

Technical University Munich
Brewery Technology Science

University Prof. Dr. L. Narziß
University Prof. Dr. W. Back - Department of drinkstechnology -

8050 Freising - Weihenstephan

Germany

**APPROVAL
MART ANOXOMAT**

*Automatic System for the
cultivation of beerspoiling anaerobic and
microaerobic organism*

Approval Anoxomat System WS8000

The Anoxomat is an automatic system for a rapid cultivation of microaerobic and anaerobic organism.

(System and functions are explained in the "User Manual" of the Anoxomat System WS 80, WS 8000, WS 8080, WS 9000 Mart B.V. Microbiology Automation).

In order to make this system especially functionable for the beerbrewery-microbiology testing and to optimize the use, an Anoxomat WS 8000 has been given for a certain period of time at the disposal of the Technical University of Munich to Prof. Dr. L. Narziß and Univ. Prof. Dr. W. Back, Department of Drinkstechnology.

The experiments were done with special organism specifically used by the brewery-industry during their day-to-day routine in the laboratory.

For this day to day routine in sampling-processing we have changed the program-sequence of the WS 8000 into an optimal sequence for the beerbrewery laboratory.

Machines and Materials used in the experiments

- **Anoxomat WS8000** with 3 connections for 3 anaerobic jars.
- **Vacuumpump**: capacity 1,5 M3 per hour
0,13 kW
- **Mart anaerobic jars AJ 9023 and AJ 9028** (for 12 - 36 petridishes)
- **Gasmixture**: 90% CO₂ + 10% H₂
- **AGAR**: NBB - A (beerspoiling bacteria)
- **AGAR**: OFS (drinkspoiling bacteria)

Results and conclusions

Microaerobic: the optimum program-sequence for cultivating microaerobic organism in an anaerobic jar:

Evacuationstep	Pressure reducing in a jar from ca. 980 mbar	Evacuated Jarvolume
1	800	20%
2	580	50%
3	580	50%

Total program time:

1 jar: 45 seconds
2 jars: 75 seconds
3 jars: 90 seconds

2. Cultivating of an anaerobic environment

10 x evacuation-steps, using a palladium coated catalyst.

Evacuationphase	Pressure reducing in jar from 980 mbar	Evacuated volume in jar
1	590 mbar	50%
2	590 mbar	50%
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10	590 mbar	50%

Total program time:

with connection of 1 jar: 2 min.
with connection of 2 jars: 4 min.
with connection of 3 jars: 6 min.

When repeating the number of evacuationsteps and at the same time reducing the evacuated jarvolume in connection with the standard programm settings, the membramfilter is lying fest on the agar. By doing this during the evacuationphase no air or whatsoever can come under the membramfilter. Only so, you will create the best conditions for an optimal agardiffusion.

One should always take care that the agar surface of the petridish is relatively scarce of humidity (slightly dried up in the incubator) in order to prevent crosscontamination of the different petridishes in the anaerobic jar.

Also take the following measurements always with cultivation with the Anoxomat:

- the Agar-surface should not be too humid. It is to be advised to take a 4-5 hours period for drying the fresh agar with 37°C (a longer dry-period may influence the prove of beerspoiling organism negatively) or a one-day storage at roomtemperature.

- the forming of too high level of condensation at filling the petridishes can be avoid by warming up the fluid agar at 48° - 50°C

- the anaerobic jars should not be packed completely full with petridishes (otherwise this may cause the forming of condensation inside the jar)

- all tubes should be cleaned regularly with hot water or alcohol (if it is appropriate for the material). When connecting the tubes to the instrument, these tubes should be completely dry inside.

- it is to be advised to check regularly the tubes and connectors on bacterial growth by taking wipe-samples.

- by intensive use of the tubes, little haircracks may arise. Therefore replace them once every year.

Prove of beer-spoiling bacteria

The prove of the for a beerbrewery very important microaerobic and other anaerobic bacteria on the agarsurface will be reached in the Anoxomat and in the expected period of time.

The especially oxygen-sensitive beerspoiling bacteria *Megasphaera* and *Pectinatus* can be proven fast and reliable with the Anoxomat-programm "anaerobic" and with an adequate treatment of the agar in advance.

Conclusions

The Anoxomat creates with the a.m. described programsteps a microaerobic environment within 90 seconds (when 3 jars are connected) and an anaerobic environment within 6 minutes (when 3 jars are connected). This short period of time is a major advantage especially for the evidence of anaerobic sensitive bacteria.

In order to prevent cross-contamination of the bacteria in the anaerobic jar, one should always be aware that the agarsurface before the programm is started, is slightly dry and there is no sweat anymore present. Also take into consideration a.m. advises.

The evidence of brewery relevant bacteria is very reliable, complete and in the expected period of time. Especially the non-complicated and fast control with the sample-processing, also with a high number of samples (> 24 petridishes), the Anoxomat is excellent in the day-to-day routine processing in the beerbrewery laboratory.

University of Technology of Munich
Faculty Beerbrewery technology
D-8050 Weihenstephan
Germany

(Univ. Prof. Dr. Werner Back)

List of bacteria cultivated with the Anoxomat

Lactobacillus:

L. lindneri L2/L7/L23
L. brevis L32/L219/L222
L. brevisimilis L43
L. frigidus L150

Pediokokken:

P. damnosus P59/P60

Others:

Pectinatus frisingensis
Megasphaera cerevisiae

Furthermore testsamples from the brewery were processed with the Anoxomat. Herewith a large number of beerspoiling Pediokokken and Lactobacillus were found.