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

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Abstract

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

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

Introduction

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

A wide range of compounds is formed out of lignin

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Materials and Methods

Thermoanaerobacter mathranii (DSM 11426)

Medium preparation

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

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

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

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

Cultivation

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

Inhibitor screening

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

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

Halomonas halophila (DSMZ 4770)

Medium preparation

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

Cultivation

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

Inhibitor screening

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

Clostridium saccharoperbutylacetonicum (DSMZ 14923)

Medium preparation

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

Cultivation

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

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

Inhibitor screening and tests of synergistic effects

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

OD measurements

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

Results and discussion

Inhibition effects of aliphatic acids, alcohols and furan aldehydes

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

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

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

Inhibition effects of phenolic compounds

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

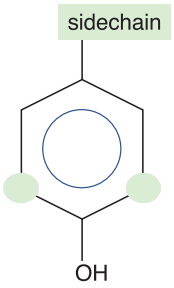


Figure 1: *Structural variations (green) of the phenylpropane units of lignins in Nature and our test programme.*

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

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

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

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

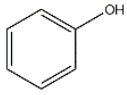
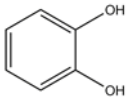
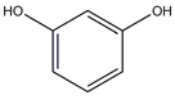
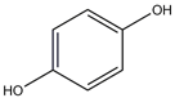
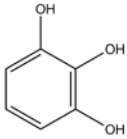
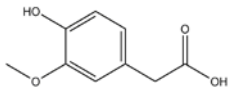
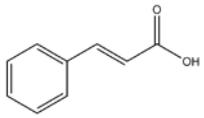
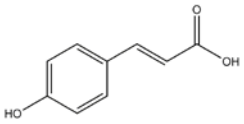
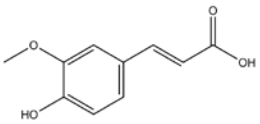
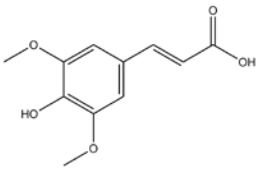
possible inhibitors	concen-tration [mmol/l]	growth [%]	microorganism/strain
phenol 	3.00	94	*Thermoanaerobacter mathranii* DSM 11426
	3.00	88	*Clostridium saccharoperbutylacetonicum* DSM 14923
	3.00	83	*Halomonas halophila* DSMZ 4770
	7.00	122	*Thermoanaerobacter mathranii* DSM 11426
	7.00	93	*Clostridium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

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

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

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

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

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

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

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

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

Is the inhibition synergistic or additive?

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

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

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

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

Conclusions

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

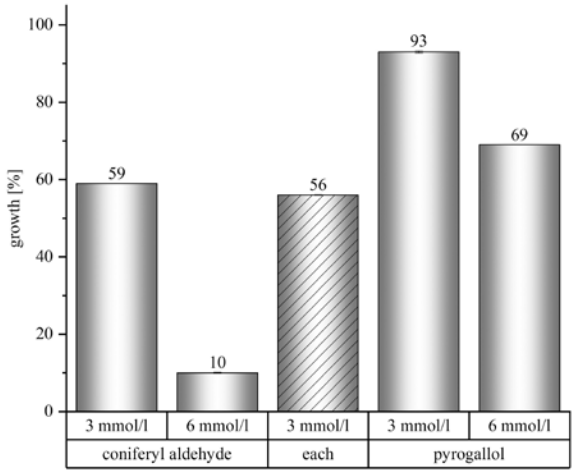


Figure 2: *Scenario 1 – growth of Clostridium saccharoperbutylacetonicum after addition of a harmless, harmful phenolic compound (pyrogallol and coniferyl aldehyde) and their mixture. The effect is additive, not synergistic.*

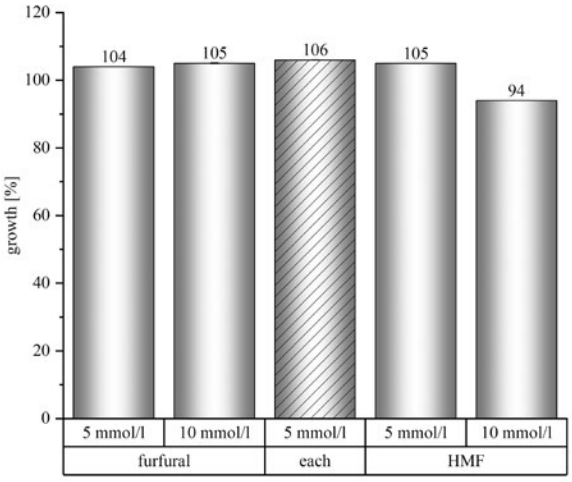


Figure 3: *Scenario 2 – growth of Clostridium saccharoperbutylacetonicum after the addition of two harmless compounds and their mixture: furfural and HMF. Two harmless compounds combined yield no inhibitory effect at all (see also SI Figure S1)*

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

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

Acknowledgement

The work is a part of the UVEFAZ project and is funded by the Austrian research funding association (FFG) and Lenzing AG.

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Appendix

Supporting Information

Colour code for growth ranges of microorganisms

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

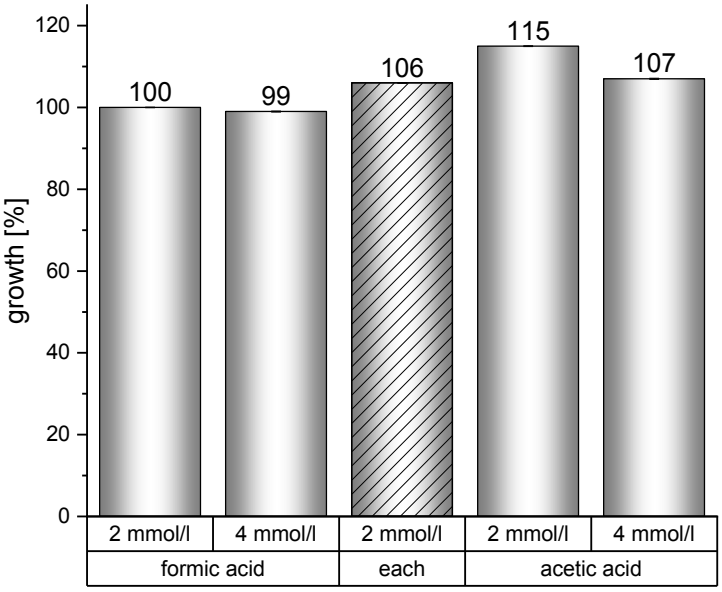


Figure S1: *Scenario 2 – growth of Clostridium saccharoperbutylacetonicum after the addition of two other harmless compounds and their mixture formic acid and acetic acid. Two harmless compounds combined yield no inhibitory effect at all.*

Table S1: *Inhibitory effects of aliphatic acids.*

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

Table S2: *Inhibitory effects of alcohols.*

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

Table S3: *Inhibitory effects of furan aldehydes.*

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

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

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

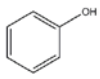
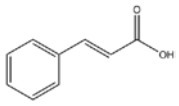
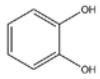
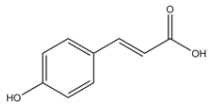
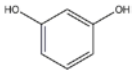
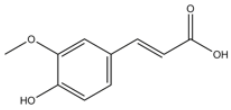
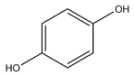
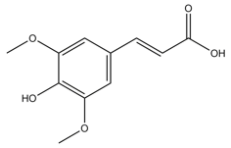
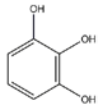
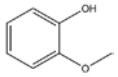
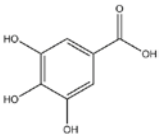
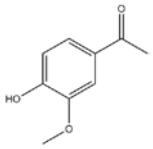
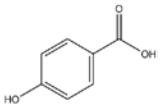
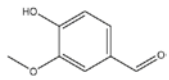
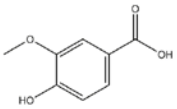
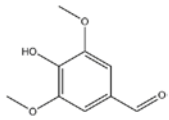
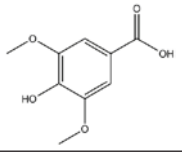
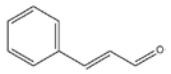
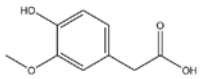
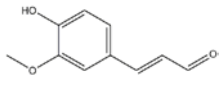
Phenol		trans-Cinnamic acid	
Catechol		p-Coumaric acid	
Resorcinol		Ferulic acid	
Hydroquinone		Sinapic acid	
Pyrogallol		Guaiacol	
Gallic acid		Apocynin	
p-Hydroxybenzoic acid		Vanillin	
Vanillic acid		Syringaldehyde	
Syringic acid		Cinnamaldehyde	
Homovanillic acid		Coniferylaldehyde	

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

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

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

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

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

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

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