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

Kateryna Huemer[a], Pascal Olschowski[a], Tom Distler[a], Karin Lanthaler[b], Hansjörg Weber[c], Hedda K. Weber[d,e]

[a] Wood K plus - Kompetenzzentrum Holz GmbH, Altenberger Straße 69, 4040 Linz, Austria
[b] Fluent in Science, fluentinscience@gmail.com, Wales, UK
[c] Institute of Organic Chemistry, TU Graz, Stremayrgasse 9, 8010 Graz, Austria
[d] Institute of Bioproducts and Paper Technology, TU Graz, Inffeldgasse 23/I, 8010 Graz Austria
[e] Green Swanlings e.U., Entenplatz 1A, 8020 Graz, Austria
Corresponding author: K. Huemer, k.huemer@kplus-wood.at

Abstract

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

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

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

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

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

Introduction

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

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

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

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

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

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

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

Materials and Methods

Thermoanaerobacter mathranii (DSM 11426)

Medium preparation

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

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

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

Cultivation

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

Inhibitor screening

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

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

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

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

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

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

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

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

Halomonas halophila (DSMZ 4770)

Medium preparation

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

Cultivation

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

Monitoring of potential inhibitors during fermentation of H. halophila

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

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

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

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

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

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

NMR spectroscopy

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

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

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

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

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

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

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

Measuring optical density

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

Results and discussion

Inhibitor screening T. mathranii

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

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

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

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

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

*These compounds were metabolized by microorganisms

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

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

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

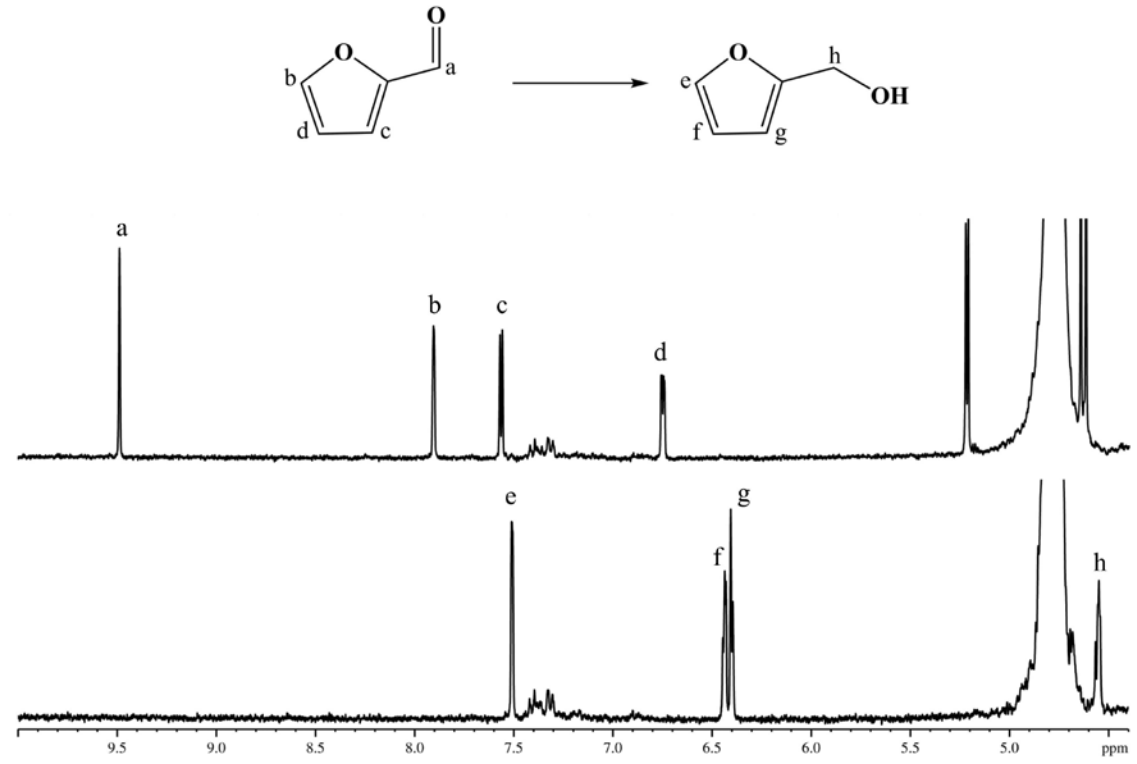

Figure 1: *^{1}H NMR spectra (D_2O, 300 MHz) of medium with furfural before the incubation with T. mathranii (top), and after fermentation for 72 h at 65 °C (bottom).*

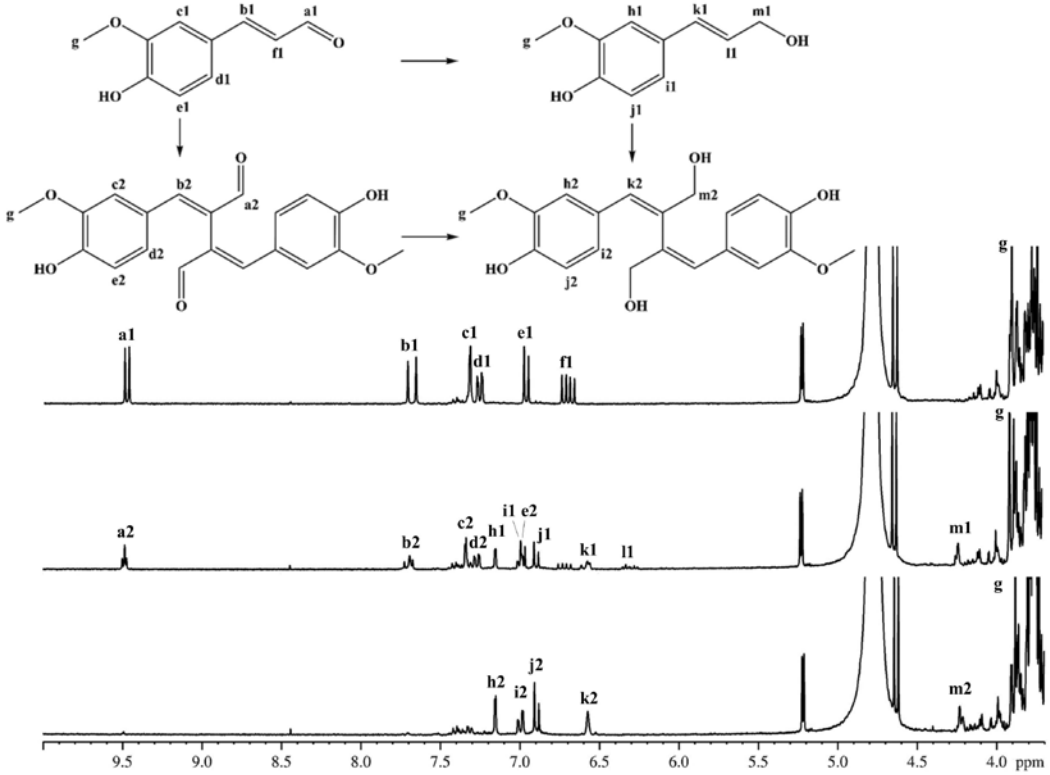

Figure 2: *^{1}H NMR spectra (D_2O, 300 MHz) of medium with coniferyl aldehyde without the addition of T. mathranii (top), medium with coniferyl aldehyde after addition of T. mathranii after incubation at 65 °C for 13 h (middle) and 72h (bottom).*

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

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

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

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

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

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

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

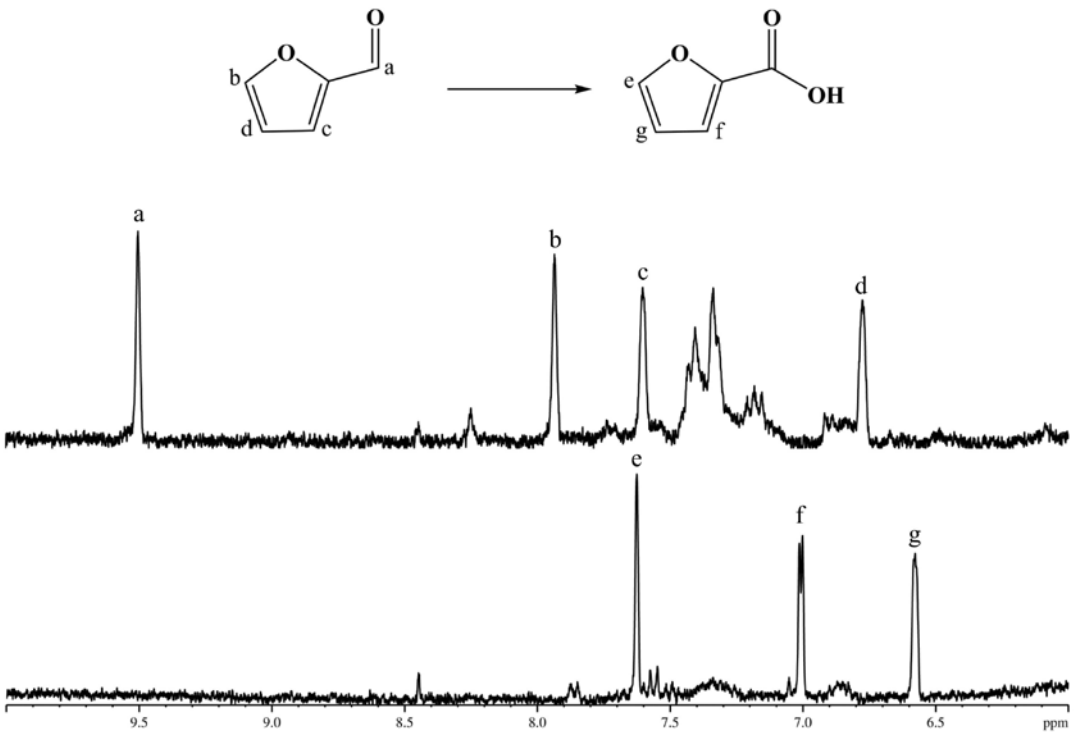

Figure 3: *^{1}H NMR spectra (D_2O, 300 MHz) of medium with furfural without the addition of H. halophila (top), medium with furfural after addition of H. halophila after incubation for 143 h at 30 °C (bottom)*

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

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

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

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

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

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

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

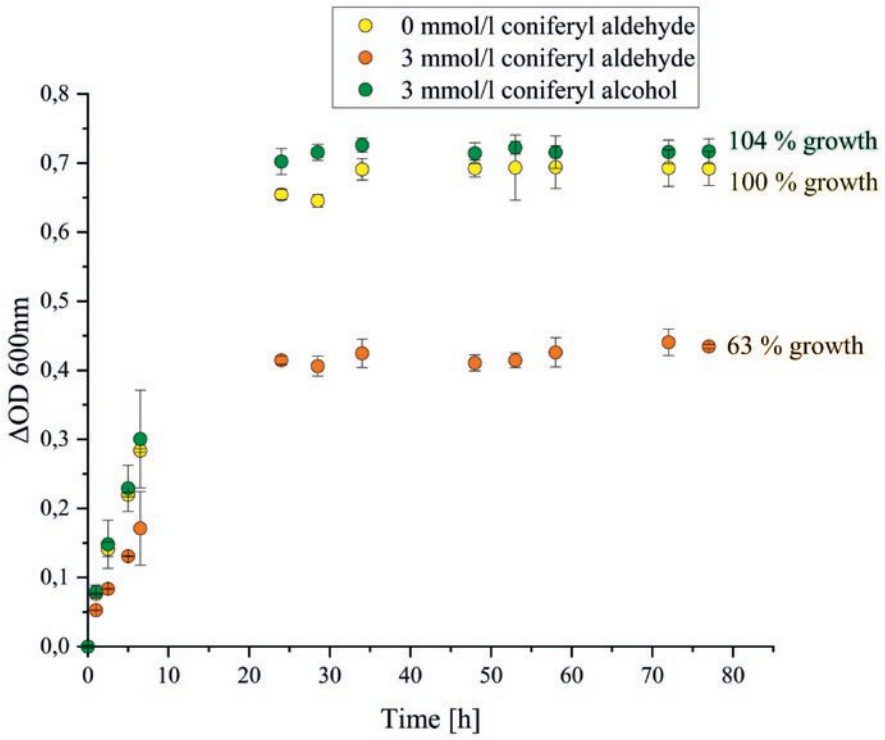

Figure 4: *Growth behaviour of T. mathranii during incubation in medium only (yellow curve) and in a medium with coniferyl aldehyde (orange curve) or coniferyl alcohol (green curve)*

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

Conclusions

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

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

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

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

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

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

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