst, when employed in conjunction with the recently developed solvent system TBMP with methanol as a co-solvent, is deemed highly promising for application in a biorefinery context. The developed enhanced POM-Ionosolv approach was also tested with several other lignocellulosic biomass like spruce, pine, miscanthus, and birch wood revealing

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similar results. The findings of this study indicate that the enhanced POM-Ionosolv concept, incorporating a novel IL and methanol as a co-solvent, merits further exploration. To fully explore the potential of this promising production concept, it is essential to investigate the recycling and reuse of the catalyst and the reaction solvation. Finally, it is recommended that the reaction conditions should be optimized in order to increase the yield of methyl formate.

The second part of this dissertation is concerned with the valorization of algal biomass. This is achieved by means of the OxFa process, which serves to enhance the extraction of protein and to produce formic acid as a valuable by-product. Following a rigorous screening process that involved two catalysts (HPA-5 ($\text{H}_8\text{PV}_5\text{Mo}_7\text{O}_{40}$) and HPA-2 ($\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$)) and three distinct algal types (red (porphyra dioica), brown (fucus vesiculosus), green (ulva fenestrata)), the selection was made to be focused on the substrate porphyra dioica and HPA-2 as the catalyst, which exhibited the highest protein content (36.4 % dw) and yield of formic acid (8.6 %); these findings are discussed in further detail below. A further optimization using a Box-Behnken Design of Experiments identified temperature as a significant parameter for protein recovery and formic acid yield, while the catalyst/substrate ratio was also significant for the formic acid yield. The optimized reaction conditions, as outlined in Section 5.3, were found to be: 80 °C, 30 h, 0.5 $g_{\text{catalyst}}/g_{\text{substrate}}$, 1000 rpm, 200 g water), a yield of formic acid of 16.4 % was achieved with a protein recovery of 59.6 %. In a subsequent stage, the most promising extraction technique was identified by screening and optimizing three different techniques (alkali extraction (AE), ultrasound-assisted alkali-based extraction (UAE) and IL extraction (ILE)). The UAE was identified as the most effective technique, with a protein recovery of 87.2 % being achieved. Analysis of the extracted proteins showed a suitable composition for biopolymer films. This study is a first step toward a biopolymer film and formic acid production in the future. The next steps would be testing the extracted proteins for the biopolymer film application and an upscale of this process.

The findings from Parts I and II of this dissertation have enhanced the valorization of diverse biomass categories within biorefinery-like concepts and POM-catalyzed processes. For the POM-Ionosolv approach, a novel catalyst-IL system was identified, and novel insights into catalyst-IL interactions were investigated. Furthermore, the utilization of macroalgae as a source of protein and formic acid was investigated, marking a significant initial step in the development of a novel process. The utilisation of both processes has the potential to generate a novel value chain in the future. The increasing social shift away from animal products would free up the lignocellulosic resource 'meadow areas' for further processing into platform chemicals in the future. The extraction of plant proteins is contingent upon the

growth conditions of the meadow. In future, meadow areas and thus very inhomogeneous substrates would be an interesting field for the processes tested in this dissertation.

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Appendix

Supporting Information of Sequential fractionation and POM catalyzed oxidation of lignocellulosic biomass improves cellulose purity and formic acid yield

In the following pages the Supporting Information for the publication Sequential fractionation and polyoxometalate-catalyzed oxidation of lignocellulosic biomass improves cellulose purity and formic acid yield^[285] is shown.

Sequential fractionation and POM catalyzed oxidation of lignocellulosic biomass improves cellulose purity and formic acid yield

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Keywords: *lignocellulosic biomass, polyoxometalate, homogenous catalysis, ionic liquid, green chemistry, biomass fractionation*

CHNS data of solids after fractionation

Table S1: CHNS data of solids after fractionation.

<i>Solvent</i>	<i>Solvent/water ratio /wt. %</i>	<i>Temperature /°C</i>	<i>N /wt. %</i>	<i>C /wt. %</i>	<i>H /wt. %</i>	<i>S /wt. %</i>
<i>EtOH</i>	60/40	125	0.09	44.715	6.155	0.025
<i>EtOH</i>	30/70	125	<0.2	45.225	6.100	<0.2
<i>EtOH</i>	60/40	170	<0.2	46.21	6.25	<0.2
<i>EtOH</i>	30/70	170	<0.2	45.975	6.1925	<0.2
<i>MeOH</i>	60/40	125	0.000	46.310	6.213	0.128
<i>MeOH</i>	30/70	125	0	48.003	6.253	0.428
<i>MeOH</i>	60/40	170	0.317	44.365	6.305	0.82
<i>MeOH</i>	30/70	170	<0.2	43.693	6.348	<0.2
<i>TEA</i>	60/40	125	1.485	41.407	6.749	3.645
<i>TEA</i>	30/70	125	0.125	46.120	6.170	0.205
<i>Beech wood</i>	Unprocessed	-	<0.2	46.78	6.34	<0.2

Reaction conditions: 200 g liquid phase (different ratios of solvent and water), 18 g beech wood chips ($x \leq 630 \mu\text{m}$), 30 bar nitrogen pressure at temperature, 125 °C/ 170 °C temperature, 1200 rpm stirrer speed, 6 h reaction time.

GPC analysis of dissolved oligomers after step one

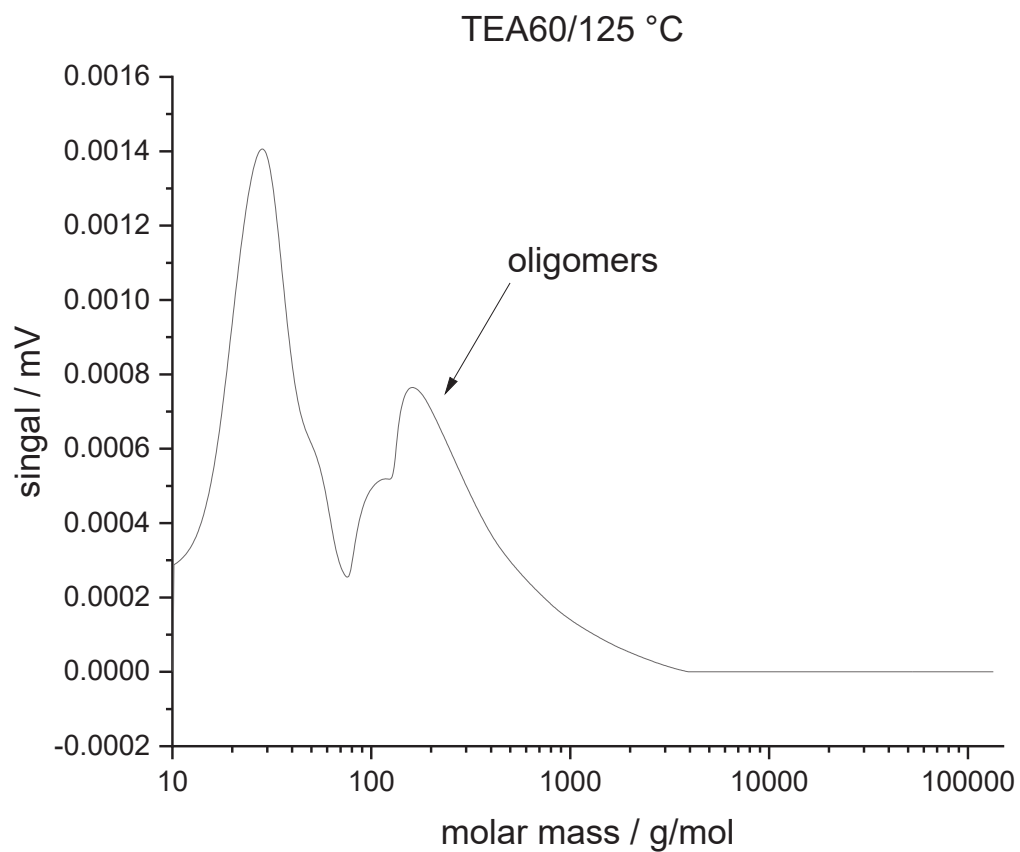


Figure S1: GPC analysis for the experiment TEA60/125°C as an example GPC analysis for the ionosolv-fractionation method.

Table S2: GPC analysis metrics number average molecular weight M_n and weight average molecular weight M_w for the experiment TEA60/125 °C as an example for the ionosolv-fractionation method.

Variable	TEA60/125 °C
M_n	4.18079e+01
M_w	1.78716e+02

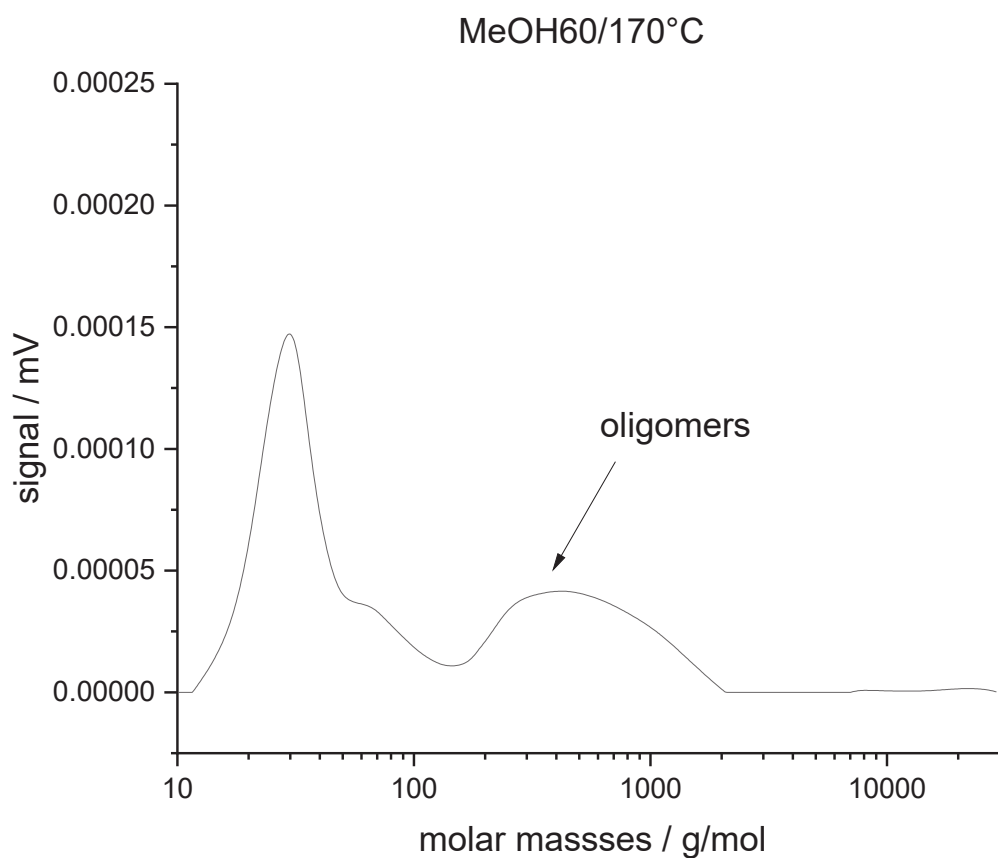


Figure S2: GPC analysis of MeOH60/170°C as an example GPC measurement for the methanolic organosolv-method.

Table S3: GPC analysis metrics number average molecular weight M_n and weight average molecular weight M_w for the experiment MeOH60/170 °C as an example for the methanolic organosolv-fractionation method.

Variable	MeOH60/170 °C
M_n	4.68454e+01
M_w	3.36044e+02

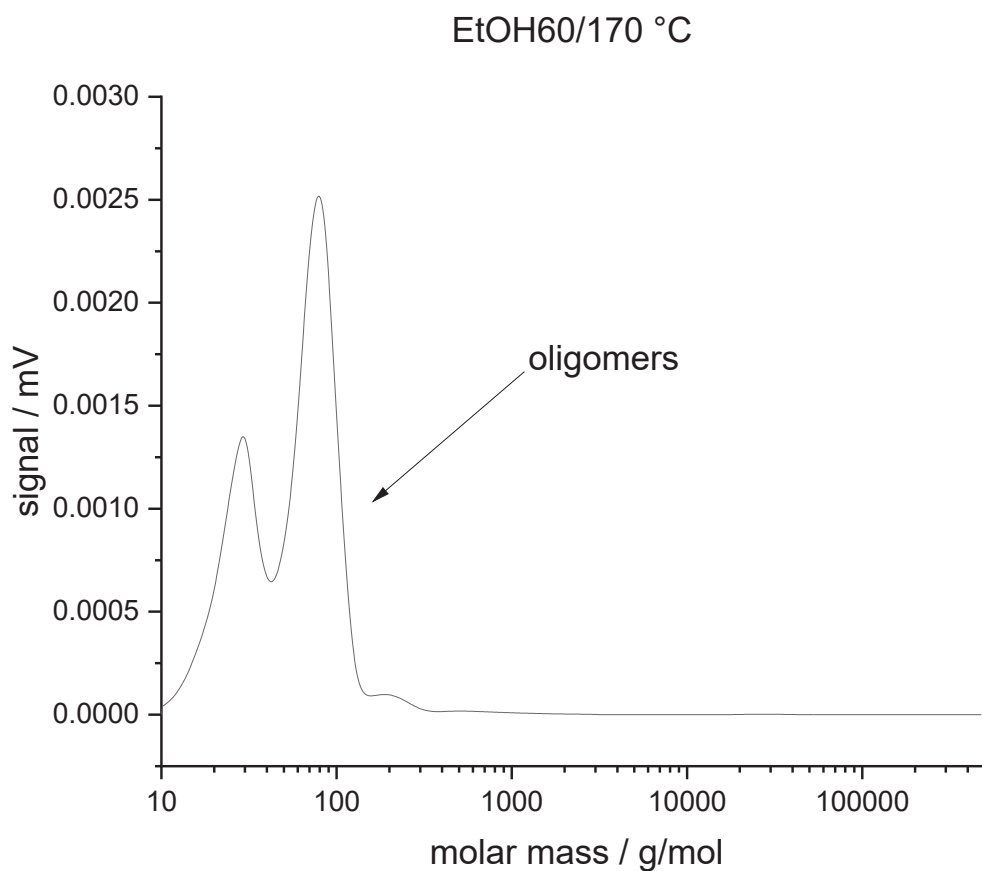


Figure S3:GPC analysis for the EtOH60/170°C experiment as an example measurement for the ethanolic organosolv-method.

Table S4:GPC analysis metrics number average molecular weight Mn and weight average molecular weight Mw for the experiment EtOH60/170 °C as an example for the ethanolic organosolv-fractionation method.

Variable	EtOH60/170 °C
Mn	4.39967e+01
Mw	1.01634e+02

GC-MS measurements

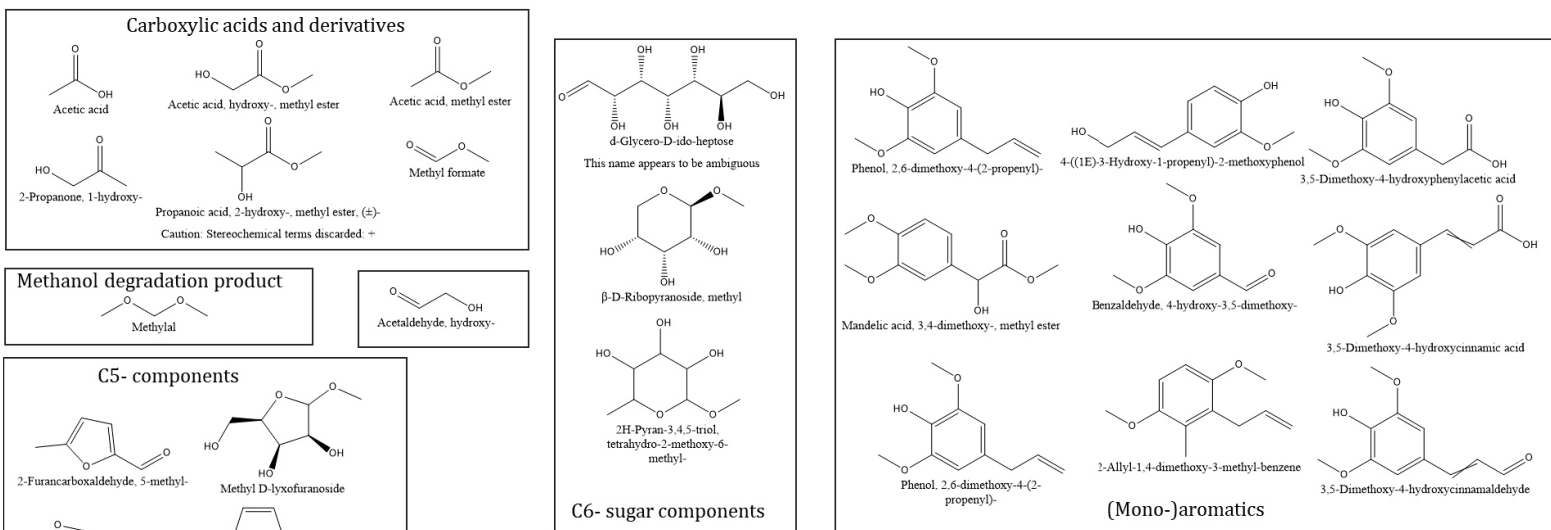


Figure S4: Products found with GC-MS in the methanolic organosolv-fractionation which could not be quantified due to a too small amount.

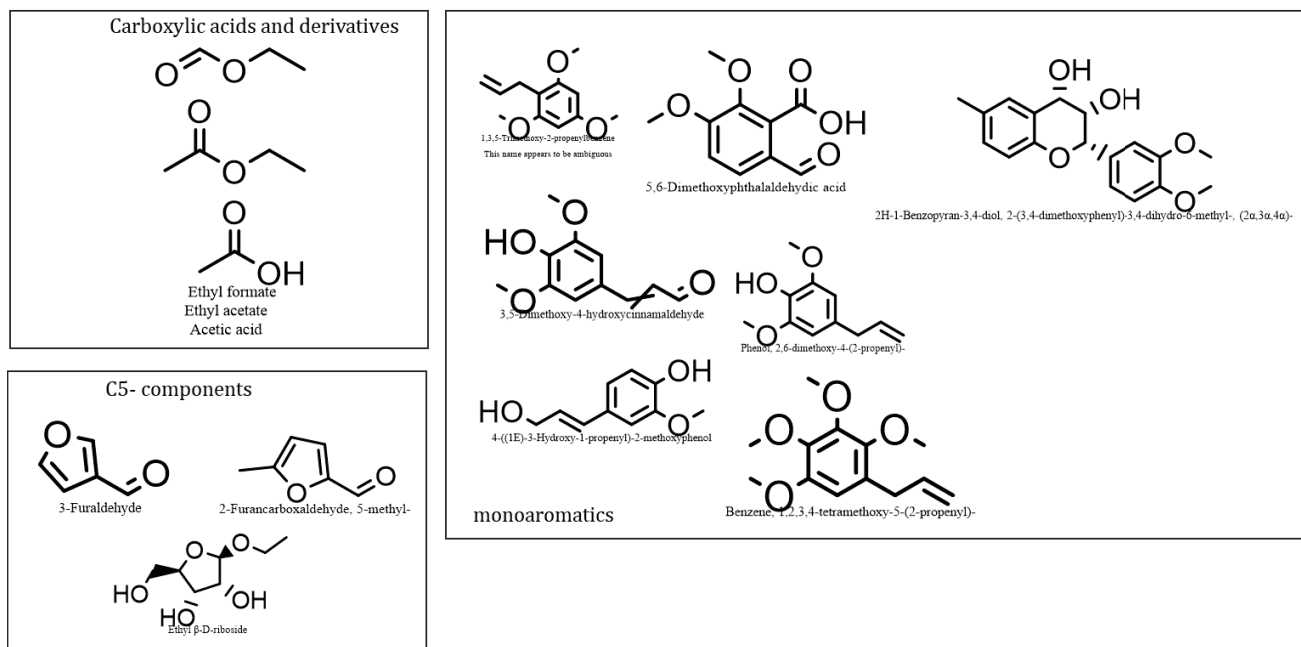


Figure S5: Products found with GC-MS in the ethanolic organosolv-fractionation which could not be quantified due to a too small amount.

Blank experiments

Table S5: Experiments without any substrate but with catalyst and solvent to investigate the stability of the solvent system under oxidative conditions - blank experiments.

Solvent system	Temperature stable at 170 °C*?	Stable with HPA-5*?	Identified products	Amount of product / mol	Yield from solvent / %
TEA60/125 °C	Not tested	Yes, negligible instability	CO ₂ Formic acid	0.00002 0.00027	0.01 0.15
TEA30/125 °C	Not tested	Yes, negligible instability	CO ₂ Formic acid	0.00009 0.00016	0.10 0.18
MeOH60/170 °C	Yes	Yes, negligible instability	CO ₂ Methyl formate formic acid	0.00006 0.0000052 0.0000023	0.033 0.003 0.001
MeOH30/170 °C	Yes	Yes, negligible instability	Methyl formate Formic acid	0.000006 0.000007	0.007 0.008
EtOH60/170 °C	Yes	Yes, negligible instability	CO ₂ Ethyl formate Formic acid	0.00002 0.000005 0.000005	0.007 0.002 0.002
EtOH30/170 °C	Yes	Yes, negligible instability	CO ₂	0.00003	0.02
* and 1200 rpm, 6 h reaction time, 30 bar nitrogen pressure at temperature **at reaction conditions (125 °C, 6.5 h, $0.5 \frac{mmol}{100 g_{liquid\ phase}}$ HPA-5, 30 bar oxygen pressure at temperature, 1200 rpm stirrer speed)					

Catalyst characterization

ICP-OES of HPA-5

Table S6: Results form ICP-OES analysis of the HPA-5 batch used in this study.

Ratio	Mo	P	V
HPA-5	7	1.33	4.70

The ratios P/V/Mo were determined by ICP-OES analysis. The data were normalized to the targeted Mo content. H₃PO₄ was used in a slight excess during the synthesis, therefore the high P content may be attributed to residual H₃PO₄.

FT-IR of HPA-5

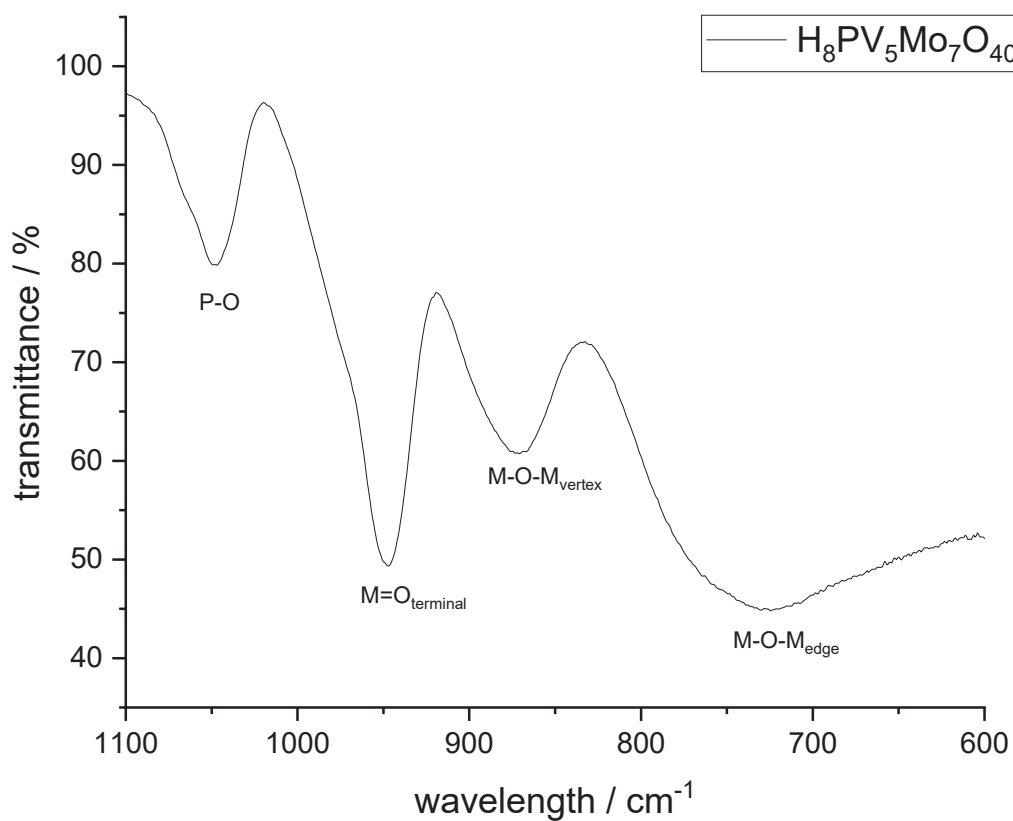


Figure S6: FT-IR (ATR) spectrum of HPA-5 with characteristic vibrations at 1045 cm⁻¹ (P-O), 948 cm⁻¹ (M=O_{terminal}), 864 cm⁻¹ (M-O-M_{vertex}), 730 cm⁻¹ (M-O-M_{edge}).^[1]

References

- [1] J. Albert, D. Lüders, A. Bösmann, D. M. Guldi, P. Wasserscheid, *Green Chem* **2014**, *16*, 226.

Supporting Information of Boosting POM-Ionosolv biorefining of lignocellulosic biomass by using redox-balanced polyoxometalate catalysts in methanolic ionic liquid reaction media

In the following pages the Supporting Information for the publication Boosting POM-Ionosolv biorefining of lignocellulosic biomass by using redox-balanced polyoxometalate catalysts in methanolic ionic liquid reaction media^[284] is shown.

Supporting Information

Boosting POM-Ionosolv biorefining of lignocellulosic biomass by using redox-balanced polyoxometalate catalysts in methanolic ionic liquid reaction media

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Investigation to improve the material systems

Morphology of processed solids

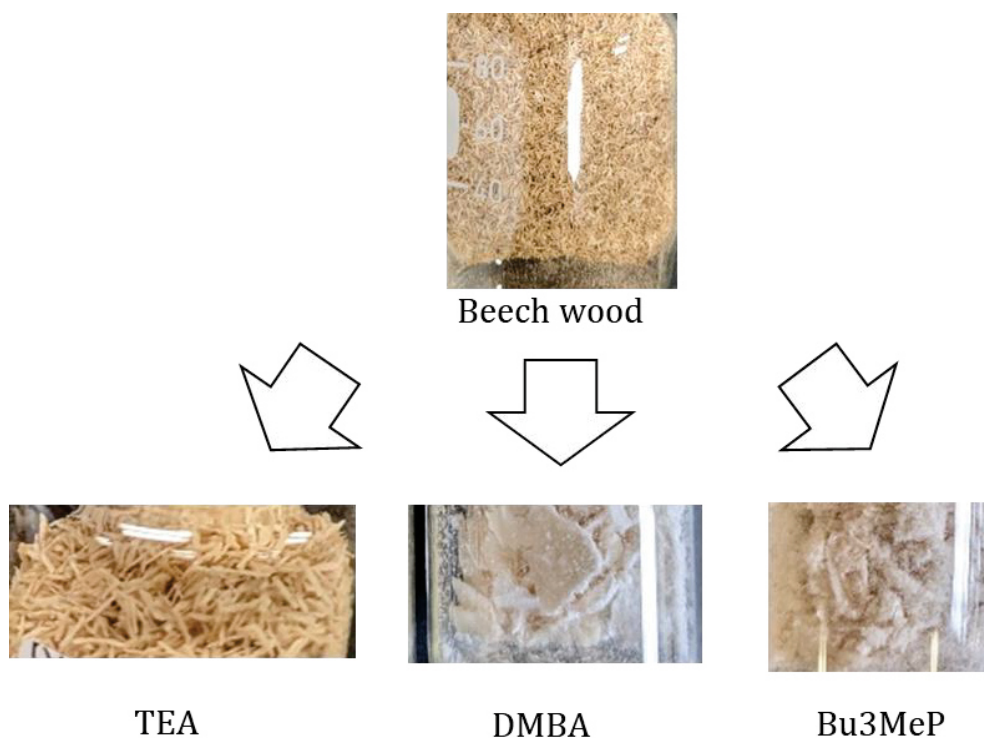


Figure S1: Pictures of beech wood and the processed solid residues with different IL/MeOH solvent systems to show the different morphologies. *Reaction conditions: 120 °C, 24 h, 1000 rpm, 30 bar of oxygen at reaction temperature, 10 g solvent ([TEA][HSO₄]/MeOH 30 wt.%/70 wt.% (TEA), [DMBA][HSO₄]/MeOH 30 wt.%/70 wt.% (DMBA), [TBMP][MeSO₄]/MeOH 30 wt.%/70 wt.% (TBMP)), 0.5 g beech wood.*

Stability of methanolic ionic liquid solvent

Stability of TEA ([TEA][HSO₄]/MeOH 30 wt.%/70 wt.%) with HPA-5

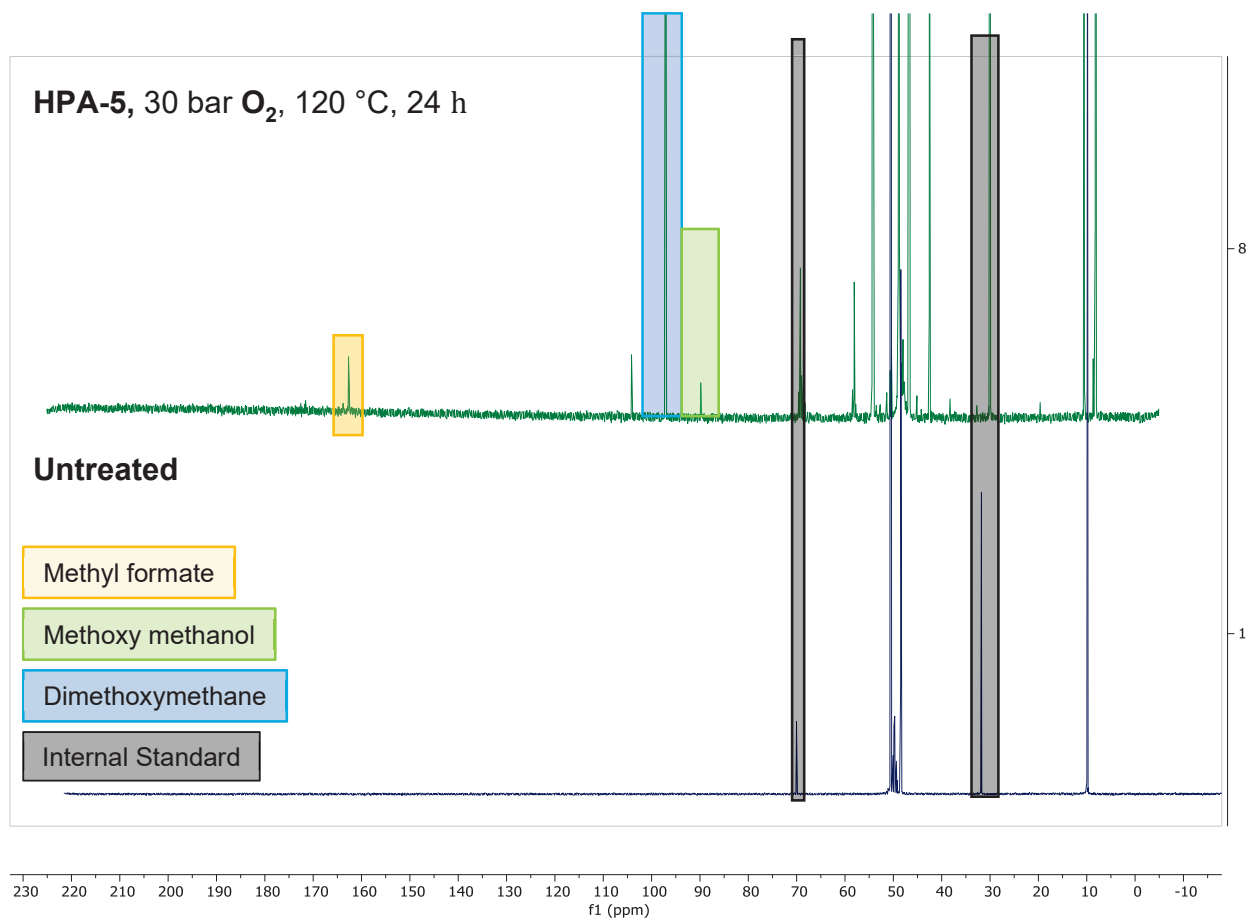


Figure S2: ¹³C NMR spectra of untreated TEA and with HPA-5 under reaction conditions treated TEA. TEA is an abbreviation for the solvent mixture of [TEA][HSO₄]/MeOH 30 wt.%/70 wt.%. The identified methanol oxidation products (methyl formate (163 ppm), methoxy methanol (91 ppm, 56 ppm) and dimethoxymethane (98 ppm, 51 ppm) and the internal standard (tert-butanol) are marked.

Stability of DMBA ([DMBAA][HSO₄]/MeOH 30 wt.%/70 wt.%) with HPA-5

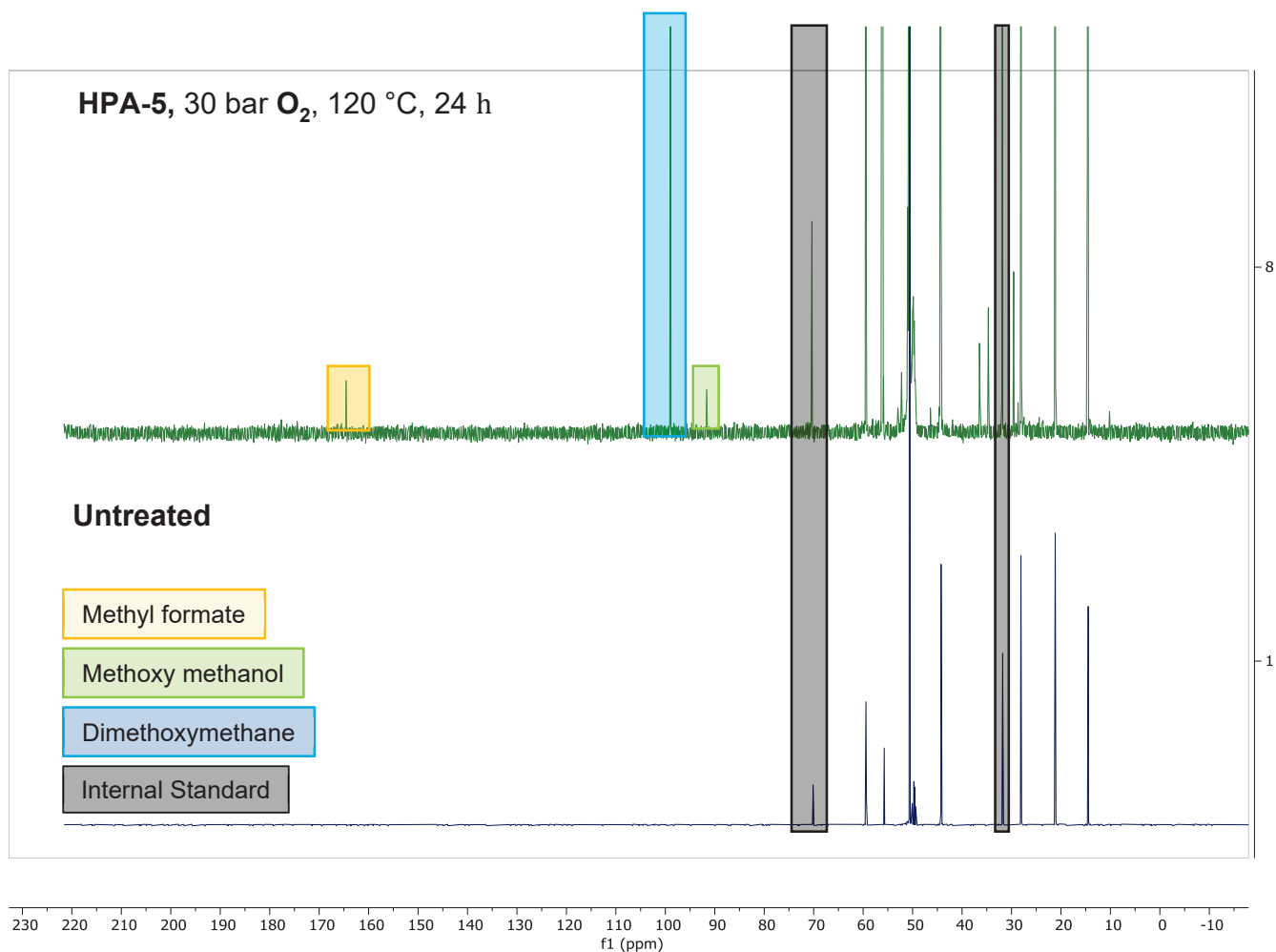


Figure S3: ¹³C NMR spectra of untreated DMBA and with HPA-5 under reaction conditions treated DMBA. DMBA is an abbreviation for the solvent mixture of [DMBAA][HSO₄]/MeOH 30 wt.%/70 wt.%. The identified methanol oxidation products (methyl formate (163 ppm), methoxy methanol (91 ppm, 56 ppm) and dimethoxymethane (98 ppm, 51 ppm) and the internal standard (tert-butanol) are marked.

Stability of TBMP ([TBMP][MeSO₄]/MeOH 30 wt.%/70 wt.%) with HPA-5

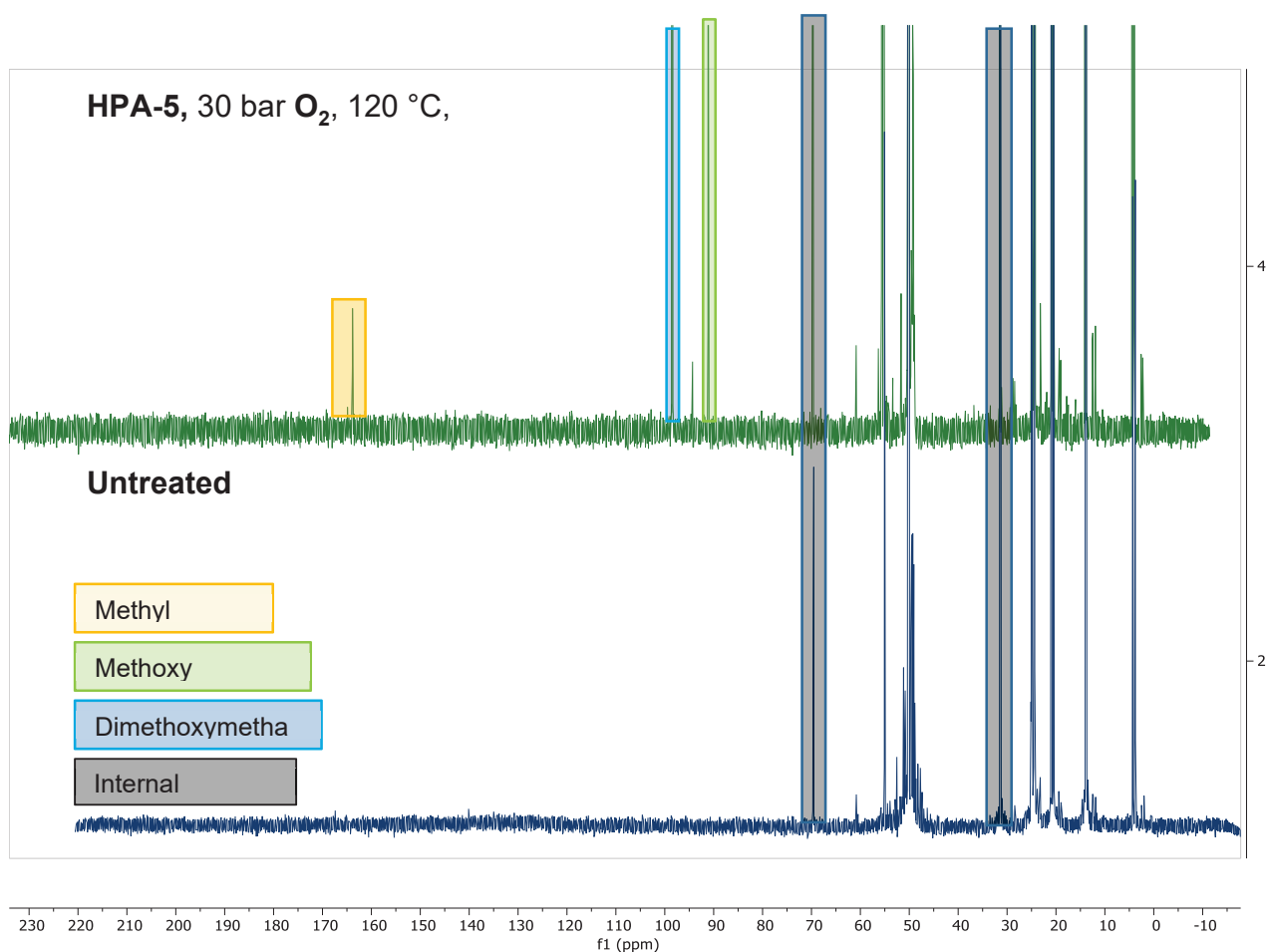


Figure S4: ¹³C NMR spectra of untreated Bu₃MeP and with HPA-5 under reaction conditions treated Bu₃MeP. Bu₃MeP is an abbreviation for the solvent mixture of [Bu₃MeP][MeSO₄]/MeOH 30 wt.%/70 wt.%. The identified methanol oxidation products (methyl formate (163 ppm), methoxy methanol (91 ppm, 56 ppm) and dimethoxymethane (98 ppm, 51 ppm) and the internal standard (tert-butanol) are marked.

Redox activity of various Keggin-type POM catalysts

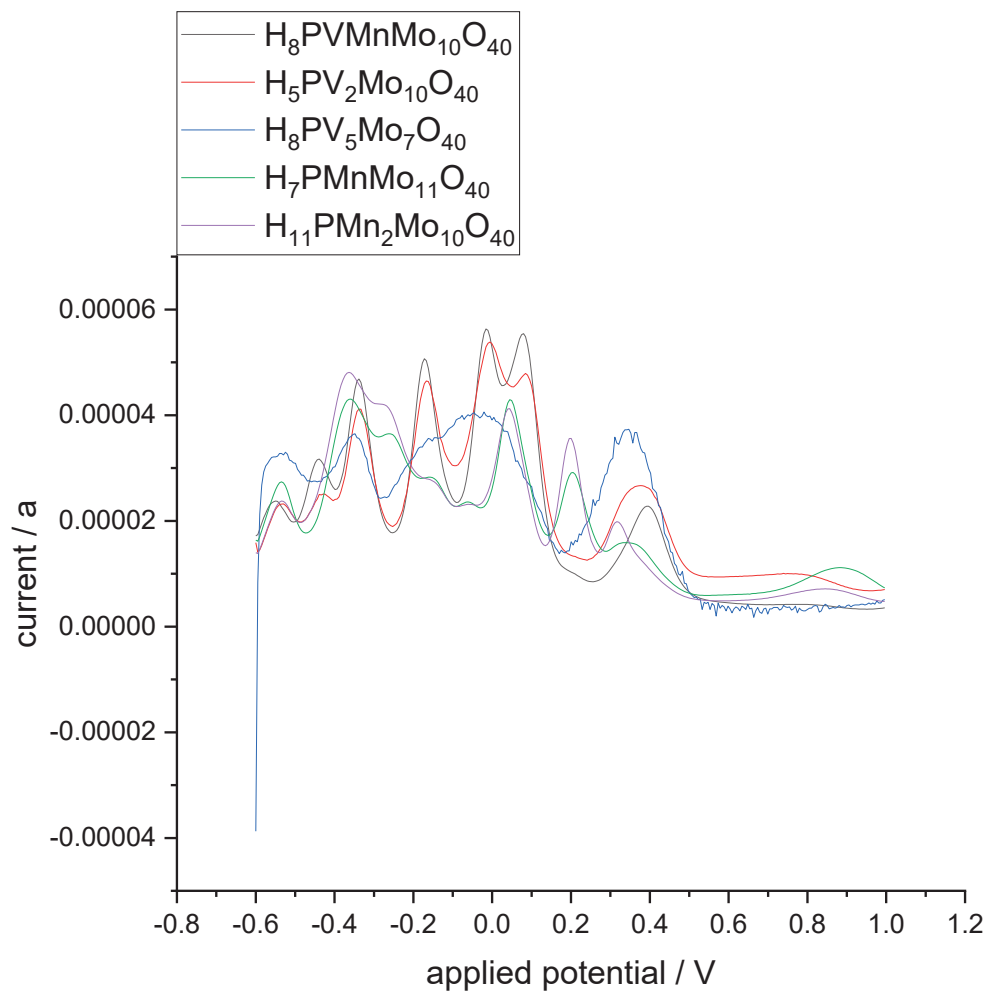


Figure S5: Square wave voltammetry data of the different catalysts in water (1 mmol L^{-1}).^{1,2}

Stability of TEA ([TEA][HSO₄]/MeOH 30 wt.%/70 wt.%) with different catalysts

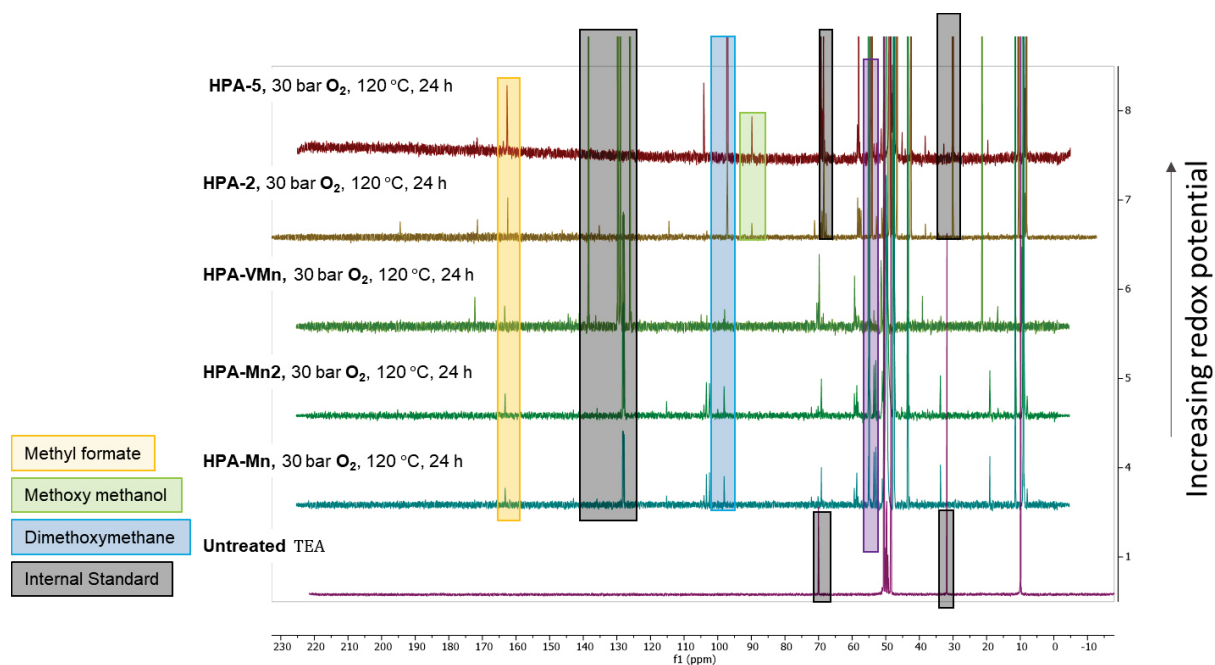


Figure S6: ¹³C NMR spectra of untreated TEA and with HPA-5, HPA-2, HPA-VMn, HPA-Mn2, and HPA-Mn under reaction conditions treated TEA. TEA is an abbreviation for the solvent mixture of [TEA][HSO₄]/MeOH 30 wt.%/70 wt.%. The identified methanol oxidation products (methyl formate (163 ppm), methoxy methanol (91 ppm, 56 ppm) and dimethoxymethane (98 ppm, 51 ppm) and the internal standard (tert-butanol and acetonitrile) are marked.

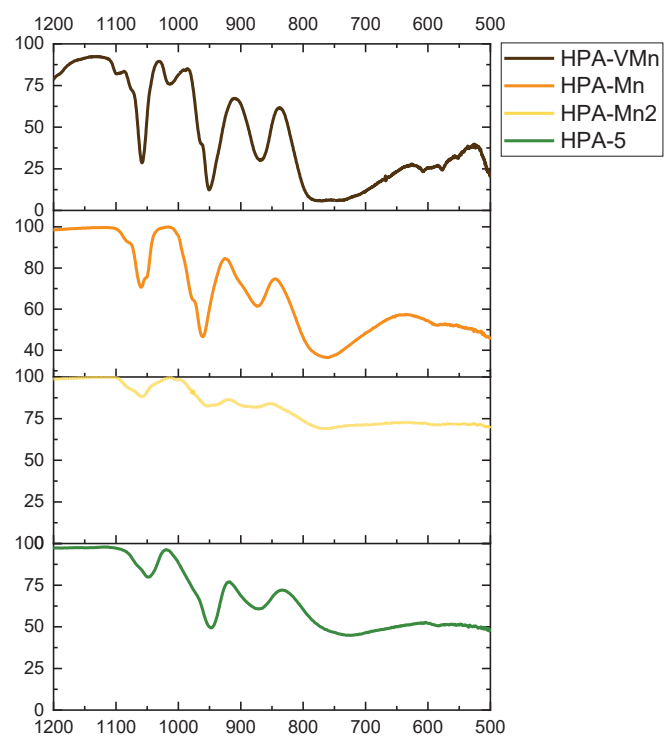


Figure S7: FT-IR spectra of different catalysts in TEA.

Stability of DMBA ([DMBA]/[HSO₄]/MeOH 30 wt.%/70 wt.%) with different catalysts

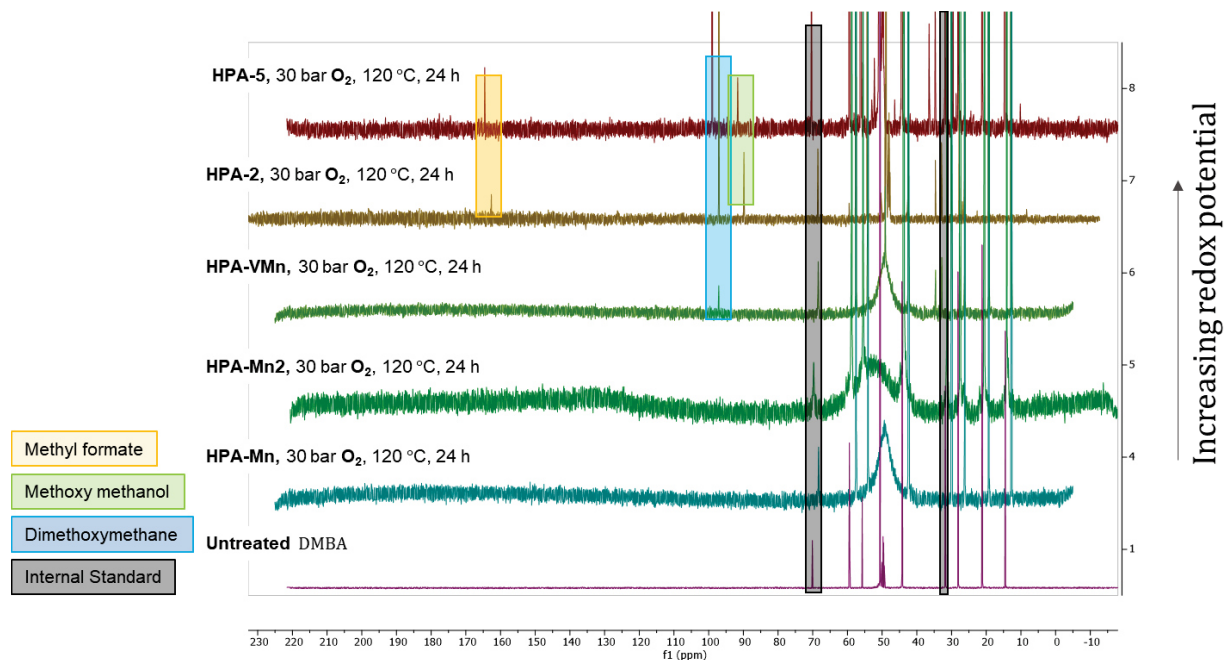


Figure S8: ¹³C NMR spectra of untreated DMBA and with HPA-5, HPA-2, HPA-VMn, HPA-Mn2, and HPA-Mn under reaction conditions treated DMBA. DMBA is an abbreviation for the solvent mixture of [DMBA]/[HSO₄]/MeOH 30 wt.%/70 wt.%. The identified methanol oxidation products (methyl formate (163 ppm), methoxy methanol (91 ppm, 56 ppm) and dimethoxymethane (98 ppm, 51 ppm) and the internal standard (tert-butanol and acetonitrile) are marked.

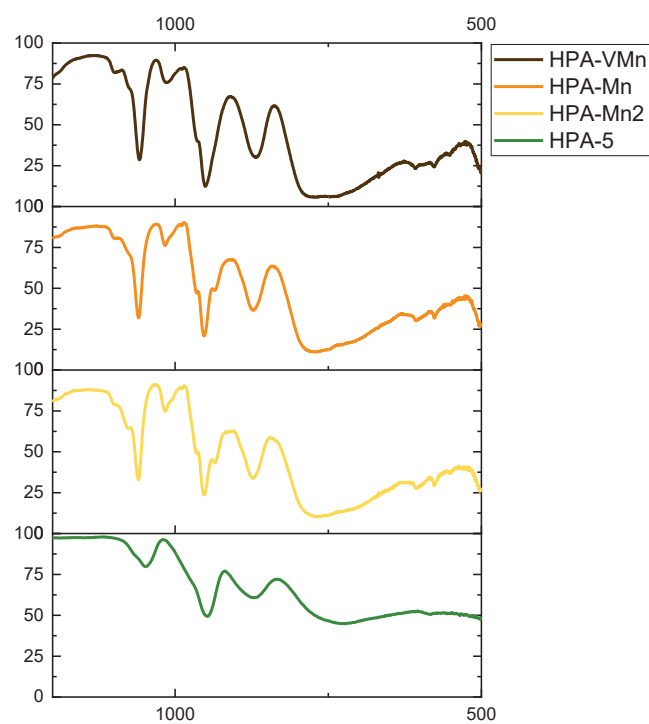


Figure S9: FT-IR spectra of different catalysts in DMBA.

Stability of TBMP ([TBMP][MeSO₄]/MeOH 30 wt.%/70 wt.%) with different catalysts

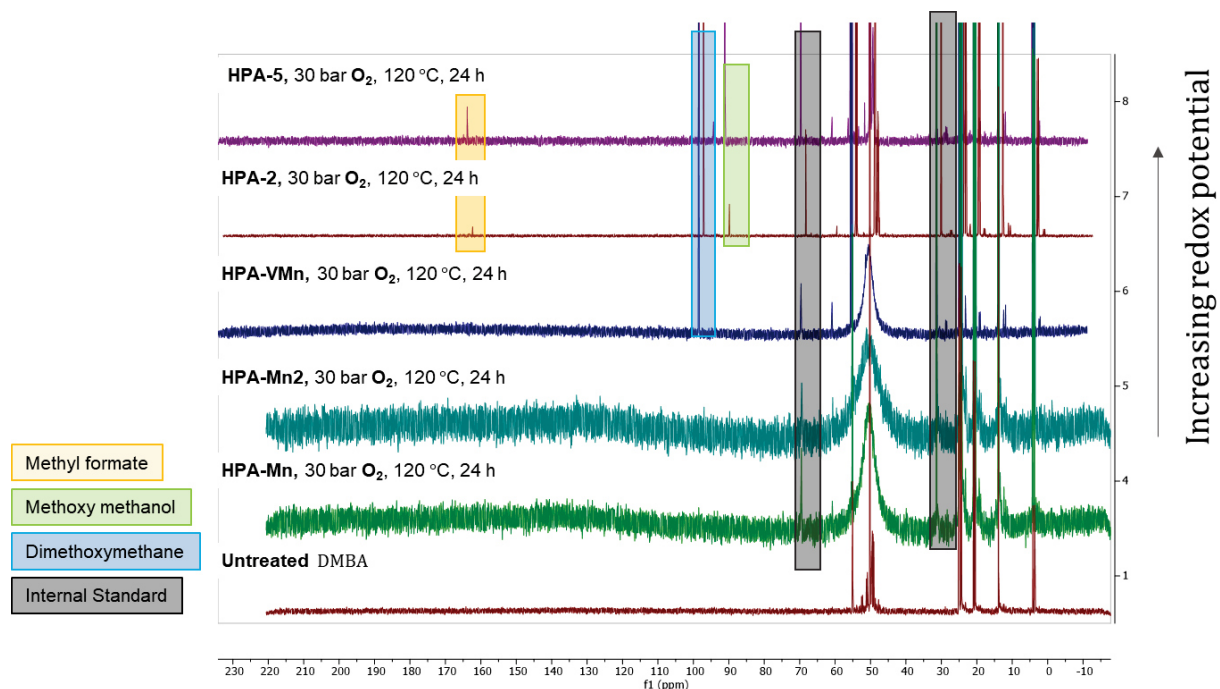


Figure S10: ¹³C NMR spectra of untreated TBMP and with HPA-5, HPA-2, HPA-VMn, HPA-Mn2, and HPA-Mn under reaction conditions treated TBMP. TBMP is an abbreviation for the solvent mixture of [TBMP][MeSO₄]/MeOH 30 wt.%/70 wt.%. The identified methanol oxidation products (methyl formate (163 ppm), methoxy methanol (91 ppm, 56 ppm) and dimethoxymethane (98 ppm, 51 ppm) and the internal standard (tert-butanol and acetonitrile) are marked.

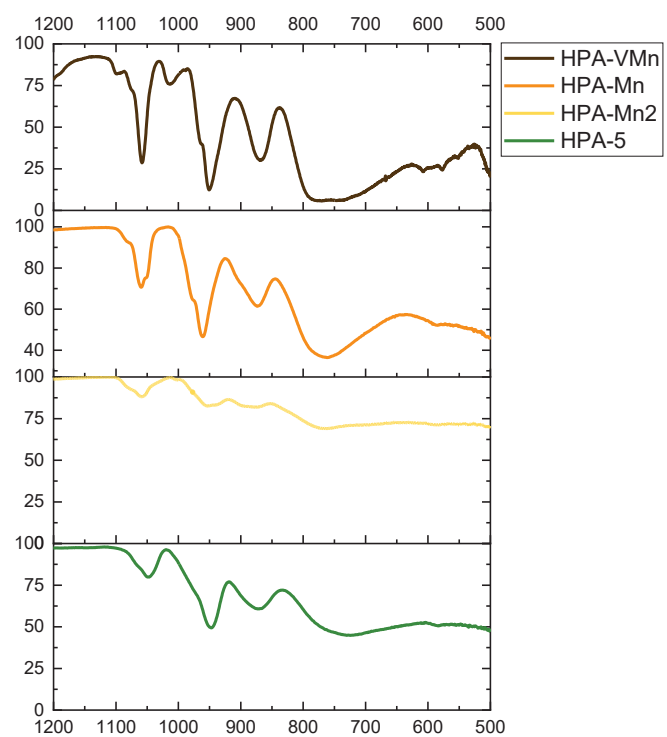


Figure S11: FT-IR spectra of different catalysts in TBMP.

Investigating catalytic behavior under reaction conditions

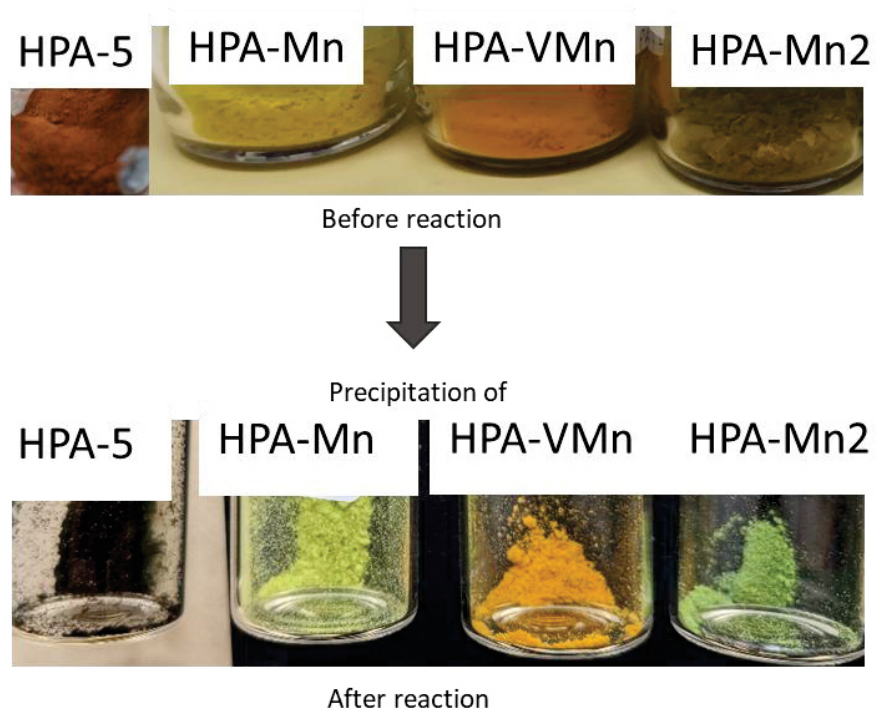


Figure S12: Catalysts before the reaction and the respective inorganic precipitates after the reaction.

Table S1: ICP-OES data as comparison between HPA-VMn as catalyst and the inorganic precipitate after the reaction with HPA-VMn and the methanolic TBMP solvent. *Reaction conditions: 120 °C, 24 h, 1000 rpm, 30 bar of oxygen at reaction temperature, 10 g solvent [TBMP]/[MeSO₄]/MeOH 30 wt.%/70 wt.% (methanolic TBMP solvent), 0.180 g HPA-VMn catalyst (1 mmol/g catalyst).*

		Mn	Mo	V	P
	Stoichiometry ideally for H ₈ PVMnMo ₁₀ O ₄₀	1	10	1	1
HPA-VMn (H ₈ PVMnMo ₁₀ O ₄₀)	measurement [g/kg]	30.1	501	27.5	20.3
	stoichiometry	1.05	10	1.03	1.25
Inorganic precipitate before reaction	measurement [g/kg]	<0.05	41	1.8	5.5
	stoichiometry	<0.02	10	0.82	4.16
Inorganic precipitate after reaction	measurement [g/kg]	<0.05	38.94	1.14	5.96
	stoichiometry	<0.02	10	0.55	4.74

Detailed information about the crystal structure

Table S2: Crystal data and structure refinement for CCDC 2422752.

Crystal data and structure refinement for CCDC 2422752	
Identification code	exp_270_autored
Empirical formula	C ₃₉ Mo ₁₂ O ₄₀ P ₄
Molecular weight/ g/mol	2383.55
Temperature/K	120.00(11)
Crystal system	monoclinic
Space group	P2 ₁ /c
a/Å	23.7676(2)
b/Å	43.1368(5)
c/Å	21.8129(2)
α/°	90
β/°	91.5590(10)
γ/°	90
Volume/Å ³	22355.6(4)
Z	12
ρ _{calc} /cm ³	2.125
μ/mm ⁻¹	17.520
F(000)	13164.0
Crystal size/mm ³	0.17 × 0.16 × 0.03
Radiation	Cu Kα (λ = 1.54184)
2θ range for data collection/°	4.54 to 161.106
Index ranges	-29 ≤ h ≤ 28, -54 ≤ k ≤ 54, -22 ≤ l ≤ 27
Reflections collected	149069
Independent reflections	46582 [R _{int} = 0.0548, R _{sigma} = 0.0511]
Data/restraints/parameters	46582/1417/2683
Goodness-of-fit on F ²	1.056
Final R indexes [I ≥ 2σ (I)]	R ₁ = 0.0725, wR ₂ = 0.2018
Final R indexes [all data]	R ₁ = 0.0968, wR ₂ = 0.2246
Largest diff. peak/hole / e Å ⁻³	1.91/-1.78

Table S3: Selected bond lengths of the IL-POM salt obtained from the single crystal structure data.

Bond	P-O _p / Å	O _p -M / Å	M-O _T / Å	M-O _b / Å
This work	1.531-1.540	2.424-2.431	1.674	1.884, 1.946
H ₈ [PVMnMo ₁₀ O ₄₀] ¹	1.537	2.422	1.913	1.663
H ₄ [PVMo ₁₁ O ₄₀] ⁶	1.536	2.442	1.640	1.873

X-ray photoelectron spectroscopy (XPS)

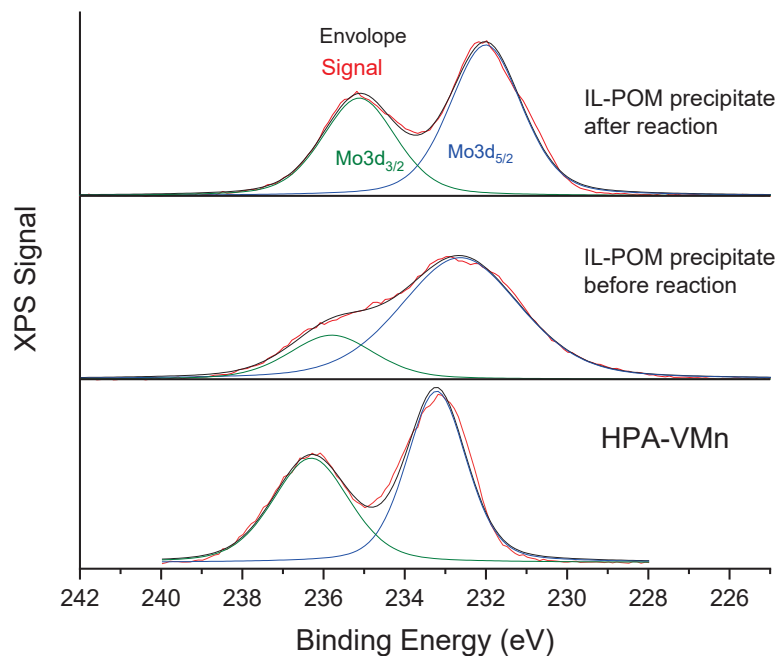


Figure S13: Comparison of the XPS signals of the Mo 3d bands of the inorganic precipitate (IL-POM precipitate) before and after the reaction and the initial HPA-VMn POM catalyst. *Reaction conditions: 120 °C, 24 h, 1000 rpm, 30 bar of oxygen at reaction temperature, 10 g solvent [TBMP]/[MeSO₄]/MeOH 30 wt.%/70 wt.% (methanolic TBMP system), 0.180 g catalyst HPA-VMn (1 mmol/g catalyst).*

Table S4: Relative surface concentrations of Mo, V, Mn and distribution of oxidation states of V and Mn determined via XPS.

Sample	Mo	V	V ³⁺	V ⁴⁺	V ⁵⁺	Mn	Mn ²⁺	Mn ³⁺	Mn ⁴⁺
HPA-VMn	9	0.50	0	0	100	0.50	0	25.7	74.7
IL-POM precipitate before reaction	9	0.98	0	16.7	83.3	0.32	1.7	57.7	40.7
IL-POM precipitate after reaction	9	0.37	14.1	45.6	40.4	0.00	-	-	-

Characterization of the inorganic precipitate

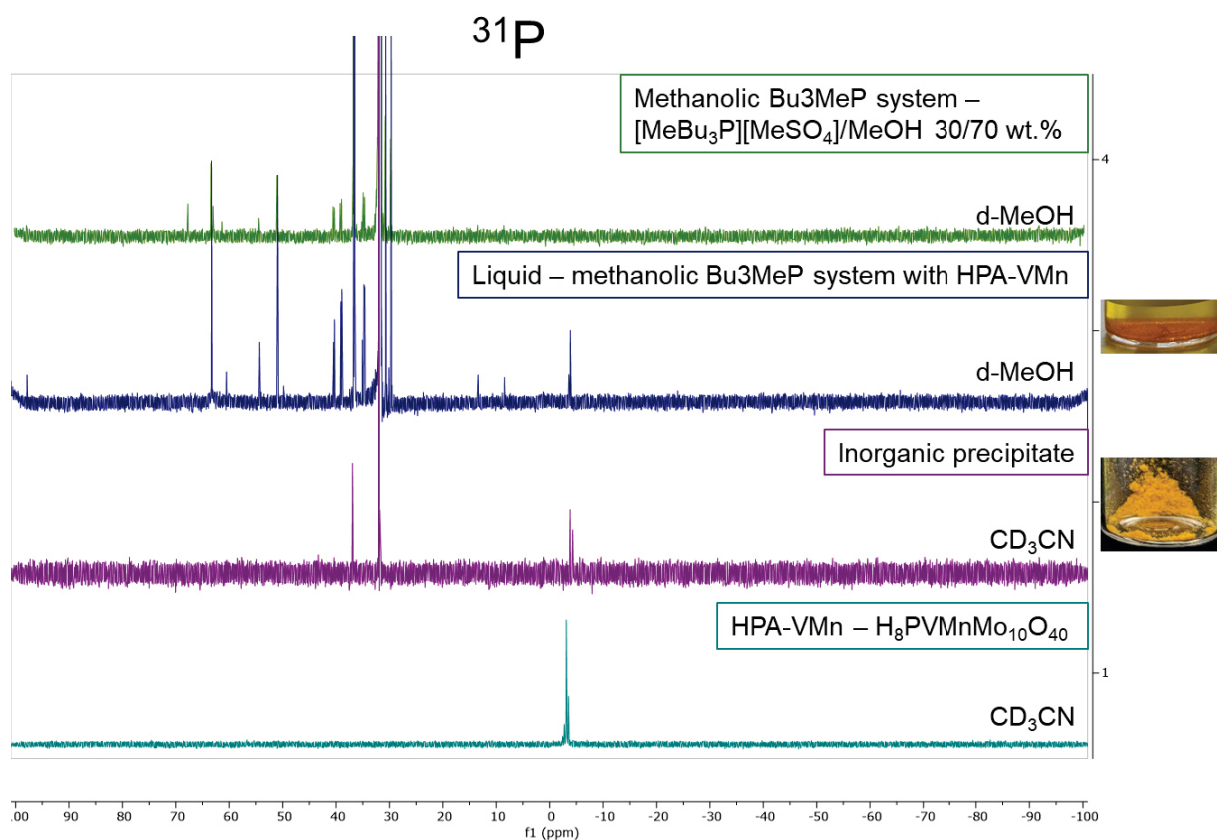


Figure S14: ^{31}P NMR spectra of inorganic precipitate from TBMP and HPA-VMn (violet) diluted in deuterated acetonitrile, the liquid methanolic TBMP solution with dissolved HPA-VMn (blue) with deuterated methanol and HPA-VMn diluted in deuterated acetonitrile (turquoise) before reaction. In the ^{31}P NMR spectra the initial methanolic TBMP system is displayed in green as a comparison to the other NMR spectra.

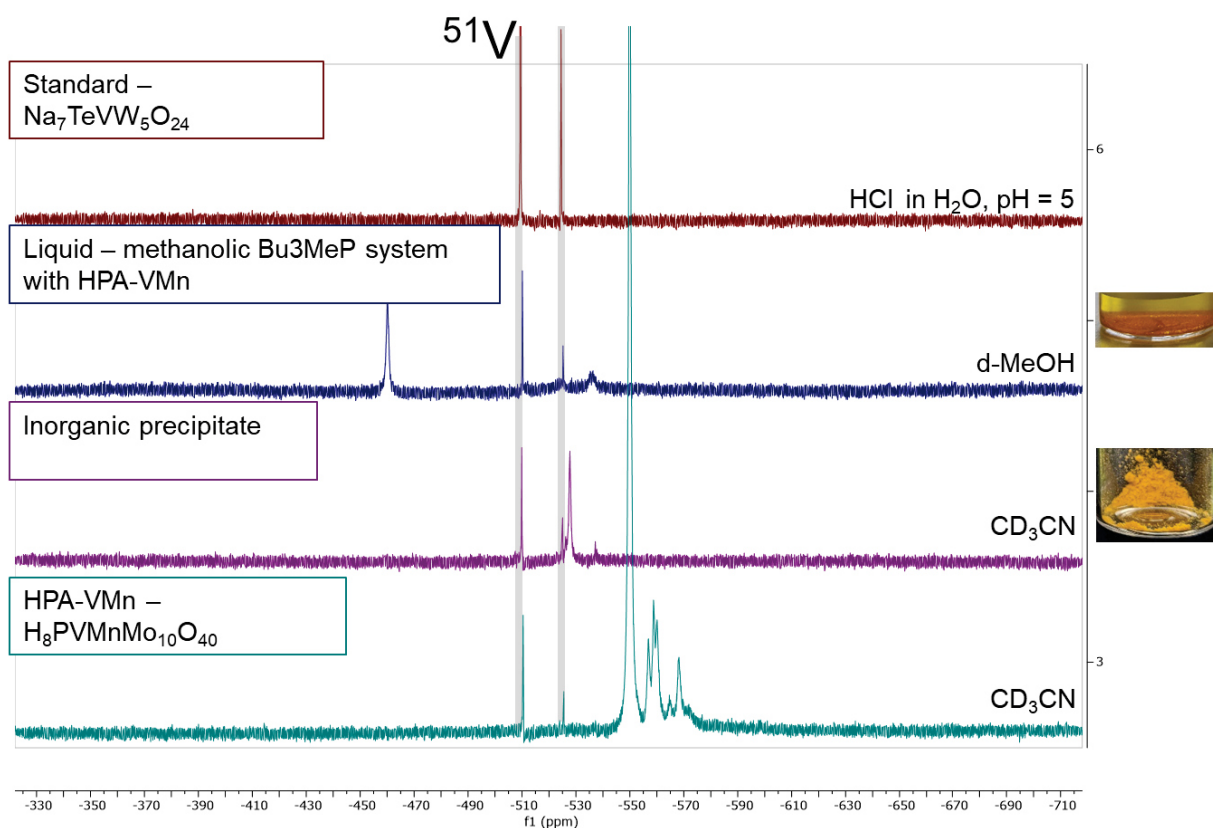


Figure S15: ^{51}V NMR spectra of inorganic precipitate from TBMP and HPA-VMn (violet) diluted in deuterated acetonitrile, the liquid methanolic TBMP solution with dissolved HPA-VMn (blue) with deuterated methanol and HPA-VMn diluted in deuterated acetonitrile (turquoise) before reaction. For a comparison an internal Standard of $\text{Na}_7\text{TeVW}_5\text{O}_{24}$ in deuterated water at pH = 5 was used (red) and marked in grey in the other spectra.



22 °C



95 °C

Figure S16: Comparison of pictures at 22 °C and 95 °C of the methanolic TBMP system with HPA-VMn as catalyst. The left picture shows an heterogeneous system with a solid inorganic precipitate and the right picture a homogeneous system where the precipitate is dissolved.

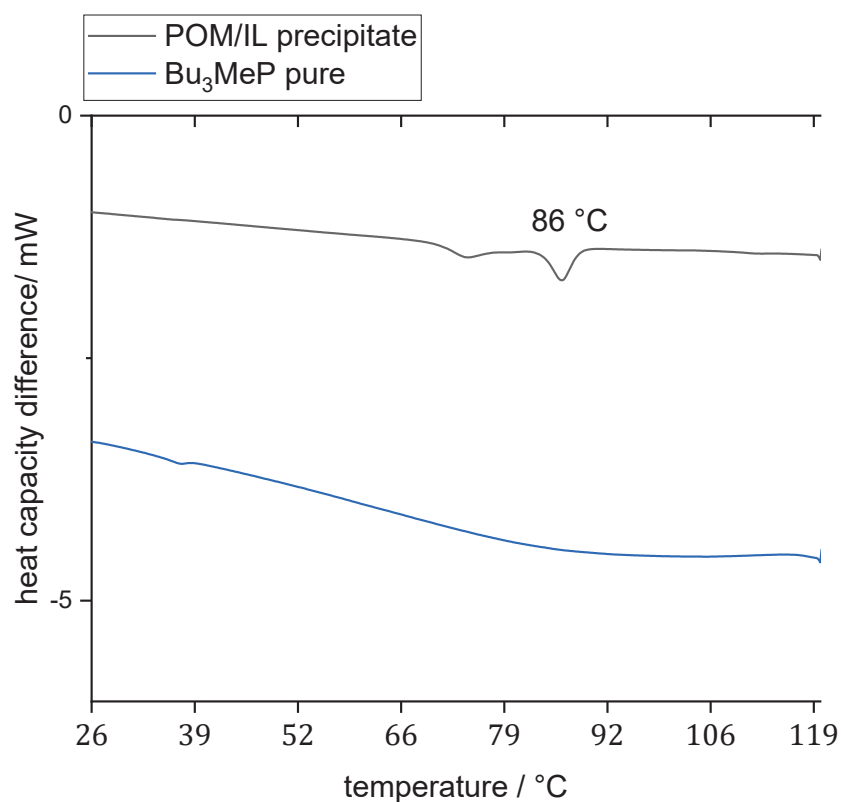


Figure S17: Comparison of DSC measurements of the POM/IL precipitate and the pure IL TBMP.

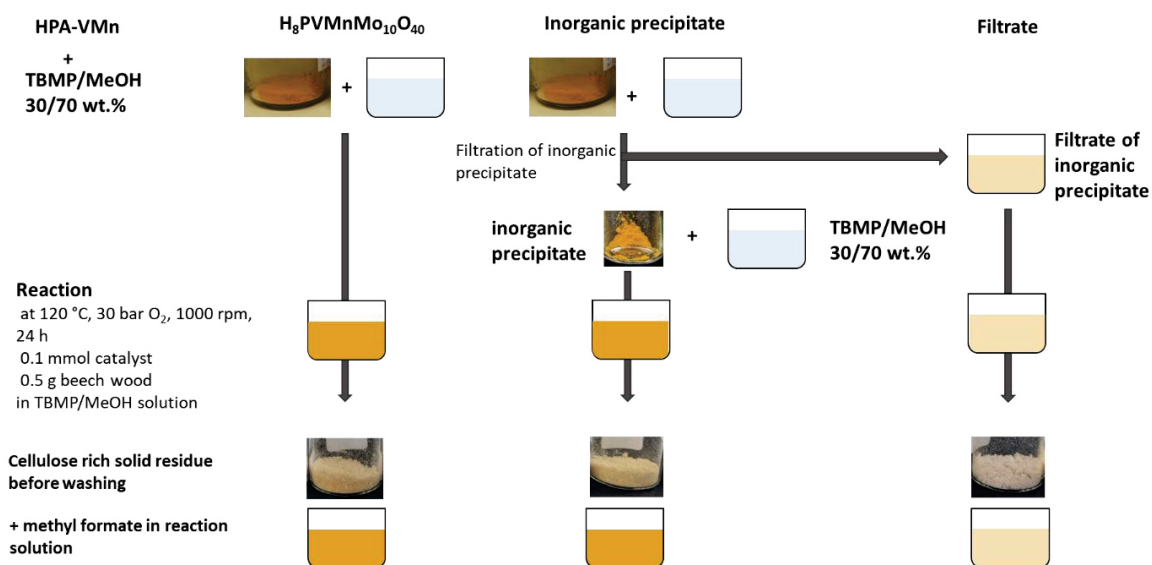


Figure S18: Schematic experiment flow sheet for better understanding.

Time dependent optimization study

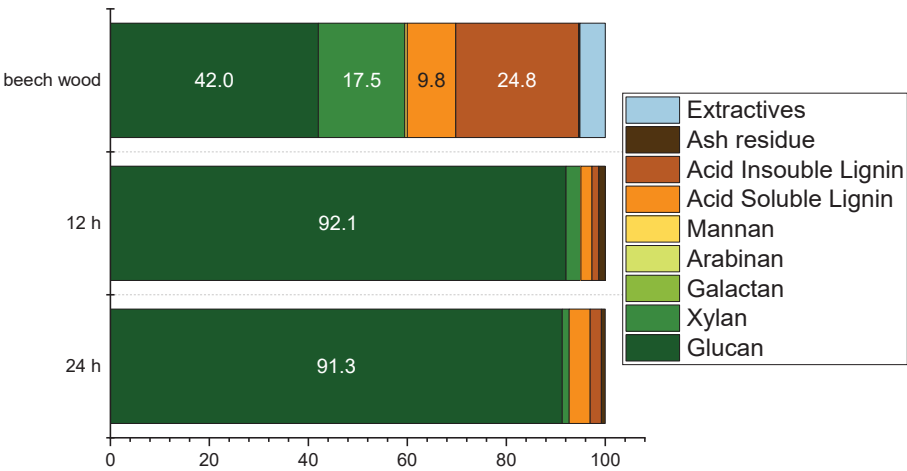


Figure S19: Compositional analysis via NREL of beech wood compared to cellulose-enriched solid residue after 12 h and 24 h of reaction time.

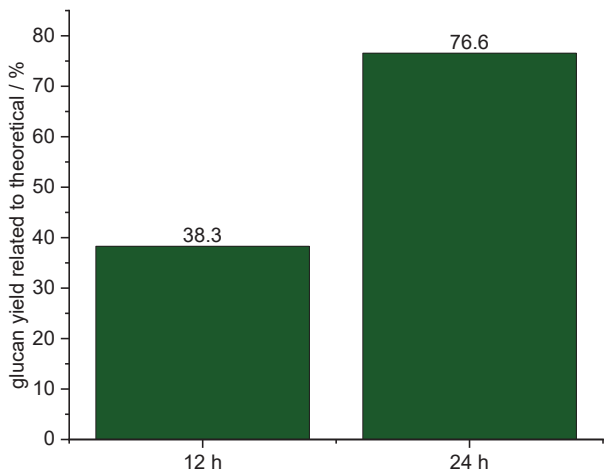


Figure S20: Enzymatic saccharification of the cellulose-rich solid after 12 h and 24 h of reaction time.

Separation and purification of reaction products

a) Processed solids

Table S5: Elemental content of V, Mo, P, S on solid after different washing procedures.

Washing agent/ element content found on solid	Vanadium / wt.% determined via ICP-OES	Molybdenum / wt.% determined via ICP- OES	Phosphorous / wt.% determined via ICP- OES	Sulphur / wt.% determined via CHNS
MeOH	0.24	14.41	1.69	0.27
MeOH + acetone	0.01	0.06	0.05	0.23
MeOH + DMSO	<0.03	<0.03	<0.03	30.43

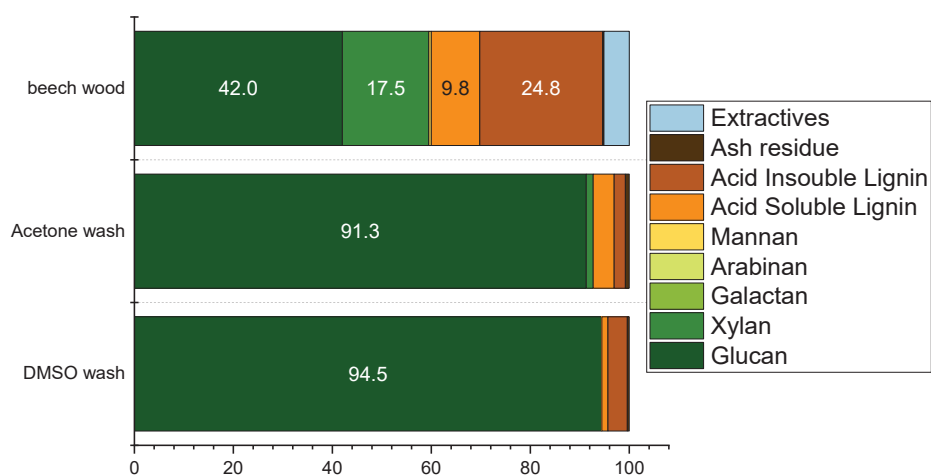


Figure S21: Comparison of compositional analysis of pure beech wood and the different washing agents acetone and DMSO.

b) Liquid reaction product

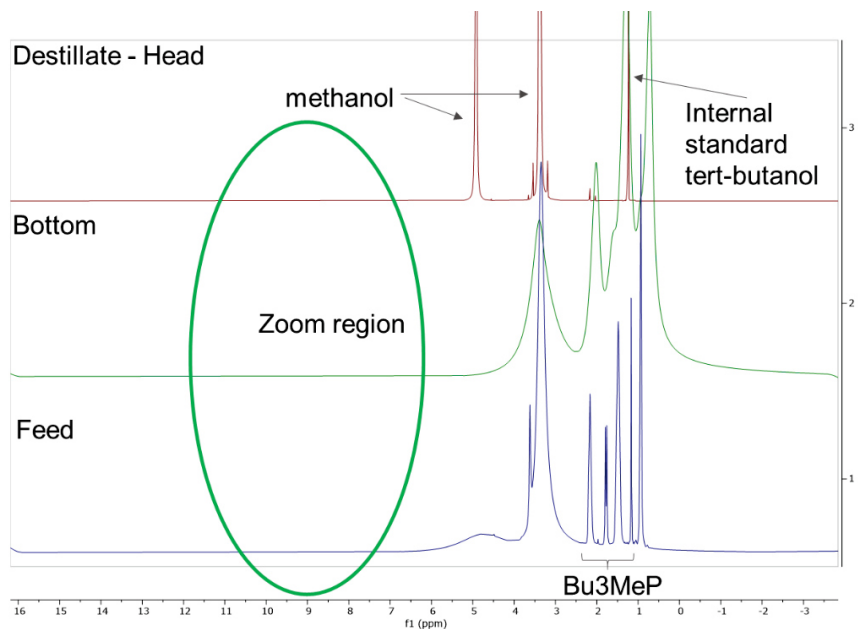


Figure S22: Comparison of the feed, the bottom of the distillation and the head in the ^1H NMR spectrum.

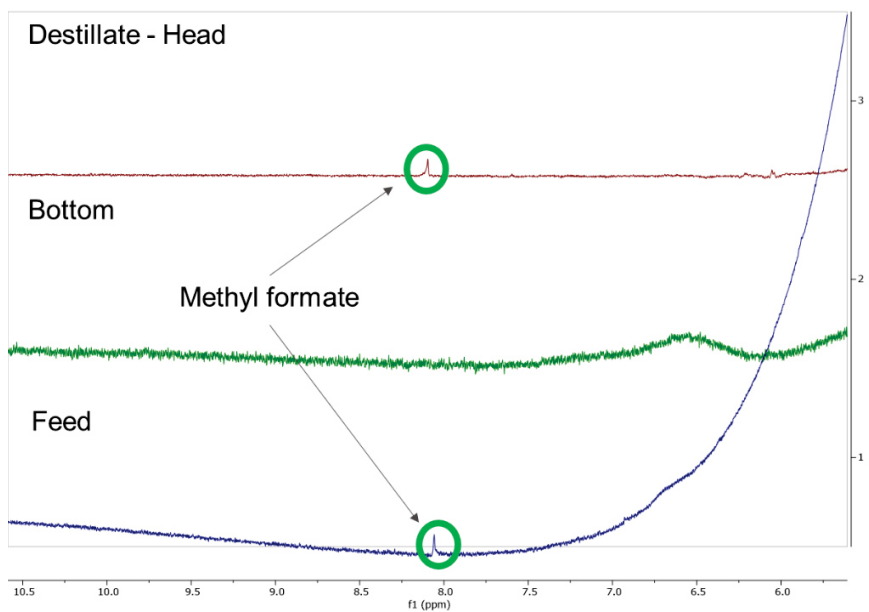


Figure S23: Zoom region of the comparison of the feed, the bottom of the distillation and the head in the ^1H NMR spectrum.

Substrate Screening

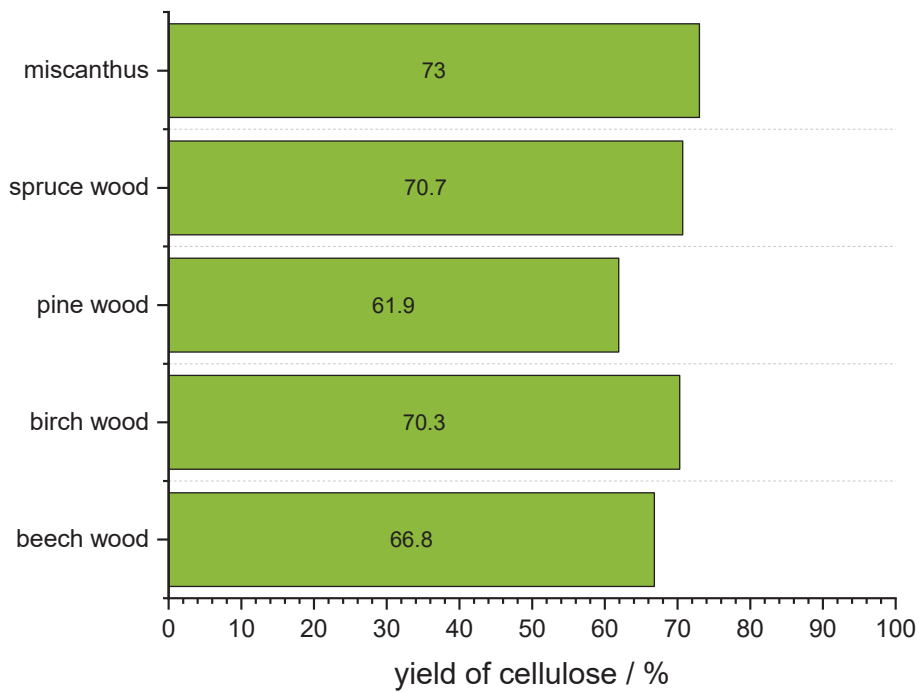


Figure S24: Yield of cellulose which could be extracted to the cellulose-enriched pulp from the initial substrate.

Table S6: All controlled experiments for the substrate screening in TBMP with HPA-VMn.

	yield MF / %	error	yield CO ₂ / %	error	yield CO / %	error	conversion in terms of mass / %	error
Pine wood								
1	15.01		3.83		0.46		71.56	
2	14.57		6.5		1.08		70.6	
3	17.9		5.06		0.83		68.8	
average	15.83	1.48	5.13	1.09	0.79	0.25	70.32	1.14
spruce wood								
1	15.47		4.66		0.79		66.03	
2	5.89		6.81		0.93		69.19	
3	8.67		4.93		0.7		68.96	
average	10.01	4.02	5.47	0.96	0.81	0.09	68.06	1.44
Birch wood								
1	16.29		4.27		0.66		70.32	
2	17.08		6.45		0.95		75.02	
3	18.98		5.74		0.88		73.98	
average	17.45	1.13	5.49	0.91	0.83	0.12	73.11	2.02
Miscanthus								
1	23.06		5.38		0.77		61.44	
2	17.23		3.06		0.43		67.8	
3	16.27		5.26		0.84		67.4	
average	18.85	3.00	4.57	1.07	0.68	0.18	65.55	2.91
beech wood								
1	21.7		4.36		0.8		72.1	
2	15.34		4.08		0.91		73.2	
3	16.05		5.31		0.83		69.65	
4	16.98		4.76		0.99		60.98	
5	21.46		5.51		0.95		59.02	
6	22.25		4.61		1.34		58.07	
7	19.97		3.43		0.84		75.07	
8	20.23		3.32		0.84		73.3	
9	19.83		3.44		0.79		73.88	
10	21.9		3.09		0.72		74.00	
average	19.83	2.44	4.19	0.82	0.91	0.16	69.27	6.24

Table S7: Control experiments for TBMP with beech wood.

	conversion in terms of mass/ %	yield MF/ %	yield CO ₂ /%	yield CO/ %
Without catalyst				
1	69%		0.0%	1%
2	64%		4.3%	2%
3	65.63%		3.0%	2.00%
4	66.22%		6.9%	2.12%
5	66.23%		2.1%	1.70%
6	64.14%		2.7%	2.30%
7	65.16%		2.03%	1.34%
8	66.12%		1.88%	1.13%
9	67.16%		2.19%	1.13%
average	65.9%		2.8%	1.6%
error	1.3%		1.8%	0.4%
HPA-5				
1	69.0%		13.9%	3.60%
2	69.80%			
3	68.60%		3.6%	3.84%
4	69.10%		10.2%	6.89%
5	70.30%		2.8%	6.51%
6	67.50%		5.5%	2.49%
7	66.90%		9.1%	3.67%
8	69.90%		2.1%	3.96%
9	70.50%		3.7%	3.75%
10				
average	69.1%		6.3%	4.3%
error	1.1%		3.9%	1.6%

Table S8: Control experiments for DMBA with beech wood.

	conversion in terms of mass/ %	yield MF/ %	yield CO ₂ / %	yield CO/ %
HPA-5				
1	58%	2.9%	2.86%	0.59%
2	63%	1.8%	2.94%	0.54%
3	72.2%	6.1%	4.28%	0.93%
Average	64.4%	2.7%	2.6%	0.7%
Error	5.8%	2.2%	1.5%	0.2%
Without catalyst				
1	71%	4.3%	2.83%	0.0091
2	62 %	5.1%	4.91%	1.32%
3	No data (n.d)	4.8%	4.69%	1.25%
4	n.d	10.6%	3.21%	1.04%
5	n.d	5.0%	2.77%	0.86%
Average	66.3%	5.9%	3.7%	1.1%
Error	4.2%	2.3%	0.9%	0.2%

Table S9: Control experiments for TEA with beech wood.

	conversion in terms of mass/ %	yield MF/ %	yield CO ₂ / %	yield CO/ %
Without catalyst				
1	46.26%	0.55%	0.01%	0.00%
2	50%	0.1%	3.50%	0.00%
3	35%	0.2%	1.65%	0.00%
4	73.70%	0.0%	0.54%	0.00%
5	44.80%	0.1%	n.d.	0.00%
Average	49.87%	0.18%	1.32%	0.00%
Error	12.85%	0.19%	1.46%	0.00%
Without catalyst				
1	33.60%	0.16%	0.57%	0%
2	24%	0.2%	1%	0.00%
3	16.20%	0.18%	0.42%	0.14%
4	15.80%	0.23%	0.44%	0.12%
5	17.95%	0.3%	0.44%	0.13%
6	18.98%	0.3%	0.37%	0.13%
7	19.00%	0.2%	0.37%	0.13%
8	17.60%	0.27%	0.41%	0.15%
9	17.64%	0.28%	0.38%	0.14%
10	16.88%	0.33%	0.42%	0.13%
Average	19.80%	0.19%	0.52%	0.11%
Error	5.13%	0.41%	0.29%	0.05%

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Supporting Information of ProFA – valorization of macroalgaee biomass as a source of proteins and formic acid

In the following pages the Supporting Information for the publication ProFA – valorization of macroalgae biomass as a source of proteins and formic acid^[287] is shown.

ProFA - Valorization of macroalgae biomass as source of proteins and formic acid

Supporting Information

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Synthesis and brief characterization of the catalysts

Heteropoly acids can be produced from their respective metalates by condensation in an acidic environment. For the synthesis of HPA-x ($H_{3+x}[PV_xMo_{12-x}O_{40}] \cdot y H_2O$), the solution is usually prepared from solutions of their salts (V_2O_5 and MoO_3). The catalyst was synthesized in two steps and the procedure is explained below. For HPA-2 and HPA-5 the stoichiometry has been adjusted accordingly, but the synthesis recipe remains the same. These are shown in Table S3.

Table S1: Needed components for the synthesis of HPA-x ($H_{3+x}[PV_xMo_{12-x}O_{40}] \cdot y H_2O$) catalysts as example HPA-2 and HPA-5.

Step	Material	m or V for HPA-2	m or V for HPA-5
A	Dest. H ₂ O	350 mL	750 mL
	V ₂ O ₅	8 g	20.0 g
	H ₂ O ₂ (30 % in H ₂ O)	66 mL	165 mL
	H ₃ PO ₄ (25 % in H ₂ O)	1.2	3.0 g
B	Dest. H ₂ O	500 mL	500 mL
	MoO ₃	63.38 g	44.3 g
	H ₃ PO ₄ (25 % in H ₂ O)	23.6 g	16.9 g

Step A - Synthesis of Vanadium (V) solution

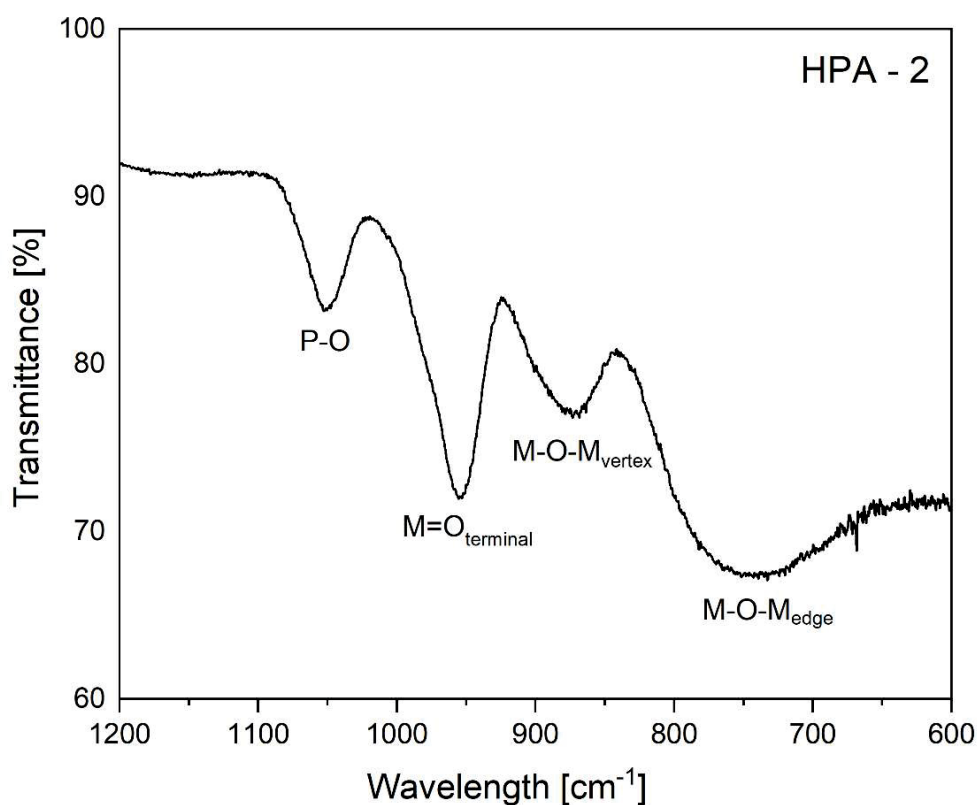
V₂O₅ and H₂O₂ (30 % in H₂O) were added to dest. water at 4 - 7 °C and stirred at 420 rpm. The mixture was stirred till it turned dark red due to the formation of Vanadium(V) peroxy compounds. The solution was then allowed to warm up to room temperature but was maintained below the temperature of 35 °C. With an increase in temperature, the color of the solution changed from dark red to brown orange along with gas evolution (in both cases). Once the gas evolution was completed, H₃PO₄ (25 % in H₂O) was added to the mixture turning the mixture into dark brown in color and was stirred for 45 minutes.

Step B - Synthesis of HPA-2 solution

MoO₃ was added along with H₃PO₄ (25 % in H₂O) to distilled water. The solution was stirred and heated to boiling in a round bottom flask with a reflux condenser. After some time, the solution turned yellow in colour to which the solution from Step A was added gradually. The solution thus formed was (dark) cherry-red in color. The final solution was evaporated in a rotary evaporator at 85 °C and reduced pressure (180 mbar to 40 mbar). The solid was dried overnight under the same conditions (85 °C, 180 mbar to 40 mbar).

Characterization of HPA-5 and HPA-2

HPA-2



FigureS1: FT-IR spectra of a Keggin POM on the example of HPA-2.

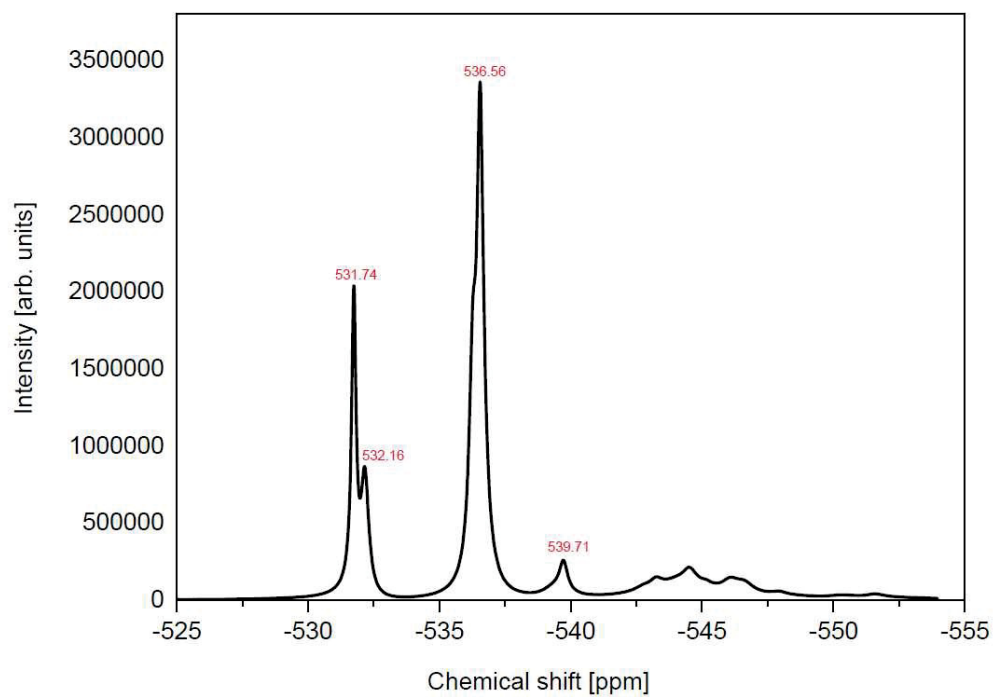


Figure S2: ^{51}V -NMR spectra of HPA-2.

HPA-5

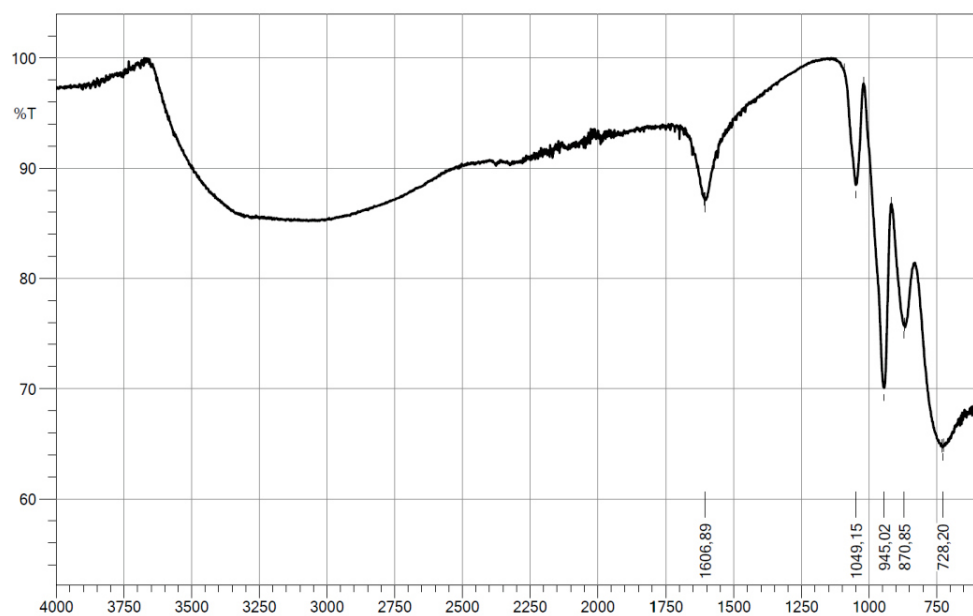


Figure S3: FT-IR spectra of a Keggin POM on the example of HPA-5.

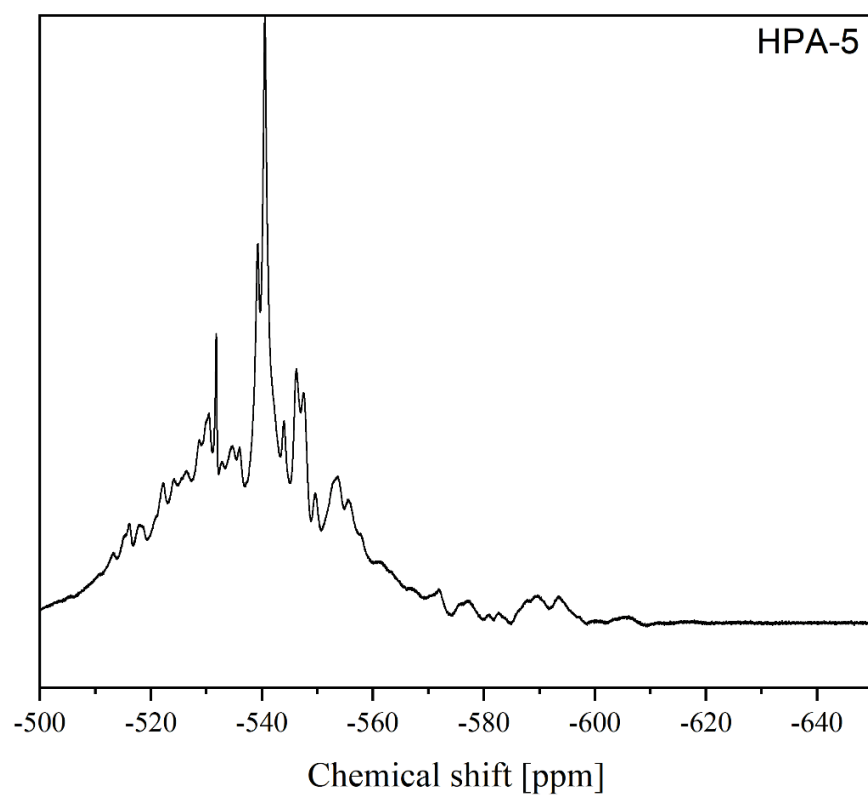


Figure S4: ^{51}V -NMR spectra of HPA-5.

Amino acid profiles of the macroalgae species

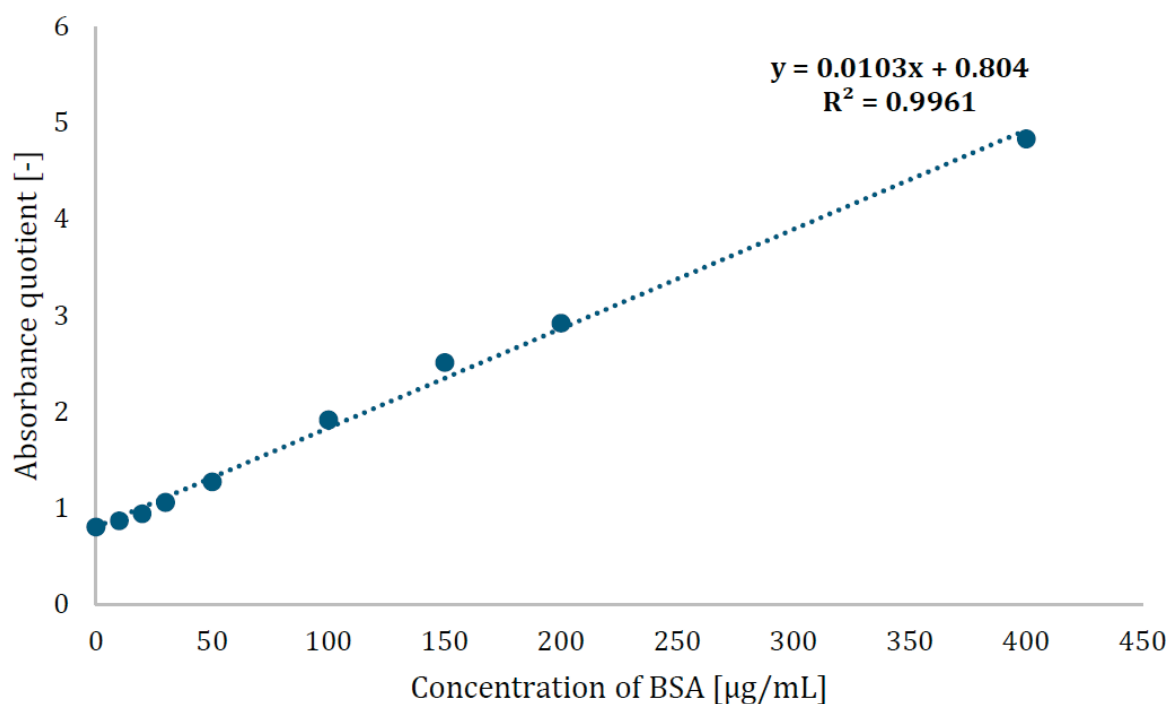


Figure S5: Bradford Assay calibration curve.

The most abundant amino acid in the porphyra dioica sample are alanine (14.6 %) and aspartic acid (12.7 %) (Supporting Information, Figure S2), so a hydrophobic and an hydrophilic amino acid. Ulva fenestrata and fucus vesiculosus, on the contrary, has high amino acid values of the amino acids glutamic acid (12.2 % and 25.2 %) and aspartic acid (14.5 % and 16.2 %), i.e. a high proportion (approx. 27 % and 42 %) of hydrophilic acids (Supporting Information, Figure S3 and Figure S4). The other amino acids present in the fucus vesiculosus are below 8 %. This means that this brown algae is not a favored substrate for the production of biopolymer films for the current stage.

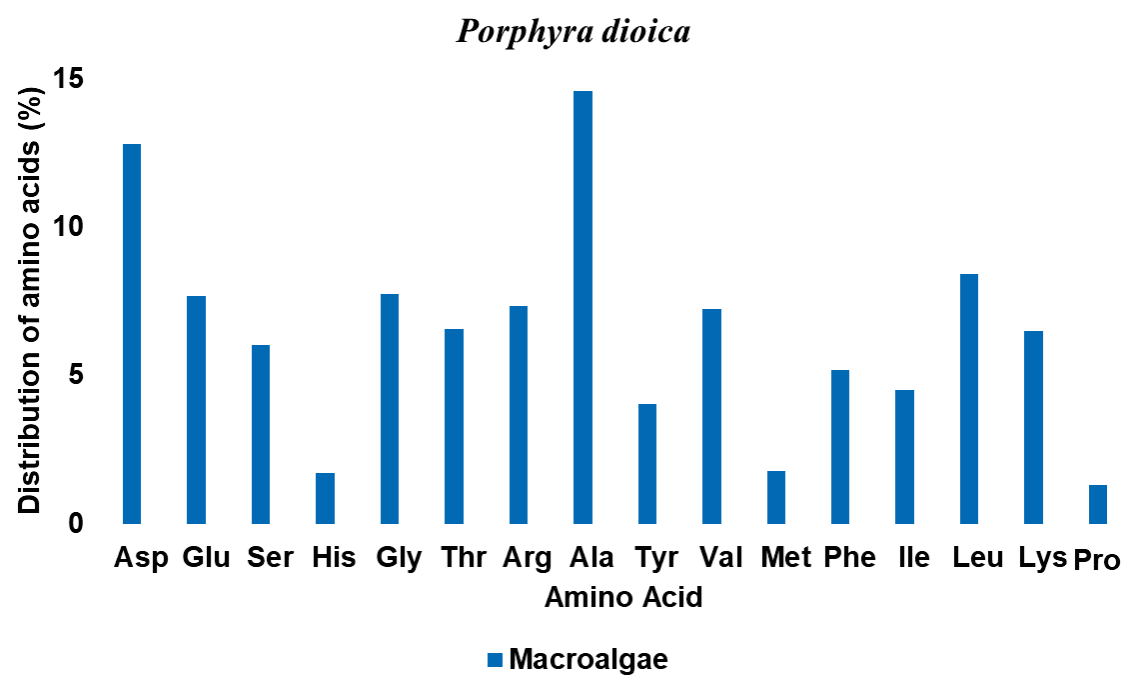


Figure S6: Amino acid profile of *Porphyra dioica*.

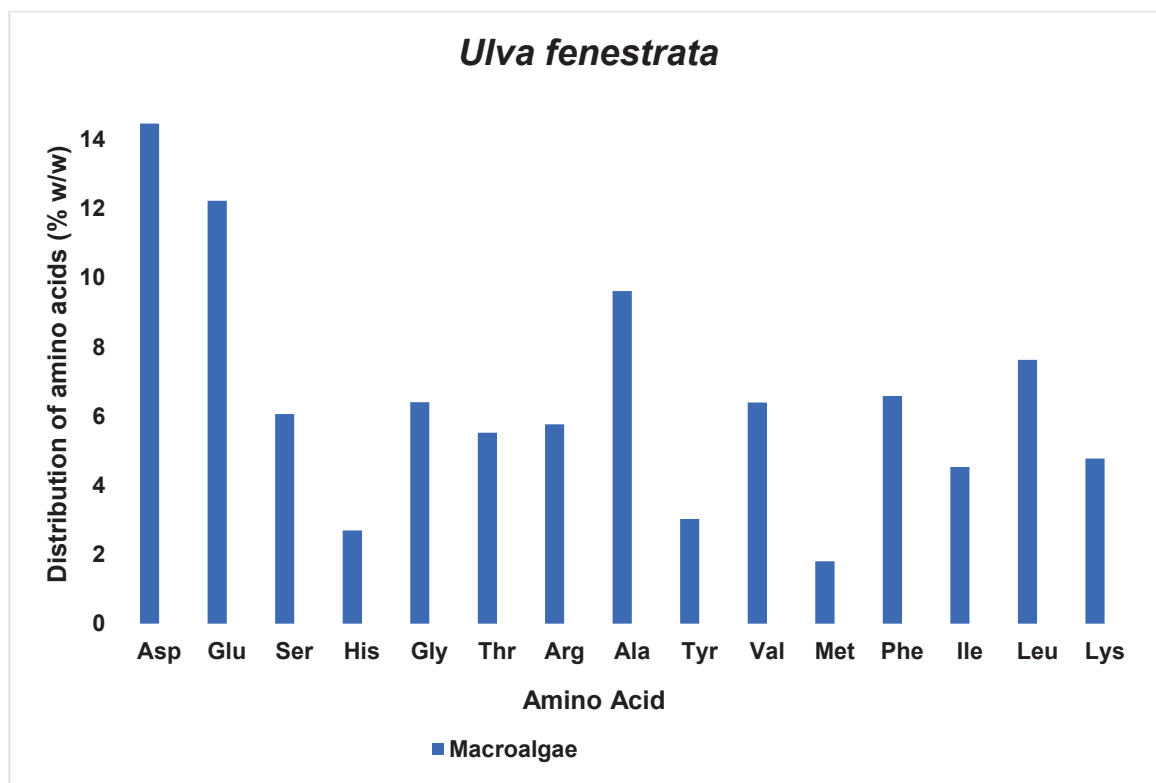


Figure S7: Amino acid profile of *ulva fenestrata*.

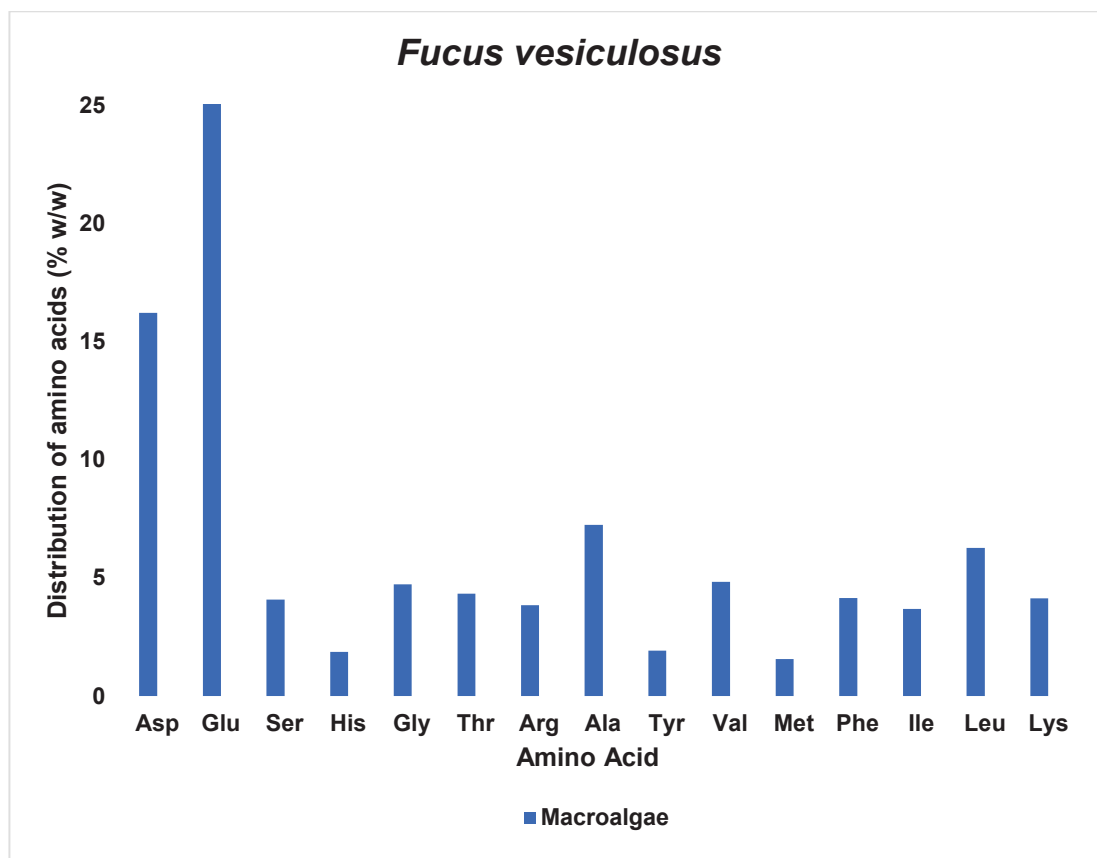


Figure S8: Amino acid profile of fucus vesiculosus.

Elemental analysis of the macroalgae species

Table S1: Elemental analysis of the macroalgae samples.

Entry	Substrate	C (wt.%)	N (wt.%)	H (wt.%)	S (wt.%)	O (wt.%)
1	Porphyra dioica	33	4.7	4.8	1.7	55.8
2	Fucus vesiculosus	32	1.4	3.6	2.1	60.9
3	Ulva fenestrata	37	2.7	4.4	5.3	60.6

Table S2: Elemental analysis of the solid residues after the OxFA.

Entry	Solid residue of	C (wt.%)	N (wt.%)	H (wt.%)	S (wt.%)	O (wt.%)
1	ulva fenestrata	29	4.3	4.6	0.23	61,9
2	fucus vesiculosus	43	5.4	3.4	0.32	47,9
3	porphyra dioica	29	3.7	6.7	0.36	60,2

Electrochemical data from the two screened catalysts

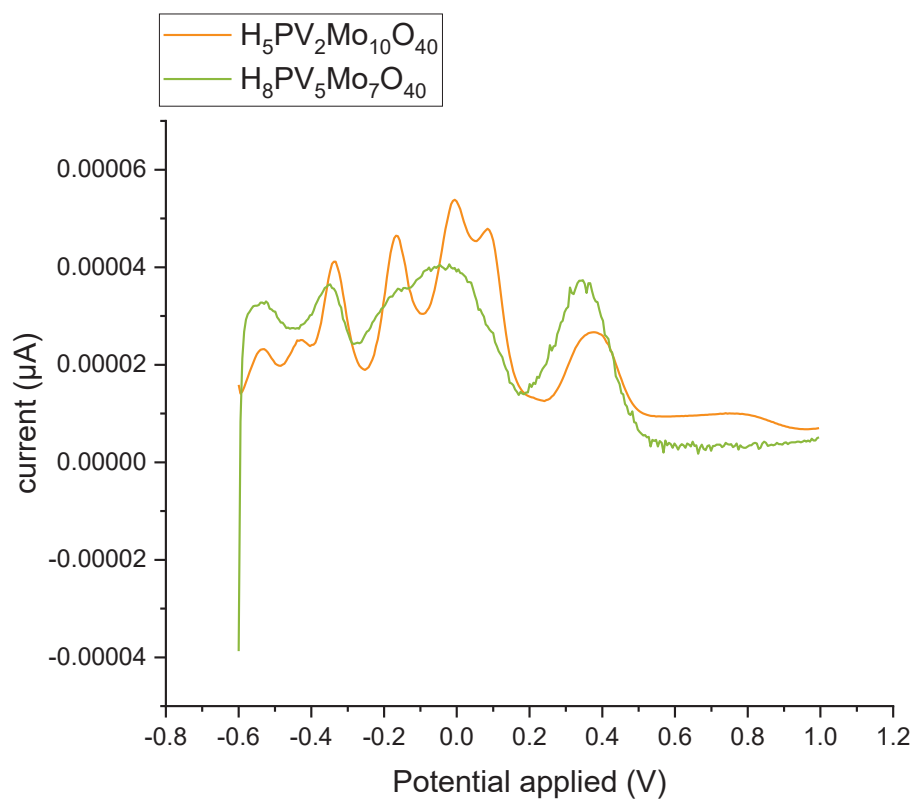


Figure S9: SWV data of the POMs HPA-2 ($\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$, orange) and HPA-5 ($\text{H}_8\text{PV}_5\text{Mo}_7\text{O}_{40}$, green). The signals between -200 mV and 200 mV merge with increasing V(V) content. ^{1,2}

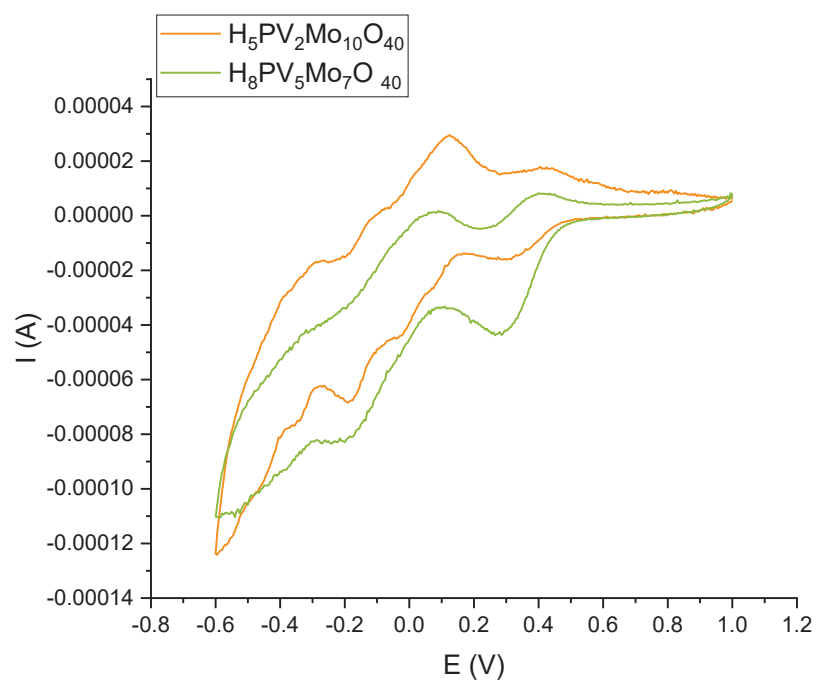


Figure S10: CV data of the POMs HPA-2 ($\text{H}_5\text{PV}_2\text{Mo}_{10}\text{O}_{40}$, orange) and HPA-5 ($\text{H}_8\text{PV}_5\text{Mo}_7\text{O}_{40}$, green). The signals between -200 mV and 200 mV merge with increasing V(V) content.^{1,2}

Amino acid profiles of macroalgae and solid residue

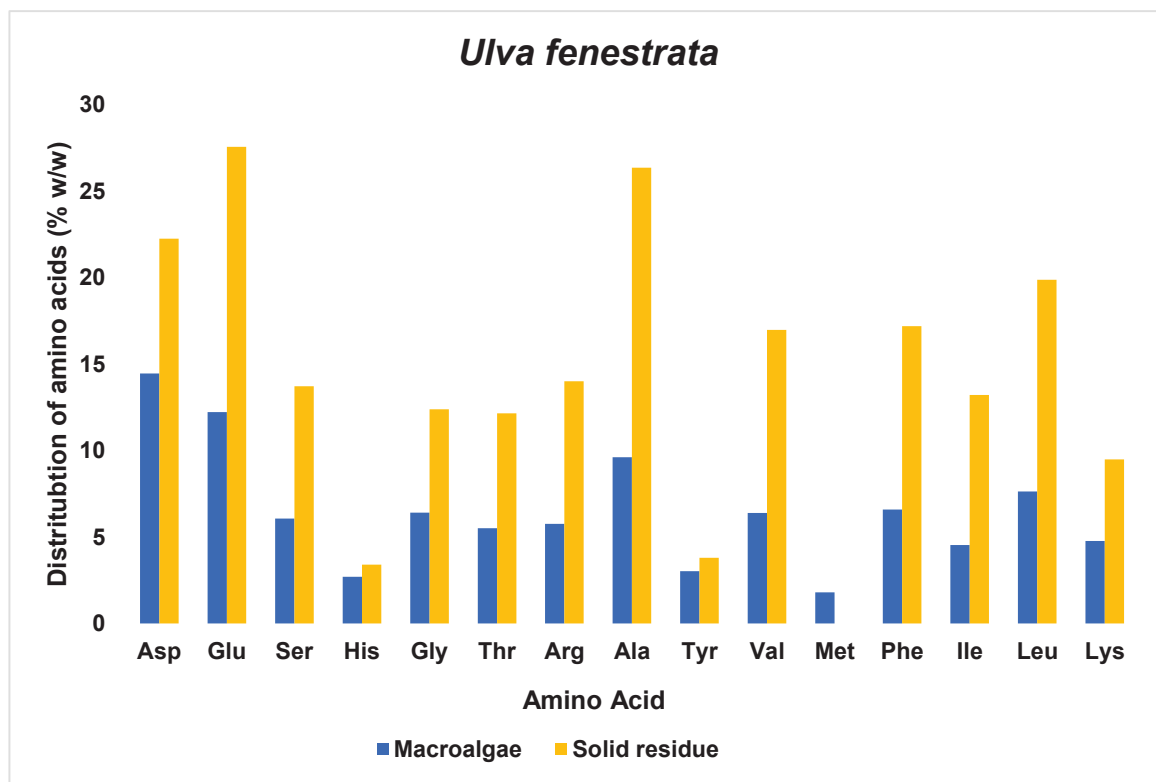


Figure S11: The amino acid profile for the macroalgae *ulva fenestrata* and its solid residue after the OxFA process. Reaction conditions: $H_5PV_2Mo_{10}O_{40}$ at initial temperature 90 °C, non-isothermal reaction, 30 bar oxygen pressure, 1000 rpm stirrer speed, 24 h reaction time. The solvent was 200 g of water with 10 g suspended macroalgae and 1.952 g of dissolved catalyst (1 mmol, $H_5PV_2Mo_{10}O_{40}$).

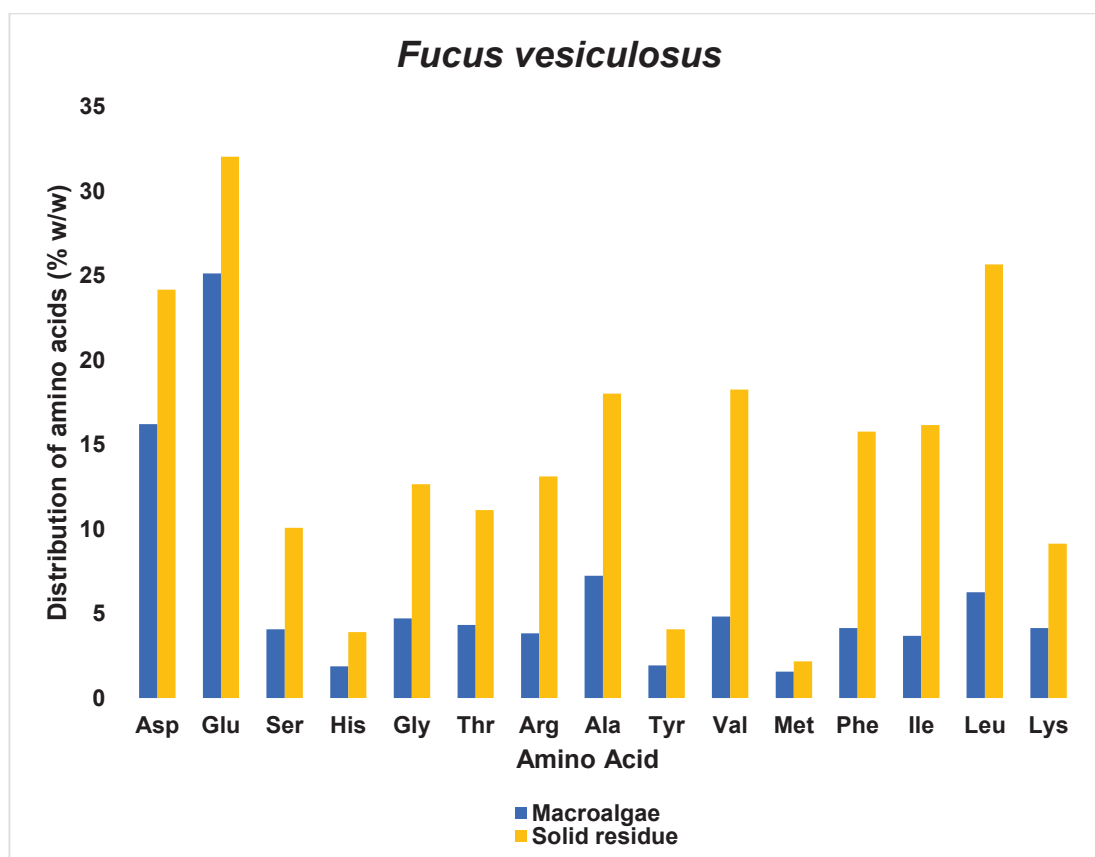


Figure S12: The amino acid profile for the macroalgae *Fucus vesiculosus* and its solid residue after the OxFA process. Reaction conditions: H5PV2Mo10O40 at initial temperature 90 °C, non-isothermal reaction, 30 bar oxygen pressure, 1000 rpm stirrer speed, 24 h reaction time. The solvent was 200 g of water with 10 g suspended macroalgae and 1.952 g of dissolved catalyst.

Characterization of 2nd batch of porphyra dioica in comparison with the 1st batch

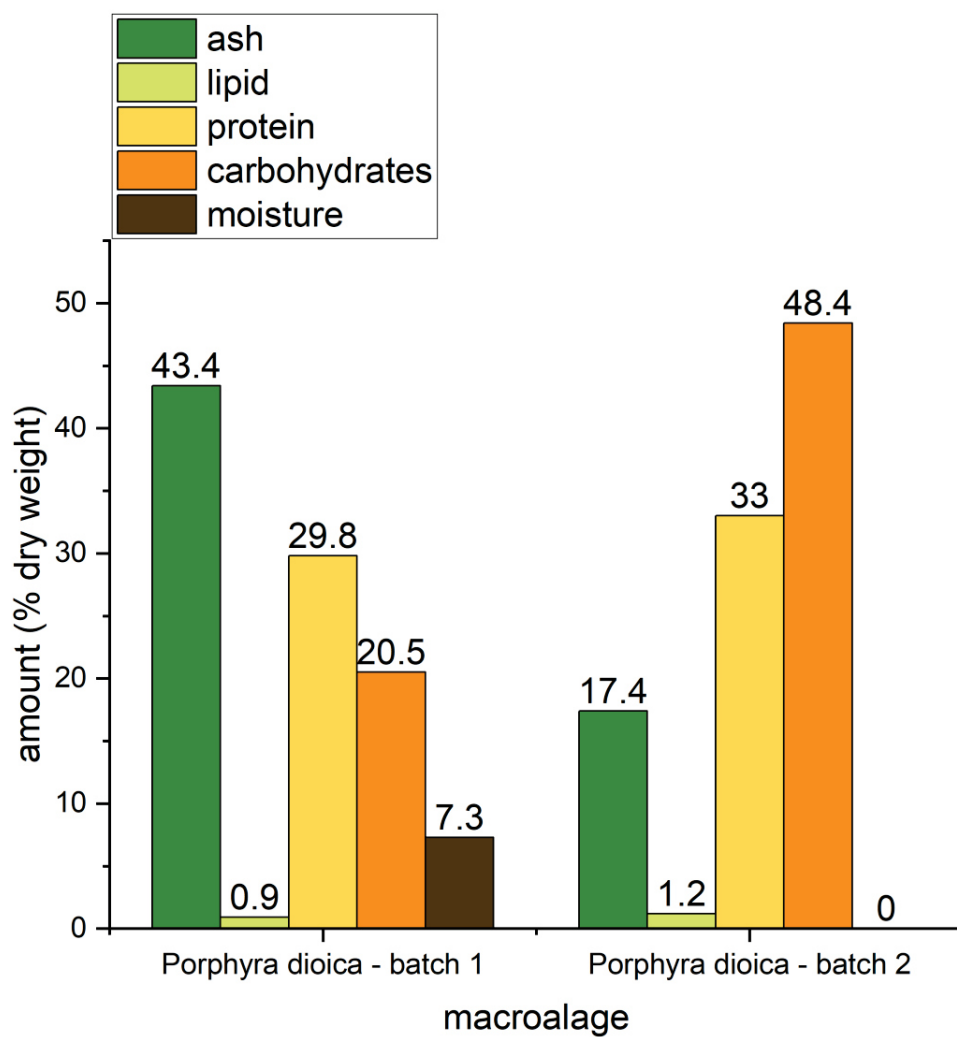


Figure S13: Comparison of the composition of the two different batches of porphyra dioica used.

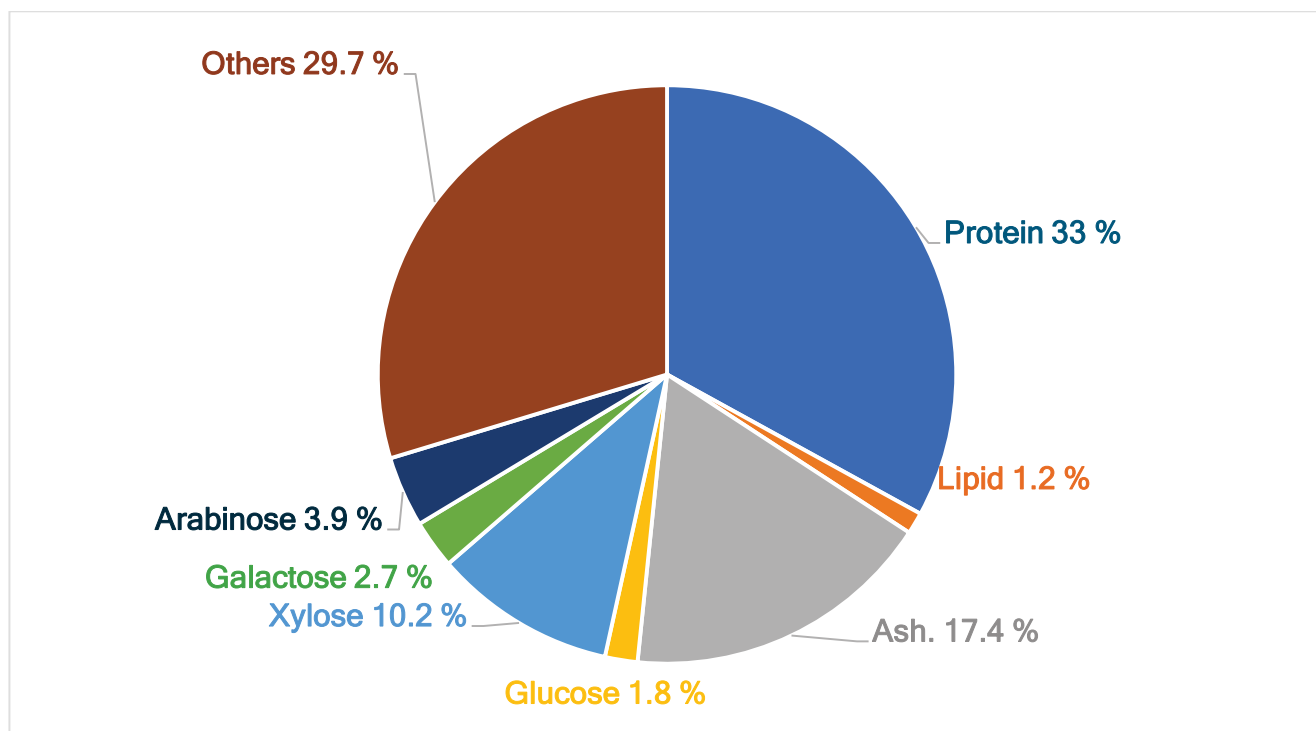


Figure S14: Composition of the porphyra dioica batch 2 with the detailed information about the carbohydrate composition determined via the NREI TP 510-42618.³

Table S3: Comparison of the two batches porphyra dioica via CHNS-elemental analysis.

Entry	Substrate		C (wt.%)	N (wt.%)	H (wt.%)	S (wt.%)	O (wt.%)
1	Porphyra batch 1	dioica	33	4.7	4.8	1.7	55.8
2	Porphyra batch 2	dioica	36	5	6	1	52

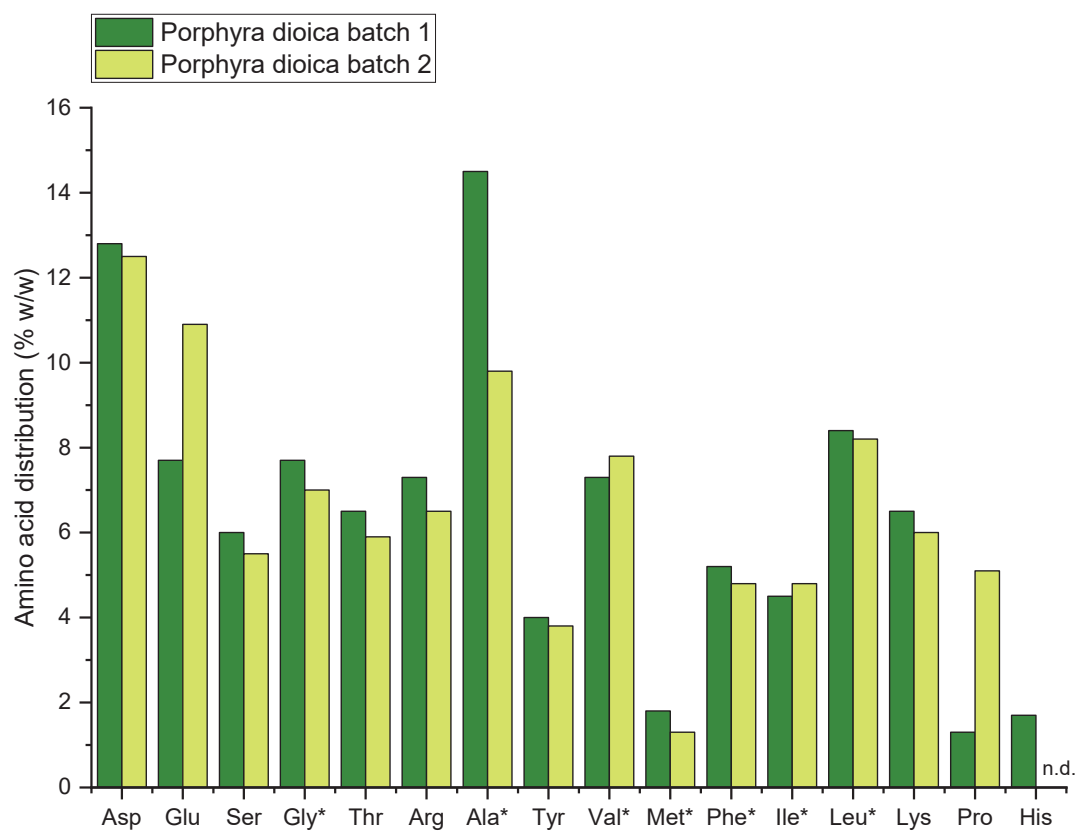


Figure S15: Amino acid distribution for the two used porphyra dioica batches. Hydrophilic acids are labelled with *.

Additional information about the DoE

Table S4: Box-Behnken experimental design for *Porphyra dioica*.

Entry	Duration (h)	Temperature (°C)	Catalyst/substrate ratio ($\frac{g_{catalyst}}{g_{substrate}}$)
1	18	100	0.5
2	24	100	0.275
3	24	120	0.5
4	24	100	0.275
5	24	80	0.5
6	18	100	0.05
7	24	80	0.05
8	30	100	0.5
9	24	100	0.275
10	18	80	0.275
11	30	120	0.275
12	24	120	0.05
13	18	120	0.275
14	30	100	0.05
15	30	80	0.275
Fixed reaction conditions: 30 bar oxygen pressure, 1000 rpm stirrer speed, 10 g of macroalgae <i>Porphyra dioica</i> as substrate, 200 g water as solvent.			

Protein recovery

Table S5: Detailed statistical evaluation from ANOVA for protein recovery.

Source	Sum of Squares	Mean Square	F-value	p-value	significant?
Model	4629.46	4629.46	90.15	< 0.0001	yes
A-temp	4629.46	4629.46	90.15	< 0.0001	
Residual	667.55	51.35			
Lack of Fit	660.59	60.05	17.26	0.0560	no
Pure Error	6.96	3.48			
Cor Total	5297.01				

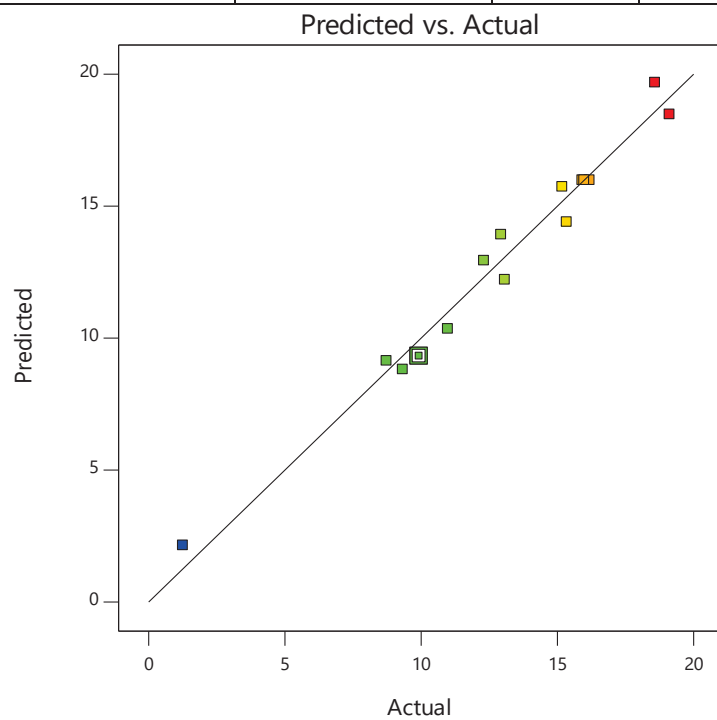


Figure S16: The fit comparison of the actual value to the predicted value for protein recovery. The values represented are protein recovery / %

Yield of formic acid

Table S6: Detailed statistical evaluation from ANOVA for the yield of formic acid.

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	285.01	31.67	31.68	0.0007	significant
A-temp	17.04	17.04	17.05	0.0091	
B-time	2.92	2.92	2.92	0.1480	
C-ratio	174.17	174.17	174.26	< 0.0001	
AB	4.79	4.79	4.79	0.0803	
AC	18.09	18.09	18.10	0.0081	
BC	1.95	1.95	1.95	0.2216	
A²	59.71	59.71	59.74	0.0006	
B²	0.0343	0.0343	0.0344	0.8602	
C²	9.21	9.21	9.21	0.0289	
Residual	5.00	0.9995			
Lack of Fit	4.96	1.65	87.25	0.0114	significant
Pure Error	0.0379	0.0189			
Cor Total	290.01				

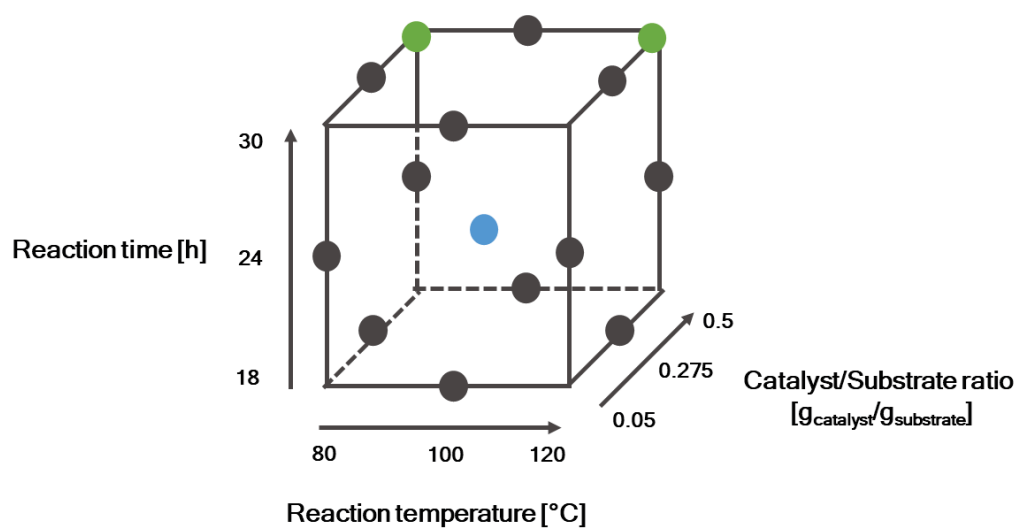


Figure S17: The Box-Behnken-Design including the verification runs which are represented in the edges of the cube in green colour.

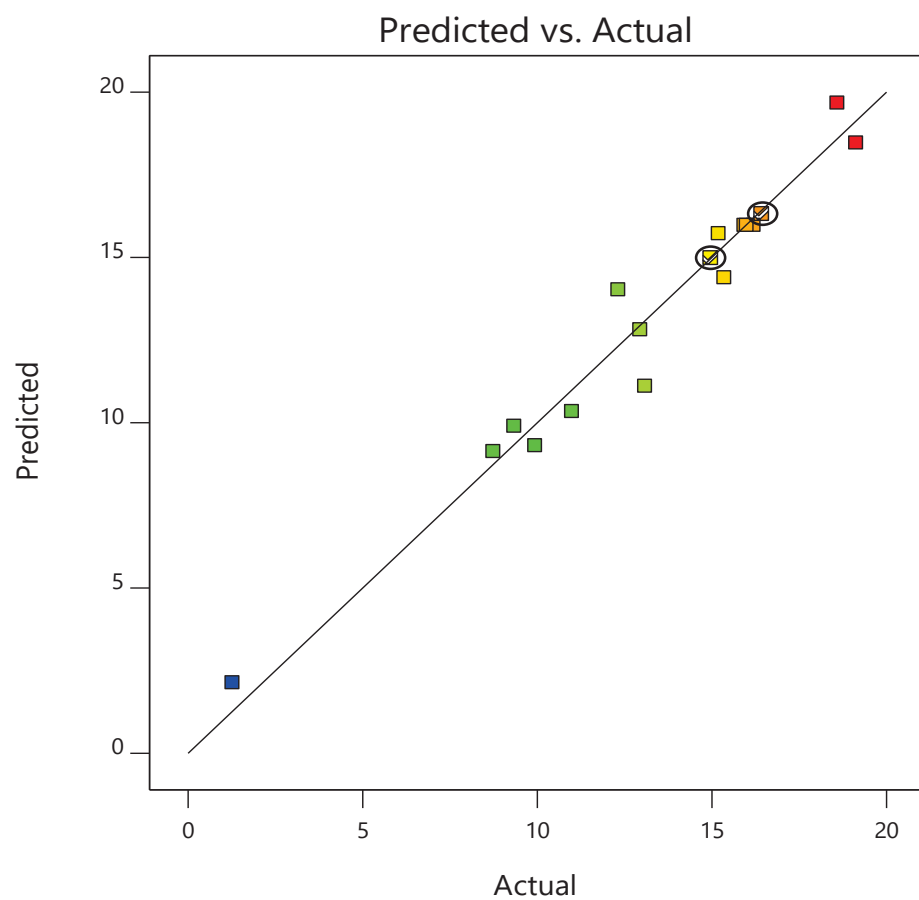


Figure S18: The fit comparison of the actual value to the predicted value for the verification experiments. The circled points indicate the verification experiments. The values presented are yield of formic acid / %.

Additional Information for the protein extraction

SDS-PAGE profiles

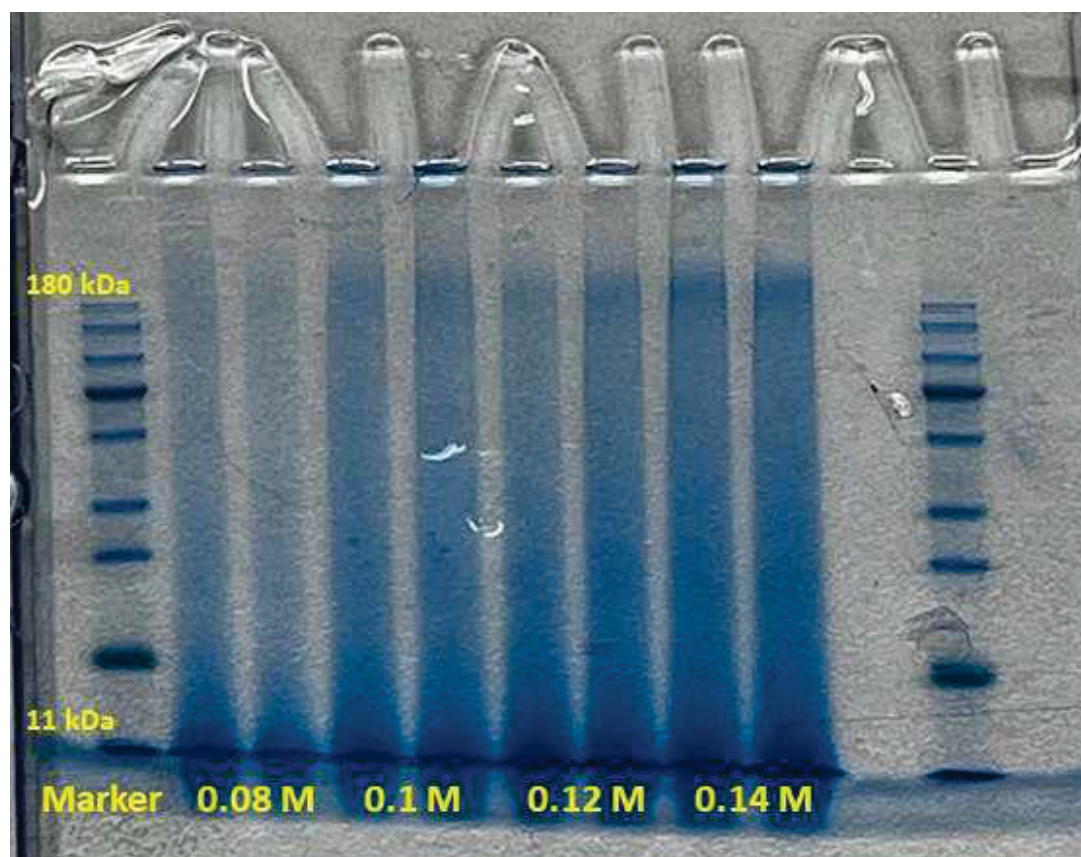


Figure S19: The SDS-PAGE profile for alkaline hydrolysis.

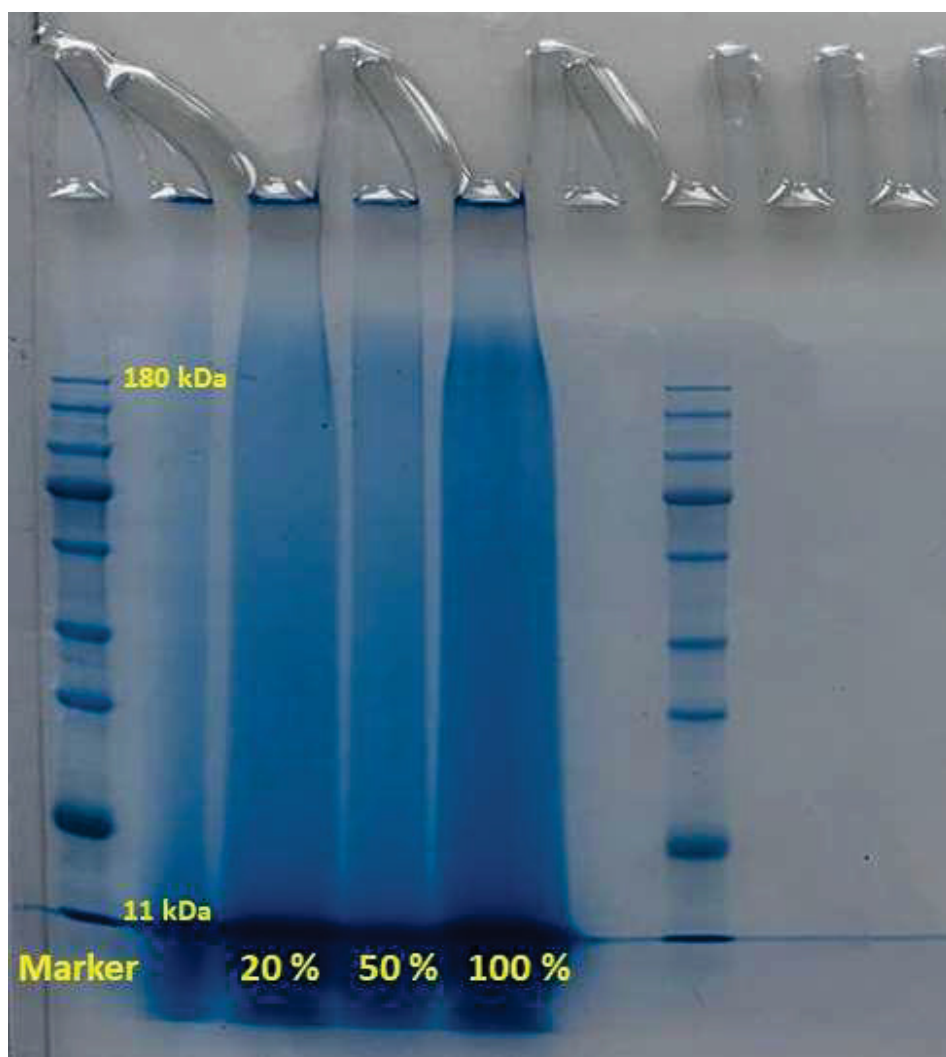


Figure S20: The SDS-PAGE gel electrophoresis profile for the ultrasonic alkaline extraction.

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