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Production of Microbial Hydrocolloids from Renewable Resources

Steven Koenig

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Dedication

This work is dedicated to *friendship*.

Abstract of 'Bacterial Conversion of Lignocellulose Hydrolysate to Exopolysaccharides'

A bank of exopolysaccharide-producing microorganisms was successfully subjected to a multi-step screening process to single out strains with robust growth and exopolysaccharide production in the presence of the commonly encountered growth inhibitors in lignocellulose hydrolysate: furfural, hydroxymethylfurfural, vanillin, formic acid, acetic acid and laevulinic acid. The best strains were tested in more detail regarding growth and production and the strain *Paenibacillus* 2H7 was selected for comparative fermentations at the 500 ml scale. The furfural concentration correlated with the onset of the exponential phase and from the insights of the small-scale fermentations an improved fermentation strategy for the 7 l scale was derived.

D-Xylose Screening 191 exopolysaccharide-producing bacteria were tested for their D-xylose utilization capabilities and 135 (71 %) were found to grow well with D-xylose as the only carbon source. The good-growing strains were transferred to new approximately one and a third 96-well plates and used in the next screening steps.

High-Content Screening with D-Xylose 95 D-xylose-consuming strains were grown on a D-xylose-rich medium for 48 h and D-xylose consumption, exopolysaccharide aldose monomer composition and production were assessed. 66 strains (69 %) consumed at least half the D-xylose. 13 strains (14 %) were found to produce at least 560 $mg \cdot l^{-1}$ of exopolysaccharide. A comparison of the exopolysaccharide aldose monomer composition with data from Rühmann *et al.* [1] showed major and minor differences in two strains each. The other strains were not affected. It is hypothesized that apparent exopolysaccharide compositional changes were not caused by a changed composition of a single exopolysaccharide, but rather the production of different exopolysaccharides at different levels.

High-Throughput Screening for Inhibitor Tolerance The 135 D-xylose-utilizing strains were screened in the presence of one and only one lignocellulose hydrolysate inhibitor at 2.0 $g \cdot l^{-1}$ to remove non-growing strains from the respective high-content screening (see next step). Vanillin was by far the strongest inhibitor shutting down microbial growth in 79 % of the strains. Furfural and hydroxymethylfurfural totally inhibited 20 % and 7 % of the strains, respectively. Acids did not appreciably inhibit microbial growth. The comparison to lignocellulose hydrolysate showed that using just the 27 and 28, respectively, best strains of vanillin and acetic acid sufficed to predict 57 % of the top 28 strains of lignocellulose hydrolysate.

High-Content Screening with Inhibitors 27 or 28 of the best growing strains per inhibitor were screened to assess inhibitor degradation, exopolysaccharide aldose monomer composition and production. Except for laevulinic acid, all inhibitors were consumed to varying degrees: furfural, hydroxymethylfurfural and formic acid were degraded completely after 48 h in the majority of strains. Acetic acid was degraded in some, produced in other strains, while vanillin's inhibitory potential led to decreased degradation. Laevulinic acid remained untouched in most cases (> 75 %). Changes in the exopolysaccharide compositions based on the inhibitor were not observed.

Strain Selection Among the seven final candidate strains selected based on the previous results, the strain *Paenibacillus* 2H7 exhibited the most robust growth and also the most robust

exopolysaccharide production in the presence of different inhibitors and was selected for the fermentations. Based on 16S rDNA sequencing, the closest relatives were determined to be different unclassified *Paenibacillus* spp., *P. cineris* and *P. favisporus*.

Parallel Fermentation with Lignocellulose Hydrolysate *Paenibacillus* 2H7 was used in 2 x 4 500 ml parallel fermentations in lignocellulose hydrolysate and a reference with pure D-glucose and D-xylose. The onset of the exponential growth phase in the lignocellulose hydrolysate fermenters was correlated with the furfural concentration and lagged approximately 36 h behind the reference fermentations. The exopolysaccharide molar masses were around $1 \cdot 10^7\,\mathrm{g \cdot mol^{-1}}$ and unaffected by the hydrolysate as long as the pH value was controlled at 7.0. The fermentation courses of all eight fermenters were comparable to each other from the onset of the exponential growth phase on. The results at the 500 ml scale were used to devise improvements to the fermentation parameters at the 7 l scale: lower initial lignocellulose hydrolysate concentration, fed-batch instead of batch and higher initial bacteria concentration.

Abstract of 'Fermentative Production of Scleroglucan and Schizophyllan'

In order to examine the differences between scleroglucan and schizophyllan, eight fermentations of *S. rolfsii* and *S. commune* at the 500 ml scale from 48 h to 144 h were conducted and the exopolysaccharides purified. The exopolysaccharide concentrations at the end of the fermentations increased over time to $3.2\,\mathrm{g \cdot l^{-1}}$ and $1.4\,\mathrm{g \cdot l^{-1}}$ for scleroglucan and schizophyllan, respectively, with one exception: *S. commune* produced $2.2\,\mathrm{g \cdot l^{-1}}$ schizophyllan after 120 h.

The exopolysaccharides exhibited poor solubility which hampered all further analyses. In addition, precipitations of samples showed a sharp drop in the exopolysaccharide concentrations after the 24 h sample due to an adaptation of the protocol to thicker fermentation broths: the samples were diluted 1:10 with ultra-pure water. All other tests—dynamic viscosity and thixotropy, molar mass, periodate test and metabolite analyses—were without clear and reliable results. Suggestions for repetitions of the experiments are discussed and the development of a quantitative fluorometric assay for the β-1,3-β-1,6-glucans scleroglucan and schizophyllan is briefly covered.

Zusammenfassung von „Bakterielle Umwandlung von Lignocellulosehydrolysat zu Exopolysacchariden“

Eine Mikroorganismenbank mit Exopolysaccharidproduzenten wurde erfolgreich einem mehrschrittigen Screeningverfahren unterzogen, um jene Stämme zu finden, die robustes Wachstum und robuste Exopolysaccharidproduktion in Gegenwart von Wachstumsinhibitoren aufweisen, die für gewöhnlich in Lignocellulosehydrolysaten vorkommen: Furfural, Hydroxymethylfurfural, Vanillin, Ameisensäure, Essigsäure und Lävulinsäure. Die besten Stämme wurden in Bezug auf Wachstum und Produktion detaillierter untersucht und der Stamm *Paenibacillus* 2H7 wurde für vergleichende Fermentationen im 500-ml-Maßstab ausgewählt. Die Furfuralkonzentration korrelierte mit dem Einsetzen der exponentiellen Wachstumsphase und aus den Erkenntnissen dieser Fermentationen im kleinen Maßstab wurde eine verbesserte Fermentationsstrategie für den 7-l-Maßstab abgeleitet.

D-Xylose-Screening 191 Exopolysaccharidproduzenten wurden hinsichtlich ihrer D-Xylose-Verwertungsfähigkeit untersucht und 135 (71 %) zeigten gutes Wachstum mit D-Xylose als einziger Kohlenstoffquelle. Die gutwachsenden Stämme wurden auf etwa eineindrittel 96-Well-Platten überführt und in den nächsten Screeningrunden benutzt.

High Content Screening mit D-Xylose 95 D-Xyloseverwerter wurden 48 h in D-xylosehaltigem Medium inkubiert und der D-Xyloseverbrauch, die Exopolysaccharid-Aldosemonomerzusammensetzung und -produktion untersucht. 66 Stämme (69 %) verbrauchten mindestens die Hälfte an D-Xylose. 13 Stämme (14 %) produzierten mindestens $560\,\mathrm{mg \cdot l^{-1}}$ Exopolysaccharid. Ein Vergleich der Exopolysaccharid-Aldosemonomerzusammensetzungen mit Daten von Rühmann *u. a.* [1] zeigte größere und kleinere Unterschiede in jeweils zweien der Stämme. Die übrigen Stämme waren nicht betroffen. Es wird die Hypothese aufgestellt, dass die augenscheinlichen Veränderungen der Exopolysaccharidzusammensetzungen nicht durch eine Veränderung der Zusammensetzung eines Exopolysaccharids verursacht wurden, sondern durch die unterschiedlich starke Produktion verschiedener Exopolysaccharide.

Hochdurchsatzscreening für die Inhibitortoleranz Die 135 D-Xyloseverwerter wurden in Anwesenheit von $2{,}0\,\mathrm{g \cdot l^{-1}}$ genau eines Lignocellulosehydrolysatinhibitors untersucht, um nichtwachsende Stämme aus den jeweiligen High Content Screenings zu entfernen (siehe nächster Schritt). Vanillin war der mit Abstand stärkste Inhibitor und stoppte der Wachstum von 79 % der Stämme vollständig. Furfural und Hydroxymethylfurfural hemmten das Wachstum von 20 % beziehungsweise 7 % der Stämme vollständig. Die Säuren hemmten das mikrobielle Wachstum nicht nennenswert. Der Vergleich mit Lignocellulosehydrolysat zeigte, dass die Verwendung von nur 27 beziehungsweise 28 der besten Vanillin- und Essigsäurestämme bereits ausreichte, um 57 % der besten 28 Lignocellulosehydrolysatstämme vorherzusagen.

High Content Screening mit Inhibitoren 27 oder 28 der am besten wachsenden Stämme pro Inhibitor wurden untersucht, um den Inhibitorabbau, die Exopolysaccharid-Aldosemonomerzusammensetzung und -produktion zu bewerten. Bis auf Lävulinsäure wurden sämtliche Inhibitoren in unterschiedlichem Ausmaße abgebaut: Furfural, Hydroxymethylfurfural und Ameisensäure wurden im Gros der Stämme nach 48 h vollständig abgebaut. Essigsäure wurde in einigen Stämmen abgebaut, in anderen aufgebaut, während Vanillins inhibitorisches Potential zu einem verringerten Abbau führte. Lävulinsäure blieb in den meisten Fällen unberührt (> 75 %). Es

wurden keine Veränderungen der Exopolysaccharidzusammensetzungen in Abhängigkeit vom Inhibitor festgestellt.

Stammauswahl Von den sieben finalen Stammkandidaten, die aus den Voruntersuchungen ausgewählt wurden, zeigte *Paenibacillus* 2H7 das robusteste Wachstum und auch die robusteste Exopolysaccharidproduktion in Gegenwart verschiedener Inhibitoren und wurde daher für die Fermentationen ausgewählt. Basierend auf einer 16S-rDNA-Sequenzierung wurden unklassifizierte Paenibacillen, *P. cineris* und *P. favisporus* als nächste Verwandte identifiziert.

Parallelfermentation mit Lignocellulosehydrolysat *Paenibacillus* 2H7 wurde in 2 x 4 500-ml-Parallelfermentationen in Lignocellulosehydrolysat und einer Referenz mit reiner D-Glucose und reiner D-Xylose eingesetzt. Der Beginn der exponentiellen Wachstumsphase in den Lignocellulosehydrolysatfermentern korrelierte mit der Furfuralkonzentration und war gegenüber den Referenzfermentationen etwa 36 h verzögert. Die molaren Massen der Exopolysaccharide lagen bei etwa $1 \cdot 10^7\ \mathrm{g \cdot mol^{-1}}$ und waren vom Lignocellulosehydrolysat unbeeinflusst, solange der pH-Wert auf 7,0 geregelt war. Die Fermentationsverläufe aller acht Fermenter waren ab dem Beginn der exponentiellen Phase untereinander vergleichbar. Die Ergebnisse im 500-ml-Maßstab wurden benutzt, um Verbesserungen an den Fermentationsparametern für den 7-l-Maßstab vorzunehmen: geringere anfängliche Lignocellulosehydrolysatkonzentration, Fed-Batch-Verfahren statt Batchverfahren und eine höhere anfängliche Bakterienkonzentration.

Zusammenfassung von „Fermentative Herstellung von Scleroglucan und Schizophyllan“

Um die Unterschiede zwischen Scleroglucan und Schizophyllan herauszuarbeiten, wurden acht Fermentationen mit *S. rolfsii* und *S. commune* im 500-ml-Maßstab über 48 h bis 144 h durchgeführt und die Exopolysaccharide aufgereinigt. Die Exopolysaccharidkonzentrationen am Ende der Fermentationen stiegen über die Zeit auf 3,2 $\mathrm{g \cdot l^{-1}}$ und 1,4 $\mathrm{g \cdot l^{-1}}$ für Scleroglucan beziehungsweise Schizophyllan mit einer Ausnahme: *S. commune* produzierte nach 120 h 2,2 $\mathrm{g \cdot l^{-1}}$ Schizophyllan.

Die Exopolysaccharide zeigten eine schlechte Löslichkeit, was sämtliche weiteren Analysen behinderte. Zusätzlich fielen die Exopolysaccharidkonzentrationen aus den Probenfällungen nach der 24-h-Probe stark ab, was an der Anpassung des Protokolls für dickflüssigere Fermentationsbrühen lag: die Proben wurden 1:10 mit hochreinem Wasser verdünnt. Alle anderen Untersuchungen – dynamische Viskosität und Thixotropie, molare Masse, Periodattest und Metabolitanalysen – blieben ohne klare und verlässliche Ergebnisse. Vorschläge für Wiederholungen der Experimente werden diskutiert und auf die Entwicklung eines quantitativen fluorometrischen Nachweises der β-1,3-β-1,6-Glucane Scleroglucan und Schizophyllan wird kurz eingegangen.

Chapter 1

Introduction

Renewable resources, lignocellulose hydrolysate and exopolysaccharides are in the focus of this thesis. Therefore, the terms are introduced in the necessary detail; the interested reader is referred to more detailled works of other authors throughout this introduction. Afterwards, the graduate programme 'BayReChem 2050', the main funding body of the project behind this work, and its focus on renewable resources is introduced.

1.1 Renewable Resources

The results of this work are based on the utilization of renewable resources, which is introduced in the first subsection. One such renewable resource is lignocellulosic biomass. It is used a basis for lignocellulose hydrolysate, a man-made product from lignocellulosic biomass. As lignocellulose hydrolysate is the starting material used in this work, commonly encountered issues stemming from the production method and remedies for them are presented herein.

1.1.1 Definition: What Makes a Resource Renewable?

The definition given by Weiss [2] is limited to '[…] the total range of living organisms providing man with food, fibers, drugs, etc., for his needs […]'. Probably, the most elaborate definition of what constitutes renewable resources is given by Armstrong and Hamrin [3] and does not include life forms other than plants:

> The term 'renewable' is generally applied to those energy resources and technologies whose common characteristic is that they are non-depletable or naturally replenishable.
>
> Renewable resources include solar energy, wind, falling water, the heat of the earth (geothermal), plant materials (biomass), waves, ocean currents, temperature differences in the oceans and the energy of the tides. Renewable energy technologies produce power, heat or mechanical energy by converting those resources either to electricity or to motive power. […]

Thus, one property of renewable resources is a non-depletable supply. This must not be mistaken for an infinite supply and one should be wary not to overuse such resources and its renewing character must be managed properly [4].

An infamous example of mismanagement of a renewable resource is the hunting of the American bison in North America in the 19th century. It is estimated that around 30 million to 75

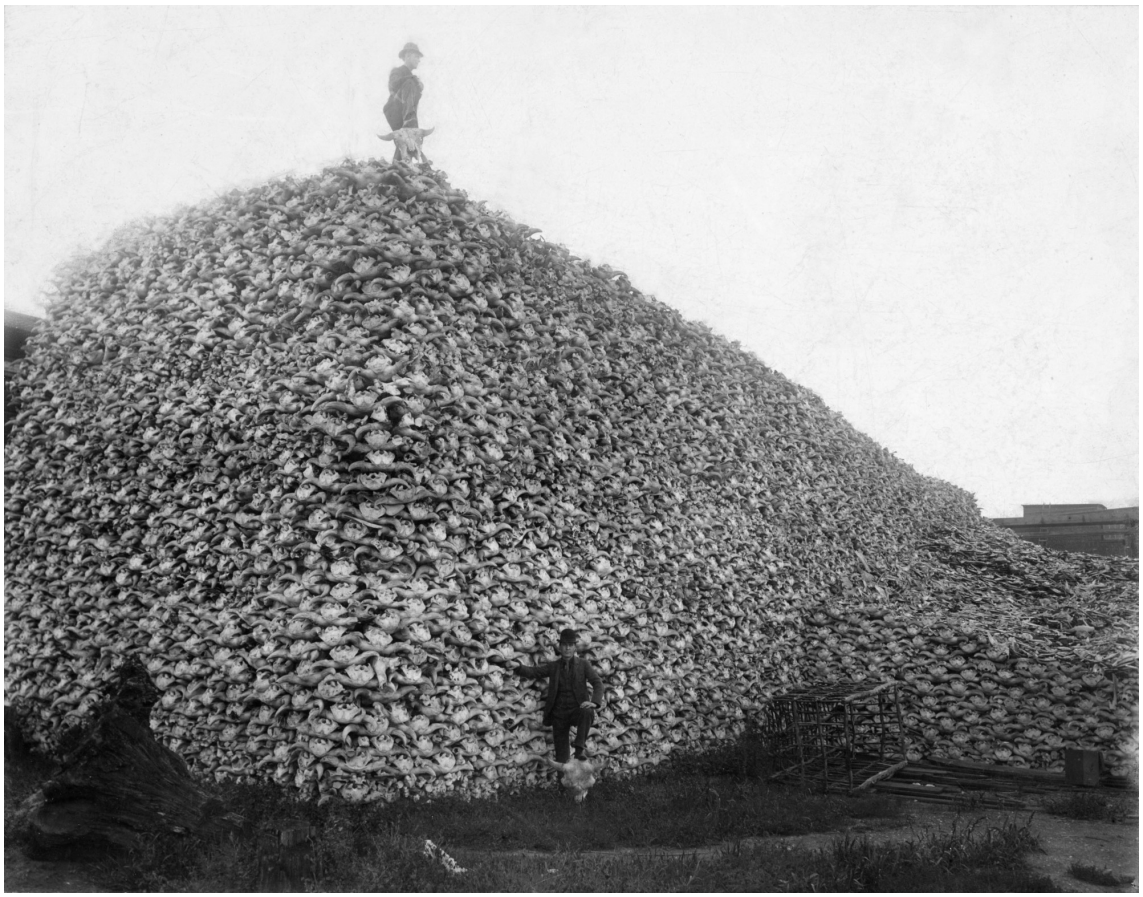


Figure 1.1: Photograph from the mid-1870s of a pile of American bison skulls waiting to be ground for fertilizer [5]. At the outset of the 19th century, between 30 million and 75 million bisons roamed North America. After a century of reckless hunting and exploitation of this renewable resource, less than 300 individuals remained [6]. Nowadays, the American buffalo is listed as 'Near Threatened' on the IUCN Red List of Threatened Species [7].

million bisons roamed North America in 1800. After just a hundred years, the number was reduced to less than 300 due to reckless hunting and exploitation of this renewable animal resource [6]. Figure 1.1 on the facing page depicts a pile of bison skulls from the mid-1870s. The number of skulls is estimated to be on the order of 100 000 and serves as a graphic example for the shortsighted mindset present at that time. The aftermath is still visible even today, more than a century later: the species is listed as 'Near Threatened' on the IUCN Red List of Threatened Species [7].

Therefore, when exploiting renewable resources, man must always keep in mind that the rates of renewal and consumption must be sustainable on a long term. Not only commercial considerations, but also the responsibility for the future generations mandate efficient use of renewable resources.

1.1.2 Lignocellulosic Biomass: The Most Abundant Renewable Carbon Resource

The production of lignocellulose is estimated to exceed $1 \cdot 10^{12}\,\mathrm{t \cdot a^{-1}}$ and is the major renewable carbon source on planet Earth [10, 11]. In contrast, the world crude oil production in 2014 was $4.24 \cdot 10^{9}$ t [12], less than one percent of the annual lignocellulose production. However, as outlined above, renewable resources must be managed wisely and as of now fossil fuels are clearly dominating the fuel market.

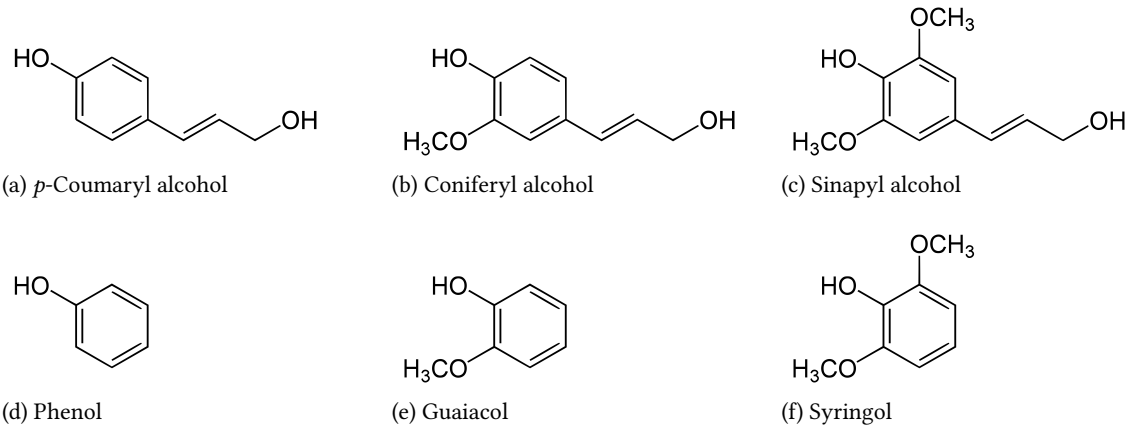
The main components of lignocellulosic biomass are the two eponymous ones lignin and cellulose and a third one called hemicellulose. All three are densely packed: cellulose fibrils are encased by hemicellulose which is chemically bound to lignin [13]. Examples of lignocellulosic biomass are wood and grass, but also wastes and residues from forestry, agriculture or municipalities.

In wood, the three aforementioned components are the main constituents as well. Wood can be classified as 'softwood' or 'hardwood'. The differences between softwood and hardwood lie within the relative proportions of lignin, cellulose and hemicellulose and structural details [13]. Examples of softwoods are pine and spruce and examples of hardwoods are aspen, oak and willow [9]. Compositions of different lignocellulosic biomasses were reported by Sun and Cheng [14].

The word 'lignin' derives from the Latin word *lignum* meaning 'wood'. Lignin is a polymer synthesized from phenylpropanoid precursors [8, 13]. Examples for these precursors are given in figure 1.2 on the next page. Based on the precursors, two lignin classes can be distinguished: guaiacyl lignins and guaiacyl-syringyl lignins. The structural difference of guaiacyl lignins and guaiacyl-syringyl lignins is an additional methoxy group at position 5 in syringyl lignins. While softwoods contain guaiacyl lignins, hardwoods contain guaiacyl-syringyl lignins. Overall, softwoods contain more lignin than hardwoods [9].

Cellulose consists of unbranched linear D-glucose monomers exclusively. The single D-glucose units are β-1,4-linked and the resulting polymer can be a highly crystalline or an amorphous material [15]. Crystalline cellulose is more difficult to degrade microbially than amorphous cellulose [16]. Nonetheless, microbial degradation of crystalline cellulose is possible [17, 18].

On the other hand, hemicelluloses are irregular branched polysaccharides which consist of pentoses, hexoses and uronic acids such as L-arabinose, D-xylose, D-galactose, D-glucose, D-mannose, D-glucuronic acid and D-galacturonic acid [9]. The hemicellulose of hardwoods is more highly acetylated than in softwoods and contains a higher proportion of D-xylose, while softwood hemicelluloses contain higher proportions of D-glucose and D-mannose than hardwood hemicellulose [19].



(a) *p*-Coumaryl alcohol (b) Coniferyl alcohol (c) Sinapyl alcohol

(d) Phenol (e) Guaiacol (f) Syringol

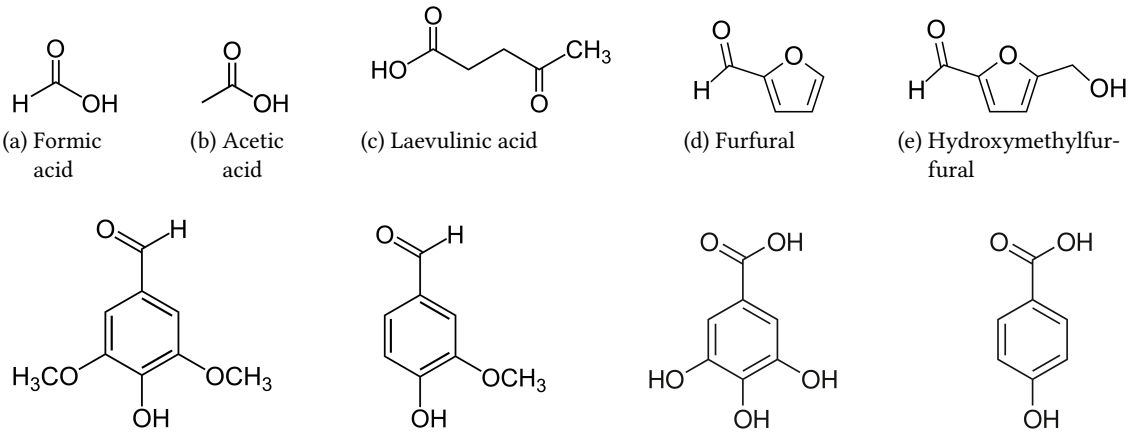
Figure 1.2: Phenylpropanoid precursors and eponymous molecules of lignin. a, b and c: Phenylpropanoid precursors of lignin [8]. d, e and f: Structures of the eponymous molecules for phenolics (d), guaiacyl lignins (e) and syringyl lignins (f). The structural difference between guaiacyl lignins and guaiacyl-syringyl lignins is an additional methoxy group in syringyl lignins, which can also be seen in the structures of the building blocks of lignin, coniferyl alcohol (b) and sinapyl alcohol (c) [9].

1.1.3 Lignocellulose Hydrolysate: A Man-Made Degradation Product

With its plethora of sugars, especially D-glucose and D-xylose, lignocellulosic biomass is a good candidate for microbial utilization. In order to make these resources available for fermentation processes, the recalcitrant structures of lignocellulosic biomass need to be broken and the sugar monomers freed. The resulting product of such a process is called lignocellulose hydrolysate. Different treatments exist to convert lignocellulosic biomass into lignocellulose hydrolysate and gain the highest amount of sugars. Unfortunately, dilute acid hydrolysis, a commonly employed treatment, is accompanied by the formation of inhibitors of microbial growth [14, 32]. For this work, the inhibitors formed during preparation of such dilute acid lignocellulose hydrolysates are the most important ones and are covered in some detail below. Neither the chemical explanations for inhibitor formation nor inhibition mechanisms or general detoxification strategies are covered. Advantages and disadvantages of the different (pre-)treatment methods are covered by Brodeur *et al.* [33], while inhibitor formation, inhibition mechanisms and general detoxification strategies are described in detail in the two reviews by Palmqvist and Hahn-Hägerdal [13, 34].

Inhibitors of Microbial Growth

A selection of different inhibitors formed during dilute acid hydrolysis of lignocellulosic biomass is presented in figure 1.3 on the facing page. Subfigures a, b and c show the structures of formic, acetic and laevulinic acid, respectively. While formic acid is a degradation product of furfural [20, 21] and hydroxymethylfurfural, laevulinic acid is a degradation product of only hydroxymethylfurfural [22]. Acetic acid is freed from the acetylated hemicellulose. Subfigures d and e depict the structures of the furan derivatives furfural and hydroxymethylfurfural, respectively. Furfural is a degradation product of C5 sugars [21] and hydroxymethylfurfural is a degradation product of C6 sugars [23–26]. The phenolic compounds syringaldehyde, vanillin, gallic acid and 4-hydroxybenzoic acid are depicted in subfigures f, g, h and i, respectively. They are degradation



(a) Formic acid (b) Acetic acid (c) Laevulinic acid (d) Furfural (e) Hydroxymethylfurfural

(f) Syringaldehyde (g) Vanillin (h) Gallic acid (i) 4-Hydroxybenzoic acid

Figure 1.3: Selected inhibitors of microbial growth released and/or formed during lignocellulose hydrolysate production. Formic acid (a) is a degradation product of furfural (d) [20, 21] and hydroxymethylfurfural (e). Acetic acid (b) is freed from acetylated hemicellulose. Laevulinic acid (c) is a degradation product of hydroxymethylfurfural (e) [22]. Furfural (d) is degradation product of C5 sugars, e.g. D-xylose [21]. Hydroxymethylfurfural (e) is a degradation product of C6 sugars, e.g. D-glucose [23–26], D-mannose or D-galactose. Phenolic compounds such as syringaldehyde (f), vanillin (g), gallic acid (h) or 4-hydroxybenzoic acid (i) are degradation products of lignin [27–29] or carbohydrates [30, 31].

products of lignin [27–29] or carbohydrates [30, 31].

Microbial Detoxification

The use of lignocellulose hydrolysate as a substrate for microorganisms is hampered by the presence of inhibitors. It is likely that the negative effect on microbial growth increases from aliphatic organic acids to furan derivatives to phenolics [34, 35]. The inhibitory effect of mixtures of different types of inhibitors increases in a synergistic manner [36].

Since most of the inhibitors occur naturally as well, it is no surprise that microorganisms have evolved mechanisms to detoxify these substances. Accounts of lignin degradation were shown not only for fungi [37] but also for bacteria [38, 39]. Three enzymes from *Sphingobium* sp. SYK6 were shown 'to release lignin monomers from complex lignin structures coming from differently prepared real lignin substrates' [38]. Furan derivatives are detoxified by bacteria as well [40–44]. Hydroxymethylfurfural can be converted to a furan dicarboxylic acid and then to furoic acid, which uses the furfural degradation pathway [42]. In this pathway, furfural is first oxidized to furoic acid, bound to coenzyme A as 2-furoyl-CoA and converted to 5-hydroxy-2-furoyl-CoA. Following these steps, 2-oxo-glutaroyl-CoA is formed via keto-enol tautomerization and hydrolysis, which then enters the citric acid cycle as 2-oxo-glutaric acid [42]. The microbial utilization of formic acid [45–47] and acetic acid [48, 49] is well-established and almost trivial. Laevulinic acid, on the other hand, does not have a known natural source. Thus, microbial utilization of laevulinic acid cannot be taken for granted and the very limited amount of literature on microbial laevulinic acid utilization seems to emphasize this [50–53]. While all these publications describe the utilization of laevulinic acid, the biochemical background remains in the dark.

1.2 Exopolysaccharides—Valuable Microbial Slime

The microbial product in the focus of this work are exopolysaccharides and is introduced in this section. The start makes a definition of the term 'exopolysaccharide', followed by details on the general structure of exopolysaccharides. The next subsection deals with the analytical methods used to assess various exopolysaccharide properties. Since the production of exopolysaccharides is a central topic of this work, the fermentative production is detailed thereafter.

The final subsection is dedicated to two very different commercially successful exopolysaccharides: the bacterial exopolysaccharide xanthan, which is approved for use in food, and the fungal exopolysaccharides scleroglucan and schizophyllan, which are used as thickening agents or in drilling fluids [54].

1.2.1 Definition

Exopolysaccharides are polysaccharides which are either produced extracellularly or intracellulary and exported out of the cell. 'EPS' is commonly used as an abbreviation for 'exopolysaccharide' [55–59], but also for 'extracellular polymeric substance' [60–62]. While all exopolysaccharides are also extracellular polymeric substances, the reverse is not true. In addition to 'pure' polysaccharides, exopolysaccharides may be further modified with e.g. acetic acid or pyruvic acid [63]. Like other polymers, exopolysaccharides can be categorized based on their monomers as either homo- or heteropolymers as well. Homopolymers exclusively consist of one species of monomer following the IUPAC definition [64], while heteropolymers are made up of different monomers and correspond to copolymers as defined by IUPAC [64]. The commercial examples below (on page 13) cover both types.

A comprehensive definition was given by Sutherland [65] and includes additional features such as the contribution of the polysaccharide to microbial structure:

> The surface of the microbial cell is a rich source of carbohydrate-containing molecules. Some of these are unique types, confined to a limited range of microorganisms. These are the components of the microbial cell walls [...]. However, in addition to these wall components, polysaccharides may be found either associated with other surface macromolecules or totally dissociated from the microbial cell. These are **exopolysaccharides**, extracellular polysaccharides [...].
>
> [...]
>
> Definition of exopolysaccharides is more difficult than definition of the carbohydrate-containing polymers found in microbial walls. [...] The term *glycocalyx*, introduced by Costerton, fails to differentiate between the different chemical entities found at the microbial surface. [...]
>
> The exopolysaccharides do not in themselves normally contribute to microbial structure; the other components of the cell surface are unaltered if exopolysaccharides are absent. [...]
>
> [...]
>
> The presence of exopolysaccharides associated with microbial cells grown on solid surfaces is frequently recognisable from the mucoid colony morphology. [...] In liquid medium, exopolysaccharide-producing cultures may become very viscous or,

> exceptionally, may solidify as a gel. The exopolysaccharide may form part of a *capsule* firmly attached to the bacterial cell surface. Alternatively, it may be observed as loose *slime* secreted by microorganisms but not directly attached to the cell. [...]

The book by Sutherland [65] is recommended to all readers who wish to get an in-depth introduction on exopolysaccharides.

1.2.2 Structure

Different sugar monomers occur in exopolysaccharides: plain carbohydrates with the empirical formula $C_nH_{2n}O_n$ such as D-glucose, D-galactose or D-xylose, and also amino sugars, deoxy sugars, uronic acids and acetylated amino sugars such as D-glucosamine, L-fucose, D-galacturonic acid and *N*-acetyl-D-glucosamine, respectively. Figure 1.4 on the following page lists some of these monomers, but one should not be under the impression that all exopolysaccharides could be generated with the monomers given in this figure: alginate for example consists of α-D-guluronic acid and β-D-mannuronic acid [81–83], both monomers are missing from figure 1.4 on the next page.

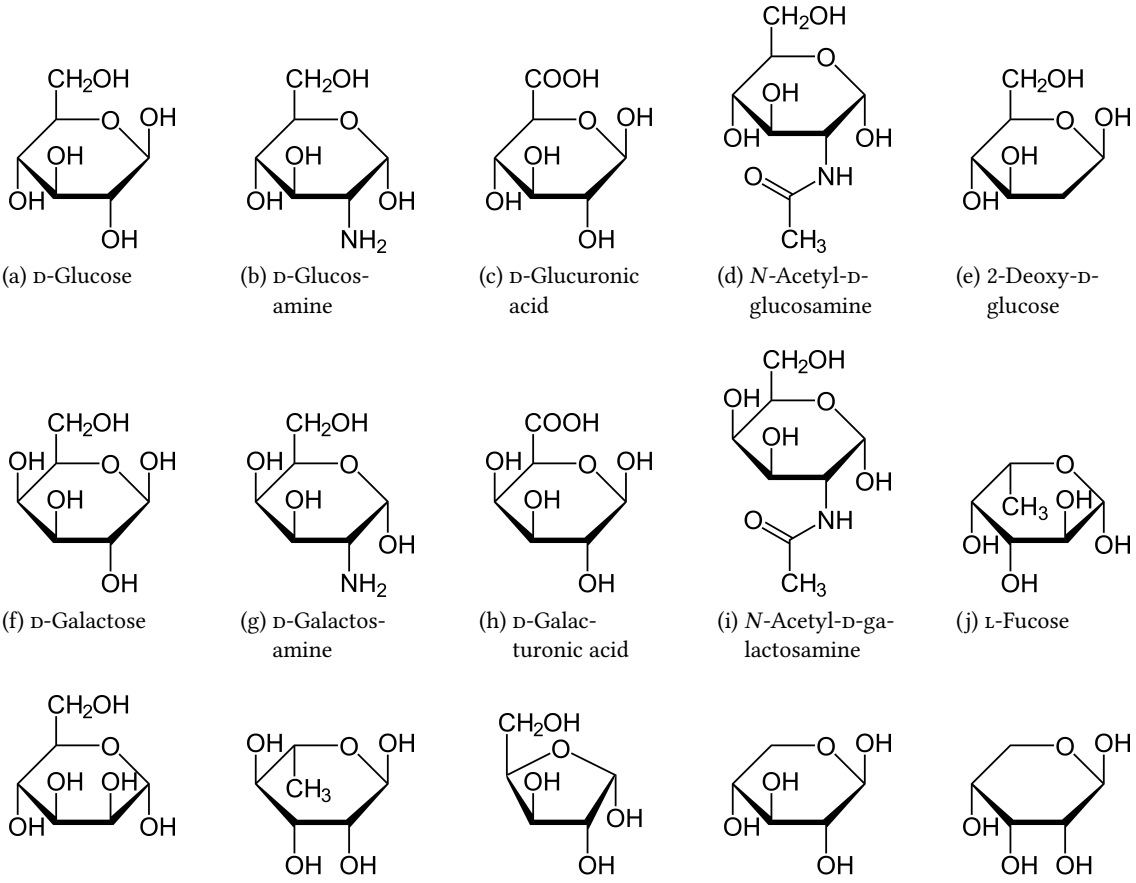
The predominant linkage in exopolysaccharides is the glycosidic bond and the exact type of the linkage directly influences the exopolysaccharide properties. This is generally true for all polysaccharides, not only exopolysaccharides: alginates form gels with Ca^{2+} ions [84], cellulose forms highly crystalline regions [15] and xanthan is approved as thickener in food [85]. Further examples and more details on different exopolysaccharides can be found in a review by Kumar *et al.* [86].

1.2.3 Analytical Methods

Characterization of exopolysaccharides encompasses many different analytical techniques, most of them requiring expensive equipment, and is time-consuming. The overall monomer composition can be analysed by hydrolyzing the polymer under acidic conditions to free the monomers. Then, the monomers can be analysed directly via HPLC [87, 88] or can be converted into volatile alditol acetates and analysed via gas chromatography [89, 90]. Aldose monomers can be derivatized with 3-methyl-1-phenyl-2-pyrazoline-5-one and analysed via HPLC-MS [1, 91–93]. Linkages can be analysed using methylation analysis via GC-MS [90, 94]. Pyruvylation can be quantified enzymatically [95] and acetylation via hydroxamic acid [96, 97].

Polymer size and molar mass can be analysed via size-exclusion chromatography. Often, no calibration standard for the polymer in question is available, hence molar masses can only be given in relation to a reference such as dextran or polystyrene. Using multi-angle laser light scattering, it is possible to determine the polymer molar mass without a reference when the refractive index increment, $\frac{dn}{dc}$ is known. The $\frac{dn}{dc}$ can be determined with a refractometer. For very bulky molecules, size-exclusion columns cannot separate the largest molecules anymore; hence, other methods such as field-flow fractionation should be used instead [98].

In the next step, more application-relevant properties, i.e. rheological properties such as the dynamic viscosity at specific shear rates, concentrations, temperatures or ionic strengths, adhesion properties, binding properties for certain metal ions, emulsification properties or mechanical properties of test specimen are characterized. For most of these analyses, special equipment is needed.



(a) D-Glucose (b) D-Glucosamine (c) D-Glucuronic acid (d) *N*-Acetyl-D-glucosamine (e) 2-Deoxy-D-glucose

(f) D-Galactose (g) D-Galactosamine (h) D-Galacturonic acid (i) *N*-Acetyl-D-galactosamine (j) L-Fucose

(k) D-Mannose (l) L-Rhamnose (m) L-Arabinose (n) D-Xylose (o) D-Ribose

Figure 1.4: Overview on possible carbohydrate monomers of exopolysaccharides. Carbohydrate monomers found in exopolysaccharides. Subfigures (a) to (e) depict D-glucose [66–74] and its derivatives D-glucosamine [75, 76], D-glucuronic acid [67, 74], *N*-acetyl-D-glucosamine [75] and 2-deoxy-D-glucose, while subfigures (f) to (j) depict D-galactose [66, 68–74] and its derivatives D-galactosamine [76], D-galacturonic acid [77], *N*-acetyl-D-galactosamine [78] and L-fucose (6-deoxy-L-galactose) [69]. Here, L-fucose and not D-fucose is depicted for its abundance in microbial exopolysaccharides. Subfigures (k) and (l) depict two other C6 sugars commonly found: D-mannose [66–69, 72–74] and L-rhamnose (6-deoxy-L-mannose) [66, 68, 70, 71]. Subfigures (m) to (o) depict the C5 sugars L-arabinose [79], D-xylose [68, 69, 73] and D-ribose [80]. This list is not exhaustive; further carbohydrates including variants of the sugars depicted here such as the corresponding furanose or pyranose forms cannot be excluded.

1.2.4 Fermentative Exopolysaccharide Production

Microbial exopolysaccharides are excreted into the medium by the respective microorganism. Therefore, fermentation processes are used for exopolysaccharide production. In a fermentation process, the respective microorganism is grown in a sterile medium with aeration, agitation, temperature and pH control. Since the microorganisms used are usually aerobic, the dissolved oxygen concentration is monitored or controlled. The off-gas oxygen and carbon dioxide contents can be monitored as well. Additional medium components can be fed during the course of the fermentation.

Several exopolysaccharides increase the broth viscosity over time. This negatively affects mixing, which in turn affects all mixing-dependent parameters such oxygen transfer, mass transfer, and the time needed to measure the effect of acid/base additions to the medium. With poor mixing, dissolved oxygen levels decrease which could impair microbial growth and/or production.

The measurement of other parameters such as the attenuance at 600 nm D_{600} for the estimation of the microbial cell concentration, the cell dry mass, the concentration of the carbon source used or other molecules of interest such as inhibitors to microbial growth or the molar mass distribution are realized via off-line analysis of dedicated samples at sensible sampling times. With off-line samples, more elaborate analysis techniques can be used to give insights that are not usually obtainable from on-line measurements.

At the end of the fermentation, the contents of the fermenter are harvested and subjected to downstream processing—the various techniques used to separate the product from all impurities. Commonly, cells are separated by centrifugation and the supernatant is purified further. In the case of exopolysaccharides, the supernatant may be precipitated directly using alcohol or purified using cross-flow filtration to leave only big molecules such as exopolysaccharide chains in the solution and remove all small molecules. Cross-flow filtration may be used to concentrate the product and save alcohol for the precipitation. The precipitated product is dried and may be ground to defined particle sizes for faster dissolution.

1.2.5 Commercial Exopolysaccharides

In this section, three very different microbial exopolysaccharides are introduced: xanthan and scleroglucan/schizophyllan. All three polymers are exploited commercially and cover bacterial heteropolymers and fungal homopolymers. A general review of hydrocolloids for thickening by Saha and Bhattacharya [99] is recommended for further reading.

Xanthan

Bacteria of the genus *Xanthomonas*, especially *Xanthomonas campestris* produce the heteropolymer xanthan. The general structure of xanthan is shown in figure 1.5 on the following page. Xanthan is a branched heteropolymer with a β-1,4-linked D-glucose backbone, which is the same structure as in cellulose. Sidechains are attached to every other backbone monomer to the C3 position of the backbone. The sidechain consists of a β-D-mannose linked via a 1,4-bond to β-D-glucuronic acid. The β-D-glucuronic acid is 1,2-linked to an α-D-mannose, which is linked via a 1,3-bond to the backbone. Up to 50 % of the terminal β-D-mannose carries a ketal of pyruvate and the hydroxy groups at the C4 and C6 positions [63]. Compared to most other exopolysaccharides, the biochemical pathways and genes involved in xanthan synthesis are well-known [100].

The rheology of aqueous solutions of xanthan has been studied in detail [100–106]. Solutions

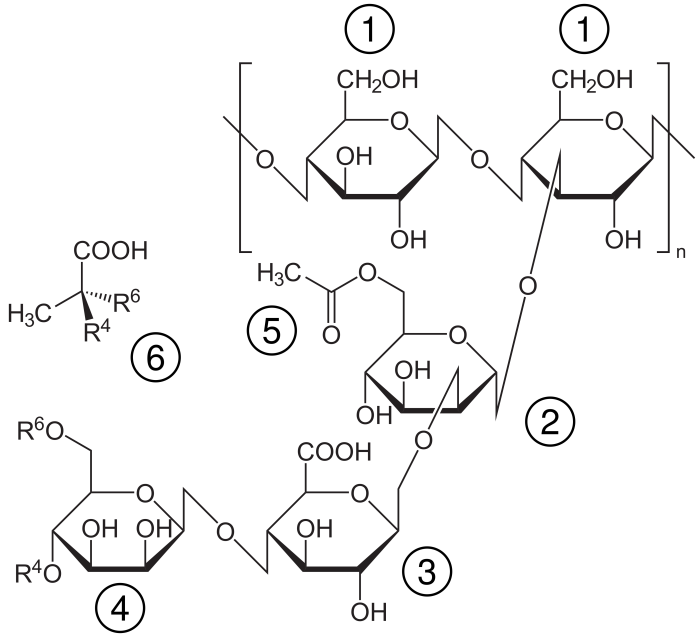


Figure 1.5: Structure of the repeating unit of xanthan. Xanthan consists of a dimeric β-1,4-linked D-glucose backbone (①) with branches consisting of a trimer at every second D-glucose residue. The trimer is connected via an α-1,3-glycosidic link to D-mannose (②). The branch consists of a β-D-mannose (④), which is 1,4-linked to a β-D-glucuronic acid (③), which in turn is connected via a 1,2-link to the α-D-mannose (②). The α-D-mannose (②) can be *O*-acetylated (⑤) at the C6 position. The terminal β-D-mannose (④) can carry a pyruvate ketal (⑥) formed from the hydroxy groups in the C4 and C6 positions and pyruvic acid [63].

of xanthan are highly viscous at room temperature and exhibit shear-thinning behaviour [102–104, 106] and quickly regain their viscosity after shearing [107]. Xanthan solutions are stable with regards to ionic strength [102] and temperatures up to 80 °C [105]. The high viscosity during fermentation of the strictly aerobic strains reduces the oxygen transfer rate [100], which can also decrease the polymer molar mass [108]. For more in-depth information on the production, recovery and properties of xanthan, the review by García-Ochoa *et al.* [109] is recommended to the reader.

Xanthan is approved for food use in the European Union [85]. The thickening properties of xanthan in foods have been examined. Nowadays, it is used routinely in a wide array of foodstuffs such as beer (foam stabilization), cheese (syneresis inhibition), ice cream (stabilization, crystallization control), mayonnaise and salad dressings (emulsifier), sauces (thickener) and syrups (pseudoplasticity), but also in industrial applications such as explosives (gelling agent), textile dyeing (pseudoplasticity) and water clarification (flocculant) [100, 110–114].

Scleroglucan/Schizophyllan

Scleroglucan is produced by fungi belonging to the genus *Sclerotium*, most notably *Sclerotium rolfsii*. It is chemically identical to schizophyllan [115] which is produced by fungi of the genus *Schizophyllum*, most notably *Schizophyllum commune* [116]. In this section, 'scleroglucan' is used synonymously with 'schizophyllan'.

Scleroglucan is an unbranched homopolymer of D-glucose consisting of β-1,3-links with single β-1,6-linked D-glucose residues per three D-glucose units, on average [115, 117, 118]. The polymer can be dissolved in water or DMSO and molar masses are usually on the order of 10^5 Da to 10^7 Da [119–121]. A peculiar feature of scleroglucan is the formation of very stable triple helices in aqueous solutions in the pH value range of 2 to 12 [119, 122–126]. Solutions are highly viscous (several Pa·s [120]), shear-thinning and quickly regain their viscosity after shearing [127]. Also, the solutions are stable at high salt concentrations of up to 350 g·kg^{-1} [120] and temperatures of up to 135 °C [119, 126, 128].

Scleroglucan is used in the production of crude oil and in cosmetics. The recovery factor of oil reservoirs is usually in the range of 30 % to 40 %. Drilling fluids with scleroglucan can enhance the recovery factor of oil reservoirs to 50 % [129–131]. As an ingredient in cosmetics, scleroglucan serves as a moisturizer to relieve dry skin conditions, atopic diseases and itching [132]. The use as part of a drug delivery system has also been researched [133] or patented [134]. For further information, the recently published review on the production and industrial applications of β-1,3-glucans by Zhu *et al.* [135] and a review on the biosynthesis, production and industrial applications of scleroglucan by Schmid *et al.* [54] are recommended.

1.3 Aims of the Thesis

1.3.1 Bacterial Conversion of Lignocellulose Hydrolysate to Exopolysaccharides

Exopolysaccharides are a diverse class of polymers with the potential to replace commonly used petrol-based polymers. But, competition with fuel or food resources might arise using the usual carbon sources used in fermentation processes such as D-glucose or sucrose. In order to avoid this, lignocellulose hydrolysate will be used as a rather cheap resource supplied by an industry partner. Due to toxic by-products in the lignocellulose hydrolysate, microbial growth and/or production might be inhibited.

Therefore, the aims of this project are:

- Exopolysaccharide Screening: Using a state-of-the-art method developed in-house [1] the aldose monomer compositions of the polymers of strains from a strain collection care analysed.
- Substrate Screening: Lignocellulose hydrolysates contain not only D-glucose, but other sugars such as D-xylose as well. As resource efficiency is key in industrial processes, only strains exhibiting a broad substrate spectrum are considered.
- Inhibitor Screening: Single isolated toxic by-products of lignocellulose hydrolysates and lignocellulose hydrolysate itself are tested with the strains from the strain collection to pick out those which are most resistant.
- Strain Selection: From all the data gathered on the strains, some strains are chosen for in-depth experiments using classical microbiological and molecular biological methods for characterization.
- Fermentation & Downstream Processing: At least one strain is fermented in bioreactors of varying size to determine basic process and scale-up data.

The final aim strived for is the robust production of one exopolysaccharide at $g \cdot l^{-1}$ scale.

1.3.2 Fermentative Production of Scleroglucan and Schizophyllan

Schizophyllan and Scleroglucan are a products already being marketed. The polymer scleroglucan appears to be identical to schizophyllan, yet identity claims are generally based on fractions of the polymers only. In order to get an answer to the question whether scleroglucan and schizophyllan are truly the same or not, this project's aims are:

- Fermentations of *S. commune* and *S. rolfsii* for the production of the polymers schizophyllan and scleroglucan, respectively.
- Purification and analyses of the polymers to compare scleroglucan and schizophyllan.
- If necessary, analytical methods are developed.

Chapter 2

Materials and Methods

2.1 Materials

Whenever a manufacturer or vendor is mentioned, the country is given only if it is not Germany. This applies to the whole document. The manufacturer/vendor abbreviations used and their respective meanings are given in table 2.1.

Table 2.1: Manufacturer/vendor abbreviations used throughout the document.

Abbreviation	Manufacturer/Vendor
AAG	Alfa Aesar GmbH + Co. KG, Karlsruhe
ACG	AppliChem GmbH, Darmstadt
AHT	Andreas Hettich GmbH & Co. KG, Tuttlingen
AHW	A. Hartenstein GmbH, Würzburg
AJA	Analytik Jena AG, Jena
APG	Anton Paar GmbH, Graz, Austria
APM	Avantor Performance Materials B.V., Deventer, Netherlands
AT	Agilent Technologies, Waldbronn
BBM	B. Braun Melsungen AG, Melsungen
BCG	Beckman Coulter GmbH, Krefeld
BDG	Bruker Daltonik GmbH, Bremen
BG	BINDER GmbH, Tuttlingen
BGK	BRAND GmbH + Co. KG, Wertheim
BGS	BlueSens gas sensor GmbH, Herten
BIC	Brookhaven Instruments Corporation, Holtsville, NY, USA
BRL	Bio-Rad Laboratories GmbH, Munich
BS	Boekel Scientific, Feasterville, PA, USA
CFS	Cargill France SAS, Saint-Germain-en-Lage, France
CRG	Carl Roth GmbH + Co. KG, Karlsruhe
CZM	Carl Zeiss Microscopy GmbH, Jena
DC	Dionex Corporation, Sunnyvale, CA, USA
DG	DASGIP GmbH, Jülich
DI	Denver Instrument, Bohemia, NY, USA
DMG	Deutsche METROHM GmbH & Co. KG, Filderstadt
DWO	Dispomed Witt oHG, Gelnhausen

continued on the next page

Table 2.1: *continued from the previous page*

Abbreviation	Manufacturer/Vendor
EA	Eppendorf AG, Hamburg
EBG	Edmund Bühler GmbH, Hechingen
ELW	ELGA Lab Water, Celle
EMD	EMD Biosciences, Inc., La Jolla, CA, USA
GBO	Greiner Bio-One GmbH, Frickenhausen
GFL	GFL Gesellschaft für Labortechnik mbH, Burgwedel
GHE	GE Healthcare Europe GmbH, Freiburg
GHU	GE Healthcare UK Ltd., Buckinghamshire, United Kingdom
HA	Harvard Apparatus, Holliston, MA, USA
HBA	Hamilton Bonaduz AG, Bonaduz, Switzerland
HG	Hellma GmbH & Co. KG, Müllheim
HIG	Heidolph Instruments GmbH & Co. KG, Schwabach
HPM	HP Medizintechnik GmbH, Oberschleißheim
HSW	Henke-Sass, Wolf GmbH, Tuttlingen
IA	Infors AG, Bottmingen/Basel, Switzerland
ICZ	Ingenieurbüro CAT, M. Zipperer GmbH, Staufen
IFM	ifm electronic, Essen
IG	Implen GmbH, München
IIG	Intas-Science-Imaging Instruments GmbH, Göttingen
IKA	IKA-Werke GmbH & Co. KG, Staufen
KMG	Spencer Kimball, Peter Mattis and the GIMP Development Team
KSF	Dr. Klaus Schopp Forschung + Technik, Karlsruhe
KSG	KERN & SOHN GmbH, Balingen
LH	Liebherr Hausgeräte, Ochsenhausen
MBB	Mallinckrodt Baker B. V., Deventer, Netherlands
MCG	Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz
MDG	Motic Deutschland GmbH, Wetzlar
MG	Memmert GmbH + Co. KG, Schwabach
MK	Merck KGaA, Darmstadt
MNG	Macherey-Nagel GmbH & Co. KG, Düren
MTG	Mettler-Toledo GmbH, Gießen
NEB	New England Biolabs GmbH, Frankfurt am Main
OC	Ohaus Corp., Pine Brook, NJ, USA
PC	Pall Corporation, Ann Arbor, MI, USA
PHK	Peter Huber Kältemaschinenbau GmbH, Offenburg
PL	Phenomenex Ltd., Aschaffenburg
PSS	PSS Polymer Standards Service GmbH, Mainz
QGD	QIAGEN GmbH Deutschland, Hilden
RLD	Rapidozym Gesellschaft für Laborhandel und DNA Diagnostika mbH, Berlin
SAC	Sigma-Aldrich Chemie GmbH, Steinheim
SAG	Sartorius AG, Göttingen
SAK	Sarstedt AG & Co. KG, Nümbrecht
SBE	SensoQuest Biomedizinische Elektronik GmbH, Göttingen
SDK	Showa Denko K.K., Kawasaki, Japan

continued on the next page

Table 2.1: *continued from the previous page*

Abbreviation	Manufacturer/Vendor
SEG	Serva Electrophoresis GmbH, Heidelberg
SIA	SI Analytics GmbH, Mainz
SKK	Shimadzu, Kyoto, Japan
SLI	Sartorius Lab Instruments GmbH & Co. KG, Göttingen
SSB	Sartorius Stedim Biotech GmbH, Göttingen
SSS	Sartorius Stedim Systems, Göttingen
TBG	Tosoh Bioscience GmbH, Stuttgart
TEL	Thermo Electron LED GmbH, Langenselbold
TFS	Thermo Fisher Scientific Inc., Waltham, MA, USA
UNK	?
VAG	VACUUBRAND GmbH + Co. KG, Wertheim
VWR	VWR International bvba/sprl, Leuven, Belgium
ZLG	Zefa-Laborservice GmbH, Harthausen

2.1.1 Chemicals

Table 2.2: Chemicals used for the production of data presented in this work. Chemicals are roughly sorted alphabetically in ascending order. For most chemicals given here, lot information and the article number are available on request. Abbreviations: M./V.: Manufacturer or vendor.

Chemical	Grade	M./V.
Acetic acid	100 %, Ph. Eur., extra pure	CRG
Acetonitrile	≥99.9 %, Reag. Ph. Eur.	VWR
Agar-agar	for microbiology	CRG
Agencourt AMPure XP	-	BCG
Ammonia	30 % to 33 %, extra pure	CRG
Ammonium chloride	-	MBB
Aniline blue	certified by the Biological Stain Commission, dye content: 65 %	SAC
Antifoam B	-	APM
L-(−)-Arabinose	≥99 %	EMD
2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt	≥98 %	SAC
Boric acid	for electrophoresis, ≥99.5 %	SAC
Bovine serum albumin	≥96 %	SAC
Calcium chloride dihydrate	≥99 %, p.a., ACS	CRG
Calcium chloride dihydrate	ACS, Reag. Ph Eur	MK
Calibration buffer pH 10	10.00 ± 0.02	VWR
Calibration buffer pH 4	4.00 ± 0.02	VWR
Calibration buffer pH 7	7.00 ± 0.02	VWR
Casein peptone	pancreatic digest, for microbiology	CRG
Chloroform/Isoamyl alcohol (24:1)	for extraction of nucleic acids	CRG

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Table 2.2: *continued from the previous page*

Chemical	Grade	M./V.
Citric acid	≥99.5 %, p.a., ACS	CRG
Cobalt chloride hexahydrate	98 %	AAG
Copper sulphate pentahydrate	≥99.0 %	SAC
Dimethylsulphoxide	≥99.8 %, p.a.	CRG
Dimethylsulphoxide	≥99.5 %	KSF
dNTP mix	10 mM	RLD
Ethidium bromide	10 $g \cdot l^{-1}$	CRG
Ethylenediaminetetraacetic acid	≥99 %, p.a., ACS	CRG
Ferric sulphate heptahydrate	ACS reagent, reag. ISO, reag. Ph. Eur., ≥99.0 %	SAC
Formic acid	≥98.0 %, p. a.	ACG
Formic acid	98 %	SAC
Fumaric acid	≥99.5 %, for biochemistry	CRG
Furfural	ACS, ≥98.0 %	MK
D-(+)-Galactosamine hydrochloride	≥98 %, for biochemistry	CRG
D-(+)-Glucosamine hydrochloride	≥99 %	EMD
D-(+)-Glucose monohydrate	for microbiology	CRG
D-(+)-Glucose monohydrate	Ph. Eur.	SEG
Glycine	USP, Ph. Eur., ≥98.5 %	SEG
HF Buffer	-	NEB
Hydrochloric acid	2 M	CRG
5-(Hydroxymethyl)furfural	97 %	AAG
Itaconic acid	-	ACG
Lithium nitrate	99 %	AAG
Magnesium sulphate heptahydrate	ACS, Reag. Ph Eur, ≥99.5 %	MK
Magnesium sulphate heptahydrate	ACS, Reag. Ph. Eur., ≥99.5 %	MK
Malic acid	≥99.0 %	ACG
Manganese chloride dihydrate	≥99.0 %	MK
Methanol	≥99.9 %	VWR
3-Methyl-1-phenyl-2-pyrazoline-5-one	99 %	SAC
3-(*N*-Morpholino) propane sulphonic acid	≥99.5 %	CRG
Oxalic acid	≥99.0 %	SAC
Phenol	for extraction of nucleic acids	CRG
5x Phusion HF Reaction Buffer	-	NEB
Potassium chloride	≥99.5 %, p.a., ACS, ISO	CRG
Potassium dihydrogen phosphate	≥99 %, p.a., ACS	CRG
Pullulan (342 Da)	analytical	PSS
Pullulan (1 kDa)	analytical	PSS
Pullulan (5 kDa)	analytical	PSS
Pullulan (10 kDa)	analytical	PSS
Pullulan (20 kDa)	analytical	PSS
Pullulan (50 kDa)	analytical	PSS
Pullulan (110 kDa)	analytical	PSS
Pullulan (200 kDa)	analytical	PSS

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Table 2.2: *continued from the previous page*

Chemical	Grade	M./V.
Pullulan (400 kDa)	analytical	PSS
Pullulan (800 kDa)	analytical	PSS
Pullulan (1.3 MDa)	analytical	PSS
Pullulan (2.5 MDa)	analytical	PSS
α-L-(+)-Rhamnose hydrate	for microbiology, ≥99.0 %	SAC
RPMI 1640 Vitamins solution (100 x)	suitable for cell culture	SAC
Scleroglucan Cs 11	-	CFS
Sodium acetate	≥99.5 %, p.a., ACS, ISO	CRG
Sodium chloride	-	ACG
Sodium chloride	≥99 %, Ph. Eur., USP	CRG
Sodium dodecyl sulphate	≥99 %, research grade	SEG
Sodium glyoxylate monohydrate	≥93 %	SAC
Sodium hydroxide	≥99 %, p.a., ISO	CRG
Sodium molybdate	≥99.5 %, p.a.	CRG
Sodium periodate	≥99.8 %, ACS	SAC
Sodium succinate hexahydrate	≥99.0 %	SAC
Sodium tartrate	≥99 %, extra pure	CRG
Sulphuric acid	96 %, p.a., ISO	CRG
Sulphuric acid	1 M	CRG
TRIS	≥99.3 %, Buffer Grade	CRG
TRIS hydrochloride	≥99 %, p.a.	CRG
Vanillin	99 %	SAC
D-(+)-Xylose	≥98.5 %, for biochemistry	CRG
D-(+)-Xylose	for biotechnological purposes, ≥98.0 % (sum of enantiomers, HPLC)	SAC
Yeast extract	for bacteriology	CRG
Zinc chloride	ACS, ISO, Reag. Ph. Eur., ≥98.0 %	MK

Lignocellulose Hydrolysate

Lignocellulose hydrolysate was supplied by an industrial partner. The lignocellulose hydrolysate was prepared from lignocellulosic biomass. Besides a general material safety datasheet no further information on the lignocellulose hydrolysate were supplied. The composition of the lignocellulose hydrolysate was analysed in-house and is described in detail in section 3.4.1 on page 69.

2.1.2 Consumables

Table 2.3: List of consumables used in this work. Abbreviations: Art. no.: Article number; M./V.: Manufacturer or vendor.

Type	Name	Art. no.	M./V.
96 well plates	96-well PCR plate, non-skirted, elevated wells, colorless, PP, DNase-free, RNase-free, DNA-free	781350	BGK
96 well plates	F bottom; 340 µl	655161	GBO
96 well plates	V bottom; 1.1 ml	745565	HA
96 well plates	V bottom; 2 ml	780271	GBO
Cellophane membrane	Model 583 Gel Dryer	165-0963	BRL
Cross-flow filtration membrane	Hydrosart; pore size: 0.45 µm	3051860601W–SG	SSB
Cross-flow filtration membrane	Hydrosart; pore size: 10 kDa	3051443901E–SW	SSB
Cross-flow filtration membrane	Hydrosart; pore size: 100 kDa	3051446801E–SG	SSB
Cryo vials	CryoPure; 1.8 ml; polypropylene	72.379	SAK
Cuvettes	PMMA, semi-micro	P951	CRG
Cuvettes	PS, semi-micro, path length 10 mm	759015	BGK
Filter membrane	Cellulose nitrate (CN); pore size: 0.45 µm; sterile	11407–50—-ACN	SSB
Filter membrane	Regenerated cellulose (RC); pore size: 0.2 µm; non-sterile	18407–47——N	SSB
Filter plates (96 well)	AcroPrep 96 Filter Plate with 1.0 µm Glass Fibre media	5032	PC
Filter plates (96 well)	AcroPrep Advance 350 0.2 µm Supor	8019	PC
Filter plates (96 well)	AcroPrep Advance 350 10K Omega	8034	PC
Filter plates (96 well)	CHROMAFIL Multi 96, Regenerated cellulose (RC); pore size: 0.2 µm	738656.M	MNG
Filter plates (96 well)	Micro SpinColumns, G-25 Packing Material	745612	HA
Gel extraction kit	NucleoSpin Gel and PCR Clean-up Kit	740609.250	MNG
Gel extraction kit	QIAEX II Gel Extraction Kit	20021	QGD
HPLC column	EC 100/2 Nucleodur C18 Gravity, 1.8 µm	E14080976	MNG
HPLC column	EC 100/2 Nucleodur C18 Gravity, 1.8 µm	E14010920	MNG
HPLC column	Metrosep A Supp 16 - 250/2.0	6.1031.230	DMG
HPLC column	Rezex ROA-Organic Acid H+ (8 %)	00H-0138-K0	PL
Miniprep kit	QIAprep Spin MiniPrep Kit	27106	QGD
Pasteur pipettes	plastic	612-1681	VWR
PCR tube caps	strips of eight caps	732-1518	VWR

continued on the next page

Table 2.3: *continued from the previous page*

Type	Name	Art. no.	M./V.
PCR tubes	PCR tubes with attached flat caps	732-0548	VWR
PCR tubes	strips of eight tubes	732-1517	VWR
Petri dishes	Diameter: 92 mm; height: 16 mm	82.1472.001	SAK
pH electrode	InLab Expert DIN	51343103	MTG
pH electrode	InLab Micro Pro	51343162	MTG
Pipette tips	epT.I.P.S. Standard; uncoloured; 1250 µl	613-3506	VWR
Pipette tips	green; 1250 µl	0030 000.935	EA
Pipette tips	uncoloured; 1000 µl	70.762	SAK
Pipette tips	uncoloured; 20 µl	70.1116	SAK
Pipette tips	uncoloured; 200 µl	70.760.002	SAK
Pipette tips	uncoloured; 10 ml	104902603	ZLG
Pipette tips	uncoloured; 5 ml	294902595	ZLG
Pipette tips	yellow; 300 µl	0030 000.897	EA
Plastic tubes	15 ml	62.554.502	SAK
Plastic tubes	50 ml	62.547.254	SAK
Plastic tubes	PP; with attached cap; 1.5 ml	72.690.001	SAK
Plastic tubes	PP; with attached cap; 2 ml	72.695	SAK
Plastic tubes	safe-lock; 1.5 ml	72.706	SAK
Plastic tubes	safe-lock; 2.0 ml	72.695.500	SAK
Plate mats	PCR-sealing mat for 96-well plates, TPE	781405	BGK
Plate mats	Whatman Capmats 96 Wells, Round, Silicone Rubber	7704-0105	GHU
Plate seals	Axygen breathable sealing film, sterile	BF-400-S	VWR
Plate seals	Axygen sealing film, aluminium, non-sterile	732-7505	VWR
Sealing film	Parafilm M	291-1213	VWR
SEC column	TSK-GEL Alpha-M	18344	TBG
SEC guard column	TSK-GEL Alpha-M	18345	TBG
Spin filter	modified PES, 10 kDa	516-0230	VWR
Sterilization tape	dry air, 180 °C	STKH	AHW
Sterilization tape	steam, 121 °C	STKD	AHW
Syringe filters	?	?	UNK
Syringe filters	Cellulose acetate (CA); pore size: 0.2 µm	514-0060	VWR
Syringe filters	Cellulose acetate (CA); pore size: 0.2 µm; sterile	514-0061	VWR
Syringe filters	Cellulose acetate (CA); pore size: 0.45 µm	514-0062	VWR
Syringe filters	Cellulose acetate (CA); pore size: 0.45 µm; sterile	514-0063	VWR
Syringes	50 ml; LuerLock	SE24	AHW

continued on the next page

Table 2.3: *continued from the previous page*

Type	Name	Art. no.	M./V.
Syringes	10 ml	4606108V	BBM
Syringes	2.0 ml; free from latex, free from silicone oil	4020-000V0	HSW
Syringes	20 ml	SE23	AHW
Syringes	5.0 ml; free from latex, free from silicone oil	4050-000V0	HSW
Syringes	50 ml; LuerLock	22050	DWO

2.1.3 Glassware

All glassware used was made from borosilicate glass.

2.1.4 Equipment

Table 2.4: List of equipment used in this work. Abbreviations: M./V.: Manufacturer or vendor.

Type	Model	M./V.
Analytical balance	PA214C	OC
Autoclave	Varioklav 135 S	HPM
Autoclave	Varioklav 135 S	TEL
Balance	TB-215 D	DI
Balance	440-47N	KSG
Balance	TE1502 S	SAG
Balance	TE6101	SAG
Balance	AW 320	SKK
Capillary electrophoresis system	2100 Bioanalyzer	AT
Carbon dioxide sensor	BCP-CO2	BGS
Cell density meter	Ultrospec 10	GHE
Centrifuge	ROTANTA 460 R	AHT
Centrifuge	Heraeus Fresco 21	TFS
Centrifuge	Heraeus Pico 17	TFS
Centrifuge	Sorvall RC 6+	TFS
Climate cabinet	KBF 240	BG
Conductivity meter	Lab 970	SIA
Conductivity probe	LF413T-ID	SIA
Deep freezer (−20 °C)	GNP 3056 Premium	LH
Deep freezer (−80 °C)	6380	GFL
Deep freezer (−80 °C)	Forma 906	TFS
Double-bladed lancet needle	PRL	AHW
Fermenter	BIOSTAT Cplus, 10 l	SSB
Fermenter module (gas mixing)	MX4/4	DG
Fermenter module (off-gas analysis)	GA4	DG
Fermenter module (pumps)	MP8	DG

continued on the next page

Table 2.4: *continued from the previous page*

Type	Model	M./V.
Fermenter module (sensors)	PH4PO4RD4	DG
Fermenter module (temperature, agitation)	TC4SC4B	DG
Gel caster with trays	for Mini-Sub Cell GT	BRL
Gel documentation system	Gel iX Imager	IIG
Gel electrophoresis chamber	Mini-Sub Cell GT System	BRL
Heating cabinet	U 27	MG
Heating cabinet	Function Line T12	TFS
Heating cabinet	Heratherm OGS100	TFS
Heating stirrer plate	MR Hei-Standard	HIG
HPLC autosampler	WPS-3000TRS	TFS
HPLC autosampler	WPS-3000TSL	TFS
HPLC binary pump	HPG-3400RS	TFS
HPLC binary pump	LPG-3400SD	TFS
HPLC column compartment	TCC-3000RS	TFS
HPLC degassing module	SRD-3400	TFS
HPLC diode array detector	DAD-3000RS	TFS
HPLC photo diode array detector	PDA-3000	TFS
HPLC post-column splitter	Acurate	TFS
HPLC refractive index detector	RI-101	SDK
HPLC system	UltiMate 3000 RS	TFS
Incubation hood for TiMiX 5 Control	TH15	EBG
Incubator	BK 6160	TFS
Incubator	Function Line B12	TFS
Lyophilizer	Alpha 2-4 LDplus	MCG
Magnetic stirrer	MR 3001 K	HIG
Magnetic stirrer	M2	ICZ
Magnetic stirrer	Telesystem 15	TFS
Magnetic stirrer	VMS-C7	VWR
Mass spectrometer	HCT	BDG
Membrane pump	SartoJet	SSB
Micro balance	MSE3.6P-000DM	SLI
Microscope	AxioCam Erc 5s	CZM
Microscope	BA310	MDG
Motor	RZR 2051control	HIG
Oxygen sensor	BCP-O2	BGS
pO_2 probe	VisiFerm DO 225	HBA
PCR cycler	MJ Mini	BRL
PCR cycler	labcycler Gradient	SBE
pH electrode	InLab Expert Pro pH 0-14, 0-100 °C	MTG
pH electrode	InLab Micro Pro pH 0-14, 0-100 °C	MTG
pH meter	FE20	MTG
pH meter	FG2	MTG
pH probe	405-DPAS-SC-K8S	MTG

continued on the next page

Table 2.4: *continued from the previous page*

Type	Model	M./V.
Photometer	P330	IG
Pipette (10 µl)	Transferpette S	BGK
Pipette (100 µl)	Transferpette S	BGK
Pipette (1 000 µl)	Transferpette S	BGK
Pipette (10 000 µl)	Transferpette S	BGK
Pipette (5 000 µl)	Transferpette S	BGK
Pipette (12 x 200 µl)	Transferpette S-12	BGK
Pipette (12 x 300 µl)	Research pro	EA
Pipette (8 x 100 µl)	Transferpette S-8	BGK
Pipette (8 x 1 200 µl)	Research pro	EA
Pipette (8 x 200 µl)	Transferpette S-8 electronic	BGK
Pipette (8 x 50 µl)	Transferpette S-8	BGK
Power supply	PowerPac Basic	BRL
Power supply	PowerPac HC	BRL
Pressure gauge (analogue)	DN10	SAG
Pressure gauge (digital)	PF2954	IFM
Quartz cuvette	104.002-QS	HG
Refrigerator	KT 1440	LH
Replicator	96 pins	BS
Rheometer	MCR 300	APG
Rocking platform	single tier	VWR
SEC-MALLS autosampler	1260 Infinity Standard Autosampler	AT
SEC-MALLS column oven	SECcurity TCC6000 column compartment	PSS
SEC-MALLS degasser	SECcurity vacuum degasser	PSS
SEC-MALLS detector	BI-MwA	BIC
SEC-MALLS pump	1260 Infinity isocratic pump	AT
SEC-MALLS RID	1260 Infinity	AT
SEC-MALLS system	SECcurity	PSS
Shaker	Tmix	AJA
Shaker	TiMix 5 Control	EBG
Shaker	MaxQ 2000	TFS
Shaker incubator	KS 4000 ic control	IKA
Shaker incubator	HT Minitron	IA
Stirrer (PTFE, crescent)	K074.1	CRG
Stirrer plate	RO 15 power	IKA
Ultrapure water system	PURELAB Classic	ELW
UV/Vis Photometer	Multiskan Spectrum	TFS
UV/Vis Photometer	Varioskan	TFS
Vacuum drying oven	VDL 53	BG
Vacuum pump	DC 2004 VARIO	VAG
Vacuum pump	RC 6	VAG
Vortex mixer	444-1372	VWR
Water bath	CC 1	PHK

2.1.5 Enzymes

Table 2.5: List of enzymes used in this work. Abbreviations: Art. no.: Article number; M./V.: Manufacturer or vendor.

Enzyme	Art. no.	M./V.
Glucose oxidase from *Aspergillus niger*	P6782-25MG	SAC
Horseradish peroxidase	G2133-50KU	SAC
Lysozyme	8259.2	CRG
Phusion DNA Polymerase	M0530 L	NEB
Proteinase K	EO0491	TFS
Proteinase K	P8102 S	NEB
Proteinase K	P8107 S	NEB
RNase A/T1 mix	EN0551	TFS

2.1.6 Nucleotides

Table 2.6: List of nucleotides used in this work. Abbreviations: Art. no.: Article number; M./V.: Manufacturer or vendor.

Nucleotide	Art. no.	M./V.
2-log ladder	N0469 S	NEB
Primer 27f	-	TFS
Primer 1492r	-	TFS
Primer 1525r	-	TFS
Quick-Load Purple 2-log DNA ladder	N0550 G	NEB

2.1.7 Sequences

Table 2.7: List of sequences used in this work.

Name	Sequence
27f	AGAGTTTGATCMTGGCTCAG
1492r	GGTTACCTTACGACTT
1525r	AAGGAGGTGWTCCARCC

2.1.8 Software

Table 2.8: List of software used in this work. Abbreviations: M./V.: Manufacturer or vendor.

Category	Name & Version	M./V.
Chromatography Software	Chromeleon 6.80 SR8 Build 2623 (156243)	DC
Chromatography Software	PSS WinGPC UniChrom 8.10, Build 2830	PSS
Fermenter Control and Data Acquisition	DASGIP Control 4.5, Rev. 237	DG

continued on the next page

Table 2.8: *continued from the previous page*

Category	Name & Version	M./V.
Fermenter Data Acquisition	MFCS/DA 3.0 (Level 43)	SSS
Fermenter Data Export	MFCS/DA 3.0 (Level 33)	SSS
Image Manipulation Software	GIMP 2.8.0	KMG
Image Manipulation Software	GIMP 2.8.10	KMG
Mass Spectrometer Control Software	HyStar 3.2.44.0	BDG
Mass Spectrum Analysis Software	DataAnalysis 4.0 SP 4 (Build 281)	BDG
Mass Spectrum Quantification Software	QuantAnalysis 2.0 SP 4 (Build 281)	BDG
UV Imaging Software	Intas GDS 3.28 16.01.2009	IIG

2.1.9 Microorganisms

The plates 'EPS1' and 'EPS2' were constructed from the exopolysaccharide producer collection at the Chair of Chemistry of Biogenic Resources by a co-worker. The bacteria were isolated from diverse habitats and screened for their ability to produce exopolysaccharides. The genera and species designations are given in tables 2.9 and 2.10 on the facing page.

2.2 Biochemical Methods

2.2.1 Agarose Gel Preparation

1 % agarose in TAE buffer was dissolved by boiling and stored at 65 °C until needed. The gel caster with the tray was levelled using a bubble level. The comb was fixed into the tray and agarose solution was poured into the tray as per requirement. Bubbles were removed using 1 µl pipette tips. The gel was let to cool, during which time the electrophoresis chamber (Mini-Sub Cell GT System, BRL) was prepared. Fresh TAE buffer was used and care was taken to cover the whole gel with buffer. In general, 3 µl to 5 µl of sample with loading dye was added to one well using a micropipette. Samples containing loading dye were prepared on a small sheet of parafilm by adding the loading dye first and then mixing it with the sample. Then, the whole sample was aspirated and added to the gel.

Loading Dye (5 x) The loading dye (5 x) contained 75 mM Tris, 50 mM EDTA, 50 % glycerol, 0.025 % bromophenol blue and 0.025 % xylene cyanol.

2.2.2 Agarose Gel Electrophoresis

The lid of the electrophoresis chamber was closed carefully and the banana plugs were connected to the banana jacks of the power source. The runs usually lasted for 40 min to 50 min with a constant voltage of 110 V and were programmed using a regulated power supply (PowerPac Basic, BRL).

2.2.3 Ethidium Bromide Staining

The staining solution for the ethidium bromide stain was composed of 250 ml TAE buffer and five drops of a 10 $g \cdot l^{-1}$ ethidium bromide solution. After completion of electrophoresis, the tray with the gel was removed from the electrophoresis chamber and the gel was carefully placed in

Table 2.9: The plate layout of the plate 'EPS1'. Wells A1 and E12 were left empty on purpose. Abbreviations: Agr: *Agrobacterium*; Anc: *Ancylobacter*; Art: *Arthrobacter*; Bac: *Bacillus*; Cur: *Curtobacterium*; Dye: *Dyella*; Er~: similar to *Erwinia*; Her: *Herbaspirillum*; KaA: associated with *Kaistobacter*; Koz: *Kozakia*; P/R: *Paracoccus*/*Rhodobacter*; Pae: *Paenibacillus*; Par: *Paracoccus*; Pse: *Pseudomonas*; Rah: *Rahnella*; Rao: *Raoultella*; SbC: close to *Sphingobacterium*; She: *Shewanella*; Sin: *Sinorhizobium*; Sp~: similar to *Sphingomonas*; Sph: *Sphingomonas*; Xan: *Xanthomonas*; µb~: similar to *Microbacterium*; µbA: associated with *Microbacterium*; µba: *Microbacterium*; µco: *Micrococcus*; ϱba: *Rhodobacter*; ϱco: *Rhodococcus*.

	1	2	3	4	5	6	7	8	9	10	11	12
A		Pse	Bac	Bac	Pse	Pse	Pse	Pse	Pse	Pse	Pse	Pse
B	Cur	Bac	Pse	Cur	Rah	Art	Art	Art	Art	Art	Art	Bac
C	Art	Art	Art	Art	Rao	Pse	Koz	Er~	Pse	Bac	Art	Pse
D	Bac	Pse	Agr	Bac	µco	Art	µco	Par	Pse	Pse	SbC	µb~
E	Dye	Sph	Her	Sph	Her	Sp~	Sp~	Her	Her	Sph	KaA	
F	Sph	ϱba	P/R	Art	Agr	Anc	µbA	µba	Agr	Art	µba	µba
G	Agr	Sph	Pae	Pae	Agr	Pse	Pae	Pae	She	µba	Art	Agr
H	µba	Pse	Xan	Sin	ϱco	ϱco	Pae	Art	ϱco	Art	Agr	Agr

Table 2.10: The plate layout of the plate 'EPS2'. Abbreviations: Agr: *Agrobacterium*; Anc: *Ancylobacter*; Art: *Arthrobacter*; Bac: *Bacillus*; BeI: *Beijerinckia indica*; BM~: similar to *Beijerinckia mobilis*; Br~: similar to *Burkholderia*; Bre: *Brevundimonas*; Bur: *Burkholderia*; Cau: *Caulobacter*; Cel: *Cellulosimicrobium*; Glu: *Gluconacetobacter*; Her: *Herbaspirillum*; Noc: *Nocardiopsis*; Pae: *Paenibacillus*; Pse: *Pseudomonas*; Rah: *Rahnella*; Rhi: *Rhizobium*; Sph: *Sphingomonas*; Xan: *Xanthomonas*; µb~: similar to *Microbacterium*; µbA: associated with *Microbacterium*; µba: *Microbacterium*; ϱco: *Rhodococcus*; ϱd~: similar to *Rhodanobacter*.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Rhi	Agr	Rhi	µb~	Anc	Pse	Rhi	µbA	µba	Her	Sph	Sph
B	Her	Sph	Sph	Rhi	Pae	Pae	Her	Her	Bac	µba	µba	µba
C	µba	µba	µba	µba	µba	µba	µba	µba	µba	µba	µba	Pse
D	Bac	Bac	ϱco	Pse	Bac	Bac	Bac	Bre	µba	ϱco	Pse	Bre
E	Bur	µba	µba	µba	µba	Br~	ϱd~	Bur	Bac	Bac	Bac	Bac
F	Agr	Art	BM~	Bur	Pae	Glu	Glu	Rah	Rah	Rah	Rah	Art
G	BeI	Bac	Bac	Bac	Bac	Art	ϱco	ϱco	ϱco	Noc	Cel	Cel
H	Sph	Pae	Cau	Bac	Xan	Agr	Xan	Xan	Xan	Xan	Agr	Agr

the staining solution for 20 min ± 5 min. In order to remove excess ethidium bromide from the gel, the gel was placed into a destaining solution for at least 10 min afterwards. The destaining solution consisted of 250 ml TAE buffer.

2.2.4 Agarose Gel UV Imaging

The destained gel was removed from the destaining solution and viewed using the gel documentation system (Gel iX Imager, IIG). Using the accompanying software (Intas GDS, IIG) image taking parameters were set and the images recorded. Images were saved to the computer in an unmodified form. Histogram equalisation and annotation was performed with the GNU Image Manipulation Program (KMG) afterwards.

2.2.5 Agarose Gel Band Cutting

The agarose gel was prepared as described under section 2.2.1 on page 28. In addition to the sample(s), one of the outermost lanes called 'sacrificial lane' contained the sample as well. The electrophoresis was conducted as described under section 2.2.2 on page 28. Before ethidium bromide staining, the sacrificial lane was cut off the gel using a scalpel and only that lane was subjected to ethidium bromide staining and UV imaging. When UV illuminated, the desired band was marked, the UV light turned off and the remainder of the gel aligned with the sacrificial lane. That way, the bands of the remaining gel were cut out without using either ethidium bromide or UV light minimizing DNA damage. After cutting the bands, the remainder of the gel was subjected to ethidium bromide staining and UV imaging as well to see if all bands were cut out correctly.

2.2.6 Gel Extraction

Two kits for the purification of double-stranded DNA from agarose gels were used, the NucleoSpin Gel and PCR Clean-Up kit (MNG) and the QIAEX II Gel Extraction kit (QGD). As an alternative to the NucleoSpin kit's purification method, magnetic beads were used as well (Agencourt AMPure XP, BCG). The descriptions given here are short versions of the complete instructions.

Gel Extraction (Macherey-Nagel Kit)

A tube with 200 µl of the buffer NTI per 100 mg of gel was prepared, the gel slice transferred into it and the tube was incubated for 10 min at 50 °C and 800 min^{-1}. Up to 700 µl of sample was transferred onto a silica column on top of a collection tube. The tube was centrifuged for 30 s at 11 000 × *g* and room temperature. The flow-through was discarded and the procedure repeated with the remaining sample.

After the sample was filtered, 700 µl of the buffer NT3 was transferred onto the column. The tube was centrifuged for 30 s at 11 000 × *g* and room temperature. The flow-through was discarded and the procedure repeated once. Then, the tube was centrifuged for 1 min at 11 000 × *g* and room temperature to remove buffer NT3 completely. Residual ethanol was removed by incubation at 70 °C for 2 min to 5 min.

The column was transferred onto a 1.5 ml tube and 15 µl to 30 µl of buffer NE was added to the top of the column. The tube was incubated at room temperature for 1 min and centrifuged for 1 min at 11 000 × *g* and room temperature. The supernatant was transferred into a new tube and contained the purified DNA.

Purification with Magnetic Beads After gel dissolution, 1.8 times the volume of the PCR product of resuspended magnetic beads was added to the dissolved gel. The beads were mixed thoroughly by pipetting and incubated for 5 min at room temperature.

The tube was put on a magnetic rack and incubated until the liquid became clear and all the beads had gathered at the bottom. The supernatant was discarded. Then, 1.0 ml buffer NT3 was added to the pellet and incubated for 30 s at room temperature. The supernatant was removed and another 1.0 ml buffer NT3 was added and the procedure repeated.

The beads were dried for approximately 3 min at room temperature and the tube removed from the magnetic rack. The DNA was eluted by adding 40 µl buffer NE and resuspending the beads in it. The tube was put on a magnetic rack and incubated until the liquid became clear and all the beads had gathered at the bottom. The supernatant was transferred to a new tube and contained the purified DNA.

Gel Extraction (Qiagen Kit)

A tube with 300 µl of the buffer QX1 per 100 mg of gel was prepared and the slice transferred into it. A QIAEX II tube was vortexed for 30 s and the appropriate volume of QIAEX II was transferred into the tube with the gel slice. The tube was incubated for 10 min at 50 °C and was vortexed every 2 min. Then, the sample was centrifuged for 30 s at 17 000 × g and room temperature and the supernatant was discarded.

500 µl of buffer QX1 was added to the pellet, the tube vortexed and centrifuged for 30 s at 17 000 × g and room temperature. The supernatant was discarded and the pellet was resuspended in 500 µl buffer PE, the tube vortexed and centrifuged for 30 s at 17 000 × g and room temperature. The supernatant was removed and the PE buffer step repeated.

The sample was air-dried for 10 min to 15 min and 20 µl of 10 mM Tris · HCl at pH value 8.5, TE buffer or water was added. The pellet was resuspended by vortexing and incubated at room temperature for 5 min.

The tube was centrifuged for 30 s at 17 000 × g and room temperature and the supernatant was transferred into a new tube and contained the purified DNA.

2.2.7 D-Glucose Assay

D-Glucose was determined using an in-house protocol [1] based on 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), commonly abbreviated as 'ABTS', and enzymatic oxidation of D-glucose. This in-house protocol was based on the Amplex Red Glucose/Glucose Oxidase Assay Kit [136].

Upon oxidation of ABTS with hydrogen peroxide a dye formed and absorption at λ_1 = 418 nm and λ_2 = 480 nm was determined. D-Glucose concentrations were calculated from these absorption values using linear calibration of standards 0 µM, 5 µM, 10 µM, 25 µM, 50 µM, 100 µM, 250 µM and 500 µM D-glucose[1].

Assay Mix

For one 96-well plate, 5.0 ml assay mix was produced. The following components made up the assay mix:

- Ultra-pure water: 4.25 ml

[1] Mass concentrations: 0 mg·l^{-1}, 0.9 mg·l^{-1}, 1.8 mg·l^{-1}, 4.5 mg·l^{-1}, 9 mg·l^{-1}, 18 mg·l^{-1}, 45 mg·l^{-1} and 90 mg·l^{-1}.

- Potassium phosphate (500 mM, pH value: 6.0): 400 µl
- D-Glucose oxidase from *Aspergillus niger* (100 $U \cdot ml^{-1}$): 200 µl
- ABTS solution (50 mM): 150 µl
- Horseradish peroxidase (1000 $U \cdot ml^{-1}$): 1.00 µl

Protocol

50 µl of appropriately diluted[2] sample were transferred into a well of a 96-well plate (F bottom, GBO), then 50 µl of assay mix was added to each well. The plate was sealed with an adhesive aluminium cover and incubated for 30 min at 30 °C and 150 min^{-1} on a shaker incubator. The cover was removed and the absorptions at 418 nm and 480 nm were measured on a photometer (Varioskan, TFS). Data were exported to broken[3] Excel files. The differences of absorptions at 418 nm and 480 nm of various concentrations tested were used to create the calibration curve. The absorption at 480 nm is subtracted from the absorption at 418 nm to make up for the background absorption of the sample.

Reaction Scheme

D-Glucose oxidase catalyses the oxidation of D-glucose using molecular oxygen to D-(+)-glucono-1,5-lactone and hydrogen peroxide (reaction 2.1). Then, the oxidation of reduced ABTS with hydrogen peroxide to reduced ABTS and water is catalysed by horseradish peroxidase (reaction 2.2). Oxidized ABTS can be determined photometrically at 418 nm.

$$2\ \text{D-glucose} + O_2 \rightleftharpoons 2\ \text{D-glucono-1,5-lactone} + 2\ H_2O_2 \tag{2.1}$$

$$H_2O_2 + 2\ \text{ABTS}_{\text{red.}} \rightleftharpoons 2\ H_2O + 2\ \text{ABTS}_{\text{ox.}} \tag{2.2}$$

2.3 Computational Methods

2.3.1 16S rDNA Sequence Generation

Sequencing data was downloaded from the sequencing company as AB1 files. Files were put into different directories based on the species/strain used. AB1 files were converted to FASTQ files to enable processing with established tools used for NGS data. FASTQ files were quality-trimmed using DynamicTrim.pl from SolexaQA 2.0 [137] using a probability cut-off of 0.05. If the filename indicated the sequence to be in reverse direction, the reverse complement was generated. The quality-trimmed FASTQ files were converted to FASTA format and stored as single records into one FASTA file. This FASTA file was used as alignment input for clustalw 2.1 [138]. The alignment was manually examined and, in case of doubts, the original AB1 files were viewed in UGENE 1.9.8

[2] Undiluted samples with 17 $g \cdot l^{-1}$ D-glucose (approximately 10 mM) give values of the 100 µM standard. Therefore, samples were generally diluted by a factor of 100 to 1000 and, if in doubt, the same sample was measured several times at different dilutions.

[3] It is important to note here that upon opening the files in Excel 2010 a popup message explained this to the user. Unfortunately, LibreOffice is not able to open broken Excel files effectively vendor-locking users to Microsoft's Office suite.

[139] to find base-calling errors. In case of suspected errors[4], the sequences were checked and edited by hand and the alignment rerun. The final sequence was put together manually using a FASTA file which contained all records and was generated with fastagrep.pl [140]. The records were laid out to contain the whole sequence in one and only one line. Parts of this process were automated using a Python script (see listing A.1 on page 123).

2.4 Exopolysaccharide Analytical Methods

2.4.1 Determination of Molar Mass

Samples of crude fermentation broth were centrifuged for 5 min at 17000 × g and 20 °C and the clear supernatant was transferred to an HPLC vial and analysed. Samples purified by cross-flow filtration were diluted 9:10 with 1 M $LiNO_3$ to give a final $LiNO_3$ concentration of 0.1 M.

Samples were analysed on a PSS SECcurity SEC system which featured a SECcurity vacuum degasser, a 1260 Infinity isocratic pump, a 1260 Infinity Standard autosampler, a SECcurity TCC6000 column oven, a BI-MwA multi-angle laser light scattering detector and a 1260 Infinity refractive index detector. The complete system was purchased from PSS Polymer Standards Service GmbH, Mainz and contained parts from Agilent Technologies, Waldbronn (pump, autosampler, refractive index detector) and Brookhaven Instruments Corporation, Holtsville, New York, USA (MALLS detector) as well. Separation was facilitated by using a TSK-GEL Alpha-M column with guard column (TBG). 100 µl of sample was injected, the mobile phase consisted of 0.1 M $LiNO_3$, the flow rate was 0.6 $ml \cdot min^{-1}$ and the column temperature was 50 °C. Data were recorded using PSS WinGPC UniChrom V 8.10, Build 2830 (PSS) on a personal computer running Microsoft Windows 7, data were processed using the same software on a Microsoft Windows XP laptop.

For all unknown polymers the molar mass could only be estimated from a pullulan reference curve consisting of the following standards: 342 Da (1.00), 1.08 kDa (1.23), 6.10 kDa (1.05), 9.60 kDa (1.09), 21.1 kDa (1.09), 47.1 kDa (1.07), 107 kDa (1.13), 200 kDa (1.11), 344 kDa (1.15), 708 kDa (1.27), 1.22 MDa (1.37) and 2.35 MDa (1.49). The molar masses refer to the molar mass at the refractive index peak and the parenthesized values denote the polydispersity index of the corresponding standard derived from the refractive index signal.

2.4.2 Determination of Rheological Properties

All rheometric measurements were conducted on an MCR 300 (APG) using cone-plate geometry and a measurement temperature of 20 °C. All measurements were performed using 0.5 % exopolysaccharide solutions in 1 % KCl in ultra-pure water. Total measurement times were limited to 10 min due to sample evaporation and a lack of know-how to prevent this.

Dynamic Viscosity

Dynamic viscosities were determined at 1 s^{-1} and 1000 s^{-1}. The sample was applied carefully using cut 1000 µl tips. Measurements at 1 s^{-1} comprised 300 points each measured for 2 s for a total measurement time of 10 min. Measurements at 1000 s^{-1} comprised 120 points each measured for 5 s. The viscosity was determined by using the arithmetic mean of the last 60 s of each measurement at 1 s^{-1} and the points from 180 s to 240 s at 1000 s^{-1}. Total measurement times,

[4] Insertions, deletions or different base in at most one of three sequences covering the same stretch of DNA. Low quality of base call was used as another indicator.

number of points, measurement time per point and—as a consequence—the ranges used for the determination of the dynamic viscosity were varied.

Thixotropy

Thixotropy, time-dependent shear-thinning, and the regain of the initial viscosity was determined using a three-step protocol: the sample was sheared for 120 points each measured for 2 s at $1\,s^{-1}$, then for 24 points of 5 s at $100\,s^{-1}$ and again for 240 s at $1\,s^{-1}$. In order to achieve greater time resolution, the third step was split into 20 points of 0.5 s each and 115 points 2 s each.

The first step was used to calculate the dynamic viscosity before shearing from the values from 180 s to 240 s. The time needed to regain 10 %, 25 %, 50 %, 75 % and 90 % of the initial viscosity after the shearing of the second step stopped was calculated from the data of the third step.

2.4.3 Determination of Aldose Monomer Composition

The aldose monomer composition of aqueous exopolysaccharide solutions was determined as described by Rühmann *et al.* [1, 93]. The exopolysaccharide solutions were subjected to an acidic hydrolysis step to yield monomeric sugars. After neutralization, the aldoses were derivatized with 3-methyl-1-phenyl-2-pyrazoline-5-one or 'PMP' for short. The samples were prepared for HPLC-MS analysis after derivatization and analysed using a reverse phase HPLC column, UV detector and an ESI-MS/MS. PMP derivatization was first mentioned by Honda *et al.* [91], HPLC-MS quantification by McRae and Monreal [92].

Hydrolysis

20 µl purified exopolysaccharide solution was transferred into an empty 96-well PCR plate (article number: 781350, BGK). For hydrolysis 20 µl 4 M trifluoroacetic acid was added and the plate was sealed with a TPE mat (article number: 781405, BGK). Mixing was achieved by putting the plate into a custom-made frame and inverting manually. After mixing, the liquid settled at the bottom of the tube due to centrifugation for 3 min at 2000 × *g* and 20 °C. The plate was put into the same custom-made steel frame, but now the screws were tightened. The exopolysaccharides were hydrolyzed for 90 min at 121 °C in a sand bath.

During hydrolysis, the next step was prepared: the volume of 3.2 % ammonia solution, necessary for neutralization, was determined. 20 µl of 4 M trifluoroacetic acid and 65 µl to 75 µl 3.2 % ammonia solution[5] were mixed. The correct pH value was verified with 12.5 µl phenol red solution ($1.0\,g \cdot l^{-1}$ in 20 % ethanol). Pink colour indicated a pH value of 8 or higher, which was necessary for the derivatization.

Following hydrolysis, the plate was removed from the heating cabinet and cooled. The steel frame was removed shortly thereafter to allow faster cooling. When cooled to room temperature, the liquid settled at the bottom of the tube due to centrifugation for 3 min at 2000 × *g* and 20 °C. The mat was removed and every sample neutralized with 3.2 % ammonia solution. The plate was fixed onto the custom-made frame and mixed manually. After mixing, the liquid settled at the bottom of the tube due to centrifugation for 3 min at 2000 × *g* and 20 °C. 5.0 µl was diluted in 45 µl ultra-pure water for the D-glucose determination using the D-glucose assay (see section 2.2.7 on page 31).

[5] Since ammonia has a relatively high vapour pressure the concentration in the liquid phase changes over time. This approach serves to counter this effect and ensure successful derivatization.

Derivatization

25 µl neutralized sample was transferred into an empty 96-well PCR plate (as before). For derivatization, 75 µl PMP solution (125 mg 3-methyl-1-phenyl-2-pyrazoline-5-one in 10.5 ml solvent made up of 8.00 ml methanol, 3.95 ml ultra-pure water and 50.0 µl 32 % ammonia) was added to each well and the plate was sealed with a new TPE mat. The plate was fixed onto the custom-made frame and mixed manually. After mixing, the liquid settled at the bottom of the tube due to centrifugation for 3 min at 2000 × *g* and 20 °C. The plate was incubated at 70 °C for 100 min in a PCR cycler (labcycler Gradient, SBE) and was cooled to room temperature after the run.

After derivatization, the plate was removed from the cycler. Mixing was achieved by putting the plate into a custom-made frame and mixing manually. After mixing, the liquid settled at the bottom of the tube due to centrifugation for 3 min at 2000 × *g* and 20 °C. 22 µl of each well was transferred to a 96-well microplate (F bottom, GBO) and mixed with 143 µl 19.2 mM acetic acid. Mixing was achieved by pipetting the sample up and down several times. The samples were transferred to an 0.2 µm PES 96-well filtration plate (AcroPrep Advance 350 0.2 µm Supor, PC) and centrifuged for 10 min at 1500 × *g* and 20 °C. The flow-through was collected in a new 96-well microplate, the plate sealed with a mat (Whatman Capmats 96 Wells, round, silicone rubber, GHU) and placed into the tray of the HPLC autosampler.

HPLC-MS Analysis

The samples were analysed on an UltiMate 3000 RS HPLC system (TFS) which featured an SRD-3400 degassing module, an HPG-3400RS binary pump, a WPS-3000TRS autosampler, TCC-3000RS column department and a DAD-3000RS diode array detector. After the diode array detector, the sample was passed through an Acurate post-column splitter (1:20, TFS) to an HCT ESI-MS (BDG). System control and data collection was done by a PC running Microsoft Windows XP, Chromeleon 6.80 SR8 Build 2623 (156243) (DC), HyStar 3.2.44.0, DataAnalysis 4.0 SP 4 (Build 281) and QuantAnalysis 2.0 SP 4 (Build 281) (all BDG).

10 µl sample was injected, the mobile phase consisted of a gradient of 85 % 5 mM ammonium acetate (pH value 5.60 ± 0.02) and 15 % acetonitrile and pure acetonitrile. The gradient is given in table 2.11 on the following page. Samples were separated at 0.6 $ml \cdot min^{-1}$ and 50 °C on an EC 100/2 Nucleodur C18 Gravity, 1.8 µm column (MNG). Derivates were detected at 245 nm, the first three minutes of each run were not analysed on the MS to prevent overloading with excess PMP. Mass spectrometer operation parameters are summarized in table 2.12 on the next page.

Calibration Standards Calibration standard 1 comprised of D-mannose, D-glucosamine, D-ribose, L-rhamnose, D-galactosamine, *N*-acetyl-D-glucosamine, cellobiose, D-glucose, D-galactose, D-xylose, 2-deoxy-D-glucose and 2-deoxy-D-ribose in a TFA matrix. Calibration standard 2 comprised of D-glucuronic acid, D-galacturonic acid, gentiobiose, lactose, *N*-acetyl-D-galactosamine, L-arabinose and L-fucose in a TFA matrix. If D-glucose dimers other than cellobiose or gentiobiose had to be expected, calibration standard 3 was used. Calibration standard 3 comprised of isomaltose, kojibiose, laminaribiose, maltose, nigerose and sophorose. The TFA matrix consisted of 4 M trifluoroacetic acid neutralized with 32 % ammonia solution to a pH value of 8.0, diluted to 1.6 M and mixed with undiluted calibration standards to give a final trifluoroacetic acid concentration of 0.8 M[6]. Standards 1 and 2 were prepared at concentrations of 200 $mg \cdot l^{-1}$, 50 $mg \cdot l^{-1}$, 40 $mg \cdot l^{-1}$, 30 $mg \cdot l^{-1}$, 20 $mg \cdot l^{-1}$, 10 $mg \cdot l^{-1}$, 5 $mg \cdot l^{-1}$, 4 $mg \cdot l^{-1}$, 3 $mg \cdot l^{-1}$ and 2 $mg \cdot l^{-1}$, standard 3

[6]Hydrolyzed samples contained 2.0 M trifluoroacetic acid. Neutralization with approximately 70 µl 3.2 % ammonia solution dilutes the acid down to approximately 0.73 M.

Table 2.11: HPLC-MS gradient for aldose monomer composition analysis. Elution of analytes was facilitated by using a gradient of mobile phase A (85 % 5 mM ammonium acetate (pH value 5.60 ± 0.02) and 15 % acetonitrile) and mobile phase B (pure acetonitrile). Changes between points are linear.

Time since injection in min	Percentage of A in %	Percentage of B in %
0.00	99	1
5.00	95	5
7.00	95	5
8.00	82	18
8.30	60	40
10.30	60	40
10.50	99	1
12.00	99	1

Table 2.12: ESI-MS operational parameters for aldose monomer composition analysis. Separated samples coming from HPLC were further analysed on the mass spectrometer. In order to prevent overloading the first three minutes of every run were directed to waste and a flow splitter was used to reduce the load by a factor of 20. This table summarizes the operational parameters of the ESI-MS.

Parameter	Setting
Scan mode	ultra (26 000 $m/z \cdot s^{-1}$)
Scan start	50 m/z
Scan stop	1 000 m/z
ICC target	200000
ICC maximum accumulation time	50 ms
ICC number of averages	4
Ion source capillary voltage	4 kV
Ion source dry temperature	325 °C
Ion source nebulizer pressure	2.76 bar
Ion source dry gas flow	6 $l \cdot min^{-1}$
MS mode	Auto
Auto MS smart target	600 m/z
MS/MS fragmentation amplitude	0.5 V

was prepared at concentrations of 50 $mg \cdot l^{-1}$, 40 $mg \cdot l^{-1}$, 30 $mg \cdot l^{-1}$, 20 $mg \cdot l^{-1}$, 10 $mg \cdot l^{-1}$, 5 $mg \cdot l^{-1}$, 4 $mg \cdot l^{-1}$ and 3 $mg \cdot l^{-1}$.

2.4.4 Quantification of the β-1,6-linked D-Glucose Content in β-1,3-β-1,6-Glucans

Periodate specifically reacts with β-1,3-β-1,6-glucans [141, 142]. While the β-1,3-glucose backbone remains intact, the β-1,6-linked D-glucose residues react with periodate: per mole β-1,6-linked D-glucose two moles periodate are consumed and one mole formic acid is formed. Periodate can be quantified spectrophotometrically at 290 nm and formic acid via HPLC. Also, the resulting pH shift can be measured. Then, the absolute periodate consumption and the absolute formic acid formation as well as the ratio of the two can be used to compare different polymer samples.

The protocol presented here is in part based on an experimental in-house protocol by Broder Rühmann.

Periodate Calibration Curve Sodium periodate was dissolved in ultra-pure water to prepare a calibration curve with the following concentrations: 20 000 µM, 15 000 µM, 10 000 µM, 8 000 µM, 6 000 µM, 4 000 µM, 1 500 µM, 1 000 µM, 800 µM, 600 µM, 400 µM, 150 µM and 0 µM.

Periodate Reaction and Sampling First, 5 ml of polymer solution was put into a 15 ml reaction tube with an aluminium foil sheath. The reaction was started by the addition of 5 ml 20 mM sodium periodate solution and mixing by vigorous pipetting. Then, samples of the reaction mixtures were taken immediately and after 1 d, 2 d, 3 d, 4 d and 5 d to determine the absorption at 290 nm and later the concentration of formic acid.

For every sample, 300 µl reaction mixture was transferred into a UV micro-cuvette and the absorption at 290 nm against ultra-pure water measured. Then, 980 µl of reaction mixture was transferred into 1.5 ml reaction tubes which were prepared with 20 µl 1 M sodium thiosulfate solution to stop the reaction. The stopped sample was incubated at room temperature over night to ascertain complete removal of periodate. On the next day, the samples were stored at −20 °C until the HPLC measurement. The pH value of the reaction mixture was measured only for the first and last samples. Between sampling, the reaction mixtures were kept in the dark.

HPLC Measurements Formic acid was determined via HPLC from thawed samples. The samples were filtered through modified PES spin filters with 10 kDa cut-off to remove polymeric substances and then analysed via HPLC using the same setup as described under section 2.8.4 on page 56. The following concentrations were used for the formic acid calibration curve: 2 000 µM, 1 600 µM, 1 200 µM, 800 µM, 400 µM, 200 µM, 160 µM, 120 µM, 80 µM and 40 µM.

2.4.5 Determination of Polymer Mass

Polymer mass was determined by gravimetry. The empty mass of a pre-dried tube was measured on an appropriate balance: for samples in the single µg region, a micro balance (MSE3.6P-000DM, SLI) was used and for samples of several tens of µg to single g, an analytical balance (PA214C, OC) was used.

The exopolysaccharide precipitate was collected and air-dried under a fume hood for up to 30 min. In case of small scale precipitations (less than 50 ml), the precipitate was transferred into pre-weighed 1.5 ml tubes by adding a small amount of precipitant, resuspending the precipitate in it and transferring the precipitate and precipitant with cut 1.0 ml tips and a pipette. The tube was

centrifuged for 60 s at 12000 × *g* and room temperature. The supernatant was removed carefully and the precipitate dried to constant mass.

At larger scales, the precipitate was collected from the stirrer or by letting the precipitate settle for at least one day, decanting the supernatant and collecting the precipitate in 50 ml tubes. The precipitate was let to settle for at least one day; the supernatant decanted and the precipitate collected in pre-weighed 1.5 ml tubes. If necessary, the 50 ml tubes were rinsed with precipitant and the remaining precipitate resuspended and transferred into pre-weighed 1.5 ml tubes. The tube was centrifuged for 60 s at 12000 × *g* and room temperature. The supernatant was removed carefully and the precipitate dried to constant mass.

The precipitate was either dried by vacuum drying at 60 °C (VDL 53, BG), freeze-drying at −40 °C (Alpha 2-4 LDplus, MCG) or drying at 60 °C in a heating cabinet (Function Line T12, TFS).

2.5 Microbiological Methods

2.5.1 Sterilization

Liquids Liquids were autoclaved for 20 min at 121 °C using the programme 'Flüssigkeit[7] RO' of the autoclave (135 S, HPM or 135 S, TEL). When the liquids were autoclaved in closed vessels, the vessel was slightly opened to allow pressure compensation, e. g. the cap was slightly unscrewed. Correct autoclaving was ensured by using an autoclave tape.

Sterile Filtration Sensitive material such as vitamin solutions were sterile filtered through 0.2 µm or 0.45 µm cellulose acetate syringe filters (VWR). Larger volumes (minimum: 2 l) were sterile filtered using an autoclaved vacuum filtration unit and an 0.45 µm cellulose nitrate filter (SSB).

Fermenters DASGIP fermenters were autoclaved for 30 min at 121 °C using the programme 'Flüssigkeit RO' of the autoclave. The 10 l fermenter was sterilized by an *in situ* sterilization process.

Plastic Items Dry autoclavable plastic items such as pipette tips were autoclaved for 20 min at 121 °C using the programme 'Instrumente[8] ST' of the autoclave. Correct autoclaving was ensured by using autoclave tape.

Glassware Glassware and other suitable instruments were sterilized for at least 3 h at 200 °C in a drying cabinet (Function Line T12, TFS). Correct autoclaving was ensured by using a baking tape.

2.5.2 Agars and Media

All masses and volumes given in this section denote the masses and volumes, respectively, needed for the production of 1 l solution.

[7] German for 'liquid'.
[8] German for 'instruments'.

Sterile pH Value Adjustment

Measuring the pH value of a sterile solution with an unsterile electrode would instantly make the solution unsterile. Therefore, whenever it was necessary to adjust the pH value of a sterile solution the following procedure was used.

The pH value was adjusted by taking a sterile aliquot, adjusting the pH in the sterile aliquot using 2 M NaOH and adding the corresponding amount of 2 M NaOH under sterile conditions to the remaining medium. Example: Draw an aliquot of 10 ml from 500 ml medium. Adjust the pH value with 45 µl 2 M NaOH to the desired pH value. Adjust the pH value of the remaining medium by adding $49 \cdot 45\,\mu l = 2205\,\mu l \approx 2.2$ ml 2 M NaOH under sterile conditions.

AMA Medium

- Nitrogen source & salts: 5.00 g casein peptone, 2.40 g $MgSO_4 \cdot 7\,H_2O$, 600 mg KCl, 1.00 g $NaNO_3$ and 30.0 mg NH_4Cl
- Carbon source: 15.0 g D-glucose and 300 mg $CaCl_2 \cdot 2\,H_2O$
- 500 mg KH_2PO_4
- Post-autoclaving additives: 2.00 ml trace elements solution
- pH value: 6.5

Nitrogen source & salts were prepared in 60 % of the final volume, carbon source in 20 % of the final volume and phosphate in 19.8 % of the final volume. Nitrogen source & salts, carbon source and phosphate were autoclaved separately. All autoclaved solutions were mixed under sterile conditions. Post-autoclaving additives were added from sterile stocks. pH was adjusted afterwards.

This medium was developed by Jochen Schmid and is a mix of the Modified Artificial Seawater Medium [143] and the Slime Medium (on the next page).

Modified EPSmax13 Medium

- Carbon source: as desired (see table 2.13 on page 42)
- KCl and yeast extract: 1.00 g yeast extract and 500 mg KCl
- Salt solution (10-fold): 3.00 g $NaNO_3$, 2.00 g K_2HPO_4, 500 mg $MgSO_4 \cdot 7\,H_2O$, 50.0 mg $FeSO_4 \cdot 7\,H_2O$ and 700 mg citric acid monohydrate

The salt solution was prepared as a sterile stock and stored at room temperature. KCl & yeast extract and carbon source were autoclaved separately and later mixed under sterile conditions. 100 ml salt solution (10-fold) was added to 900 ml of C source with KCl & yeast extract. The original medium was called 'EPSmax13' and contained 40.0 $g \cdot l^{-1}$ D-glucose as the C source. It was developed for maximum production of scleroglucan by *S. rolfsii* [144].

Lysogeny Broth

- 10.0 g Casein tryptone
- 5.00 g Yeast extract
- 10.0 g NaCl

This medium is abbreviated with 'LB[9]'. Contrary to the original recipe [145], the LB medium used in this work did not contain D-glucose. All components were autoclaved together.

Slime Media

- Carbon source: as desired (see table 2.13 on page 42)
- 5.00 g Casein peptone
- Salts & buffer: 20.0 g MOPS, 1.33 g $MgSO_4 \cdot 7\,H_2O$ and 20.0 ml NaOH (2 M)
- Post-autoclaving additives 20.0 ml KH_2PO_4 (83.5 g), 1.00 ml $CaCl_2 \cdot 2\,H_2O$ (50.0 g), 2.00 ml vitamins solution (RPMI 1640) and 1.00 ml trace elements solution
- pH value: 7.0

This set of media was used for most exopolysaccharide production experiments and is based on the screening medium used in [1]. KH_2PO_4 solution and $CaCl_2 \cdot 2\,H_2O$ solution were autoclaved separately and used as sterile stocks. Trace elements solution was sterile filtered and stored at 4 °C as sterile stock. Carbon source, peptone and salts & buffer were autoclaved separately. All autoclaved solutions were mixed under sterile conditions, then post-autoclaving additives were added from sterile stocks. pH was adjusted afterwards.

Nomenclature The basic slime medium given above was adjusted to suit different needs. Most often carbon source and peptone content were varied. For high-throughput screening less MOPS was used and for fermentation no MOPS was used at all.[10] In order to easily specify the contents of the medium used, a shorthand notation was developed and used.

- The carbon source and its respective concentration were coded by a number following the medium abbreviation directly, such as 'SM19' for 'Slime Medium with 24.0 $g \cdot l^{-1}$ D-glucose and 6.00 $g \cdot l^{-1}$ D-xylose'. The carbon source codes used in this work are summarized in table 2.13 on page 42.
- The peptone concentration was indicated by adding a space after the medium abbreviation, followed by a 'P' and then followed by the percentage of peptone used (in relation to the default medium value of 5.00 $g \cdot l^{-1}$).

[9] This abbreviation is often claimed to stand for 'Luria Broth', 'Lennox Broth' or 'Luria-Bertani medium'. In [145] the creator of this medium, Giuseppe Bertani, explains that the abbreviation stands for 'Lysogeny Broth': 'The acronym has been variously interpreted, perhaps flatteringly, but incorrectly, as Luria broth, Lennox broth, or Luria-Bertani medium. For the historical record, the abbreviation LB was intended to stand for "lysogeny broth."'

[10] Since small molecules were not removed completely by gel filtration, the overall amount of small molecules was reduced by lowering the MOPS concentration. Fermentations were carried out with pH control and, therefore, did not need MOPS.

- For screening purposes, a reduced MOPS concentration of 10.0 $g \cdot l^{-1}$ was used and indicated by an 'S'. If the medium contained 100 % peptone, the 'S' was given after a space after the medium shorthand, such as 'SM19 S'. If the medium contained a different amount of peptone, the 'S' was given directly after the peptone percentage, such as 'SM19 P30S'.
- In fermentations, MOPS-free slime medium was used, designated by an 'F'. If the medium contains 100 % peptone, the 'F' was given after a space after the medium shorthand, such as 'SM19F'. If the medium contained a different amount of peptone, the 'F' was given directly after the peptone percentage, such as 'SM19 P200F'.

Y(E)PD Medium

- 40.0 g D-Glucose
- Nitrogen source: 20.0 g casein peptone and 10.0 g yeast extract

D-Glucose and nitrogen source were autoclaved separately. The autoclaved solutions were mixed under sterile conditions.

Trace Elements Solution

- 1.80 g $MnCl_2 \cdot 4\,H_2O$
- 2.50 g $FeSO_4 \cdot 7\,H_2O$
- 258 mg Boric acid
- 31.0 mg $CuSO_4 \cdot 5\,H_2O$
- 21.0 mg $ZnCl_2$
- 75.0 mg $CoCl_2 \cdot 6\,H_2O$
- 23.0 mg $MgMoO_4$ or 25.7 mg Na_2MoO_4
- 2.10 g Sodium tartrate dihydrate

The solution was sterile filtered and stored at 4 °C in the dark.

Vitamins Solution (RPMI 1640)

The vitamins solution was bought sterile (SAC). Sterile aliquots of 1.0 ml to 2.0 ml were stored at −20 °C. Ingredients according to the manufacturer [146]:

- 0.02 g D-Biotin
- 0.3 g Choline chloride
- 0.1 g Folic acid
- 3.5 g myo-Inositol
- 0.1 g Niacinamide

Table 2.13: Carbon source code list. Carbon sources are indicated using the medium shorthand and the carbon source code. Carbon source codes are not limited to one and only one carbon source and, thus, may stand for an arbitrary number of different carbon sources used in a mix. The advantage of this approach is that all the carbon sources used in a medium were indicated by one and only one number.

Code	Carbon Source(s)	Concentration in $g \cdot l^{-1}$
0	no C source	0.00
2	D-xylose	30.00
17	D-xylose	10.00
18	D-glucose	10.00
19	D-glucose	24.00
	D-xylose	6.00
LCH[a]	D-glucose	24.00
	D-xylose	6.00

[a] LCH stands for 'lignocellulose hydrolysate' and was a dark brown liquid. It contained numerous other substances, some of them known inhibitors of microbial growth. Table 2.13 lists the *main carbon sources* of a medium with 30 vol% lignocellulose hydrolysate. See section 2.1.1 on page 21 for further data on the lignocellulose hydrolysate employed.

- 0.1 g p-Aminobenzoic acid
- 0.025 g D-Pantothenic acid · 0.5 Ca
- 0.1 g Pyridoxal · HCl
- 0.02 g Riboflavin
- 0.1 g Thiamine · HCl
- 0.0005 g Vitamin B12
- 0.2 g KCl
- 0.2 g KH_2PO_4
- 8.0 g NaCl
- 1.15 g Na_2HPO_4

Agar Preparation

Agar plates were prepared by the addition of 1.5 % of agar to the medium prior to autoclaving. For media whose components were autoclaved separately, the agar was either autoclaved separately as well or added to one of the components. The component to which the agar was added was chosen based on the reactivity of agar with the component, therefore, the agar was usually added to a sugar or salt(s) solution.

The completed agar solution was poured into petri dishes [147] under a laminar flow cabinet and openly stacked on each other in a pyramid style to allow faster cooling/solidification

and prevent water condensation on the lid of the dishes. After cooling, the dishes were closed, labelled, stacked vertically, bagged and stored at 4 °C in the dark until needed.

Plate Preparation for Fungi

Mycelial growth into the agar makes it nearly impossible to cleanly slice off part of the fungi without also having parts of the agar 'contaminate' the sample. In order to prevent mycelial ingrowth, agar plates were covered with a cellophane membrane (BRL) which allows small molecules to pass, but not mycelia. Cellophane membranes came with a piece of paper between each sheet as a separator. A cellophane membrane together with one separator was cut out using scissors and a petri dish as a template. The cut out pieces were gathered in a glass petri dish. Once ultra-pure water was added to the petri dish and everything was soaked in the water, the lid was closed and the plate was wrapped into aluminium foil and autoclaved as a liquid. After autoclaving, sterile cellophane membranes were removed from the petri dish using a flamed pair of tweezers. The cellophane membrane was transferred onto the surface of an agar plate such that the whole surface was covered and as much of the surface as possible was in direct contact with the membrane. After preparing the plates, they were stored at 4 °C.

2.5.3 96-Well Inoculation & Propagation

An autoclaved 96-pin replicator (BS) was disinfected using 80 % ethanol, flamed thoroughly, let cool, put back into the ethanol and flamed shortly again. The replicator was submerged into the contents of the wells of the source plate. The wells were homogenized by mixing carefully with the replicator. The replicator was then removed from the source plate and submerged into the contents of the wells of the destination plate. If necessary, complete transfer was facilitated by mixing carefully with the replicator. During all steps, the replicator was handled cautiously in order to avoid contact with the walls of the wells. Alternatively, defined volumes of the source plate were transferred to new plates using multi-channel pipettes. When inoculating from deep-frozen cultures, the replicator was carefully pushed into the frozen broth and subsequently transferred to the destination plate.

2.5.4 Cultivation

Agar Plates

Bacteria Bacteria were inoculated by streaking a suspension using an inoculation loop. In order to obtain single colonies, a fractionating pattern as depicted in figure 2.1 on the following page was used. Bacteria growing in tiny and tightly packed colonies were separated by transferring colonies to 1.0 ml sterile 0.9 % NaCl solution, vortexing vigorously and dilution streaking of the suspension[11]. Incubation usually took place at 30 °C. For the purpose of colony counting, 100 µl of an appropriately diluted bacterial suspension was evenly distributed on agar plates.

Fungi New cultures were started by placing a sclerotium from *Sclerotium rolfsii* or a small amount of deep-frozen mycelium from *Schizophyllum commune* into the centre of a YEPD agar plate and incubating it until the plate was covered completely with mycelium. Such a plate was considered as 'fresh'. Using a double-bladed lancet needle, approximately 1 cm^2 of mycelium was cut out and transferred to a new plate.

[11] This technique shall be called 'eppi dilution'.

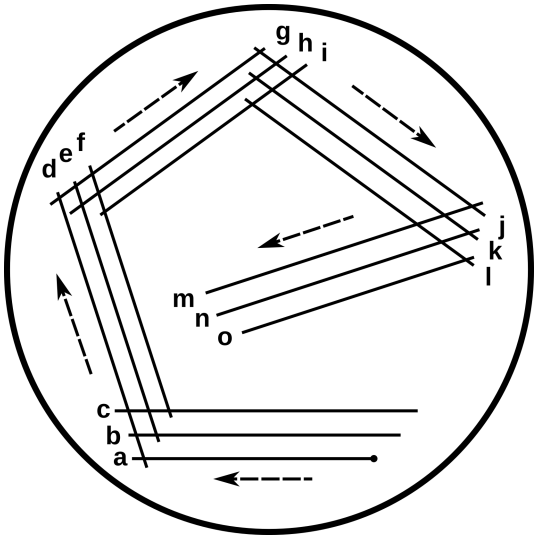


Figure 2.1: Dilution streaking of bacterial suspension on agar plates. Single colonies were obtained by dilution streaking of bacterial suspensions. The sterile inoculation loop was submerged in the bacterial suspension and rows a to c were streaked. The loop was flame-sterilized and rows d to f were streaked. Flame-sterilization and streaking were repeated until most of the plate was covered. Adapted from: [148].

Liquid Cultures

Liquid cultures were prepared in baffled or unbaffled Erlenmeyer flasks. Generally, the flask volume chosen was at least five times, preferably ten times, the liquid volume to ensure sufficient oxygen transfer [149]. Cultures were shaken on orbital shakers, usually at 150 min^{-1}, 30 °C and an eccentricity e of 1.9 cm.

For high-throughput experiments, cultivation was carried out in 96-well plates (2.0 ml; V bottom, sterile, GBO) using 1.0 ml culture per well and plates were incubated at 1 000 min^{-1} and 30 °C on a shaker with incubation hood (TiMiX 5 Control with TH15, EBG) and an eccentricity e of 1.5 mm.

2.5.5 Fermentation

Fermentations were carried out in two different systems: two parallel fermenter blocks with four fermenters each were used for small-scale parallel fermentations, while a 10-litre fermenter was used for single fermentations and scale-up experiments.

Parallel Fermentation of Fungi

Preculture Conditions Precultures of *Sclerotium rolfsii* and *Schizophyllum commune* were prepared in modified EPSmax13 medium with 40.0 $\mathrm{g \cdot l}^{-1}$ D-glucose as carbon source. 50 ml medium was incubated in 250 ml Erlenmeyer flasks without baffles and were agitated by magnetic stir bars on a magnetic stirrer (Telesystem 15, TFS) inside an incubation cabinet (KBF 240, BG). Preculture parameters were 30 % agitation power, 30 °C, 350 min^{-1} at low viscosity and 200 min^{-1} at higher viscosity.

Five days prior to inoculation of the fermenters with *S. rolfsii*, six YPD plates with 4 cm^2 of fresh mycelium were prepared and incubated for two days at 30 °C. Three days prior to inoculation of the fermenters with *S. rolfsii*, 250 ml modified EPSmax13 medium with 40.0 $g \cdot l^{-1}$ D-glucose as carbon source and 75 $mg \cdot l^{-1}$ ampicillin in a 2-litre Erlenmeyer flask were inoculated with a quarter of an agar plate cut into tiny pieces. On the day of inoculation of the fermenters, 50 ml of the preculture was drawn from the flask to a syringe using a sterile tubing.

Three days prior to inoculation of the fermenters with *S. commune*, 300 ml modified EPSmax13 medium with 40.0 $g \cdot l^{-1}$ D-glucose as carbon source and 50 $mg \cdot l^{-1}$ ampicillin was inoculated with 10 ml of preculture from a six day old preculture with 34 $mg \cdot l^{-1}$ chloramphenicol. On the day of inoculation of the fermenters, 50 ml of the preculture was drawn from the flask to a syringe using a sterile tubing.

Fermenter Setup Each fermenter was equipped with a pO_2 probe, a sampling tube, an inoculation port, an acid port, a foam probe, an anti-foam port, a base port, a septum, an air inlet with tube and sparger, an exhaust gas cooler, a pH probe and a temperature sensor. The exact layout used is given in figure 2.2 on the next page. Fermenter 2 deviated from the default layout: the septum and the pH probe were swapped.

Improvised foam breakers were prepared from cable ties as described by Riedel *et al.* [150]. Sterilizing the fermenters left several cable ties loose, so they slid down on top of the stirrer. The following foam breakers were affected: the two bottommost foam breakers of the fermenters 2 and 4, and all foam breakers of the fermenters 7 and 8. Some foam breakers were too long and thus, could not rotate: all foam breakers of the fermenters 2, 7 and 8; the two bottommost foam breakers of fermenter 4; and only the bottommost foam breakers of the fermenters 3 and 5. The dimensions of a single fermenter are given in figure 2.3 on page 47.

Calibrations of pH probe, pO_2 probe, peristaltic pumps and off-gas analyser were carried out in accordance with manufacturers' instructions. After off-gas calibration, the value of fermenter 1 was slightly lower than that of the other fermenters.

Fermentation Parameters Each fermenter contained 450 ml of 10:9 concentrated modified EPSmax13 medium and was inoculated with 50 ml of three day old preculture. Fermenters 1, 3 and 5 were inoculated with *S. rolfsii*, fermenters 2, 4, 6, 7 and 8 were inoculated with *S. commune*. After inoculation, 225 µl ampicillin solution were added so that the final concentration in the fermenter was 50 $mg \cdot l^{-1}$. At the start of fermentation, the initial pH value was measured by a pH electrode (405-DPAS-SC-K8S, MTG) and controlled to reach 6.0 with 1.0 M HCl and 1.0 M NaOH, control was disabled afterwards. Dissolved oxygen was controlled at 20 % relative to oxygen saturated medium and measured using an EasyFerm pO_2 probe (HBA). Dissolved oxygen was kept constant with a cascade: first, agitation would increase from 400 min^{-1} to 1 200 min^{-1}, then the gas flow would increase from 0.2 vvm to 0.6 vvm [12]. Temperature was maintained at 30 °C and was measured using a Pt100 temperature sensor. Anti-foam B 1:10 in ultra-pure water was added automatically once foam reached a foam probe. Agitation was limited to 400 min^{-1} 43 h after inoculation. Five days after inoculation, gas flow was limited to 0.2 vvm. Fermenters were harvested after 48 h (fermenters 1 and 2), 72 h (fermenters 3 and 4), 96 h (fermenters 5 and 6), 120 h (fermenter 7) and 144 h (fermenter 8).

Sampling Plan For the first 48 h, samples were drawn every three hours. Three different sample types were distinguished: small, large and large with rheometry. Small samples were taken every

[12] 0.2 vvm to 0.6 vvm corresponds to 6.0 $l_N \cdot h^{-1}$ to 18 $l_N \cdot h^{-1}$ in this setup.

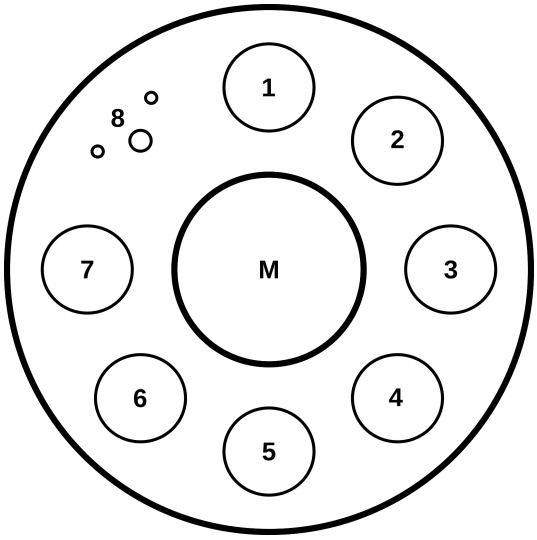


Figure 2.2: DASGIP fermenter top view. In the default layout, DASGIP fermenters were equipped with a motor for the stirrer (M) and seven ports for a pO_2 probe (1), ports for acid, sampling and inoculation (2), ports for base, anti-foam and foam probe (3), a septum (4), the air inlet with tube and sparger (5), an exhaust gas cooler (6) and a pH probe (7). A sheath for the temperature sensor and two connections for ground cables were present as well (8).

three hours, large samples every six hours and large samples with rheometry every twentyfour hours. Sample volume was 1.5 ml to 2.0 ml for small samples and 6.0 ml to 7.0 ml for large samples. After 48 h, small samples were taken every six hours, large samples every twelve hours and large samples with rheometry every twentyfour hours.

Small samples were taken for analyzing D-glucose consumption using the D-glucose assay and also certain metabolites using HPLC later. The metabolites were L-malic acid, succinic acid, citric acid, fumaric acid, glyoxalic acid, itaconic acid and oxalic acid. Small samples up until and including the 24 h sample were not diluted and directly centrifuged for 10 min at 10 000 × g and 20 °C, the supernatant transferred into a new tube and stored at −20 °C. Later, samples were diluted 1:10 with 13.5 ml ultra-pure water in 15 ml tubes and centrifuged for 30 min at 5 000 × g and 20 °C; the supernatant was transferred into a new tube and stored at −20 °C.

Large samples up until and including the 18 h sample were not diluted and directly centrifuged for 30 min at 5 000 × g and 20 °C and 4.5 ml supernatant were precipitated in 9.0 ml isopropanol. Later, samples were diluted 1:10 with 45 ml ultra-pure water in 50 ml tubes and centrifuged for 30 min at 5 000 × g and 20 °C. Three times 15 ml supernatant were precipitated in 30 ml isopropanol each.

At all times large samples with rheometry were diluted 1:10 with 45 ml ultra-pure water in 50 ml tubes and centrifuged for 30 min at 5 000 × g and 20 °C, 5.0 ml supernatant transferred into a tube for later rheometry measurements and stored at −20 °C, two times 15 ml supernatant precipitated in 30 ml isopropanol. The precipitates of all precipitation duplicate and triplicate samples were combined for the determination of polymer mass.

Cell dry mass at the end of the fermentation was estimated from the pelleted mycelia after centrifugation. Pelleted mycelia were stored at −20 °C in pre-weighed 50 ml tubes. The samples were dried at 60 °C to constant mass.

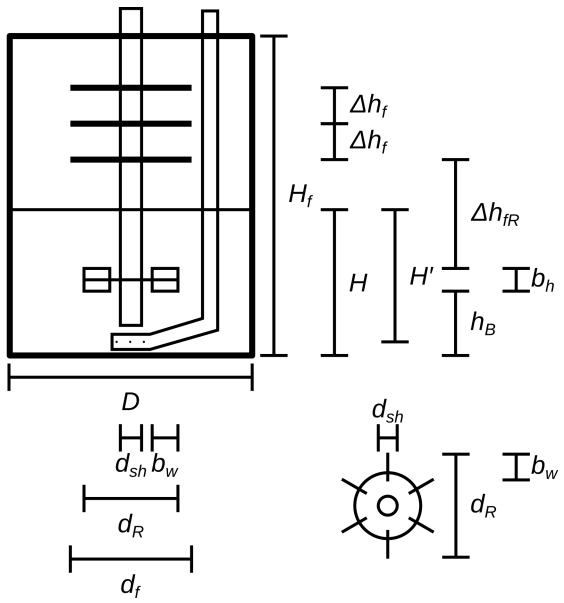


Figure 2.3: Schematic drawings of a DASGIP 1-litre fermenter filled up to approximately 500 ml (upper left corner) and a six-blade Rushton impeller (lower right corner). The fermenter inner diameter D was 9.9 cm, the total height H_f 14.4 cm. The medium height H was approximately 6.7 cm and the medium height as measured from the sparger H' was approximately 5.7 cm. The space between the stirrer and the fermenter bottom h_B was approximately 2.6 cm. The space between the bottom foam breaker and the stirrer Δh_{fR} was 7.8 cm. The distance of each foam breaker to neighbouring foam breakers Δh_f was 1.0 cm. The shaft diameter d_{sh} was 7.9 mm. The stirrer blade width b_w was 1.2 cm and the stirrer blade height b_h was 1.2 cm. The stirrer diameter d_R was 4.5 cm. The foam breaker diameter d_f was approximately 4.8 cm. Sampling tube and probes are not depicted. In reality, foam breakers were positioned at an angle of 90° to neighbouring foam breakers.

Parallel Fermentation of Bacteria

Preculture Conditions From a two day old LB agar plate of *Paenibacillus* 2H7, a single colony was used to inoculate 10 ml LB medium in a 25 ml Erlenmeyer flask without baffles. After 24 h incubation at 30 °C, 150 min^{-1} and an eccentricity e of 1.9 cm, 1.00 ml of this preculture was used to inoculate 100 ml SM with 24.0 $g \cdot l^{-1}$ D-glucose and 6.00 $g \cdot l^{-1}$ D-xylose with 100 % peptone (SM19 P100) in a 1-litre Erlenmeyer flask with baffles. The preculture was incubated for 24 h at 30 °C, 150 min^{-1} and an eccentricity e of 1.9 cm and then used to directly inoculate the fermenters with an initial D_{600} of 0.05.

Fermenter Setup The fermenter setup was the same as for the fungal fermentations (see section 2.5.5 on page 45) with the following differences: pH probe and pO_2 probe were swapped, the base port was connected to (2) and the sampling tube was used for inoculation and flushed with sterile air afterwards instead of using a dedicated inoculation port. The numbers correspond to the numbers given in figure 2.2 on page 46. Dimensions were the same as for the fungal fermentations given in figure 2.3 on the previous page with the following deviations: the space between the stirrer and the fermenter bottom h_B was approximately 3.6 cm; the space between the stirrer and the bottommost foam breaker Δh_{fR} was 4.0 cm; foam breakers were positioned at an angle of 60° to each other in line with stirrer disks.

Fermentation Parameters Fermenters 1 to 4 (block 1) contained 500 ml SM19 P100 without MOPS (SM19 P100F), fermenters 5 to 8 (block 2) contained 500 ml SM0 P100F with 30 % lignocellulose hydrolysate equalling 24.0 $g \cdot l^{-1}$ D-glucose and 6.00 $g \cdot l^{-1}$ D-xylose, minor amounts of organic acids and inhibitors (SMLCH P100F). Before inoculation, the pH value was set to 7.0 and controlled with 42.5 % phosphoric acid and 2 M sodium hydroxide during the course of the fermentation. Dissolved oxygen was monitored, but not controlled, using a VisiFerm pO_2 probe (HBA). Temperature was maintained at 30 °C. Agitation was constant at 400 min^{-1} and aeration at 0.4 vvm [13]. 1.0 ml antifoam (antifoam B, 1:10 in ultra-pure water) was added before inoculation. Additional antifoam was added manually. All fermenters were inoculated with 3.7 ml one day old preculture with D_{600} = 6.8.

48 h after inoculation, the pH control for block 1 was set to 6.4 and fermentations were ended after 91.5 h. 87.5 h after inoculation, the pH control for block 2 was set to 6.4 and fermentations were stopped after 134 h.

Sampling Plan For the first sample, approximately 2.0 ml was drawn from each fermenter. D_{600} was determined using a 1:20 dilution in ultra-pure water. 180 µl sample was diluted 1:10 with ultra-pure water and centrifuged for 5 min at 17000 × g and 4 °C. 1.0 ml of the supernatant was transferred to a new tube for the D-glucose assay. The undiluted sample was also centrifuged for 5 min at 17000 × g and 4 °C and 1.0 ml of the supernatant was transferred into a new tube for inhibitor, D-xylose and polymer analysis. Both supernatants were stored at −20 °C.

From the second sample on, approximately 2.0 ml was drawn from each fermenter. D_{600} was determined using a 1:20 dilution in ultra-pure water. The sample was centrifuged for 5 min at 17000 × g and 20 °C. 100 µl of the supernatant was diluted 1:10 with ultra-pure water in a new tube for the D-glucose assay. A part of the remaining supernatant was transferred to a fresh tube for inhibitor, D-xylose and polymer analysis. Both supernatants were stored at −20 °C. Excess supernatant was discarded and the pellet was used for the determination of cell dry mass.

[13] Corresponds to 12.0 $l_N \cdot h^{-1}$.

If the sample was still turbid after centrifugation it was diluted 1:5 with ultra-pure water and centrifuged for 5 min at 17 000 × g and 20 °C. 500 µl supernatant was diluted 1:2 with ultra-pure water in a new tube for the D-glucose assay. A part of the remaining supernatant was transferred to a fresh tube for inhibitor, D-xylose and polymer analysis. Both supernatants were stored at −20 °C. Excess supernatant was discarded and the pellet was used for the determination of cell dry mass.

Samples were drawn after 1.0 h, 14 h, 25.2 h, 37.5 h, 48 h, 62.2 h, 72.2 h and 88 h for both blocks; one final sample before harvest for block 1 after 91.5 h and further samples for block 2 after 96.3 h, 112 h, 120 h and 134 h.

Polymer Purification The fermentation broths of each block were collected and centrifuged for 10 min at 17 000 × g and 20 °C. The supernatants were cross-flow filtered through 0.45 µm membranes and samples for precipitation drawn from the feed, the retentate and the permeate.

7-litre Fermentation

Preculture Conditions Five days prior to inoculation, two SM1 P100 plates were inoculated from a cryo stock of *Paenibacillus* 2H7 from 2015-02-26 and incubated at 30 °C. Two days prior to inoculation, two times 10 ml LB medium in 25 ml Erlenmeyer flasks without baffles were inoculated with a single colony from the plates prepared two days earlier and incubated at 30 °C, 150 min^{-1} and an eccentricity e of 1.9 cm. One day prior to inoculation, two baffled Erlenmeyer flasks with 100 ml of SM19 P100 were inoculated with 5 ml of a one day old LB culture giving D_{600} of the preculture of 0.1.

Fermenter Setup The fermenter (BIOSTAT Cplus, SSB) was equipped with a pH probe, pO_2 probe, temperature probe, foam probe, ports for base, lignocellulose hydrolysate, medium concentrate and anti-foam, air inlet with tube and sparger, exhaust gas cooler, exhaust gas sensors for CO_2 and O_2 (BCP-CO2 and BCP-O2, respectively, BGS) and three baffles. The fermenter had a maximum working volume of 10 l, a total volume of 14 l and was equipped with one six-bladed Rushton type impeller. Stirrer blade width b_w was 35.0 mm, stirrer blade height b_h was 23.0 mm and the stirrer diameter d_R was 11.4 cm. Three improvised foam breakers were attached to the shaft, the space between the bottommost foam breaker and the stirrer Δh_{fR} was 20 cm, the space between each foam breaker to neighbouring foam breakers Δh_f was 2.0 cm and foam breakers were positioned at an angle of 60° to each other in line with the stirrer disks. The medium height as measured from the sparger H' was 24 cm, the distance between the stirrer and the medium surface was 12 cm. All pumps were calibrated prior to fermentation. The pump 'ACID' had a maximum feed rate of 16.3 ml·min^{-1}, the pumps 'BASE', 'AFOAM' and 'LEVEL' had maximum feed rates of 15.0 ml·min^{-1}. MFCS/DA 3.0 (SSS) was used for data acquisition.

Fermentation Parameters At the start of fermentation, the fermenter contained 5.0 l SM0 P100F with 5.0 % lignocellulose hydrolysate[14] and 1.0 ml·l^{-1} anti-foam B (1:10 in ultra-pure water, stirred). The medium contained phosphate for 7.0 l from the beginning of the fermentation; all other compounds were fed through 10 x medium concentrate, so that at the start of fermentation, the fermenter contained 500 ml of medium concentrate already. Aeration was set and controlled to 0.4 vvm [15], temperature to 30.0 °C and agitation to 400 min^{-1}. The initial pH

[14] 250 ml of 5.0 l.

[15] At the beginning of fermentation this corresponded to 120 $l_N \cdot h^{-1}$, after feeding 168 $l_N \cdot h^{-1}$.

Table 2.14: 7-litre Fermentation feeding programme of lignocellulose hydrolysate. Lignocellulose hydrolysate was fed into the fermenter slowly, starting at four hours after the inoculation. Initial hydrolysate concentration was 5 % or 250 ml of 5 l and was increased over the next 24 h time to 30 % or 2.1 l of 7.0 l. Feed rates between the points given in the table were interpolated linearly. 100 % pump output gave 16.3 $ml \cdot min^{-1}$.

Time after inoculation in h	Pump output in %	Total volume in l
0.00	0.0	0.25
4.00	0.0	0.25
4.02	0.0	0.25
16.00	6.0	0.59
16.02	6.0	0.59
28.00	25.0	2.02
28.02	25.0	2.02
31.00	25.0	2.10
31.02	0.0	2.10

Table 2.15: 7-litre Fermentation feeding programme of medium concentrate. Medium concentrate comprising of peptone, magnesium sulphate, calcium chloride, trace elements and vitamins at 10 x the final concentration were added during the course of fermentation to make up for the additional volume added by the lignocellulose hydrolysate. The starting volume was 5.0 l litre which contained 500 ml medium concentrate. Feeding started four hours after the hydrolysate feed and ended after eight hours. Feed rates between the points given in the table were interpolated linearly. 100 % pump output gave 15.0 $ml \cdot min^{-1}$.

Time after inoculation in h	Pump output in %	Total volume in ml
0.00	0.0	500
20.00	0.0	500
20.02	0.0	500
28.00	5.6	700
28.02	5.6	700
31.00	5.6	700
31.02	0.0	700

value was set to 7.21 and controlled using 2.0 M sodium hydroxide. Lignocellulose hydrolysate was fed according to table 2.14 on the facing page, medium concentrate was fed according to table 2.15 on the facing page. The acid pump was used for pumping lignocellulose hydrolysate, the base pump for 2.0 M sodium hydroxide, the anti-foam pump for anti-foam and the level pump for 10 x medium concentrate. The fermenter was inoculated with two one day old precultures at D_{600} of 5.8 and 6.0. 170 ml preculture was centrifuged for 10 min at 4000 × g and 20 °C. The resulting 86 ml of jelly-like 'pellet' was used to inoculate the fermenter with an initial D_{600} of 0.2. Between 15.5 h and 24 h after inoculation, the bottommost foam breaker slid down. An additional 4.0 ml anti-foam was fed manually into the fermenter 24 h after inoculation. 25 h after inoculation, aeration was manually set to 2.8 l·min^{-1} to keep aeration rate constant at 0.4 vvm.

Sampling Plan Samples were drawn after 0.1 h, 15.5 h, 24.2 h, 39.5 h, 48.0 h, 53.8 h, 72.5 h and 80.0 h. For the first sample, 28 ml was drawn from the fermenter. D_{600} was determined using a 1:10 dilution in ultra-pure water for this and later samples, if not stated otherwise. For the second and third sample, the sampling tube was initially flushed with fresh sample and then the real sample was drawn. 1.0 ml was transferred into 1.5 ml tubes three times—one of them pre-weighed—and centrifuged for 5 min at 17000 × g and room temperature. Supernatants were transferred into new tubes for monomer & inhibitor analysis, molar mass determination and D-glucose determination. The slimy phase on top of the pellet in the pre-weighed tube was carefully transferred to another pre-weighed empty 1.5 ml tube. If slime stuck to the pipette tip, it was removed by flushing the tip with ultra-pure water, collecting it in the pre-weighed tube with the slime, centrifuging for 5 min at 17000 × g and room temperature and discarding the supernatant.

For the fourth sample, a relatively stable foam formed inside the broth and was removed by centrifugation for 1 min at 2000 × g and room temperature. From this sample on, the samples were diluted 1:2 with ultra-pure water to facilitate formation of a clearly distinguishable pellet, top phase and supernatant. Also, D_{600} determinations were carried out with 1:20 dilutions from this sample on.

For the fifth sample, the 1:20 dilution for the determination of D_{600} was prepared gravimetrically in addition to another measurement using the usual approach of pipetting the fermentation broth. From the sixth sample on, dilution for centrifugation was 1:3 with ultra-pure water. The fermentation broth was harvested 81.8 h after inoculation: 2 l was heat-inactivated at 60 °C for 30 min, another 2 l was autoclaved and the remaining 3 l was diluted 1:10 with ultra-pure water and centrifuged for 15 min at 12000 × g and 30 °C. Autoclaved broth, heat-inactivated broth and the supernatant of the 1:10 diluted broth were stored at 4 °C for purification later.

2.5.6 Cryopreservation

Cryo-stocks in single vials (CryoPure, SAK) were prepared by adding equal amounts of sterile 60 % glycerol and microorganism suspension into the vial, e.g. 750 µl sterile 60 % glycerol and 750 µl microorganism suspension. Cryo-stocks in 96-well format were prepared by pipetting 50 µl of sterile 60 % glycerol into each well first. Subsequently, 100 µl of the respective microorganism suspension was added to each well. If the glycerol and the suspension were thoroughly mixed through the addition of the suspension an adhesive aluminium foil was used to seal the plate. Otherwise, the plate was shaken gently by hand to facilitate sufficient mixing. The prepared plates were then stored at −80 °C.

2.5.7 Determination of Attenuance at 600 nm

The term 'attenuance' is recommended by IUPAC. It is reserved for the quantity which takes into account the effects of absorbance, scattering and luminescence. The former name was 'extinction'. The symbol for attenuance is 'D' [64]. In short, it is the same value which is colloquially referred to as 'optical density' (OD_λ) in microbiology labs.

Determination of Linear Range

Attenuance measurements exhibit a limited linear range and undiluted samples easily exceed this range. Therefore, undiluted samples need to be diluted prior to measurement. The linear range was determined by measuring one sample at different dilutions and calculating the undiluted value from the diluted value and the dilution factor. The linear range is marked by a stable undiluted value. If no such measurements were possible or feasible, default dilution factors were used.

Cuvettes

In order to estimate the growth and cell concentration in pre-culture shake flasks and fermenters, the attenuance at 600 nm D_{600} was measured using an Ultrospec 10 Cell Density Meter (GHE). Samples were measured in PS semi-micro cuvettes with an optical path length of 1.0 cm. The sample was drawn from the culture and diluted appropriately. The attenuance at 600 nm was measured against pure diluant—ultra-pure water, if not stated otherwise. A dilution is considered appropriate if the attenuance of the diluted sample is within the linear range.

96-Well Plates

150 µl of the culture was transferred into a well of a 96-well plate (F bottom, GBO). The attenuance D_{600} was measured using a plate reader (Multiskan Spectrum or Varioskan, TFS).

2.6 Molecular Biological Methods

2.6.1 Extraction of Genomic DNA

Gram Positive Bacteria

4 ml of liquid culture containing the Gram positive bacterium was centrifuged for 10 min at 21 000 × g and 4 °C (Heraeus Fresco 21, TFS). The pellet was washed two times with ultra-pure water and centrifuged for 10 min at 21 000 × g and 4 °C. The pellet was resuspended in 200 µl Tris · HCl buffer (50 mM Tris · HCl, 10 mM EDTA, pH value 8). 25 µl lysozyme solution (25 mg · ml^{-1}) was added to the buffered suspension. Incubation for 60 min to 90 min at 450 min^{-1} and 37 °C (Tmix, AJA) followed. Then, the lysed cells were incubated with 50 µl SDS solution (10 %) for 10 min at room temperature. 2 µl RNase A/T1 mix (TFS) was added, followed by incubation for 60 min at 450 min^{-1} and 37 °C. 3 µl proteinase K (TFS or NEB) was added, followed by incubation for 60 min at 450 min^{-1} and 60 °C. In order to reduce shear stress, from this point on, only cut tips were used. 300 µl acetate buffer (3 M sodium acetate, pH value 4.8) was added and the sample carefully inverted several times. After centrifugation for 10 min at 21 000 × g and room temperature, the clear supernatant was transferred into a fresh 1.5 ml tube. DNA extraction was facilitated by adding 150 µl phenol (CRG) and 150 µl C/I mix (CRG), inverting

carefully, spinning down and transferring the top phase to a new tube for three times. 1 ml of 96 % ethanol at −20 °C was added and the tube inverted carefully. The tube was stored overnight at −20 °C. On the following day, the tube was centrifuged for 15 min at 21 000 × *g* and 4 °C. The supernatant was discarded and the DNA air dried in-place for approximately 10 min; care was taken to not overdry the DNA. Finally, the DNA was dissolved in 50 µl ultra-pure water and stored at 4 °C. This protocol is based on [151].

Gram Negative Bacteria

2 ml of a liquid culture was centrifuged for 10 min at 21 000 × *g* and 4 °C (Heraeus Fresco 21, TFS). 267 µl lysis buffer (40 mM Tris acetate at pH value 7.8, 20 mM sodium acetate, 1 mM EDTA, 1 % SDS) was added to the pellet. The pellet was resuspended by vigorous pipetting, not by vortexing, to prevent foam formation. 3 µl RNase A/T1 mix (TFS) was added, followed by incubation for 60 min at 450 min^{-1} and 37 °C (Tmix, AJA). 90 µl 5 M NaCl solution was added, proteins and cell debris precipitated and the solution became viscous. After centrifugation for 10 min at 21 000 × *g* and 4 °C, the clear supernatant was transferred to a new tube. One volume of C/I mix (CRG) was added and the tube inverted gently for at least 50 times until the liquid became milky. After centrifugation for 10 min at 21 000 × *g* and 4 °C, the clear supernatant was transferred to a new tube. 1 ml of 96 % ethanol at −20 °C was added and the tube inverted carefully. The tube was stored overnight at −20 °C. On the next day, the tube was centrifuged for 15 min at 21 000 × *g* and 4 °C. The supernatant was discarded. The DNA was further purified by performing the following procedure twice: the pellet was washed with 1 ml of 70 % ethanol, centrifuged for 15 min at 21 000 × *g* and 4 °C after which the supernatant was discarded. The DNA was air dried for approximately 10 min; care was taken to not overdry the DNA. Finally, the DNA was dissolved in 50 µl ultra-pure water and stored at 4 °C. This protocol is based on [152].

Additional Steps for Exopolysaccharide Producers

While highly viscous cultures were a minor obstacle, lysis of exopolysaccharide producers was more difficult. The former resulted in small or no pellets at all and was tackled by using different media or a younger culture or dilution prior to centrifugation. Lysis of exopolysaccharide producers was improved by additional lysis steps.

Instead of using 4 ml of microorganism suspension directly, 2 ml was transferred into a 50 ml tube, diluted to 35 ml with ultra-pure water, well mixed and centrifuged for 20 min at 4580 × *g* and 4 °C (ROTANTA 460 R, AHT). The supernatant was discarded, the pellet resuspended in 1 ml ultra-pure water and additional 2 ml of microorganism suspension was transferred into the tube, diluted to 35 ml with ultra-pure water, well mixed and centrifuged for 20 min at 4580 × *g* and 4 °C. The supernatant was discarded, the pellet resuspended in 1.5 ml ultra-pure water and centrifuged for 10 min at 21 000 × *g* and 4 °C (Heraeus Fresco 21, TFS). The supernatant was discarded and the pellet resuspended in 1.5 ml ultra-pure water. The tube was subjected to quick-freezing for 1 min in liquid nitrogen and heating for 10 min at 60 °C in a water bath for three times, the same method used by Michael Loscar for cell lysis. The tube was centrifuged for 5 min at 21 000 × *g* and room temperature and the supernatant was discarded. At this point, the cell wall was weak enough and the Gram positive protocol was used starting with the resuspension of the pellet in 200 µl Tris · HCl buffer.

2.6.2 Estimation of DNA Concentration

DNA concentration of the purified samples (see section 2.2.6 on page 30 and section 2.2.6 on page 31) was estimated on a nanophotometer (P330, IG). 1.0 µl ultra-pure water was used as reference. After every measurement, both the lid and cell were wiped with a lint-free tissue. In order to ensure complete sample removal, 1.0 µl ultra-pure water was placed on the measurement cell and the lid was attached to the cell. Then cell and lid were wiped off with a lint-free tissue.

The following equation[16] was used by the instrument for quantification [153]:

$$c_{\mathrm{DNA}} = f_{\mathrm{ssDNA}} \cdot l \cdot d \cdot (\Delta A(260\,\mathrm{nm}) - \Delta A(320\,\mathrm{nm})) \tag{2.3}$$

c_{DNA} is the DNA concentration in $\mathrm{ng \cdot \mu l^{-1}}$, f_{ssDNA} a substance specific factor for nucleic acid in $\mathrm{ng \cdot \mu l^{-1}}$, l the lid factor dependent on the lid used in the range 5 to 250, d the dilution factor, $\Delta A(260\,\mathrm{nm})$ the absorbance at 260 nm in relation to the reference, and $\Delta A(320\,\mathrm{nm})$ the absorbance at 320 nm in relation to the reference.

2.6.3 16S rDNA Polymerase Chain Reaction

For every reaction, a reaction volume of 20 µl was used. All components and the master mix were kept on ice until usage. The master mix was prepared by adding ultra-pure water first. Then, 4.0 µl high fidelity buffer (B0518 S, NEB), 0.4 µl 100 pM forward primer 27f, 0.4 µl 100 pM reverse primer 1492r or 1525r (all primers: TFS), 0.4 µl 10 mM dNTP mix, 0.2 µl high-fidelity polymerase (Phusion DNA Polymerase, NEB) and 1.0 µl sample.

Reaction tubes were placed in a PCR cycler (labcycler Gradient, SBE or MJMini, BRL), the lid closed and the program started. It consisted of the following steps:

- 95 °C for 10 min
- Repeat 35 times:
  - 95 °C for 60 s
  - 54 °C for 60 s
  - 72 °C for 90 s
- 72 °C for 10 min

The lid temperature was 105 °C and ultra-pure water was used as the default negative control. If available, a positive control was employed as well; usually genomic DNA which had already worked in a previous reaction.

Sequencing PCR products were cleaned up as described in section 2.2.6 on page 30 and sequenced by GATC Biotech AG, Konstanz.

[16] The equation presented in the manual appears to be wrong: the units of c_{DNA} would be $\mathrm{ng \cdot cm \cdot \mu l^{-1}}$. Therefore, I decided to change the unit of f_{ssDNA} from $\mathrm{ng \cdot cm \cdot \mu l^{-1}}$ to $\mathrm{ng \cdot \mu l^{-1}}$.

2.7 Purification Methods

2.7.1 Cell Separation

Harvested fermentation broths were diluted up to ten-fold with ultra-pure water to allow sedimentation of microorganisms. Prior to centrifugation, the diluted broth was shaken vigorously to ensure homogeneity. The diluted broths were centrifuged for 15 min at 12000 × *g* and 30 °C. The supernatant was collected in appropriate vessels and, if not used on the same day, was stored at 4 °C in the dark. Pellets were usually discarded.

2.7.2 Cross-flow Filtration

Cross-flow filtration was conducted using a membrane pump (SartoJet, SSB) and membranes on the basis of stabilized cellulose derivatives (Hydrosart, SSB) with a pore size of 0.45 µm or nominal molecular weight cut-off of 100 kDa or 10 kDa were used. Feed pressure was monitored using a digital pressure gauge, permeate and retentate pressures were monitored using analogue pressure gauges. Up to three membranes (of the same size) were used simultaneously.

The feed solution was stirred at 200 min^{-1} with a crescent blade stirrer mounted on a motor (RZR 2051 control, HIG). The minimal volume used was 0.5 l, the maximum volume was 5.0 l. Concentration was facilitated by manually adding more feed to the feed vessel. Diafiltration was facilitated by manually adding more washing solution to the feed vessel and was monitored by conductivity measurements.

Viscous exopolysaccharide solutions were diluted appropriately with ultra-pure water to allow filtration within the pressure limits and sensible Reynolds number regions.

2.7.3 High-Throughput Exopolysaccharide Purification

Cultures in deep 96-well plates were centrifuged for 30 min at 3710 × *g* and 20 °C (Sorvall RC 6+, TFS). 200 µl of supernatant was transferred to a 1.0 µm glass fibre filter plate (PC) and the plate centrifuged for 10 min at 3000 × *g* and 20 °C. 30 µl of the filtrate was transferred onto a prepared gel filtration plate (Micro SpinColumns, G-25 packing material, HA) and the plate centrifuged for 2 min at 1000 × *g* and 20 °C. At least 30 µl of filtrate was gathered in the collection plate.

Preparation of Gel Filtration Plate On the day prior to use, the gel filtration plate was washed two times with 150 µl of PMP buffer (5 mM ammonium acetate at pH value 5.60 ± 0.05) per well by centrifugation for 2 min at 2000 × *g* and 20 °C. An additional 150 µl of PMP buffer was added to each well and the plate centrifuged for 2 min at 1000 × *g* and 20 °C. For overnight storage at 4 °C, an additional 150 µl of PMP buffer was added to each well and the plate was covered and put into a sealable bag.

On the day of use, the gel filtration plate was washed two times with 150 µl of PMP buffer per well by centrifuging for 2 min at 2000 × *g* and 20 °C. An additional 150 µl of PMP buffer was added to each well and the plate centrifuged for 2 min at 1000 × *g* and 20 °C. The plate was used thereafter.

After use, the plate was washed three times with 150 µl ultra-pure water to remove small molecules by centrifuging for 2 min at 2000 × *g* and 20 °C. For storage, 100 µl 20 % ethanol was added to each well and the plate was covered and put into a sealable bag.

2.7.4 Precipitation of Exopolysaccharides

For precipitation, 2-propanol was used as a precipitant at a volumetric ratio of 2:1 (precipitant to exopolysaccharide solution). Exopolysaccharide solutions with a volume of 500 ml or more were precipitated by slowly pouring the exopolysaccharide solution into a vessel with the precipitant. During precipitation, the vessel was agitated at $200\,\mathrm{min}^{-1}$ with a crescent blade stirrer mounted on a motor (RZR 2051 control, HIG). Exopolysaccharide solutions with a volume between 50 ml and 500 ml were precipitated by slowly pouring the exopolysaccharide solution into an adequately sized beaker stirred by hand with a spatula. Lower volumes were precipitated in 50 ml tubes or 15 ml tubes.

2.8 Miscellaneous Methods

2.8.1 Calibration of pH Meter

The pH meter (FE20 or FG2, MTG) was calibrated using a calibration solution with pH value 7.00 ± 0.02 and, depending on the desired range, 4.00 ± 0.02 or 10.00 ± 0.02. The electrode was removed from the storage solution and rinsed with ultra-pure water. The remaining liquid was dabbed carefully with a tissue. The electrode was lowered into the calibration solution and the calibration started. After calibration of the first solution, the electrode was rinsed with ultra-pure water and dabbed again and lowered into the next calibration solution. After calibration, the electrode was rinsed with ultra-pure water and the remaining liquid was dabbed carefully with a tissue. Then the electrode was lowered into the storage solution.

2.8.2 Determination of pH Value

pH values were measured using an FE20 pH meter (MTG) and either an InLab Micro Pro pH electrode or an InLab Expert DIN pH electrode (both MTG). If the electrode had not been calibrated on the same day in the desired range, calibration was performed (see section 2.8.1). Before each measurement, the electrode was rinsed with ultra-pure water and the remaining liquid was dabbed carefully with a tissue. The electrode was lowered into the sample solution and the measurement started. When the signal became stable, the measured value was noted down. After measurement, the electrode was rinsed with ultra-pure water and the remaining liquid was dabbed carefully with a tissue. Then the electrode was lowered into the storage solution.

2.8.3 Determination of the Buffer Capacity

The initial pH value of the analyte, pH_0, was determined and noted down. A defined amount of the analyte was transferred into a beaker and titrated with 0.5 M NaOH to $pH_1 = pH_0 + 1$. The amount of substance of sodium hydroxide necessary for the pH shift was divided by the volume of the substance used. The buffer capacity is the result of that division in $\mathrm{mmol \cdot l^{-1}}$.

2.8.4 Determination of Acetic Acid, Formic Acid and Laevulinic Acid

Microorganisms were grown in the presence of an acid—acetic acid, formic acid or laevulinic acid—and had to be removed. Cultures in deep 96-well plates were centrifuged for 30 min at $3710 \times g$ and 20 °C. 20 µl supernatant was mixed with 180 µl ultra-pure water and filtered in a 10 kDa PES filtration plate (AcroPrep Advance 350 10K Omega, PC) by centrifuging for 30 min

at 1200 × g and 20 °C. If the filtrate volume was less than 155 µl, only 80 µl filtrate was taken and diluted with 80 µl ultra-pure water.

The samples were analysed on an UltiMate 3000 RS HPLC system (TFS) which featured an SRD-3400 degassing module, an HPG-3400RS binary pump, a WPS-3000TRS autosampler, a TCC-3000RS column department, a PDA-3000 photo-diode array detector and an RI-101 refractive index detector (SDK). System control and data collection were done by a PC running Microsoft Windows XP and Chromeleon 6.80 SR8 Build 2623 (156243) (DC).

The sample injection volume was 10 µl and the mobile phase consisted of 2.5 mM sulphuric acid. Samples were separated at 0.5 $ml \cdot min^{-1}$ and 70 °C on a Rezex ROA-Organic Acid H+ (8 %) column (PL). Acids were detected at 210 nm. Samples and calibration standards were run for 25 min. Standard 1 comprised of 5.0 $g \cdot l^{-1}$ acetic acid and formic acid, standard 2 comprised of 5.0 $g \cdot l^{-1}$ laevulinic acid. Standards were injected at different volumes: 10 µl, 6 µl, 3 µl, 1 µl, 0.6 µl, 0.3 µl and 0.1 µl corresponding to concentrations of 5.0 $g \cdot l^{-1}$, 3.0 $g \cdot l^{-1}$, 1.0 $g \cdot l^{-1}$, 0.5 $g \cdot l^{-1}$, 0.3 $g \cdot l^{-1}$, 0.1 $g \cdot l^{-1}$ and 0.05 $g \cdot l^{-1}$, respectively.

2.8.5 Determination of Conductivity

Conductivity was measured using a Lab 970 conductivity meter equipped with an LF413T-ID conductivity probe (both SIA). The probe was submerged into the liquid such that the hole was completely covered and no air bubbles remained in it. The measurement was started and after the displayed value had stabilized, it was noted down.

2.8.6 Determination of Furfural, Hydroxymethylfurfural and Vanillin

Microorganisms were grown in the presence of an inhibitor—furfural, hydroxymethylfurfural (5-(hydroxymethyl)-2-furaldehyde) or vanillin—and had to be removed. Cultures in deep 96-well plates were centrifuged for 30 min at 3710 × g and 20 °C. 30 µl supernatant was mixed with 120 µl ultra-pure water and 150 µl acetonitrile diluting the sample 10-fold and adjusting the acetonitrile concentration to 50 %. Acetonitrile was necessary to facilitate filtration of furfural without measurable retention. 200 µl of diluted sample was filtered using a 10 kDa PES filtration plate (AcroPrep Advance 350 10K Omega, PC) by centrifuging for 30 min at 1200 × g and 20 °C.

PMP Derivatization

PMP derivatization was conducted as described in section 2.4.3 on page 35. After derivatization, the plate was removed from the cycler. Mixing was achieved by putting the plate into a custom-made frame and mixing manually. After mixing, the liquid was gathered at the bottom of the tube by centrifugation for 3 min at 2000 × g and 20 °C. 40 µl of each well was transferred to a 96-well microplate (F bottom, GBO) and mixed with 260 µl 19.2 mM acetic acid in 40 % acetonitrile. Mixing was achieved by pipetting up and down several times. 280 µl of the samples was transferred to an 0.2 µm RC 96-well filtration plate (CHROMAFIL Multi 96, MNG) and centrifuged for 15 min at 700 × g and 20 °C. 155 µl flow-through was collected in a new 96-well microplate, the plate sealed with a mat (Whatman Capmats 96 Wells, round, silicone rubber, GHU) and put into the tray of the HPLC autosampler.

HPLC-MS Analysis

The setup used for analysis was the same as described under section 2.4.3 on page 35 including mass spectrometer operational parameters. For separation of the inhibitors, the gradient was

Table 2.16: HPLC-MS gradient for inhibitor analysis. Elution of analytes was facilitated by using a gradient of mobile phase A (85 % 5 mM ammonium acetate at pH value 5.60 ± 0.02 and 15 % acetonitrile) and mobile phase B (pure acetonitrile). Changes between points are linear.

Time since injection in min	Percentage of A in %	Percentage of B in %
0.00	99	1
5.00	95	5
7.00	95	5
8.00	82	18
9.00	70	30
9.30	70	30
9.70	60	40
11.30	60	40
11.50	99	1
13.00	99	1

extended by a minute and an intermediate acetonitrile concentration was used, see table 2.16. This gradient allowed the separation of aldose derivatives as well.

Calibration Standards Calibration standard '3Mix' comprised of furfural, hydroxymethylfurfural and vanillin in ultra-pure water. Standards were prepared at concentrations of 100 $mg \cdot l^{-1}$, 50 $mg \cdot l^{-1}$, 40 $mg \cdot l^{-1}$, 30 $mg \cdot l^{-1}$, 20 $mg \cdot l^{-1}$, 10 $mg \cdot l^{-1}$, 5 $mg \cdot l^{-1}$, 4 $mg \cdot l^{-1}$, 3 $mg \cdot l^{-1}$ and 2 $mg \cdot l^{-1}$.

2.8.7 Determination of UV/Vis Absorption Spectra

UV/Vis absorption at single wavelengths or spectra were measured on a photometer (Multiskan Spectrum or Varioskan, TFS) in 96-well plates (F bottom, GBO), PS or PMMA cuvettes (VWR) with a path length 10 mm or quartz cuvettes (104.002-QS, HG).

Chapter 3

Bacterial Conversion of Lignocellulose Hydrolysate to Exopolysaccharides

In a multi-step process, bacterial exopolysaccharide producers were screened singling out the most promising strains for conversion of lignocellulose hydrolysate to exopolysaccharide in a fermentation.

The strains of the exopolysaccharide producers collection (plates 'EPS1' and 'EPS2') were subjected to a growth screening on D-xylose in section 3.1 on the next page. Strains growing on D-xylose were taken to the next round in newly prepared plates: 'Xyl1' and 'Xyl2'.

The strains on Xyl1 were grown in the presence of D-xylose again in section 3.2 on the following page. D-Xylose consumption, exopolysaccharide concentrations and the exopolysaccharide aldose monomer compositions were quantified using PMP derivatization and HPLC-MS analysis. Exopolysaccharide aldose monomer composition data were compared with those published by Rühmann [154].

During lignocellulose hydrolysate preparation, numerous substances which can act as inhibitors to microbial growth might form. The tolerance towards six of these inhibitors—furfural, hydroxymethylfurfural, vanillin, acetic acid, formic acid and laevulinic acid—was examined in section 3.3 on page 65. Plates Xyl1 and Xyl2 were subjected to a growth screening: the growth in the presence of inhibitors was compared to the growth of a reference without inhibitors. The best-growing strains were transferred to new plates: 'ISp' for furfural, hydroxymethylfurfural and vanillin; 'ISr' for acetic acid, formic acid and laevulinic acid.

Industrially produced lignocellulose hydrolysate was used in a growth screening of the plates Xyl1 and Xyl2 to quantify how well the results from single inhibitor studies match the results with the real, far more complex, substrate in section 3.4 on page 69.

In section 3.5 on page 72, the plates ISp and ISr were grown with the respective inhibitors in each well. D-Glucose consumption, inhibitor degradation, exopolysaccharide production and aldose monomer compositions were analysed and used as a basis for the strain selection, described in section 3.6 on page 79.

Parallel fermentations of the strain *Paenibacillus* 2H7 at 500 ml scale were carried out to get reliable growth data in section 3.7 on page 83. Four fermenters were set up with a medium containing D-glucose and D-xylose at the same concentrations as a 30 % solution of lignocellulose hydrolysate; another four fermenters were set up using a medium with lignocellulose hydrolysate as carbon source. The data at 500 ml scale were used to run a 7 l fermentation with an improved fermentation strategy.

3.1 Growth on D-Xylose

Microbial growth of the exopolysaccharide producing strains of the plates 'EPS1' and 'EPS2' was screened in a 96-well format by incubation in 1.0 ml SM2 P30 for 48 h at 1000 min^{-1} and 30 °C. D_{600} was measured and 135 of 191 strains showed $D_{600} \geq 0.2$ and, thus, were deemed as 'growing well'. For well growing cultures, the maximum D_{600} was 1.63 and the median D_{600} was 0.83. From the 135 strains which showed good growth, new 96-well plates called 'Xyl1' and 'Xyl2' were prepared for storage at −80 °C for subsequent experiments. The plate layouts of these new plates are given in tables 3.1 and 3.2 on the facing page.

3.2 High-Content Screening with D-Xylose

While the previous step was used to reduce the number of candidates to test, information on exopolysaccharide production, exopolysaccharide monomer composition and D-xylose uptake of cells were still lacking. These information were obtained through a high-content screening of plate Xyl1. 1.0 ml SM17 P30S in a 96-well plate was inoculated from plate Xyl1 for 48 h at 30 °C and 1000 min^{-1}. Then, this plate was used to inoculate a new plate with 1.0 ml SM17 P30S using the replicator. This new plate was then incubated for 48 h at 30 °C and 1000 min^{-1}. The culture was subjected to high-throughput exopolysaccharide purification (see page 55), hydrolysis (see page 34), derivatization (see page 35) and HPLC-MS analysis (see page 35). Calibration standards 1, 2 and 3 were used; all without a TFA matrix.

In order to assess D-xylose consumption and to exclude the possibility that peptone was used as the sole carbon source, a part of the centrifuged culture was not subjected to gel filtration. Instead, the samples were diluted 1:50 and derivatized directly.

3.2.1 Controls & Deviations

The strain in well E10 showed no growth. An LB agar plate of that well incubated for three days at 30 °C showed growth of many tiny colonies without any apparent contamination. After growth had been observed in the empty control well E12, an LB agar plate of that well was incubated for three days at 30 °C as well and colonies with two distinguishable colony types were found. Morphology and colour of the colonies did not match contaminants described previously in this laboratory.

Correct neutralization was verified by the development of a pink colour of all samples upon addition of a phenol red solution to the remaining derivatized sample. Pink colour indicated a pH value of 8 or higher, which was necessary for the derivatization.

3.2.2 Aldose Monomer Composition

Out of the 95 strains screened 13 exhibited combined aldose monomer concentrations above the threshold of 560 $mg \cdot l^{-1}$[1]. The data of the 13 'hits' are summarized in table 3.3 on page 63, the results of all 95 strains are given in table A.1 on page 127. D-Galactose and L-rhamnose were found in all 13 exopolysaccharides; D-glucose in all but one of the exopolysaccharides produced.

[1]The threshold was calculated by multiplying the dilution factor 5.6 with a reliably detectable exopolysaccharide concentration of 100 $mg \cdot l^{-1}$. Since only single monomers are detected, the exopolysaccharide concentration is calculated as the sum of the single monomers. At 100 $mg \cdot l^{-1}$, even monomers making up 10 % only can still be determined reliably. Thus, the threshold of 100 $mg \cdot l^{-1}$ was chosen to make up for different exopolysaccharide compositions and contains a considerable safety margin.

Table 3.1: Plate Xyl1 was constructed from the exopolysaccharide producer strain collection plates EPS1 and EPS2. The well E12 was kept empty on purpose. Strains were selected based on their growth in a D-xylose containing medium. All of these strains from the genera *Arthrobacter*, *Bacillus*, *Microbacterium*, *Paenibacillus*, *Pseudomonas*, *Rhodococcus* and *Sphingomonas* are contained in this plate. For details, refer to tables 2.9 and 2.10 on page 29.

	1	2	3	4	5	6	7	8	9	10	11	12
A	*EPS1.B6*	*EPS1.B7*	*EPS1.B8*	*EPS1.B9*	*EPS1.B10*	*EPS1.B11*	*EPS1.C3*	*EPS1.C4*	*EPS1.C11*	*EPS1.D6*	*EPS1.F4*	*EPS1.F10*
B	*EPS1.G11*	*EPS1.H8*	*EPS1.H10*	*EPS2.F2*	*EPS2.F12*	*EPS2.G6*	*EPS1.A3*	*EPS1.A4*	*EPS1.B2*	*EPS1.B12*	*EPS1.C10*	*EPS1.D1*
C	*EPS1.D4*	*EPS2.D1*	*EPS2.D2*	*EPS2.D6*	*EPS2.D7*	*EPS2.E9*	*EPS2.E10*	*EPS2.E11*	*EPS2.E12*	*EPS2.G2*	*EPS2.G3*	*EPS2.G4*
D	*EPS2.G5*	*EPS2.H4*	*EPS1.F8*	*EPS1.F11*	*EPS1.F12*	*EPS1.G10*	*EPS2.A9*	*EPS2.C1*	*EPS2.C2*	*EPS2.C3*	*EPS2.C4*	*EPS2.C5*
E	*EPS2.C6*	*EPS2.C7*	*EPS2.C9*	*EPS2.E2*	*EPS2.E3*	*EPS2.E4*	*EPS2.E5*	*EPS1.D12*	*EPS2.A4*	*EPS1.F7*	*EPS2.A8*	-
F	*EPS1.G3*	*EPS1.G4*	*EPS1.G7*	*EPS1.G8*	*EPS1.H7*	*EPS2.B5*	*EPS2.B6*	*EPS2.F5*	*EPS2.H2*	*EPS1.A2*	*EPS1.A5*	*EPS1.A6*
G	*EPS1.A8*	*EPS1.A9*	*EPS1.A10*	*EPS1.A12*	*EPS1.B3*	*EPS1.C6*	*EPS1.C9*	*EPS1.C12*	*EPS1.D9*	*EPS1.D10*	*EPS1.G6*	*EPS1.H2*
H	*EPS2.A6*	*EPS2.C12*	*EPS2.D4*	*EPS1.H5*	*EPS1.H6*	*EPS1.H9*	*EPS2.D3*	*EPS1.E4*	*EPS1.E10*	*EPS2.A11*	*EPS2.B3*	*EPS2.H1*

Table 3.2: Plate Xyl2 was constructed from the exopolysaccharide producer strain collection plates EPS1 and EPS2. The wells D5 to D12 and E1 to H12 were kept empty on purpose. Strains were selected based on their growth in a D-xylose containing medium. All of these strains from the genera *Agrobacterium*, *Herbaspirillum*, *Xanthomonas* and several other, sometimes only represented by one strain, are contained in this plate. For details, refer to tables 2.9 and 2.10 on page 29.

	1	2	3	4	5	6	7	8	9	10	11	12
A	*EPS1.D3*	*EPS1.F5*	*EPS1.F9*	*EPS1.G1*	*EPS1.G5*	*EPS1.G12*	*EPS1.H11*	*EPS1.H12*	*EPS2.F1*	*EPS2.H6*	*EPS1.E3*	*EPS1.E5*
B	*EPS1.E8*	*EPS1.E9*	*EPS2.A10*	*EPS2.B1*	*EPS2.B7*	*EPS1.H3*	*EPS2.H5*	*EPS2.H7*	*EPS2.H8*	*EPS1.A1*	*EPS2.E1*	*EPS2.F4*
C	*EPS2.H3*	*EPS2.G11*	*EPS2.G12*	*EPS1.B1*	*EPS1.B4*	*EPS1.E1*	*EPS1.C8*	*EPS1.C7*	*EPS1.D7*	*EPS1.D11*	*EPS1.B5*	*EPS2.F8*
D	*EPS2.F9*	*EPS2.F11*	*EPS1.C5*	*EPS2.B4*	-	-	-	-	-	-	-	-
E	-	-	-	-	-	-	-	-	-	-	-	-
F	-	-	-	-	-	-	-	-	-	-	-	-
G	-	-	-	-	-	-	-	-	-	-	-	-
H	-	-	-	-	-	-	-	-	-	-	-	-

The exopolysaccharide concentration in the culture supernatant was calculated by summing up all monomer concentrations from the aldose monomer analysis. The median, minimum and maximum exopolysaccharide concentrations above the threshold were 1441 $mg \cdot l^{-1}$, 949 $mg \cdot l^{-1}$ and 2879 $mg \cdot l^{-1}$, respectively.

3.2.3 D-Xylose Consumption

SM17 P30S contained 10.0 $g \cdot l^{-1}$ D-xylose and 1.50 $g \cdot l^{-1}$ peptone. Since peptone may be used as a carbon source as well, the fact that the strains grew on the aforementioned medium does not conclusively prove that D-xylose was consumed. Therefore, the D-xylose concentration in the supernatant was determined using PMP derivatization.

D-Xylose was consumed by a majority of the strains: 87 of the 95 strains consumed at least one sixth of the D-xylose within 48 h, 66 at least half the D-xylose and 36 strains consumed at least 85 % of the D-xylose within 48 h. The remaining strains consumed less than one sixth of the D-xylose within 48 h. The median and the lower and upper quartiles of the residual D-xylose concentration were 3470 $mg \cdot l^{-1}$, 505 $mg \cdot l^{-1}$ and 5675 $mg \cdot l^{-1}$, respectively. Detailed data are given in table A.2 on page 131.

3.2.4 Influence of the Carbon Source on the Exopolysaccharide Aldose Monomer Composition

Exopolysaccharide aldose monomer compositions of the 13 'hits' are visualized in figure 3.1 on page 64. Major differences between the D-xylose-fed and the D-glucose-fed polymers were found for the strains *Xyl1.D11* and *Xyl1.E3*, minor differences were found for the strains *Xyl1.C1* and *Xyl1.C4*. The exopolysaccharide compositions of the other strains were affected very slightly or in almost undetectable amounts by the carbon source used.

Exopolysaccharide Composition Differences in *Xyl1.D11* On D-xylose, the *Xyl1.D11* exopolysaccharide contained around 20 % D-mannose and around 10 % D-glucuronic acid while on D-glucose, the monomers were dominated by D-glucose making up around 85 % and completely displacing both, D-mannose and D-glucuronic acid.

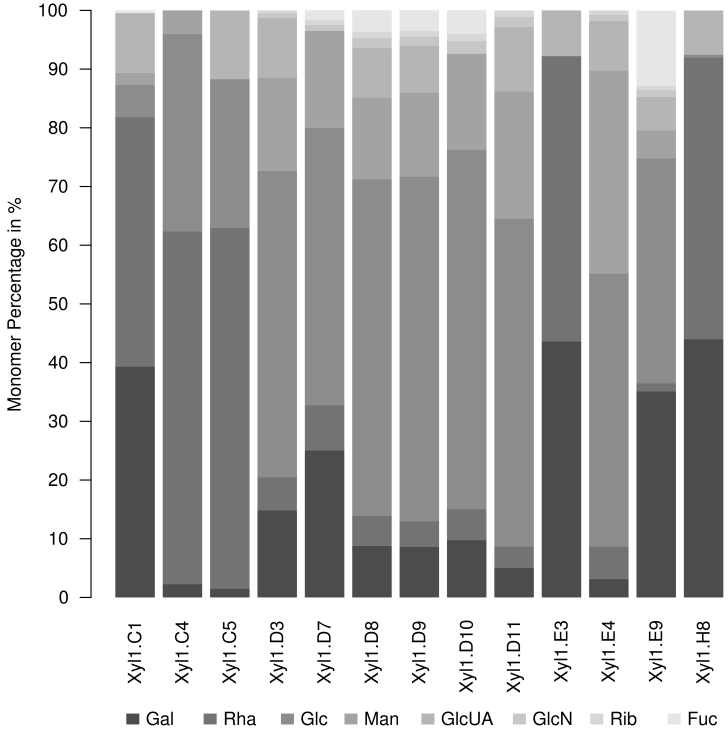
Exopolysaccharide Composition Differences in *Xyl1.E3* The exopolysaccharide of *Xyl1.E3* contained around 40 % D-galactose and 50 % L-rhamnose when the strain was grown on D-xylose, but 75 % D-galactose and slightly less than 10 % L-rhamnose when the strain was grown on D-glucose. Since D-glucose was detected in low amounts (less than 5 %), it is likely to be an artifact stemming from the method instead. For details on the method used for the generation of the D-glucose data, see 'Differences Between the Methods Behind D-Xylose and D-Glucose Data' on page 65.

Exopolysaccharide Composition Differences in *Xyl1.C1* *Xyl1.C1*'s exopolysaccharide contained 40 % L-rhamnose and 10 % D-glucuronic acid when the strain was grown on D-xylose, but 50 % L-rhamnose and less than 5 % D-glucuronic acid when the strain was grown on D-glucose.

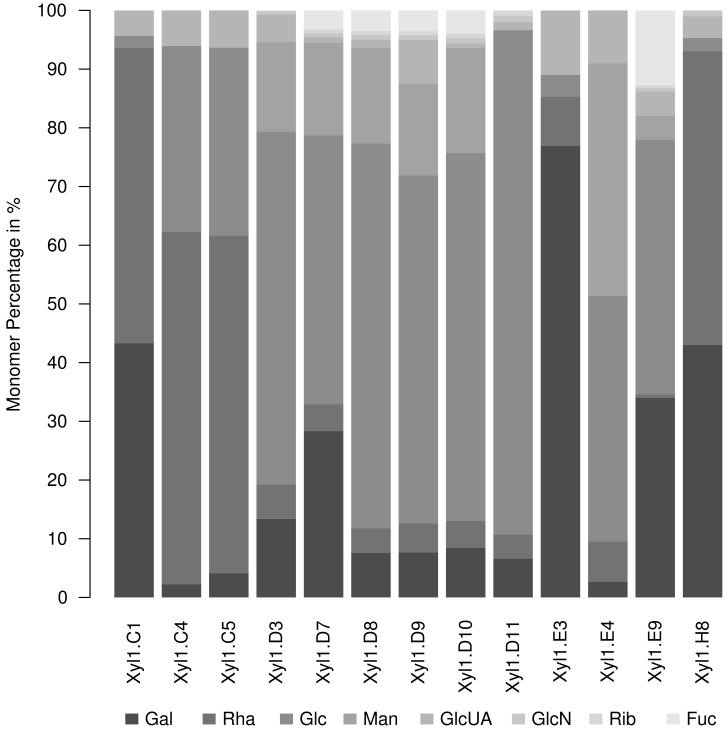
Exopolysaccharide Composition Differences in *Xyl1.C4* The exopolysaccharide of *Xyl1.C4* contained less than 5 % D-mannose when the strain was grown on D-xylose. However, this was not observed when the strain was grown on D-glucose. Instead, between 5 % to 10 % D-glucuronic acid were found—with the other monomer percentages virtually unchanged.

Table 3.3: The exopolysaccharide producers of plate Xyl1 were incubated for 48 h in SM17 P30S, a medium which contained 10.0 $g \cdot l^{-1}$ D-xylose as the sole carbon source. 13 strains exhibited exopolysaccharide concentrations exceeding the threshold value of 560 $mg \cdot l^{-1}$ in the supernatant. The aldose compositions of the exopolysaccharides are summarized in this table and visualized in figure 3.1 on the following page. The concentrations of the D-glucose dimers isomaltose and nigerose were too low for quantification. Therefore, the presence of these dimers is indicated qualitatively. The following analytes were not found in any sample and, thus, left out from the table: *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, L-arabinose, cellobiose, 2-deoxy-D-glucose, 2-deoxy-D-ribose, D-galactosamine, D-galacturonic acid, gentiobiose, kojibiose, lactose, laminaribiose, maltose and sophorose. D-Xylose was present in every sample, but also left out from this table, because it was not quantified after the gel filtration and, therefore, D-xylose could not be attributed to the medium or the polymer. Abbreviations: Fuc: L-fucose; Gal: D-galactose; Glc: D-glucose; GlcN: D-glucosamine; GlcUA: D-glucuronic acid; Man: D-mannose; Rha: L-rhamnose; Rib: D-ribose; Ism: isomaltose; Nig: nigerose; y: yes; n: no; ?: inconclusive. All values are in $mg \cdot l^{-1}$.

Strain	Fuc	Gal	Glc	GlcN	GlcUA	Man	Rha	Rib	Sum	Ism	Nig
Xyl1.C1	8	659	92	0	170	34	712	0	1675	n	n
Xyl1.C4	0	22	319	0	0	38	570	0	949	n	n
Xyl1.C5	0	17	286	0	132	0	694	0	1129	n	n
Xyl1.D3	0	427	1504	24	292	457	161	14	2879	n	n
Xyl1.D7	33	503	950	20	0	332	154	17	2009	n	n
Xyl1.D8	43	104	680	20	100	165	61	13	1186	y	?
Xyl1.D9	43	106	723	19	98	176	53	12	1230	y	?
Xyl1.D10	39	95	598	21	0	160	52	12	977	?	n
Xyl1.D11	0	49	543	16	107	210	36	11	972	y	n
Xyl1.E3	0	628	0	0	112	0	701	0	1441	n	n
Xyl1.E4	0	49	722	16	132	536	86	12	1553	n	n
Xyl1.E9	229	626	683	20	103	84	25	13	1783	y	n
Xyl1.H8	0	724	9	0	124	0	789	0	1646	n	n



(a) Exopolysaccharide aldose monomer compositions of the 13 'hits' of the high-content screening on D-xylose. Data source: table 3.3 on the previous page.



(b) Exopolysaccharide aldose monomer compositions of the 13 'hits' of the high-content screening on D-xylose when the strains were grown on D-glucose instead. Data source: Rühmann [154].

Figure 3.1: Exopolysaccharide aldose monomer compositions of the 13 'hits' of the high-content screening when grown on D-xylose or D-glucose. The concentrations of the single monomers were converted to percentages of all monomers found. The sequence of the single sugars in the figures and its legends is based on the frequency of their occurrence in the 13 exopolysaccharides when the strains were grown on D-xylose. D-Galactose and L-rhamnose were found in all exopolysaccharides and, thus, were the first two monomers, D-glucose was found in twelve exopolysaccharides only making it the third monomer, etc. Abbreviations, in the same order as in the legends: Gal: D-galactose; Rha: L-rhamnose; Glc: D-glucose; Man: D-mannose; GlcUA: D-glucuronic acid; GlcN: D-glucosamine; Rib: D-ribose; Fuc: fucose.

Differences Between the Methods Behind D-Xylose and D-Glucose Data Aldose monomer composition data using D-glucose as the carbon source were produced using virtually the same method used to produce the D-xylose data in this work [154]. The medium was SM18 P30S. Since D-glucose was present in the medium and some of it passed through size exclusion chromatography, the D-glucose freed by hydrolysis was indistinguishable from the D-glucose of the medium during HPLC-MS analysis. Therefore, the D-glucose concentration prior to hydrolysis was determined using the D-glucose assay (see section 2.2.7 on page 31) and used to correct the HPLC-MS results. For the inoculation of the main culture plate from the preculture plate, 10 µl of the preculture was used to inoculate the main culture. To the best of my knowledge, there were no further deviations.

3.3 High-Throughput Screening for Inhibitor Tolerance

The growth of the strains on plates Xyl1 and Xyl2 was screened in 96-well format by incubation in 1.0 ml of SM18 P30S and one inhibitor at $2.0\,\mathrm{g\cdot l^{-1}}$: furfural, hydroxymethylfurfural, vanillin, acetic acid, formic acid and laevulinic acid. As a reference, cultures were grown on medium without inhibitors as well. The precultures were inoculated from the corresponding cryostocks in 1.0 ml of SM18 P30S without inhibitors and incubated for 48 h at 30 °C and $1000\,\mathrm{min^{-1}}$. All main culture plates were inoculated from the same preculture plates by transferring 10 µl of the preculture to 990 µl of the medium. The main cultures were incubated for 48 h at 30 °C and $1000\,\mathrm{min^{-1}}$. At the end of the incubation, D_{600} of each well was determined to assess microbial growth in the presence of one inhibitor in relation to the growth without inhibitors.

For each inhibitor, 27 or 28 of the best-growing strains of this screening were gathered in two new plates designated as 'ISp' and 'ISr'[2] and were used for the next step—the high-content screening on inhibitors (see section 3.5 on page 72).

3.3.1 Controls & Deviations

The introduction of $2.0\,\mathrm{g\cdot l^{-1}}$ acid—be it acetic, formic or laevulinic—would have had an impact on the medium pH value or, at least, on the remaining buffer capacity. In order to compensate for the introduction of acid, the three acid inhibitors were prepared as stock solutions with $100\,\mathrm{g\cdot l^{-1}}$ acid, which were neutralized with 10 M sodium hydroxide. The final pH values were 7, 11 and 12 for acetic acid, formic acid and laevulinic acid, respectively. Since the ideal buffering pH value of a weak acid is at its pK_a even striving for a neutral pH easily resulted in excess OH^- which, in turn, was responsible for seemingly[3] dramatically high pH values. The medium contained some buffering agents which kept the medium at the desired pH value. The initial pH value of the preculture medium was 6.72, not 7.0.

Due to a calculation error, the concentration of $MgSO_4 \cdot 7\,H_2O$ was $2.66\,\mathrm{g\cdot l^{-1}}$, twice the concentration given for slime media (see section 2.5.2 on page 40).

[2] The original names were 'IS1r2pmp' for 'ISp' and 'IS1r2rez' for 'ISr' and were shorthand notations, too. The original names were used in the laboratory notebook, files and on 96-well plates. The names correspond to '**I**nhibitor **S**creening **1**, **r**ound **2**' and the means for the determination of the inhibitor concentrations: 'pmp' for all aldehydes (furfural, hydroxymethylfurfural and vanillin) and 'rez' for the acids. See also section 2.8.4 on page 56 and section 2.8.6 on page 57. 'round 2' was the internal name used for the high-content screening with inhibitors (see section 3.5 on page 72). The even shorter shorthand names were chosen for convenience and their brevity.

[3] The concentration of OH^- at the pH values 11 and 12 is 1 mM and 10 mM, respectively. The buffer concentration of SM18 P30S was 48 mM and only the 50th part of the medium was made up of the neutralized acids, so that the buffer easily absorbed the excess OH^- ions.

Judging with bare eyes, strains *Xyl1.F2* and *Xyl1.F4* did not seem to grow in the preculture. In the main culture, however, both grew. The negative control, *Xyl1.E12*, did not grow in any culture. In the reference main culture, strains *Xyl1.A4*, *Xyl1.E11*, *Xyl1.F11*, *Xyl1.G4*, *Xyl1.G6*, *Xyl1.G8*, *Xyl1.G10* and *Xyl2.C1* did not grow. Therefore, the calculations of this screening step could not be carried out with these strains and, thus, they were not considered for subsequent analyses.

3.3.2 Inhibitor Tolerance

The median background attenuance at 600 nm was calculated from empty wells of each plate and subtracted from every other well. The median background values, inter-quartile ranges and the amount of background values used are summarized in table A.3 on page 132. In the next step, the relation of each attenuance to the corresponding attenuance in the reference plate was calculated. The resulting percentages were grouped into eight classes. The classes, their descriptive short names and their boundaries are given in table 3.4 on the facing page. The data are visualized in figure 3.2 on page 68.

Example: The median background attenuance at 600 nm was 0.008. The strain was part of the furfural and the formic acid screenings. The raw attenuances at 600 nm were 1.116, 0.892 and 1.345 for the reference, furfural and formic acid, respectively. In the first step, the median background attenuance was subtracted yielding 1.108, 0.884 and 1.337 for the reference, furfural and formic acid, respectively. The relation of the attenuance of the furfural screening to the reference and the formic acid screening to the reference are 78.9 % and 121 %, respectively. The result of the furfural screening was grouped into class V (slightly inhibited growth), while the result of the formic acid screening was grouped into class VIII (excessive growth).

The response to **furfural** was mixed: 25 of 127 strains did not grow at all, 50 strains were inhibited moderately at least, only 28 strains were slightly inhibited, 18 showed normal growth and six strains showed excessive growth.

Compared to furfural, **hydroxymethylfurfural** was less inhibiting: only nine strains showed no growth, while 42 strains exhibited at least moderate inhibition, another 39 showed only slight inhibition, 31 grew normally and six strains showed excessive growth.

Vanillin was the most potent inhibitor of the ones tested: 100 of the 127 strains tested or 79 % showed no growth at all, only one strain grew normally, while 14 strains were at least moderately inhibited and twelve strains slightly inhibited only.

Acetic acid showed a low inhibitory potential: two strains did not grow, another two showed rudimentary growth, 24 strains were inhibited more or less, while 83 strains showed normal growth and 16 excessive growth.

Formic acid inhibited microbial growth to a lesser extent than acetic acid: three strains did not grow, one strain grew rudimentarily, 20 strains were inhibited at least slightly, while 80 strains showed normal growth and 23 strains excessive growth.

No strains were susceptible to **laevulinic acid**: all strains grew. Six were strongly, ten moderately and 24 slightly inhibited, while 85 strains grew to their normal attenuance values and two showed excessive growth.

3.3.3 Preparation of Plates for the High-Content Screening with Inhibitors

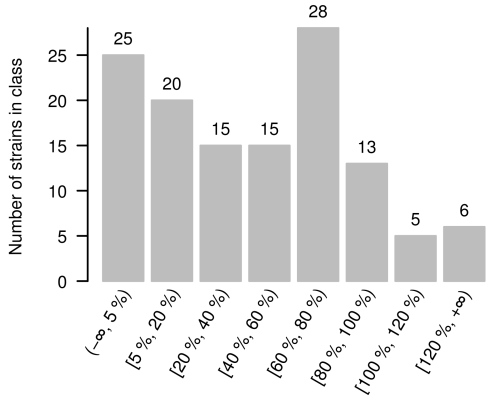
Two 96-well plates for the high-content screening were prepared. Using the growth data, each strain's growth in the presence of each inhibitor was ranked from best to worst. The top 27 strains of furfural, hydroxymethylfurfural and vanillin were collected in plate ISp and the top 28 strains of acetic acid, formic acid and laevulinic acid were collected in plate ISr. Plate layouts

Table 3.4: Definitions of the growth classes used in the inhibitor tolerance and lignocellulose hydrolysate tolerance experiments. Class numbers are used when longer names would pose space problems. The descriptions are used in the running text to make comprehension easier. The intervals define relative attenuance ranges. Relative attenuances at 600 nm were calculated by subtracting the median attenuance of empty wells from the attenuance of every other well on a per-plate basis. The relations of each attenuance in inhibitor presence to the corresponding attenuance in the reference plate gave the relative attenuances. These relative attenuances were used for classing.

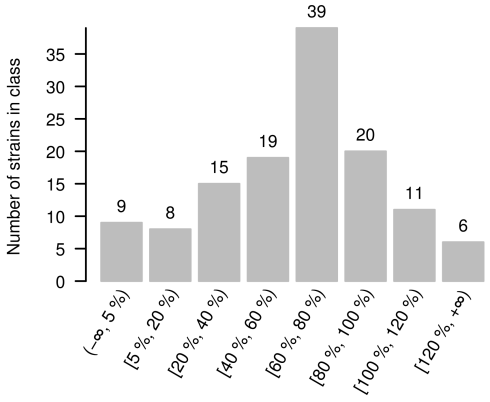
Class	Description	Interval
I	no growth	$(-\infty, 5\,\%)$
II	rudimentary growth	$[5\,\%, 20\,\%)$
III	strongly inhibited growth	$[20\,\%, 40\,\%)$
IV	moderately inhibited growth	$[40\,\%, 60\,\%)$
V	slightly inhibited growth	$[60\,\%, 80\,\%)$
VI	normal growth	$[80\,\%, 100\,\%)$
VII		$[100\,\%, 120\,\%)$
VIII	excessive growth	$[120\,\%, +\infty)$

Table 3.5: Strains appearing in the top 27/28 of at least four inhibitors. In this table all strains in at least four top 27/28 are highlighted. Abbreviations: Fur.: furfural; HMF: hydroxymethylfurfural; Van.: vanillin; Acet.: acetic acid; Form.: formic acid; Laev.: laevulinic acid.

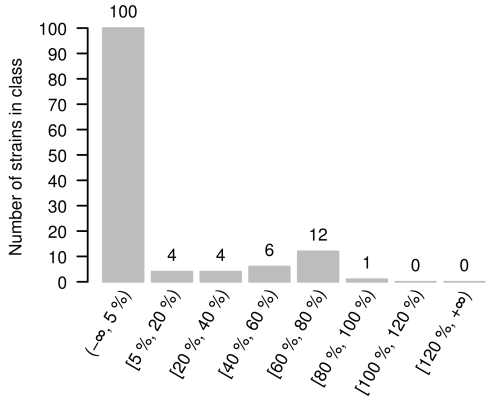
Strain	Part of Top 27/28 of Inhibitor … ?					
	Fur.	HMF	Van.	Acet.	Form.	Laev.
Xyl1.A10	yes	yes	yes	no	yes	no
Xyl1.C4	no	yes	yes	yes	yes	no
Xyl1.C5	no	yes	yes	yes	yes	no
Xyl1.G5	yes	yes	yes	yes	yes	yes
Xyl1.G11	yes	yes	yes	yes	yes	no
Xyl2.A1	no	yes	yes	no	yes	yes
Xyl2.A6	yes	yes	yes	yes	no	yes
Xyl2.A9	yes	no	yes	yes	no	yes
Xyl2.B7	yes	yes	yes	no	no	yes
Xyl2.B8	yes	yes	no	yes	yes	yes
Xyl2.C4	yes	yes	yes	yes	no	yes
Xyl2.C5	yes	yes	yes	yes	yes	no



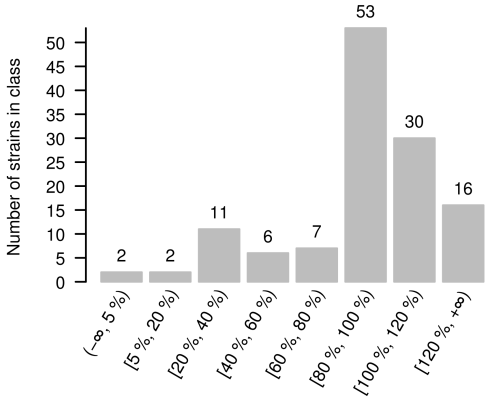
(a) Tolerance towards furfural



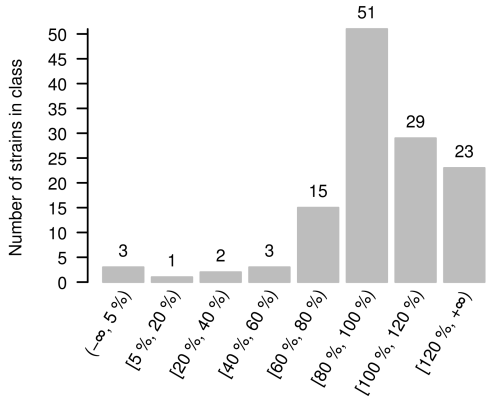
(b) Tolerance towards hydroxymethylfurfural



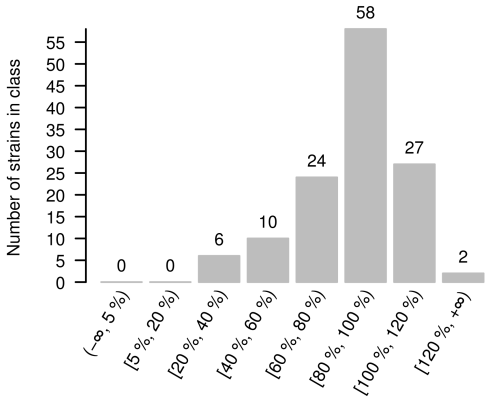
(c) Tolerance towards vanillin



(d) Tolerance towards acetic acid



(e) Tolerance towards formic acid



(f) Tolerance towards laevulinic acid

Figure 3.2: Tolerance towards six different inhibitors. The strains of the plates Xyl1 and Xyl2 were grown with and without an inhibitor at a concentration of $2.0\,g \cdot l^{-1}$. After subtraction of the background attenuance, the attenuance in the presence of an inhibitor was divided by the attenuance without any inhibitor. The strains of both plates were classed into eight classes (see table 3.4 on the previous page) and the results plotted as bar graphs. The data are available in table A.4 on page 133.

are given and explained in detail in tables A.5 and A.6 on page 133. Ranks of all strains and for all inhibitors and lignocellulose hydrolysate are given in table A.7 on page 135. As the selection process allowed each strain to appear once for every of the six inhibitors, some strains appeared more than once. Strains appearing in at least four of the six inhibitor top 27/28 are highlighted in table 3.5 on page 67. Given the low number of strains growing in the presence of vanillin, it should be noted here that only one of these strains, *Xyl2.B8*, was *not* among the top 27 strains of the vanillin screening.

3.4 High-Throughput Screening for Lignocellulose Hydrolysate Tolerance

On the availability of lignocellulose hydrolysate for experimentation, several of its parameters were analysed—most importantly: concentrations of D-glucose, D-xylose and furfural and the D_{600}. Microbial growth in the presence of lignocellulose hydrolysate as carbon source was tested analogously to the single inhibitor screening (see section 3.3 on page 65). The medium used as reference was SM19 P30, while the medium with lignocellulose hydrolysate was SMLCH P30. The results of this screening were purely informational and not used in the selection process for plates ISp and ISr. Instead, they were used to assess how reliable the results from the single inhibitor screening were for the prediction of the impact of the lignocellulose hydrolysate used on microbial growth.

3.4.1 Lignocellulose Hydrolysate Analyses Results

As outlined earlier (see section 2.1.1 on page 21), data on the lignocellulose hydrolysate supplied were scarce. The complete results of in-house analyses are given in table 3.6 on the following page. The lignocellulose hydrolysate was acidic as evident from the pH value (4.86) and acetic acid concentration ($9.86\,g\cdot l^{-1}$). Furthermore, the hydrolysate contained roughly $75\,g\cdot l^{-1}$ D-glucose, $20\,g\cdot l^{-1}$ D-xylose, $2.7\,g\cdot l^{-1}$ furfural and $500\,mg\cdot l^{-1}$ hydroxymethylfurfural.

3.4.2 Controls & Deviations

Before inoculation, each well of the main culture plates contained 995 µl medium, instead of 990 µl. The preculture was incubated for 49.5 h. In the preculture of Xyl1, the negative control Xyl1.E12 did not grow. The strains *Xyl1.E10* and *Xyl1.F10* did not show any growth as well. In the preculture of Xyl2, the negative controls Xyl2.D5 to Xyl2.D12 did not grow. However, growth was visible in wells Xyl2.F1, Xyl2.F10, Xyl2.F11 and Xyl2.G5. Therefore, when transferring from the preculture plate Xyl2 to the main culture plates, the bottom half was not used.

All strains which did not show growth in the preculture of the reference did not grow in the main culture as well. Therefore, the calculations of this screening step could not be carried out with these strains and thus, they were not considered for subsequent analyses. The main cultures were incubated for 48.5 h.

Conductivities of SM19 P30 and SMLCH P30 at 22.5 °C were $4.48\,mS\cdot cm^{-1}$ and $7.53\,mS\cdot cm^{-1}$, respectively.

3.4.3 Lignocellulose Hydrolysate Tolerance

The results were analysed analogously to the results of the single inhibitor experiments (see section 3.3.2 on page 66). The median background attenuances ± half the corresponding inter-

Table 3.6: Results of in-house analyses of the lignocellulose hydrolysate supplied by an industrial partner.

Parameter	Value
pH value	4.86
Buffer capacity	39.1 mM
Conductivity[a]	6.72 $mS \cdot cm^{-1}$
Pelletable particles[b]	0.044 %
D_{600}[c]	0.808
D_{600} (clarified)[c]	0.559
Acetic acid concentration	9.86 $g \cdot l^{-1}$
Formic acid concentration	70 $mg \cdot l^{-1}$
D-Glucose concentration	75 $g \cdot l^{-1}$
D-Xylose concentration	20 $g \cdot l^{-1}$
Furfural concentration	2.7 $g \cdot l^{-1}$
Hydroxymethylfurfural concentration	500 $mg \cdot l^{-1}$

[a] Measurement temperature: 20 °C.

[b] Pelletable particles were examined by determining the dry mass of the pellet of approximately 5.2 g lignocellulose hydrolysate. Particles were sedimented by centrifugation for 10 min at 17 000 × *g* and room temperature.

[c] Reference: ultra-pure water. Lignocellulose hydrolysate was clarified by centrifuging for 10 min at 17 000 × *g* and room temperature.

quartile ranges were 0.0400 ± 0.0009, 0.0397 ± 0.0010, 0.1280 ± 0.0015 and 0.1266 ± 0.0021 for reference plates Xyl1 and Xyl2 and the lignocellulose hydrolysate plates Xyl1 and Xyl2, respectively. For Xyl1, three wells were used for background calculation and for Xyl2, eight wells were used for background calculation. Details are given in table A.3 on page 132.

In the next step, the relation of each attenuance to the corresponding attenuance in the reference plate was calculated. The resulting percentages were classified into eight classes. The data are given, explained in detail and visualized in figure 3.3 on the facing page.

Most strains were not affected by 30 vol% lignocellulose hydrolysate: 67 of 133 strains showed normal growth, 22 were slightly inhibited, nine moderately inhibited, four strongly inhibited and seven showed excessive growth. 20 strains showed no growth, while four exhibited at least rudimentary growth.

Comparison with Single Inhibitor Experiments The strains highlighted previously (see table 3.5 on page 67) were also tested in the lignocellulose hydrolysate screening and the results are given in table 3.7 on the facing page. *Xyl1.A10*, *Xyl1.C4*, *Xyl1.C5*, *Xyl1.G5*, *Xyl2.A1* and *Xyl2.C5* or half of the highlighted strains did not reach the top 28 of the lignocellulose hydrolysate screening. On the other hand, ten of the top 28 strains of the lignocellulose hydrolysate screening did not reach the top 27/28 of *any* single inhibitor and are given in table 3.8 on page 72.

In order to assess how well the single inhibitor experiments matched the lignocellulose hydrolysate screening, the top 28 strains were compared. There were eight matches (28.6 %) between furfural and lignocellulose hydrolysate, seven (25.0 %), ten (35.7 %), two (7.1 %), eleven (39.3 %) and nine matches (32.1 %) between hydroxymethylfurfural, vanillin, formic acid, acetic

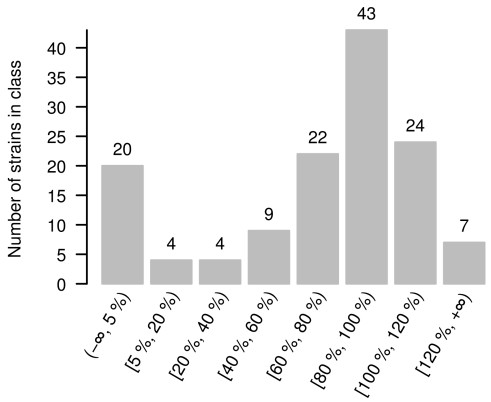


Figure 3.3: Tolerance towards lignocellulose hydrolysate. The strains in the plates Xyl1 and Xyl2 were grown without lignocellulose hydrolysate (SM19 P30) and with lignocellulose hydrolysate (SMLCH P30). SM19 P30 contained the same amount of D-glucose and D-xylose as SMLCH P30. After subtraction of the background attenuance, the attenuance in the presence of lignocellulose hydrolysate was divided by the attenuance without lignocellulose hydrolysate. The strains of both plates were classified into eight classes (see table 3.4 on page 67) and the results plotted as bar graphs. The data are available in table A.4 on page 133.

Table 3.7: Comparison of single inhibitor and lignocellulose hydrolysate results of high-performing strains. In this table, the ranks of the strains highlighted earlier for reaching the top 27/28 of at least four inhibitors (see table 3.5 on page 67) are compared with the corresponding ranks in the lignocellulose hydrolysate screening. Complete data for all strains is given in table A.7 on page 135. Abbreviations: Fur.: furfural; HMF: hydroxymethylfurfural; Van.: vanillin; Acet.: acetic acid; Form.: formic acid; Laev.: laevulinic acid; LCH: lignocellulose hydrolysate.

Strain	Rank in experiment with						
	Fur.	HMF	Van.	Acet.	Form.	Laev.	LCH
Xyl1.A10	27	16	21	22	36	37	42
Xyl1.C4	25	31	7	1	16	43	95
Xyl1.C5	2	34	12	2	18	34	92
Xyl1.G5	6	14	24	4	6	6	45
Xyl1.G11	21	20	27	23	25	71	16
Xyl2.A1	19	33	8	52	17	10	65
Xyl2.A6	8	6	1	13	31	23	4
Xyl2.A9	44	19	3	9	33	28	3
Xyl2.B7	22	25	19	89	52	27	28
Xyl2.B8	14	10	120	11	22	2	11
Xyl2.C4	7	9	25	8	29	5	25
Xyl2.C5	3	12	28	12	11	74	40

Table 3.8: Comparison of lignocellulose hydrolysate high-performing strains excluded from further screening. For the next screening step, strains had to achieve a rank among the top 27/28 in single inhibitor experiments. The strains in this table were not included in any further screening due to a low rank, but were among the top performers on lignocellulose hydrolysate. Single inhibitor screening data for *Xyl2.C1* are missing, because it did not grow in these screenings. Complete data for all strains is given in table A.7 on page 135. Abbreviations: Fur.: furfural; HMF: hydroxymethylfurfural; Van.: vanillin; Acet.: acetic acid; Form.: formic acid; Laev.: laevulinic acid; LCH: lignocellulose hydrolysate.

Strain	Rank in experiment with						
	Fur.	HMF	Van.	Acet.	Form.	Laev.	LCH
Xyl1.C3	29	94	29	74	61	89	27
Xyl1.C6	36	63	60	73	88	69	17
Xyl1.C9	45	60	62	80	85	33	21
Xyl1.C11	33	69	63	49	98	48	20
Xyl1.F7	116	104	90	125	63	100	7
Xyl1.H5	75	85	99	65	116	118	19
Xyl2.B2	89	76	115	57	90	83	13
Xyl2.B3	122	124	116	111	66	102	14
Xyl2.C1	-	-	-	-	-	-	2
Xyl2.C2	31	68	35	50	96	62	23

acid and laevulinic acid and lignocellulose hydrolysate, respectively. Combining the matches of the single inhibitors, 18 (64.3 %) matches were found. Using only two inhibitors as indicators, the combination of vanillin and acetic acid gave the best results with 16 matches (57.1 %). Given the fact that only 27 strains were considered as growing in the vanillin screening, one should note that ten of these are also present in the top 28 of the lignocellulose hydrolysate screening.

3.5 High-Content Screening with Inhibitors

The previous round served to screen the exopolysaccharide producers growing on D-xylose for their ability to grow in the presence of single inhibitors or lignocellulose hydrolysate. The next step was the high-content screening of a consolidated set of strains to have a closer look at the exopolysaccharide concentrations and aldose monomer compositions of the exopolysaccharides produced.

1.0 ml SM18 P30S in 96-well plates was inoculated from the plates ISp and ISr for 48 h at 30 °C and 1000 min^{-1}. Then, these plates were used to inoculate new plates with 990 µl SM18 P30S with the respective inhibitor at a final concentration of 2.0 $g \cdot l^{-1}$ using 10 µl of the preculture. These plates were incubated for 48 h at 30 °C and 1000 min^{-1}.

The cultures were subjected to a high-throughput exopolysaccharide purification (see page 55), hydrolysis (see page 34) , derivatization (see page 35) and HPLC-MS analysis (see page 35). Calibration standards 1 and 2, both with a TFA matrix, were used.

Since D-glucose was part of the carbon source, D-glucose assay samples were taken after centrifugation of the cultures, after gel filtration and after neutralization. The samples were diluted 1:10 with ultra-pure water. Inhibitor concentrations were assessed using an adapted PMP derivatization method (see page 57) or HPLC-UV detection (see page 56).

3.5.1 Controls & Deviations

The introduction of aldehydes or neutralized acids slightly shifted pH values from 7.0 to 6.9 for furfural and vanillin and 6.8 for the acids. The pH value of the medium with hydroxymethylfurfural was not affected. Precultures and main cultures were incubated for 47.2 h and 48.3 h, respectively.

The major deviation was the stretching of the analyses over several days. Initially, all analyses were planned to be finished two days after the end of the incubation of the main cultures, which proved to be unfeasible. Since storage might have had an influence on the exopolysaccharide concentration or aldose monomer composition, in the following text, the time of most steps is given relative to the end of incubation.

Five wells of ISp and one well of ISr[4] of the main culture appeared to be empty upon visual inspection and no pellet had formed after centrifugation. All blank or control wells[5] were empty. Thirtytwo wells[6] were still turbid after centrifugation indicating low or no sedimentation of bacteria. Pellets were found in all remaining wells.

Since the inhibitor analysis of ISr started only on the next day, 700 µl of the supernatants were stored at 4 °C overnight in a deep well plate, which was covered with parafilm. For well F9, comparatively much of the pellet was transferred.

The aldehyde inhibitor standards were not filtered through the 10 kDa plate. Filtrate volumes of all wells of ISp were sufficient for the next step. For ISr, more than 50 % of the filtrate volumes were insufficient for the next step. Hence, a 1:10 dilution with ultra-pure water of all wells was made and then centrifuged. At least 155 µl filtrate gathered in the receiving plate for all but twelve wells[7]. The insufficient filtrate of the aforementioned wells was diluted 1:2 with ultra-pure water to give 160 µl. 155 µl of each well was transferred into a new 96-well plate, sealed with a mat and stored at 4 °C.

Acid samples were run for 25 min on an HPLC. Initially, 50 min were planned for the samples to be safe from ghost peaks arising from slow eluting peaks from prior samples.

D-Glucose assays of the supernatants were conducted on the following day. Slightly lower volumes were transferred to the wells ISp.E6 and ISp.F6 (tested with furfural harbouring the strains *Xyl1.F8* and *Xyl1.F9*, respectively), because these two samples were sticky. All samples for the D-glucose assay were stored at 4 °C in sealed 96-well plates.

Sugar standards for exopolysaccharide aldose monomer composition analysis were split between the analyses of ISp and ISr. Sugar standard 1 was analysed with ISp, sugar standard 2 was analysed with ISr.

Two days after the end of incubation, the stored supernatants were centrifuged for 15 min at 3710 × g and 20 °C again. The 1.0 µm glass filtration of both plates was conducted on the same day. The supernatants of ten wells[8] were not filtered completely. There was at least 70 µl

[4]No pellet after centrifugation: B6, C6, E11, F7, G7 of ISp and B12 of ISr. This corresponds to the strains *Xyl1.F2*, *Xyl1.F3*, *Xyl2.A7* and *Xyl2.A8* for furfural, *Xyl2.B7* for vanillin and *Xyl2.A6* for laevulinic acid.

[5]Blank or control wells: H1 to H12 of both plates and G4, G8 and G12 of ISp.

[6]Low or no sedimentation of bacteria after centrifugation: A3, A6, B3, C3, D6, E6, F3, F6 and G3 of ISp and A1, A4, A7, A8, B1, C10, C12, D1, D3, D6, D8, D10, E1, E3 to E5, E10, F4, F5, F7, G1, G4 and G7 of ISr. This corresponds to the strains *Xyl1.F1*, *Xyl1.F4*, *Xyl1.F8* and *Xyl1.F9* for furfural, *Xyl1.F4*, *Xyl1.F8*, *Xyl1.F9*, *Xyl2.A1* and *Xyl2.A6* for hydroxymethylfurfural, *Xyl1.C4*, *Xyl1.C5*, *Xyl1.F1*, *Xyl1.H8*, *Xyl2.A6*, *Xyl2.A7*, *Xyl2.A8* and *Xyl2.B1* for acetic acid, *Xyl1.C4*, *Xyl1.C5*, *Xyl1.F2*, *Xyl1.F4*, *Xyl1.F8*, *Xyl2.A1*, *Xyl2.A2*, *Xyl2.B10*, *Xyl2.C12*, *Xyl2.D2* and *Xyl2.D3* for formic acid and *Xyl1.D8*, *Xyl1.D9*, *Xyl1.D10* and *Xyl2.A7* for laevulinic acid.

[7]Insufficient filtrate volumes after ten-fold dilution: A1, A7, B1, C10, D3, D10, E4, E5, E10, F4, F5 and G4 of ISr. This corresponds to the strains *Xyl1.C4*, *Xyl1.C5* and *Xyl1.H8* for acetic acid, *Xyl1.C4*, *Xyl1.C5*, *Xyl2.A1*, *Xyl2.C12*, *Xyl2.D2* and *Xyl2.D3* for formic acid and *Xyl1.D8*, *Xyl1.D9* and *Xyl1.D10* for laevulinic acid.

[8]Glass filtration incomplete: A3, B3, C3, D6, E6 and F6 of ISp and D1, D6, E1 and G1 of ISr. This corresponds to

of filtrate in every well. The filtered supernatants of ISr were stored at 4 °C again, while the filtered supernatants of ISp were used on the same day for exopolysaccharide aldose monomer composition analysis.

The sealing mat of ISp during hydrolysis was fastened too firmly. As a result, the mat was destroyed and some volume was missing in the following wells after hydrolysis: A3 to A9, G1. Neutralization, however, was successful in all wells.

Four days after the end of incubation, the supernatants of ISr which were stored after glass-filtration were subjected to exopolysaccharide aldose monomer composition analysis. Gel filtration plates were washed and equilibrated only 3 h prior to use and not overnight as stated in section 2.7.3 on page 55. Since the sugar standards were put into wells H1 to H10, the original contents of H9 and H10 (uninoculated media) were discarded and replaced by two of the standards.

Five days after the end of incubation, the remaining D-glucose assays were run: one plate after gel filtration, one plate after neutralization for each, ISp and ISr.

3.5.2 Inhibitor Degradation

Generally, all inhibitors with the exception of laevulinic acid were degraded. The results are summarized in table 3.9 on the facing page and the summarized data visualized in figure 3.4 on the facing page. Full raw data are given in table A.8 on page 138 for ISp and table A.9 on page 140 for ISr. The strains which did not appear to grow did not show inhibitor degradation, except for F7 (more than 50 %) and G7 (around 30 %) of ISp, both tested with furfural harbouring the strains *Xyl2.A7* and *Xyl2.A8*, respectively. The residual inhibitor concentrations of thirtytwo wells[9] were not indicative of inhibitor degradation. Some of the residual inhibitor concentrations exceeded the initial concentration, especially when the inhibitor was an acid.

Performance of Highlighted Strains In section 3.3.3 on page 66, several strains have been highlighted for their inclusion in the top 27/28 of at least four different inhibitors. The results are summarized in table 3.10 on page 76. In comparison to the medians of the set of all strains tested in this step, furfural and hydroxymethylfurfural were degraded to the same extent, while vanillin was degraded to a lesser extent. Acetic acid appeared to be either degraded or produced more as evidenced by the lower median and higher upper quartile. Formic acid was degraded completely in the majority of the strains, in two cases formic acid was not or only slightly consumed. No laevulinic acid was degraded by the highlighted strains.

3.5.3 D-Glucose Consumption

D-Glucose consumption differed between inhibitors, summary statistics are given in table 3.11 on page 76. Sorting the inhibitors from best D-glucose consumption to worst yielded the following order: formic acid, vanillin, acetic acid, laevulinic acid, hydroxymethylfurfural, furfural. Nonetheless, D-glucose was consumed in all but four cases to an extent of at least 50 %.

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the strains *Xyl1.F4*, *Xyl1.F8* and *Xyl1.F9* for furfural, *Xyl1.F4*, *Xyl1.F8* and *Xyl1.F9* for hydroxymethylfurfural, *Xyl1.F1* for acetic acid and *Xyl1.F2*, *Xyl1.F4* and *Xyl1.F8* for formic acid.

[9] No inhibitor degradation: A12, B10, B12, C2, E10 and G2 of ISp; A6, A11, B6, B9, C6, C7, C9, C10, C12, D9, D10, D12, E6, E7, E9, E10, E12, F6, F8, F9, F10, F12, G8, G9, G11 and G12 of ISr. This corresponds to the strains *Xyl1.C10* and *Xyl1.D12* for hydroxymethylfurfural, *Xyl1.G5*, *Xyl1.G11*, *Xyl2.C5* and *Xyl2.C7* for vanillin, *Xyl1.D12*, *Xyl1.E1*, *Xyl1.F6*, *Xyl1.G5*, *Xyl2.A2*, *Xyl2.A5*, *Xyl2.C4* and *Xyl2.C5* for acetic acid, *Xyl1.E2* for formic acid and *Xyl1.A5*, *Xyl1.A6*, *Xyl1.A7*, *Xyl1.A8*, *Xyl1.A9*, *Xyl1.A11*, *Xyl1.D8*, *Xyl1.D9*, *Xyl1.D10*, *Xyl1.D12*, *Xyl1.G5*, *Xyl2.A1*, *Xyl2.A7*, *Xyl2.A9*, *Xyl2.B7*, *Xyl2.B8*, *Xyl2.C4* for laevulinic acid.

Table 3.9: Summary statistics of the inhibitor concentrations after 48 h incubation. The plates ISp and ISr were incubated with 1.0 ml SM18 P30S with 2.00 $g \cdot l^{-1}$ of inhibitor for 48 h at 30 °C and 1 000 min^{-1}. Afterwards, the inhibitor concentrations were determined using PMP derivatization and HPLC-MS analysis (furfural, hydroxymethylfurfural and vanillin) or HPLC-UV analysis (acids). In this table, summary statistics of each single inhibitor are given. For the calculation, negative concentrations were set to zero and the concentrations of the aldehyde inhibitors were clipped at 2.00 $g \cdot l^{-1}$. Since acid production might have occurred, acid inhibitor concentrations exceeding 2.00 $g \cdot l^{-1}$ were not changed. The results were rounded to two decimals. The data are visualized in figure 3.4. The complete raw data are given in table A.8 on page 138 and table A.9 on page 140.

Inhibitor	Inhibitor concentration in $g \cdot l^{-1}$ after 48 h		
	Lower quartile	Median	Upper quartile
Furfural	0.01	0.03	0.06
Hydroxymethylfurfural	0.00	0.01	0.03
Vanillin	0.04	0.05	1.34
Acetic acid	0.00	0.95	2.53
Formic acid	0.00	0.00	0.00
Laevulinic acid	1.84	2.41	2.90

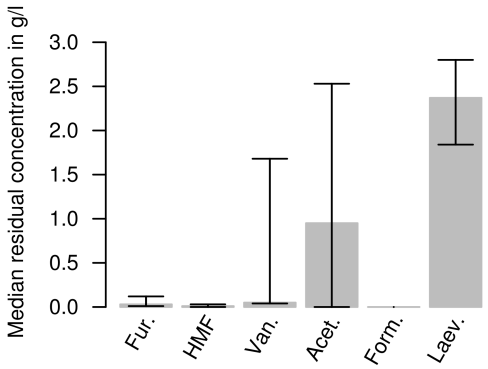


Figure 3.4: Median inhibitor concentrations after 48 h incubation. The plates ISp and ISr were incubated with 1.0 ml SM18 P30S with 2.00 $g \cdot l^{-1}$ of inhibitor for 48 h at 30 °C and 1 000 min^{-1}. Afterwards, the inhibitor concentrations were determined using PMP derivatization and HPLC-MS analysis (furfural, hydroxymethylfurfural and vanillin) or HPLC-UV analysis (acids). In this figure, the medians (bars) and the lower and upper quartiles (error bars) of the inhibitor concentration after incubation are depicted. For the calculation, negative concentrations were set to zero and the concentrations of the aldehyde inhibitors were clipped at 2.00 $g \cdot l^{-1}$. Since acid production might have occurred, acid inhibitor concentrations exceeding 2.00 $g \cdot l^{-1}$ were not changed. The results were rounded to two decimals. Summarized data are available in table 3.9. The complete raw data are given in table A.8 on page 138 and table A.9 on page 140. Abbreviations: Fur.: furfural; HMF: hydroxymethylfurfural; Van.: vanillin; Acet.: acetic acid; Form.: formic acid; Laev.: laevulinic acid.

Table 3.10: Summary of the inhibitor degradation of the previously highlighted strains (see section 3.3.3 on page 66). No values reported here were clipped at 2.0 $g \cdot l^{-1}$. Abbreviations: Fur.: furfural; HMF: hydroxymethylfurfural; Van.: vanillin; Acet.: acetic acid; Form.: formic acid; Laev.: laevulinic acid.; n.t.: not tested.

Strain	Inhibitor concentration in $g \cdot l^{-1}$ after 48 h					
	Fur.	HMF	Van.	Acet.	Form.	Laev.
Xyl1.A10	0.04	0.00	0.05	0.31	n.t.	n.t.
Xyl1.C4	n.t.	0.00	0.03	0.00	0.00	n.t.
Xyl1.C5	n.t.	0.00	0.04	1.88	0.00	n.t.
Xyl1.G5	0.02	0.01	1.96	4.15	1.86	4.11
Xyl1.G11	0.04	0.37	1.98	0.97	0.00	n.t.
Xyl2.A1	n.t.	0.00	0.04	n.t.	0.00	2.62
Xyl2.A6	0.01	0.01	1.05	0.00	n.t.	1.98
Xyl2.A9	0.02	n.t.	1.44	0.00	n.t.	2.41
Xyl2.B7	0.02	0.01	3.84	n.t.	n.t.	2.58
Xyl2.B8	0.16	0.01	n.t.	0.45	0.00	2.66
Xyl2.C4	0.01	0.00	1.91	4.30	n.t.	3.44
Xyl2.C5	0.01	0.00	2.98	3.39	1.67	n.t.
Lower quartile	0.01	0.00	0.05	0.08	0.00	2.49
Median	0.02	0.00	1.44	0.71	0.00	2.62
Upper quartile	0.04	0.01	1.97	3.01	0.83	3.05

Table 3.11: Summary statistics of the residual D-glucose after 48 h incubation. The plates ISp and ISr were incubated with 1.0 ml SM18 P30S with 2.00 $g \cdot l^{-1}$ of inhibitor for 48 h at 30 °C and 1000 min^{-1}. Afterwards, the residual D-glucose concentrations were determined using a D-glucose assay. In this table, summary statistics of each single inhibitor are given. For the calculation, negative concentrations were set to zero. The results were rounded to two decimals. The complete raw data are given in table A.10 on page 143 and table A.11 on page 145.

Inhibitor	D-Glucose concentration in $g \cdot l^{-1}$ after 48 h		
	Lower quartile	Median	Upper quartile
Reference	9.56	10.12	10.26
Furfural	0.26	2.83	6.02
Hydroxymethylfurfural	0.00	1.83	4.54
Vanillin	0.00	0.00	1.31
Acetic acid	0.00	0.10	5.41
Formic acid	0.00	0.00	0.00
Laevulinic acid	0.00	1.10	5.51

Non-Growing Strains The strains without visible turbidity and pellets after centrifugation most likely did not grow. Four of them were part of the furfural screening and in two cases no furfural was degraded, in one case 30 % and in another case 50 % of the furfural was degraded. Oddly, no D-glucose was consumed in two cases, one exhibiting 50 % furfural degradation. In the other two cases vanillin and laevulinic acid were used and no degradation was observed. Among all six cases, at most 10 % D-glucose was consumed. Aldose monomer composition analysis yielded apparent cumulative monomer concentrations of up to $392\,\mathrm{mg}\cdot\mathrm{l}^{-1}$. In all cases however, D-glucose constituted the majority (at least 50 %) of the monomers. The only other monomer found was D-mannose.

3.5.4 Aldose Monomer Composition

The D-glucose which passed through 96-well gel filtration was quantified using the D-glucose assay. Another D-glucose assay after neutralization was used to assess the D-glucose freed during hydrolysis. Assuming that no D-glucose was lost during hydrolysis, the difference of the D-glucose concentration after neutralization and after gel filtration should be greater than or equal to zero. This was not the case for nine wells[10].

Top-Performing Strains The summary statistics in table 3.12 on the next page show that the lower quartiles and medians of the cumulative monomer concentrations are in a narrow range of $25\,\mathrm{mg}\cdot\mathrm{l}^{-1}$ to $104\,\mathrm{mg}\cdot\mathrm{l}^{-1}$ and $48\,\mathrm{mg}\cdot\mathrm{l}^{-1}$ to $169\,\mathrm{mg}\cdot\mathrm{l}^{-1}$, respectively, but the difference in the upper quartiles covered almost one order of magnitude: $117\,\mathrm{mg}\cdot\mathrm{l}^{-1}$ to $823\,\mathrm{mg}\cdot\mathrm{l}^{-1}$.

Sixteen strains[11] exhibited cumulative monomer concentrations exceeding $1.0\,\mathrm{g}\cdot\mathrm{l}^{-1}$. The two strains per inhibitor with the highest cumulative monomer concentrations were *Xyl2.A2* ($352\,\mathrm{mg}\cdot\mathrm{l}^{-1}$) and *Xyl1.G5* ($253\,\mathrm{mg}\cdot\mathrm{l}^{-1}$) for furfural, *Xyl1.C5* ($1079\,\mathrm{mg}\cdot\mathrm{l}^{-1}$) and *Xyl1.C4* ($1053\,\mathrm{mg}\cdot\mathrm{l}^{-1}$) for hydroxymethylfurfural, *Xyl1.C5* ($449\,\mathrm{mg}\cdot\mathrm{l}^{-1}$) and *Xyl1.C4* ($448\,\mathrm{mg}\cdot\mathrm{l}^{-1}$) for vanillin, *Xyl2.A8* ($2055\,\mathrm{mg}\cdot\mathrm{l}^{-1}$) and *Xyl1.C5* ($1875\,\mathrm{mg}\cdot\mathrm{l}^{-1}$) for acetic acid, *Xyl1.C4* ($1930\,\mathrm{mg}\cdot\mathrm{l}^{-1}$) and *Xyl1.C5* ($1807\,\mathrm{mg}\cdot\mathrm{l}^{-1}$) for formic acid and *Xyl2.A7* ($1009\,\mathrm{mg}\cdot\mathrm{l}^{-1}$) and *Xyl1.A9* ($881\,\mathrm{mg}\cdot\mathrm{l}^{-1}$) for laevulinic acid. Altogether, this makes for seven different strains; *Xyl1.C5* appears four times and *Xyl1.C4* appears three times. Since the exopolysaccharide aldose monomer compositions of *Xyl1.C4* and *Xyl1.C5* appear to be highly similar and *Xyl1.C5* exhibited the higher cumulative aldose monomer composition of the two strains, *Xyl1.C5* was chosen as an example for the comparison of the exopolysaccharide aldose monomer compositions with and without inhibitors in figure 3.5 on the following page.

Comparison with Compositions on D-Glucose without Inhibitors The aldose monomer compositions of *Xyl1.C5* grown in inhibitor presence are shown alongside data published by Rühmann [154] without inhibitor in figure 3.5 on the next page. In all cases, D-glucose and L-rhamnose made up the majority of the polymer with around 90 %, while D-glucuronic acid and D-galactose made up 6 % and 2 %, respectively, on average.

[10] Less D-glucose after hydrolysis: A4, A6, A10, A12, C6 and G2 of ISp and B12, C8 and F10 of ISr. This corresponds to the strains *Xyl1.F1* and *Xyl1.F3* for furfural, *Xyl1.D12* and *Xyl2.B7* for hydroxymethylfurfural, *Xyl1.F10* and *Xyl2.C5* for vanillin, *Xyl2.A12* for acetic acid and *Xyl1.D12* and *Xyl2.A6* for laevulinic acid.

[11] Cumulative monomer concentrations greater than $1.0\,\mathrm{g}\cdot\mathrm{l}^{-1}$: none in the presence of furfural, *Xyl1.C4* and *Xyl1.C5* in the presence of hydroxymethylfurfural, none in the presence of vanillin, *Xyl1.C4*, *Xyl1.C5*, *Xyl1.H8*, *Xyl2.A5*, *Xyl2.A7*, *Xyl2.A8* in the presence of acetic acid, *Xyl1.C4*, *Xyl1.C5*, *Xyl1.F5*, *Xyl2.A1*, *Xyl2.A2*, *Xyl2.B10* and *Xyl2.D3* in the presence of formic acid and *Xyl2.A7* in the presence of laevulinic acid.

Table 3.12: Summary statistics of the monomer concentration after 48 h incubation. The plates ISp and ISr were incubated with 1.0 ml SM18 P30S with 2.00 $g \cdot l^{-1}$ of inhibitor for 48 h at 30 °C and 1000 min^{-1}. Afterwards, the exopolysaccharide aldose monomer composition was determined. In this table, summary statistics for each single inhibitor are given. The results were rounded to two decimals. The complete raw data are given in table A.12 on page 148.

Inhibitor	Monomer concentration in $mg \cdot l^{-1}$ after 48 h		
	Lower quartile	Median	Upper quartile
Furfural	33	85	117
Hydroxymethylfurfural	45	87	235
Vanillin	25	75	333
Acetic acid	60	156	511
Formic acid	15	48	823
Laevulinic acid	104	169	295

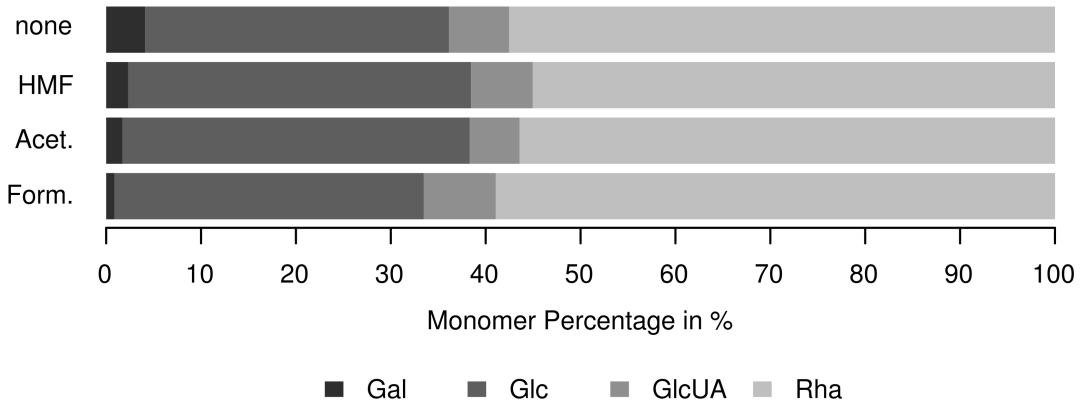


Figure 3.5: Aldose monomer compositions of *Xyl1.C5* with and without inhibitors. In order to reliably assess the aldose monomer compositions, the cumulative aldose monomer composition threshold was 900 $mg \cdot l^{-1}$. Results with lower cumulative aldose monomer compositions were not used. Compositional data of inhibitor-free growth was adapted from Rühmann [154]. Abbreviations: none: no inhibitor, data by Rühmann [154]; HMF: hydroxymethylfurfural; Acet.: acetic acid; Form.: formic acid; Gal: D-galactose; Glc: D-glucose; GlcUA: D-glucuronic acid; Rha: L-rhamnose;.

3.6 Strain Selection

A set of seven strains was selected based not only on data presented within this work, but also taking into consideration data produced by other members of the working group and the expected scientific and industrial potential. The seven strains selected were:

- *Xyl1.F6*: *Paenibacillus*, also *EPS2.B5*, screened on acetic acid
- *Xyl1.G11*: *Pseudomonas*, also *EPS1.G6*, screened on furfural, hydroxymethylfurfural, vanillin, acetic acid and formic acid
- *Xyl1.H10*: *Sphingomonas*, also *EPS2.A11*, screened on vanillin and laevulinic acid
- *Xyl2.A6*: *Agrobacterium*, also *EPS1.G12*, screened on furfural, hydroxymethylfurfural, vanillin, acetic acid and laevulinic acid
- *Xyl2.B8*: *Paenibacillus*, also *EPS2.H7*, screened on furfural, hydroxymethylfurfural, acetic acid, formic acid and laevulinic acid
- *Xyl2.C4*: *Curtobacterium*, also *EPS1.B1*, screened on furfural, hydroxymethylfurfural, vanillin, acetic acid and laevulinic acid
- *Xyl2.C11*: *Rahnella*, also *EPS1.B5*, screened on vanillin

3.6.1 Strain Data Overview

All strains grew in the presence of D-xylose, the three strains on plate Xyl1—*Xyl1.F6*, *Xyl1.G11*, *Xyl1.H10*—were part of the high-content screening on D-xylose and they produced 60 $mg_{EPS} \cdot l^{-1}$, 105 $mg_{EPS} \cdot l^{-1}$ and 43 $mg_{EPS} \cdot l^{-1}$, respectively. All of the selected strains were part of at least one of the top 27/28 in the growth screening in the presence of inhibitors. The cumulative aldose monomer concentrations of all strains are summarized in table 3.13 on the following page.

3.6.2 Further Analyses

In order to familiarize with each strain, they were grown on agar plates of AMA, LB, SM1 P30S and ST1, incubated for two to four days at 30 °C and colony colour and morphology were noted down. Also, the strains were incubated in 10 ml LB and SM1 P30S in 50 ml baffled Erlenmeyer flasks for three days, the broth diluted to 50 ml and centrifuged for 15 min at 3000 × *g* and 20 °C. The supernatant was precipitated in a 2:1 ratio using isopropanol or ethanol (see section 2.7.4 on page 56) without any visible precipitation.

Genomic DNA of all strains was isolated in order to run a 16S PCR on it and as a starting material for genome sequencing. Due to issues with the 16S PCR and gel purification, the 16S rDNA sequence was only available after the strain had been selected. Although the genomes of the strains *Xyl1.G11*, *Xyl2.A6* and *Xyl2.C4* were extracted, they were not considered for further analysis due to purity concerns after incubation on agar plates.

The remaining strains were incubated in 20 ml SM1 P100 in 100 ml baffled Erlenmeyer flasks at 30 °C and 150 min^{-1} for four days. The broths were diluted to 50 ml and centrifuged for 30 min at 4000 × *g* and 30 °C. The supernatant was still turbid—only slightly for *Xyl1.H10* and *Xyl2.B8*—in all cases, but pellets had formed nonetheless. The solutions were precipitated with isopropanol at a 2:1 ratio (see section 2.7.4 on page 56). The only supernatant giving directly visible filamentous

Table 3.13: Summary of the cumulative aldose monomer concentrations of the exopolysaccharides of the selection of seven strains for more detailed analysis. Data are taken from table A.12 on page 148. Abbreviations: Fur.: furfural; HMF: hydroxymethylfurfural; Van.: vanillin; Acet.: acetic acid; Form.: formic acid; Laev.: laevulinic acid; n.t.: not tested.

Strain	Aldose monomer concentration in $mg \cdot l^{-1}$					
	Fur.	HMF	Van.	Acet.	Form.	Laev.
Xyl1.F6	n.t.	n.t.	n.t.	33	n.t.	n.t.
Xyl1.G11	33	41	38	54	28	n.t.
Xyl1.H10	n.t.	n.t.	9	n.t.	n.t.	91
Xyl2.A6	219	924	33	680	n.t.	107
Xyl2.B8	135	143	n.t.	139	216	131
Xyl2.C4	187	273	217	350	n.t.	110
Xyl2.C11	n.t.	n.t.	384	n.t.	n.t.	n.t.

Table 3.14: Exopolysaccharide aldose monomer compositions of the four remaining strains. The four strains *Xyl1.F6*, *Xyl1.H10*, *Xyl2.B8* and *Xyl2.C11* were incubated in 20 ml SM1 P100 for 4 d at $150\,min^{-1}$. The exopolysaccharides were recovered using centrifugation and isopropanol precipitation. After drying, solutions with $1.0\,g_{EPS} \cdot l^{-1}$ to $10\,g_{EPS} \cdot l^{-1}$ were prepared and the aldose monomer composition determined. The recovery was calculated by dividing the cumulative concentration of all aldose monomers by the exopolysaccharide concentration used. For *Xyl2.B8*, filamentous precipitate floating on top and a pellet were found. The filamentous precipitate is indicated by ^{top}. The data are visualized in figure 3.6 on the facing page. Abbreviations: Gal: D-galactose; GalN: D-galactosamine; Glc: D-glucose; GlcN: D-glucosamine; GlcNAc: *N*-acetyl-D-glucosamine; GlcUA: D-glucuronic acid; Man: D-mannose; Rha: L-rhamnose; Rib: D-ribose; Sum: cumulative concentration of all aldose monomers; n.d.: not detected.

Strain	Aldose monomer concentration in $mg \cdot l^{-1}$										Recovery
	Gal	GalN	Glc	GlcN	GlcNAc	GlcUA	Man	Rha	Rib	Sum	
Xyl1.F6	181	n.d.	1	n.d.	n.d.	23	n.d.	197	n.d.	402	40 %
Xyl1.H10	45	n.d.	284	n.d.	n.d.	n.d.	168	49	24	570	14 %
Xyl2.B8	32	19	1423	274	14	193	905	44	n.d.	2904	29 %
Xyl2.B8^{top}	n.d.	n.d.	158	21	n.d.	n.d.	103	n.d.	n.d.	282	28 %
Xyl2.C11	163	n.d.	4	n.d.	n.d.	26	42	184	n.d.	419	42 %

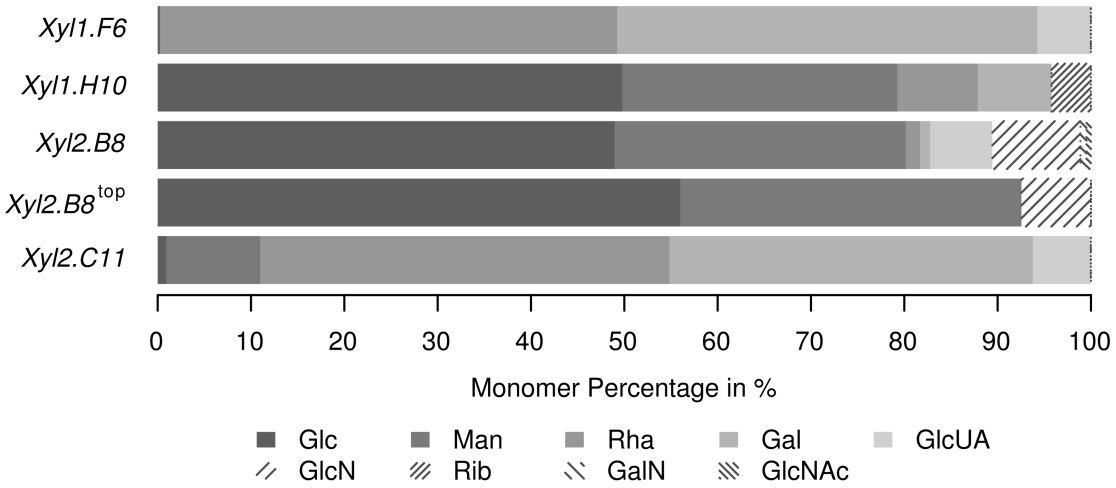


Figure 3.6: Exopolysaccharide aldose monomer compositions of the four remaining strains. The four strains *Xyl1.F6*, *Xyl1.H10*, *Xyl2.B8* and *Xyl2.C11* were incubated in 20 ml SM1 P100 for 4 d at 150 min^{-1}. The exopolysaccharides were recovered using centrifugation and isopropanol precipitation. After drying, solutions with 1.0 $g_{EPS} \cdot l^{-1}$ to 10 $g_{EPS} \cdot l^{-1}$ were prepared and the aldose monomer composition determined. For *Xyl2.B8*, filamentous precipitate floating on top and a pellet were found. The filamentous precipitate is indicated by 'top'. The concentrations of the filamentous precipitate and the pellet of *Xyl2.B8* differed by a factor of 10. Therefore, some monomer concentrations were below the detection limit in the 'top' sample. The data for this figure are given in table 3.14 on the facing page.

precipitates was *Xyl2.B8*'s. There was noticeable precipitate in all samples. The filamentous precipitates of *Xyl2.B8* floating on the top and the pellet at the bottom were analysed separately.

After the precipitate had settled for a month, the supernatant was removed carefully, the pellets air-dried for 2 h and then dried in a vacuum drying oven at 45 °C for 20 h. The dried pellets were weighed and used to calculate the approximate exopolysaccharide concentrations in the broths:

- *Xyl1.F6*: $7.3\,\mathrm{g\cdot l^{-1}}$
- *Xyl1.H10*: $0.2\,\mathrm{g\cdot l^{-1}}$
- *Xyl2.B8*: $2.0\,\mathrm{g\cdot l^{-1}}$
- *Xyl2.C11*: $8.4\,\mathrm{g\cdot l^{-1}}$

These pellets were dissolved in ultra-pure water to yield final concentrations of $1.0\,\mathrm{g\cdot l^{-1}}$ for *Xyl1.F6*, *Xyl2.B8* (top) and *Xyl2.C11*, $4.0\,\mathrm{g\cdot l^{-1}}$ for *Xyl1.H10* and $10\,\mathrm{g\cdot l^{-1}}$ for *Xyl2.B8*. The exopolysaccharide aldose compositions are given in table 3.14 on page 80 and are shown in figure 3.6 on the preceding page.

3.6.3 Selection of *Paenibacillus* 2H7

The strain *Xyl2.B8*—tentatively named '*Paenibacillus* 2H7' after its location on the EPS plates, EPS2.H7—was chosen as the exopolysaccharide production strain for the subsequent parallel fermentation. The strain showed robust growth on different media and exopolysaccharide production in the presence of different inhibitors. The robustness of the exopolysaccharide production was derived from table 3.13 on page 80. The aldose monomer concentration of *Xyl2.B8* in the presence of each of the five inhibitors tested lies within a range of $131\,\mathrm{mg\cdot l^{-1}}$ to $216\,\mathrm{mg\cdot l^{-1}}$, while every other strain tested with five inhibitors either produced low amounts of exopolysaccharide only (*Xyl1.F6*) or showed a greater variance in the exopolysaccharide concentration (*Xyl2.A6*, *Xyl2.C4*). The product could be purified with relative ease and the product concentration was sufficient; the desired minimal concentration was $1.0\,\mathrm{g\cdot l^{-1}}$. The exopolysaccharide aldose monomer composition showed a total of eight different monomers dominated by D-glucose and D-mannose. The presence of acidic and basic monomers could contribute to unusual rheological properties making the resulting polymer a candidate for possible future industrial uses. Therefore, the exopolysaccharide appeared to have a high potential and *Paenibacillus* 2H7 was selected for further studies.

16S rDNA Sequence

Genomic DNA was extracted and used as template for a 16S rDNA amplification. The complete sequence is given in figure A.1 on page 155 and was used in a BLAST search using MegaBLAST [157, 158] in the database 'nt' of NCBI. The ten highest scoring hits are given in table 3.15 on the facing page, E values were 0 for at least the 100 highest scoring sequences. Among these, the dominating genera were *Paenibacillus* (90) and *Bacillus* (6), the most frequently found species were *P. cineris* (11), *P. favisporus* (11) and *P. azoreducens* (6).

Table 3.15: 16S rDNA BLAST results for *Paenibacillus* 2H7. The 16S rDNA sequence of *Paenibacillus* 2H7 was used in a MegaBLAST query and the first ten results are given in this table. E values were 0.0 in all cases.

Description	Identifier	Score in bits	Reference
Paenibacillus sp. A25 16S rRNA, partial	KF479541.1	2634	n.a.
Paenibacillus sp. SSG-1 16S rRNA, partial	KF750627.1	2623	[155]
Paenibacillus sp. C82 16S rRNA gene, partial	JX011004.1	2619	n.a.
Paenibacillus cineris 16S rRNA gene, partial	LN890143.1	2617	n.a.
Paenibacillus sp. B19 16S rRNA gene, partial	KF479580.1	2617	n.a.
Paenibacillus cineris JN237 16S rRNA gene, partial	KF150476.1	2617	n.a.
Paenibacillus favisporus T2 16S rRNA gene, partial	JN867753.1	2612	n.a.
Paenibacillus sp. 3492BRRJ 16S rRNA gene, partial	JF309261.1	2612	n.a.
Paenibacillus sp. 07-G-dH 16S rRNA gene, partial	HM776458.1	2610	[156]
Paenibacillus sp. FJAT-21993 16S rRNA gene, partial	KP728976.1	2608	n.a.

3.7 Parallel Fermentation with Lignocellulose Hydrolysate

The strain *Paenibacillus* 2H7 was selected for the first series of parallel fermentations. The aim was to compare the influence of the carbon source on microbial growth, exopolysaccharide production and exopolysaccharide quality. The carbon sources used were lignocellulose hydrolysate and a mix of pure D-glucose and D-xylose. All fermentations were run in parallel quadruplicates and were inoculated from the same preculture. In order to get a basic understanding of the strain's fermentation behaviour, only pH value and temperature were controlled. Stirrer speed, aeration rate and gas composition were kept constant; dissolved oxygen was only recorded. During the fermentation, samples were drawn for the determination of the cell dry mass, the concentrations of D-glucose, D-xylose and the two major inhibitors in the lignocellulose hydrolysate[12] and the aldose monomer composition of the exopolysaccharide produced. All relevant parameters of the fermentation are described in section 2.5.5 on page 48. Fermenters 1 to 4 are referred to as 'block 1', fermenters 5 to 8 are referred to as 'block 2'.

3.7.1 Controls & Deviations

Autoclaving the fermenters adversely affected the pH probe of fermenter 7: comparison with the other three fermenters with the same setup showed that the actual pH value was around 0.25 lower than the value shown. Therefore, all setpoints were increased by 0.25 for this fermenter. All sterile controls showed no signs of contamination.

Additional anti-foam was added manually after 63.2 h (1 ml) and, for block 2 only, 96.5 h (0.5 ml). No additional anti-foam was added through automated means. Overall, 2 ml of anti-foam was added to block 1 and 2.5 ml of anti-foam was added to block 2.

The middle foam breaker of fermenter 1 slid down and came to a halt on the bottommost foam breaker.

Sampling and Analytics Samples for cell dry mass were drawn only from the second sample onwards, therefore, values for the first sample are missing. Molar mass values for the first sample

[12] Furfural and hydroxymethylfurfural.

are missing for all fermenters. The value of sample two is missing for fermenters 3 and block 2. The values of the samples three and four (inclusively) are missing for block 2. The reason for missing molar mass values is the absence of an RI peak in the high molar mass range.

Fermenter 3 showed delayed growth, which can also be seen in the data (see figure 3.7 on the facing page): the quantile lines of the dissolved oxygen and the carbon dioxide data of block 1 appear to shadow the median line. Since the deviation diminished on later samples, the data of fermenter 3 was still used.

Exopolysaccharide aldose monomer data were determined, but deemed erroneous and, thus, are not shown: the overall aldose monomer sum slowly *decreases* over time, proportional to the D-glucose concentration. Also, D-glucose was by far the most abundant monomer which is in contradiction to the results of the purified exopolysaccharide.

D-Xylose concentration determination was tried using PMP derivatization (see section 2.4.3 on page 35, data not shown), but yielded dissatisfactory results with a vague trend downwards. Also, the values were inconsistent with the actual concentrations used. Thus, these results were not used.

3.7.2 Fermentation Courses

Cell dry mass, furfural concentration (block 2 only), polymer molar mass at RI peak, D_{600}, D-glucose concentration, dissolved oxygen and carbon dioxide concentration over time are given in figure 3.7 on the facing page and described in the following paragraphs.

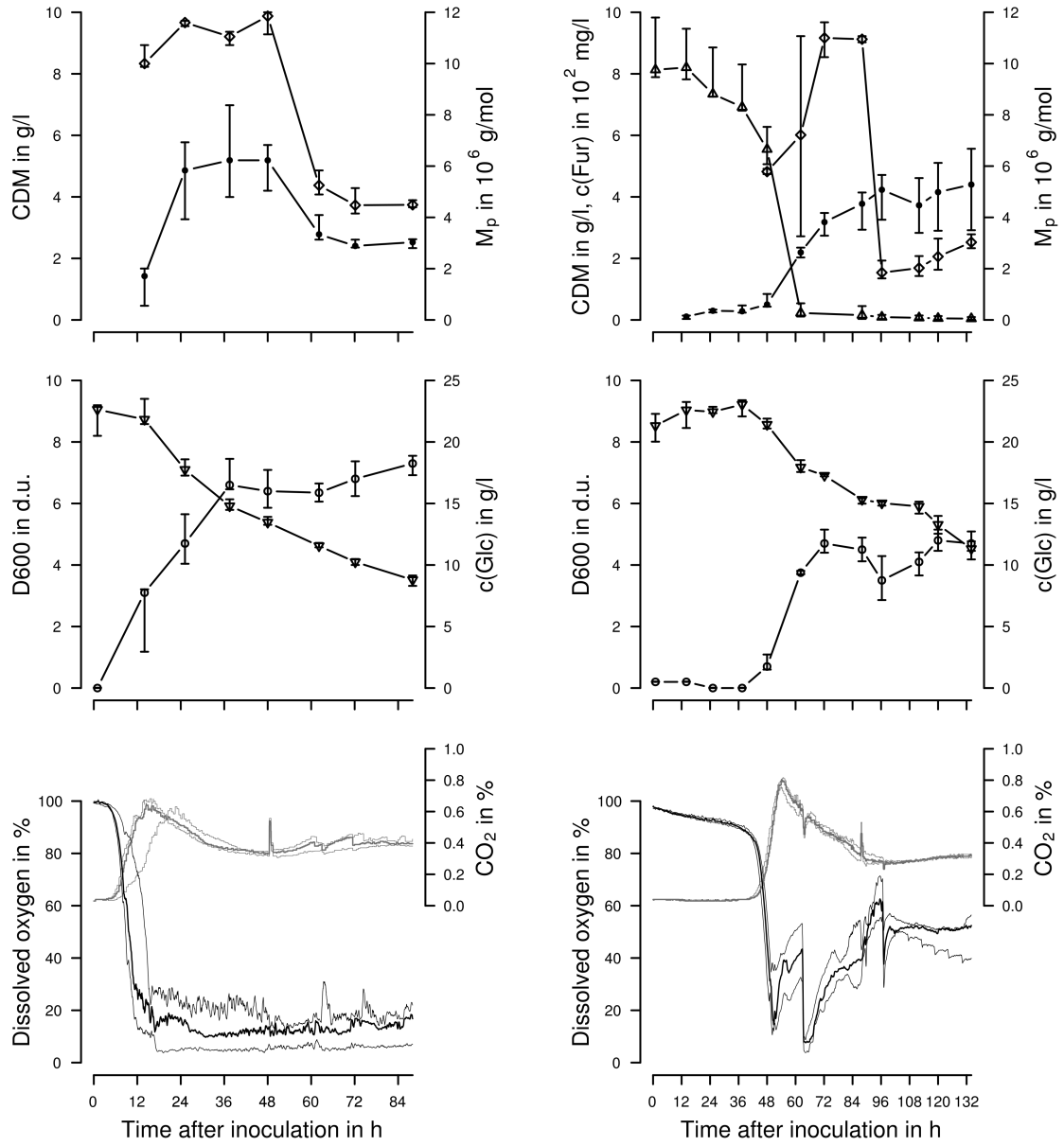
Microbial Growth After an initial increase, cell dry mass in block 1 decreases and hits a plateau. The drop coincides with the pH change. In block 2, cell dry mass stabilized at around $4\,\mathrm{g}\cdot\mathrm{l}^{-1}$ and no drop in response to the pH change was observed.

D_{600} values increase over time and appear to hit a plateau in both blocks. In block 2, a slight drop appears after the pH change.

Directly after inoculation, the dissolved oxygen concentration decreases until a minimum at around 10 % in block 1. The carbon dioxide concentration in the off-gas shows a strong increase at the beginning until around 15 h, slowly decreases to and remains at around 0.4 % until the end of the fermentations. The CO_2 spike at approximately 48 h coincides with the pH change. In block 2, there is virtually no CO_2 generation for the first 36 h followed by a similar pattern as seen in block 1: a relatively sharp increase followed by a slow decrease, levelling off at around 0.3 % until the end of fermentations; the pH change coincides with the CO_2 spike at around 86 h. The dissolved oxygen decreases only very slowly, almost linearly, for the first 36 h followed by a sharp decrease mimicking the pattern seen in block 1. At around 63 h and 96 h, anti-foam was added which coincides with two negative peaks in the CO_2 course and two sharp drops of the dissolved oxygen levels which recovered slowly when compared to CO_2.

Production of Acidic and/or Basic Compounds The pH value was maintained at preset levels during the fermentation. Acid and base were available to counter any changes in the pH value. Initially, *Paenibacillus* 2H7 produced acid in all cases (acidifying phenotype) and went through a phase of pH stability in all cases except fermenter 3. During pH stability, the addition of acid or base was not needed.

Block 1 The stability phase occurred from 70.5 h to 75.8 h, 74.0 h to 77.5 h and 68.0 h to 73.7 h for fermenters 1, 2 and 4, respectively. Since the pH shift occurred at 48 h in block 1, the stability



(a) Fermenters 1 to 4: Reference.

(b) Fermenters 5 to 8: Lignocellulose Hydrolysate.

Figure 3.7: Comparison of reference fermentations and lignocellulose hydrolysate fermentations. *Paenibacillus* 2H7 was fermented in parallel at 500 ml scale without (block 1) and with lignocellulose hydrolysate (block 2) to assess its impact on the process. The setup is detailed in section 2.5.5 on page 48. Points of cell dry mass (•), furfural concentration (▵), polymer molar mass at RI peak (◇), D_{600} (○) and D-glucose concentration (▽) and thick lines of dissolved oxygen (—) and carbon dioxide concentrations (—) represent the median value. Error bars and thin lines of dissolved oxygen and carbon dioxide concentrations represent the quantiles at 10 % and 90 %. Lines between points serve as visual aids only. Dissolved oxygen and carbon dioxide data were smoothed using the rolling average of 50 samples prior to all further processing. Generally, sample sizes are $n = 4$. See section 3.7.1 on page 83 for details. The complete processing steps of the raw data are documented in listing A.4 on page 156.

phase started 20 h to 26 h afterwards. After the pH stability, base was no longer needed to control the pH, but acid. This phenotype will be referred to as 'de-acidifying phenotype'.

Block 2 The stability phase occurred from 55.5 h to 59.3 h, 53.5 h to 56.0 h, 55.3 h to 59.0 h and 59.5 h to 62.0 h for fermenters 5, 6, 7 and 8, respectively. Since the pH shift occurred at 87.5 h in block 2, the stability phase ended 25.5 h to 31.5 h before the pH shift. Between the stability phase and the pH shift, the pH value fluctuated in fermenters 5 to 7. In fermenter 8, only acid was needed to control the pH. After the pH shift, only base was needed to control the pH in all fermenters.

D-Glucose Consumption In both blocks, around half the initial D-glucose was still present at the end of the fermentations: around 9 $g \cdot l^{-1}$ in block 1 and around 11 $g \cdot l^{-1}$ in block 2. In block 1, D-glucose consumption appears to be almost linear from the third sample on. Given the growth delay in block 2, a similar behaviour is observed from the sixth sample on.

Furfural Consumption Furfural was observed only in block 2. Over the first 36 h, only miniscule amounts of furfural vanished. The rapid decline in furfural concentrations between 36 h and 48 h coincides with the sharp decline of the dissolved oxygen and the sharp increases in CO_2, D_{600}, cell dry mass and the starting point consumption of D-glucose.

Polymer Molar Mass The polymer molar mass at the RI peak increased up to $1 \cdot 10^7$ $g \cdot mol^{-1}$ in block 1 and $9 \cdot 10^6$ $g \cdot mol^{-1}$ in block 2. After the pH change, the molar mass dropped to approximately half the previous value in block 1 and a fifth of the previous value in block 2. In block 1, the decrease continued in a mild form until the end of the fermentations and the final value was around $4 \cdot 10^6$ $g \cdot mol^{-1}$. In block 2, the decrease was more pronounced, but the polymer molar mass increased slightly until the end of the fermentations and the final was around $3 \cdot 10^6$ $g \cdot mol^{-1}$.

3.7.3 Polymer Purification and Yield

Centrifugation and cross-flow filtration were employed for polymer purification as described in section 2.5.5 on page 49. The 0.45 µm membranes used were too coarse for the polymer: the feed solution of block 1 contained 1.6 $g_{EPS} \cdot l^{-1}$ and only 0.2 $g_{EPS} \cdot l^{-1}$ were found in the retentate, while 1.3 $g_{EPS} \cdot l^{-1}$ were found in the permeate. The feed solution of block 2 contained 2.4 $g_{EPS} \cdot l^{-1}$ and only 0.8 $g_{EPS} \cdot l^{-1}$ were found in the retentate, while 1.6 $g_{EPS} \cdot l^{-1}$ were found in the permeate.

Permeates and retentates of each block were re-united after the filtration and stored at 4 °C. Upon re-filtration using 100 kDa and 10 kDa membranes, around 20 % of the polymer of block 1 and around 70 % of the polymer of block 2 could not be recovered.

3.8 Discussion

3.8.1 Growth on D-Xylose

The amount of 135 well-growing strains of all the 191 strains tested corresponds to 71 %. In a similar screening with the aim of finding polyhydroxybutanoate producers from environmental isolates using D-xylose as the sole carbon source conducted by Lopes *et al.* [159], only 24 % of the strains tested grew on D-xylose. While Lopes *et al.* [159] took into consideration all the strains,

in the screening discussed here, only pre-selected strains were used. For these strains the ability to produce exopolysaccharides was an established fact and most of these strains belonged to genera known for plant pathogenicity. So, the ability to grow in D-xylose is not uncommon for the strains tested in this screening.

3.8.2 High-Content Screening with D-Xylose

Exclusion of Xyl2

In the high-content screening with D-xylose, the plate Xyl1 was tested, while Xyl2 was not. As seen in table 3.2 on page 61, more than half of Xyl2 was empty, because—as outlined in section 3.1 on page 60—not all of the strains of the plates EPS1 and EPS2 showed good enough growth on D-xylose. The complete method for the aldose monomer analysis is quite time-consuming and geared towards the analysis of complete 96-well plates [1]. Therefore, Xyl2 was left out from this step of the screening.

The direct screening of the plates EPS1 and EPS2 was not an option either. The high-throughput purification involves a 96-well size exclusion chromatography (see section 2.7.3 on page 55) to reduce the overall amount of small molecules (and not just monomeric aldoses) to enable exact quantification and to protect both the HPLC and the MS. Using strains which do not grow on D-xylose would have meant an additional $10\,\mathrm{g \cdot l^{-1}}$ D-xylose in the medium. Therefore, the D-xylose growth screening was used to sort out non-growing strains.

Non-Growing Strains

The strain *Xyl1.E10* showed growth on an LB agar plate, although it did not grow in the screening. In the D-xylose growth screening, this strain (*EPS1.F7*) grew to a D_{600} of 0.63, three-fold the limit of 0.2 and easily visible to the bare eye. One possible explanation is that during the preparation of plate Xyl1, not *EPS1.F7*, but *EPS2.F7* was transferred. *EPS2.F7* was clearly not growing on D-xylose, with a D_{600} of 0.00. Since only a single strain was affected in this screening, this matter was not investigated in detail. Since *EPS1.F7* is designated as *Paenibacillus* and *EPS2.F7* as *Gluconacetobacter*, a 16S rDNA analysis would be a suitable method.

Contamination of the Well Xyl1.E12

Growth in the 'empty' well Xyl1.E12 most likely occurred due to a contamination. As further investigations revealed, the 80 % ethanol for the replicator had not been replaced for around a month. After incubating 1 ml of the ethanol on an LB agar plate for 48 h at 30 °C, several hundred colonies had formed with at least two distinct colony morphologies. The replicator was flamed, but given such a high concentration of spores, it is not unlikely to have inoculated the empty well. As a consequence for all later experiments, only fresh ethanol was used and put into an autoclaved container and the replicator was also autoclaved regularly.

D-Xylose Consumption

The median residual D-xylose concentration was $3\,470\,\mathrm{mg \cdot l^{-1}}$ after 48 h of incubation. Four possible reasons for this relatively high value are discussed in the following: low initial cell count, low oxygen transfer rate (OTR), exopolysaccharide production and the strain-specific growth and D-xylose consumption characteristics. Additionally, the screening used the same medium

Table 3.16: D-Xylose consumption by genus. The strains of the plate Xyl1 were incubated in SM17 P30S for 48 h at 1000 min^{-1} and 30.0 °C. The initial D-xylose concentration was 10.0 $g \cdot l^{-1}$. D-Xylose consumption varies among the strains in the range from 'no consumption' to 'complete consumption'. In this table, the consumption is summarized by genus to show that the bacterial genus appears to have a high impact on the D-xylose consumption. The parenthesized number after the genus name indicates the number of strains belonging to the respective genus on plate Xyl1.

Strain (frequency)	D-Xylose concentration in $g \cdot l^{-1}$		
	Lower quartile	Median	Upper quartile
Arthrobacter (18)	4.36	4.80	5.88
Bacillus (20)	3.63	4.93	6.58
Microbacterium (20)	0.46	0.64	3.84
Paenibacillus (9)	0.11	1.07	4.13
Pseudomonas (18)	0.00	0.10	0.96
Rhodococcus (4)	2.32	6.25	10.16
Sphingomonas (5)	8.40	8.55	9.55

for all the different bacteria. Some of them might grow faster, consume more D-xylose or produce more exopolysaccharide in an optimized medium. As the goal of this work was to find a robust strain, no further efforts were devoted to medium optimization at such an early point.

In the work of Rühmann [154], the main culture was inoculated from 10.0 µl of the preculture, while the main culture of this screening was inoculated using the replicator. Therefore, the initial number of bacteria transferred to the main culture could have been too low for complete D-xylose consumption.

Microbial growth in deep-well plates at 1000 min^{-1} and an eccentricity of only 5 mm might have been limited by an insufficient OTR [160–163]. Extrapolating[13] the data presented by Duetz and Witholt [162], the OTR in a deep-well plate using 500 µl filling volume would be 84 $mmol_{O_2} \cdot l^{-1} \cdot h^{-1}$. According to Duetz *et al.* [160] and Duetz and Witholt [161], the OTR ratio of a well with a filling volume of 500 µl and 1000 µl is between 2:1 and 3:1. This would equate to an OTR in the range of 28 $mmol_{O_2} \cdot l^{-1} \cdot h^{-1}$ to 42 $mmol_{O_2} \cdot l^{-1} \cdot h^{-1}$, which is comparable to the OTR achieved in 250 ml Erlenmeyer flasks with 25 ml medium at 300 min^{-1} and an eccentricity of 5.0 cm [163].

The experimental maximum OTRs of 200 µl filling volume given by Hermann *et al.* [163] for 96-well plates shaken at 1000 min^{-1} and an eccentricity of 3 mm and 900 min^{-1} and an eccentricity of 6 mm were 15 $mmol_{O_2} \cdot l^{-1} \cdot h^{-1}$ and 24 $mmol_{O_2} \cdot l^{-1} \cdot h^{-1}$, respectively. At 200 µl filling volume, the OTR without shaking was 7 $mmol_{O_2} \cdot l^{-1} \cdot h^{-1}$ [163]. No values for 1000 µl filling volume were reported. Given these numbers, low OTR might have occurred, but taking into consideration that 30 strains consumed at least 85 % of the D-xylose, it seems unlikely to be the sole reason behind low D-xylose consumption for all strains.

Exopolysaccharide production might increase the dynamic viscosity, which in turn would reduce liquid movement due to shaking and reduce the OTR. Since the 13 strains which produced

[13] Every time the eccentricity halves, the minimum shaking frequency must be increased by another 100 min^{-1} to reach the same OTR as before. The OTR was treated as increasing linearly from 0 $mmol_{O_2} \cdot l^{-1} \cdot h^{-1}$ to 120 $mmol_{O_2} \cdot l^{-1} \cdot h^{-1}$ at an eccentricity of 25 mm starting from 200 min^{-1} and going to 1000 min^{-1}. The step from 6.25 mm to 5.0 mm was treated as being the OTR at 6.25 mm subtracted by 15 $mmol_{O_2} \cdot l^{-1} \cdot h^{-1} \cdot \frac{6.25\,mm - 5.0\,mm}{0.5 \cdot 6.25\,mm}$.

substantial amounts of exopolysaccharide (see table 3.3 on page 63) consumed D-xylose to levels of 665 $mg \cdot l^{-1}$ at most, it is highly unlikely that exopolysaccharide production adversely affected D-xylose consumption. On the contrary, *all* exopolysaccharide producing strains readily consumed D-xylose leading to the hypothesis that good D-xylose consumption generally positively affects exopolysaccharide production in certain types of strains.

Therefore, the reason for differences in exopolysaccharide production and D-xylose consumption has to lie within the bacteria used. The data presented in table 3.16 on the facing page support this statement. The table lists the median residual D-xylose concentrations for each bacterial genus and while some genera such as *Microbacterium*, *Paenibacillus* or *Pseudomonas* were able to consume D-xylose almost completely, others such as *Sphingomonas* did show low D-xylose consumption at best.

Differences in Exopolysaccharide Production

Only thirteen strains produced reliably detectable amounts of exopolysaccharide which were measured as aldose monomers (see section 3.2.2 on page 60). While the strains of the exopolysaccharide bank are known to produce different amounts of exopolysaccharide [154], the low exopolysaccharide production on D-xylose was still peculiar. The median cumulative aldose monomer concentration across all strains tested was at 102 $mg \cdot l^{-1}$, lower and upper quartiles at 68 $mg \cdot l^{-1}$ and 208 $mg \cdot l^{-1}$, respectively. The same strains grown on D-glucose [154], exhibited similar productivity with median, lower and upper quartiles at 86 $mg \cdot l^{-1}$, 45 $mg \cdot l^{-1}$ and 318 $mg \cdot l^{-1}$, respectively, and 20 strains reaching at least 560 $mg \cdot l^{-1}$ cumulative aldose monomer concentration. The maximum concentrations were also not affected. Therefore, it can be concluded that the productivity on a plate scale was mostly unaffected.

Differences in Exopolysaccharide Aldose Monomer Composition

The influence of the carbon source on the aldose monomer composition of the exopolysaccharides has been examined using D-glucose versus D-xylose. As seen in figure 3.1 on page 64, only two of the 13 strains showed major differences in the exopolysaccharide aldose monomer composition when grown on D-xylose compared to D-glucose. Two strains showed minor differences and the remaining strains appeared to be unaffected.

To the best of my knowledge, there are no reports on the screening and comparison of the exopolysaccharide aldose monomer composition of exopolysaccharide producers grown on D-glucose and D-xylose in the published literature so far. However, several authors have found differences in the exopolysaccharide composition of different microorganisms grown on different carbon sources [66–74, 164, 165].

Kai *et al.* [72] studied the fungus *Pestalotiopsis microspora* and the composition of its exopolysaccharide grown on D-glucose, D-mannose, D-galactose, D-xylose, *N*-acetyl-D-glucosamine, L-rhamnose, L-arabinose and D-arabinose using gas chromatography and ^{13}C NMR. The exopolysaccharide contained at most three monomers: D-mannose, D-glucose and D-galactose. The resulting exopolysaccharides can be divided into four groups, based on the carbon source used: group 1 with D-glucose, D-mannose, L-rhamnose and D-arabinose; group 2 with D-galactose; group 3 with D-xylose and *N*-acetyl-D-glucosamine and group 4 with L-arabinose.

The group 1 exopolysaccharides contained 90 % D-glucose and 10 % D-mannose, while the group 2 exopolysaccharide contained slightly more D-mannose, around 15 %. The exopolysaccharides of group 3 were the only ones to contain D-galactose at 10 % to 15 %. D-Glucose was at

55 % to 60 % and the remaining 25 % to 35 % were made from D-mannose. The group 4 exopolysaccharide contained D-glucose exclusively. All of these results are in mol%.

The fungus *Phellinus linteus* L13202 was studied by Lee *et al.* [69] and the growth, exopolysaccharide production and composition on different carbon sources, among them D-glucose and D-xylose. Unfortunately, the fungus did not grow in D-xylose, therefore, no exopolysaccharide was produced and hence, no compositional data reported.

Another fungus, *Ganoderma applanatum* was studied by Lee *et al.* [73]. While they did not test D-xylose as a carbon source, they found up to 10 % of D-xylose in the polymer.

The lactobacillal exopolysaccharides of *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 and *Lactobacillus casei* CG11 were studied by Grobben *et al.* [70, 71] and Cerning *et al.* [68], respectively. The carbon sources studied were D-glucose and D-fructose [70, 71], and D-glucose and lactose [68].

It was found that on D-fructose, the exopolysaccharide of *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 consisted of D-glucose and D-galactose in the ratio 1:2.4, while in an equimolar mix of D-glucose and D-fructose and on D-glucose alone, the composition shifted to D-glucose, D-galactose and L-rhamnose in the ratio 1:7.0:0.8 [70]. After the separation of a high molar mass peak from a low molar mass peak, the high molar mass exopolysaccharide from the same strain grown on D-fructose was composed of D-glucose, D-galactose and L-rhamnose at a ratio of 1.3:4.3:1.0. On D-glucose the composition shifted to 1:4.7:1 [71].

Comparing the growth of *Lactobacillus casei* CG11 on D-glucose and lactose, Cerning *et al.* [68] found that the exopolysaccharide compositions differed: on D-glucose, the polymer consisted of three quarters (75.7 %) D-glucose, a fifth (20.5 %) L-rhamnose and traces of D-galactose (2.1 %) and D-mannose (1.7 %). On lactose, the lion's share (63.0 %) was still made up of D-glucose, while almost the same amounts of L-rhamnose (16.1 %) and D-galactose (13.2 %) were found. The remainder consisted of D-mannose (6.8 %) and D-xylose (1.0 %).

Fischer *et al.* [164] examined the exopolysaccharide of *Azospirillum brasilense* Cd under 'normal' (minimal medium) conditions and with the addition of wheat root exudate. The exopolysaccharide produced with pure minimal medium contained 47 % D-glucose, 28 % L-fucose, 11 % D-galactose, 6 % L-rhamnose and only traces of D-xylose, D-mannose and L-arabinose. However, the exopolysaccharide produced with wheat root exudates looked different: D-glucose was still the major component with 37 %, but there were considerably less L-fucose (14 %) and L-rhamnose (trace); on the other hand, D-xylose (17 %) and L-arabinose (11 %) made up more than a fourth of the polymer. The D-galactose (15 %) and D-mannose (trace) contents were virtually unchanged.

The exopolysaccharide of four strains of *Pseudomonas syringae* pv. *glycinea* grown on saccharose, D-glucose and on soybean leaves were analysed by Osman *et al.* [165]. On saccharose, a levan was produced, while they found an alginate on D-glucose and on leaves.

In 1986, Bryan *et al.* [66] studied *Klebsiella* sp. K32 and *Acinetobacter calcoaceticus* BD4 on defined media with D-glucose, D-mannose, L-rhamnose, succinate, glutamate or ethanol as the carbon source and a complex medium with D-glucose. In all cases, the relevant monomers of *Klebsiella* exopolysaccharide were D-galactose, D-mannose and L-rhamnose. The molar ratio of L-rhamnose to D-galactose was 1:2 on ethanol, almost 2:1 on D-glucose, D-mannose or L-rhamnose and approximately 1:1 on the complex medium. D-Mannose was present only when grown on L-rhamnose and only in trace amounts. The effect on *Acinetobacter calcoaceticus* BD4 was more pronounced: when grown on L-rhamnose, the molar ratio of L-rhamnose:D-glucose:D-mannose was approximately 1:1:2 on L-rhamnose, 4:2:1 on succinate and 10:5:1 on glutamate or ethanol.

Raza *et al.* [74] used the strain *Paenibacillus polymyxa* SQR-21 to assess the impact of different carbon and nitrogen sources on the exopolysaccharide production. They only analysed

the exopolysaccharide composition of the exopolysaccharide produced on an optimized medium. The monomers were D-mannose, D-galactose, D-glucose and D-glucuronic acid at a ratio of 2.7:2.5:2.2:1.

Tait *et al.* [67] studied the exopolysaccharide production, monomer composition, acetylation and pyruvylation of *Xanthomonas campestris* S459 with regard to several deficiencies: D-glucose, ammonium, sulphur, phosphorus, magnesium and iron. Under all deficiencies tested, the monomer composition remained constant. The acetyl content of the xanthan remained stable under all conditions tested, except for the ammonium deficiency: the xanthan had the highest acetyl content under this deficiency. Under the same conditions, the lowest pyruvyl content was achieved. The only other deficiency which led to a slight reduction in pyruvyl content was sulphur deficiency.

None of the effects observed by Kai *et al.* [72] can be found in the data presented in this work. Lee *et al.* [69] found no growth for the fungus studied on D-xylose, so that these results cannot be used. Lee *et al.* [73] studied fungi as well and found different exopolysaccharide compositions based on the carbon source, but did not test the carbon sources in question: D-glucose and D-xylose. The studies of Cerning *et al.* [68] and Grobben *et al.* [70, 71] with *Lactobacillus* spp. used D-glucose, but not D-xylose. The same is true for the studies on *Azospirillum brasilense* Cd by Fischer *et al.* [164], *Pseudomonas syringae* pv. *glycinea* by Osman *et al.* [165], *Klebsiella* sp. K32 and *Acinetobacter calcoaceticus* BD4 by Bryan *et al.* [66], and *Xanthomonas campestris* S459 by Tait *et al.* [67]. In the work of Raza *et al.* [74], the monomer composition was analysed for the optimized medium with D-galactose as the sole carbon source.

However, from the studies which analysed the monomer composition for each carbon source, it can be concluded that the exact culture conditions, more specifically: the carbon source, *can* have a strong influence on the monomer composition. But, the actual extent to which each exopolysaccharide depends on the carbon source and other culture conditions, must be studied in detail for every strain.

It should be kept in mind that aldose monomer composition analyses are just one building block for fostering understanding of microbial exopolysaccharide production capabilities. As Rütering *et al.* [166] have shown recently, strains harbouring the corresponding genes [167] may produce two different types of exopolysaccharide. Therefore, if one only took into account the exopolysaccharide composition, the conclusion that the composition of *the* exopolysaccharide changed can be misleading.

For none of the strains tested in this screening, additional steps to pinpoint the true nature of apparent differences in the exopolysaccharide aldose monomer compositions were made. Also, no analyses regarding ketoses or modifications with pyruvic or acetic acid were run to keep the workload manageable. From the data available, it cannot be concluded whether different exopolysaccharides were produced at different levels or if the apparent aldose monomer compositions changed for other reasons. However, the results of the high-throughput screening on D-xylose presented in this work are in line with the available scientific literature.

3.8.3 High-Throughput Screening for Inhibitor Tolerance

Non-Growing Strains

Eight strains of the reference did not grow, hence, they had to be excluded from this screening step. The affected strains were neither in the same row nor in the same column, which would have hinted at an operator mistake during pipetting. While the cause is not known and given that in the subsequent very similar experiment with lignocellulose hydrolysate all of the affected

reference strains grew, a hitherto unknown effect could have caused this: the re-use of deep-well plates.

Some of the deep-well plates used were not new. After use, the previous plate user autoclaved the plate, cleaned it in a lab dishwasher and autoclaved the dry one in a plastic bag again for re-use. Sometimes, additional manual cleaning of single wells was necessary due to stubborn remains from autoclaving. Manual cleaning made extensive use of detergents. Co-workers stated that they also encountered irreproducible growth behaviour in re-used deep-well plates and suspected the cleaning procedure to leave detergents in single wells. The wells in question were filled with cleaning solutions and let to stand overnight. Due to adsorption, even rinsing the plastic afterwards and using the dishwasher might not have removed enough of the detergents. The apparently erratic occurrence of non-growing strains would be in line with the manual cleaning of specific wells only. Unfortunately, no further studies on the phenomenon were performed.

Excessively Growing Strains

Excessive growth was observed for all inhibitors, but vanillin. Generally, excessive growth could be attributed to the utilization of the additional carbon source present. The utilization of formic acid [45–47], acetic acid [48, 49], furfural [40–44] and hydroxymethylfurfural [41–44] are well described. Laevulinic acid utilization was reported by Steinbüchel and Gorenflo [52].

Growth was measured using the quick, but insensitive method of measuring the attenuance at 600 nm. Changes in cell morphology, probably induced by inhibitor presence, could lead to both, over- and underestimation of the growth on the inhibitor. The non-linearity of the attenuance signal was ignored. Assuming that the cell morphology in the inhibitor tests was unaltered compared to the reference, the non-linearity would be cancelled out almost completely at normal growth. Low growth in the presence of an inhibitor would give a better impression than in the linear case, excessive growth in the presence of an inhibitor would give a worse impression than in the linear case.

Since all the growth experiments in inhibitor presence were part of a screening, none of them were reproduced to keep the effort manageable. The probability for false positives and negatives could be high and some of the ‘winners’ of the inhibitor tolerance screenings could have been selected by chance. For 32 of the 53 excessively growing strains, the reference attenuances were below the average of the plates EPS1 and EPS2 of the growth screening on D-xylose (0.6). That at least half of all strains (26.5) grew to the average value is rooted in the definition of the arithmetic mean. The small surplus is explained with random errors. Therefore, excessive growth could also have been caused by a less-than-normal growth of the reference and not only a more-than-normal growth of the strain in inhibitor presence for at most six strains. Nonetheless, the ability to grow in inhibitor presence could still be shown for the strains used in the high-content screening.

Comparison of Inhibitor Results

As outlined above (see chapter 3 on page 59), the screening approach was used to quickly reduce the number of strains to test in subsequent screening rounds. For the inhibitor tolerance screening, six different inhibitors were tested. For future re-uses of the strategy employed in this screening, a further reduction of the workload or a higher degree of automation is desirable. Therefore, the results of the different inhibitors are compared in detail in this section to point out potential for refinements.

While only 27 strains showed at least rudimentary growth in the vanillin test series, 17 of these were also present in the top 27/28 of at least one other inhibitor. The exact numbers are: 10

of 27 for furfural, 7 of 27 for hydroxymethylfurfural, 10 of 28 for formic acid, 10 of 28 for acetic acid and 7 of 28 for laevulinic acid. Under the assumption that vanillin tolerance is independent from tolerance towards other inhibitors, on average, six[14] of the 27 'vanillin strains' should appear among the best 27 or 28 strains, respectively. This number, six, is smaller than any of the numbers found for the other inhibitors. Although this cannot be considered as solid evidence, it can be used as a hint for reducing the screening effort.

Combining the top strains of acetic acid and vanillin test series, 29 different strains were found. Matches with the top strains of the remaining inhibitors were better than with vanillin alone: 16 of 27 for furfural, 14 of 27 for hydroxymethylfurfural, 13 of 28 for formic acid and 13 of 28 for laevulinic acid.

Taking the strains of the hydroxymethylfurfural test series into account as well, the numbers are even better: 22 of 27 for furfural, 17 of 28 for formic acid and 14 of 28 for laevulinic acid. Three, ten and fourteen strains of the top strains of furfural, formic acid and laevulinic acid, respectively, were unique across all inhibitors. Subtracting these unique strains, the combination of acetic acid, vanillin and hydroxymethylfurfural finds all but two, all but one and all strains of the furfural, formic acid and laevulinic acid test series, respectively. Reducing the amount of inhibitors from six to acetic acid, vanillin and hydroxymethylfurfural would reduce the workload considerably, while still retaining 82 %[15] of the hits form the screening.

It should be noted at this point that nine of the fourteen unique strains of the laevulinic acid test series were among the laevulinic acid top 14 strains. One interpretation of this is that the metabolic capabilities for laevulinic acid utilization are usually not tied to acetic acid, vanillin or hydroxymethylfurfural utilization.

Inhibitory Effects of Vanillin

Most strains were totally inhibited by vanillin: 100 of the 127 strains tested. This also means that the 100 non-growing strains cannot be differentiated further. In hindsight, given that only one strain managed to grow normally, halving the vanillin concentration to $1.0\,g\cdot l^{-1}$ could have allowed to distinguish between the non-growing strains better and paint a more realistic picture of the metabolic capabilities of more strains.

Prior to the screening, the vanillin concentration of $2.0\,g\cdot l^{-1}$ seemed to be reasonable to quantify the effect of all phenolic inhibitors encountered in hydrolysates. While Jönsson *et al.* [37] reported the vanillin concentration of a willow hydrolysate to be $430\,mg\cdot l^{-1}$, Clark and Mackie [168] found the total low molar mass phenolics content in a *Pinus radiata* hydrolysate to be around $2.0\,g\cdot l^{-1}$. Nishikawa *et al.* [169] studied the growth and productivity of *Klebsiella pneumoniae* on D-xylose in the presence of different phenolic inhibitors. For vanillin, they found 91.5 % growth compared to the reference after 48 h. The inhibitory effects of vanillin on ethanol production in *Saccharomyces cerevisiae* were studied by Ando *et al.* [170] and found to be less severe than those of 4-hydroxybenzoic acid or 4-hydroxybenzaldehyde at $1.0\,g\cdot l^{-1}$. They found 60 % inhibition of *Saccharomyces cerevisiae* at a vanillin concentration of $2.0\,g\cdot l^{-1}$.

[14] The amount of strains growing in vanillin presence (27) per all strains tested (127) times the amount of strains in the top 27/28 (27 or 28) rounded to the next integer. $\frac{27}{127}\cdot 27 \approx 5.74 \approx 6$ or $\frac{27}{127}\cdot 28 \approx 5.95 \approx 6$.

[15] All 82 hits of acetic acid, vanillin and hydroxymethylfurfural and 53 of the 83 hits of the other three inhibitors: $\frac{82+53}{82+83} = \frac{135}{165} \approx 82\,\%$.

Acetonitrile Use in HPLC-MS Method

The methods for aldose monomer composition analysis and aldehyde inhibitor analysis rely on the same basic principle. But, due to the hydrophobicity of the inhibitors, especially furfural, and their PMP derivates, it was necessary to use acetonitrile and use a different filter plate to keep the analytes in the solution (data not shown).

3.8.4 High-Throughput Screening for Lignocellulose Hydrolysate Tolerance

The single inhibitor screening was conducted as a means to estimate the tolerance towards real world hydrolysate based on single experiments with chemically defined inhibitors. One of the 28 top strains of the lignocellulose hydrolysate trial did not grow in the single inhibitor screenings, therefore, only 27 of the 28 strains of lignocellulose hydrolysate can be compared with the chemically defined inhibitors. Combining all the top 27/28 strains of the single inhibitor experiments, 17 of the top 27 strains of the lignocellulose hydrolysate screening were found. The same result can be achieved by combining the top 27/28 strains of acetic acid, vanillin and hydroxymethylfurfural only. Leaving out hydroxymethylfurfural would reduce the matches by only one. Eight to nine[16] matches could have been expected by chance assuming independence of single inhibitor tolerances and lignocellulose hydrolysate tolerance, but almost double the amount of strains was found.

This means that the single inhibitor experiments correctly predicted the majority of lignocellulose hydrolysate tolerant strains. As the presence of lignocellulose hydrolysate can interfere with e.g. the high-throughput HPLC-MS analysis of the polymer composition, usage of single inhibitors instead of lignocellulose hydrolysate circumvents these issues during the screening. Reducing the amount of inhibitors from furfural, hydroxymethylfurfural, vanillin, acetic acid, formic acid and laevulinic acid to only vanillin and acetic acid, the required effort could be reduced to one third by sacrificing only a negligible amount of correctly predicted strains.

3.8.5 High-Content Screening with Inhibitors

Non-Pelleted Strains

As described in section 3.5.1 on page 73, the supernatant in some wells remained more or less turbid after centrifugation. The question is whether this behaviour correlates with inhibitor levels, D-glucose consumption and/or exopolysaccharide production. Generally, acid inhibitors appear to be worse for pelletability than aldehyde inhibitors: only nine strains were reported in plate ISp, while twenty-three were reported in ISr. Sedimentation depends on the dynamic viscosity and exopolysaccharides can easily increase the dynamic viscosity more than thousandfold compared to pure water. Therefore, if the inhibitor in question is less stressful to the microorganism, exopolysaccharides can be produced, which in turn can impede pelletization and filtration.

On the other hand, there were some wells without any apparent microbial growth, a prerequisite for pellets after centrifugation. Although in some cases furfural degradation was found, D-glucose consumption was at most 10 %. Furfural degradation is explained on page 96. The D-glucose consumption combined with the inhibitor degradation could be explained with strains investing energy into furfural degradation instead of cell division (cf. section 3.8.7 on page 98), D-glucose consumption alone could be an artifact stemming from random errors during assay

[16] The 39 different strains of the acetic acid, vanillin and hydroxymethylfurfural experiments correspond to 30.7 % of the 127 strains tested. By chance alone, 8.29 or 30.7 % of the top 27 strains would have been shared with the combination of the top 27/28 of the three single inhibitor experiments.

preparation. Interestingly, up to 392 $mg \cdot l^{-1}$ of monomers were found after aldose monomer composition analysis with the majority made up of D-glucose and the remaining part of D-mannose. This can be explained with the high residual D-glucose concentrations before gel filtration: the gel filtration only has a limited capacity for separating small molecules from the macromolecules. With up to 10 $g \cdot l^{-1}$ D-glucose, some of this could easily get past this step and react during hydrolysis and derivatization. Since derivatization conditions were basic, the D-mannose could be a product of D-glucose in the Lobry de Bruyn-Alberda van Ekenstein transformation [171]. D-Fructose would be another product, but would not react during derivatization, because it is a ketose, which makes it undetectable using PMP.

Non-Pelleted Strains vs. Inhibitor Concentrations The non-pelleted strains of plate ISp do not show peculiarities, but on plate ISr, two strains do. Strain *Xyl2.A7* was incubated with laevulinic acid and showed a laevulinic acid concentration exceeding the initial concentration of 2.0 $g \cdot l^{-1}$ by more than 30 %. It is unlikely that the strain produced additional laevulinic acid and possible other explanations are given on this page. Strain *Xyl2.D3* was incubated with formic acid, which was consumed completely, but produced copious amounts of acetic acid (3.4 $g \cdot l^{-1}$).

Non-Pelleted Strains vs. D-Glucose Concentrations Eighteen strains completely consumed the carbon source and only four consumed less than half the D-glucose. There is no apparent correlation between D-glucose consumption and pelletability.

Non-Pelleted Strains vs. Exopolysaccharide Aldose Monomers vs. Poor Filtration Performance In section 3.5.4 on page 77, sixteen strains with cumulative exopolysaccharide aldose monomer concentrations exceeding 1.0 $g \cdot l^{-1}$ are listed, all exclusively from acid inhibitor experiments. Twelve of these strains are also among the non-pelleted strains. Overall, 38 different strains showed low or no sedimentation or poor filtration performance. Of these, there was considerable overlap: 22[17] of the 38 or 58 % exhibited low or no sedimentation and poor filtration during either 10 kDa filtration or glass filtration. Among these 22 strains were seven[18] strains with cumulative exopolysaccharide aldose monomer concentrations exceeding 1.0 $g \cdot l^{-1}$. Taken together, this corroborates the earlier statement that conditions favourable to exopolysaccharide production correlate with poor pelletization and also filtration.

Residual Inhibitor Concentrations

Aldehyde Inhibitors Aldehyde inhibitor analysis was planned for low inhibitor concentrations and the low values found (see table 3.9 on page 75) support this approach. Nonetheless, high values occurred and are beyond the highest concentration of the calibration curve, 50.0 $mg \cdot l^{-1}$. Without the ten-fold dilution, this translates to a maximum of 500 $mg \cdot l^{-1}$. Therefore, high values can exceed the initial concentration of 2.0 $g \cdot l^{-1}$ as was the case for the strains *Xyl2.B7*, *Xyl2.C5*,

[17] Strains with low or no sedimentation and poor filtration performance: A3, B3, C3, D6, E6 and F6 of ISp and A1, A7, B1, C10, D1, D3, D6, D10, E1, E4, E5, E10, F4, F5, G1 and G4 of ISr. This corresponds to the strains *Xyl1.F4*, *Xyl1.F8* and *Xyl1.F9* for furfural, *Xyl1.F4*, *Xyl1.F8* and *Xyl1.F9* for hydroxymethylfurfural, *Xyl1.C4*, *Xyl1.C5*, *Xyl1.F1* and *Xyl1.H8* for acetic acid, *Xyl1.C4*, *Xyl1.C5*, *Xyl1.F2*, *Xyl1.F4*, *Xyl1.F8*, *Xyl2.A1*, *Xyl2.C12*, *Xyl2.D2* and *Xyl2.D3* for formic acid and *Xyl1.D8*, *Xyl1.D9* and *Xyl1.D10* for laevulinic acid.

[18] Strains with low or not sedimentation, poor filtration performance and cumulative exopolysaccharide aldose monomer concentrations of over 1.0 $g \cdot l^{-1}$: A1, A7, B1, D3, E5, F5 and G4 of plate ISr. This corresponds to the strains *Xyl1.C4*, *Xyl1.C5* and *Xyl1.H8* for acetic acid and *Xyl1.C4*, *Xyl1.C5*, *Xyl2.A1* and *Xyl2.D3* for formic acid.

Xyl2.C7 and *Xyl2.D1* with vanillin, *Xyl1.D12* for hydroxymethylfurfural and the reference wells ISp.G4 and ISp.G12, which supports this explanation.

The furfural reference well ISp.G8 contained only $1.0\,g\cdot l^{-1}$ which is considerably less than the $2.8\,g\cdot l^{-1}$ for hydroxymethylfurfural or the $3.2\,g\cdot l^{-1}$ for vanillin. Furfural undergoes autoxidation with oxygen [21] and given the vigorous shaking ($1000\,min^{-1}$), the relatively low concentration can be explained by autoxidation. The extent to which furfural degraded in other wells was not examined.

Acid Inhibitors In the case of the acid inhibitor analyses, the calibration curve ended at $5.0\,g\cdot l^{-1}$ leaving room for further production of the inhibitor from D-glucose by the microorganism. But, other factors could interfere with the correct determination of the acid concentration. Due to a human error, the run time of each injection was reduced to 25 min from 50 min. This could have led to ghost peaks from the previous run. If a peak occurred at the retention time of the acid, it would be indistinguishable and counted as additional acid. Also, unidentified microbial products could elute at the same time as the acid in question and affect the peak area. On the other hand, the reference values for formic acid, acetic acid and laevulinic acid were $2.42\,g\cdot l^{-1}$, $2.43\,g\cdot l^{-1}$ and $2.30\,g\cdot l^{-1}$, respectively. Since the samples were diluted ten-fold or even twenty-fold and the lowest concentration of the calibration curve was at $50\,mg\cdot l^{-1}$, this could also have introduced considerable errors.

Since laevulinic acid production seems unlikely [172], the increases in laevulinic acid are assumed to be false positives. Bacterial consumption of laevulinic acid was first shown in 1969 by Harada *et al.* [173] using laevulinic acid as the sole carbon source. Jang and Rogers [51] demonstrated the consumption of laevulinic acid using D-glucose as the primary carbon source and laevulinic acid as the secondary carbon source. Keenan *et al.* [53] showed laevulinic acid consumption with a different strain and D-xylose as primary carbon source.

Therefore, the laevulinic acid degradation seen for the strains *Xyl1.A3*, *Xyl1.H7*, *Xyl1.H9*, *Xyl1.H10*, *Xyl1.H11* and *Xyl2.A4* can be plausibly attributed to microbial degradation. According to the preliminary annotation in the strain collection, three of these strains belong to the genus *Sphingomonas* and one each to the genera *Agrobacterium*, *Arthrobacter* and *Rhodococcus*.

Statistical Calculations on Raw Values The raw values given in tables A.8 on page 138 and A.9 on page 140 contain negative values and may list values for all three inhibitors. For statistical calculations, negative values and 'n.d.' were treated as zero and only values of the respective test series were used, i.e. the vanillin values of the furfural and hydroxymethylfurfural test series were not used for the calculation of the vanillin median and quartiles.

D-Glucose Freed by Hydrolysis

Nine wells[19] exhibited D-glucose concentrations after hydrolysis below the respective concentrations before hydrolysis. A deeper look at these inconsistencies revealed that for the strains *Xyl1.F1* (furfural trial) and *Xyl1.D12* (hydroxymethylfurfural trial), the difference was below 5 % of the gel filtration value. This is considered to be caused by random errors. Taking the HPLC-MS D-glucose concentrations into account, *Xyl1.F3* (furfural trial), *Xyl2.C5* (vanillin trial), *Xyl2.A12* (acetic acid trial) and *Xyl2.A6* (laevulinic acid trial) exhibited an increase in D-glucose concentration after the hydrolysis. The difference to the HPLC-MS value for *Xyl1.F10* (vanillin trial) was

[19] *Xyl1.F1* and *Xyl1.F3* for furfural, *Xyl1.D12* and *Xyl2.B7* for hydroxymethylfurfural, *Xyl1.F10* and *Xyl2.C5* for vanillin, *Xyl2.A12* for acetic acid and *Xyl1.D12* and *Xyl2.A6* for laevulinic acid.

less than 0.2 % of the gel filtration value. Like before, this is seen as an artifact caused by random errors.

This leaves *Xyl2.B7* (hydroxymethylfurfural trial) and *Xyl1.D12* (laevulinic acid trial). The corresponding D-glucose assay and HPLC-MS D-glucose values were in both cases more than 10 % smaller than the D-glucose assay value after gel filtration. Therefore, for these two wells, the most likely interpretation is that the concentration determined after the gel filtration is too high and the actual concentration was lower.

Inhibitor Impact on Exopolysaccharide Production

In section 3.5.4 on page 77, sixteen strains[20] exhibiting cumulative exopolysaccharide aldose monomer concentrations greater than $1.0\,\mathrm{g\cdot l^{-1}}$ are listed. Only two of these strains are from plate ISp, while the majority is from plate ISr. Since phenolic compounds have been found to be more inhibitory to microbial growth than furan derivates or weak acids [35, 37, 168] and aldehydes have been found to be more inhibitory than weak acids [174–177], these different outcomes are attributed to the inhibitor used.

The differences in exopolysaccharide aldose monomer compositions found when *Xyl1.C5* was grown with different inhibitors or without were shown in figure 3.5 on page 78. Since one and only one factor was changed between experiments, differences in exopolysaccharide aldose monomer compositions can be attributed to the different inhibitors. Still, the same caveats mentioned above (see section 3.8.2 on page 89) for comparing apparent differences in the compositions with regard to inhibitors or carbon sources used apply.

High Similarity Between *Xyl1.C4* and *Xyl1.C5*

From one step to the next, more and more hints for a high similarity between the outstanding strains *Xyl1.C4* and *Xyl1.C5* accrued: the exopolysaccharide aldose monomer compositions during the high-content screening on D-xylose (see table 3.3 on page 63); the exopolysaccharide aldose monomer compositions found by Rühmann [154] on D-glucose (see figure 3.1 on page 64); the ranks in the inhibitor and lignocellulose hydrolysate tolerance screenings (see table 3.5 on page 67 and table 3.7 on page 71) and the sedimentation and filtration issues, the residual inhibitor concentrations, the cumulative monomer concentrations and the exopolysaccharide aldose monomer compositions in the presence of inhibitors during the inhibitor high-content screening (see table 3.10 on page 76 and section 3.5.4 on page 77). Since most results stem from single measurements, the results of single experiments may not agree fully with each other, but taking into account all the available data, there is strong evidence that the two strains *Xyl1.C4* and *Xyl1.C5* are very similar, if not the same.

3.8.6 Strain Selection

It should be noted that the apparent differences in the exopolysaccharides of *Xyl2.B8* at the bottom and top (see section 3.6.2 on page 79) are a side effect of the different concentrations used: monomers below a certain threshold could not be detected in the 'top' polymer (D-galactose, D-galactosamine, *N*-acetyl-D-glucosamine, D-glucuronic acid, L-rhamnose). Apart from that, re-

[20] None in the presence of furfural or vanillin, *Xyl1.C4* and *Xyl1.C5* in the presence of hydroxymethylfurfural, *Xyl1.C4*, *Xyl1.C5*, *Xyl1.H8*, *Xyl2.A5*, *Xyl2.A7*, *Xyl2.A8* in the presence of acetic acid, *Xyl1.C4*, *Xyl1.C5*, *Xyl1.F5*, *Xyl2.A1*, *Xyl2.A2*, *Xyl2.B10* and *Xyl2.D3* in the presence of formic acid and *Xyl2.A7* in the presence of laevulinic acid.

coveries and monomer concentrations of the two *Xyl2.B8* samples are in good agreement with each other.

The exopolysaccharide of *Xyl2.B8* consists of the major constituents D-glucose and D-mannose, the minor constituents are D-glucosamine and D-glucuronic acid and traces of L-rhamnose, D-galactose, D-galactosamine and *N*-acetyl-D-glucosamine. Major and minor monomers combined provided more than 96 % of the total detected aldose monomer mass. It is not known whether during hydrolysis or derivatization side reactions such as the Lobry de Bruyn-Alberda van Ekenstein transformation occured possibly interconverting different sugars [171]. For uronic acids, high degradation during hydrolysis has been reported [1], which means that the actual uronic acid content of the polymer most likely is higher. Other monomers may also be more sensitive to degradation distorting the exopolysaccharide aldose monomer composition.

The molar ratio of the major and minor monomers D-glucose, D-glucosamine, D-glucuronic acid and D-mannose of the exopolysaccharide of *Paenibacillus* 2H7 was 7.9 : 1.5 : 1.0 : 5.0. The exopolysaccharides of *Paenibacillus* spp. are generally diverse [178]. The exopolysaccharide of *Paenibacillus polymxa* SQR-21 consisted of D-mannose, D-galactose and D-glucose at a ratio of 1.23 : 1.14 : 1. Han and Clarke [179] found one exopolysaccharide of a *Paenibacillus polymyxa* to be a levan exclusively composed of β-2,6-linked D-fructose. The polymer reported by Lee *et al.* [180] contained D-glucose, D-galactose, D-glucuronic acid, D-mannose and L-fucose. For two different exopolysaccharides of *Paenibacillus polymyxa* EJS-3 Liu *et al.* [181] reported different ratios of D-glucose, D-fructose and D-mannose, the major component being D-fructose in all cases. Madden *et al.* [182] found L-fucose, D-mannose, D-galactose and D-glucose at a ratio of 0.2 : 1.5 : 1.0 : 2.2 and uronic acid.

Apart from strains of *Paenibacillus polymyxa*, Wang *et al.* [183] and Li *et al.* [184] studied other members of the genus *Paenibacillus*. The exopolysaccharide of *P. elgii* B69 consisted of D-glucose, D-glucuronic acid, D-xylose, and D-mannose at a ratio of 1 : 0.53 : 1.15 : 0.46 [184]. The exopolysaccharide of *Paenibacillus* sp. TKU023 was found to contain mainly D-glucose and also D-mannose [183]. Rühmann [154] reported nine *Paenibacillus* spp. which produce exopolysaccharide containing D-glucosamine.

Although diverse results have been published, reports on finding D-glucosamine as a part of the polymer are scarce and none—to the best of my knowledge—contain 16S rDNA sequences of the strains tested. Therefore, this is the first report of a *Paenibacillus* with known 16S rDNA sequence producing exopolysaccharide containing D-glucosamine.

3.8.7 Parallel Fermentation with Lignocellulose Hydrolysate

The primary aim of the parallel fermentation of *Paenibacillus* 2H7 was obtaining reliable growth data as a basis for further fermentations and process optimizations. As outlined below, this aim was reached successfully and it was possible to optimize the process at a 7 l scale to counter the lignocellulose hydrolysate's detrimental effect on microbial growth (data not shown).

Inhibitor Degradation

The strains in block 2 exhibited a considerable lag phase of approximately 36 h. Afterwards, the fermentation continued in a similar fashion as in block 1. Inhibitor analyses revealed that the end of the lag phase coincided with the beginning of the degradation of furfural (see figure 3.7 on page 85). While only furfural is reported here, hydroxymethylfurfural was detected as well. But, as outlined in section 3.4.1 on page 69, the analysis of pure lignocellulose hydrolysate found around 1.8 $g \cdot l^{-1}$ furfural and only around 260 $mg \cdot l^{-1}$ hydroxymethylfurfural. The

actual concentration in the fermentation broth was expected to be 30 % of these values, so that hydroxymethylfurfural would have been negligible.

The initial furfural and hydroxymethylfurfural concentrations were around 800 $mg \cdot l^{-1}$ and 100 $mg \cdot l^{-1}$, respectively. The hydroxymethylfurfural data is not included in this work for two reasons:

- The course of the hydroxymethylfurfural concentration shows the same behaviour as the other aldehyde inhibitor analysed, furfural.
- While the slow decrease of furfural could be measured reliably, hydroxymethylfurfural vanishes completely from one sample to the next.

The fact that the microorganisms were the driving force behind furfural degradation after 36 h, is derived from the seemingly exponential degradation of furfural after 36 h, which coincided with the beginning of the exponential growth phase of the strain. Since more microorganisms also means an increase in catalytic power, growth and furfural degradation correlate with each other. The relevant process parameters—aeration rate, oxygen content of the air, stirrer speed, temperature and pH—were kept constant, so that it is unlikely that the furfural degradation accelerated. Since furfural is known to undergo autoxidation [21, 185], it is not clear whether the degradation of furfural seen in the first 36 h was caused by the microorganisms or the oxygen supplied continuously. Liu *et al.* [186] reported a similar lag phase for ethanol fermentations of *S. cerevisiae* on lignocellulose hydrolysate, which was attributed to the presence of furfural and hydroxymethylfurfural [174].

Acidification Causes Carbon Dioxide Spikes

The carbon dioxide spikes at 48 h in block 1 and at 87.5 h in block 2 coincided with the acidification caused by the more acidic new setpoint of the pH controller. More acidic conditions will increase the amount of carbonic acid in the equilibrium of carbonic acid, carbonates and carbon dioxide. The equilibrium readjusts according to Le Châtelier's principle releasing carbon dioxide.

Influence of the pH Shift

The effect of pH shifts on the outcome of microbial exopolysaccharide fermentations has been studied by several authors [176, 187–190]. Therefore, the parallel fermentations included a pH shift as well to examine the effects of the shift on the microorganism and the product.

Zaldivar and Ingram [176] studied the effect of different weak acids from lignocellulose hydrolysates and found that the 'toxicity of all acids except gallic acid was reduced by an increase in initial pH (from pH 6.0 to pH 7.0 to pH 8.0)'. This indicates that doing the reverse would increase the toxicity of the acids. Lee *et al.* [188] found the optimal pH value for exopolysaccharide production of *Paenibacillus polymyxa* KCTC 8648P to be 7.0, at 6.0 the final exopolysaccharide concentration dropped by 25 %.

Esgalhado *et al.* [189] studied the growth and productivity of *Xanthomonas campestris* NRRL B-1459 and found different pH and temperature optima for cell growth and xanthan production: the pH value for xanthan production was 7.0 to 8.0, higher than that for cell growth (6.0 to 7.5). The addition of weak acids to well-aerated cultures at a controlled pH value of 6.0 led to decreased growth, but increased xanthan production [190]. Oxygen supply in the parallel fermentation was low as evident by the dissolved oxygen course of block 1 and most likely block 2, too. See 'Effects of Anti-Foam Addition' on the next page for more details.

Degeest *et al.* [187] showed that a pH drop from 6.2 to 3.0 inhibited enzymatic degradation of high molar mass polymer, which could occur during prolonged fermentation of *Streptococcus thermophilus* LY03.

Given the dramatic drop in peak molar mass, the increase of the inhibitory potential of weak acids due to protonation [191–196] and the increase in protonated weak acids in lignocellulose hydrolysate presence, the pH shift towards a more acidic pH is seen as detrimental and should be avoided in subsequent processes. If a pH shift is used, the pH value should adapt to the new value slowly, ideally by the action of the microorganism alone, and not via a change in the setpoint of the pH controller and subsequent introduction of external acid. The pH shift took approximately 10 min in block 1 and 20 min in block 2, which put considerable stress on the microorganisms and also demonstrates the different buffering capacities of the reference medium and the lignocellulose hydrolysate medium.

Comparison of Carbon Dioxide Courses

The carbon dioxide courses in both blocks are very similar (see figure 3.7 on page 85). This observation is difficult to make, because the timescales are different due to the lag phase in block 2, so that block 2 appears to be compressed on the x-axis. Another effect can be attributed to the uncalibrated carbon dioxide sensors, so that the absolute carbon dioxide values between the two blocks appear to differ: the maximum values were 0.67 % to 0.70 % and 0.78 % to 0.82 % for block 1 and 2, respectively. Given the almost identical general shape of the carbon dioxide curve and that D-glucose was consumed slower in block 2, the carbon dioxide production should actually be lower in block 2 than in block 1. But, since D-xylose and other usable carbon sources were not quantified, more data are needed for a definite statement.

Effects of Anti-Foam Addition

Anti-foam was added after 63.2 h and 96.5 h. While only very minor influences were seen in block 1 (small drop of CO_2), the effects were more pronounced in block 2: upon anti-foam addition, the CO_2 dropped slightly and the dissolved oxygen dropped dramatically. The effect was weaker the second time, because only half the amount of anti-foam was added. What exactly caused these disturbances can only be hypothesized: the numerous different compounds present in lignocellulose hydrolysate interact with anti-foam to lower oxygen solubility and raise carbon dioxide solubility.

Given that the carbon dioxide courses and D-glucose consumption in both blocks are very similar, it is unlikely that the dissolved oxygen course does not match the carbon dioxide course after approximately 50 h. To the best of my knowledge, this phenomenon—lignocellulose hydrolysate influencing the dissolved signal/sensor—has not yet been described in the literature.

Incomplete D-Glucose Utilization

As is evident from figure 3.7 on page 85, approximately half the D-glucose was consumed over the course of the fermentation in both blocks. The consumption dropped after acidificiation in block 2 and in both cases an approximately linear consumption was found. The D_{600} remained constant in block 1 and with the exception of the two samples after acidification in block 2 as well, microbial growth is interpreted to have stalled and D-glucose was not used for cell division, but for maintenance only. Cell dry mass courses cannot be used to assess microbial growth in this fermentation, see ‘Cell Dry Mass Courses’ on the facing page for details. Therefore, a lack of

oxygen is seen as the most likely reason for slow and low D-glucose consumption and the growth stop. On the other hand, quorum sensing might have been at work [197] and cannot be ruled out with the available data.

Cell Dry Mass Courses

The cell dry mass course of block 1 appears to reach a maximum before the acidification and to drop to a constant value afterwards. During the sampling process starting with the third sample, the bacteria did not form a uniform pellet, but a comparatively small slime phase gathered on top. A similar behaviour was found in block 2: starting with the sixth sample, which fermentation-wise corresponds to the third sample of block 1, the two phases formed as well. In the following text, the pellet is also referred to as 'bottom phase', while the slime phase is also referred to as 'top phase'.

Since the slime phase sedimented on top of the pellet upon centrifugation, the density of its components must have been higher than that of the surrounding medium, but lower than that of the pellet. The most likely explanation for the slimy appearance of the top phase is the presence of exopolysaccharides in relatively high concentration. Since exopolysaccharides are completely dissolved in the medium, sedimenting them using a standard bench-top centrifuge should be nigh impossible. Instead, the following hypothesis is put forth as an explanation of this phenomenon: during the fermentation, at some point growth slows and the bacteria start producing exopolysaccharides. During production, the exopolysaccharide is still attached to the microorganism. Upon centrifugation, some of the strains will be attached to enough exopolysaccharide chains to have a slightly different density and hydrodynamic properties. The highly viscous top phase further impedes sedimentation, such that two distinct phases form upon centrifugation.

After the pH shift in block 1, the top phase was considerably smaller explaining the apparent sudden drop in cell dry mass in block 1. Assuming the above hypothesis to be true, most of the drop could be explained by the lack of top phase. This phenomenon was accounted for in the 7 l fermentation and studied in more detail, see '7 l Fermentation' on the next page. The molar mass drop suggests a concomitant degradation of the polymer, which could be initiated by the pH drop and carried out by enzymatic action. As the pH drop was not as sudden in block 2, the effects on the cell dry mass were not as severe as in block 1. In both blocks, the top phase never vanished completely.

The different behaviours of *Paenibacillus* 2H7 in the reference medium and in the lignocellulose hydrolysate medium surfaced in the cell dry mass course as well: the top phase in block 2 was considerably larger after the pH drop and the cell dry mass recovered completely from the pH drop. Also, the peak molar mass began to recover as well in block 2, while in block 1, it appears to have remained constant at best. The acidifying phenotype, it seems, is connected to exopolysaccharide build-up, while the de-acidifying phenotype is not.

Molar Mass Courses and Determination

As outlined before, the peak molar mass of the polymer was seriously affected by the pH drop and did not recover in block 1, but started to recover slowly in block 2. It should be noted that the molar mass values given are not absolute, but only in relation to the pullulan standards used. Since the standard's highest molar mass was 2.35 MDa, all values exceeding this were extrapolated from the calibration curve. Nonetheless, the values are comparable to each other and clearly show that the maximum molar mass was reached before the pH shift in both blocks.

Polymer Purification

The cross-flow filtration unit and membranes used for the removal of small molecules and concentration of the polymer was cleaned before and after each run as described by the manufacturer. Still, flushing the system with NaOH does not reliably kill spores. After the first unsuccessful filtration attempt with an overly coarse membrane, the supernatant of the unautoclaved fermentation broth was stored in a canister at 4 °C for two and a half weeks.

Unfortunately, a spore-forming strain of a co-worker known to grow at 4 °C and degrade exopolysaccharides infected the broth through contact with the cross-flow filtration unit and membranes. The peak molar mass of the feed solution was halved compared to the final fermentation sample. This was also evidenced by the clear occurrence of a bacterial pellet upon recentrifugation and the different precipitation behaviour. Usually, the exopolysaccharide would form long threads around the stirrer, but the degraded polymer precipitated in a 'cat hair-like' fashion.

Exopolysaccharide Composition over Time

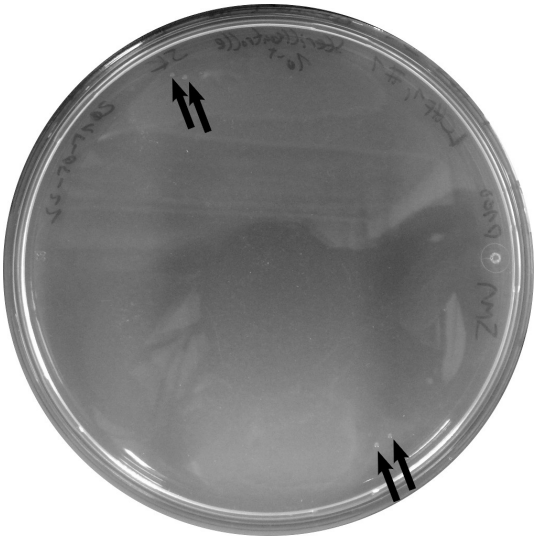
The analytical approach for the exopolysaccharide aldose monomer composition analysis relies on the relative absence of small molecules, especially monomeric aldoses. The gel filtration step only has a limited power to separate the polymers from the monomers. In both blocks, the residual D-glucose was above $10\,\mathrm{g \cdot l^{-1}}$ and taking into account other small molecules as well, a considerable proportion passed through the gel filtration. The monomeric D-glucose after gel filtration was determined, but the sheer amount of D-glucose affected the derivatization negatively. The only other monomer not supplied as a carbon source found in block 1 was D-mannose and the concentrations increased from the start of the fermentation to the end (data not shown). In block 2, D-galactose, usually found in lignocellulose hydrolysate [198], and L-rhamnose were present in all samples. The concentrations were too low and no conclusive statement can be made from these measurements. Therefore, it remains unknown whether the exopolysaccharide composition changed over time as reported by Bryan *et al.* [66] for *Klebsiella sp.* K32 or not as reported for *Acinetobacter calcoaceticus* BD4 by the same author.

7 l Fermentation

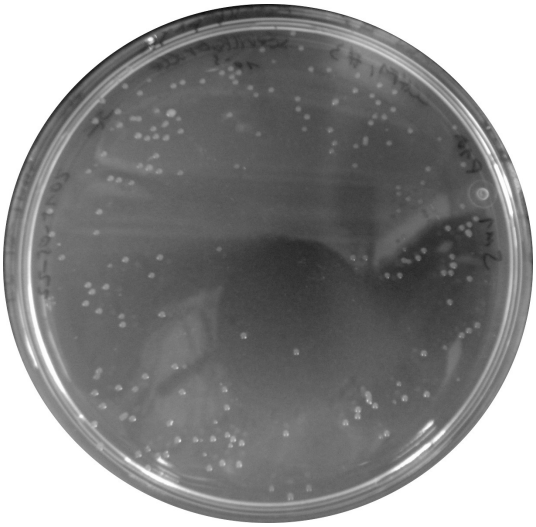
The experiences from the parallel fermentation were used to shorten the lag phase: the fermenter was inoculated with a D_{600} of 0.2 instead of 0.05 and at the start of the fermentation, only 5 % lignocellulose hydrolysate was supplied. After four hours of incubation and adaptation, the fermenter was fed lignocellulose hydrolysate and the corresponding other medium components over the course of the next 24 h, increasing linearly. The final medium contained 30 % and the strain grew from the beginning.

The reason, why the 7 l fermentation is not part of the results section, is a contamination with a *Bacillus subtilis*. The contamination became visible on a sterile control of the 53.8 h sample. In figure 3.8 on the facing page, four SM1 P100 plates at different timepoints of the fermentation are shown. At the end of the fermentation, the contaminant and *Paenibacillus* 2H7 were present in approximately the same number. The 16S rDNA sequence of the contamination is given in figure A.2 on page 164. The two best hits in a BLAST search using MegaBLAST in the database 'nt' of NCBI were *Bacillus subtilis* subsp. *inaquosorum* strain 19A_1.1 and *Bacillus subtilis* BSn5. Samples up to and including the 48.0 h sample are considered virtually unaffected by the contaminant.

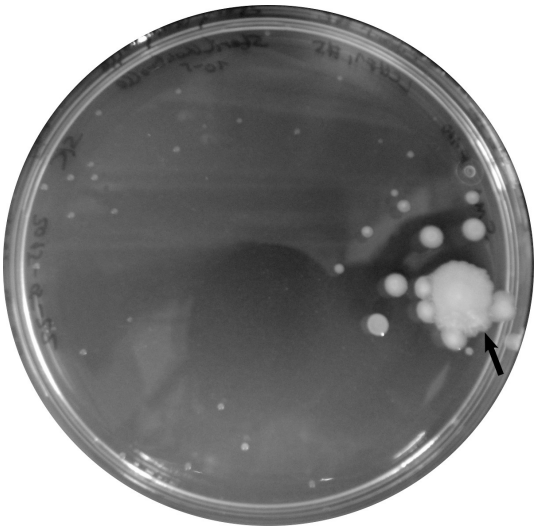
The most likely infection route was the sampling valve at the bottom of the fermenter. A simple test was devised to confirm this assumption. After the fermentation, sterilization and



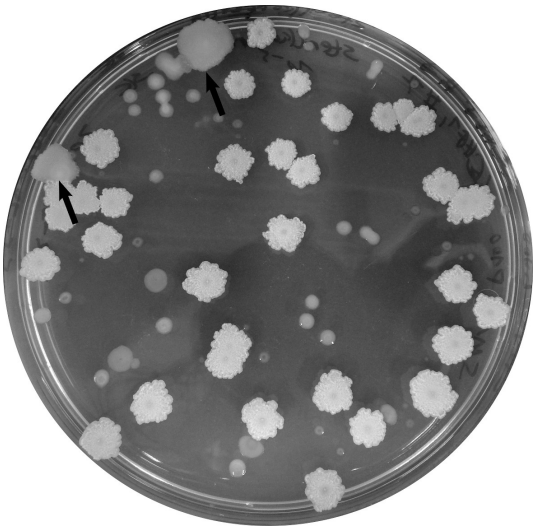
(a) 15.5 h. Arrows: colonies of *Paenibacillus* 2H7.



(b) 39.5 h. All colonies are exclusively from *Paenibacillus* 2H7.



(c) 53.8 h. Arrow: mixed colony of the contaminant and *Paenibacillus* 2H7. The big and more opaque round colonies surrounding the contaminant colony are from *Paenibacillus* 2H7 exhibiting a different morphology when near the contaminant.



(d) 80.0 h. The rosette-like colonies are pure contaminant colonies. The arrows point to mixed colonies of contaminant and *Paenibacillus* 2H7. Other colonies are from *Paenibacillus* 2H7 mostly exhibiting the same morphology as seen in the 53.8 h sample.

Figure 3.8: Sterile controls of the 7 l fermentation. *Paenibacillus* 2H7 was fermented at 7 l scale using a fed-batch process. The initial volume was 5.0 l with 5 % lignocellulose hydrolysate. Starting 4 h after inoculation, lignocellulose hydrolysate and medium concentrate were fed linearly increasing for 24 h to a final lignocellulose hydrolysate concentration of 30 %. After 80.0 h the fermentation was stopped and the fermentation broth harvested. Sterile controls were prepared from 100 µl of $1{:}10^5$ diluted sample on SM1 P100 agar plates. The plates were incubated for 3 d at 30 °C.

cleaning, the outlet of the sampling valve was blocked and filled with sterile SM1 P100. After incubation of approximately 40 min, the medium was gathered in a 50 ml tube and put on a rocking platform at room temperature for approximately 7 h at 120 min^{-1}. The D_{600} after incubation was 0.24. 100 µl was streaked in dilutions of 10^0 to 10^{-7}. Around 10^5 CFU·ml^{-1} and at least four different colony morphologies were found.

While the fermenter was sterilized in place, the tubing could not be sterilized and given prior fermentations in this fermenter with spore-forming bacteria, the issue was aggravated by the high viscosity of the broth. While the tube would drain at the beginning of the fermentation, the viscous broth stayed in the tubing allowing the growth of the contaminating species. Upon the next sampling, the valve was opened and closed again allowing the entry of the contaminant.

Nonetheless, the contamination allowed one interesting observation: the growth of *Paenibacillus* 2H7 was influenced by the contamination and the difference is apparent on the sterile controls as well: colonies grew to larger sizes and had a different colour. While they were translucent white before, when growing in vicinity to the contaminant colony, they would turn to a more yellowish to beige phenotype. Also, the rheological behaviour of the fermentation broth at the end of the fermentation did not resemble anything the author has seen before in a biologically produced polymer solution. Therefore, deliberate co-cultivations might pose yet another method for process optimization [199].

In order to verify the assumption that the actual cell dry mass does not decrease, but the slime phase does, the two phases were separated after centrifugation and weighed alone. Although the fermentation was contaminated, weighing both separately allowed to gather some valuable information:

- The bottom phase dry mass remained low throughout the fermentation. The low masses made accurate determinations difficult, but until the contamination, the bottom phase dry mass did not exceed 1.0 g·l^{-1}.
- After the contamination, bottom phase dry mass increased until 1.8 g·l^{-1} at the end of fermentation.
- The top phase dry mass exceeded the bottom phase dry mass from the second sample on: until the contamination, the top phase amassed 20.8 g·l^{-1} dry matter.
- With the contamination taking over, the top phase dry mass fell down to 10.2 g·l^{-1} at the end of the fermentation. The highest value was 35.4 g·l^{-1}, when the contamination was first visible on plate.

The rise of the bottom phase dry mass and the fall of the top phase dry mass coincide suggesting that *Paenibacillus* 2H7 ceased exopolysaccharide production, so that more cells could settle in the bottom phase.

3.9 Outlook

3.9.1 Expansion of Analytics

The lignocellulose hydrolysate contained acetic acid at a concentration of 4.6 g·l^{-1} (externally supplied value) or 9.7 g·l^{-1} (custom analysis). In the medium SMLCH, this would equal 1.4 g·l^{-1} to 2.9 g·l^{-1} acetic acid. Under the impression of the aforementioned different phenotypes of *Paenibacillus* 2H7 (see section 3.8.7 on page 99), the quantification of acetic acid, but also formic

acid would give new insights into the metabolism of *Paenibacillus* 2H7, which could be leveraged for further process optimizations.

The main drawback of the PMP-based HPLC-MS method is the need to remove all monomeric aldoses for the most exact analysis of the aldose monomer composition of the fermentation samples, with and without lignocellulose hydrolysate. The specificity for aldehydes is another, but a far less severe limitation. For single fermentations and, thus, low sample numbers, the more laborious method of precipitating the exopolysaccharide of single samples and dissolving the precipitate in water becomes feasible.

For high-throughput applications, the following adaptations could be investigated:

- Enhance gel filtration separation characteristics by sample dilution or higher column volumes.
- Downscaling the precipitation and re-dissolution process.
- Selective aldehyde removal via unspecific oxidation [200, 201].

The gel filtration plates used were run with 30 µl of sample. Scaling up the column volume and/or diluting the sample to make the high small molecule load manageable, could solve the issues at hand with the least amount of changes to the overall process. Unfortunately, the gel filtration plates are not available in a deep-well format by default, so that diluting and numerous parallel runs of the diluted sample would be needed. The final sample would need to be concentrated, probably by another gel filtration. Alternatively, small molecules could also be removed via 96-well microdialysis [202].

While precipitation at 1 ml scale should not pose any problems, the re-dissolution is expected to be challenging. Although the dissolution does not need to be perfect for the hydrolysis, each well should be homogeneous and given that some polymers can be difficult to redissolve after precipitation, considerable effort might be needed to get this approach to work reliably.

The major downside of any reactive method is the introduction of additional small molecules. If the reaction does not yield gaseous or solid products, the number of small molecules most likely cannot be reduced. If these products do not interfere with hydrolysis and derivatization, the relatively hydrophobic PMP-derivates could be selectively adsorbed, washed and desorbed prior to HPLC-MS analysis removing all less hydrophobic contaminants. The inverse process—adsorbing all hydrophilic molecules—is conceivable as well. Since side reactions could also affect the polymers, this approach would require extensive work to be reliable.

3.9.2 Process Optimization

The conversion of lignocellulose hydrolysate to exopolysaccharide—the grand goal of this work—must be as efficient as possible. In order to achieve the highest possible efficiency, not only the fermentation process itself needs to be improved from a process engineering point of view, but also the strain, the pre-treatment of the substrates used and the purification of the final product.

Pre-Treatment of Lignocellulose Hydrolysate

The fewer interfering substances are in the lignocellulose hydrolysate, the better. If the lignocellulose hydrolysate contained less inhibitors many of the challenges faced in this work would be alleviated. While appearing as very promising, ionic liquids were not employed in this work for their prohibitive costs. But, recently, low-cost ionic liquids have been developed enabling the complete removal of hemicelluloses and removal of over 80 % of the lignin. [203] Using these

ionic liquids, the resulting lignocellulose hydrolysate will cause considerably less issues during fermentation and downstream processing.

Fermentation

The 7 l fed-batch fermentation must be repeated using proper equipment to prevent contamination and be able to gather data on the fermentation of pure *Paenibacillus* 2H7. Further fermentations, probably in the form of 'scout fermentations' at 500 ml scale, could give further insights into the behaviour of *Paenibacillus* 2H7. Parameters for optimization include: temperature, pH value (constant, shifting), dissolved oxygen concentration, impeller design, stirrer speed, co-fermentation and the medium (list not exhaustive). Especially impeller design and stirrer speed strongly influence power consumption and can have beneficial or detrimental effects on microorganism growth and/or exopolysaccharide quality.

Generally, anti-foam should not be used, if not absolutely necessary [204]. In the fermentations carried out for this work, foaming was not an issue and anti-foam was mainly used to avoid foam from entering the sensitive gas analytics. Still, anti-foam was used and found to have unexplained effects on the apparent dissolved oxygen levels in the lignocellulose hydrolysate 500 ml fermentations (see section 3.8.7 on page 100). These hitherto unreported effects of anti-foam addition to the lignocellulose hydrolysate fermentations would need to be investigated thoroughly in the case of anti-foam use. Further unexplored effects on the process as a whole, including downstream processing, cannot be ruled out. The added complexity could be avoided in a large scale fermentation by employing foam centrifuges. At the scales available, the aforementioned cable ties [150] work satisfactorily. An alternative could be using metal foam breakers which are attached to the same shaft as the stirrer.

Product Purification

Purified precipitated exopolysaccharides of block 2 retained the brown colour of the lignocellulose hydrolysate. Therefore, the purification of the product needs to be improved. Three starting points for such enhancements are:

- Adaptation of the cross-flow filtration process, e.g. use of different membranes and buffers.
- Sugaring-out lignin and inhibitors directly in the lignocellulose hydrolysate [205–207].
- Genetic engineering of *Paenibacillus* 2H7 to degrade lignin. Outlined in more detail in section 3.9.3 on page 108.

Cross-flow filtration was carried out using Hydrosart membranes with nominal molar mass cut-offs of 10 kDa, 100 kDa and 0.45 µm and was only used to remove small molecules by diafiltrating with ultra-pure water. A more elaborate process could separate lignin from exopolysaccharide by using different solvent conditions to affect the hydrodynamic radii of both. One such process might look like the following and, of course, depends on the unknown physico-chemical characteristics of lignin and exopolysaccharide:

1. Diafiltration with ultra-pure water to remove all small non-target molecules, exopolysaccharide and lignin of medium size or greater are retained. The maximum membrane pore size is 100 kDa.

2. Diafiltration to exchange the solvent with one with low exopolysaccharide solubility (and—ideally—high lignin solubility) to facilitate the compression of the exopolysaccharide chains, while the more bulky lignin molecules do not change considerably or even relax. The maximum membrane pore size for this step is 10 kDa, so that neither exopolysaccharide nor lignin are removed.

3. Selective removal of compressed exopolysaccharide chains and retention of lignin. The membrane pore size must be carefully chosen, possibly a 100 kDa membrane proves worthwhile. The permeate contains virtually lignin-free exopolysaccharide.

4. Diafiltration to exchange the solvent with one with good exopolysaccharide solubility—most likely ultra-pure water or a slightly buffered solution—to relax the exopolysaccharide chains again. The maximum membrane pore size for this step is 10 kDa, so that no exopolysaccharide is removed.

5. Concentration of the relaxed polymer solution with the same membrane as before to reduce the volume and, in turn, the volume for precipitation.

The corresponding experiments regarding the hydrodynamic radius could be carried out using SEC-MALLS using the same conditions as during the cross-flow filtration.

A relatively recent method, called 'sugaring-out', was published by Wang *et al.* [205] and could be used to selectively remove lignin and inhibitors from lignocellulose hydrolysates. Initially, the process was studied with an acetonitrile-water solution and syringic acid, furfural, *para*-coumaric acid, ferulic acid and hydroxymethylfurfural. Upon the introduction of monomeric D-glucose or D-xylose at concentrations of $15\,g\cdot l^{-1}$ and $25\,g\cdot l^{-1}$, respectively, at 1 °C, two phases formed. Most of the sugar was retained in the bottom phase and acetonitrile concentrations in the top phase were greater than 90 %, the concentrations in the bottom phase were between 16 % to 26 %. The process was already developed further in [206] and mentions applications suitable for lignocellulose hydrolysate detoxification: 'The extraction system [...] can be used [...] to extract inhibitors and by-products, for instance organic acids, plant phenolics, furfural and 5-hydroxymethylfurfural (HMT), from fermentation broths and biomass hydrolysates [..]'. Additionally, a 1:1 (mass) mix of D-glucose and D-xylose showed synergistic effects. The use of a sugared-out bottom phase for fermentation is described as well.

Since some acetonitrile will always remain in the bottom phase, the microorganism used must be able to consume it. Acetonitrile consumption has been shown for several genera [208], including *Arthrobacter* [209, 210], *Bacillus* [211], *Brevibacterium* [212], *Candida* [213], *Chromobacterium* [214], *Geotrichum* [215], *Klebsiella* [216], *Kluyveromyces* [217], *Nocardia* [218, 219], *Pseudomonas* [214, 220, 221] and *Rhodococcus* [222–224]. Therefore, chances are good that *Paenibacillus* 2H7 can utilize acetonitrile and using the novel method of sugaring-out for lignocellulose hydrolysate detoxification might pose a feasible alternative to the other ideas presented here.

3.9.3 Strain Engineering

Molecular Characterization

The strain used, *Paenibacillus* 2H7, has not been characterized on a molecular level yet. Using RAST [225–227], access to the whole genome sequence would allow to quickly find known genes and clusters thought to be responsible for exopolysaccharide production and detoxification of inhibitors. Investing more time and effort, the complete genetic basis including regulation and export of the exopolysaccharide production of *Paenibacillus* 2H7 and possible quorum sensing

mechanisms could be elucidated. If necessary, probably to expand the substrate spectrum, additional tolerance genes might be introduced. On the other hand, known production strains are more genetically accessible and are easier regulation-wise. Therefore, it might be a viable alternative to confer the exopolysaccharide production capabilities and inhibitor tolerance mechanisms to a well-described host instead.

Lignin Degradation

Residual lignin degraded the quality of the final product and could not be removed completely by the approach used. In addition to the two methods suggested above (see section 3.9.2 on page 106), the degradation of lignin may be assumed by *Paenibacillus* 2H7 through genetic engineering. By using laccases and peroxidases, the strain could be enabled to degrade lignin [228]. Ideally, all the lignin would be degraded below a certain maximum molar mass, such that high-purity exopolysaccharide could be produced from the fermentation broth by one centrifugation, one cross-flow filtration/diafiltration and one precipitation step.

3.9.4 Product Characterization

In order for the product to be used, it must be studied in more detail. Using relatively pure exopolysaccharide, re-solubilization studies should be performed to determine the effect different purification steps have on the solubility of the polymer purification, e.g. precipitation, drying and milling. Highly pure and easily soluble exopolysaccharide should be used for more detailed analyses on the monomeric composition and possible modifications such as acetylation or pyruvylation. The ketose content and different ketoses must also be studied as only around 40 % of the mass used for monomer analysis were also found as monomers.

While the monomeric composition allows to draw conclusions on the exopolysaccharide, the repeating unit and types of linkages need to be elucidated for a deeper understanding of the molecular basis of its properties. Accurate molar mass determinations can be facilitated by the determination of the dn/dc value followed by SEC-MALLS of known concentrations of the exopolysaccharide. An alternative for too large polymers is field flow fractionation. Using SEC-MALLS or FFF-MALLS, super-structures could be discovered as well.

For possible applications, an extensive rheological characterization of the product would be beneficial. Since no such experiments have been carried out, studies will have to start from scratch: dynamic viscosities of concentration series of the polymer in ultra-pure water and 1 % KCl, shear-stability and thixotropic behaviour, temperature and pH stability. If gels are formed at high concentrations or with certain ions, then the gels need to be characterized as well. If the properties suggest use in a certain field or for a certain application, further studies specific to that field or application would follow.

Chapter 4

Fermentative Production of Scleroglucan and Schizophyllan

Schizophyllan and scleroglucan are thought to be identical as outlined in the introduction (see section 1.2.5 on page 15). On the other hand, a closer look at the relevant literature revealed that the basis for claiming chemical identity usually are analyses of carefully selected fractions of the polymers [122, 124, 125, 128, 229–232]. Therefore, it was hypothesized that schizophyllan and scleroglucan are not identical. In order to gather evidence supporting this hypothesis the following plan was adopted:

- Fermentatively produce both polymers using the respective fungi in one parallel fermentation.
- Analyse the fungal exopolysaccharides (precipitates of supernatants) with respect to parameters used to claim identity: molar mass distribution via SEC-MALLS, dynamic viscosity and thixotropy, solubility, frequency of β-1,6-linked D-glucose.
- If present, resolve differences in the polymer over the course of the fermentation.
- If present, resolve differences between the polymers at the same fermentation duration or different fermentation durations.

The results were disheartening and are presented together with the discussion in section 4.1. The discussion focuses on the methods employed and how the experiments could have been conducted better. The author sees the publication and discussion of 'negative' results as an integral part of the scientific method, one that presently is woefully neglected by too many journals and tossed aside by scientists as a consequence of the dominant publish or perish 'culture'. Hopefully, the following track of failures will aid others in getting things right at first go.

4.1 Parallel Fermentation of *S. rolfsii* and *S. commune*

4.1.1 Controls & Deviations

As stated under section 2.5.5 on page 45, one sample type was 'large with rheometry'. No rheometric measurements were conducted with these samples.

Due to issues with the foam sensor of fermenter 6 approximately 150 ml of anti-foam was pumped into the fermenter overnight triggering the overflow protocol of the fermenter. All

samples of fermenter 6 were heavily contaminated by anti-foam and could not be considered for comparisons. Also, the pO_2 probe of fermenter 6 was overgrown resulting in too low oxygen readings.

The large samples at 60 h and 84 h were skipped and instead were taken at 66 h and 90 h, respectively. The 108 h and 132 h samples were shifted to 104 h and 128 h, respectively.

The major deviation encountered during the fungal fermentations is the poor solubility of the products. This is discussed in more detail in section 4.1.4 on page 113. Since all fermentations were carried out only once, no statistical analyses were conducted.

4.1.2 Cell Dry Masses at the End of the Fermentation

Cell dry masses increased with fermentation time for both fungi as can be seen in figure 4.1 on the facing page. *S. commune* showed a slight dent at 96 h which is attributed to the high amount of anti-foam in this fermenter.

4.1.3 Exopolysaccharide Courses

The exopolysaccharide concentrations at the end of the fermentations were determined by precipitating the remaining fermentation broth and are shown in figure 4.2 on the facing page. The highest concentrations were 3.25 $g \cdot l^{-1}$ after 96 h and 2.21 $g \cdot l^{-1}$ after 120 h for *S. rolfsii* and *S. commune*, respectively. The concentrations increased over time, except for the 144 h fermentation of *S. commune*. The reason for the low performance of this fermenter is unclear. Compared to fermentations of *Sclerotium rolfsii* ATCC 201126 and *Sclerotium glucanicum* NRRL 3006 reported by Fariña *et al.* [233] and Taurhesia and McNeil [234], respectively, the overall exopolysaccharide concentrations achieved were low: 20.6 $g \cdot l^{-1}$ after 72 h and 8 $g \cdot l^{-1}$ after 96 h, respectively, vs. 1.9 $g \cdot l^{-1}$ after 72 h and 3.4 $g \cdot l^{-1}$ after 96 h. While the real reason(s) must remain speculative, it is unlikely that these striking differences were caused by random events. Possible reasons include mixing issues, slow adaptation to the fermentation conditions, too high or low nutrient levels including O_2, degradation, fungal growth on the fermenter walls, the stirrer shaft and foam-breakers which slid down and general hardware-related issues such as miscalibrated instruments. The last explanation is deemed unlikely as neither operators prior nor operators after the fungal fermentations noticed any such issues.

The courses of the exopolysaccharide concentrations over the whole fermentations are depicted in figure 4.3 on page 112. The sharp drop after the 24 h sample was caused by using another sample purification method: the raw broth was first diluted 1:10 with ultra-pure water. Precipitate purity was never analysed[1], but it is assumed that most of the precipitate of the undiluted samples did not constitute exopolysaccharide. High residual exopolysaccharide in the biomass seems unlikely, as the biomass of fermenter 8 was washed and the precipitate of the supernatant amounted to only 4.8 % of the total precipitate mass of that fermenter (data not shown). One possible explanation for the apparent drop after starting 1:10 dilutions is that all components get diluted 1:10 and that might be enough to keep these components in solution at approximately 65 % isopropanol.

On the other hand, the concentrations obtained via the last samples were generally higher than the concentrations obtained via the precipitation of the whole fermentation broth at the end of the fermentation. Given the aforementioned drop after applying a 1:10 dilution, the only 1:3

[1] Since only some milliliters of the fermentation broth were sampled, the absolute precipitate masses were too low for most analyses: at most 20.5 mg, arithmetic mean: 2.3 mg, median: 1.7 mg.

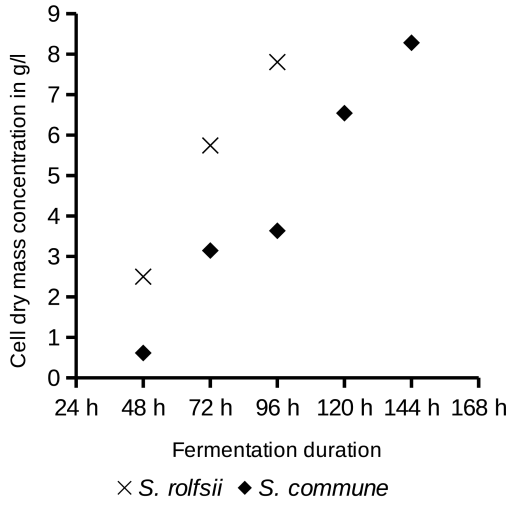


Figure 4.1: Cell dry masses at the end of the fermentations of *S. rolfsii* and *S. commune*. The dry masses increase over time. The 96 h value of *S. commune* was lower than expected which is attributed to the high amount of anti-foam in this fermenter. Each point represents a single measurement.

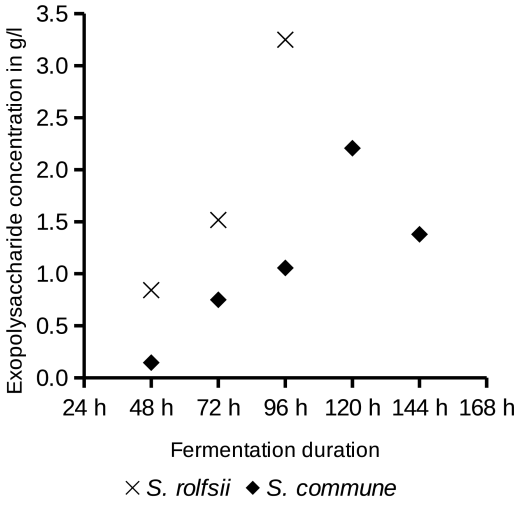


Figure 4.2: Exopolysaccharide concentrations at the end of the fermentations of *S. rolfsii* and *S. commune*. The exopolysaccharide concentrations increase with fermentation time, the only exception being the 144 h fermentation of *S. commune*. The reason for the low exopolysaccharide production in the 144 h process is unknown. The final concentrations are in good agreement with the concentrations obtained from the final samples (see table A.13 on page 165). Each point represents a single measurement.

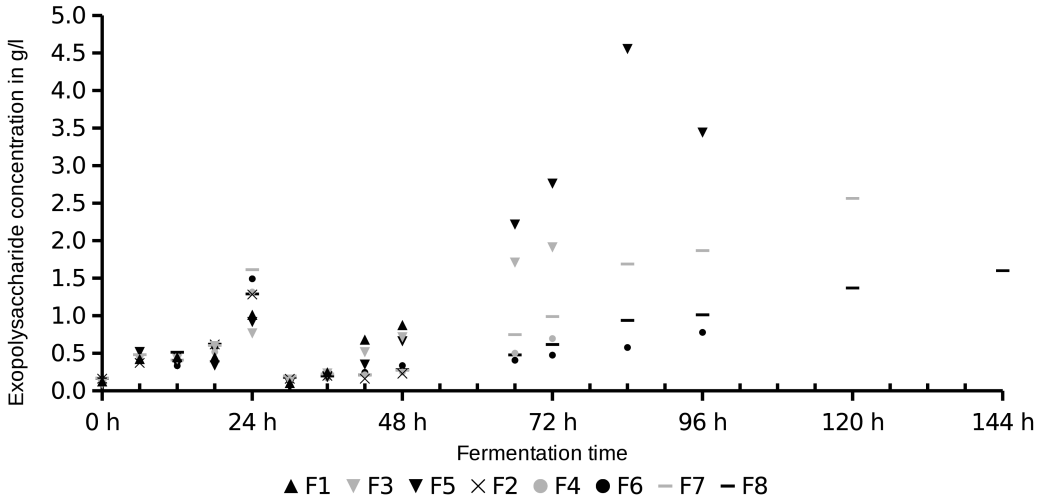


Figure 4.3: Exopolysaccharide concentration courses of the fermentations of *S. rolfsii* and *S. commune*. Exopolysaccharide concentrations increase up to 24 h and drop to approximately one tenth of the previous value. This is most likely an artifact caused by using a different sample purification protocol: all samples up to and including the 24 h sample were used directly, while all samples thereafter were 1:10 diluted with ultra-pure water. It is assumed that a considerable part of the undiluted samples' precipitates are no exopolysaccharide. The final concentrations are in good agreement with the concentrations obtained from precipitating the harvested raw broth (see table A.13 on page 165). Fermenters 1, 3 and 5 were inoculated with *S. rolfsii*, fermenters 2, 4, 6, 7 and 8 with *S. commune*. Each point represents a single measurement. Abbreviations: Fn: fermenter no. n with $n \in \mathbb{N} \wedge 0 < n < 9$.

diluted fermentation broth should have given considerably higher concentrations than the last samples. An adequate explanation is missing.

These shortcomings combined point to the necessity to conduct pre-tests to find reliable protocols for measuring the exopolysaccharide concentration of samples and at the end of the fermentation. Work on one such method spawned a publication [235] and is outlined in detail in section 4.1.9 on page 118. For future fermentations, such analytical methods should be used in any case.

Nonetheless, the final samples are in good agreement with concentrations obtained through precipitation of the complete fermentation broth at the end of the fermentation (see table A.13 on page 165).

4.1.4 Precipitate Solubility

The solubility of the precipitates was of utmost importance for all further analyses, because all of them required the exopolysaccharides to be well-dissolved in the respective solvents. Over the course of the development of the analytical methods after the fermentations had been conducted, it became apparent that none of the precipitates would dissolve completely. Some samples gave a clearer solution, others even gave large undissolving gel clumps.

In order to facilitate better dissolution 20 ml of the solution was prepared in a 50 ml tube and mixed with 50 glass pearls (diameter: 4.0 mm). The tube was shaken at 250 min^{-1} and 60 °C for one night to up to four days. The idea was to use the principle behind ball mills: the exopolysaccharide flakes start to dissolve immediately forming a swollen outer layer which shields the inner undissolved exopolysaccharide from the solvent. By shearing off that outer layer the solvent gains access to the still undissolved exopolysaccharide on the inside. Visual inspection revealed that the dissolution was improved by this method, but still far from perfect. The increasing viscosity is most likely to be responsible for slowing down the glass pearls to ineffective speeds.

In the end, any dissolution enhancing method tackles the wrong problem: the exopolysaccharide should not be poorly dissolvable to start with. Therefore, efforts should be directed towards the production of easily dissolvable exopolysaccharides instead. That would mean to conduct pre-tests to answer the following questions, among others:

- Are the precipitation parameters—precipitant, volume ratio, mixing unit—optimal?
- Do other substances co-precipitate with the exopolysaccharide? If so, which and how could they be separated prior to precipitation? Dialysis? Cross-flow filtration? Enzymatic treatments?
- Do co-precipitates influence the redissolution?
- Do longer polymer chains precipitate before shorter ones? If so, would a fractionated precipitation process be sensible and feasible? Could one obtain first molar mass information that way?
- How does the polymer concentration influence the precipitation?
- What does a good precipitate appear like? Small and ‘dry’ flakes or long and ‘wet’ threads?
- Does a redissolution of the gathered precipitate in ultra-pure water followed by another precipitation increase the purity? What about the overall yield?

- How does the drying process influence redissolution behaviour? Would freeze-drying of highly concentrated redissolved solutions yield better redissolvable polymer?
- How can the polymer be redissolved in a homogeneous manner? How can the homogeneity be assessed?

The analyses in the upcoming sections were conducted without answering these questions first, because the product had already been produced and *only* needed to be analysed. Therefore, the results are to be taken with great care.

4.1.5 Dynamic Viscosity and Thixotropy

The total amount of exopolysaccharide from the 48 h fermenter 2 (*S. commune*) was insufficient for rheological characterization. Therefore, data on the exopolysaccharide of that fermenter are missing. Dynamic viscosity data are given in figure 4.4 on the facing page, thixotropy data in figure 4.5 on page 116.

Dynamic Viscosity The general trend of measurements at $1\,s^{-1}$ does not deviate from $1\,000\,s^{-1}$. There seemed to be no correlation between fermentation time and dynamic viscosity for *S. rolfsii*, but—apart from the contaminated fermenter 6—the viscosity of schizophyllan seems to increase until it reaches a plateau after 120 h at around $2.5\,Pa\cdot s$.

Thixotropy Due to the low time resolution of only 0.5 s the values for 10 % and 25 % viscosity regain are all equal. After 96 h, scleroglucan appears to regain viscosity in less than a tenth of the time it took the 48 h and 72 h products. There does not seem to be a strong correlation between fermentation time and viscosity regain for schizophyllan[2]: 90 % of the dynamic viscosity was regained after 2.5 s to 4.3 s.

4.1.6 Molar Mass Determination

The analytical method was first tested with a reference scleroglucan (Actigum Cs 11) and it was found that in aqueous systems the samples would elute in the exclusion volume meaning that the sample molecules were too big to enter the cavities of the chromatographic media. This is in line with published research and attributed to the stable triple helices that native β-1,3-glucans form in aqueous solution [119, 122–126]. Tests in DMSO with a different column showed reproducible symmetrical peaks. For a comparison, see figure 4.6 on page 117. The molar mass distributions of the exopolysaccharides could not be analysed due to very low to no solubility of the precipitates in DMSO. Therefore, no data on the molar mass distributions is available.

4.1.7 Periodate Test

The periodate test was used to assess whether the polymers contained β-1,6-linked D-glucose units and whether the content differs over time or between polymers. The molar ratios of periodate consumed to formic acid formed of all fermenters except two and six are shown in figure 4.7 on page 118. Under the assumptions that all products were of the same purity and that the polymer concentrations used were equal, there are no considerable differences between the different

[2]The exopolysaccharide of fermenter 6 was most likely highly contaminated with anti-foam. Therefore, rheologic properties might have been affected and the data was not be compared to the uncontaminated fermenters.

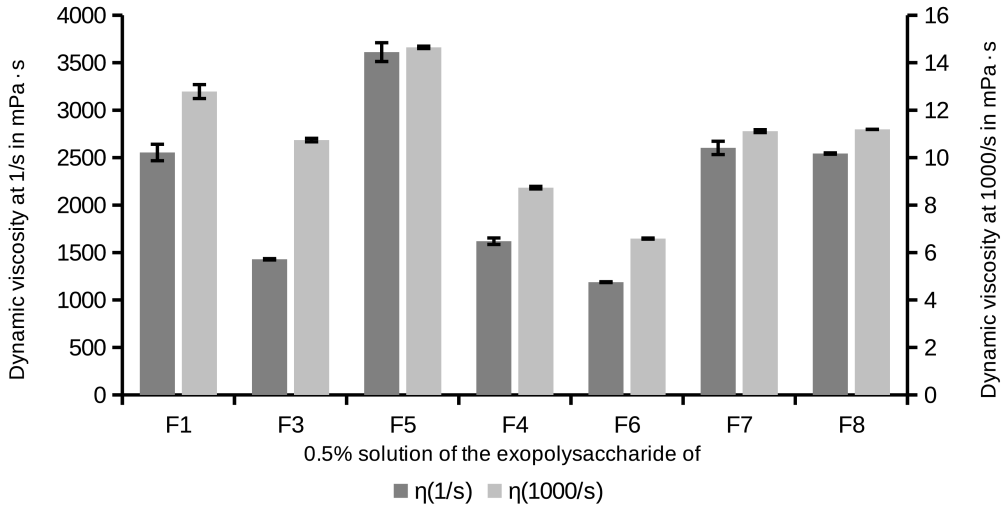


Figure 4.4: Dynamic viscosities at 1 s^{-1} and 1 000 s^{-1} of scleroglucan and schizophyllan harvested at different times. Fermenters 1, 3 and 5 were inoculated with *S. rolfsii* for 48 h, 72 h and 96 h, respectively, fermenters 4, 6, 7 and 8 with *S. commune* for 72 h, 96 h, 120 h and 144 h, respectively. Fermenter 2 is not listed as only insufficient amounts of exopolysaccharide were produced. The exopolysaccharide of fermenter 6 was most likely highly contaminated with anti-foam which resulted in a relatively low viscosity. Bars depict the arithmetic mean of three measurements except for fermenter 7. For fermenter 7, the sample size was two. Error bars depict the standard deviation from the arithmetic mean. Abbreviations: Fn: fermenter no. n with $n \in \mathbb{N} \wedge 0 < n < 9$; η: dynamic viscosity.

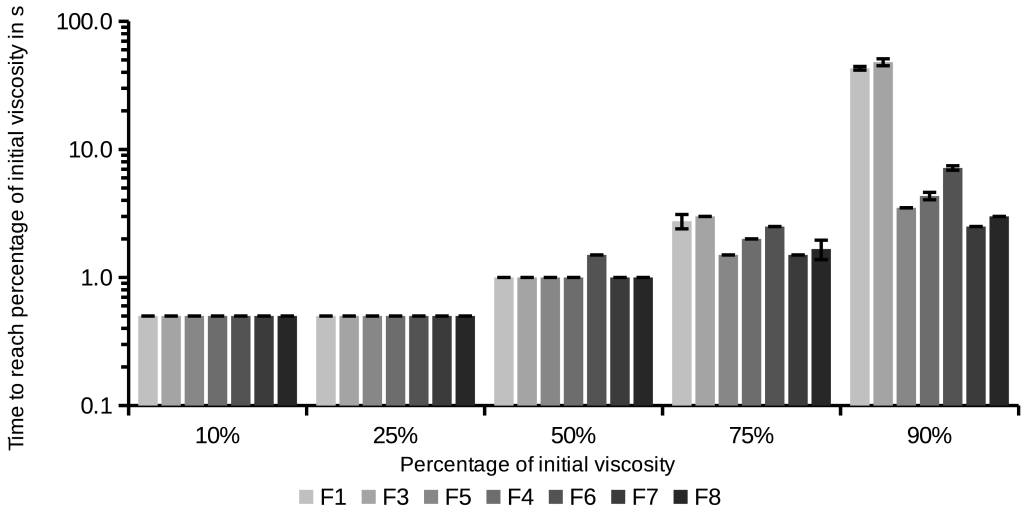
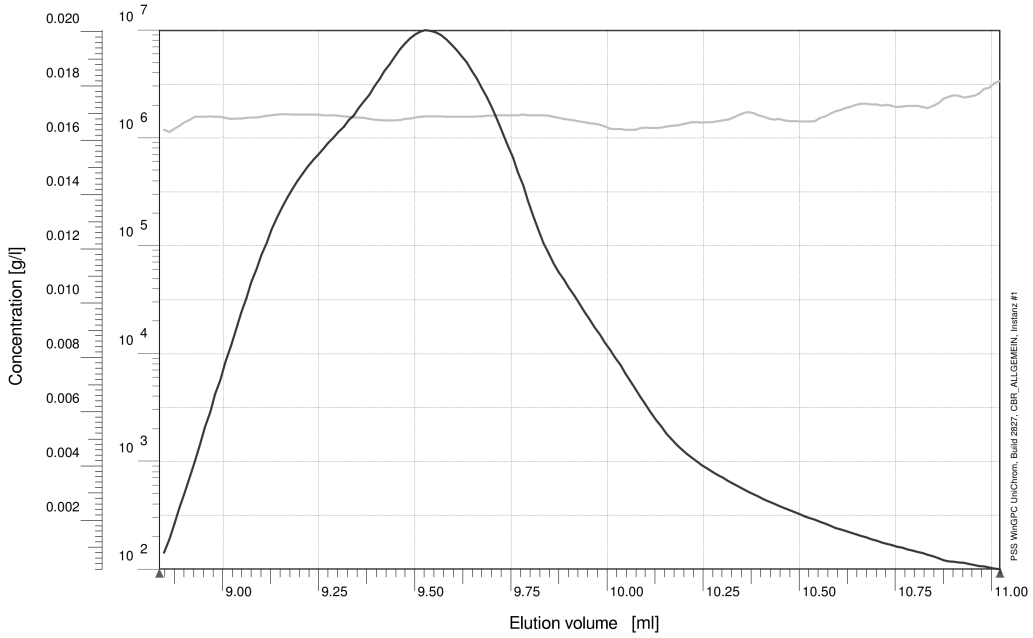
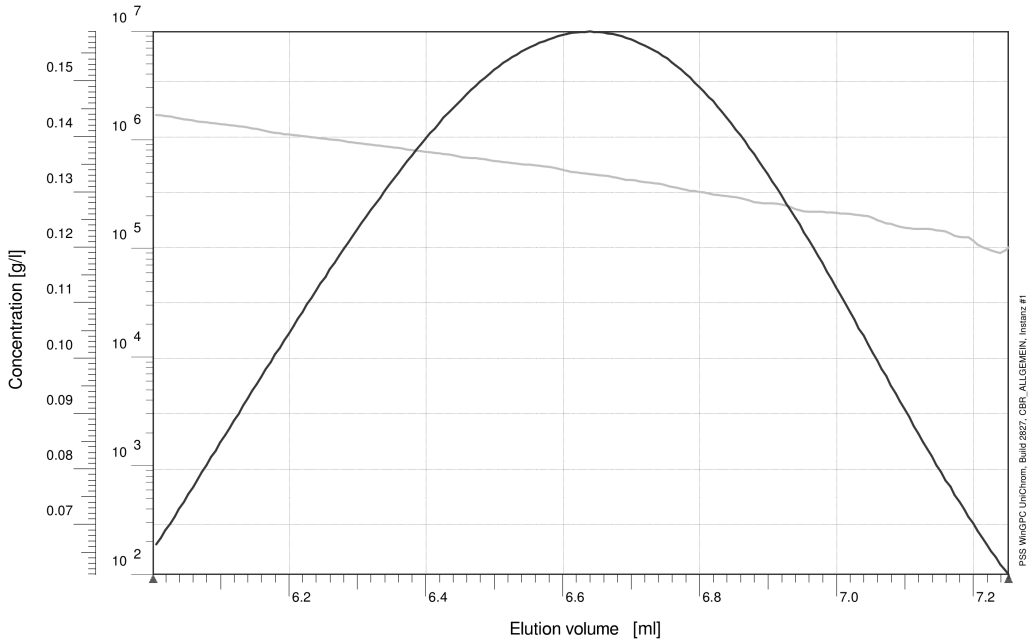


Figure 4.5: Thixotropic behaviour of fungal fermentation polymers. Time to regain the initial dynamic viscosity after shearing at $100\,s^{-1}$ for 2 min. Fermenters 1, 3 and 5 were inoculated with *S. rolfsii* for 48 h, 72 h and 96 h, respectively, fermenters 4, 6, 7 and 8 with *S. commune* for 72 h, 96 h, 120 h and 144 h, respectively. Fermenter 2 is not listed as only insufficient amounts of exopolysaccharide were produced. The exopolysaccharide of fermenter 6 was most likely highly contaminated with anti-foam which resulted in a relatively low viscosity and might have affected viscosity regain as well. The time resolution was 0.5 s and therefore, the 10 % and 25 % times are all the same. The x-axis uses logarithmic scale. Bars depict the arithmetic mean of three measurements except for fermenter 1. For fermenter 1, the sample size was two. Error bars depict the standard deviation from the arithmetic mean. Abbreviations: F*n*: fermenter no. *n* with $n \in \mathbb{N} \wedge 0 < n < 9$.



(a) Actigum Cs 11 in aqueous solution separated on Suprema columns.



(b) Actigum Cs 11 in DMSO separated on a TSKgel column.

Figure 4.6: Reference scleroglucan separations in water and DMSO. The reference scleroglucan Actigum Cs 11 was dissolved in two different solvents and separated using two different columns. In (a), the scleroglucan was dissolved in 0.1 M $LiNO_3$ and separated on three Suprema columns. In (b), the scleroglucan was dissolved in DMSO and separated on one TSKgel column. The steep increase of the aqueous sample is interpreted as being in the exclusion volume. Dark curves: concentration (left-most y-axis); light curves: molar mass in $g \cdot mol^{-1}$ (second y-axis from left).

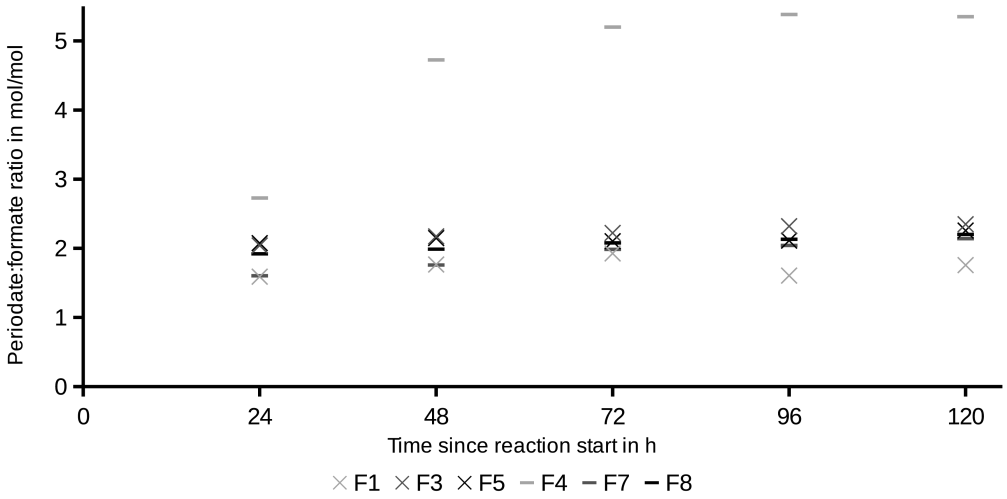


Figure 4.7: Periodate test: ratios of periodate consumption to formic acid formation. Molar ratios of the periodate consumed and the formic acid formed after defined reaction times of the exopolysaccharides and periodate. Fermenters 1, 3 and 5 were inoculated with *S. rolfsii* and fermenters 4, 7 and 8 with *S. commune*. Fermenter 2 is not listed as only insufficient amounts of exopolysaccharide were produced, fermenter 6 is missing, because of the excessive amounts of anti-foam which were pumped into the fermenter and subsequently contaminated the precipitate. Abbreviations: Fn: fermenter no. n with $n \in \mathbb{N} \wedge 0 < n < 9$.

polymers and they all appear to gather around the theoretical value of 2.0 [142]. The only exception is the polymer of fermenter 4, a schizophyllan, which consumed all the available periodate and produced the least amount of formic acid. The reason for that behaviour is not known and the author welcomes any help for finding an adequate explanation.

Similarly, periodate consumption (table A.14 on page 166) or formic acid formation (table A.15 on page 166) alone did not give any clear indication that the scleroglucan and schizophyllan are different or that the polymers harvested at different times are different.

4.1.8 Metabolite Analysis

Trials to quantify L-malic acid, succinic acid, citric acid, fumaric acid, glyoxalic acid, itaconic acid and oxalic acid on a Rezex ROA-Organic Acid H+ (8 %) column were unsuccessful as several components eluted at the same time: citric acid and succinic acid; succinic acid (second peak), glyoxalic acid and D-glucose; itaconic acid and fumaric acid. L-malic acid and oxalic acid were the only two metabolites which did not share elution time with other tested substances. No other method was available and the results were seen as secondary, which is why the metabolite analyses were not pursued any further.

4.1.9 Aniline Blue Assay for the Quantitative Determination of β-1,3-β-1,6-Glucans

As a consequence of the difficulties encountered during the determination of the exopolysaccharide concentration from fermentation broths (see section 4.1.3 on page 110), a precipitation-free method was needed. This subsection deals with what eventually led to the article ‘Quantitative

assay of β-(1,3)–β-(1,6)–glucans from fermentation broth using aniline blue' [235] and serves to give an outline of the assay's background, its development, and use. Finally, the supplemental material contains a complete protocol for the quantitative assay including a qualitative variant (see appendix A.3.3 on page 167). Generally, data in this section are not shown; the reader is referred to the publication for details.

Fluorescence Properties of Aniline Blue

The search for a specific reaction of β-1,3-β-1,6-glucans led to several different assays [236–240], but most covered only β-1,3-glucans, not β-1,3-β-1,6-glucans. Nitschke *et al.* [238] reported an assay for β-1,3-β-1,6-glucans based on Congo red. Since the assays of Ko and Lin [236] and Shedletzky *et al.* [237] used aniline blue, aniline blue was given precedence over Congo red.

As early as 1949 Arens [241] described that aniline blue showed fluorescence in conjunction with callose. Callose is a β-1,3-glucan present in plants, but Faulkner *et al.* [242] found that the specificity of aniline blue was not limited to callose. Laminarin and pachyman, also β-1,3-glucans, and cellulose, a β-1,4-glucan, also exhibited fluorescence.

Smith and McCully [243] were the first to take a closer look at the fluorescence properties of aniline blue and substantiated the findings of Faulkner *et al.* [242] regarding the specificity. They also found that commercial aniline blue preparations contained considerably varying fluorophore contents. Finally, Evans and Hoyne [244] published the complete structure and further fluorescence properties of the fluorophore. The fluorophore was later named 'Sirofluor' and its interactions with different polymers were reported by Evans *et al.* [245]. Scleroglucan and schizophyllan were among the polymers tested and both exhibited strong fluorescence with Sirofluor.

The high-throughput assay for β-1,3-glucan synthases by Shedletzky *et al.* [237] and the assay for β-1,3-glucan in foodstuffs by Ko and Lin [236] were used as the basis for further experiments. Therefore, aniline blue and not pure Sirofluor was used.

Optimization of the Aniline Blue Fluorescence

While the first test was only a proof of concept, the fluorescence intensity was already dependent on the scleroglucan concentration. Subsequent optimization steps included finding optima for the aniline blue concentration, the excitation and emission wavelengths, the buffer pH and additives, incubation times, and upper and lower bounds for the calibration curve.

The first test run with actual samples from fungal fermentations revealed a major flaw in the assay: the sample-to-reagent ratio was 2:1 allowing the sample matrix to have a strong influence on the assay conditions. While the rather pure reference scleroglucan Actigum Cs 11 could be detected reliably, readings from actual samples gave only unusable results.

Final Aniline Blue Assay

After further optimization work, the final assay [235] had the following properties:

- Calibration range[3]: $30\,\mathrm{mg \cdot l^{-1}}$ to $6\,\mathrm{g \cdot l^{-1}}$ with $R^2 > 99.8\,\%$
- Sample-to-reagent ratio: 1:9
- Excitation wavelength: 405 nm
- Emission wavelength: 495 nm

[3] The calibration curve was constructed using the scleroglucan Actigum CS 11 from Cargill.

- Robustness[4]
  - D-Glucose: $50\,g \cdot l^{-1}$
  - Oxalic acid: $22.5\,g \cdot l^{-1}$
  - Potassium chloride: $13.3\,g \cdot l^{-1}$
  - Bovine serum albumine: $0.667\,g \cdot l^{-1}$
- Reagent composition: 183 mM glycine, 229 mM NaOH, 130 mM HCl, $618\,mg \cdot l^{-1}$ aniline blue in ultra-pure water. Final pH ≈ 9.9.

The calibration range should be highlighted here as it is superior by at least one order of magnitude to the ones reported by Nitschke *et al.* [238] and Ko and Lin [236].

Application of the Aniline Blue Assay for Fermentation Broth Samples

The last and most important step for the future use of the aniline blue assay was the quantification of samples drawn from cultures of *S. rolfsii* and *S. commune*. The fungi were cultivated for two days to five days and the concentrations calculated from precipitations compared to concentrations from the aniline blue assay conducted at different stages of the purification process: raw broth, blended broth, neutralized broth, diluted broth, heat-inactivated broth, and supernatant after the final centrifugation before the precipitation.

For both fungi, suitable correction factors[5] were found and allowed to achieve values with the aniline blue assay mimicking precipitation results. For *S. rolfsii*, the correction factor was 2.46 using the aniline blue concentration determined with the supernatant after the final centrifugation. For *S. commune*, the correction factor was 3.83 using the aniline blue concentration determined with the blended broth.

4.2 Outlook

With the scarce data available, only fungal growth and changing exopolysaccharide concentrations could be reliably shown. The questions which led to the fermentations and the subsequent analyses could not be answered. As the product showed poor solubility, it was resilient against analysis. Therefore, the fermentations would have to be repeated, ideally with a sample size of at least three, better five, for each fungus and fermentation time.

But, in order for this to be successful, the analytical methods will need to be established and, more importantly, the exopolysaccharides purified in a way which facilitates excellent redissolution in water and DMSO. Some pointers into the future direction of these works can be derived directly from section 4.1.4 on page 113:

- Precipitation optimization including precipitant, volume ratio and mixing unit
- Steps to reduce impurities prior to precipitation such as dialysis or cross-flow filtration
- Fractionated precipitation trials

[4] Concentrations denote the maximum concentration of the respective component in the sample without a statistically significant influence on the fluorescence intensity. Results were seen as statistically significant, when $p < 0.05$. p was calculated from the two-tailed Student's t-test for independent samples of references and samples.

[5] The correction factor is calculated by dividing the concentration from the precipitation by the concentration from the aniline blue assay.

- Drying process trials including freeze-drying of precipitate re-dissolved in water

On the other hand, the results of the aniline blue assay point towards a way to reliably quantify β-1,3-β-1,6-glucans possibly superior to precipitation altogether.

Removing the major roadblocks would allow to run fermentations and produce exopolysaccharides which give clear and reproducible results for the evaluation of the properties of scleroglucan and schizophyllan.

Appendix A

Supplemental Material

A.1 Materials and Methods

A.1.1 Computational Methods

Listing A.1: 16S_sequence.py: Python script to read in 16S sequencing results from AB1 files, quality-trim the sequences and align forward and reverse sequences with clustalw to generate aligned sequences. The final 16S sequence was generated manually.

```
#!/usr/bin/env python
# -*- coding: utf-8 -*-
#
# 16S Sequence Analysis Script
#
# Author: Steven Koenig <steven.koenig.wzs@kreuvf.de>
# Licence: GNU General Public Licence, Version 3
#
# Requirements: [[TO DO]]
#
# Description:
# This script scans any directory given for *.ab1 files. All *.ab1 files found
# are expected to contain sequences of PCR products of the same region of
# interest in forward and reverse direction with enough overlap to assemble all
# into one long stretch of DNA.
#
# In the first step all *.ab1 files are converted to *.fastq. Then, the *.fastq
# files are quality-trimmed and the resulting sequences are converted to FASTA
# format. Reverse sequences are converted to their reverse complement and all
# the sequences are passed to ClustalW for aligning. The raw FASTQ files and the
# quality-trimmed FASTQ files are deleted after script execution.
#
# You may also give --no-delete to keep all generated intermediate files.
#
# This script expects your files to be organized like that:
# Organism_0000
# ├── Org0000_fwd_0.ab1
# ├── Org0000_fwd_1.ab1
# ├── Org0000_fwd_2.ab1
# ├── Org0000_fwd_3.ab1
# ├── Org0000_rev_0.ab1
# ├── Org0000_rev_1.ab1
# └── Org0000_rev_2.ab1
# Organism_0001
# ├── Org0001_rev_0.ab1
# ├── Org0001_rev_1.ab1
# └── Org0001_rev_2.ab1
# Organism_0002
```

```
# ├── Org0002_fwd_0.ab1
# ├── Org0002_rev_0.ab1
# └── Org0002_rev_1.ab1
#
# The long term aim is to re-implement DynamicTrim.pl in Python to kick out the
# SolexaQA dependency.

import os
import re
from Bio import SeqIO
from subprocess import call

# Check input
# No argument: exit with help
# One or more arguments: verify that every argument is a directory
# For every directory: verify that it contains at least one *.ab1
# One and only one *.ab1 file: warn about missing alignment, stop after trimming
# Only rev or only fwd *.ab1: warn

for file in os.listdir("."):
    if file.endswith(".ab1"):
        SeqIO.convert(file, "abi", re.findall('^(.*)(\.ab1)$', file)[0][0]+".fastq", "fastq")

records = list()

for file in os.listdir("."):
    if file.endswith(".fastq"):
        call(["DynamicTrim.pl", file, "-probcutoff", "0.05", "-sanger"])
        os.remove(file)
        os.rename(file + ".trimmed", file)
        os.remove(file + ".trimmed_segments")
        handle = open(file, "r")
        if re.findall('rev', file):
            record = SeqIO.read(handle, "fastq")
            rc_record = record.reverse_complement(id=record.id+"_rc")
            records.append(rc_record)
        else:
            records.append(SeqIO.read(handle, "fastq"))
        handle.close()
        os.remove(file)

handle = open("processed_sequences.fasta", "w")
SeqIO.write(records, handle, "fasta")
handle.close()

handle = open("processed_sequences.fasta", "r")
call(["clustalw", "-infile=processed_sequences.fasta", "-align", "-output=FASTA", "-outfile=alignment.fasta"])
handle.close()
os.remove(re.findall('^(.*)(\.fasta)$', "processed_sequences.fasta")[0][0]+".dnd")

print(call(["fastagrep.pl", "-w", "0", "''", "alignment.fasta"]))
```

Listing A.2: select-paired.patch: Enable processing of FASTQ files from ZIEL. Patch in unified diff format to be applied to select_paired.pl. The unpatched version did not process FASTQ files from ZIEL. A short investigation revealed that the regular expressions for finding entries did not match with the entry lines in the FASTQ files. Additionally, this patch adds some debug output, because the order of arguments was confusing at first.

```
--- select_paired.pl    2013-02-11 11:17:50.949078659 +0100
+++ select_paired.pl_new    2013-02-11 12:35:53.545109421 +0100
@@ -1,4 +1,12 @@
 #!/usr/bin/perl -X
+# Adjusted to work with our files
```

```
+# Sample for ID:
+# >M00202:10:000000000-A2EE7:1:1101:14828:1747 1:N:0:2
+# Warning: whole sequence needs to be on exactly one line!
+# Steven Koenig, Technische Universität München, Germany.
+# steven.koenig.wzs@kreuvf.de
+#
+# Based on:
 # Konrad Paszkiewicz, University of Exeter UK.
 # k.h.paszkiewicz@exeter.ac.uk

@@ -28,6 +36,12 @@
 my $outfile2 = shift or die $usage;

 our $outfile3 = shift or die $usage;
+print "infile1   : $infile1
+outfile1  : $outfile1
+infile2   : $infile2
+outfile2  : $outfile2
+singletons: $outfile3
 open(OUTFILE2, ">$outfile2") or die "Cannot open $outfile2\n";
 open(OUTFILE3, ">$outfile3") or die "Cannot open $outfile3\n";

-my $name1;
-my $name2;
+my $name1_1;
+my $name1_2;
+my $name2_1;
+my $name2_2;

 while(<FILE1>){
-        if(/^(\>.*)\/\d$/){
+        if(/^(\>[^ :]+:[^ :]+:[^ :]+:[^ :]+:\d+:\d+) \d(:\w:\d:\d)$/){
                 $hash1{$1}=1;
-                $name1=$1;
+                $name1_1=$1;
+                $name1_2=$2;
         }else{
-                $hash1{$name1}=$_;
+                $hash1{$name1_1}=$_;
         }
 }
 close(FILE1);

 while(<FILE2>){
-        if(/^(\>.*)\/\d$/){
+        if(/^(\>[^ :]+:[^ :]+:[^ :]+:[^ :]+:\d+:\d+) \d(:\w:\d:\d)$/){
                 $hash2{$1}=1;
-                $name2=$1;
+                $name2_1=$1;
+                $name2_2=$2;
         }else{
-                $hash2{$name2} = $_;
+                $hash2{$name2_1} = $_;
         }
 }
 close(FILE2);

-for $name1 ( keys %hash1 ) {
-        if(exists $hash2{$name1}){
-                print OUTFILE1 "$name1/1\n$hash1{$name1}";
-                print OUTFILE2 "$name1/2\n$hash2{$name1}";
+for $name1_1 ( keys %hash1 ) {
+        if(exists $hash2{$name1_1}){
+                print OUTFILE1 "$name1_1 1$name1_2\n$hash1{$name1_1}";
+                print OUTFILE2 "$name1_1 2$name1_2\n$hash2{$name1_1}";
         }else{
-                print OUTFILE3 "$name1/1\n$hash1{$name1}";
```

```
+                print OUTFILE3 "$name1_1 1$name1_2\n$hash1{$name1_1}";
         }
 }

-for $name2 (keys %hash2){
-        if(!exists $hash1{$name2}){
-                print OUTFILE3 "$name2/2\n$hash2{$name2}";
+for $name2_1 (keys %hash2){
+        if(!exists $hash1{$name2_1}){
+                print OUTFILE3 "$name2_1 2$name2_2\n$hash2{$name2_1}";
         }
 }
```

Listing A.3: create_contig_table.sh: Extract contig information from assemblies. bash script to generate a file which contains node, length and coverage (as given by velvet) from an assembly.

```
#!/bin/sh

# Velvet contigs information table
# Reads in contigs.fa produced by velvetg and outputs unsorted table with contig information
# Outputs one file called contigs.table in the same directory as the contigs.fa
#
# Author: Steven Koenig
# Created: 2013-02-06

E_MISSINGFILE=2
FILENAME="contigs.table"

if [ $# -eq 0 ]
then
    echo 'No filename given.'
    echo 'Usage: ./create_contig_table.sh filename'
    exit $E_MISSINGFILE
fi

FILEPATH=`echo $1 | rev | sed -r 's|^[^/]*/|/|' | rev`

if [ "$FILEPATH" = "$1" ]
then
    FILEPATH=""
fi

echo 'Node  Length  Coverage' > $FILEPATH$FILENAME
grep -E 'length_[0-9]+' $1 | sed -r 's/^>NODE_([0-9]+)_length_([0-9]+)_cov_([0-9]+\.[0-9]+)$/\1\t\2\t\3/' >> $FILEPATH$FILENAME
```

A.2 From Lignocellulose Hydrolysate to Exopolysaccharide

A.2.1 High-Content Screening with D-Xylose

Table A.1: Exopolysaccharide monomer compositions of D-xylose high-content screening. The exopolysaccharide producers on the plate Xyl1 were incubated for 48 h in SM17 P30S, a medium which contained 10.0 $g \cdot l^{-1}$ D-xylose as the sole carbon source. The aldose composition of the exopolysaccharides are summarized in this table. The concentrations of the D-glucose dimers isomaltose, laminaribiose, nigerose and sophorose were too low for quantification. Therefore, the presence of these dimers is indicated qualitatively. The following analytes were not found in any sample and, thus, left out from the table: *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, cellobiose, 2-deoxy-D-ribose, gentiobiose, kojibiose, lactose and maltose. D-Xylose was present in every sample, but it was not quantified after the gel filtration and, therefore, D-xylose could not be attributed to the medium or the polymer. Since L-arabinose could not be distinguished from D-xylose the combined values are given here, but were *not* included in the sum. Abbreviations: Fuc: L-fucose; Gal: D-galactose; GalN: D-galactosamine; GalUA: D-galacturonic acid; 2-D-Glc: 2-deoxy-D-glucose; Glc: D-glucose; GlcN: D-glucosamine; GlcUA: D-glucuronic acid; Man: D-mannose; Rha: L-rhamnose; Rib: D-ribose; Sum: sum of all values to the left of the same row; Xyl/Ara: D-xylose and L-arabinose; Ism: isomaltose; Lam: laminaribiose; Nig: nigerose; Sop: sophorose; y: yes; n: no; ?: inconclusive. All values are in $mg \cdot l^{-1}$.

Strain	Fuc	Gal	GalN	GalUA	Gen	2-D-Glc	Glc	GlcN	GlcUA	Man	Rha	Rib	Sum	Xyl/Ara	Ism	Lam	Nig	Sop
Xyl1.A1	0	7	0	0	0	0	33	13	0	24	24	0	101	202	n	n	n	n
Xyl1.A2	5	11	0	0	0	0	38	11	29	34	97	0	225	106	n	n	n	n
Xyl1.A3	0	33	0	0	0	0	179	12	32	27	100	0	383	172	n	n	n	n
Xyl1.A4	1	19	0	0	0	7	39	14	0	11	0	0	91	389	n	n	n	n
Xyl1.A5	0	9	0	0	0	0	34	12	29	23	94	0	201	101	n	n	n	n
Xyl1.A6	0	8	0	0	0	0	31	12	28	21	79	0	179	143	n	n	n	n
Xyl1.A7	0	9	0	0	0	0	31	11	27	20	77	0	175	168	n	n	n	n
Xyl1.A8	0	9	0	0	0	0	33	12	27	23	78	0	182	130	n	n	n	n
Xyl1.A9	0	97	0	0	0	0	98	13	45	44	76	0	373	167	n	n	n	n
Xyl1.A10	0	8	0	0	0	0	29	12	0	19	0	0	68	298	n	n	n	n
Xyl1.A11	3	10	0	0	0	0	13	14	0	32	0	0	72	253	n	n	n	n
Xyl1.A12	0	7	9	0	0	0	16	35	0	21	9	0	97	537	n	n	n	n
Xyl1.B1	0	7	8	0	0	0	26	31	0	15	9	0	96	187	n	n	n	n

continued on the next page

Table A.1: *continued from the previous page*

Strain	Fuc	Gal	GalN	GalUA	Gen	2-D-Glc	Glc	GlcN	GlcUA	Man	Rha	Rib	Sum	Xyl/Ara	Ism	Lam	Nig	Sop
Xyl1.B2	0	9	0	0	0	0	7	0	0	13	0	0	29	203	n	n	n	n
Xyl1.B3	0	9	0	0	0	0	7	0	0	12	0	0	28	111	n	n	n	n
Xyl1.B4	0	40	0	0	0	0	99	0	0	0	0	0	139	145	n	n	n	n
Xyl1.B5	0	11	0	0	0	0	0	0	0	0	0	0	11	155	n	n	n	n
Xyl1.B6	0	9	0	0	3	0	6	13	0	14	11	0	56	191	n	n	n	n
Xyl1.B7	0	15	0	0	0	6	42	14	0	25	0	0	102	248	n	n	n	n
Xyl1.B8	0	17	0	0	0	6	34	13	0	20	10	0	100	256	n	n	n	n
Xyl1.B9	0	8	0	0	0	0	56	12	44	43	107	0	270	155	n	n	n	n
Xyl1.B10	3	7	0	0	0	0	25	14	0	12	0	8	69	196	n	n	n	n
Xyl1.B11	3	8	0	0	0	0	24	14	0	13	0	0	62	119	n	n	n	n
Xyl1.B12	0	111	0	0	0	0	81	0	0	12	35	0	239	147	n	n	n	n
Xyl1.C1	8	659	0	0	0	0	92	0	170	34	712	0	1675	13	n	n	n	n
Xyl1.C2	0	8	0	0	0	0	20	13	23	22	12	0	98	89	n	n	n	n
Xyl1.C3	0	9	0	0	0	0	66	15	29	29	16	0	164	40	n	n	n	n
Xyl1.C4	0	22	0	0	0	0	319	0	0	38	570	0	949	15	n	n	n	n
Xyl1.C5	0	17	0	0	0	0	286	0	132	0	694	0	1129	13	n	n	n	n
Xyl1.C6	0	7	0	0	0	0	20	13	0	0	0	0	40	150	n	n	n	n
Xyl1.C7	19	18	0	50	0	0	40	20	0	0	20	0	167	43	n	n	n	n
Xyl1.C8	1	19	0	0	0	0	24	13	0	70	0	0	127	95	n	n	n	n
Xyl1.C9	0	66	0	0	0	0	28	15	26	20	77	0	232	220	n	n	n	n
Xyl1.C10	0	31	0	0	0	0	58	17	0	39	0	0	145	189	n	n	n	n
Xyl1.C11	0	6	0	0	0	6	37	14	0	33	0	0	96	198	n	n	n	n
Xyl1.C12	0	6	0	0	0	0	25	13	0	22	0	0	66	223	n	n	n	n
Xyl1.D1	0	18	0	0	0	0	20	13	0	58	0	0	109	106	n	n	n	n
Xyl1.D2	0	6	0	0	0	0	7	22	0	13	0	0	48	103	n	n	n	n
Xyl1.D3	0	427	0	0	0	0	1504	24	292	457	161	14	2879	87	n	n	n	n
Xyl1.D4	0	20	0	0	0	0	18	12	0	45	11	0	106	98	n	n	n	n

continued on the next page

Table A.1: *continued from the previous page*

Strain	Fuc	Gal	GalN	GalUA	Gen	2-D-Glc	Glc	GlcN	GlcUA	Man	Rha	Rib	Sum	Xyl/Ara	Ism	Lam	Nig	Sop
Xyl1.D5	0	73	0	0	0	0	6	11	0	36	15	0	141	98	n	n	n	n
Xyl1.D6	0	14	0	0	0	0	57	12	29	14	94	0	220	49	n	n	n	n
Xyl1.D7	33	503	0	0	0	0	950	20	0	332	154	17	2009	27	n	n	n	n
Xyl1.D8	43	104	0	0	0	0	680	20	100	165	61	13	1186	86	y	n	?	n
Xyl1.D9	43	106	0	0	0	0	723	19	98	176	53	12	1230	44	y	n	?	n
Xyl1.D10	39	95	0	0	0	0	598	21	0	160	52	12	977	52	?	n	n	n
Xyl1.D11	0	49	0	0	0	0	543	16	107	210	36	11	972	19	y	n	n	n
Xyl1.D12	0	7	0	0	0	0	18	15	0	23	0	0	63	144	n	n	n	n
Xyl1.E1	0	9	0	0	0	0	18	31	24	17	16	0	115	55	n	n	n	n
Xyl1.E2	0	9	0	0	0	0	26	11	0	23	12	0	81	38	n	n	n	n
Xyl1.E3	0	628	0	0	0	0	0	0	112	0	701	0	1441	16	n	n	n	n
Xyl1.E4	0	49	0	0	0	0	722	16	132	536	86	12	1553	62	n	n	n	n
Xyl1.E5	0	27	0	0	0	0	12	12	0	32	30	0	113	41	n	n	n	n
Xyl1.E6	0	16	0	0	0	0	18	11	0	53	0	0	98	32	n	n	n	n
Xyl1.E7	0	57	15	0	0	0	26	16	0	68	57	0	239	74	n	n	n	n
Xyl1.E8	0	31	0	0	0	0	19	14	26	83	31	0	204	113	n	n	n	n
Xyl1.E9	229	626	0	0	0	0	683	20	103	84	25	13	1783	45	y	n	n	n
Xyl1.E10	4	10	0	0	0	0	11	11	0	12	9	0	57	154	n	n	n	n
Xyl1.E11	0	10	0	0	0	0	38	0	0	43	10	0	101	9	n	n	n	n
Xyl1.E12	0	6	13	0	0	5	9	17	0	0	0	0	50	125	n	n	n	n
Xyl1.F1	1	19	0	0	0	0	24	20	0	21	9	8	102	11	n	n	y	n
Xyl1.F2	0	15	0	0	0	0	13	21	0	21	9	0	79	9	n	n	n	n
Xyl1.F3	0	12	0	0	0	0	13	16	0	20	0	0	61	10	n	n	n	n
Xyl1.F4	0	14	0	0	0	0	16	19	0	28	0	0	77	37	n	n	n	n
Xyl1.F5	0	36	0	0	0	0	96	17	26	32	0	0	207	48	y	n	n	n
Xyl1.F6	0	8	0	0	0	0	22	15	0	15	0	0	60	81	n	n	n	n
Xyl1.F7	0	12	0	0	0	0	30	15	0	14	0	0	71	79	n	n	n	n

continued on the next page

Table A.1: *continued from the previous page*

Strain	Fuc	Gal	GalN	GalUA	Gen	2-D-Glc	Glc	GlcN	GlcUA	Man	Rha	Rib	Sum	Xyl/Ara	Ism	Lam	Nig	Sop
Xyl1.F8	0	37	0	57	0	0	29	13	0	64	11	0	211	31	n	n	n	n
Xyl1.F9	0	27	0	0	0	0	30	15	0	23	0	0	95	10	n	n	y	n
Xyl1.F10	0	31	0	0	0	0	43	0	0	16	11	7	108	9	n	n	n	n
Xyl1.F11	0	34	0	58	0	0	49	0	0	68	12	7	228	9	n	n	n	n
Xyl1.F12	0	7	13	0	0	5	25	20	0	10	9	12	101	84	n	n	n	n
Xyl1.G1	0	6	12	0	0	0	64	15	0	0	0	0	97	48	n	n	n	n
Xyl1.G2	0	8	13	0	0	0	43	20	0	11	0	0	95	72	n	n	n	n
Xyl1.G3	0	6	0	0	0	0	44	0	0	10	0	0	60	74	n	n	n	n
Xyl1.G4	0	7	0	0	0	0	19	0	0	0	0	0	26	9	n	n	n	n
Xyl1.G5	0	9	0	0	0	0	67	13	98	135	16	0	338	37	n	n	y	n
Xyl1.G6	0	7	0	0	0	0	24	0	0	0	0	12	43	9	n	n	n	n
Xyl1.G7	0	8	0	0	0	0	26	13	0	11	12	0	70	9	n	n	n	n
Xyl1.G8	2	9	0	0	0	0	27	0	0	0	0	0	38	9	n	n	n	n
Xyl1.G9	6	7	0	0	0	0	40	10	0	0	14	0	77	10	n	n	n	n
Xyl1.G10	7	6	0	0	0	0	38	10	0	11	14	0	86	9	n	n	n	n
Xyl1.G11	0	11	12	0	0	7	38	15	0	11	0	11	105	9	n	n	n	n
Xyl1.G12	0	9	0	0	0	0	37	0	0	17	11	0	74	11	n	n	n	n
Xyl1.H1	0	9	16	0	0	0	54	24	0	0	0	0	103	10	n	n	n	n
Xyl1.H2	0	11	17	0	0	0	44	39	0	28	25	0	164	29	n	n	n	n
Xyl1.H3	0	6	0	0	0	0	62	0	24	48	14	12	166	31	n	n	n	y
Xyl1.H4	0	7	0	0	0	0	9	0	0	11	0	0	27	468	n	n	n	n
Xyl1.H5	0	9	0	0	0	0	10	11	0	11	15	0	56	109	n	n	n	n
Xyl1.H6	0	16	0	0	0	0	60	0	0	11	22	14	123	109	n	n	n	n
Xyl1.H7	1	7	0	0	0	0	8	0	0	11	0	0	27	481	n	n	n	n
Xyl1.H8	0	724	0	0	0	0	9	0	124	0	789	0	1646	13	n	n	n	n
Xyl1.H9	0	7	0	0	0	0	8	10	0	12	0	0	37	305	n	n	n	n
Xyl1.H10	0	6	0	0	0	0	10	13	0	14	0	0	43	472	n	n	n	n

continued on the next page

Table A.1: *continued from the previous page*

Strain	Fuc	Gal	GalN	GalUA	Gen	2-D-Glc	Glc	GlcN	GlcUA	Man	Rha	Rib	Sum	Xyl/Ara	Ism	Lam	Nig	Sop
Xyl1.H11	0	10	0	0	0	0	24	0	0	0	0	0	34	399	n	n	n	n
Xyl1.H12	0	7	15	0	0	0	12	28	0	0	0	0	62	510	n	n	n	n

Table A.2: The residual D-xylose after 48 h incubation in SM17 P30S of the strains of Xyl1 in $g \cdot l^{-1}$. The initial D-xylose concentration was 10.0 $g \cdot l^{-1}$. Values exceeding 10.0 $g \cdot l^{-1}$ are considered artifacts. D-Xylose consumption of the 'empty' well E12 stems from a contamination.

	1	2	3	4	5	6	7	8	9	10	11	12
A	4.88	3.99	4.54	7.55	3.25	3.86	4.69	4.30	4.03	4.75	4.85	4.70
B	8.95	6.30	5.90	5.60	5.80	6.35	7.35	6.95	3.84	4.44	4.07	3.98
C	0.39	4.77	1.35	0.57	0.61	6.45	2.98	6.30	7.15	5.10	7.05	7.20
D	5.55	5.40	0.60	6.35	6.60	2.44	0.61	0.57	0.59	0.67	0.11	5.90
E	3.08	2.33	0.42	0.00	3.47	0.28	4.96	5.75	0.47	9.65	0.10	6.50
F	0.12	0.10	0.10	1.07	4.13	4.77	5.20	2.63	0.11	0.00	0.00	2.46
G	1.22	2.25	2.27	0.10	1.09	0.00	0.00	0.00	0.00	0.10	0.00	0.10
H	0.10	0.42	0.55	10.20	2.24	2.35	10.15	0.34	8.55	9.55	8.40	10.95

A.2.2 High-Throughput Screening for Inhibitor/Lignocellulose Hydrolysate Tolerance

Table A.3: Background attenuance on a per-plate basis. The median of the background attenuance of each plate was calculated from empty wells and subtracted from every other well. The values given are the median ± half the inter-quartile range. The background values were calculated from eight independent measurements for all single inhibitor experiments with Xyl1 and the laevulinic acid experiment with Xyl2, from nine independent measurements for all other single inhibitor experiments. For lignocellulose hydrolysate experiments, three wells were used for background calculation of Xyl1 and eight wells were used for background calculation of Xyl2.

Test Series	Background attenuance in plate	
	Xyl1	Xyl2
Reference	0.0435 ± 0.0018	0.0585 ± 0.0038
Furfural	0.0418 ± 0.0006	0.0429 ± 0.0015
Hydroxymethylfurfural	0.0415 ± 0.0003	0.0498 ± 0.0053
Vanillin	0.0784 ± 0.0044	0.0795 ± 0.0015
Acetic acid	0.0407 ± 0.0012	0.0526 ± 0.0045
Formic acid	0.0425 ± 0.0011	0.0533 ± 0.0064
Laevulinic acid	0.0519 ± 0.0036	0.0671 ± 0.0275
Lignocellulose Hydrolysate Tolerance Experiment		
Reference	0.0400 ± 0.0009	0.0397 ± 0.0010
Lignocellulose hydrolysate	0.1280 ± 0.0015	0.1266 ± 0.0021

Table A.4: Classed data of inhibitor/lignocellulose hydrolysate tolerance. Attenuance percentages of cultures grown in the presence of single inhibitors or lignocellulose hydrolysate relative to the reference without inhibitors. The median background value of each plate was subtracted and then the aforementioned percentage calculated. In order to assess the impact each inhibitor or lignocellulose hydrolysate had on microbial growth, data were classed and visualized in figure 3.2 on page 68 or figure 3.3 on page 71, respectively. The classes are: class I: (−∞, 5 %); class II: [5 %, 20 %); class III: [20 %, 40 %); class IV: [40 %, 60 %); class V: [60 %, 80 %); class VI: [80 %, 100 %); class VII: [100 %, 120 %); class VIII: [120 %, +∞).

Inhibitor	Number of strains in class							
	I	II	III	IV	V	VI	VII	VIII
Furfural	25	20	15	15	28	13	5	6
Hydroxymethylfurfural	9	8	15	19	39	20	11	6
Vanillin	100	4	4	6	12	1	0	0
Acetic acid	2	2	11	6	7	53	30	16
Formic acid	3	1	2	3	15	51	29	23
Laevulinic acid	0	0	6	10	24	58	27	2
Lignocellulose hydrolysate	20	4	4	9	22	43	24	7

Table A.5: Plate layout of ISp. Strains from A1 to G4, A5 to G8 and A9 to G12 correspond to the top 27 of the hydroxymethylfurfural screening, the furfural screening and the vanillin screening, respectively. Empty wells were reserved for the following special purposes. G4: Uninoculated medium with hydroxymethylfurfural; G8: uninoculated medium with furfural; G12: uninoculated medium with vanillin; H1 to H10: sugar standards according to the HPLC-MS method (see section 2.4.3 on page 34); H11: water; H12: uninoculated medium.

	1	2	3	4	5	6	7	8	9	10	11	12
A	*Xyl1.A7*	*Xyl1.C4*	*Xyl1.F4*	*Xyl2.B7*	*Xyl1.A10*	*Xyl1.F1*	*Xyl1.G5*	*Xyl2.A9*	*Xyl1.A10*	*Xyl1.F10*	*Xyl1.H10*	*Xyl2.C5*
B	*Xyl1.A10*	*Xyl1.C5*	*Xyl1.F8*	*Xyl2.B8*	*Xyl1.B4*	*Xyl1.F2*	*Xyl1.G11*	*Xyl2.B7*	*Xyl1.C1*	*Xyl1.G5*	*Xyl2.A1*	*Xyl2.C7*
C	*Xyl1.A12*	*Xyl1.C10*	*Xyl1.F9*	*Xyl2.C4*	*Xyl1.B5*	*Xyl1.F3*	*Xyl2.A2*	*Xyl2.B8*	*Xyl1.C4*	*Xyl1.G7*	*Xyl2.A6*	*Xyl2.C9*
D	*Xyl1.B7*	*Xyl1.C12*	*Xyl1.G5*	*Xyl2.C5*	*Xyl1.B10*	*Xyl1.F4*	*Xyl2.A5*	*Xyl2.C4*	*Xyl1.C5*	*Xyl1.G9*	*Xyl2.A9*	*Xyl2.C10*
E	*Xyl1.B10*	*Xyl1.D2*	*Xyl1.G11*	*Xyl2.C12*	*Xyl1.B11*	*Xyl1.F8*	*Xyl2.A6*	*Xyl2.C5*	*Xyl1.C7*	*Xyl1.G11*	*Xyl2.B7*	*Xyl2.C11*
F	*Xyl1.B11*	*Xyl1.D4*	*Xyl2.A1*	*Xyl2.D2*	*Xyl1.C2*	*Xyl1.F9*	*Xyl2.A7*	*Xyl2.C12*	*Xyl1.E3*	*Xyl1.H3*	*Xyl2.B12*	*Xyl2.D1*
G	*Xyl1.C2*	*Xyl1.D12*	*Xyl2.A6*	-	*Xyl1.D2*	*Xyl1.F10*	*Xyl2.A8*	-	*Xyl1.E7*	*Xyl1.H8*	*Xyl2.C4*	-
H	-	-	-	-	-	-	-	-	-	-	-	-

Table A.6: Plate layout of ISr. Strains from A1 to G4, A5 to G8 and A9 to G12 correspond to the top 28 of the formic acid screening, the acetic acid screening and the laevulinic acid screening, respectively. Empty wells were reserved for the following special purposes. H1 to H8: acid standards for the HPLC method (see section 2.8.4 on page 56); H9: uninoculated medium; H10: uninoculated medium with formic acid; H11: uninoculated medium with acetic acid; H12: uninoculated medium with laevulinic acid.

	1	2	3	4	5	6	7	8	9	10	11	12
A	*Xyl1.C4*	*Xyl1.F10*	*Xyl1.G11*	*Xyl2.B10*	*Xyl1.A10*	*Xyl1.D12*	*Xyl1.H8*	*Xyl2.A8*	*Xyl1.A3*	*Xyl1.C12*	*Xyl1.G5*	*Xyl2.A4*
B	*Xyl1.C5*	*Xyl1.F12*	*Xyl1.H1*	*Xyl2.C5*	*Xyl1.B3*	*Xyl1.E1*	*Xyl1.H12*	*Xyl2.A9*	*Xyl1.A5*	*Xyl1.D1*	*Xyl1.H4*	*Xyl2.A6*
C	*Xyl1.E1*	*Xyl1.G1*	*Xyl1.H3*	*Xyl2.C6*	*Xyl1.B8*	*Xyl1.E2*	*Xyl2.A2*	*Xyl2.A12*	*Xyl1.A6*	*Xyl1.D8*	*Xyl1.H7*	*Xyl2.A7*
D	*Xyl1.F2*	*Xyl1.G2*	*Xyl2.A1*	*Xyl2.C7*	*Xyl1.B10*	*Xyl1.F1*	*Xyl2.A4*	*Xyl2.B1*	*Xyl1.A7*	*Xyl1.D9*	*Xyl1.H9*	*Xyl2.A9*
E	*Xyl1.F4*	*Xyl1.G3*	*Xyl2.A2*	*Xyl2.C12*	*Xyl1.C4*	*Xyl1.F6*	*Xyl2.A5*	*Xyl2.B8*	*Xyl1.A8*	*Xyl1.D10*	*Xyl1.H10*	*Xyl2.B7*
F	*Xyl1.F5*	*Xyl1.G5*	*Xyl2.B4*	*Xyl2.D2*	*Xyl1.C5*	*Xyl1.G5*	*Xyl2.A6*	*Xyl2.C4*	*Xyl1.A9*	*Xyl1.D12*	*Xyl1.H11*	*Xyl2.B8*
G	*Xyl1.F8*	*Xyl1.G9*	*Xyl2.B8*	*Xyl2.D3*	*Xyl1.C12*	*Xyl1.G11*	*Xyl2.A7*	*Xyl2.C5*	*Xyl1.A11*	*Xyl1.E2*	*Xyl2.A1*	*Xyl2.C4*
H	-	-	-	-	-	-	-	-	-	-	-	-

Table A.7: Tolerance ranks of the strains in Xyl1 and Xyl2. Some strains did not grow in the experiments with the inhibitors furfural, hydroxymethylfurfural, vanillin, acetic acid, formic acid and laevulinic acid, but in the experiment with lignocellulose hydrolysate. For others, the situation was vice versa. Therefore, complete growth data is not available for every strain. Missing data is indicated by '-'. Abbreviations: Fur.: furfural; HMF: hydroxymethylfurfural; Van.: vanillin; Acet.: acetic acid; Form.: formic acid; Laev.: laevulinic acid; LCH: lignocellulose hydrolysate.

Strain	Rank on						
	Fur.	HMF	Van.	Acet.	Form.	Laev.	LCH
Xyl1.A1	90	70	36	79	69	36	97
Xyl1.A2	50	46	39	62	83	30	62
Xyl1.A3	55	40	40	61	78	19	103
Xyl1.A4	-	-	-	-	-	-	55
Xyl1.A5	54	39	41	56	71	24	54
Xyl1.A6	48	42	42	59	72	14	60
Xyl1.A7	28	37	43	60	58	22	64
Xyl1.A8	40	45	44	58	65	21	57
Xyl1.A9	58	64	38	53	54	20	66
Xyl1.A10	27	16	21	22	36	37	42
Xyl1.A11	53	41	45	55	50	18	77
Xyl1.A12	18	50	46	46	74	58	30
Xyl1.B1	98	81	47	83	126	32	58
Xyl1.B2	56	32	48	81	84	40	87
Xyl1.B3	99	48	49	26	38	38	76
Xyl1.B4	94	27	50	71	70	41	93
Xyl1.B5	97	28	51	70	81	53	91
Xyl1.B6	103	84	52	67	73	35	90
Xyl1.B7	13	35	53	93	44	46	48
Xyl1.B8	32	57	54	16	89	49	32
Xyl1.B9	49	38	55	51	59	29	86
Xyl1.B10	5	8	56	14	45	47	71
Xyl1.B11	11	11	57	36	93	90	59
Xyl1.B12	93	59	58	45	37	93	47
Xyl1.C1	111	62	11	39	125	72	9
Xyl1.C2	10	13	59	76	94	59	53
Xyl1.C3	29	94	29	74	61	89	27
Xyl1.C4	25	31	7	1	16	43	95
Xyl1.C5	2	34	12	2	18	34	92
Xyl1.C6	36	63	60	73	88	69	17
Xyl1.C7	52	77	16	90	97	95	69
Xyl1.C8	43	75	61	86	99	64	36
Xyl1.C9	45	60	62	80	85	33	21
Xyl1.C10	20	121	30	38	39	75	18
Xyl1.C11	33	69	63	49	98	48	20
Xyl1.C12	24	65	64	28	68	26	8
Xyl1.D1	39	72	65	40	101	25	12

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Table A.7: *continued from the previous page*

Strain	Rank on						
	Fur.	HMF	Van.	Acet.	Form.	Laev.	LCH
Xyl1.D2	16	18	66	94	103	77	41
Xyl1.D3	73	115	67	82	112	92	99
Xyl1.D4	26	47	68	34	80	61	33
Xyl1.D5	51	96	69	96	118	99	70
Xyl1.D6	60	111	70	47	92	57	83
Xyl1.D7	62	108	71	110	120	117	109
Xyl1.D8	66	103	72	42	53	17	61
Xyl1.D9	59	101	73	30	40	9	72
Xyl1.D10	67	106	74	41	46	13	67
Xyl1.D11	47	78	75	44	42	31	74
Xyl1.D12	1	100	76	5	111	3	50
Xyl1.E1	78	113	77	7	24	52	39
Xyl1.E2	65	112	78	24	62	15	106
Xyl1.E3	42	54	6	32	57	63	29
Xyl1.E4	57	97	79	91	51	42	89
Xyl1.E5	84	80	80	97	106	91	94
Xyl1.E6	79	109	81	87	105	80	81
Xyl1.E7	34	61	2	35	48	45	37
Xyl1.E8	46	66	32	43	79	51	79
Xyl1.E9	83	118	82	85	124	82	105
Xyl1.E10	112	110	83	77	110	79	-
Xyl1.E11	-	-	-	-	-	-	98
Xyl1.F1	91	3	84	19	107	86	129
Xyl1.F2	95	1	85	99	8	70	43
Xyl1.F3	101	4	86	113	117	103	96
Xyl1.F4	4	5	87	101	5	105	115
Xyl1.F5	69	82	88	105	19	112	102
Xyl1.F6	109	102	89	18	108	85	1
Xyl1.F7	116	104	90	125	63	100	7
Xyl1.F8	12	15	91	106	26	114	130
Xyl1.F9	17	7	37	78	121	39	131
Xyl1.F10	85	26	14	116	4	111	-
Xyl1.F11	-	-	-	-	-	-	123
Xyl1.F12	117	90	92	121	10	121	111
Xyl1.G1	119	91	93	120	15	125	124
Xyl1.G2	115	88	94	118	13	122	120
Xyl1.G3	118	92	95	119	14	124	113
Xyl1.G4	-	-	-	-	-	-	119
Xyl1.G5	6	14	24	4	6	6	45
Xyl1.G6	-	-	-	-	-	-	88
Xyl1.G7	70	52	15	124	49	123	56
Xyl1.G8	-	-	-	-	-	-	108
Xyl1.G9	121	71	17	114	3	113	68

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Table A.7: *continued from the previous page*

Strain	Rank on						
	Fur.	HMF	Van.	Acet.	Form.	Laev.	LCH
Xyl1.G10	-	-	-	-	-	-	104
Xyl1.G11	21	20	27	23	25	71	16
Xyl1.G12	125	126	31	126	127	116	121
Xyl1.H1	126	127	96	112	28	115	100
Xyl1.H2	124	119	97	108	114	97	126
Xyl1.H3	114	44	4	115	23	108	122
Xyl1.H4	80	114	98	54	60	16	84
Xyl1.H5	75	85	99	65	116	118	19
Xyl1.H6	104	87	100	104	119	126	75
Xyl1.H7	87	116	101	37	64	12	73
Xyl1.H8	38	58	5	25	34	68	15
Xyl1.H9	88	107	102	84	115	11	132
Xyl1.H10	92	98	26	64	104	7	5
Xyl1.H11	82	99	103	69	109	8	118
Xyl1.H12	76	67	104	6	55	84	107
Xyl2.A1	19	33	8	52	17	10	65
Xyl2.A2	81	2	105	10	20	107	101
Xyl2.A3	102	43	106	98	95	98	31
Xyl2.A4	77	83	107	3	32	1	133
Xyl2.A5	110	22	108	15	30	56	24
Xyl2.A6	8	6	1	13	31	23	4
Xyl2.A7	100	23	109	20	100	4	10
Xyl2.A8	106	17	110	27	76	104	127
Xyl2.A9	44	19	3	9	33	28	3
Xyl2.A10	113	51	111	100	91	96	34
Xyl2.A11	74	89	112	109	122	110	112
Xyl2.A12	96	56	113	17	113	73	116
Xyl2.B1	107	86	114	21	35	55	26
Xyl2.B2	89	76	115	57	90	83	13
Xyl2.B3	122	124	116	111	66	102	14
Xyl2.B4	105	123	117	107	7	101	125
Xyl2.B5	123	125	118	122	123	67	128
Xyl2.B6	86	105	119	72	75	81	110
Xyl2.B7	22	25	19	89	52	27	28
Xyl2.B8	14	10	120	11	22	2	11
Xyl2.B9	120	120	121	88	43	78	78
Xyl2.B10	71	79	122	31	21	44	35
Xyl2.B11	72	49	34	92	82	76	117
Xyl2.B12	68	73	22	63	86	106	52
Xyl2.C1	-	-	-	-	-	-	2
Xyl2.C2	31	68	35	50	96	62	23
Xyl2.C3	37	74	33	66	56	65	38
Xyl2.C4	7	9	25	8	29	5	25

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Table A.7: *continued from the previous page*

Strain	Rank on						
	Fur.	HMF	Van.	Acet.	Form.	Laev.	LCH
Xyl2.C5	3	12	28	12	11	74	40
Xyl2.C6	127	117	123	127	9	119	85
Xyl2.C7	64	30	23	123	27	127	49
Xyl2.C8	30	29	124	48	102	88	80
Xyl2.C9	41	55	9	33	47	50	6
Xyl2.C10	61	122	18	75	77	60	63
Xyl2.C11	35	53	13	29	41	66	22
Xyl2.C12	9	21	125	102	1	87	46
Xyl2.D1	63	95	20	117	87	120	44
Xyl2.D2	15	24	126	103	2	94	51
Xyl2.D3	23	36	10	68	12	54	82
Xyl2.D4	108	93	127	95	67	109	114

A.2.3 High-Content Screening with Inhibitors

Table A.8: Inhibitor concentrations after 48 h, complete raw data of ISp. The plate ISp was incubated with 1.0 ml SM18 P30S with 2.00 $g \cdot l^{-1}$ of inhibitor for 48 h at 30 °C and 1000 min^{-1}. Afterwards, the inhibitor concentrations were determined using PMP derivatization and HPLC-MS analysis. In this table, the raw data of all utilized wells are given. These data were used to build the summary statistics in table 3.9 on page 75. For the calculation of the summary statistics, only non-medium values of growing strains of the corresponding test series were used. Since every sample was analysed for all three inhibitors given, all values are given here. Wells G4, G8 and G12 contained medium with 2.00 $g \cdot l^{-1}$ of inhibitor. Since these raw data were taken directly from the HPLC software, figures do not imply significance. Abbreviations: n.d.: not detected.

Well	Test series	Inhibitor concentration in $mg \cdot l^{-1}$ after 48 h		
		Furfural	Hydroxymethylfurfural	Vanillin
A1	Hydroxymethylfurfural	0.5654	16.2102	8.1903
A2	Hydroxymethylfurfural	4.4901	−6.4767	9.5261
A3	Hydroxymethylfurfural	10.0895	457.6257	6.5047
A4	Hydroxymethylfurfural	7.3651	10.6818	9.983
A5	Furfural	38.9421	n.d.	45.0688
A6	Furfural	73.7549	−12.2035	27.2587
A7	Furfural	17.8666	−6.2576	16.7666
A8	Furfural	20.3905	4.5859	27.6381
A9	Vanillin	n.d.	−1.4914	52.3396
A10	Vanillin	n.d.	−7.6508	41.3085
A11	Vanillin	n.d.	9.5028	36.6111
A12	Vanillin	n.d.	4.2088	2975.6543
B1	Hydroxymethylfurfural	8.177	1.4065	516.1782
B2	Hydroxymethylfurfural	20.6053	−18.1262	13.3525
B3	Hydroxymethylfurfural	12.3902	498.5299	12.3138

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Table A.8: *continued from the previous page*

Well	Test series	Inhibitor concentration in $mg \cdot l^{-1}$ after 48 h		
		Furfural	Hydroxymethylfurfural	Vanillin
B4	Hydroxymethylfurfural	23.1437	10.0868	14.2941
B5	Furfural	841.0768	11.8819	29.1273
B7	Furfural	41.4957	18.2835	28.2764
B8	Furfural	15.8933	−12.5393	22.8073
B9	Vanillin	30.4275	28.334	52.918
B10	Vanillin	27.9673	−2.4463	1960.5322
B11	Vanillin	30.1295	−1.4928	39.6034
B12	Vanillin	n.d.	11.8571	3655.5686
C1	Hydroxymethylfurfural	n.d.	46.1832	16.4358
C2	Hydroxymethylfurfural	n.d.	1958.1578	19.2407
C3	Hydroxymethylfurfural	5.4137	155.8943	12.9152
C4	Hydroxymethylfurfural	3.2524	−12.2767	9.2299
C5	Furfural	831.0684	−15.9823	31.0555
C7	Furfural	28.8506	−12.1669	19.967
C8	Furfural	158.7271	9.7583	28.3026
C9	Vanillin	n.d.	−13.5124	34.4418
C10	Vanillin	n.d.	−2.1384	38.4054
C11	Vanillin	n.d.	−8.921	1048.5927
C12	Vanillin	n.d.	−0.9989	51.3901
D1	Hydroxymethylfurfural	n.d.	16.3563	12.6288
D2	Hydroxymethylfurfural	n.d.	3.8401	16.0255
D3	Hydroxymethylfurfural	n.d.	7.5024	9.1261
D4	Hydroxymethylfurfural	n.d.	−5.3171	10.3122
D5	Furfural	9.9358	−15.8832	23.2332
D6	Furfural	32.8621	2.558	27.7631
D7	Furfural	25.2935	−0.1766	26.0261
D8	Furfural	14.0014	−8.63	17.7663
D9	Vanillin	n.d.	−15.6145	35.5572
D10	Vanillin	n.d.	−3.5848	63.3027
D11	Vanillin	n.d.	−11.6462	1442.368
D12	Vanillin	n.d.	12.3606	235.1482
E1	Hydroxymethylfurfural	n.d.	8.1669	13.5933
E2	Hydroxymethylfurfural	n.d.	23.0577	18.9891
E3	Hydroxymethylfurfural	n.d.	372.8809	19.3377
E4	Hydroxymethylfurfural	26.0941	−2.3895	17.1148
E5	Furfural	11.2237	−0.2278	23.7963
E6	Furfural	30.1357	n.d.	30.1814
E7	Furfural	11.9253	−13.5107	25.6695
E8	Furfural	10.9599	−3.6246	25.5985
E9	Vanillin	n.d.	4.8959	39.6286
E10	Vanillin	n.d.	−13.6706	1976.3702
E12	Vanillin	n.d.	0.2715	51.0982
F1	Hydroxymethylfurfural	n.d.	−6.6173	17.1247

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Table A.8: *continued from the previous page*

Well	Test series	Inhibitor concentration in $mg \cdot l^{-1}$ after 48 h		
		Furfural	Hydroxymethylfurfural	Vanillin
F2	Hydroxymethylfurfural	20.6469	3.7885	12.3358
F3	Hydroxymethylfurfural	n.d.	−14.885	21.3977
F4	Hydroxymethylfurfural	55.0436	1.5027	33.7957
F5	Furfural	11.5952	−18.8661	23.3776
F6	Furfural	19.8651	−17.6509	25.8751
F8	Furfural	82.4602	17.2663	28.8255
F9	Vanillin	n.d.	19.3548	48.6427
F10	Vanillin	n.d.	0.7755	35.4903
F11	Vanillin	25.7273	4.6483	37.3688
F12	Vanillin	127.6365	n.d.	3305.9377
G1	Hydroxymethylfurfural	1.6613	16.78	25.9032
G2	Hydroxymethylfurfural	33.0725	2126.8727	30.4242
G3	Hydroxymethylfurfural	9.3084	12.3587	22.7853
G4	Hydroxymethylfurfural	5.1237	2804.8755	32.053
G5	Furfural	12.6983	n.d.	29.1563
G6	Furfural	73.9162	n.d.	34.2682
G8	Furfural	1006.1831	−15.0396	35.4624
G9	Vanillin	19.2029	−14.7257	111.1418
G10	Vanillin	37.9452	−15.8579	55.8642
G11	Vanillin	8.6244	−5.9679	1907.7689
G12	Vanillin	47.0394	−13.1312	3207.8571

Table A.9: Inhibitor concentrations after 48 h, complete raw data of ISr. The plate ISr was incubated with 1.0 ml SM18 P30S with $2.00\,g \cdot l^{-1}$ of inhibitor for 48 h at 30 °C and $1000\,min^{-1}$. Afterwards, the inhibitor concentrations were determined using HPLC-UV analysis. In this table, the raw data of all utilized wells are given. These data were used to build the summary statistics in table 3.9 on page 75. For the calculation of the summary statistics, only non-medium values of growing strains of the corresponding test series were used. Since every sample was analysed for all three inhibitors given, all values are given here. Wells H10, H11 and H12 contained medium with $2.00\,g \cdot l^{-1}$ of inhibitor. Since these raw data were taken directly from the HPLC software, figures do not imply significance. Abbreviations: n.d.: not detected.

Well	Test series	Inhibitor concentration in $g \cdot l^{-1}$ after 48 h		
		Acetic acid	Formic acid	Laevulinic acid
A1	Formic acid	0.0688	n.d.	n.d.
A2	Formic acid	n.d.	n.d.	n.d.
A3	Formic acid	n.d.	n.d.	n.d.
A4	Formic acid	n.d.	n.d.	n.d.
A5	Acetic acid	0.3073	n.d.	n.d.
A6	Acetic acid	2.4904	n.d.	n.d.
A7	Acetic acid	0.5892	n.d.	n.d.
A8	Acetic acid	1.5361	−0.173	n.d.

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Table A.9: *continued from the previous page*

Well	Test series	Inhibitor concentration in $g \cdot l^{-1}$ after 48 h		
		Acetic acid	Formic acid	Laevulinic acid
A9	Laevulinic acid	n.d.	−0.1262	1.6284
A10	Laevulinic acid	n.d.	−0.1592	3.582
A11	Laevulinic acid	0.4486	−0.152	4.1116
A12	Laevulinic acid	n.d.	−0.1339	n.d.
B1	Formic acid	0.1857	n.d.	n.d.
B2	Formic acid	n.d.	n.d.	n.d.
B3	Formic acid	n.d.	n.d.	n.d.
B4	Formic acid	0.321	1.6691	n.d.
B5	Acetic acid	n.d.	−0.1441	n.d.
B6	Acetic acid	2.6416	n.d.	n.d.
B7	Acetic acid	−0.115	n.d.	n.d.
B8	Acetic acid	n.d.	n.d.	n.d.
B9	Laevulinic acid	n.d.	n.d.	2.609
B10	Laevulinic acid	n.d.	n.d.	1.9705
B11	Laevulinic acid	0.2005	n.d.	1.8381
C1	Formic acid	0.9867	n.d.	n.d.
C2	Formic acid	n.d.	n.d.	n.d.
C3	Formic acid	n.d.	n.d.	n.d.
C4	Formic acid	n.d.	−0.0906	n.d.
C5	Acetic acid	0.4655	n.d.	n.d.
C6	Acetic acid	3.6227	n.d.	n.d.
C7	Acetic acid	2.3077	−0.1381	n.d.
C8	Acetic acid	0.9403	n.d.	n.d.
C9	Laevulinic acid	n.d.	−0.1143	2.3412
C10	Laevulinic acid	n.d.	−0.3462	1.9823
C11	Laevulinic acid	n.d.	n.d.	n.d.
C12	Laevulinic acid	n.d.	−0.1259	2.6241
D1	Formic acid	0.5493	−0.0214	n.d.
D2	Formic acid	n.d.	−0.1265	n.d.
D3	Formic acid	1.6168	n.d.	n.d.
D4	Formic acid	n.d.	−0.0839	n.d.
D5	Acetic acid	0.0618	n.d.	n.d.
D6	Acetic acid	2.138	−0.0799	n.d.
D7	Acetic acid	n.d.	n.d.	n.d.
D8	Acetic acid	n.d.	−0.1132	n.d.
D9	Laevulinic acid	n.d.	−0.1152	3.2181
D10	Laevulinic acid	n.d.	n.d.	1.9681
D11	Laevulinic acid	n.d.	n.d.	n.d.
D12	Laevulinic acid	n.d.	−0.1683	2.4075
E1	Formic acid	0.8573	0.1273	n.d.
E2	Formic acid	n.d.	n.d.	n.d.
E3	Formic acid	0.7748	−0.1126	n.d.
E4	Formic acid	n.d.	n.d.	n.d.

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Table A.9: *continued from the previous page*

Well	Test series	Inhibitor concentration in $g \cdot l^{-1}$ after 48 h		
		Acetic acid	Formic acid	Laevulinic acid
E5	Acetic acid	n.d.	n.d.	n.d.
E6	Acetic acid	3.1148	−0.1702	n.d.
E7	Acetic acid	2.7294	n.d.	n.d.
E8	Acetic acid	0.45	−0.0413	n.d.
E9	Laevulinic acid	0.5372	n.d.	4.614
E10	Laevulinic acid	n.d.	n.d.	1.989
E11	Laevulinic acid	n.d.	−0.1283	n.d.
E12	Laevulinic acid	n.d.	n.d.	2.577
F1	Formic acid	n.d.	n.d.	n.d.
F2	Formic acid	0.3129	1.8585	n.d.
F3	Formic acid	n.d.	n.d.	n.d.
F4	Formic acid	−0.0068	−0.223	n.d.
F5	Acetic acid	1.8816	n.d.	n.d.
F6	Acetic acid	4.1498	−0.1331	n.d.
F7	Acetic acid	n.d.	n.d.	n.d.
F8	Acetic acid	4.2959	−0.152	n.d.
F9	Laevulinic acid	n.d.	n.d.	3.0853
F10	Laevulinic acid	0.115	n.d.	2.7112
F11	Laevulinic acid	n.d.	−0.1108	n.d.
F12	Laevulinic acid	n.d.	−0.1489	2.6596
G1	Formic acid	0.7089	0.3068	n.d.
G2	Formic acid	n.d.	−0.0737	n.d.
G3	Formic acid	0.0724	n.d.	n.d.
G4	Formic acid	3.4461	−0.2853	n.d.
G5	Acetic acid	n.d.	n.d.	n.d.
G6	Acetic acid	0.9689	−0.1295	n.d.
G7	Acetic acid	0.9727	n.d.	n.d.
G8	Acetic acid	3.3859	−0.1297	n.d.
G9	Laevulinic acid	0.2886	−0.1716	3.945
G10	Laevulinic acid	0.1012	−0.1179	1.8422
G11	Laevulinic acid	2.0348	−0.1377	2.62
G12	Laevulinic acid	0.4235	−0.1224	3.4447
H10	Formic acid	n.d.	2.4229	n.d.
H11	Acetic acid	2.4252	−0.1302	n.d.
H12	Laevulinic acid	n.d.	−0.1149	2.2968

Table A.10: D-Glucose concentrations after 48 h, complete raw data of ISp. The plate ISp was incubated with 1.0 ml SM18 P30S with 2.00 $g \cdot l^{-1}$ of inhibitor for 48 h at 30 °C and 1000 min^{-1}. Afterwards, the D-glucose concentrations were determined using a D-glucose assay. In this table, the raw data of all utilized wells are given. These data were used to build the summary statistics in table 3.11 on page 76. For the calculation of the summary statistics, only non-medium values of growing strains of the corresponding test series were used. Since these raw data were taken directly from the photometer software, figures do not imply significance. Abbreviations: $A418$: absorbance at 418 nm (dimensionless); $A480$: absorbance at 480 nm (dimensionless); DF: dilution factor (dimensionless); $c(Glc)$: D-glucose in $g \cdot l^{-1}$ after 48 h.

Well	Test series	$A418$	$A480$	DF	$c(Glc)$
A1	Hydroxymethylfurfural	1.117	0.1058	100	3.6136
A2	Hydroxymethylfurfural	0.0901	0.0443	100	0.0847
A3	Hydroxymethylfurfural	0.053	0.0423	100	−0.0436
A4	Hydroxymethylfurfural	1.4563	0.1271	100	4.776
A5	Furfural	0.2184	0.0511	100	0.5288
A6	Furfural	2.2824	0.1763	100	7.6159
A7	Furfural	0.0556	0.0412	100	−0.0301
A8	Furfural	1.3541	0.1203	100	4.4273
A9	Vanillin	0.1972	0.0503	100	0.4542
A10	Vanillin	0.5162	0.0774	100	1.5212
A11	Vanillin	0.0583	0.0421	100	−0.0235
A12	Vanillin	2.3604	0.1848	100	7.8699
B1	Hydroxymethylfurfural	0.1424	0.0481	100	0.262
B2	Hydroxymethylfurfural	0.0772	0.0439	100	0.039
B3	Hydroxymethylfurfural	0.0532	0.0444	100	−0.0506
B4	Hydroxymethylfurfural	1.7303	0.143	100	5.7195
B5	Furfural	2.8165	0.216	100	9.4231
B7	Furfural	0.0538	0.0411	100	−0.0363
B8	Furfural	1.2645	0.1153	100	4.118
B9	Vanillin	0.046	0.0416	100	−0.0667
B10	Vanillin	0.594	0.0808	100	1.7932
B11	Vanillin	0.1212	0.0504	100	0.1761
B12	Vanillin	0.0524	0.0418	100	−0.044
C1	Hydroxymethylfurfural	1.7789	0.1451	100	5.8895
C2	Hydroxymethylfurfural	2.4783	0.1871	100	8.2925
C3	Hydroxymethylfurfural	0.047	0.041	100	−0.0608
C4	Hydroxymethylfurfural	0.0519	0.0416	100	−0.0451
C5	Furfural	3.0141	0.223	100	10.1198
C7	Furfural	2.4752	0.185	100	8.2889
C8	Furfural	2.8143	0.2084	100	9.4429
C9	Vanillin	0.0528	0.0418	100	−0.0425
C10	Vanillin	0.3947	0.0657	100	1.1199
C11	Vanillin	0.4974	0.073	100	1.4686
C12	Vanillin	0.0457	0.0411	100	−0.0659
D1	Hydroxymethylfurfural	1.2158	0.1149	100	3.9415

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Table A.10: *continued from the previous page*

Well	Test series	$A418$	$A480$	DF	$c(Glc)$
D2	Hydroxymethylfurfural	1.4906	0.1279	100	4.8985
D3	Hydroxymethylfurfural	0.0533	0.0419	100	−0.0411
D4	Hydroxymethylfurfural	0.0525	0.0432	100	−0.0487
D5	Furfural	0.8914	0.0936	100	2.8335
D6	Furfural	0.052	0.0442	100	−0.0542
D7	Furfural	2.5597	0.1931	100	8.5681
D8	Furfural	0.0534	0.0434	100	−0.0462
D9	Vanillin	0.0542	0.0434	100	−0.0433
D10	Vanillin	0.4665	0.0736	100	1.3535
D11	Vanillin	1.2149	0.1173	100	3.9294
D12	Vanillin	0.0603	0.0436	100	−0.0217
E1	Hydroxymethylfurfural	0.9303	0.0956	100	2.9684
E2	Hydroxymethylfurfural	1.3193	0.1192	100	4.3041
E3	Hydroxymethylfurfural	0.344	0.0599	100	0.9558
E4	Hydroxymethylfurfural	0.0564	0.0433	100	−0.0349
E5	Furfural	0.7415	0.084	100	2.3207
E6	Furfural	0.5049	0.0756	100	1.4865
E7	Furfural	0.4324	0.0667	100	1.254
E8	Furfural	0.0533	0.0427	100	−0.044
E9	Vanillin	0.0469	0.0413	100	−0.0623
E10	Vanillin	0.7314	0.0879	100	2.2695
E12	Vanillin	0.0454	0.0412	100	−0.0674
F1	Hydroxymethylfurfural	0.5999	0.0761	100	1.832
F2	Hydroxymethylfurfural	2.2231	0.1725	100	7.413
F3	Hydroxymethylfurfural	0.552	0.0741	100	1.6642
F4	Hydroxymethylfurfural	0.0534	0.0421	100	−0.0414
F5	Furfural	0.7096	0.0825	100	2.2096
F6	Furfural	1.0803	0.1062	100	3.478
F8	Furfural	0.0584	0.0425	100	−0.0246
F9	Vanillin	0.0471	0.0423	100	−0.0652
F10	Vanillin	0.0508	0.0417	100	−0.0495
F11	Vanillin	0.0526	0.0426	100	−0.0462
F12	Vanillin	0.0469	0.0414	100	−0.0626
G1	Hydroxymethylfurfural	1.0541	0.1032	100	3.3932
G2	Hydroxymethylfurfural	2.5655	0.1938	100	8.5868
G3	Hydroxymethylfurfural	0.8516	0.0927	100	2.6913
G4	Reference	3.0141	0.2264	100	10.1074
G5	Furfural	1.322	0.1184	100	4.3169
G6	Furfural	1.2161	0.112	100	3.9532
G8	Reference	3.0176	0.2252	100	10.1246
G9	Vanillin	0.4098	0.0687	100	1.1641
G10	Vanillin	0.0461	0.0413	100	−0.0652
G11	Vanillin	0.3971	0.066	100	1.1276
G12	Reference	2.278	0.1797	100	7.5874

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Table A.10: *continued from the previous page*

Well	Test series	$A418$	$A480$	DF	$c(Glc)$
H12	Reference	2.8776	0.2149	100	9.6505

Table A.11: D-Glucose concentrations after 48 h, complete raw data of ISr. The plate ISr was incubated with 1.0 ml SM18 P30S with 2.00 $g \cdot l^{-1}$ of inhibitor for 48 h at 30 °C and 1000 min^{-1}. Afterwards, the D-glucose concentrations were determined using a D-glucose assay. In this table, the raw data of all utilized wells are given. These data were used to build the summary statistics in table 3.11 on page 76. For the calculation of the summary statistics, only non-medium values of growing strains of the corresponding test series were used. Since these raw data were taken directly from the photometer software, figures do not imply significance. Abbreviations: $A418$: absorbance at 418 nm (dimensionless); $A480$: absorbance at 480 nm (dimensionless); DF: dilution factor (dimensionless); $c(Glc)$: D-glucose in $g \cdot l^{-1}$ after 48 h.

Well	Test series	$A418$	$A480$	DF	$c(Glc)$
A1	Formic acid	0.054	0.0452	100	−0.0506
A2	Formic acid	0.0554	0.0425	100	−0.0356
A3	Formic acid	0.055	0.0412	100	−0.0323
A4	Formic acid	1.1841	0.1221	100	3.7993
A5	Acetic acid	0.0489	0.0409	100	−0.0535
A6	Acetic acid	2.2394	0.174	100	7.4671
A7	Acetic acid	0.0645	0.0504	100	−0.0312
A8	Acetic acid	1.0574	0.1051	100	3.3983
A9	Laevulinic acid	1.932	0.1551	100	6.4125
A10	Laevulinic acid	1.4044	0.1231	100	4.6009
A11	Laevulinic acid	0.0522	0.0413	100	−0.0429
A12	Laevulinic acid	0.0606	0.0459	100	−0.029
B1	Formic acid	0.0572	0.0458	100	−0.0411
B2	Formic acid	0.0548	0.0429	100	−0.0392
B3	Formic acid	0.0595	0.045	100	−0.0297
B4	Formic acid	0.0532	0.0423	100	−0.0429
B5	Acetic acid	0.0571	0.0431	100	−0.0316
B6	Acetic acid	0.0586	0.0431	100	−0.0261
B7	Acetic acid	1.6704	0.1417	100	5.5053
B8	Acetic acid	1.2036	0.1133	100	3.9027
B9	Laevulinic acid	0.0506	0.0422	100	−0.052
B10	Laevulinic acid	1.2687	0.1213	100	4.1115
B11	Laevulinic acid	2.6107	0.2044	100	8.7132
C1	Formic acid	0.2383	0.0529	100	0.595
C2	Formic acid	0.0527	0.0419	100	−0.0433
C3	Formic acid	0.0511	0.0417	100	−0.0484
C4	Formic acid	0.0556	0.0414	100	−0.0308
C5	Acetic acid	1.1538	0.1057	100	3.7485
C6	Acetic acid	0.0553	0.0411	100	−0.0308
C7	Acetic acid	1.6331	0.1388	100	5.3795

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Table A.11: *continued from the previous page*

Well	Test series	$A418$	$A480$	DF	$c(Glc)$
C8	Acetic acid	2.7702	0.2088	100	9.2802
C9	Laevulinic acid	0.0544	0.0414	100	−0.0352
C10	Laevulinic acid	0.4198	0.0756	100	1.1754
C11	Laevulinic acid	2.9216	0.2144	100	9.8132
C12	Laevulinic acid	2.5413	0.1973	100	8.4855
D1	Formic acid	0.0504	0.0432	100	−0.0564
D2	Formic acid	0.0552	0.0432	100	−0.0389
D3	Formic acid	0.0606	0.0495	100	−0.0422
D4	Formic acid	0.0542	0.0429	100	−0.0414
D5	Acetic acid	0.1252	0.0474	100	0.2017
D6	Acetic acid	0.0498	0.0425	100	−0.0561
D7	Acetic acid	0.0583	0.0449	100	−0.0338
D8	Acetic acid	1.9423	0.1571	100	6.4429
D9	Laevulinic acid	0.0559	0.0447	100	−0.0418
D10	Laevulinic acid	0.4276	0.0763	100	1.2014
D11	Laevulinic acid	0.0562	0.044	100	−0.0381
D12	Laevulinic acid	1.1581	0.1103	100	3.7474
E1	Formic acid	0.055	0.0452	100	−0.0469
E2	Formic acid	0.0541	0.0421	100	−0.0389
E3	Formic acid	1.5165	0.1325	100	4.9763
E4	Formic acid	0.0709	0.0593	100	−0.0403
E5	Acetic acid	0.0592	0.0474	100	−0.0396
E6	Acetic acid	1.4832	0.129	100	4.8674
E7	Acetic acid	0.8536	0.0904	100	2.7071
E8	Acetic acid	1.9543	0.1548	100	6.4952
E9	Laevulinic acid	0.0519	0.0426	100	−0.0487
E10	Laevulinic acid	0.3986	0.0738	100	1.1045
E11	Laevulinic acid	0.054	0.042	100	−0.0389
E12	Laevulinic acid	1.1074	0.1061	100	3.5774
F1	Formic acid	0.0555	0.0426	100	−0.0356
F2	Formic acid	0.0559	0.0427	100	−0.0345
F3	Formic acid	3.1057	0.2572	100	10.3297
F4	Formic acid	0.0637	0.0548	100	−0.0502
F5	Acetic acid	0.0645	0.0542	100	−0.0451
F6	Acetic acid	0.0624	0.0425	100	−0.01
F7	Acetic acid	0.0591	0.0479	100	−0.0418
F8	Acetic acid	0.0604	0.0429	100	−0.0188
F9	Laevulinic acid	0.0702	0.0591	100	−0.0422
F10	Laevulinic acid	2.0483	0.1609	100	6.8165
F11	Laevulinic acid	0.0557	0.0427	100	−0.0352
F12	Laevulinic acid	2.2758	0.1775	100	7.5874
G1	Formic acid	0.0609	0.0537	100	−0.0564
G2	Formic acid	0.056	0.042	100	−0.0316
G3	Formic acid	2.6202	0.1949	100	8.7827

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Table A.11: *continued from the previous page*

Well	Test series	$A418$	$A480$	DF	$c(Glc)$
G4	Formic acid	0.0726	0.0623	100	−0.0451
G5	Acetic acid	2.55	0.1916	100	8.5382
G6	Acetic acid	0.0479	0.0411	100	−0.0579
G7	Acetic acid	2.0273	0.161	100	6.7393
G8	Acetic acid	0.061	0.0434	100	−0.0184
G9	Laevulinic acid	0.2568	0.0546	100	0.6564
G10	Laevulinic acid	0.0553	0.0429	100	−0.0374
G11	Laevulinic acid	2.0081	0.1582	100	6.6794
G12	Laevulinic acid	0.0527	0.0439	100	−0.0506
H9	Reference	3.0258	0.229	100	10.1407
H10	Reference	3.1669	0.2406	100	10.6141
H11	Reference	2.7644	0.2039	100	9.2769
H12	Reference	3.1669	0.2404	100	10.6148

Table A.12: Exopolysaccharide monomer compositions of inhibitor high-content screening. The exopolysaccharide producers on the plates ISp and ISr were incubated for 48 h in SM18 P30S, a medium which contained 10.0 $g \cdot l^{-1}$ D-glucose as the main carbon source. The aldose composition of the exopolysaccharides are summarized in this table. The following analytes were not found in any sample and, thus, left out from the table: *N*-acetyl-D-glucosamine, 2-deoxy-D-glucose, 2-deoxy-D-ribose, D-galacturonic acid, *N*-acetyl-D-galactosamine and lactose. D-Glucose was present in every sample and was quantified after the gel filtration using the D-glucose assay. The residual D-glucose concentration was subtracted from the monomeric D-glucose found in the HPLC-MS screening. Therefore, it is possible to have the value '0' for D-glucose which differs from 'not detected'. For three samples, the D-glucose concentration after the gel filtration was greater than the D-glucose concentration found in the HPLC-MS screening and set to zero: ISp.A4 (453 $mg \cdot l^{-1}$ vs. 519 $mg \cdot l^{-1}$, *Xyl2.B7* with hydroxymethylfurfural), ISp.A10 (277 $mg \cdot l^{-1}$ vs. 278 $mg \cdot l^{-1}$, *Xyl1.F10* with furfural) and ISr.F10 (349 $mg \cdot l^{-1}$ vs. 391 $mg \cdot l^{-1}$, *Xyl1.D12* with laevulinic acid). Since L-arabinose could not be distinguished from D-xylose, the combined values are given here. Abbreviations: Inhibitors: Acet.: acetic acid; Form.: formic acid; Fur.: furfural; HMF: hydroxymethylfurfural; Laev.: laevulinic acid; Van.: vanillin. Aldoses: Cel: cellobiose; Fuc: fucose; Gal: D-galactose; GalN: D-galactosamine; Gen: gentiobiose; Glc: D-glucose; GlcN: D-glucosamine; GlcUA: D-glucuronic acid; Man: D-mannose; Rha: L-rhamnose; Rib: D-ribose; Xyl/Ara: D-xylose and L-arabinose; Sum: sum of all values to the left of the same row. Other: n.d.: not detected. All values are in $mg \cdot l^{-1}$.

Well	Inhibitor	Cel	Fuc	Gal	GalN	Gen	Glc	GlcN	GlcUA	Man	Rha	Rib	Xyl/Ara	Sum
ISp.A1	HMF	n.d.	n.d.	n.d.	n.d.	n.d.	217	n.d.	36	48	310	n.d.	n.d.	611
ISp.A2	HMF	n.d.	n.d.	22	n.d.	n.d.	359	n.d.	79	n.d.	593	n.d.	n.d.	1053
ISp.A3	HMF	n.d.	n.d.	20	n.d.	n.d.	12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	32
ISp.A4	HMF	n.d.	n.d.	13	n.d.	n.d.	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	13
ISp.A5	Fur.	n.d.	n.d.	n.d.	n.d.	n.d.	7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7
ISp.A6	Fur.	n.d.	n.d.	15	n.d.	n.d.	61	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	76
ISp.A7	Fur.	n.d.	n.d.	10	n.d.	n.d.	116	n.d.	93	16	18	n.d.	n.d.	253
ISp.A8	Fur.	n.d.	n.d.	10	n.d.	n.d.	82	23	n.d.	n.d.	n.d.	n.d.	n.d.	115
ISp.A9	Van.	n.d.	n.d.	n.d.	n.d.	n.d.	23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	23
ISp.A10	Van.	n.d.	n.d.	n.d.	n.d.	n.d.	0	n.d.	n.d.	n.d.	6	n.d.	n.d.	6
ISp.A11	Van.	n.d.	n.d.	n.d.	n.d.	n.d.	9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	9
ISp.A12	Van.	n.d.	n.d.	n.d.	n.d.	n.d.	125	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	125
ISp.B1	HMF	n.d.	n.d.	n.d.	n.d.	n.d.	54	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	54
ISp.B2	HMF	n.d.	n.d.	25	n.d.	n.d.	390	n.d.	70	n.d.	594	n.d.	n.d.	1079
ISp.B3	HMF	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0

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Table A.12: *continued from the previous page*

Well	Inhibitor	Cel	Fuc	Gal	GalN	Gen	Glc	GlcN	GlcUA	Man	Rha	Rib	Xyl/Ara	Sum
ISp.B4	HMF	n.d.	n.d.	13	n.d.	n.d.	130	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	143
ISp.B5	Fur.	n.d.	n.d.	n.d.	n.d.	n.d.	206	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	206
ISp.B6	Fur.	n.d.	n.d.	n.d.	n.d.	n.d.	114	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	114
ISp.B7	Fur.	n.d.	n.d.	n.d.	n.d.	n.d.	13	20	n.d.	n.d.	n.d.	n.d.	n.d.	33
ISp.B8	Fur.	n.d.	n.d.	13	n.d.	n.d.	97	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	110
ISp.B9	Van.	n.d.	n.d.	141	n.d.	n.d.	6	n.d.	18	n.d.	170	n.d.	n.d.	335
ISp.B10	Van.	n.d.	n.d.	n.d.	n.d.	n.d.	186	n.d.	10	n.d.	8	n.d.	n.d.	204
ISp.B11	Van.	n.d.	n.d.	27	n.d.	n.d.	60	n.d.	n.d.	n.d.	53	n.d.	n.d.	140
ISp.B12	Van.	n.d.	n.d.	12	n.d.	n.d.	11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	23
ISp.C1	HMF	n.d.	n.d.	n.d.	n.d.	n.d.	71	16	n.d.	n.d.	n.d.	n.d.	n.d.	87
ISp.C2	HMF	n.d.	n.d.	n.d.	n.d.	n.d.	101	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	101
ISp.C3	HMF	n.d.	n.d.	19	n.d.	n.d.	12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	31
ISp.C4	HMF	n.d.	n.d.	12	n.d.	n.d.	122	n.d.	88	25	26	n.d.	n.d.	273
ISp.C5	Fur.	n.d.	n.d.	n.d.	n.d.	n.d.	118	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	118
ISp.C6	Fur.	n.d.	n.d.	n.d.	n.d.	n.d.	29	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	29
ISp.C7	Fur.	12	n.d.	48	n.d.	n.d.	292	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	352
ISp.C8	Fur.	n.d.	n.d.	n.d.	n.d.	n.d.	113	n.d.	n.d.	22	n.d.	n.d.	n.d.	135
ISp.C9	Van.	n.d.	n.d.	n.d.	n.d.	n.d.	155	n.d.	27	n.d.	266	n.d.	n.d.	448
ISp.C10	Van.	n.d.	n.d.	n.d.	n.d.	n.d.	9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	9
ISp.C11	Van.	n.d.	n.d.	n.d.	n.d.	n.d.	33	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	33
ISp.C12	Van.	n.d.	n.d.	162	n.d.	n.d.	0	n.d.	22	n.d.	190	n.d.	n.d.	374
ISp.D1	HMF	n.d.	n.d.	15	n.d.	n.d.	77	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	92
ISp.D2	HMF	n.d.	n.d.	n.d.	n.d.	n.d.	110	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	110
ISp.D3	HMF	n.d.	n.d.	11	n.d.	n.d.	109	n.d.	76	19	13	n.d.	n.d.	228
ISp.D4	HMF	n.d.	n.d.	10	n.d.	n.d.	111	n.d.	84	22	15	n.d.	n.d.	242
ISp.D5	Fur.	n.d.	n.d.	9	n.d.	n.d.	24	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	33
ISp.D6	Fur.	n.d.	n.d.	16	n.d.	n.d.	11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	27

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Table A.12: *continued from the previous page*

Well	Inhibitor	Cel	Fuc	Gal	GalN	Gen	Glc	GlcN	GlcUA	Man	Rha	Rib	Xyl/Ara	Sum
ISp.D7	Fur.	n.d.	n.d.	n.d.	n.d.	n.d.	110	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	110
ISp.D8	Fur.	n.d.	n.d.	10	n.d.	n.d.	83	n.d.	65	15	14	n.d.	n.d.	187
ISp.D9	Van.	n.d.	n.d.	11	n.d.	n.d.	148	n.d.	27	n.d.	263	n.d.	n.d.	449
ISp.D10	Van.	n.d.	n.d.	n.d.	n.d.	n.d.	6	n.d.	n.d.	n.d.	6	n.d.	n.d.	12
ISp.D11	Van.	n.d.	n.d.	n.d.	n.d.	n.d.	60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	60
ISp.D12	Van.	n.d.	n.d.	n.d.	n.d.	n.d.	44	n.d.	n.d.	10	n.d.	n.d.	n.d.	54
ISp.E1	HMF	n.d.	n.d.	n.d.	n.d.	n.d.	83	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	83
ISp.E2	HMF	n.d.	n.d.	n.d.	n.d.	n.d.	31	19	n.d.	n.d.	n.d.	n.d.	n.d.	50
ISp.E3	HMF	n.d.	n.d.	n.d.	n.d.	n.d.	26	15	n.d.	n.d.	n.d.	n.d.	n.d.	41
ISp.E4	HMF	n.d.	n.d.	11	n.d.	n.d.	24	n.d.	n.d.	n.d.	15	n.d.	n.d.	50
ISp.E5	Fur.	n.d.	n.d.	11	n.d.	n.d.	32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	43
ISp.E6	Fur.	n.d.	n.d.	16	n.d.	n.d.	17	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	33
ISp.E7	Fur.	n.d.	n.d.	25	n.d.	n.d.	194	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	219
ISp.E8	Fur.	n.d.	n.d.	n.d.	n.d.	n.d.	84	n.d.	n.d.	13	14	n.d.	n.d.	111
ISp.E9	Van.	n.d.	25	19	n.d.	n.d.	21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	65
ISp.E10	Van.	n.d.	n.d.	n.d.	n.d.	n.d.	38	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	38
ISp.E11	Van.	n.d.	n.d.	n.d.	n.d.	n.d.	383	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	383
ISp.E12	Van.	n.d.	n.d.	168	n.d.	n.d.	2	n.d.	23	n.d.	191	n.d.	n.d.	384
ISp.F1	HMF	n.d.	n.d.	n.d.	n.d.	n.d.	17	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	17
ISp.F2	HMF	n.d.	n.d.	17	n.d.	n.d.	60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	77
ISp.F3	HMF	n.d.	n.d.	87	n.d.	n.d.	129	n.d.	n.d.	81	190	n.d.	n.d.	487
ISp.F4	HMF	n.d.	n.d.	11	n.d.	n.d.	42	n.d.	n.d.	n.d.	15	n.d.	n.d.	68
ISp.F5	Fur.	n.d.	n.d.	n.d.	n.d.	n.d.	22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	22
ISp.F6	Fur.	n.d.	n.d.	16	n.d.	n.d.	8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	24
ISp.F7	Fur.	n.d.	n.d.	n.d.	n.d.	n.d.	82	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	82
ISp.F8	Fur.	n.d.	n.d.	14	n.d.	n.d.	37	n.d.	n.d.	11	23	n.d.	n.d.	85
ISp.F9	Van.	n.d.	n.d.	142	n.d.	n.d.	3	n.d.	19	n.d.	167	n.d.	n.d.	331

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Table A.12: *continued from the previous page*

Well	Inhibitor	Cel	Fuc	Gal	GalN	Gen	Glc	GlcN	GlcUA	Man	Rha	Rib	Xyl/Ara	Sum
ISp.F10	Van.	n.d.	n.d.	n.d.	n.d.	n.d.	14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	14
ISp.F11	Van.	n.d.	n.d.	15	n.d.	n.d.	23	13	n.d.	19	6	n.d.	n.d.	76
ISp.F12	Van.	n.d.	n.d.	15	n.d.	n.d.	13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	28
ISp.G1	HMF	n.d.	n.d.	n.d.	n.d.	n.d.	16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	16
ISp.G2	HMF	n.d.	n.d.	n.d.	n.d.	n.d.	121	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	121
ISp.G3	HMF	14	n.d.	72	n.d.	n.d.	838	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	924
ISp.G5	Fur.	n.d.	n.d.	8	n.d.	n.d.	38	20	n.d.	n.d.	n.d.	n.d.	n.d.	66
ISp.G6	Fur.	n.d.	n.d.	n.d.	n.d.	n.d.	29	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	29
ISp.G7	Fur.	n.d.	n.d.	n.d.	n.d.	n.d.	94	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	94
ISp.G9	Van.	n.d.	n.d.	37	n.d.	n.d.	62	n.d.	n.d.	27	31	n.d.	n.d.	157
ISp.G10	Van.	n.d.	n.d.	146	n.d.	n.d.	2	n.d.	18	n.d.	175	n.d.	n.d.	341
ISp.G11	Van.	n.d.	n.d.	n.d.	n.d.	n.d.	209	n.d.	n.d.	n.d.	8	n.d.	n.d.	217
ISr.A1	Form.	n.d.	n.d.	20	n.d.	n.d.	651	n.d.	161	n.d.	1098	n.d.	n.d.	1930
ISr.A2	Form.	n.d.	n.d.	n.d.	n.d.	n.d.	12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	12
ISr.A3	Form.	n.d.	n.d.	n.d.	n.d.	n.d.	14	14	n.d.	n.d.	n.d.	n.d.	n.d.	28
ISr.A4	Form.	64	n.d.	n.d.	n.d.	n.d.	801	n.d.	72	511	24	n.d.	n.d.	1472
ISr.A5	Acet.	n.d.	n.d.	9	n.d.	n.d.	12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	21
ISr.A6	Acet.	n.d.	n.d.	n.d.	n.d.	n.d.	97	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	97
ISr.A7	Acet.	n.d.	n.d.	744	n.d.	n.d.	2	n.d.	79	n.d.	891	n.d.	n.d.	1716
ISr.A8	Acet.	26	n.d.	194	n.d.	n.d.	1835	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2055
ISr.A9	Laev.	n.d.	n.d.	20	n.d.	n.d.	229	n.d.	n.d.	25	21	n.d.	n.d.	295
ISr.A10	Laev.	n.d.	n.d.	n.d.	n.d.	n.d.	175	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	175
ISr.A11	Laev.	n.d.	n.d.	12	n.d.	n.d.	104	n.d.	62	11	15	n.d.	n.d.	204
ISr.A12	Laev.	n.d.	n.d.	61	n.d.	n.d.	33	n.d.	n.d.	n.d.	25	n.d.	n.d.	119
ISr.B1	Form.	n.d.	n.d.	16	n.d.	n.d.	589	n.d.	137	n.d.	1065	n.d.	n.d.	1807
ISr.B2	Form.	n.d.	n.d.	11	n.d.	n.d.	14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	25
ISr.B3	Form.	n.d.	n.d.	n.d.	n.d.	n.d.	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5

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Table A.12: *continued from the previous page*

Well	Inhibitor	Cel	Fuc	Gal	GalN	Gen	Glc	GlcN	GlcUA	Man	Rha	Rib	Xyl/Ara	Sum
ISr.B4	Form.	n.d.	n.d.	n.d.	n.d.	n.d.	16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	16
ISr.B5	Acet.	n.d.	n.d.	n.d.	n.d.	n.d.	27	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	27
ISr.B6	Acet.	n.d.	n.d.	n.d.	17	n.d.	91	45	n.d.	21	n.d.	n.d.	n.d.	174
ISr.B7	Acet.	n.d.	n.d.	n.d.	25	n.d.	112	45	n.d.	n.d.	n.d.	n.d.	n.d.	182
ISr.B8	Acet.	n.d.	n.d.	n.d.	n.d.	n.d.	91	13	n.d.	n.d.	n.d.	n.d.	n.d.	104
ISr.B9	Laev.	n.d.	n.d.	n.d.	n.d.	n.d.	198	n.d.	39	47	467	n.d.	n.d.	751
ISr.B10	Laev.	n.d.	n.d.	n.d.	n.d.	n.d.	168	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	168
ISr.B11	Laev.	n.d.	n.d.	15	n.d.	n.d.	140	n.d.	n.d.	32	n.d.	n.d.	n.d.	187
ISr.B12	Laev.	n.d.	n.d.	n.d.	n.d.	n.d.	107	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	107
ISr.C1	Form.	n.d.	n.d.	n.d.	19	n.d.	68	48	n.d.	n.d.	n.d.	n.d.	n.d.	135
ISr.C2	Form.	n.d.	n.d.	n.d.	n.d.	n.d.	15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	15
ISr.C3	Form.	n.d.	n.d.	n.d.	n.d.	n.d.	6	n.d.	n.d.	n.d.	n.d.	n.d.	7	13
ISr.C4	Form.	n.d.	n.d.	n.d.	n.d.	n.d.	7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7
ISr.C5	Acet.	n.d.	n.d.	19	n.d.	n.d.	100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	119
ISr.C6	Acet.	n.d.	n.d.	n.d.	n.d.	n.d.	107	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	107
ISr.C7	Acet.	15	n.d.	62	n.d.	n.d.	379	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	456
ISr.C8	Acet.	n.d.	n.d.	n.d.	n.d.	n.d.	42	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	42
ISr.C9	Laev.	n.d.	n.d.	n.d.	n.d.	n.d.	193	n.d.	37	51	467	n.d.	n.d.	748
ISr.C10	Laev.	n.d.	n.d.	14	n.d.	n.d.	65	n.d.	21	24	n.d.	n.d.	n.d.	124
ISr.C11	Laev.	n.d.	26	n.d.	n.d.	n.d.	128	n.d.	16	n.d.	n.d.	n.d.	n.d.	170
ISr.C12	Laev.	n.d.	n.d.	100	n.d.	n.d.	905	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1005
ISr.D1	Form.	n.d.	n.d.	15	n.d.	n.d.	16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	31
ISr.D2	Form.	n.d.	n.d.	n.d.	n.d.	n.d.	11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	11
ISr.D3	Form.	n.d.	n.d.	213	n.d.	n.d.	269	n.d.	40	241	433	n.d.	n.d.	1196
ISr.D4	Form.	n.d.	n.d.	214	n.d.	n.d.	179	n.d.	25	n.d.	n.d.	n.d.	n.d.	418
ISr.D5	Acet.	n.d.	n.d.	n.d.	n.d.	n.d.	7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7
ISr.D6	Acet.	n.d.	n.d.	18	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	18

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Table A.12: *continued from the previous page*

Well	Inhibitor	Cel	Fuc	Gal	GalN	Gen	Glc	GlcN	GlcUA	Man	Rha	Rib	Xyl/Ara	Sum
ISr.D7	Acet.	n.d.	n.d.	179	n.d.	n.d.	68	n.d.	n.d.	n.d.	62	n.d.	n.d.	309
ISr.D8	Acet.	n.d.	n.d.	n.d.	n.d.	n.d.	63	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	63
ISr.D9	Laev.	n.d.	n.d.	n.d.	n.d.	n.d.	168	n.d.	31	44	402	n.d.	n.d.	645
ISr.D10	Laev.	n.d.	13	32	n.d.	n.d.	167	n.d.	29	36	20	n.d.	n.d.	297
ISr.D11	Laev.	n.d.	n.d.	52	n.d.	n.d.	24	n.d.	n.d.	n.d.	20	n.d.	n.d.	96
ISr.D12	Laev.	n.d.	n.d.	11	n.d.	n.d.	76	13	n.d.	n.d.	n.d.	n.d.	n.d.	100
ISr.E1	Form.	n.d.	n.d.	21	n.d.	n.d.	16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	37
ISr.E2	Form.	n.d.	n.d.	n.d.	n.d.	n.d.	11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	11
ISr.E3	Form.	51	n.d.	195	n.d.	11	1205	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1462
ISr.E4	Form.	n.d.	n.d.	222	n.d.	n.d.	13	n.d.	n.d.	448	n.d.	n.d.	n.d.	683
ISr.E5	Acet.	n.d.	n.d.	24	n.d.	n.d.	662	n.d.	94	n.d.	1030	n.d.	n.d.	1810
ISr.E6	Acet.	n.d.	n.d.	n.d.	n.d.	n.d.	28	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	28
ISr.E7	Acet.	36	n.d.	134	n.d.	12	1149	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1331
ISr.E8	Acet.	n.d.	n.d.	19	n.d.	n.d.	120	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	139
ISr.E9	Laev.	n.d.	n.d.	n.d.	n.d.	n.d.	205	n.d.	35	51	479	n.d.	n.d.	770
ISr.E10	Laev.	n.d.	n.d.	12	n.d.	n.d.	51	n.d.	20	10	n.d.	n.d.	n.d.	93
ISr.E11	Laev.	n.d.	n.d.	49	n.d.	n.d.	24	n.d.	n.d.	n.d.	18	n.d.	n.d.	91
ISr.E12	Laev.	n.d.	n.d.	20	n.d.	n.d.	85	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	105
ISr.F1	Form.	57	n.d.	257	n.d.	34	1269	n.d.	36	35	n.d.	n.d.	n.d.	1688
ISr.F2	Form.	n.d.	n.d.	n.d.	n.d.	n.d.	23	n.d.	16	n.d.	n.d.	n.d.	n.d.	39
ISr.F3	Form.	n.d.	n.d.	n.d.	n.d.	n.d.	150	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	150
ISr.F4	Form.	n.d.	n.d.	227	n.d.	n.d.	18	n.d.	n.d.	454	n.d.	n.d.	n.d.	699
ISr.F5	Acet.	n.d.	n.d.	32	n.d.	n.d.	686	n.d.	99	n.d.	1058	n.d.	n.d.	1875
ISr.F6	Acet.	n.d.	n.d.	n.d.	n.d.	n.d.	356	n.d.	16	n.d.	n.d.	n.d.	n.d.	372
ISr.F7	Acet.	26	n.d.	70	n.d.	n.d.	557	14	n.d.	n.d.	n.d.	13	n.d.	680
ISr.F8	Acet.	n.d.	n.d.	n.d.	n.d.	n.d.	333	n.d.	17	n.d.	n.d.	n.d.	n.d.	350
ISr.F9	Laev.	n.d.	n.d.	231	n.d.	n.d.	288	n.d.	51	67	244	n.d.	n.d.	881

continued on the next page

Table A.12: *continued from the previous page*

Well	Inhibitor	Cel	Fuc	Gal	GalN	Gen	Glc	GlcN	GlcUA	Man	Rha	Rib	Xyl/Ara	Sum
ISr.F10	Laev.	n.d.	n.d.	n.d.	n.d.	n.d.	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0
ISr.F11	Laev.	n.d.	n.d.	50	n.d.	n.d.	25	n.d.	n.d.	n.d.	18	n.d.	n.d.	93
ISr.F12	Laev.	n.d.	n.d.	17	n.d.	n.d.	114	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	131
ISr.G1	Form.	n.d.	n.d.	28	n.d.	n.d.	30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	58
ISr.G2	Form.	n.d.	n.d.	n.d.	n.d.	n.d.	6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6
ISr.G3	Form.	n.d.	n.d.	18	n.d.	n.d.	198	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	216
ISr.G4	Form.	n.d.	n.d.	222	n.d.	n.d.	273	n.d.	39	248	457	n.d.	n.d.	1239
ISr.G5	Acet.	n.d.	n.d.	n.d.	n.d.	n.d.	123	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	123
ISr.G6	Acet.	n.d.	n.d.	n.d.	n.d.	n.d.	19	17	n.d.	n.d.	n.d.	18	n.d.	54
ISr.G7	Acet.	24	n.d.	123	n.d.	11	1103	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1261
ISr.G8	Acet.	n.d.	n.d.	n.d.	n.d.	n.d.	249	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	249
ISr.G9	Laev.	n.d.	n.d.	n.d.	n.d.	n.d.	23	n.d.	n.d.	45	n.d.	n.d.	n.d.	68
ISr.G10	Laev.	n.d.	n.d.	n.d.	n.d.	n.d.	213	n.d.	n.d.	19	n.d.	n.d.	n.d.	232
ISr.G11	Laev.	n.d.	n.d.	30	n.d.	n.d.	117	n.d.	n.d.	36	61	n.d.	n.d.	244
ISr.G12	Laev.	n.d.	n.d.	n.d.	n.d.	n.d.	99	n.d.	n.d.	n.d.	11	n.d.	n.d.	110

A.2.4 Strain Selection

```
CATGCAGTCGAGCGGACTTGAATGGAGAGCTTGCTCTCCTGATGGTTAGC    50
GGCGGACGGGTGAGTAACACGTAGGCAACCTGCCTGCAAGACCGGGATAA   100
CCCACGGAAACGTGAGCTAATACCGGATATCTCATTTCCTCGCCTGAGGG   150
AATGACGAAAGACGGAGCAATCTGTCACTTGCGGATGGGCCTGCGGCGCA   200
TTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGA   250
CCTGAGAGGGTGAACGGCCACACTGGGACTGAGACACGGCCCAGACTCCT   300
ACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGA   350
GCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGCC   400
AGGGAAGAACGTCCGGTAGAGTAACTGCTACCGGAGTGACGGTACCTGAG   450
AAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGG   500
GCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTCATT   550
TAAGTCTGGTGTTTAAGGCCAAGGCTCAACCTTGGTTCGCACTGGAAACT   600
GGGTGACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGA   650
AATGCGTAGATATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGGC   700
TGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATA   750
CCCTGGTAGTCCACGCCGTAAACGATGAATGCTAGGTGTTAGGGGTTTCG   800
ATACCCTTGGTGCCGAAGTTAACACATTAAGCATTCCGCCTGGGGAGTAC   850
GGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCAGT   900
GGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTG   950
ACATCCCTCTGACCGGTACAGAGATGTACCTTTCCTTCGGGACAGAGGAG  1000
ACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAA  1050
GTCCCGCAACGAGCGCAACCCTTGATTTTAGTTGCCAGCACTTCGGGTGG  1100
GCACTCTAGAATGACTGCCGGTGACAAACCGGAGGAAGGCGGGGATGACG  1150
TCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTACTACAATGGC  1200
CAGTACAACGGGAAGCGAAGCCGCGAGGTGGAGCCAATCCTATCAAAGCT  1250
GGTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGTCGGAATT  1300
GCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGTCTTG  1350
TACACACCGCCCGTCACACCACGAGAGTTTACAACACCCGAAGTCGGTGA  1400
GGTAACCGCAAGGAGCCAGCCGCCGAAGGTGGGGTAGATGATTTGGGGG  1449
```

Figure A.1: 16S rDNA sequence of *Paenibacillus* 2H7. Genomic DNA of *Paenibacillus* 2H7 was amplified according to section 2.6.3 on page 54. Sequencing yielded two forward sequences and two reverse sequences. The sequences were sanitized, aligned and the concensus sequence built from them as described in section 2.3.1 on page 32.

A.2.5 Parallel Fermentation with Lignocellulose Hydrolysate

Listing A.4: make-pf-plot-data.r: R script to read in and process several data files with fermentation and results from off-line measurements and save the final data for faster plot generation.

```
# # # # # # # # # # # # # # # # # # # # #
# Generate graphs of LCHF0
# LCH-PF, two figures, each with four y-axes
# # # # # # # # # # # # # # # # # # # # #
library(splitstackshape) # cSplit
library(lubridate) # as.duration
library(plyr) # round_any, join
library(zoo) # rollapply
library(readxl) # read_excel


# # # # # # # # # # # # # # # # # # # # #
# Data structure and type definitions
# Conversion of sample number to sampling time in seconds
# blk1smptim = block sample times
blk1smptim <- read.table(text =
"sample.block.1 t
0   3600
1   50400
2   90600
3   135000
4   172800
5   223800
6   259800
7   316800
8   329400", # D600 only sample at the end of the process
header = TRUE, colClasses = c("numeric", "numeric"))
blk1smptim$t <- as.duration(blk1smptim$t)

blk2smptim <- read.table(text =
"sample.block.2 t
0   3600
1   50400
2   90600
3   135000
4   172800
5   223800
6   259800
7   316800
8   346800
9   403200
10  432000
11  482400",
header = TRUE, colClasses = c("numeric", "numeric"))
blk2smptim$t <- as.duration(blk2smptim$t)

# Dilution factors
# lchfa1df = LCHFA1 dilution factor
lchfa1df <- 5.05 # 61 µl for neutralization
# lchfa223df = LCHFA2_2 and LCHFA2_3 dilution factor
lchfa223df <- 400

# # # # # # # # # # # # # # # # # # # # #
# Function definitions
# Function for reading in fermentation data
getFermDat <- function(df, file){
    # Read in data
    df <- as.data.frame(
        cSplit(
            read.table(file, header = TRUE,
                # Names for description of the expected content only
                col.names = c("Date and time",
```

```
                    "Time after inoculation in hh:mm:ss", "pH value",
                    "DO in %", "CO2 in A.U."),
                colClasses = c("NULL", "character", "numeric", "numeric",
                    "numeric"),
                sep = "\t", dec = '.'),
            splitCols = "Time.after.inoculation.in.hh.mm.ss", sep =":",
            drop = TRUE))
    # Give temporary names
    colnames(df) <- c("pH value", "DO in %", "CO2 in A.U.", "HH", "MM", "SS")
    return(df)
    }

# Function for preparing fermentation data
prepFermDat <- function(df, shorthand){
    # Remove unnecessary pH column
    df <- df[ , c(2, 3, 4, 5, 6)]
    # Reduce time to duration in seconds and
    # round down to 30 s to have matching times when joining columns
    # Rounding down due to F2 data which has seconds of
    # 14, 44, 14, 45, 15, 44, 15, 45 ... giving ~25% of duplicate values
    df$SS <- round_any(
        as.duration(
            df$SS + (df$MM * 60) + (df$HH * 3600))
        , 30, floor)
    # Remove now unnecessary hours and minutes columns, reorder columns
    df <- df[ , c(5, 1, 2)]
    # Name columns sensibly
    # Explanations:
    #   [[1]]   t: process time in s
    #   [[2]]   DO: dissolved oxygen in percent of max. calibrated at process start
    #   [[3]]   CO2: CO2 in off-gas in percent
    colnames(df) <- c("t", paste('DO', shorthand, sep='.'),
        paste('CO2', shorthand, sep='.'))
    # Rolling average over 50 of DO values, last 50 values take median
    df[[2]] <- rollapply(
        df[[2]], 50, mean, fill=c(mean(head(df[[2]], 50)),
        NA, median(tail(df[[2]], 50))))
    return(df)
    }


# # # # # # # # # # # # # # # # # # # # #
# Input fermentation/on-line data
#   Data of 8 fermenters: time, pH, DO, CO2

# Vector with filenames
infiles <- c('f1.txt', 'f2.txt', 'f3.txt', 'f4.txt',
    'f5.txt', 'f6.txt', 'f7.txt', 'f8.txt')
# String with relative path to files
directory <- 'ferm-dat/'
# Create 'indon' as empty list; indon = in data, online
indon <- list()

# Loop for the actual input
for (i in 1:8) {
    indon[[i]] <- getFermDat(indon[[i]], paste(directory, infiles[[i]], sep = ''))
}


# # # # # # # # # # # # # # # # # # # # #
# Input off-line sample data
#   Data of 8 fermenters: time, D600, CDM, Glucose concentration (glucose
#   assay), Molar mass at RI peak, Furfural (found in block 2, only; data for
#   all 8 available)
#   Not considered: EPS concentration (at the end, from precipitation), Xylose
#   concentration (PMP, too unreliable), EPS concentration and monomer
#   compositions (PMP, too unreliable)
```

```
130
131 # Input D600
132 d600 <- as.data.frame(
133     cSplit(
134         read.table('ferm-dat/d600.txt', header = TRUE, sep = "\t", dec = ",",
135             col.names = c("Time after inoculation in hh:mm", "D600.F1", "D600.F2", "↩
                D600.F3", "D600.F4", "D600.F5", "D600.F6", "D600.F7", "D600.F8"),
136             na.strings = "-",
137             colClasses = c("character", rep("numeric", 8)), nrows = 13),
138         splitCols = "Time.after.inoculation.in.hh.mm", sep = ":", drop = FALSE))
139 colnames(d600) <- c("t", "D600.F1", "D600.F2", "D600.F3", "D600.F4", "D600.F5", "D600.↩
    F6", "D600.F7", "D600.F8", "HH", "MM")
140
141 # Input CDM
142 cdm <- read_excel("lchf0-cdm.xlsx", sheet = "BTM", col_names = TRUE, skip = 1)
143
144 # Input glucose assay data (LCHFA2); skip rows with visually appealing header
145 lchfa2 <- as.data.frame(
146     read_excel("glc-results.xlsx", sheet = "conc",
147         col_names = c("junk", "UID", "A.418.-A.480.", "Glc.conc."),
148         col_types = c("text", "text", "text", "numeric"),
149         skip = 4))
150
151 # Input EPS monomer data; skip rows with type, unit, analyte, signal
152 epsamc <- as.data.frame(
153     read_excel("eps-amc.xlsx", sheet = "PMP",
154         col_names = TRUE,
155         col_types = c("text", "text", rep("numeric", 21)),
156         na = "n.a.", skip = 4))
157
158 # Input molar mass at RI peak data
159 mp <- as.data.frame(
160     read_excel("sec-malls-results.xlsx", sheet = "Tabelle1",
161         col_names = c("Sample.ID", "Peak elution time", "Mn in g/mol", "Mw in g/mol", "↩
            Mp in g/mol", "Injection datetime"),
162         col_types = c("text", rep("numeric", 4), "text"),
163         na = "-", skip = 1))
164
165 # Input furfural data
166 fur <- as.data.frame(
167     read_excel("glc-xyl-fur-hplc.xlsx", sheet = "PMP",
168         col_names = c("junk", "UID", "FermGlc.PMP", "FermXyl.PMP", "HMF", "Fur"),
169         col_types = c("text", "text", rep("numeric", 4)),
170         na = "n.a.", skip = 4))
171
172 # # # # # # # # # # # # # # # # # # # # #
173 # Process data
174 #   Transform time into seconds and round to nearest 30 for comparison, get
175 #   rid of unneeded columns, name columns according to their origin
176 for (i in 1:8) {
177     indon[[i]] <- prepFermDat(indon[[i]], paste('F', i, sep = ''))
178 }
179
180 # Transform D600 data
181 #   Time data to duration in seconds
182 #   Remove no longer necessary columns
183 d600$t <- as.duration((d600$MM * 60) + (d600$HH * 3600))
184 d600 <- d600[, 1:9]
185 # Split into block 1 and 2
186 blk1d600 <- d600[ , 1:5]
187 blk2d600 <- d600[ , c(1, 6:9)]
188 # Remove NAs
189 blk1d600 <- blk1d600[complete.cases(blk1d600),]
190 blk2d600 <- blk2d600[complete.cases(blk2d600),]
191 # Remove last block 1 sample
192 # it's unnecessary, because there is no other data to correlate it with
193 blk1d600 <- blk1d600[1:(nrow(blk1d600)-1),]
```

```
194
195 # Transform CDM data
196 #   Columns fermenter, sample, concentration stay; others will be removed
197 cdm <- cdm[, c(1, 2, 8)]
198 colnames(cdm) <- c("Fermenter", "Sample", "CDM")
199 # Remove NA-only rows
200 cdm <- cdm[rowSums(is.na(cdm)) != ncol(cdm),]
201 # Reshape format to Sample, Fermenter n ...
202 cdm <- as.data.frame(reshape(cdm, idvar = "Sample", timevar = "Fermenter", direction = ↛
      "wide"))
203 # Replace "Sample" column with sample times
204 # blk2smptim used, because 8th blk1 sample is D600 only
205 # +1, because R starts indexing content at 1
206 cdm[,1] <- blk2smptim[(cdm[,1]+1),2]
207 # Adapt column names
208 colnames(cdm) <- c("t", "CDM.F1", "CDM.F2", "CDM.F3", "CDM.F4", "CDM.F5", "CDM.F6", "↛
      CDM.F7", "CDM.F8")
209 # Split into block 1 and 2
210 blk1cdm <- cdm[ , 1:5]
211 blk2cdm <- cdm[ , c(1, 6:9)]
212 # Remove NAs
213 blk1cdm <- blk1cdm[complete.cases(blk1cdm),]
214 blk2cdm <- blk2cdm[complete.cases(blk2cdm),]
215
216 # Transform glucose assay data
217 # Remove column 'junk' and absorption differences
218 lchfa2 <- lchfa2[ , c(2, 4)]
219 # Split into two parts:
220 #   PMP glucose (LCHFA2_0 + LCHFA2_1)
221 #   glucose in fermenter (LCHFA2_2 + LCHFA2_3)
222 pmpglc <- lchfa2[1:152, ]
223 fermglc <- lchfa2[153:nrow(lchfa2), ]
224
225 # Transform PMP glucose data
226 # Keep rows from before hydrolysis only, renumber rows
227 pmpglc <- pmpglc[c(1:68),]
228 rownames(pmpglc) <- NULL
229 # Reverse dilution (5 µl sample + 45 µl ddH2O)
230 pmpglc[, 2] <- 10 * pmpglc[, 2]
231 # Split UID: LCHFA2_0.B9_F1.0 --> LCHFA2    0.B9    F1  0
232 pmpglc <- as.data.frame(cSplit(pmpglc,
233     splitCols = "UID", sep ="_", drop = TRUE, type.convert = FALSE))
234 pmpglc <- as.data.frame(cSplit(pmpglc,
235     splitCols = "UID_3", sep =".", drop = TRUE, type.convert = FALSE))
236 # Throw away columns: experiment identifier, plate + coordinates
237 pmpglc <- pmpglc[ , c(1, 4, 5)]
238 colnames(pmpglc) <- c("PMPGlc", "Fermenter", "Sample")
239 # Auto-convert columns now
240 pmpglc[, 3] <- type.convert(pmpglc[, 3])
241 pmpglc[, 2] <- type.convert(pmpglc[, 2])
242 # Set negative values to zero
243 pmpglc[ ,1] <- with(pmpglc, ifelse(PMPGlc < 0, 0, PMPGlc))
244 # Reorder columns: Sample, Fermenter, Glc. conc.
245 pmpglc <- pmpglc[ , c(3, 2, 1)]
246 # Reshape content: columns for every fermenter
247 pmpglc <- as.data.frame(reshape(pmpglc, idvar = "Sample", timevar = "Fermenter", ↛
      direction = "wide"))
248 # Renumber rows
249 rownames(pmpglc) <- NULL
250
251 # Transform Fermentation glucose data
252 # Renumber rows
253 rownames(fermglc) <- NULL
254 # Remove rows with standards and 10 l fermentation
255 fermglc <- fermglc[-c(9:10, 19:20, 29:30, 39:41, 50:52, 61:63, 72:73, 82:84, 180:212), ↛
      ]
256 # Renumber rows
```

```
257 rownames(fermglc) <- NULL
258 # Fix wrong labelling in raw data: sample F7.2 in (E|F)2 is actually F7.1
259 fermglc[c(113, 125), 1] <- "LCHFA2_3.E2_F7.1"
260 # Split UID: LCHFA2_2.A1_F1.0 --> LCHFA2    2.A1    F1.0
261 fermglc <- as.data.frame(cSplit(fermglc,
262     splitCols = "UID", sep ="_", drop = TRUE, type.convert = FALSE))
263 # Throw away columns: UID_1, UID_2
264 fermglc <- fermglc[ , c(1, 4)]
265 # Rename column
266 colnames(fermglc) <- c("FermGlc", "Fermenter.Sample")
267 # Create one column for every sample
268 #   Max. two values per sample --> mark via duplicate
269 #   reshape data to get two rows and lots of columns
270 fermglc['dpl'] <- as.numeric(duplicated(fermglc[ , c(2)]))
271 fermglc <- as.data.frame(reshape(fermglc, idvar = "dpl", timevar = "Fermenter.Sample", ↛
        direction = "wide"))
272 # Add row with means ignoring NAs
273 fermglc <- rbind(fermglc, sapply(fermglc, mean, na.rm = 1))
274 # Remove unneeded rows
275 fermglc <- fermglc[3, ]
276 # Re-reshape into long format
277 fermglc <- as.data.frame(reshape(fermglc))
278 # Re-number rows
279 rownames(fermglc) <- NULL
280 # Drop now useless dpl column
281 fermglc <- fermglc[ , 2:3]
282 # Rename columns
283 colnames(fermglc)[2] <- "FermGlc"
284 # Split into fermenter and sample
285 fermglc <- as.data.frame(cSplit(fermglc,
286     splitCols = "Fermenter.Sample", sep =".", drop = TRUE, type.convert = FALSE))
287 # Rename columns
288 colnames(fermglc)[2:3] <- c("Fermenter", "Sample")
289 # Auto-convert columns now
290 fermglc[, 3] <- type.convert(fermglc[, 3])
291 # FAINARU RISHEIPU (final reshape): Sample  FermGlc.F1  FermGlc.F2 ...
292 fermglc <- as.data.frame(reshape(fermglc, idvar = "Sample", timevar = "Fermenter", ↛
        direction = "wide"))
293 # Re-number rows
294 rownames(fermglc) <- NULL
295 # Apply dilution factor
296 fermglc[, 2:ncol(fermglc)] <- lchfa223df*fermglc[, 2:ncol(fermglc)]
297 # Convert unit from mg/l to g/l
298 fermglc[, 2:ncol(fermglc)] <- 0.001*fermglc[, 2:ncol(fermglc)]
299 # Replace sample number with sample time
300 # blk2smptim used, because 8th blk1 sample is D600 only
301 # +1, because R starts indexing content at 1
302 fermglc[,1] <- blk2smptim[(fermglc[,1]+1),2]
303 # Rename sample column
304 colnames(fermglc)[1] <- "t"
305
306 # Transform EPS monomer data
307 #   Use appropriate column names
308 #   Remove unused rows and colums
309 #   Remove NA only columns
310 #   Subtract glucose (from glucose assay)
311 colnames(epsamc) <- c(
312     "Sample number", "Sample name", "Man", "GlcUA", "GlcN", "GalUA",
313     "Rib", "Rha", "Gen", "GalN", "GlcNAc", "Lac",
314     "Cel", "Glc", "GalNAc", "Gal", "Ara", "Xyl",
315     "Fuc", "2dGlc", "2dRib", "HMF", "Fur")
316 # Throw away junk rows start (standards)
317 epsamc <- epsamc[21:nrow(epsamc), ]
318 # Throw away junk rows at the end (standards at the end, 10 l fermentation)
319 epsamc <- epsamc[1:(nrow(epsamc)-17), ]
320 # Throw away junk cols (Ara, Xyl)
321 epsamc <- epsamc[, -(17:18)]
```

```
# Throw away junk cols (sample number, HMF, Fur)
epsamc <- epsamc[, 2:(ncol(epsamc)-2)]
# Set sample #10 of fermenter 8 GalUA value to "NA": no GalUA detected in MS!
epsamc[67,5] <- NA
# Remove all columns which contain only NA
epsamc <- Filter(function(x)!all(is.na(x)), epsamc)
# Split sample name column twice to get columns for fermenter and sample
epsamc <- as.data.frame(cSplit(epsamc,
    splitCols = "Sample name", sep ="_", drop = TRUE, type.convert = FALSE))
epsamc <- as.data.frame(cSplit(epsamc,
    splitCols = "Sample name_2", sep =".", drop = TRUE, type.convert = FALSE))
# Throw away column: plate + coordinates
epsamc <- epsamc[ , -6]
# Reorder columns: sample, fermenter and then sugars in alphabetical order
epsamc <- epsamc[ , c(7, 6, 5, 4, 2, 1, 3)]
colnames(epsamc) <- c("Sample", "Fermenter", "AMCGal", "AMCGlc", "AMCGlcN", "AMCMan", "↛
    AMCRha")
# Unify fermenter naming: capital "F" followed by fermenter number
epsamc[ , 2] <- paste("F", epsamc[ , 2], sep = "")
# Auto-convert columns now
epsamc[, 1] <- type.convert(epsamc[, 1])
epsamc[, 2] <- type.convert(epsamc[, 2])
# Add new dummy column for monomer sums
epsamc$AMCSum <- rep(NA, nrow(epsamc))
# Reshape content: columns for every fermenter
epsamc <- as.data.frame(reshape(epsamc, idvar = "Sample", timevar = "Fermenter", ↛
    direction = "wide"))
# Renumber row names
rownames(epsamc) <- NULL
# Reorder columns: first step puts "Sample" at the end
epsamc <- epsamc[ , order(names(epsamc))]
epsamc <- epsamc[ , c(ncol(epsamc), 1:(ncol(epsamc)-1))]
# Apply dilution factor
epsamc[, 2:ncol(epsamc)] <- lchfa1df*epsamc[, 2:ncol(epsamc)]
# Subtract monomeric glucose before hydrolysis
for (i in 1:8) {
    amccol <- paste("AMCGlc.F", i, sep = "")
    pmpcol <- paste("PMPGlc.F", i, sep = "")
    epsamc[ , amccol] <- epsamc[ , amccol] - pmpglc[ , pmpcol]
}
# Calculate sums
#   Copy dataframe
#   Replace NAs by 0s
#   Finally calculate sums
temp.df <- epsamc
temp.df[is.na(temp.df)] <- 0
for (i in 1:8) { # i = Fermenters
    galcol <- paste("AMCGal.F", i, sep = "")
    glccol <- paste("AMCGlc.F", i, sep = "")
    glcncol <- paste("AMCGlcN.F", i, sep = "")
    mancol <- paste("AMCMan.F", i, sep = "")
    rhacol <- paste("AMCRha.F", i, sep = "")
    sumcol <- paste("AMCSum.F", i, sep = "")
    for (j in 1:nrow(epsamc)) {
        epsamc[j, sumcol] <-
            temp.df[j, galcol] + temp.df[j, glccol] +
            temp.df[j, glcncol] + temp.df[j, mancol] +
            temp.df[j, rhacol]
    }
}
remove(temp.df)
# Replace sample number with sample time
# blk2smptim used, because 8th blk1 sample is D600 only
# +1, because R starts indexing content at 1
epsamc[,1] <- blk2smptim[(epsamc[,1]+1),2]
# Change column name accordingly
colnames(epsamc)[1] <- "t"
```

```
387
388 # Transform molar mass data
389 # Throw away unneeded columns
390 mp <- mp[ , c(1, 5)]
391 # Throw away columns with standards, LiNO3, LCHF1 samples, co-worker samples
392 mp <- mp[-c(1:13, 15, 23, 32, 41, 50:51, 64, 77, 90, 103:nrow(mp)) ,]
393 # Split Sample ID: 1.0 -> 1 0
394 mp <- as.data.frame(cSplit(mp,
395     splitCols = "Sample.ID", sep =".", drop = TRUE, type.convert = FALSE))
396 # Rename columns
397 colnames(mp) <- c("Mp", "Fermenter", "Sample")
398 # Unify fermenter naming: capital "F" followed by fermenter number
399 mp[ , 2] <- paste("F", mp[ , 2], sep = "")
400 # Reorder columns
401 mp <- mp[ , c(3, 2, 1)]
402 # Reshape: Sample   Mp.F1   Mp.F2 ...
403 mp <- as.data.frame(reshape(mp, idvar = "Sample", timevar = "Fermenter", direction = "⇛
      wide"))
404 # Convert column
405 mp[ ,1] <- type.convert(mp[ ,1])
406 # Renumber rows
407 rownames(mp) <- NULL
408 # Replace sample number with sample time
409 # blk2smptim used, because 8th blk1 sample is D600 only
410 # +1, because R starts indexing content at 1
411 mp[,1] <- blk2smptim[(mp[,1]+1),2]
412 # Change column name accordingly
413 colnames(mp)[1] <- "t"
414
415 # Transform furfural data
416 # Remove unnecessary columns (junk, FermGlc.PMP, FermXyl.PMP, HMF)
417 fur <- fur[ , c(2, 6)]
418 # Remove unnecessary rows (standards, 10 l samples)
419 fur <- fur[-c(1:13, 90:nrow(fur)), ]
420 # Split UID: B01_F1.0 -> B01    F1  0
421 fur <- as.data.frame(cSplit(fur,
422     splitCols = "UID", sep ="_", drop = TRUE, type.convert = FALSE))
423 fur <- as.data.frame(cSplit(fur,
424     splitCols = "UID_2", sep =".", drop = TRUE, type.convert = FALSE))
425 # Remove unnecessary column
426 fur <- fur[, -2]
427 # Rename columns
428 colnames(fur)[2:3] <- c("Fermenter", "Sample")
429 # Convert column
430 fur[ , 3] <- type.convert(fur[ , 3])
431 # Set negative values to 0 (necessary for plotting)
432 fur[ ,1] <- with(fur, ifelse(Fur < 0, 0, Fur))
433 # Convert unit: from mg/l to g/l
434 fur[ ,1] <- fur[ ,1]/1000
435 # Reshape: Sample   Fur.F1  Fur.F2  ...
436 fur <- as.data.frame(reshape(fur, idvar = "Sample", timevar = "Fermenter", direction = ⇛
      "wide"))
437 # Renumber rows
438 rownames(fur) <- NULL
439 # Replace sample number with sample time
440 # blk2smptim used, because 8th blk1 sample is D600 only
441 # +1, because R starts indexing content at 1
442 fur[,1] <- blk2smptim[(fur[,1]+1),2]
443 # Change column name accordingly
444 colnames(fur)[1] <- "t"
445
446 # # # # # # # # # # # # # # # # # # # # #
447 # Merge data
448 #   For every variable, sequence is: block 1 (blk1dat), block 2 (blk2dat)
449 # Start with fermentation data
450 blk1dat <- indon[[1]]
451 for (i in 2:4) {
```

```
    blk1dat <- join(blk1dat, indon[[i]], by = c("t"), type = "full", match = "all")
}
blk2dat <- indon[[5]]
for (i in 6:8) {
    blk2dat <- join(blk2dat, indon[[i]], by = c("t"), type = "full", match = "all")
}
# Reorder columns to cluster DO and CO2
blk1dat <- blk1dat[ , c(1, 2, 4, 6, 8, 3, 5, 7, 9)]
blk2dat <- blk2dat[ , c(1, 2, 4, 6, 8, 3, 5, 7, 9)]

# D600 data
blk1dat <- join(blk1dat, blk1d600, by = c("t"), type = "full", match = "all")
blk2dat <- join(blk2dat, blk2d600, by = c("t"), type = "full", match = "all")

# CDM data
blk1dat <- join(blk1dat, blk1cdm, by = c("t"), type = "full", match = "all")
blk2dat <- join(blk2dat, blk2cdm, by = c("t"), type = "full", match = "all")

# Fermenter glucose data
blk1dat <- join(blk1dat, fermglc[ , 1:5], by = "t", type = "full", match = "all")
blk2dat <- join(blk2dat, fermglc[ , c(1, 6:9)], by = "t", type = "full", match = "all")

# Sum of EPS aldose monomers
blk1dat <- join(blk1dat, epsamc[ , c(1, 42:45)], by = "t", type = "full", match = "all"⁄
    )
blk2dat <- join(blk2dat, epsamc[ , c(1, 46:49)], by = "t", type = "full", match = "all"⁄
    )

# Molar mass at RI peak data
blk1dat <- join(blk1dat, mp[ , 1:5], by = "t", type = "full", match = "all")
blk2dat <- join(blk2dat, mp[ , c(1, 6:9)], by = "t", type = "full", match = "all")

# Furfural data
blk1dat <- join(blk1dat, fur[ , 1:5], by = "t", type = "full", match = "all")
blk2dat <- join(blk2dat, fur[ , c(1, 6:9)], by = "t", type = "full", match = "all")

# Remove unnecessary rows of blk1dat (+4 empty)
blk1dat <- blk1dat[1:(nrow(blk1dat)-254), ]

# Remove unnecessary rows of blk2dat (every row after last sample)
blk2dat <- blk2dat[1:(nrow(blk2dat)-108), ]

# Generate statistics and join
quantiles <- c(0.1, 0.50, 0.9)
coltypes <- c("DO", "CO2", "D600", "CDM", "FermGlc", "AMCSum", "Mp", "Fur")
for (i in 1:8) {
    start <- 2 + (4 * (i - 1))
    stop <- start + 3
    newcols <- c(paste(coltypes[i], quantiles[1], sep = "."),
        paste(coltypes[i], quantiles[2], sep = "."),
        paste(coltypes[i], quantiles[3], sep = "."))
    statdat <- as.data.frame(t(apply(blk1dat[, start:stop], 1, quantile, quantiles, na.⁄
        rm = TRUE)))
    blk1dat[ , newcols] <- statdat
    statdat <- as.data.frame(t(apply(blk2dat[, start:stop], 1, quantile, quantiles, na.⁄
        rm = TRUE)))
    blk2dat[ , newcols] <- statdat
}
save(blk1dat, file = "block1-plot-data.Rda")
save(blk2dat, file = "block2-plot-data.Rda")
```

A.2.6 Discussion

```
TGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAA    50
CCGGGGCTAATACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAA   100
GGTGGCTTTGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTT   150
GGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGG   200
TGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCA   250
GCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGC   300
GTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAAC   350
AAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCC   400
ACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTT   450
GTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGA   500
TGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACT   550
TGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTA   600
GAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTG   650
ACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTA   700
GTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTT   750
AGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAA   800
GACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATG   850
TGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTC   900
TGACAATCCTAGAGATAGGACGTCCCCTTCGGGGGCAGAGTGACAGGTGG   950
TGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA  1000
ACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAA  1050
GGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCAT  1100
CATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAA  1150
GGGCAGCGAAACCGCGAGGTTAAGCCAATCCCACAAATCTGTTCTCAGTT  1200
CGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAATCGCTAGTAATC  1250
GCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGC  1300
CCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTT  1350
TAGGAGCCAGCCGCCGAA  1368
```

Figure A.2: 16S rDNA sequence of the contamination of the lignocellulose hydrolysate 7 l fermentation of *Paenibacillus* 2H7. Genomic DNA of the contaminant was amplified according to section 2.6.3 on page 54. Sequencing yielded one forward sequence and one reverse sequence. The sequences were sanitized as described in section 2.3.1 on page 32. The combined sequence was created manually.

A.3 Parallel Fermentation of *S. rolfsii* and *S. commune*

A.3.1 Exopolysaccharide Courses

Table A.13: Exopolysaccharide concentrations at the end of the fermentations of *S. rolfsii* and *S. commune*. The concentrations were determined through the usual sampling routine ('Last Sample', c_{ls}) and precipitation of the fermentation broth at the end of the fermentation ('End', c_{End}). The latter data are plotted in figure 4.2 on page 111. Each datum represents a single measurement. Abbreviations: c(EPS): exopolysaccharide concentration; c_{end}: concentration determined via the precipitation of the fermentation broth at the end of the fermentation; c_{ls}: concentration determined via the last sample.

		c(EPS) in $g \cdot l^{-1}$		
Fungus	Fermentation time	End	Last Sample	c_{end}/c_{ls} in %
S. rolfsii	48 h	0.84	0.87	104
S. rolfsii	72 h	1.52	1.91	126
S. rolfsii	96 h	3.25	3.44	106
S. commune	48 h	0.15	0.23	156
S. commune	72 h	0.75	0.70	93
S. commune	96 h	1.06	0.78	74
S. commune	120 h	2.21	2.56	116
S. commune	144 h	1.38	1.60	116

A.3.2 Periodate Test

Table A.14: Periodate consumption after different reaction times. The 0 h values were used to determine the initial periodate concentration. Therefore, they are zero *per definitionem*. Fermenters 1, 3 and 5 were inoculated with *S. rolfsii* and fermenters 4, 7 and 8 with *S. commune*. Fermenter 2 is not listed as only insufficient amounts of exopolysaccharide were produced, fermenter 6 is missing, because of the excessive amounts of anti-foam which were pumped into the fermenter and subsequently contaminated the precipitate. Fermenter 4 showed the only clear deviation consuming all the available periodate for unknown reasons. Abbreviations: Fn: fermenter no. n with $n \in \mathbb{N} \wedge 0 < n < 9$.

Reaction time in h	Periodate consumption in mmol·l^{-1}					
	F1	F3	F5	F4	F7	F8
0	0.00	0.00	0.00	0.00	0.00	0.00
24	3.98	4.29	4.54	6.44	4.81	4.53
48	5.08	4.51	4.69	9.07	5.62	4.85
72	5.68	4.60	4.69	9.84	6.25	5.23
96	5.27	4.70	4.63	10.1	6.40	5.42
120	5.80	4.82	4.93	10.1	6.65	5.67

Table A.15: Formic acid formation after different reaction times. The 0 h values were used as reference points. Therefore, they are zero *per definitionem*. Fermenters 1, 3 and 5 were inoculated with *S. rolfsii* and fermenters 4, 7 and 8 with *S. commune*. Fermenter 2 is not listed as only insufficient amounts of exopolysaccharide were produced, fermenter 6 is missing, because of the excessive amounts of anti-foam which were pumped into the fermenter and subsequently contaminated the precipitate. Abbreviations: Fn: fermenter no. n with $n \in \mathbb{N} \wedge 0 < n < 9$.

Reaction time in h	Formic acid formation in mmol·l^{-1}					
	F1	F3	F5	F4	F7	F8
0	0.00	0.00	0.00	0.00	0.00	0.00
24	2.50	2.10	2.19	2.36	3.00	2.36
48	2.88	2.07	2.18	1.92	3.20	2.44
72	2.95	2.07	2.23	1.89	3.15	2.52
96	3.28	2.03	2.19	1.88	3.13	2.54
120	3.30	2.05	2.18	1.88	3.11	2.57

A.3.3 Aniline Blue Assay for the Quantitative Determination of β-1,3-β-1,6-Glucans[1]

Equipment

- 1.5 ml reaction tubes with tight lid
- 2.0 ml reaction tubes with tight lid
- 10 ml measuring pipettes
- 15 ml reaction tubes with screw cap
- 50 ml volumetric flask
- 100 ml beaker
- 96 well fluorescence plates
- 96 well fluorescence reader
- Magnetic stirrer and magnetic stir bar (for 100 ml beaker)
- Parafilm
- Analytical balance
- Centrifuge for 1.5 ml reaction tubes
- Centrifuge for 50 ml reaction tubes
- Incubator/oven at 50 °C
- Laboratory scales
- pH meter with pH electrode
- Pipettes (20 µl to 200 µl, 100 µl to 1000 µl)
- Pipetting aid
- Scissors
- Vortexer

Chemicals

- 2 M HCl
- Aniline Blue diammonium salt (Sigma-Aldrich, article number: 415049; referred to as **AB**)
- Glycine
- Scleroglucan Cs 11 (Cargill) as reference
- Sodium hydroxide
- Ultrapure water (referred to as **ddH_2O**)

[1] The contents of this section have been reproduced from an unpublished protocol of mine written for the chair of Chemistry of Biogenic Resources. If you would like to cite this assay, you may also reference the publication [235].

Overview

Sirofluor (4,4′-[carbonylbis(benzene-4,1-diyl)bis(imino)]bisbenzenesulfonic acid) is the fluorochrome in aniline blue [244] used to stain different parts of plants [243]. It does not specifically interact with β-1,3-glucans only. Smith and McCully [243] report that in solid samples fluorescence can also be observed for cellulose (β-1,4-glucan) and lichenan (β-1,3:1,4-glucan). In buffered solutions, Evans *et al.* [245] tested a wide range of poly- and oligosacharides and found, among others, that:

- fluorescence is exclusively induced with α- and β-glucans and not with other homo- or hetero-glycans,
- not all α-D-glucans induce fluorescence: amylose (α-1,4), amylopectin and glycogen (α-1,4 and α-1,6) and cyclic α-1,4-D-oligoglucosides show weak fluorescence; dextrans (α-1,6), pullulan (α-1,4 and α-1,6) and isolichenin (α-1,3 and α-1,4) do not show fluorescence; with unpurified fluorochrome Faulkner *et al.* [242] found fluorescence for amylose, amylopectin, some dextrans and pullulan,
- the linear β-1,3-D-glucans laminarin and O-carboxymethyl-pachyman induced intense fluorescence and
- linear β-1,3-D-glucans with some single β-1,6-linked D-glucosyl units induce intense fluorescence.

This is the basis for quantification of scleroglucan and schizophyllan. Within a certain range, the fluorescence intensity depends linearly on the scleroglucan concentration and using a calibration curve the fluorescence intensity can be used to determine the scleroglucan concentration in the sample in less than 75 min.

Preparation

Reference Solution (RS)

1. Weigh an empty 100 ml beaker with a magnetic stir bar. Note the mass.
2. Add approximately 10 ml ddH_2O.
3. Add 150.0 mg scleroglucan Cs 11 powder.
4. Add water until a total mass (powder + water) of 15.00 g is reached.
5. Cover the beaker with parafilm to reduce evaporation.
6. Stir overnight at room temperature and reasonably high speed. Make sure that the whole fluid volume is thoroughly mixed at all times even after complete dissolution of scleroglucan and the subsequent increase in viscosity.
7. On the next day, stop the stirring and let the solution cool to room temperature.
8. Carefully remove the parafilm and condensed water from the walls of the beaker.
9. Weigh the beaker again and add enough ddH_2O to make up for the water lost due to evaporation.

10. Stir again for some minutes to ensure complete mixing.

11. Transfer as much of the solution as possible to a 15 ml reaction tube.

12. Reference solution may be stored in aliquots of 1.90 g (sufficient for quintuplicates) at −20 °C.

Calibration Curve

13. Prepare standards with the following concentrations using the scheme below: $6\,g\cdot l^{-1}$, $3\,g\cdot l^{-1}$, $1\,g\cdot l^{-1}$, $600\,mg\cdot l^{-1}$, $300\,mg\cdot l^{-1}$, $100\,mg\cdot l^{-1}$, $60\,mg\cdot l^{-1}$, $30\,mg\cdot l^{-1}$, $10\,mg\cdot l^{-1}$, $5\,mg\cdot l^{-1}$, $0\,g\cdot l^{-1}$.

14. Due to high viscosity prepare the standards with $6\,g\cdot l^{-1}$, $3\,g\cdot l^{-1}$ and $1\,g\cdot l^{-1}$ using a scales:

Final concentration	Mass of $10\,g\cdot l^{-1}$ standard	Mass of ddH_2O	Comment
$6\,g\cdot l^{-1}$	480 mg	320 mg	
$3\,g\cdot l^{-1}$	240 mg	560 mg	
$1\,g\cdot l^{-1}$	170 mg	1530 mg	Use 2 ml tube.

15. Thoroughly mix these standards by vortexing.

16. Centrifuge the tubes for 10 s at 8000 × *g* and room temperature prior to opening.

17. Prepare the remaining standards by pipetting liquid volumes, always put ddH_2O first:

Final concentration	Volume of ddH_2O	Standard for dilution	Volume of standard
$600\,mg\cdot l^{-1}$	400 µl	$1\,g\cdot l^{-1}$	600 µl
$300\,mg\cdot l^{-1}$	455 µl	$1\,g\cdot l^{-1}$	195 µl
$100\,mg\cdot l^{-1}$	900 µl	$1\,g\cdot l^{-1}$	100 µl
$60\,mg\cdot l^{-1}$	630 µl	$0.6\,g\cdot l^{-1}$	70 µl
$30\,mg\cdot l^{-1}$	729 µl	$0.3\,g\cdot l^{-1}$	81 µl
$10\,mg\cdot l^{-1}$	630 µl	$0.1\,g\cdot l^{-1}$	70 µl
$5\,mg\cdot l^{-1}$	550 µl	$30\,mg\cdot l^{-1}$	110 µl
$0\,mg\cdot l^{-1}$	1 ml	—	—

The schemes are tuned to fulfil the following criteria:

- Remaining masses or volumes after all dilution steps are at least 600 mg or 600 µl for up to six runs with quintuplicates.
- The standards can be pipetted by using a 100 µl, 200 µl and 1000 µl pipette without using the lowest third of the maximum volume to reduce pipetting errors.
- Dilution factors are 10 at most.

18. Calibration curve samples should be stored in aliquots of 100 mg or 100 µl (for quintuplicates) at −20 °C.

Dye Solution (DS)

19. Add 50.0 mg AB to a 15 ml reaction tube.

20. Add 9.95 g ddH_2O to the reaction tube.

21. Wait until the dye is completely dissolved. This can be sped up by shaking or inverting the tube.
    Hint: If the dye solution touches the inner part of the cap, this can result in splashes when opening the screw cap. In order to prevent these, centrifuge the tube for 3 min at 1 000 × *g* and room temperature prior to opening.
22. Dye solution may be stored in aliquots of 3.2 ml (ideally) or 1.6 ml at −20 °C.

Glycine/NaOH Buffer (GN) The glycine/NaOH buffer contains 1.0 M glycine and 1.25 M NaOH.

23. Put in some ddH_2O first.
24. Add 3.76 g glycine to a 50 ml volumetric flask.
25. Add 2.50 g sodium hydroxide to the flask.
26. Add ddH_2O and wait for the dissolution of the two components.
27. Let the solution cool to room temperature.
28. Fill with ddH_2O to the mark.
29. 1 M Glycine/NaOH buffer may be stored in aliquots of 4.7 ml (ideally) or 1.6 ml at −20 °C.

Reaction Buffer (RB) The Reaction Buffer contains approx. 209 mM glycine, 261 mM NaOH and 149 mM HCl.

30. Add 12.7 ml ddH_2O to a 50 ml reaction tube.
31. Add 3.70 ml GN to the reaction tube.
32. Add 1.31 ml 2 M HCl to the reaction tube.
33. The buffer may be stored at −20 °C.

Working Solution (WS) The final concentrations of each component in the working solution are approx. 183 mM glycine, 229 mM NaOH, 131 mM HCl and 6.18 mg aniline blue per litre. Prepare the working solution one day in advance and store in a dark place.

34. Add 2.50 ml DS to the reaction tube with 17.7 ml RB.
35. Mix thoroughly.
36. Check the pH value, it should be in the range of 9.8 to 10.0. Generally, more acidic conditions (pH = 9.5 and below) are very likely to give smaller readings while slightly more basic conditions (up to pH = 10.5) have no effect besides discolouring the solution faster. Within a range of approximately 9.5 to 10.5 readings are unaffected.
37. Let the solution stand at room temperature in the dark overnight. The colour of the solution fades from blue to green to yellow. It is believed that the yellow colour originates from the fluorophore, Sirofluor.
38. 20.2 ml WS is sufficient for one 96-well plate with a safety margin of approximately 17 %.
39. Storage at −20 °C was not tested. Storage at room temperature for up to seven days is possible.

Reaction Tubes

40. For each sample and each standard prepare one empty 2 ml tube.

Assay Instructions

Generally, make sure to have your sample in its respective tube, before adding the working solution. The addition of working solution should ideally be done for all samples at the same time. If that is not possible, try to do it without interruption and as fast as possible.

Calibration Curve

41. For 10 $g \cdot l^{-1}$, 6 $g \cdot l^{-1}$ and 3 $g \cdot l^{-1}$ add 100 mg of standard to the respective tube.

42. For all other standards add 100 µl to the respective tube.

43. The amount is sufficient for quintuplicates.

Samples

44. Add 20 µl of sample to the respective tube. This amount is sufficient for one measurement. When the colour becomes dark blue upon mixing with the sample, the pH value is most likely too low which will result in an artificially low value. In that case, neutralize the sample prior to measurement.

Addition of WS

45. Add the respective amount of WS to every tube. For the values given above this means: 900 µl for standards and 180 µl for samples. Close the lids.

46. Vortex the tubes thoroughly.

47. Centrifuge the tubes for 10 s at 8000 × g and room temperature.

Incubation

48. Incubate the tubes at 50 °C, preferably in the dark, for 30 min.

49. Turn on your microplate reader directly prior to the end of the incubation to ensure a stable light source.

50. Afterwards incubate for 30 min at room temperature for cooling.

51. Vortex the tubes thoroughly.

52. Centrifuge the tubes for 10 s at 8000 × g and room temperature prior to opening.

Transfer and Measurement

53. Transfer 180 µl of sample or standard per well into a 96 well fluorescence plate. The outer ring of wells may show slightly lower values than wells inside.

54. Measure fluorescence intensity at an excitation wavelength of 405 nm and an emission wavelength of 495 nm.

55. The calibration curve should give a line with at least $R^2 > 99.8$ % in the range from 30 $mg \cdot l^{-1}$ to 10 $g \cdot l^{-1}$.

Please note

- The numbering is consecutive for the entire document to make sure that references to single steps of this protocol e.g. in laboratory notebooks cannot be confused with each other.

- The assay is quite robust: oxalic acid concentrations up to 22.5 $g \cdot l^{-1}$, D-glucose concentrations up to 50 $g \cdot l^{-1}$, BSA concentrations of up to 667 $mg \cdot l^{-1}$ and KCl concentrations of up to 13.3 $g \cdot l^{-1}$ in the sample do not interfere with the measurement.

- Correction factors for fermentation broth samples of *S. rolfsii* and *S. commune* are 2.46 and 3.83, respectively. This is valid only under special circumstances and it is up to each user to find correction factors for the sample in question! The assay should work fine with pure polysaccharide samples.

Alternatives

Qualitative Determination The test can be downsized to qualitative determination by preparing and using one and only one standard (e.g. 100 $mg \cdot l^{-1}$) and ddH_2O instead of a calibration curve.

Appendix B

Version

This document is under version control using Git. The current version is '48d9b49'[1] from 2019-12-23 by Steven "Kreuvf" Koenig (steven.koenig.wzs@kreuvf.de). The branch this document was created from is 'bitbucket/official'.

[1] The complete version number is: 48d9b491244c0349f0ab658edd6c5bea97859bb3.

List of Abbreviations

Symbols of chemical elements, and SI units and their respective prefixes [246] are not listed. Units are listed only if they are not covered in the SI brochure or are used with a different definition. Abbreviations for manufacturers and vendors are given in table 2.1 on page 17. Abbreviations in company names and abbreviations of German legal terms within company names, and abbreviations in trademarked names are not listed.

Chemicals

2-D-Glc	2-deoxy-D-glucose
AB	aniline blue
ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid
Acet.	acetic acid
Ara	L-arabinose
C/I	chloroform/isoamyl alcohol
C5 sugar	sugar with five carbon atoms
C6 sugar	sugar with six carbon atoms
CA	cellulose acetate
Cel	cellobiose
CN	cellulose nitrate
CoA	coenzyme A
DMSO	dimethylsulphoxide
dNTP	desoxynucleoside triphosphate
DS	dye solution
EDTA	ethylenediaminetetraacetic acid or 2,2′,2″,2‴-(ethane-1,3-diyldinitrilo)-tetraacetic acid
Form.	formic acid
Fuc	L-fucose

Fur.	furfural or furan-2-carbaldehyde
Gal	D-galactose
GalN	D-galactosamine
GalUA	D-galacturonic acid
Gen	gentiobiose
Glc	D-glucose
GlcNAc	*N*-acetyl-D-glucosamine
GlcN	D-glucosamine
GlcUA	D-glucuronic acid
GN	glycine/NaOH buffer
HMF	hydroxymethylfurfural or 5-(hydroxymethyl)furan-2-carbaldehyde
Ism	isomaltose
Laev.	laevulinic acid
LB	lysogeny broth
LCH	lignocellulose hydrolysate
Man	D-mannose
MOPS	3-morpholinopropane-1-sulphonic acid
Nig	nigerose
PES	polyethersulfone
PMMA	polymethylmethacrylate
PMP	3-methyl-1-phenyl-2-pyrazoline-5-one
PP	polypropylene
PS	polystyrene
PTFE	polytetrafluoroethylene
RB	reaction buffer
RC	regenerated cellulose
Rha	L-rhamnose
Rib	D-ribose
RS	reference solution

SDS	sodium dodecyl sulphate
SEC	size exclusion chromatography
SM	slime media (see section 2.5.2 on page 40)
TAE	TRIS, acetate, EDTA
TFA	trifluoroacetic acid
TPE	thermoplastic elastomer
TRIS	tris(hydroxymethyl)aminomethane or 2-amino-2-(hydroxymethyl)propane-1,3-diol
Van.	vanillin
WS	working solution
Xyl	D-xylose

File Extensions

AB1	sequencing data in a binary format [247]
FASTA	plaintext format for storing nucleotide or protein sequence information
FASTQ	plaintext format for storing nucleotide or protein sequence and quality information

Units and Symbols Used in Formula

$\Delta A(260\,\mathrm{nm})$	absorbance at 260 nm
$\Delta A(320\,\mathrm{nm})$	absorbance at 320 nm
$A418$	absorbance at 418 nm
$A480$	absorbance at 480 nm
b_h	stirrer blade height
b_w	stirrer blade width
c(EPS)	exopolysaccharide concentration
$c(Glc)$	D-glucose concentration
c_{DNA}	DNA concentration
c_{End}	concentration determined via the precipitation of the fermentation broth at the end of the fermentation
c_{ls}	concentration determined via the last sample
D	fermenter inner diameter

d	dilution factor
D_{600}	attenuance at 600 nm
Da	Dalton, used as $\mathrm{g \cdot mol^{-1}}$
d_f	form breaker diameter
DF	dilution factor
$\frac{dn}{dc}$	refractive index increment
d_R	stirrer diameter
d_{sh}	shaft diameter
η	dynamic viscosity
f_{ssDNA}	substance specific factor for nucleic acid
g	average gravitational acceleration at sea level on Earth, approximately $9.81\,\mathrm{m \cdot s^{-2}}$
H	medium height
H'	medium as measured from the sparger
h_B	distance between stirrer and fermenter bottom
Δh_f	distance between neighbouring foam breakers
H_f	fermenter total height
Δh_{fR}	distance between bottom foam breaker and stirrer
K_a	dissociation constant of an acidic group
l	lid factor
$\mathrm{l_N}$	litre under standard conditions
M	$\mathrm{mol \cdot l^{-1}}$
OTR	oxygen transfer rate
pH	power of hydrogen
pK_a	cologarithm of K_a
$\mathrm{pO_2}$	partial pressure of oxygen (in the liquid phase)
vvm	Gas volume per minute relative to the fermenter liquid volume. Shorthand for ‘gas **v**olume per liquid **v**olume and **m**inute’. Standard conditions are assumed for the gas volume.

Roman Letters

3D	three-dimensional
ABIF	Applied Biosystems, Inc. Format
Art. no.	article number
DNA	desoxyribonucleic acid
EPS1	plate 1 with exopolysaccharide producers
EPS2	plate 2 with exopolysaccharide producers
EPS	exopolysaccharide
ESI-MS	electron spray ionization mass spectrometry
et al.	*et alia*
FFF	field-flow fractionation
FLOSS	free/libre open-source software
GC-MS	gas chromatography coupled with mass spectrometry
GIMP	GNU Image Manipulation Program
GNU	GNU's Not Unix
GPC	gel permeation chromatography
HF	high fidelity
HPLC-MS	high pressure liquid chromatography coupled with mass spectrometry
HPLC	high pressure liquid chromatography
i.e.	in exemplum
IGB	Institut für Grenzflächen- und Bioverfahrenstechnik
IS1r2pmp	plate of the inhibitor screening 1, round 2 for PMP analysis
IS1r2rez	plate of the inhibitor screening 1, round 2 for Rezex analysis (acids)
ISp	short for plate IS1r2pmp
ISr	short for plate IS1r2rez
IUCN	International Union for Conservation of Nature and Natural Resources
IUPAC	International Union of Pure and Applied Chemistry
M./V.	manufacturer or vendor
MALLS	multi angle laser light scattering

MS/MS	tandem mass spectrometry
MS	mass spectrometry
n.d.	not detected
n.t.	not tested
NGS	next-generation sequencing
NMR	nuclear magnetic resonance
PCR	polymerase chain reaction
RAST	Rapid Annotations using Subsystems Technology
rDNA	ribosomal DNA
RID	refractive index detector
RI	refractive index
RPMI	Roswell Park Memorial Institute
TUM	Technische Universität München
UV/Vis	ultraviolet and visible light
Xyl1	plate 1 with exopolysaccharide producers growing on D-xylose
Xyl2	plate 2 with exopolysaccharide producers growing on D-xylose

Microorganisms

Agr	*Agrobacterium*
Anc	*Ancylobacter*
Art	*Arthrobacter*
Bac	*Bacillus*
BeI	*Beijerinckia indica*
BM~	similar to *Beijerinckia mobilis*
Br~	similar to *Burkholderia*
Bre	*Brevundimonas*
Bur	*Burkholderia*
Cau	*Caulobacter*
Cel	*Cellulosimicrobium*
Cur	*Curtobacterium*

Dye	*Dyella*
Er~	similar to *Erwinia*
Glu	*Gluconacetobacter*
Her	*Herbaspirillum*
KaA	associated with *Kaistobacter*
Koz	*Kozakia*
Noc	*Nocardiopsis*
P. azoreducens	*Paenibacillus azoreducens*
P. cineris	*Paenibacillus cineris*
P. favisporus	*Paenibacillus favisporus*
P/R	*Paracoccus*/*Rhodobacter*
Pae	*Paenibacillus*
Par	*Paracoccus*
Pse	*Pseudomonas*
Rah	*Rahnella*
Rao	*Raoultella*
Rhi	*Rhizobium*
S. commune	*Schizophyllum commune*
S. rolfsii	*Sclerotium rolfsii*
SbC	close to *Sphingobacterium*
She	*Shewanella*
Sin	*Sinorhizobium*
Sp~	similar to *Sphingomonas*
Sph	*Sphingomonas*
Xan	*Xanthomonas*
µb~	similar to *Microbacterium*
µbA	associated with *Microbacterium*
µba	*Microbacterium*
µco	*Micrococcus*

ɋba	*Rhodobacter*
ɋco	*Rhodococcus*
ɋd~	similar to *Rhodanobacter*

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Eidesstattliche Erklärung

Ich erkläre an Eides statt, dass ich die bei der promotionsführenden Einrichtung *Campus Straubing für Biotechnologie und Nachhaltigkeit* der TUM zur Promotionsprüfung vorgelegte Arbeit mit dem Titel „Production of Microbial Hydrocolloids from Renewable Resources“ in der Professur für *Chemie Biogener Rohstoffe* unter der Anleitung und Betreuung durch *Herrn Prof. Dr. Volker Sieber* ohne sonstige Hilfe erstellt und bei der Abfassung nur die gemäß § 6 Ab. 6 und 7 Satz 2 angebotenen Hilfsmittel benutzt habe.

- ☒ Ich habe keine Organisation eingeschaltet, die gegen Entgelt Betreuerinnen und Betreuer für die Anfertigung von Dissertationen sucht, oder die mir obliegenden Pflichten hinsichtlich der Prüfungsleistungen für mich ganz oder teilweise erledigt.
- ☒ Ich habe die Dissertation in dieser oder ähnlicher Form in keinem anderen Prüfungsverfahren als Prüfungsleistung vorgelegt.
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- ☒ Ich habe den angestrebten Doktorgrad noch nicht erworben und bin nicht in einem früheren Promotionsverfahren für den angestrebten Doktorgrad endgültig gescheitert.
- ☐ Ich habe bereits am …… bei der Fakultät für …… der Hochschule …… unter Vorlage einer Dissertation mit dem Thema …… die Zulassung zur Promotion beantragt mit dem Ergebnis: …… .

Die öffentlich zugängliche Promotionsordnung der TUM ist mir bekannt, insbesondere habe ich die Bedeutung von § 28 (Nichtigkeit der Promotion) und § 29 (Entzug des Doktorgrades) zur Kenntnis genommen. Ich bin mir der Konsequenzen einer falschen Eidesstattlichen Erklärung bewusst.

Mit der Aufnahme meiner personenbezogenen Daten in die Alumni-Datei bei der TUM bin ich einverstanden.

Ort	Datum	Unterschrift