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Process Development for Silage Inoculants – Optimization of *Lactobacillus sp.* Fermentation with Parallel Bioreactor Systems

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Abstract

This application note describes the process development of a *Lactobacillus sp.* fermentation for use as silage inoculant at BIOMIN® Research Center. To determine the optimum parameters for maximal yields of active cells an Eppendorf DASGIP® Parallel Bioreactor System was utilized. Sufficient amount of data was generated for statistical evaluations in order to optimize medium composition and growth conditions.

Introduction

Silage is an animal feed ingredient produced by controlled fermentation of crops with high moisture content. The main objective of ensiling is the achievement of anaerobic conditions as quickly as possible, which causes the inhibition of undesirable microorganisms such as clostridia and enterobacteria. Thus, the nutritional value of the original crop is preserved. The optimum conditions can only be guaranteed by quick filling and proper sealing of the silo, in order to provide the necessary conditions for the following fermentation process. The fermentation process can be accelerated and improved by the addition of homofermentative and/or heterofermentative lactic acid bacteria (LAB).

The following application note describes the fermentation process development of *Lactobacillus sp.* for the application as silage inoculant using an Eppendorf DASGIP Parallel Bioreactor System. In the experiments eight parallel bioreactors were used simultaneously to test growth parameters. All experiments were carried out at the BIOMIN Research Center in Tulln, Austria. The BIOMIN Holding GmbH is a research-oriented company whose objective is to improve animal health and the economic production of animals.

Its core business is the development and manufacturing of innovative and natural feed additives and preservatives to stabilize feed materials.

The main objective of the process development was to determine the optimum parameters leading to a maximum yield of active cells. These parameters were pH, temperature, agitation, consumption of base, the media components

Figure 1: 4-fold DASGIP Parallel Bioreactor System for microbial applications

and their concentrations. The Eppendorf DASGIP Parallel Bioreactor System allows testing of different parameters in parallel fermentations at the same time. Therefore, a sufficient amount of data could be generated for statistical evaluations to optimize medium composition and growth conditions.

In this application note the optimization of temperature and pH is described.

Material and Methods

All experiments were carried out using various LAB strains in media containing for example glucose, yeast extract, peptone and salts. The fermentation time depended on the glucose concentration in the medium.

The experiments were carried out with an 8-fold DASGIP Parallel Bioreactor System with 1.5 L vessels. The initial fermentation volumes were 500 to 1000 mL. Subsequently, scale-up experiments were performed in a 20 L lab fermentor to verify the optimized parameters in pilot scale.

Key parameters during fermentation such as pH, temperature, agitation and most importantly base consumption were controlled online and documented with the software DASGIP Control 4.0*. The same parameters were also measured in pilot-scale fermentation allowing to correlate the results of both systems.

Results and Discussion

Fermentation process development could be performed successfully with the DASGIP Parallel Bioreactor System. All shown figures and data were obtained and analyzed with the Eppendorf software DASGIP Control 4.0.*

Figure 2 exemplarily summarizes the results of a parallel fermentation of *Lactobacillus sp.* for optimizing the growth temperature. Base consumption was used as proportional indicator for the growth-dependent acidification of the fermentation broth. The strain was cultivated at different temperatures ranging from 29 °C to 37 °C and the fermentation was finished after base consumption had stopped. For a detailed insight into the bacterial growth, the colony forming units (CFU) for strains obtained at different temperatures (29 °C, 30 °C, 33 °C, 34 °C and 37 °C) are illustrated in table 1.

Table 1: Evaluation of optimum growth temperature. Colony forming units (CFU) of *Lactobacillus sp.* obtained at different temperatures.

Temperature [°C]	CFU/mL fermentation broth
29	1.16×10^{10}
30	1.18×10^{10}
33	1.32×10^{10}
34	1.59×10^{10}
37	1.74×10^{10}

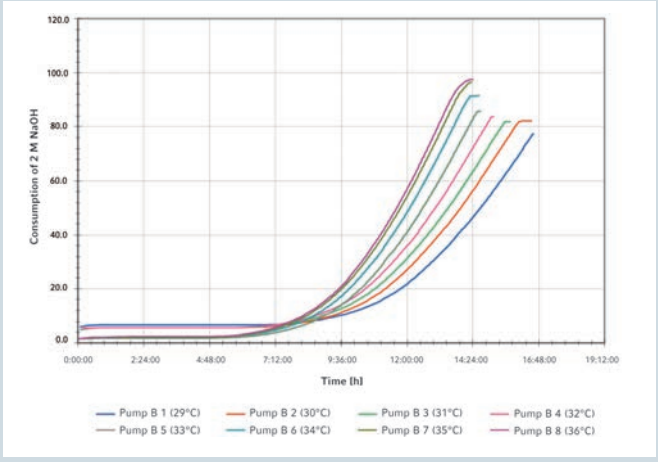

Figure 2: Growth progress depending on temperature. NaOH consumption was used as proportional indicator for the growth-dependent acidification.

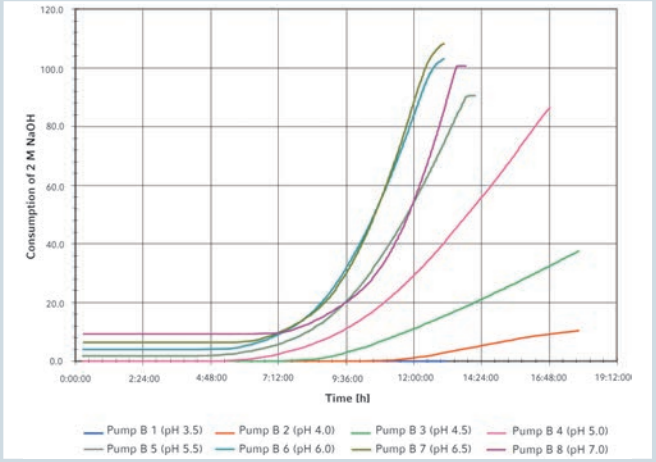

Figure 3: Growth progress depending on pH. NaOH consumption was used as proportional indicator for the growth-dependent acidification.

* DASGIP Control is now DASware® control 5. Please refer to ordering information on page 4.

Considering these values, a fermentation temperature of 37 °C turned out to be the most suitable temperature for cultivation of the used *Lactobacillus sp.*, verifying the results shown in figure 2.

In the next development step the optimum pH value was determined, again by using eight bioreactors in parallel. Figure 3 shows the base consumption during fermentation at different pH values. After fermentation the CFUs in each bioreactor were determined. The optimum was obtained at a pH value of 5.5. This result is in accordance with the base consumption (figure 3), which was also very high for pH 5.5. In table 2 the numbers of CFUs in the fermentors with different pH values are shown.

Table 2: Evaluation of optimum pH. Colony forming units (CFU) of L*actobacillus sp.* obtained at different pH values.

pH value	CFU/mL fermentation broth
3.50	2.85×10^9
4.00	1.18×10^{10}
5.50	1.69×10^{10}
6.00	1.54×10^{10}
7.00	1.02×10^{10}

Finally, the process developed with the DASGIP Parallel Bioreactor System was successfully transferred to 20 L pilot-scale fermentation, demonstrating the reliable scalability properties of the system.

Conclusion

The Eppendorf DASGIP Parallel Bioreactor System is very suitable for fermentation process development with anaerobic microorganisms. The system allows optimization of fermentation processes very effectively as different levels of certain parameters such as pH, redox potential, oxygen concentration and temperature can be tested at the same time. A particular advantage of the Eppendorf DASGIP Parallel Bioreactor System is the simultaneous calibration of pH, redox sensors and pumps which saves a lot of time during preparation of experiments.

The results obtained in the eight bioreactors are comparable among each other and show good reproducibility between different runs. Additionally, results are comparable to larger scale and therefor can be used for the efficient design of scale-up experiments.

Users at BIOMIN especially liked the attractive design of the DASGIP systems: „The whole set-up is very user-friendly, especially the self-explanatory software. Finally, the support from DASGIP service (since 2013 Eppendorf Bioprocess Center) is always on the spot and helps to handle challenges occuring, for example with installation of software updates."

Ordering information	Order no.
DASGIP® Parallel Bioreactor System for Microbial Applications, max. 250 sL/h gassing	
4-fold system with Bioblock	76DG04MBBB
8-fold system with Bioblock	76DG08MBBB
16-fold system with Bioblock	76DG16MBBB
4-fold system, benchtop	76DG04MB
8-fold system, benchtop	76DG08MB
16-fold system, benchtop	76DG16MB
DASware® control, incl. PC, OS, and licenses	
for 4-fold DASGIP® system	76DGCS4
for 8-fold DASGIP® system	76DGCS8
for 16-fold DASGIP® system	76DGCS16

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