YEAST VITALITY ANALYZED FOR THE FIRST TIME WITH PARTICLE CHARGE

CONTEXT

Yeast vitality is, among other things, an important parameter for alcoholic fermentation and beverage quality. [1/2] Using the example of beer, good yeast vitality can be seen in the color of the beer, the taste, and the stability of the foam.[1]

The determination of the vitality of the yeast is currently carried out with a time-consuming and expensive cytometer. With the help of particle charge determination and combined charge titration, a cost-effective and fast method is available. [1]

PARTICLE CHARGING IN TITRATION

Whether the interface is a smooth surface, a particle surface, or a molecular surface, the same laws apply to all of them. A charged interface attracts opposite-polar excess ions from the liquid environment, which shield the charge from the outside. The loosely bound ions of the charge cloud (double layer) can be sheared off by an electric field or by mechanical work (flow).

The potential that forms on the shear layer can be tapped with the STABINO ZETA measuring devices by mechanical shear as a flow potential signal or zeta potential ζ (***Figure 1***).

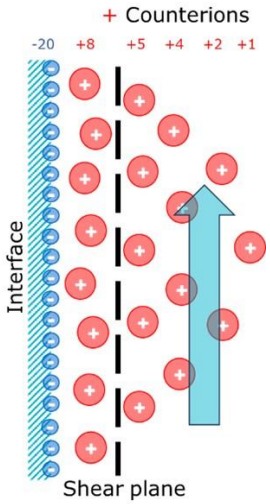

Figure 1: Schematic representation of the generation of the charge potential in the Shear plane.

In the measuring device, the sample is moved in a kind of capillary in the direction of the flow. The part of the particles adsorbed on the measuring cell walls is spatially fixed. The outer ions are sheared off from this layer, whereby an immediately induced measurement signal is picked up as a current or voltage (***Figure 2***).

This measurement signal represents the particle charge. During charge titration with charge-calibrated polyelectrolyte solutions, the charge of the particles is neutralized, and thus the total charge is determined. The mixing of the sample with the titrand solution takes just a few seconds, so that the next titration step can be performed immediately afterwards and the entire charge zero titration only takes a few minutes. This is an advantage that allows comprehensive ionic characterisation of macromolecules or particles.

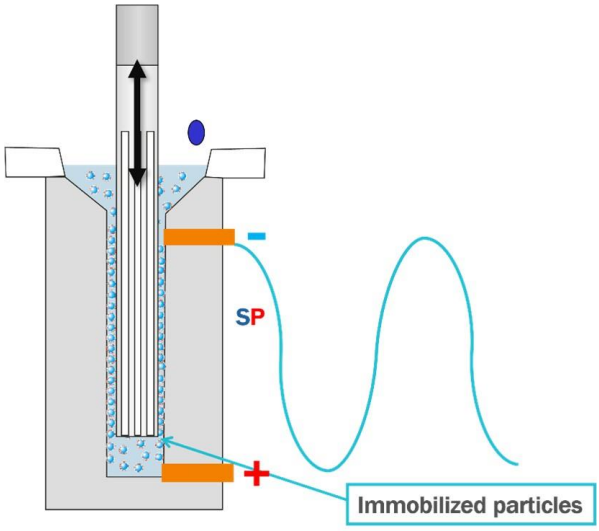

Figure 2: Electrical measurement of the particle interface potential in the STABINO ZETA by oscillatory displacement of the ion cloud around the particles adhering to the walls of the PFTE sample container and flask.

Displacement is caused by the upward and downward movement of the displacement piston (pistil). The resulting oscillating charge cloud is tapped as a sinusoidal flow potential (SP) / zeta potential (ZP) on the two electrodes and displayed as potential. Since this is an electrical measurement, a change in the potential of the particles is immediately indicated. The pistil provides both the measurement signal and rapid mixing with a titrande solution dripped from above. This allows for titration charges in minutes.

PERFORMING THE STRESS DETERMINATION ON YEASTS

For sample preparation, the yeast is transferred to a centrifuge cooled to 5° C for 5 min at 4000 rpm-1 and centrifuged. Subsequently, the yeast is redispersed twice in a physiological saline solution (0.1% peptone water) to wash the yeast. Afterwards, a live-dead determination is carried out using methylene construction. It is important to ensure that there is always the same number of cells as possible.[1]

To determine the vitality of the yeasts, stress tests with ethanol, oxidative (H_2O_2), osmotic, and due to nutrient deficiency were used.[1] Afterwards, the amount of charge of the differently stressed anionic samples was titrated by polyelectrolyte titration with a charge-calibrated cationic solution of 0.001N P-DADMAC (**poly-diallyl dimethylammonium chloride**) to the charge zero point in the STABINO ZETA[1] and compared with the reference yeast sample in each case. The yeast strain TUM 34/70 was used as the yeast strain.[1]

RESULTS

The titration curves of the 4 different stresses are shown below. At the end of the paper, the consumption of cationic Poly-DADMAC solution of stressed and respective reference samples is summarized in tabular form for an overview. For comparison, the CO_2 production was determined for all measured yeast samples.[1]

Stressing with ethanol

The stressing of the yeasts was started by adding 1 GG-% or 10 GG-% ethanol. The exposure time was one hour at a constant temperature. From the charge titration data, a significant difference between the different concentrations of alcohol can be recognised, as shown in **Figure 3**.[1]

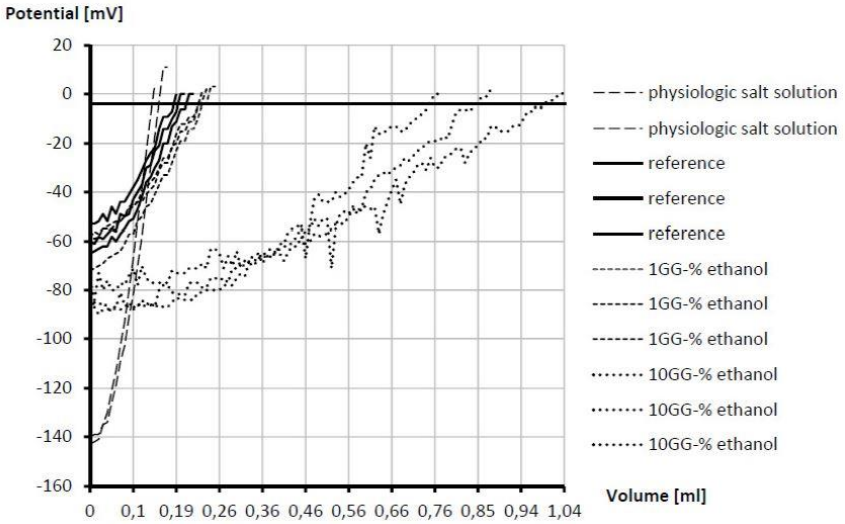

Figure 3: Stressing the yeast with 1 GG-% or 10 GG-% ethanol,[1]

With increasing stress, a decrease in potential and an increase in titration volume can be observed. These data are also in line with the measured CO_2 production.[1]

Oxidative Stress

The addition of 0.1 GG-% and 1 GG%-H_2O_2 to the yeasts triggers oxidative stress. Here, too, the exposure time was one hour at a constant temperature. From the data of the charge titration, as shown in Figure 4, a significant difference between the different concentrations of H_2O_2 can be seen.[1]

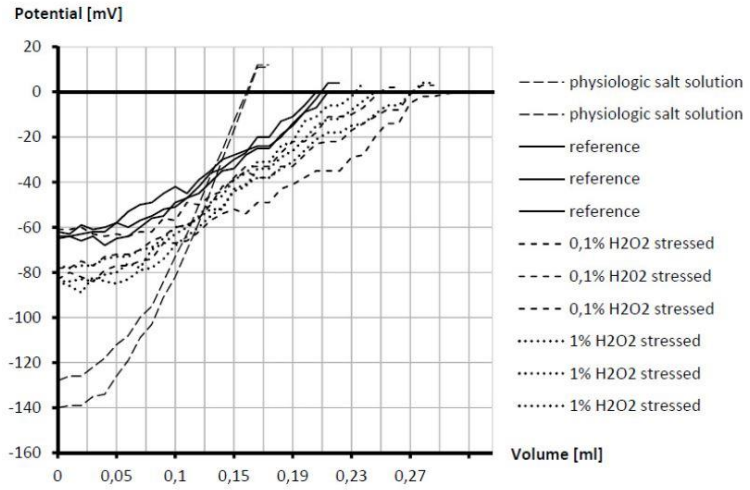

Figure 4: Stress of the yeast with 0.1 GG-% and 1 GG-% H_2O_2,[1]

Osmotic stressing

Osmotic stressing is achieved by adding 15 or 20 WEIGHT-% sorbitol. Yeast vitality dropped to 92% in this test. The degree of stress is similarly high at both concentrations, as can be seen from ***Figure 5***.[1]

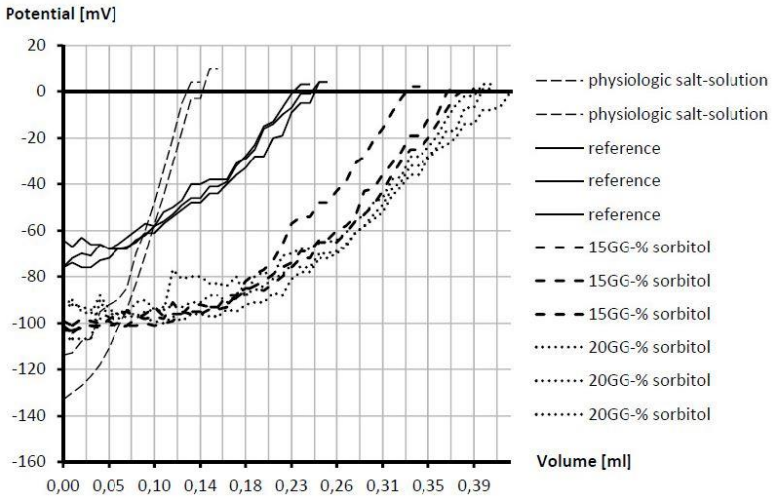

Figure 5: Stressing of yeast with 15 or 20 GG-% sorbitol,[1]

Stress due to food deprivation

The addition of distilled water simulates food deprivation. This type of stress has only a minor influence on yeast vitality (***Figure 6***).[1]

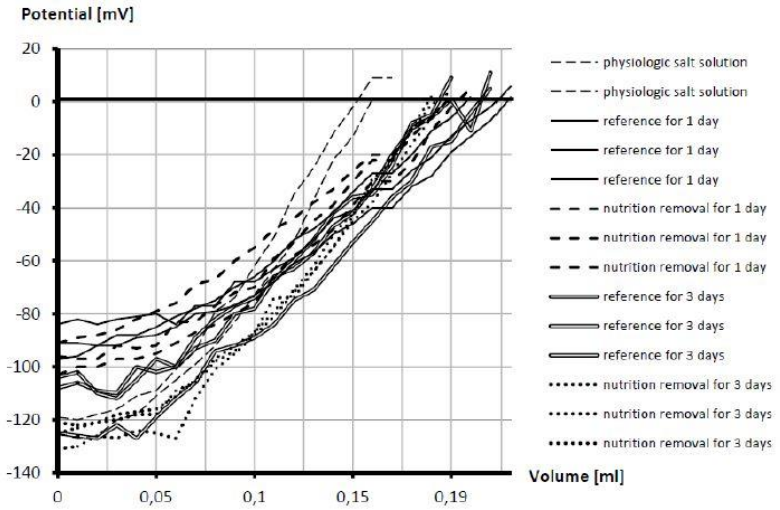

Figure 6: Stressing of yeast due to food deprivation with the addition of distilled water after one and three days, taken from [1]

Table 1: Overview of the measurement results

Stress influence	Verb. /Ml	Verb. /Ml	Verb. /Ml	Verb. MW/ml	Standard deviation	Differential consumption MW/ml
Reference	0,19	0,20	0,21	0,20	0,01	
1% **Ethanol**	0,24	0,25	0,25	0,25	0,01	0,05
10% Ethanol	0,76	0,87	1,02	0,88	0,13	**0,68**
Oxidative Reference	0,22	0,23	0,23	0,23	0,01	
0,1% H^2O	0,28	0,30	0,34	0,31	0,03	0,08
^{2}1% H^2O^2	0,26	0,27	0,30	0,28	0,02	0,05
Osmosis Referenz	0,25	0,27	0,28	0,27	0,01	
15% Sorbitol	0,38	0,43	0,44	0,42	0,03	0,15
20% Sorbitol	0,45	0,47	0,49	0,47	0,02	**0,21**
Food deprivation Reference 1 day	0,21	0,23	0,23	0,22	0,01	
withdrawal 1 day	0,21	0,20	0,20	0,20	0,00	-0,02
reference 3 days	0,20	0,22	0,22	0,21	0,01	
withdrawal 3 days	0,19	0,20	0,20	0,19	0,00	-0,02

Table 1 shows that the stressing by 10% ethanol and by osmosis was most effective. Food deprivation by distilled water showed no effect.

SUMMARY

In conclusion, the flow potential measurement in combination with charge titration represents a new and simple method for yeast vitality measurement. All measured data are consistent with the CO_2 produced.[1] In addition to the determination of the yeast vitality, the STABINO ZETA charge determination system is also suitable for rapid turbidity prediction or stability determination.[3,4]

ACKNOWLEDGMENT

The STABINO ZETA charge titration device from Microtrac makes possible what optically designed zeta potential measuring devices simply cannot do due to the lengthy titration process on the one hand and the difficulty of conducting polyelectrolyte charge titrations on the other. It takes a pioneering spirit and courage to use a lesser-known method such as the charge titration of the STABINO ZETA, the predecessor device, to enter uncharted territory as described here [1].

These pioneering spirits also include Titze, Ilberg et al.[4], who recognized the potential of the charge titration method and were the first to use it to predict turbidity stability. We have reported on this [3,4]. Therefore, We are pleased to present another groundbreaking research result with this yeast study.

LITERATURE

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- J. Titze, V. Ilberg, F. Jacob, H. Parler: 2008, Possible applications of the charge titration method for the assessment of chemical-physical beer stability Part 1 & Part 2 Brauwelt 148 No. 18/19, 23, pp. 506-509, pp. 624-627. [4]