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Enhancing Flavour Stability in Beer Using Biological Scavengers
Part 1: Methodology and Preliminary Trials

The diffusion of oxygen into the bottles and the subsequent oxidation of beer components is the main cause for deteriorating flavour during storage, limiting the shelf life of the beer. Active aerobic or facultative anaerobic microorganisms should be able to metabolise the oxygen before beer components are oxidized. In order to investigate this approach, two acetic acid bacteria (*Acetobacter pasteurianus* and *Gluconobacter oxydans*), as well as four yeasts (Rh bottom fermenting yeast, 68 obg. top-fermenting yeast, *Saccharomycodes ludwigii* and *Brettanomyces bruxellensis*), were tested. At first, these microorganisms were inoculated into a model beer. As the results were poor a second test series was conducted, in which the microorganisms were added into commercially available beer (German Pilsener). All microorganisms were capable of decreasing the initial oxygen concentration of approx. 2 ppm faster than the beer matrix itself, whereas the yeasts showed better results in a non-representative sensorial evaluation regarding prevention of oxidation smell and flavour than the bacteria. Especially long-term stability of the acetic acid bacteria seems to be an issue. Based on the results of the preliminary trials further investigation is required. Results will be presented in a second part following this paper.

Descriptors: biological scavengers, flavour stability, beer, bottle fermentation

1 Introduction

Beer like many other foods is sensitive towards oxidation, especially deteriorating the flavour. As oxygen diffuses into the bottles through the compound material of the crown cap of glass bottles and the wall of PET bottles and the closures in general, the shelf life of beer is limited. An uptake of 1 ppm oxygen is considered to be the maximum tolerable level before beer quality is affected (e.g. [1, 2]). The uptake of oxygen through a standard crown cork with PVC compound within half a year is given exemplarily with 1.4 ppm [3], through the walls of 0.5-l-monolayer PET bottles without coating with 14–20 ppm, resp. 1–7 ppm with coatings, multilayer PET bottles with 1–10 ppm and 1–1.5 ppm for PEN bottles [4], which corresponds well to the observation of limited shelf life of beer especially when filled into monolayer PET bottles. Active aerobic or facultative anaerobic microorganisms should be able to metabolise the oxygen diffusing through the package into the beer, acting as biological scavengers. One question to be answered is, whether the microorganisms can consume the oxygen fast enough to impair oxidation of the beer matrix.

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Active yeast is known to render oxygen innocuous in different ways: First of all yeasts as facultative anaerobic microorganisms are capable of metabolising oxygen directly. Secondly, yeasts form SO_2, which is a potent antioxidant in beer. Thirdly yeasts are capable to reduce staling aldehydes built during beer ageing to their corresponding alcohols with much higher taste threshold values and maybe even reverse the oxidation effect to a certain degree [5]. Consequently traditionally bottle fermented or unfiltered beers could have an advantage when it comes to flavour stability – a thesis which is supported by some traditional beers like Belgian Gueuze with, in some cases, perennial shelf life.

On the other side a number of side effects have to be taken into consideration:

- Yeasts are susceptible to autolysis, which is accompanied by a characteristic off-flavour (muffled, musty, skunkiness). Bottom fermenting yeasts are generally regarded to be more vulnerable to autolysis than top-fermenting yeasts.
- Different yeasts form different aroma compounds, which might contradict the targeted beer style.
- Over-fermenting yeasts might form substantial amounts of CO_2 leading to swelling and in worst case even to bursting of the (glass) bottles during storage.
- Formation of turbidity, which might fail to comply with consumer's expectation.

Apart from the bottom and top-fermenting yeasts used in beer production, some other non-Saccharomyces yeasts might prove effective when being used as biological scavengers. *Brettanomyces bruxellensis* is known to belong to the slow fermenting yeasts

maintaining a good vitality over a long period of time, which is the reason for being already suggested to act as an oxygen scavenger for ales and lagers stored under climatized conditions, an approach which was then considered to be not applicable due to economic and technical reasons [6]. A disadvantage is clearly the formation of esters, especially ethyl lactate and ethyl acetate in higher concentration compared to other yeasts. Nevertheless, for some beers like Berlin "Weißbier", it contributes to the typical overall flavour [7,8].

Saccharomycodes ludwigii is limited to sucrose, glucose and fructose as substrate. The yeast is not capable to use maltose as a carbohydrate source. Like *B. bruxellensis S. ludwigii* belongs to the slow fermenting yeasts [9], which again could be beneficial for its use as a biological scavenger.

Acetic acid bacteria might be an alternative to yeasts. They oxidise ethanol in the presence of oxygen to acetic acid or further to CO_2 and H_2O. Complete oxidation to CO_2 is beneficial for the applicability as biological scavengers. But even if acetic acid is formed, the increase in beer is limited, which is due to the relatively small amount of oxygen to be eliminated. The *Acetobacter genus* belongs to the group of acetic acid bacteria which completely oxidize ethanol to CO_2, whereas the *Gluconobacter genus* forms acetic acid. Nevertheless, *Gluconobacter* is considered to provide a huge oxidation potential due to its big number of oxidoreductase enzymes [10,11]. The species *Acetobacter pasteurianus* and *Gluconobacter oxydans* amongst others are known to show high conversion rates [12,13].

Before using any microorganisms in food, health risks have to be ruled out from the microbiological and toxicological point of view. A concept for the safety evaluation of microorganisms provides the QPS-status (Qualified Presumption of Safety) from the European Food Safety Authority (EFSA). Furthermore, microorganisms not listed as QPS can be used as long as there is a so-called "history of safe use", which means that these microorganisms have been used for a long time without any problems in food production. If both are not the case microorganisms have to be evaluated carefully in experiments regarding the formation of toxins, the presence of pathogenic factors, allergenicity, antibiotic resistance and others [14]. The American counterpart for QPS is the GRAS status (Generally Recognised as Safe) assigned by the US Food and Drug Administration (FDA). S. carlsbergensis, S. cerevisiae and *G. oxydans* are QPS listed microorganisms. *B. bruxellensis* and *A. pasteurianus* were used in food production ever since and hence should provide a "history of safe use". Furthermore, *A. pasteurianus*, as well as *S. ludwigii*, own GRAS-status.

The present project aims at the development of a method which allows the easy characterization of different microorganism strains regarding their applicability as a biological scavenger.

2 Materials and methods

Six different microorganism strains were tested for their applicability as biological scavengers. All were taken from the reference stocks from Versuchs- und Lehranstalt für Brauerei (VLB) in Berlin:

- Rh bottom fermenting (BF) strain of *Saccharomyces carlsbergensis* (abbr. Rh (BF))
- 68 obg. top fermenting (TF) strain of *Saccharomyces cerevisiae* (abbr. 68 obg. (TF))
- *Saccharomycodes ludwigii* 3448 (abbr. *S. ludwigii*)
- *Brettanomyces bruxellensis* (abbr. *B. bruxellensis*)
- *Acetobacter pasteurianus* (abbr. *A. pasteurianus*)
- *Gluconobacter oxydans* (abbr. *G. oxydans*)

Propagation of the strains was done on malt extract agar (MEA) (Merck KGaA, Darmstadt).

In the first set of trials, model beer was used to mimic beer without the oxygen elimination otherwise caused by the redox system of the beer matrix, which was expected to hinder the observation of the effect of the biological scavengers themselves. The model beer was based on an earlier research project [15] and adapted to the needs of this work with the following composition: distilled water 4630 g, $NaHCO_3$ 55 g (Carl Roth, Karlsruhe, Germany), citric acid 80 g (Carl Roth, Karlsruhe, Germany), ethanol 260 ml (Carl Roth, Karlsruhe, Germany), maltodextrin 01910C*DryMD 150 g (Cerestar, Krefeld, Germany), yeast nitrogen base 340 g (Fluka Analytical, Steinheim, Germany), ringer tablets 10 (Merck Darmstadt, Germany). All components except ethanol and $NaHCO_3$ were weighed in, dissolved and autoclaved at 121 °C for 15 min. $NaHCO_3$ was put in perforated plastic bags and added together with the ethanol to each bottle filled with the medium under sterile conditions. In contact with the citric acid in the bottle, $NaHCO_3$ released CO_2 for carbonation of the medium.

PET bottles (PET with a passive barrier, screw caps without chemical scavenger, provided by VLB Berlin) were chosen for safety reasons

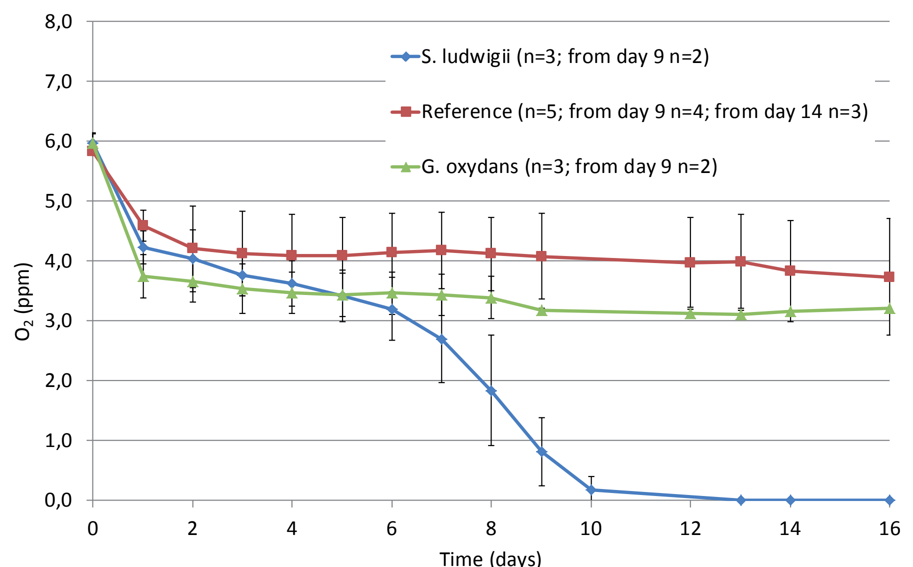

Fig. 1 Oxygen concentration in the model beer after inoculation with *S. ludwigii* and *G. oxydans* compared to the reference sample without addition of microorganisms

in case of excessive CO_2-formation by the microorganisms. In order to monitor oxygen concentration after filling and inoculation with the referring microorganism, the PET bottles were equipped with optical-chemical sensor spots (SP-Pst3-NAU-DS-YOP, PreSens Germany) which were glued on the inner wall of the PET-bottles. During measurement, the sensor spots are activated by light emitted by the handheld measuring device (Fibox4trace, PreSens Germany). The return light from the sensor spot is dampened in the presence of oxygen and measured again by the handheld, allowing repeated measuring in a non-destructive way. The function is described in detail by *Huber* et al. [16]. Disinfection of the so prepared bottles was done by ethanol 70 % (in- and outside) and then dried under the clean bench before filling with media and inoculation. The initial oxygen concentration of approx. 6 ppm was reached by aerating the medium with pressurized air using a sterile filter (Millex-PTFE, poresize 0.24 µm) and a ceramic frit (porosity 0) (both vwr, Hannover, Germany). Inoculation was done by simply transferring one colony from the referring agar plate into the bottle with an inoculating loop. The bottles were then stored at room temperature in dark conditions.

For the second set of trials bottled beer was sourced commercially from a big-sized brewery (Warsteiner Pils, Germany) in glass bottles. During transfer of the beer into the PET bottles the oxygen concentration increased to approx. 2 ppm simply by the handling under clean bench conditions. Additionally un-inoculated PET bottles were treated under the same conditions in order to measure the oxygen consumption caused only by the beer matrix as well as to serve as the comparison for the inoculated samples (same oxygen stress like the inoculated samples, but no biological scavengers). These samples refer to as “reference”. Furthermore, samples in the original glass bottles stayed unopened (referred to as “original”) and were stored under the same conditions as the PET bottles. The rest of the handling was the same as for the first set of trials with model beer as described above.

Statistical evaluation was done by comparing the oxygen concentration in the inoculated samples with the one in the reference sample using the t-Test.

3 Results and discussion

Figure 1, for reasons of clarity only as an excerpt with two microorganisms compared to the reference, and table 1 show the course of the oxygen concentration in the samples with different microorganisms when inoculated into the model beer.

After slightly higher values for all samples at the beginning, the oxygen concentration in the reference sample as expected is

Table 1 Oxygen concentration in the model beer after inoculation (as long as not stated otherwise: reference sample n = 5; all microorganisms n = 3); n.s. = not significant, * = significant (probability of error < 5 %), ** = highly significant (probability of error < 1 %), * = most significant (probability of error < 0.1 %) compared to the corresponding reference value**

	Reference		Rh (BF)		68 obg. (TF)		S. ludwigii		B. bruxellensis		A. pasteurianus		G. oxydans	
	AVE	STD	AVE	STD	AVE	STD	AVE	STD	AVE	STD	AVE	STD	AVE	STD
Day	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)
0	5.832	0.068	6.074	0.045	5.895	0.172	5.975	0.172	5.923	0.093	5.930	0.114	5.962	0.158
1	4.584	0.259	4.233	0.253	4.338	0.140	4.223	0.278	4.403	0.181	4.209	0.187	3.744	0.354
2	4.206	0.714	4.172	0.294	4.058	0.167	4.042	0.481	4.297	0.261	4.153	0.263	3.661	0.351
3	4.126	0.710	4.107	0.245	4.101	0.216	3.758	0.342	4.152	0.170	4.010	0.266	3.539	0.417
4	4.089	0.688	4.044	0.257	4.086	0.228	3.622	0.374	4.008	0.151	3.912	0.324	3.466	0.351
5	4.092	0.639	3.994	0.267	4.038	0.233	3.423	0.431	3.815	0.195	3.852	0.359	3.432	0.363
6	4.142	0.659	4.043	0.275	4.073	0.212	3.198	0.528	3.691	0.192	3.851	0.400	3.461	0.351
7	4.179	0.638	4.024	0.270	4.132	0.225	2.696	0.724	3.409	0.157	3.823	0.404	3.431	0.347
8	4.115	0.604	3.963	0.274	4.082	0.238	1.837	0.928	3.069	0.183	3.751	0.450	3.386	0.352
13	3.990^{x1}	0.783^{x1}	3.726^{x2}	0.031^{x2}	3.910^{x2}	0.084^{x2}	0.001^{x2}	0.001^{x2}	0.275^{x2}	0.144^{x2}	3.433^{x2}	0.489^{x2}	3.112^{x2}	0.064^{x2}
21	3.779^{x3}	0.905^{x3}	3.713^{x4}		3.782^{x2}	0.084^{x2}	0.000^{x2}	0.000^{x2}	0.000^{x2}	0.000^{x2}	3.059^{x4}		3.175^{x4}	
Day			Rh (BF)		68 obg. (TF)		*S. ludwigii*		*B. bruxellensis*		*A. pasteurianus*		*G. oxydans*	
0			**		n.s.		n.s.		n.s.		n.s.		n.s.	
1			n.s.		n.s.		n.s.		n.s.		n.s.		*	
2			n.s.		n.s.		n.s.		n.s.		n.s.		n.s.	
3			n.s.		n.s.		n.s.		n.s.		n.s.		n.s.	
4			n.s.		n.s.		n.s.		n.s.		n.s.		n.s.	
5			n.s.		n.s.		n.s.		n.s.		n.s.		n.s.	
6			n.s.		n.s.		n.s.		n.s.		n.s.		n.s.	
7			n.s.		n.s.		n.s.		n.s.		n.s.		n.s.	
8			n.s.		n.s.		*		*		n.s.		n.s.	

x1: n = 4; x2 = 2; x3: n = 3; x4: n = 1

Table 2 **Colony forming units per ml on malt extract agar after one and three weeks in model beer**

	Reference 1 (CFU/ml)	Reference 2 (CFU/ml)	Rh (BF) (CFU/ml)	68 obg. (TF) (CFU/ml)	*S. ludwigii* (CFU/ml)	*B. bruxellensis* (CFU/ml)	*A. pasteurianus* (CFU/ml)	*G. oxydans* (CFU/ml)
after one week	n.d.	n.d.	2.5E+03	1.2E+03	4.0E+03	1.0E+05	n.d.	2.5E+02
after three weeks	n.d.	n.d.	1.1E+03	< 1.0E+02	8.9E+04	1.0E+06	n.d.	n.d.

n.d. = not detectable (limit of quantifiction: 100/ml)

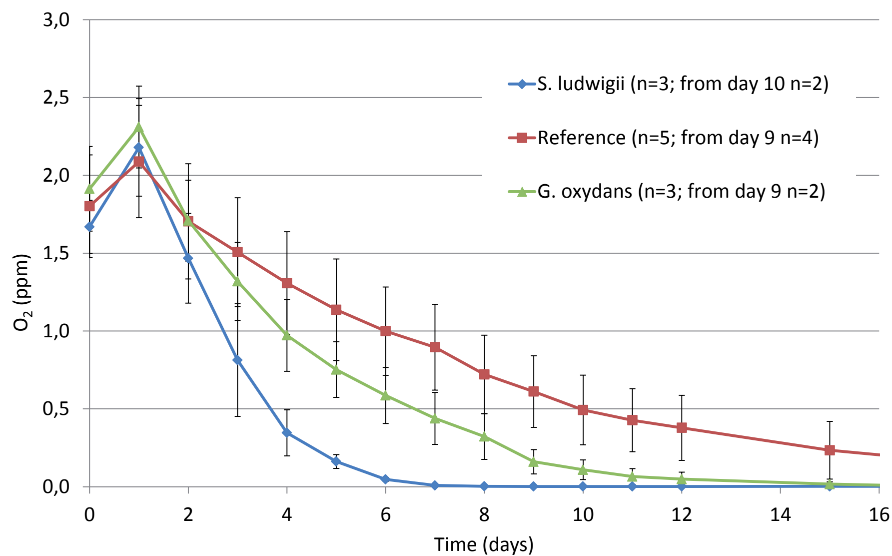

Fig. 2 **Oxygen concentration in beer after inoculation with *S. ludwigii* and *G. oxydans* compared to the reference sample without addition of microorganisms**

unchanged at around 4 ppm of oxygen over the course of three weeks, which indicates that the matrix does not consume any relevant amount of oxygen. The yeasts *S. ludwigii* and *B. bruxellensis* consume most of the oxygen within two weeks, whereas *S. ludwigii* is slightly faster. Nevertheless, it takes a lag time of eight days until a significant decrease compared to the reference is observed. The other microorganisms (yeasts Rh (BF) and 68 obg. (TF) as well as the bacteria *A. pasteurianus* und *G. oxydans*) do not eliminate the oxygen in the model beer.

Due to the limited number of samples and not too precise inoculum cell count, table 2 only provides an indication of the survival of the microorganisms in the model beer. Nevertheless and in line with the oxygen measurement the cell counts of the yeasts *S. ludwigii* and *B. bruxellensis* increase, whereas Rh (BF) and especially 68 obg. (TF) show a decreasing tendency. Both bacteria *A. pasteurianus* und *G. oxydans* are not detectable after three weeks storage time. Therefore, it can be stated that lack of survival is a major cause for not affecting the oxygen level in the model beer by the four microorganisms Rh (BF), 68 obg. (TF), *A. pasteurianus* and *G. oxydans*.

Samples were checked olfactory in a non-representative way by two people after three weeks. The model beer without inoculation already showed an unpleasant mainly mouldy smell, whereas the inoculated samples were even worse. Only slightly worse compared to the reference were the samples with *A. pasteurianus* and *G. oxydans* (papery sour), whereas the yeasts Rh (BF) und 68 obg. (TF) already showed stronger deviations, being described as sour, mouldy and papery. Worst smell resulted from the inoculation with *S. ludwigii* (strongly disgusting) and *B. bruxellensis* (disgusting).

Although the first trials with model beer were not too promising, in subsequent trials the model beer was substituted by commercially available beer. Figure 2, again as an excerpt, and table 3 show the results.

The oxygen concentration decreases even in the reference sample, which is caused by the redox system of the beer matrix, and reaches a value of 0.05 ppm after four weeks. Nonetheless the presence of the inoculated microorganisms helps to lower the oxygen concentration faster compared to the reference with *A. pasteurianus* being the fastest reaching a significant difference to the reference already on the second day, followed by Rh (BF) (significant difference on the third day) and 68 obg. (TF) and *S. ludwigii* (significant difference on the fourth day). *B. bruxellensis* is a bit slower with a significantly lower oxygen concentration on day six compared to the reference. *G. oxydans* is considerably slower, but reaches zero within three weeks, whereas the reference still shows a low oxygen concentration even after four weeks. In contrast to the high initial speed, *A. pasteurianus* does not reach zero level within four weeks. Taking a closer look on the culturable number of microorganisms after one and four weeks reveals that survival rate of *A. pasteurianus* is poor long-term in the beer as well (see table 4), which might explain the observation. The yeasts Rh (BF), 68 obg. (TF) and *S. ludwigii* are quite stable within the test period at approx. 4 power of ten per ml. *B. bruxellensis* grew quite well but could not be counted due to colonies on the culture medium not being separated in the sample after four weeks. Nevertheless, it has to be acknowledged that the microbiological tests were done only once hence the validity of the results is limited.

All pH-values of the beer after one and four weeks were close together in the range of 4.12 and 4.20 (results not shown).

Non-representative sensorics, again done by two people, revealed strong oxidation smell and taste in the reference sample (with exposure to O_2 at the beginning of the tests, but without inoculation of microorganisms) after one and four weeks, whereas the original sample (no exposure to O_2, no microorganisms) was free from any oxidation and off-flavours within the four weeks period. Samples inoculated with Rh (BF) showed slight oxidation after one and after four weeks, 68 obg. (TF) was good in smell and taste after

Table 3 **Oxygen concentration in beer after inoculation (as long as not stated otherwise: reference sample n = 5; all microorganisms n = 3); n.s. = not significant, * = significant (probability of error < 5 %), ** = highly significant (probability of error < 1 %), *** = most significant (probability of error < 0.1 %) compared to the corresponding reference value**

	Reference		Rh (BF)		68 obg. (TF)		S. ludwigii		B. bruxellensis		A. pasteurianus		G. oxydans	
	AVE	STD	AVE	STD	AVE	STD	AVE	STD	AVE	STD	AVE	STD	AVE	STD
Day	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)
0	1.802	0.330	1.726	0.191	1.799	0.273	1.670	0.171	2.108	0.400	1.891	0.161	1.914	0.271
1	2.089	0.360	1.939	0.435	1.867	0.362	2.180	0.313	2.118	0.454	2.051	0.147	2.311	0.263
2	1.705	0.370	1.264	0.459	1.415	0.349	1.468	0.289	1.659	0.456	0.841	0.101	1.710	0.260
3	1.507	0.350	0.787	0.218	1.152	0.304	0.813	0.362	1.397	0.432	0.420	0.038	1.320	0.250
4	1.307	0.330	0.412	0.119	0.687	0.142	0.346	0.148	0.946	0.387	0.249	0.045	0.973	0.231
5	1.137	0.326	0.225	0.102	0.423	0.114	0.162	0.044	0.513	0.310	0.191	0.056	0.752	0.179
6	1.000	0.283	0.171	0.060	0.174	0.058	0.047	0.012	0.202	0.149	0.115	0.046	0.586	0.180
7	0.896	0.276	0.085	0.029	0.042	0.032	0.009	0.002	0.068	0.063	0.087	0.034	0.439	0.167
8	0.722	0.252	0.029	0.011	0.003	0.001	0.003	0.001	0.003	0.005	0.064	0.020	0.323	0.147
15	0.234^{x5}	0.185^{x5}	0.004^{x6}	0.003^{x6}	0.002^{x6}	0.001^{x6}	0.002^{x6}	0.001^{x6}	0.000^{x6}	0.000^{x6}	0.064^{x6}	0.017^{x6}	0.017^{x6}	0.015^{x6}
22	0.095^{x5}	0.109^{x5}	0.005^{x6}	0.003^{x6}	0.002^{x6}	0.000^{x6}	0.002^{x6}	0.001^{x6}	0.000^{x6}	0.000^{x6}	0.055^{x6}	0.017^{x6}	0.000^{x6}	0.000^{x6}
29	0.050^{x5}	0.059^{x5}	0.005^{x6}	0.004^{x6}	0.002^{x6}	0.000^{x6}	0.002^{x6}	0.001^{x6}	0.000^{x6}	0.000^{x6}	0.031^{x6}	0.013^{x6}	0.000^{x6}	0.000^{x6}
Day			**Rh (BF)**		**68 obg. (TF)**		***S. ludwigii***		***B. bruxellensis***		***A. pasteurianus***		***G. oxydans***	
0			n.s.		n.s.		n.s.		n.s.		n.s.		n.s.	
1			n.s.		n.s.		n.s.		n.s.		n.s.		n.s.	
2			n.s.		n.s.		n.s.		n.s.		*		n.s.	
3			*		n.s.		n.s.		n.s.		**		n.s.	
4			*		*		*		n.s.		**		n.s.	
5			*		*		**		n.s.		**		n.s.	
6			**		**		**		*		**		n.s.	
7			**		**		**		**		**		n.s.	
8			**		**		**		**		*		n.s.	

x5: n = 4; x6: n = 2

Table 4 **Colony forming units per ml on malt extract agar after one and four weeks in beer**

	Reference 1 (CFU/ml)	Reference 2 (CFU/ml)	Rh (BF) (CFU/ml)	68 obg. (TF) (CFU/ml)	*S. ludwigii* (CFU/ml)	*B. bruxellensis* (CFU/ml)	*A. pasteurianus* (CFU/ml)	*G. oxydans* (CFU/ml)
after one week	n.d.	n.d.	2.0E+04	4.0E+04	3.0E+04	1.0E+06	3.0E+05	5.0E+04
after four weeks	n.d.	n.d.	1.3E+04	2.5E+04	1.0E+04	n.e.	n.d.	1.0E+02

n.d. = not detectable (limit of quantifiction: 100/ml)
n.e. = not evaluable (colonies not separated)

Table 5 **Summary of results, „+“ indicating a positive, „o“ a neutral and “–“ a negative effect regarding the use of the referring microorganism as biological scavenger in beer**

	Rh (BF)	68 obg. (TF)	S. ludwigii	B. bruxellensis	A. pasteurianus	G. oxydans
O_2-Scavenging	+	+	+	o	+	–
Oxidation	o	+	+	+	–	–
Off-Flavour	+	o	+	–	+	–

one week but revealed some off-flavour (estery, fruity, solvent-like) after four weeks. The samples inoculated with *S. ludwigii* were free from oxidation and from off-flavours after one week as well as four weeks, whereas *B. bruxellensis* prevented the beer from oxidation, but showed a whole range of off-flavours ranging from estery and fruity to solvent-like and mouldy, which somehow had to be expected. *A. pasteurianus* suppressed the formation of oxidation flavours after one week but was not successful in long-term as after four weeks strong oxidation smell and taste was present. Similar long-term effects could be observed with *G. oxydans* resulting in a strong indication for oxidation, whereas after one week only some adstringent off-flavour was present.

4 Conclusion/Summary

Adding the selected microorganisms to beer instead of model beer as biological scavengers yielded much better results in respect to

oxygen decrease and survival rate of the microorganisms. Although model beer provides the advantage of not consuming any oxygen by the matrix itself, acquired results are in no means corresponding to those obtained in real beer, hence the methodology was adapted accordingly.

Although the microorganisms used in the trials do not eliminate the oxygen at once – but faster than the beer matrix as can be seen in the reference sample – an oxidation preventing effect can be observed.

Table 5 gives an overview of the effects caused by the microorganisms observed during the trials with beer.

The acetic acid bacteria *Acetobacter pasteurianus* and *Gluconobacter oxydans* did not show a long-term capability of preventing beer from oxidation when exposed to oxygen. So the use of acetic acid bacteria does not look promising, presumably due to their deficiencies in surviving conditions in beer in the long run.

The yeasts were all quite effective in preventing the oxidation, but *B. bruxellensis* was fairly slow in growth in beer, furthermore forming strong off-flavours, which is in line with literature [7, 8]. *S. ludwigii* showed the best results regarding its use as a biological scavenger.

Screening of further yeasts using a standardized methodology is a promising approach for identifying suitable yeasts. Apart from the O_2-scavenging capability, more focus should be put on evaluating the effect on oxidation smell and off-flavours by a trained sensory panel. Furthermore tendency of the yeasts in the direction of over-fermenting capabilities has to be considered as bursting bottles have to be avoided by all means. Formation of turbidity is another issue that should be considered in future when looking for biological scavengers.

Enhancing flavour stability of beer in a natural way by adding suitable yeasts is a promising approach. Nevertheless, the yeasts should be checked first in a standardised manner for their applicability as biological scavengers. The methodology described in this work may serve as a blueprint. A second paper will present the results of a screening project with different yeasts extracted from traditional beers regarding their applicability as biological scavengers.

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