Test report

VH Berlin Standard operation procedures SOP R 10, SOP R 17 and SOP R 22
Comparison of VH Berlin standard equipment in comparison to the
ANKOM RF Gas Production System

Pre tests: 14.06.2010 - 30.06.2010
Tests: 05.07.2010 - 22.07.2010

Scope

The Versuchsanstalt der Hefeindustrie (VH, Research institute for baker's yeast) ran tests on three different VH-Berlin SOPs to determine whether these SOPs can also be performed with the ANKOM RF Gas Production System. The SOPs that are tested are the determination of fermentable sugar in molasses and other sugar containing media (SOP R 10), the determination of the alcohol yield and the inhibition of fermentation (SOP R 17) and the toxicity test which shows possible growth inhibition due to the presence or absence of components that effect the growth of yeast *Saccharomyces cerevisiae* (SOP R 22).
SOP R 10 and SOP R 17 are certified by DAkkS (Deutsche Akkreditierungsstelle GmbH) according to DIN EN ISO/IEC 17025:2005. SOP R 22 is run regularly at the VH-Berlin. The tests achieve high acceptance from our members and customers.

Material and methods

1.1 SOP R 10 Determination of fermentable sugar in molasses by measuring the gas formation

The SOP R 10 is useful for the fermentation industry, dealing with natural, non defined sugar media like molasses or thick juice. Beneath the total sugar and the inverted sugar, the fermentable sugar indicates the amount of reducible sugar, which is metabolized by microorganisms (here baker's yeast). The difference between the total sugar (or total sugar as invert (TSH)) and the fermentable sugar comprises the non-fermentable sugars. For the baker's yeast, such a sugar can be raffinose, which is a trisaccharide.

In this method, yeast is permitted to ferment sugars in a molasses sample. The amount of CO_2 evolved is used as a measure of fermentable sugar content, by comparing the volume of CO_2 obtained with that produced from the fermentation of known amount of reducible sugar, like sucrose or glucose.
Sucrose is used as the reference sugar when analysing beet molasses.
For this measuring method it is not necessary to convert the pressure rise [mbar] to CO_2 [ml], as the test requires a calibration for each test with standard sugar solutions. As the reference method (Risograph™) works with [ml CO_2], the results were displayed also in this unit.

SOP R 10 is run at the VH with the Risograph™ (type 1260-R) from National Instruments. The Risograph has up to 12 channels, in which the sugar test can be done. The comparison with the ANKOM system is run either in parallel or, if not enough space was left in the water bath, at another day. Molasses and other high sugar containing materials are usually stable over weeks / months. Microbial contamination (degradation) has not been detected.

The preparation of the test for the ANKOM system was performed analogue to the VH SOP R 10 protocol.

1.2 SOP R 17 Determination of the alcohol yield and the inhibition of fermentation of molasses

The SOP R 17 is divided into two parts. The first part is the determination of fermentation speed, which is indicated by the weight loss [g] due to CO_2 evolved and released over time. The comparison between the two curves (treated and untreated molasses) can show the presence and the effect of possible inhibitory substances (like volatile acids), which can be removed by molasses treatment (heat + acid treatment). The second part is the determination of the produced alcohol content by distillation. This part is analogue for the ANKOM system.

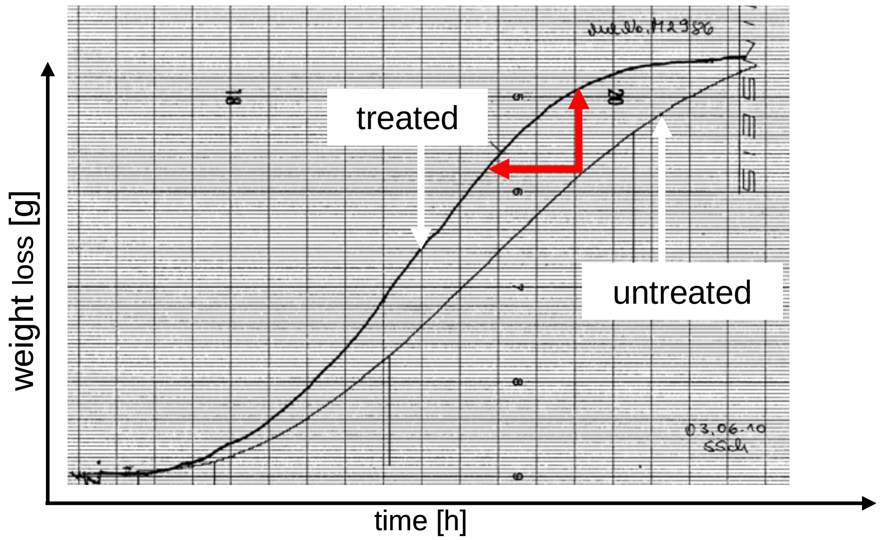

Fig. 1: Typical diagram weight loss over time for anaerobic molasses fermentation VH SOP R 17 (M 2986). Red arrows indicate the effect of inhibitory substances, detected by offset and lag time.

Changes between the VH SOP and ANKOM

The ANKOM system is measuring the pressure rise caused by CO_2 (gas) evolvement. By knowing the pressure rise, it is possible to calculate the mol CO_2 / weight loss due to gas release (the values in the figures Fig. 7, Fig. 8 and Fig. 9 are calculated as described here). The density change of the broth caused by alcohol production is not measured by the ANKOM system (forced weight loss).

Advantages could be to quantify the inhibition by correlating the curve area (integration, calculus) or by determination and correlating the maximum slope (first derivative, gas production rate) of the gas production curves (with and without treatment).

1.3 SOP R 22 Determination of toxicity factor (growth inhibition) of molasses

Changes between the VH SOP and ANKOM

The toxicity factor according to the VH SOP is tested in a 100 ml Erlenmeyer flask with a cotton plug. This open system releases the gas continuously, but due to the higher density of CO_2, there will be an oxygen barrier. Even with the magnetic stir bar, it is not expected to have a high oxygen uptake by the media. In order to compare the ANKOM system with the VH test, the optimal flask volume was checked. The oxygen uptake in this closed system is a function of the stirrer speed and the surface area. The stirrer speed has been kept constant, but the bottle size was varied (100 ml, 250 ml, 500 ml).

General consideration

One of the features of the ANKOM RF Gas Production System is the ability to configure system settings for specific tests. For our testing we used the settings as shown in Tab. 1.

Tab. 1: Settings for the ANKOM system for high gas production rates

live interval	1	[s]
pressure release	138	[mbar]
recording interval	1	[min]
valve open time	500	[ms]

General testing

Prior to the comparison to the VH-SOPs, the ANKOM RF Gas Production System was tested for capability and reproducibility. Fig. 2 shows the reproducibility of the system. Four bottles were filled with the same amount of sugar solution (0,25g sucrose / test) and were inoculated with a yeast suspension. The test was run according to SOP R 10.

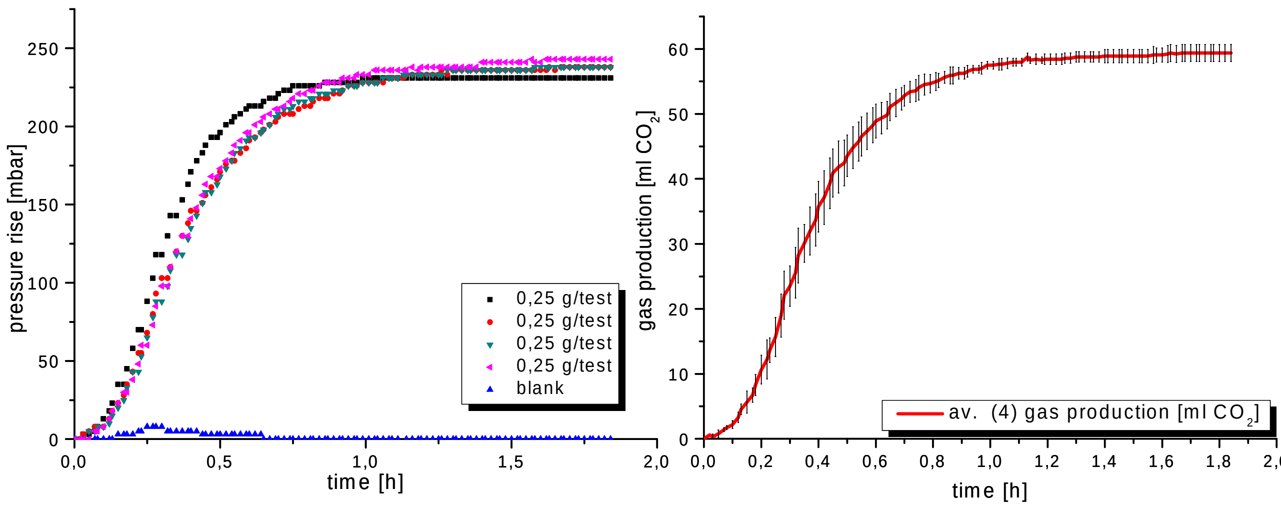

Fig. 2: (left) Single gas production curves (sucrose content 0.25 g/test) for the vessels 1-4. (right) Four averaged values (red line) with standard deviation (black error bar).

The system showed a good reproducibility and gas tightness if the system is handled properly.

The accuracy is sufficient but it is recommended to run duplicates and to use the average value. If a vessel is not airtight, the single value can be used.

The tested working range for the VH SOP R 10 is between 0 g sucrose / test and 1.0 g sucrose / test (working volume 50 ml → $c_{sucrose}$ = 20 g/l). The linearity of this SOP was tested on the ANKOM system. It is advantageous to know the fact that the method can be used even at higher concentrations than 1 g / test, because the method doesn’t need to be adapted if substrates with higher sugar concentrations (i.e. starch hydrolysate, thick juice, HFCS) need to be tested. Fig. 3 shows the results for a 4-point calibration with pure sucrose solutions up to 1.75g sugar / test. The resulting linear calibration based on the maximum CO_2 produced gave a very good correlation (R^2=0,9994).

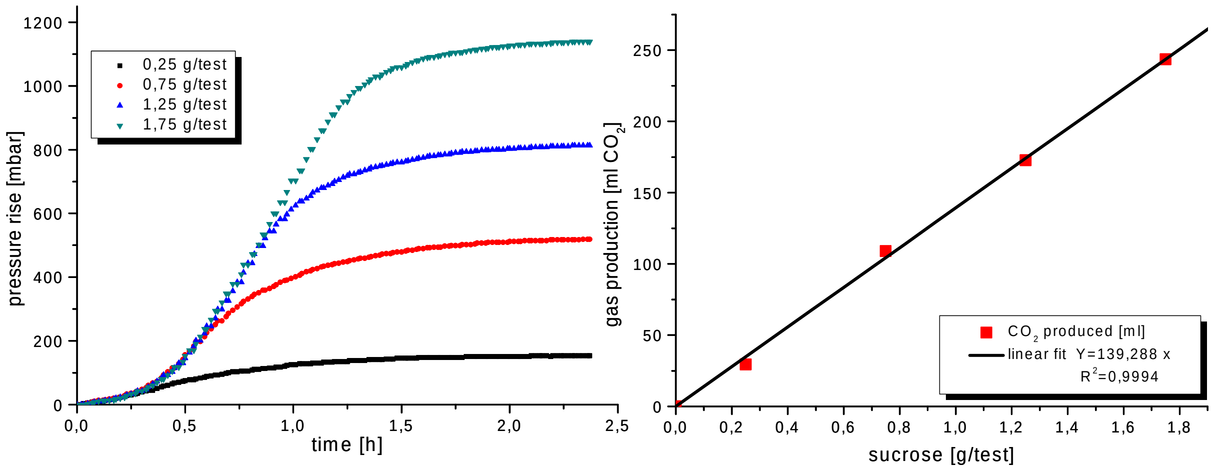

Fig. 3: (left) pressure rise over time for different amounts of sucrose (0,25 g; 0,75 g, 1,25 g, 1,75 g added to the test bottles. (right) Linear calibration over the measuring range (R^2=0,9994)

Results: Comparison between VH method and ANKOM system

1.4 SOP R 10

A comparative test of the ANKOM system with the VH SOP R 10 showed a good correlation. The results for the fermentable sugar for M 2994 and M 2988 are shown in Tab. 2.

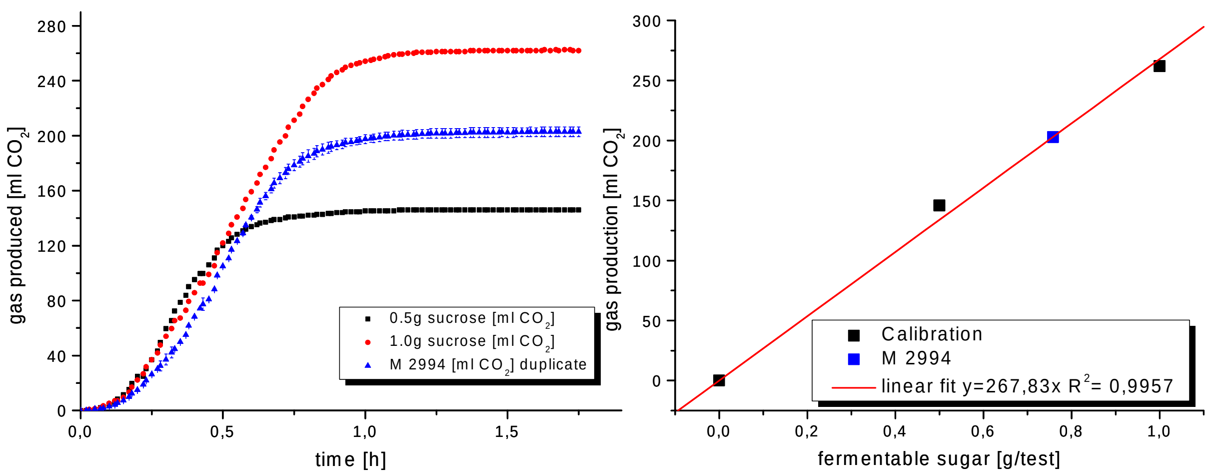

Fig. 4: (left) ml of gas produced over time for two sucrose concentrations (0.50 g / 50 ml (black); 1.00 g / 50 ml red)) and 1.52 g molasses M 2994 (duplicate) added to the test bottles. (right) Linear calibration (R^2=0,9957) and fermentable sugar content of the molasses (blue).

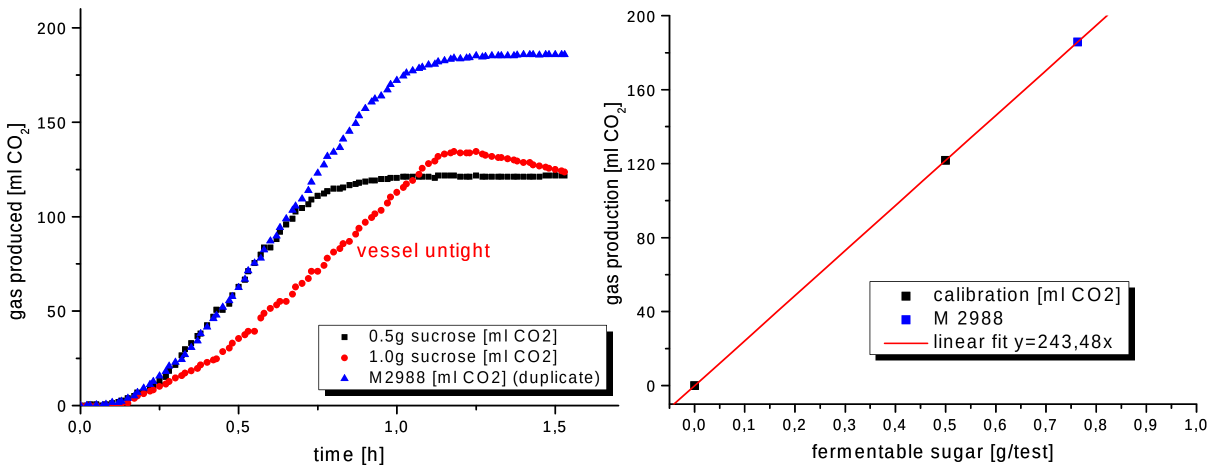

Fig. 5: (left) ml of gas produced over time for two amounts of sucrose (0.50 g / 50 ml (black); 1.00 g / 50 ml (red → vessel untight, not taken for calibration)) and 1.52 g molasses M 2988 (duplicate).. (right) 2 point linear calibration and fermentable sugar content of the molasses (blue).

The results show a very good correlation for the molasses M 2994, where the value for the fermentable sugar between ANKOM and VH only differed by 0.29%. This is equivalent to a variance of 0.58%. The VH method itself has a standard deviation of 0.28%.

The comparison between VH and ANKOM for molasses M 2988 was much weaker (see Tab. 2), which can be caused by the use of a 2 point calibration instead of a 3 point calibration.

Tab. 2: Comparison of the results SOP R 10 “fermentable sugar” for the molasses M 2994 and M 2988

		VH		ANKOM		
molasses	mass [g]	[g sugar / test]	fementable sugar [%]	[g sugar / test]	fementable sugar [%]	variance [%]
M 2994	1,52	0,762	50,16	0,758	49,87	0,58
M 2988	1,52	0,782	51,48	0,763	50,19	2,51

The results show that the VH SOP R 10, which is performed with a Risograph™ can also be accomplished with the ANKOM RF Gas Production System. In order to receive a good result, the calibration should be a three point calibration. Optimal would be to run calibration and sample in duplicates.

From our present state of research we can recommend the ANKOM system for the determination of fermentable sugars of molasses.

1.5 SOP R 17

Fig. 6, Fig. 7 and Fig. 8 show the results for the comparison of three molasses samples on SOP R 17. The results for Fig. 6 (ANKOM) are displayed as the gas production in ml, whereas the other figures are displayed as weight loss [g], which is the unit of the VH SOP. The shape of the curves, especially for Fig.

6 does not display the “amount of inhibition”, as it is shown by the balanced weight control. The problem here might be that the weight loss itself is a mixture between the loss of carbon dioxide and a weight loss caused by the alcohol formation (density change). The results for the alcohol yield are shown in Tab. 3.

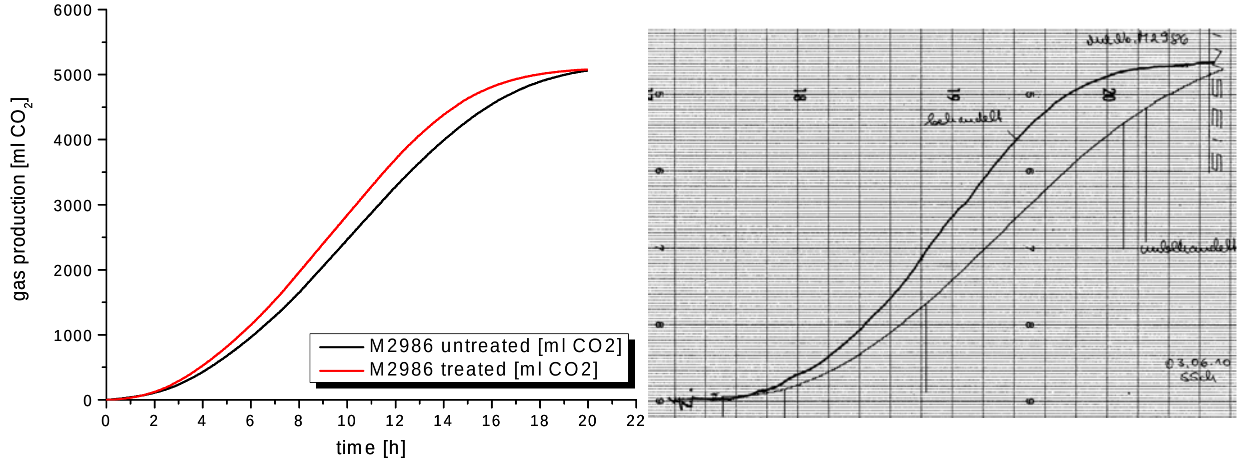

Fig. 6: (left) ml of gas produced over time for a sample of treated (black) and untreated molasses (red) measured with the ANKOM system. (right) Scan of the test results for the same molasses sample (M2986) recorded on the VH system.

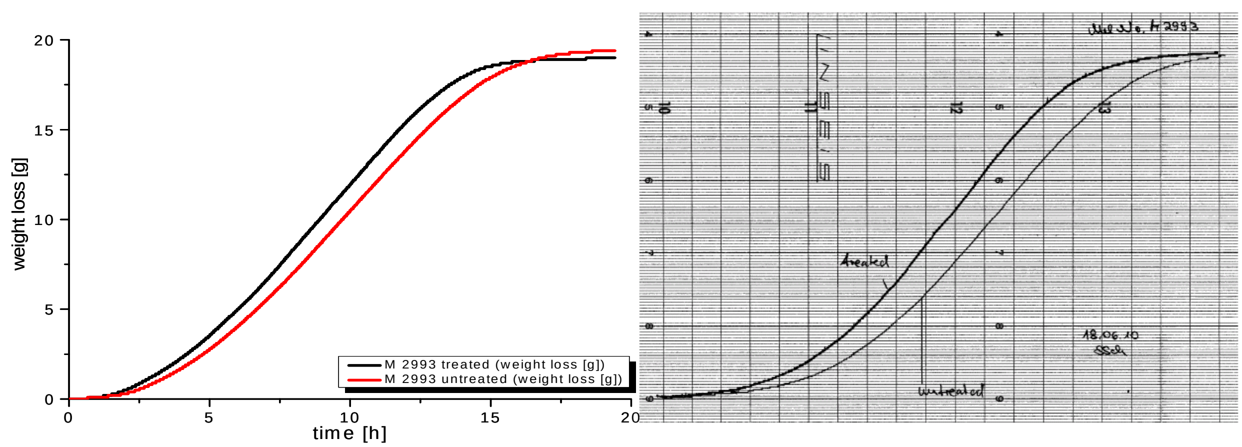

Fig. 7: (left) weight loss (calculated by gas loss) over time for a sample of treated (black) and untreated molasses (red), measured with the ANKOM system. (right) Scan of the test results for the same molasses sample (M2993) recorded on the VH system (weight loss over time).

Tab. 3: Comparison of the results SOP R 17 “Ethanol yield” for the molasses M 2986, M 2993 and M 2994

	VH			ANKOM		
molasses	inhibition	alcohol yield [l EtOH / 100kg molasses]		inhibition	alcohol yield [l EtOH / 100kg molasses]	
		treated	untreated		treated	untreated
M 2986	yes			slightly		
M 2993	slightly	30,0	30,5	slightly	30,0	30,0
M 2994	no	29,5	30,0	no	30,0	30,0

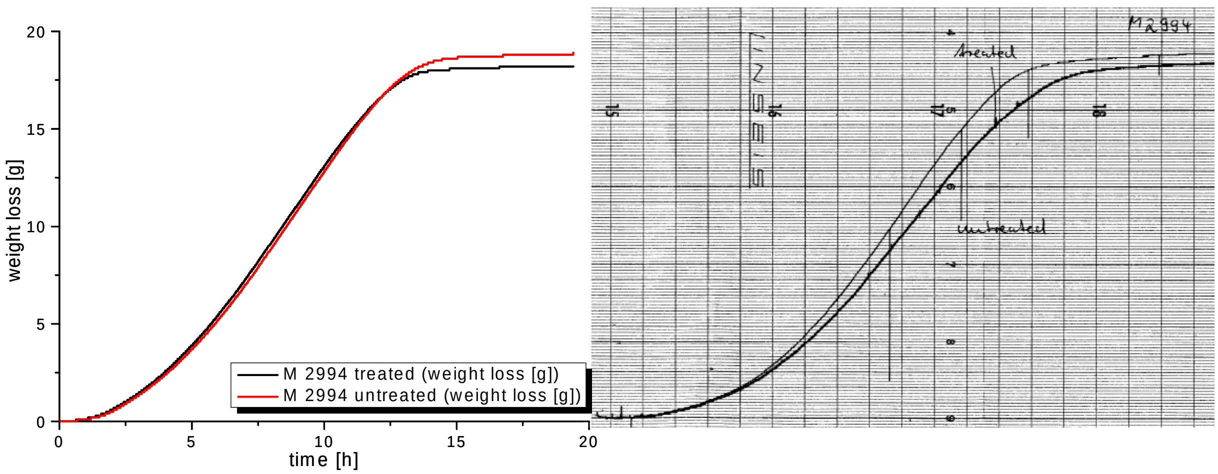

Fig. 8: (left) weight loss (calculated by gas loss) over time for a sample of treated (black) and untreated molasses (red) measured with the ANKOM system. (right) Scan of the test results for the same molasses sample (M2994) recorded on the VH system (weight loss over time).

As mentioned above, the results generated with the system from ANKOM are not 100% comparable to the VH-SOP. The alcohol yield is identical, but the curves don’t generate the same information. This can be seen on Fig. 9. The advantage of the ANKOM system would be that the results for the “toxicity” of the molasses before and after treatment can be displayed as an additional figure in the same test. It is possible to compare the areas under the curves (integral). For molasses M 2986 the area for the untreated molasses is 49488 and for the treated molasses 53875 (equal to 100 %). In relation we could say that the toxicity of the molasses could be reduced by 8 % by acid and heat treatment. Using the maximum gas production rate (first derivate / slope) is not advantageous, because the difference is minimal (maximum slope 3.4 % difference between treated and untreated molasses sample M2993) and does not contain information about the lag phase.

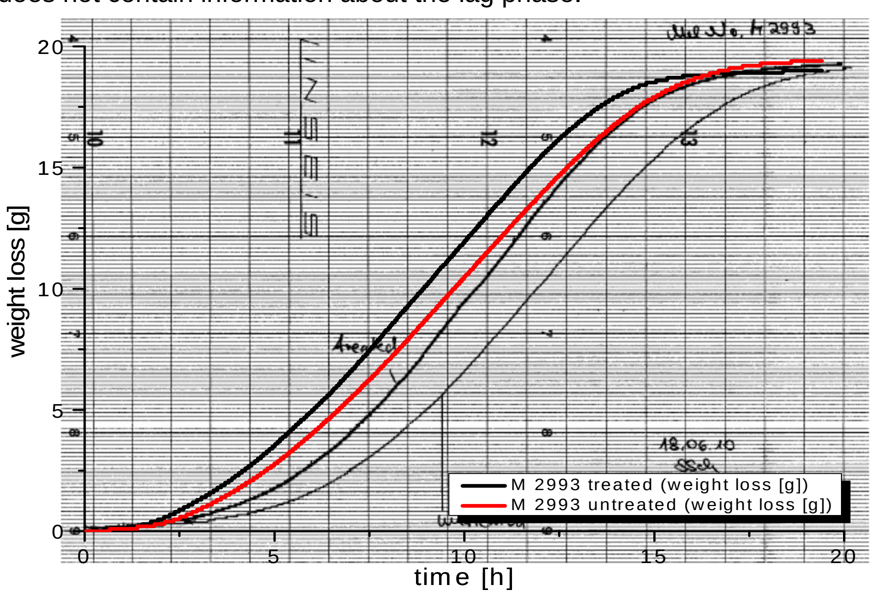

Fig. 9: Overlay of the VH and the ANKOM-curves for molasses sample M 2993

1.6 SOP R 22

Fig. 10 and Fig. 11 show the results for the comparison of different bottle volumes. Fig. 10 indicates that a bottle volume of 250 ml gives identical results to the method used by the VH. Fig. 11 additionally shows that the wet biomass content still rises for bigger bottle volumes (bigger surface area). Further tests were carried out with the 250 ml bottles, which were part of the ANKOM system.

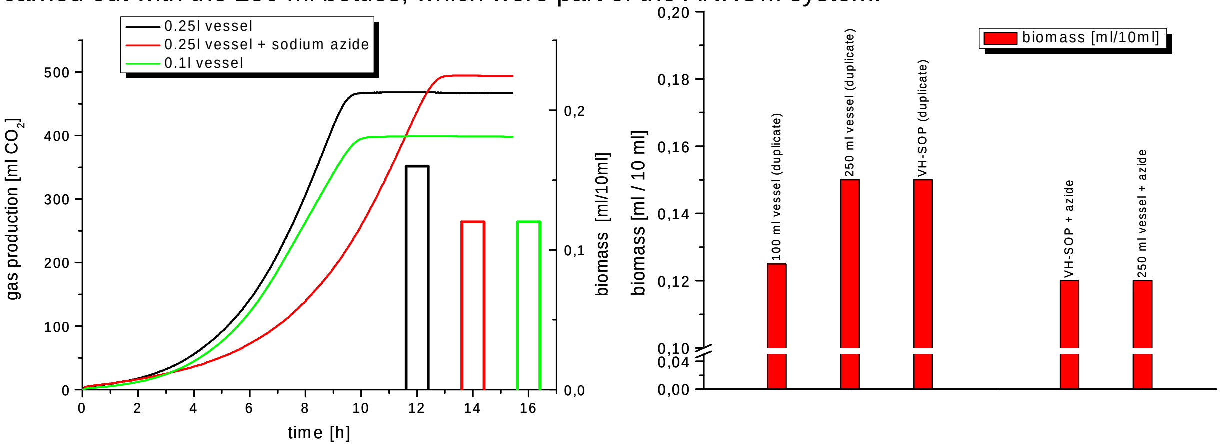

Fig. 10: (left) ANKOM results CO_2-production and biomass production [ml / 10 ml]. (right) Comparison between VH results and ANKOM results on biomass production.

The right part of Fig. 10 shows a direct comparison of the biomasses, generated with the two different systems. The amount of biomass for our standard molasses was 0.15 ml / 10 ml of fermentation broth. The biomass value is determined by centrifugation. The value for the biomass production with an inhibitor (respiration inhibitor sodium azide) added is shown in the same diagram. Only 0.12 ml / 10 ml were reached. These values were directly comparable between the two systems. The same molasses, run with a 100 ml vessel only, received a biomass content of 0.13 ml / 10 ml.

Fig. 11 left shows comparable gas production profiles. But for the test itself the bars are more interesting. The comparison between the 500 ml vessel and the 250 ml vessel showed, as expected, a slightly higher biomass growth for the big bottle.

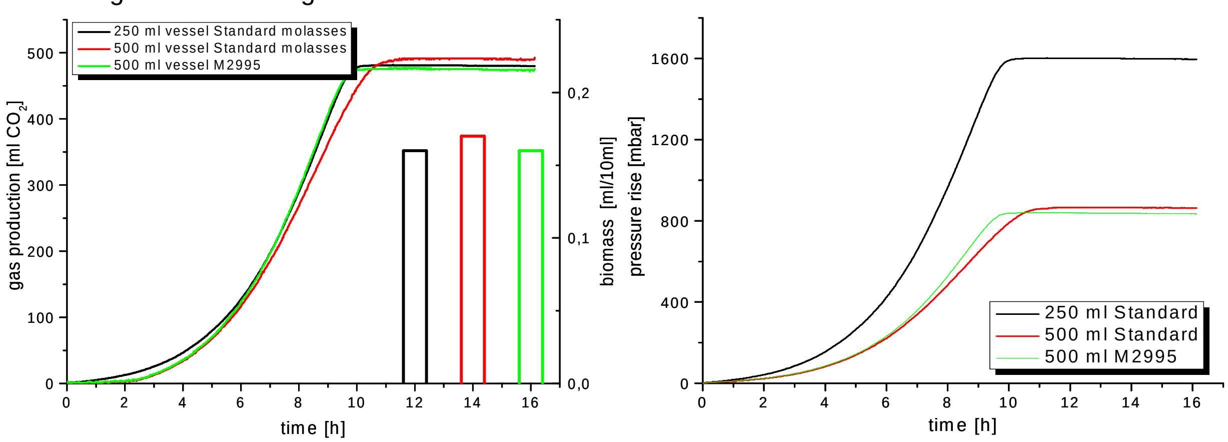

Fig. 11: (left) ANKOM results CO_2-production and biomass production [ml / 10 ml]. (right) Pressure rise of the same sugar solutions with different bottle sizes.

Fig. 12 shows a direct comparison between the ANKOM and the VH system, but without any pressure release while the experiment was running. This possibly caused a reduced biomass volume by 0.01ml in comparison to the VH measurement. If the pressure release is set to 138 mbar, the systems give similar results on the biomass production.

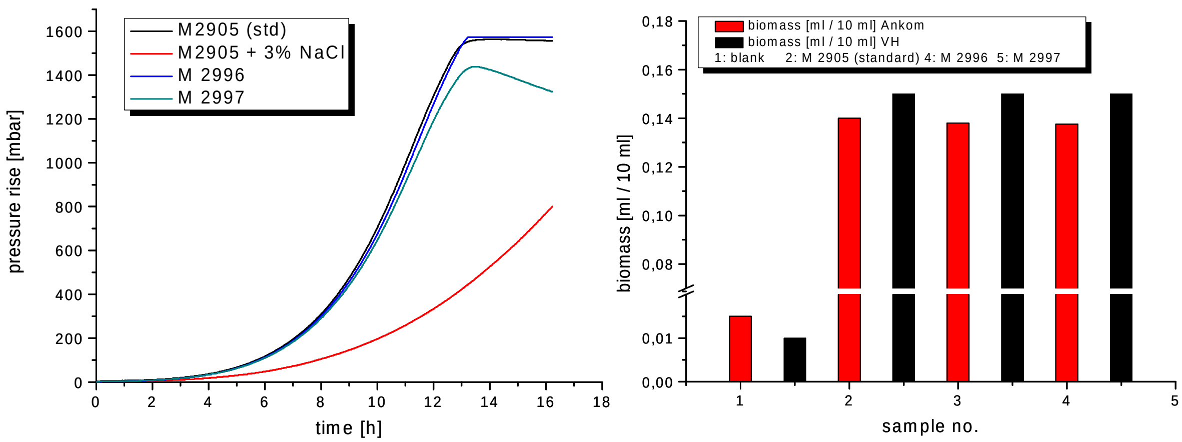

Fig. 12: (left) ANKOM results pressure rise [mbar]. (right) direct comparison of the biomass production between the VH SOP (black) and ANKOM (red).

The VH SOP R 22 primarily looks after the biomass, which was grown in a media with a certain amount of sugar. An advantage of using the ANKOM system is to see when the conversion and biomass production is finished. However, this is not the only advantage of the ANKOM system. When additional tests with inhibitors were performed, Fig. 13 shows the inhibitory effect of sodium chloride in different concentrations and Fig. 14 shows the inhibitory effect of calcium propionate in different concentrations on the a) respiration and b) on the biomass yield.

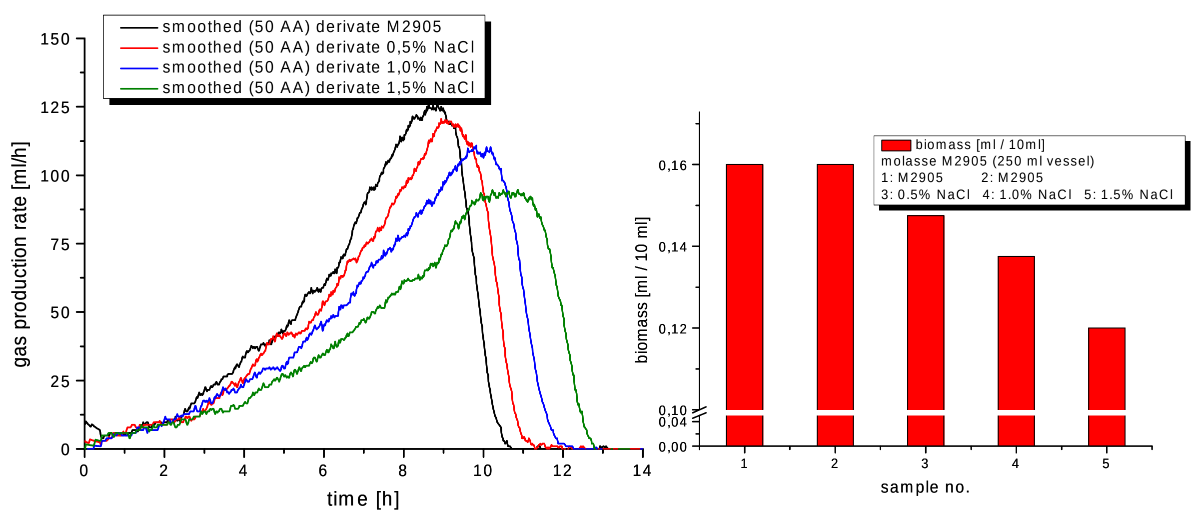

Fig. 13: (left) ANKOM results gas production rate over time as a function of NaCl-content [ml / h] (1^{st} deviation of gas production data). (right) biomass production depending on the NaCl-concentration.

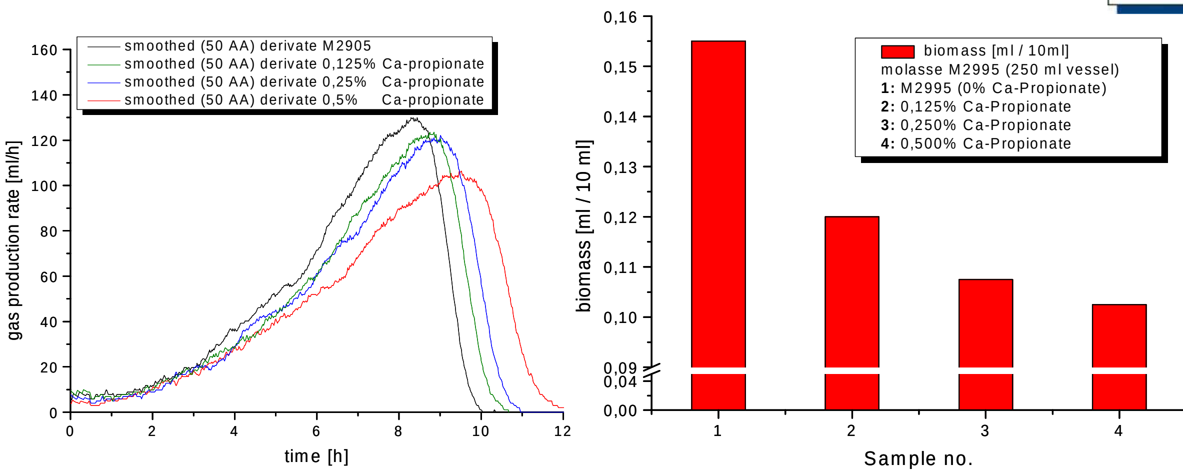

Fig. 14: (left) ANKOM results gas production rate over time as a function of Calcium propionate-content [ml / h] (1^{st} deviation of gas production data). (right) biomass production depending on the Calcium propionate -concentration.

The results show that the VH SOP R 22 can also be accomplished with the ANKOM RF Gas Production System. In order to receive comparable biomasses, it is necessary to run the tests in 250 ml bottles. Running the SOP R 22 with the ANKOM system gives additional information like the gas production rate (see Fig. 13 (left) and Fig. 14 (left)) which can help to quantify effects of media components. From our present state of research we can recommend the ANKOM system for the determination of the toxicity of molasses.

Conclusion

The comparative tests between the VH SOP R 10, R 17 and R 22 showed very good results. The ANKOM system is easily set up and the handling is good. The VH confirms that the ANKOM equipment is usable for the SOP R 10 and SOP R 22. While using the ANKOM system for R 17 showed a reduced information content of the “weight-loss-curves”. The influence on the alcohol conversion speed (inhibition by toxic, volatile components in the molasses) is not accurately shown as it is displayed with the VH-system (weight loss recorded by balanced fermenter). For the determination of the ethanol yield, both systems deliver equal results.

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Appendix: List of tables and figures