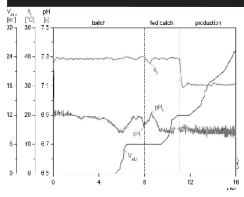
pH measurement in *Escherichia coli* fermentation with EASYFERM FOOD

Technical Note



Uninhibited cell growth due to new electrolyte FOODLYTE in the pH sensor EASYFERM FOOD

Industry: Biotechnology
Application: Fermentation / Protein Synthesis
HAMILTON product: EASYFERM FOOD VP, EASYFERM PLUS VP

In fermentations, it is very important that the cells have optimal conditions for growth and protein synthesis. To ensure this, none of the components should pose a threat to the micro organisms used in fermentation.

Electrolyte outflow from certain pH electrodes could possibly affect microorganisms used in fermentation in an undesired way.

One important design goal of the new HAMILTON pH sensor EASYFERM FOOD is to avoid any effect on micro organisms in fermentation. This is achieved by the new electrolyte FOODLYTE, which consists only of components compatible with food.

The EASYFERM FOOD electrode with the new electrolyte FOODLYTE was tested by MDT* and passed the cytotoxicity test in reactor volumes down to 500 ml (Worst Case Scenario). Therefore, this electrode is suitable for applications such as fermentation, where the risk of cell growth reduction or cell death must be excluded.

This application note is based on experiments that Dipl. Ing. Frithjof Tatge performed at the Hamburg University of Applied Sciences.

Measurement Equipment

The practical performance of the EASYFERM FOOD VP was tested in a Biostat® Q PLUS 6 Multifermenter with periphery from Sartorius Stedim Biotech. The system consists of 6 glass reactors (1l) which are equipped with DO-, temperature and pH-sensors (one EASYFERM PLUS K8 and one EASYFERM FOOD VP each). A gas mixing station ensures the required ratio of oxygen to nitrogen and a pump station allows Fed Batch operation. The measurement of oxygen and CO_2 is accomplished with sensors from BlueSens. The two supply units were connected to gas and cold water supplies for the reactors, stirrers, and sensors for DO, pH (EASYFERM PLUS K8 or EASYFERM FOOD VP), and temperature.

Measuring with EASYFERM FOOD/PLUS

Control of the exact pH-values is essential for the cultivation of *Escherichia coli* bacteria and production of proteins. Throughout the course of the procedure a constant pH value of the media at around 6.8 was obtained by addition of ammonia or phosphoric acid. The pH adjustment was based on values from the EASYFERM PLUS electrode in each reactor. EASYFERM

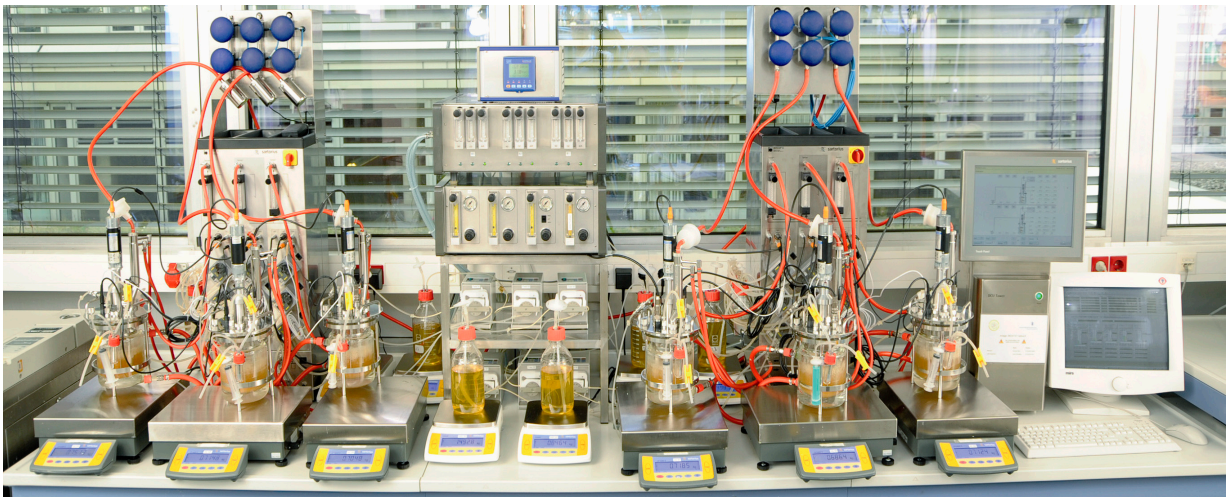


Figure 1: *Multifermenter BIOSTAT® Qplus 6 with periphery from the company Sartorius Stedim Biotech*

* MDT = Medical Device Testing GmbH Ochsenhausen, Germany

BIOSTAT® is a registered trademark of Sartorius Stedim Biotech

HAMILTON

FOOD electrodes were run in parallel to test the suitability of the new electrolyte FOODLYTE which consists only of components compatible to fermentation processes.

In a first step the pH sensors were calibrated in HAMILTON buffers pH 4.00 and pH 7.01. The sensors were then mounted into the medium filled reactor and autoclaved at 121°C for 15 minutes. After sterilization, the reactor content was cooled to 37°C and pH sensors were recalibrated.

In the "batch phase" of the reaction a constant oxygen flow (1.5 l/h) was led into the medium until the substrate (glucose) was consumed. In the following "fed batch phase" glucose was added and an oxygen air stream was fed into the reactor. Once the cell density of the *E. coli* bacteria reached 15 g/l, IPTG (Isopropyl-β-D-thiogalactopyranoside) was added to the mixture. This induced the "production phase" and the temperature decreased to between 31 and 25 °C, while the stirring speed was increased up to 1000 rpm. In this phase protein was produced and harvested for up to 20 h. At the end of the reaction the whole medium was autoclaved for a second time.

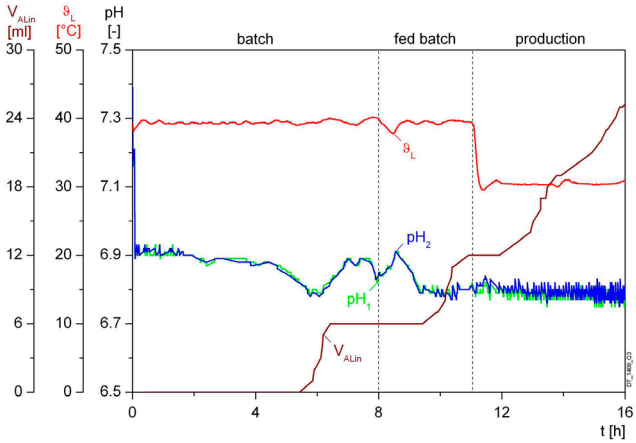


Figure 2: *Comparison of pH1 (EASYFERM FOOD 160 VP, S/N: 1003) with pH2 = Easyferm Plus 160 K8.*
ϑ_L: Temperature of the liquid phase
V_{ALin}: Added volume of ammonia for base titration

Result

EASYFERM FOOD delivered pH values of high accuracy and reproducibility that were comparable to those of the established and proven EASYFERM PLUS. All calibrations were in the accepted range.

Both EASYFERM PLUS and EASYFERM FOOD have successfully shown their ability to be used in fermentation processes. The fact that EASYFERM FOOD was designed and certified for excellent biocompatibility makes it a first choice in biotechnological, pharmaceutical and food applications.

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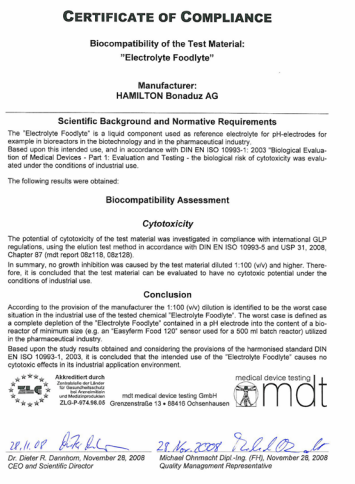
User benefits of EASYFERM FOOD:

- No inhibited cell growth due to the food safe FOODLYTE gel reference electrolyte
- Cleanability tested and certified according to EHEDG criteria
- Biocompatibility tested and certified by MDT (Medical device testing, Ochsenhausen/Germany)
- Suitable for small reactor volumes down to 500 ml
- Measurement with high accuracy and reproducibility



CERTIFICATE OF COMPLIANCE

Biocompatibility of the Test Material:
"Electrolyte Foodlyte"

Manufacturer:
HAMILTON Bonaduz AG

Scientific Background and Normative Requirements

The "Electrolyte Foodlyte" is a liquid component used as reference electrolyte for pH-electrodes for example in bioreactors in the biotechnology and in the pharmaceutical industry.
Based upon this intended use, and in accordance with DIN EN ISO 10993-1: 2003 "Biological Evaluation of Medical Devices - Part 1: Evaluation and Testing - the biological risk of cytotoxicity was evaluated under the conditions of industrial use.

The following results were obtained:

Biocompatibility Assessment

Cytotoxicity

The potential of cytotoxicity of the test material was investigated in compliance with international GLP regulations, using the elution test method in accordance with DIN EN ISO 10993-5 and USP 31, 2008, Chapter 87 (mdt report 08z118, 08z126).
In summary, no growth inhibition was caused by the test material diluted 1:100 (v/v) and higher. Therefore, it is concluded that the test material can be evaluated to have no cytotoxic potential under the conditions of industrial use.

Conclusion

According to the provision of the manufacturer the 1:100 (v/v) dilution is identified to be the worst case situation in the industrial use of the tested chemical "Electrolyte Foodlyte". The worst case is defined as a complete depletion of the "Electrolyte Foodlyte" contained in a pH electrode into the content of a bioreactor of minimum size (e.g. an "Easyferm Food 120" sensor used for a 500 ml batch reactor) utilized in the pharmaceutical industry.
Based upon the study results obtained and considering the provisions of the harmonised standard DIN EN ISO 10993-1, 2003, it is concluded that the intended use of the "Electrolyte Foodlyte" causes no cytotoxic effects in its industrial application environment.

Akkreditiert durch ZLG-P-074.96.05
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28.11.08
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Acknowledgement

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*Hamburg University of Applied Sciences

Technical data EASYFERM FOOD VP

Range of measurement:	pH 0 - 14
Zero point:	0 ± 20 mV
Sensitivity:	57 - 59 mV / pH at 25°C
Operation temperature:	0 - 135°C
Pressure range:	0 - 6 bar
Temperature sensor:	Pt 100
Measurement principle:	Potential measured against reference
Shaft material:	Glass
Membrane glass:	HAMILTON type "HB" glass
O-ring material:	FDA approved EPDM
Electrolyte:	Pressurized FOODLYTE
Diaphragm material:	HP COATRAMIC

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